
PROCEEDING
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**Shri Y. P. Gupta**
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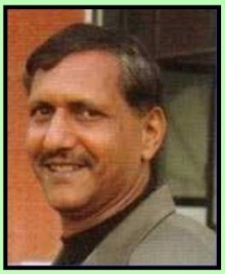
**Uttar Pradesh**

**Message**

**It gives immense pleasure to write for the proceeding of International Conference on "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development" jointly organized by MIT College of Pharmacy, and MET Faculty of Pharmacy collaboratively with AIRO Journals. Conferences of this magnitude help the graduate students and researchers to interact amongst themselves as well as experts in various areas. I feel that, with presentations and interactions with experts; students are exposed to the emerging trends in respective domains and the research work being carried out.**

**I would like to congratulate participants for contributing to this conference. I appreciate conference conveners, faculty coordinators, staff members of both the organization for organizing the International Conference focusing on Drug Discovery&Development.**



**Shri Adarsh Kumar Agarwal**
**Secretary**
**MITGI, Moradabad**
**Uttar Pradesh**

**Message**

**Education is always a sign of development and learning. It should be researchoriented helping society to create something new. Thinking in an innovative and new way is significant to cope with technological changes. This Conference provides a forum for scholarly discussion on drug development and discovery. It is a matter of great pleasure for us to host this International Conference "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development" jointly organized by MIT College of Pharmacy, and MET Faculty of Pharmacy collaboratively with AIRO Journals. It is also relevant for exploring and searching various aspects of education through the appropriate application for research. Presentation of research papers is extremely beneficial for research scholars and motivating factor for organizers to organize such conferences frequently in future. I extend my heartily congratulations to both pharmacy colleges and Airo Journals.**

**Shri Sudhir Gupta**
**Founder Chairman**
**MITGI, Moradabad**
**Uttar Pradesh**

**Message**

**MIT groups of Institutions, Moradabad holding the torch of illuminating new thoughts and fostering innovations for the generations to realize the vision through its innovative learning experience. In the same context, both our Pharmacy colleges have been extending their efforts to bring the Institute close to its purpose and mission. This conference is collaboratively organized with Airo Journalson "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development", which is again an interesting area of deliberation.**

**I welcome all the esteemed resource persons, delegates, sponsors, participants, organizing members and congratulate all for its grand success. I am confident that the participants will take optimal academic advantage of the two-day conference. It's my aspiration that this collaborative conference will be a base for new ideas towards Drug Discovery and Development.**

**I am happy and extend my sincere thanks to all the concerned for the grand success of the conference.**

**Shri Arvind K. Goel**
**Vice Chairman**
**MITGI, Moradabad**
**Uttar Pradesh**

**Message**

**It is truly fascinating to know that our two Pharmacy institutions, MIT College of Pharmacy and MET Faculty of pharmacy, were resolute in their decision to forge time, talent and even resources in order to realize this conference titled "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development" as the key to unlimited discoveries".**

**Surely, the organizers are with high hopes that academicians, scientists, pharmacists and students participating in this two days conference will be immensely enriched by interacting with the renowned speakers and presenters in this event. It is also my wish that, this type of conference may help the healthcare sector towards newer inventions. I complement my heartfelt gratitude for the efforts of everyone in creating this note-worthy engagement. On behalf of MITGI, I offer my warmest thanks to everyone.**

**Shri Neeraj Kumar Agarwal**
**Treasurer**
**MITGI, Moradabad**
**Uttar Pradesh**

**Message**

**It is my great honor and pleasure to invite you to participate in the "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development" collaboratively organized by MIT College of Pharmacy, and MET Faculty of Pharmacy collaboratively with AIRO Journals, from 15th of November to 16th November, 2024. At this conference let us celebrate what we, as a professional community, have achieved.**

**In addition, our future vision is to create even greater value to all corners of the globe. This conference will be one for us to share our thoughts and exchange ideas on how to chart our journey forward to reach new heights. Sincerely hope that this conference will deliberate on various issues that need to be addressed and it would be a memorable and productive as well. I extend my heartfelt wishes to all the participants who have contributed in making this conference a great success.**

**Shri Anil Kumar Agarwal**
**Joint Secretary,**
**MITGI, Moradabad**
**Uttar Pradesh**

**Message**

**This is indeed a matter of pleasure to witness the two-day international conference on "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development" from 15th of November to 16th November, 2024 jointly organized byMIT College of Pharmacy, and MET Faculty of Pharmacyin collaboration with AIRO Journals. In fact, success for any conference requires dedication, formalities and clear objective. This is good to know that both the institutes have collaborated well to organize it in a proper manner. The cohesive efforts of a dedicated and committed team become necessary for organizing such conferences. We are fortunate enough for having such a hardworking team with us.**

**I wish one and all my best regards for its success.**

**Shri Pradeep Jain**
**Vice chairman,**
**MITGI, Moradabad**
**Uttar Pradesh**

**Message**

**I am delighted in acknowledging the International Conference organized by MIT College of Pharmacy, and MET Faculty of Pharmacyin collaboration with AIRO Journals on "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development".**
**I appreciate the organizing committee for showing a keen interest in organizing a successful Conference and contributing new ideas and translating research into practice. I wish them for their endeavors to spread knowledge.**

**Prof. (Dr.) Neeraj Upamanyu,**
**Pro Vice Chancellor,SAGE University & Former CC member,**
**Pharmacy Council of India**

**Message**

**MITGI,Moradabad holding the torch of illuminating new ideas and fostering innovation for the generations to come. In the same context, the MIT College of Pharmacy has been extending its effort to bring the MITGI close to its purpose and mission. This international conference on "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development" has been scheduled on an interesting area of deliberation. I welcome all the delegates, sponsors, participants, organizing members and congratulate all for its grand success.**

**Prof. (Dr.) AkashVed,**
**Associate Dean& Nodal officer,**
**(AKTU, Lucknow) and**
**Central council member,**
**Pharmacy**

**Message**

**Education is always a sign of development and learning. It should be researchoriented helping society to create something new. Thinking in an innovative and new way is significant to cope with technological changes. This International Conference provides a forum for scholarly discussion on drug discovery and development. It is a great pleasure for me to join as chief Guest in this International Conference collaboratively organized by MIT College of Pharmacy and MET Faculty of Pharmacy along with MOU partner Airo Journals on "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development". Presentation of research papers is extremely beneficial for research scholars and stimulating factor for organizers to organize such conferences frequently in future. Wishing all my best regards.**

**Dr.Nitin Sharma,**
**Senior Physician,**
**Vinayak Multi-specialty**
**Hospital, Bareilly**

**Message**

**In the relentless task of nation building, MIT College of Pharmacy is committed to promote excellence in higher education for a sprightly and inclusive society through knowledge creation and dissemination of it. It is sincerely making all efforts to contribute by providing the right kind of human resources and heading towards accomplishing its mission to impart quality education. Thus, it encourages co curricular, extra-curricular and extension activities along with research and innovations. I am delighted to a part of the International Conference 2024 on "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development" collaboratively with Airo Journals. This is again a step ahead in expanding the horizon of our interest in research and innovation.**

**Prof.(Dr.)Nalini Kanta Sahoo,**
**Director and Principal,**
**M.Pharm, Ph.D, Post Doc (IUH, Vietnam), FSASS, SRAP, FSIRG**
**MIT College of Pharmacy**

**Message**

**It gives me great pleasure to express my greetings for the great success of International Conference as Convener and organizing Secretary. Education is an instrument to enhance the capabilities of human beings to become knowledgeable, creative and good citizens which resulted in my urge to develop excellent educational facility. I am extremely happy as convener, hosting International Conference on "Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development".**

**It is a justified gesture to provide a platform for graduate students and researchers to present their work and seek expert's evaluation that provides insight in the work undertaken. I hope that the presentations, discussions, appreciations and suggestions will help in improving their research work.**

**Such innovative and informative Conferences help the pharmacy students and researchers for close interaction amongst themselves, with experts and other delegates from various areas. I feel students are exposed to the emerging trends in respective domains. I would like to congratulate participants for contributing and presenting their research works in this conference. I appreciate faculty coordinators, staff members and Airo Journals for the untiring support in organizing such an International Conference focusing on drug discovery and development.**

**I extend my heartfelt wishes to all the participants who have contributed in making this conference a great success.**

**Prof. (Dr.) Sudhansu Ranjan Swain,**
**Director**
**MET Faculty of Pharmacy,**
**Moradabad**

**Message**

**Ilt gives me great pleasure to express my greetings for the great success of International Conference With the noble goal of providing students with the best technical knowledge and skills, nurturing scientific temperaments, identifying hidden talents, instilling professional ethics and human values, and giving them opportunities to realize their full potential.**

**Organizing this type of international conference not only encourage the young researchers to publish their research papers in journals of repute but also encourages the post graduate students how to write research papers with quality findings. In this conference more than 65 quality research papers are presented. This is a platform for young researchers to interact with experts from pharmacy professionals and clear their doubt regarding their research work as well as they can showcase their research work to scientific community.**

**My best wishes and extending my gratitude to the whole pharmacy Fraternity.**

Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development
November 2024

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Pharmaceutical Sciences 2024: Navigating the Future of Drug Discovery and Development
November 2024

# Physicochemical and pharmacokinetic Studies of Potential Drug Target Against Anti Diabetic Agent *Zingiber officinale*.

**Amrita Yadav1*, Uma Shankar Maurya2**

[1*]Department Of Pharmaceutical Chemistry, Goel Institute Of Pharmacy And Sciences, Faizabad Road, Near Indira Canal, Lucknow, UP, India, Pin – 226028

[2]Department Of Pharmaceutical Chemistry, Goel Institute Of Pharmacy And Sciences, Faizabad Road, Near Indira Canal, Lucknow, UP, India,Pin – 226028

***Corresponding Author Email Id:** amritayadavpharmacy@gmail.com

**Abstract:**

Diabetes mellitus is a global health issue that requires research into new treatment approaches. An anti-diabetic agent is any drug or substance that acts on glucose metabolism, enhances insulin sensitivity, or controls blood sugar levels in order to assist treat or manage diabetes mellitus. These agents may be herbal remedies, prescription medications, or lifestyle changes meant to manage the signs and consequences of diabetes. The herb ginger, Zingiber officinale, has long been known for its therapeutic benefits, which include the possibility of anti-diabetic effects due to the bioactive chemicals in the root. In this study, we investigated the interaction between natural chemicals from Z. officinale and important molecular targets implicated in the etiology of diabetes using in-silico analysis and molecular docking experiments. We identified significant protein targets, such as alpha- amylase, alpha-glucoside, and insulin receptor, and used computational algorithms to pick notable compounds from ginger, such as gingerols, shogaols, and paradols. The Z. officinale compounds may have anti-diabetic effects by modulating important enzymes involved in insulin signaling pathways and glucose metabolism. These molecular mechanisms may be the basis for these benefits. These results offer insightful information for additional experimental validation and the creation of innovative therapeutic medicines for the treatment of diabetes.

**Keyword:** Diabetes Mellitus, Zingiber officinale, ADMET, Anti-Diabetic.

## INTRODUCTION

Elevated blood glucose levels are the hallmark of diabetes, a chronic medical illness. It is mainly caused by problems with either the action or synthesis of insulin. The hormone insulin, which is secreted by the pancreas, is essential for controlling blood sugar levels(1). Blurred vision, weariness, increased thirst, and frequent urination are among symptoms. Type 1 Diabetes: Is an autoimmune disease in which the body is unable to manufacture glucose, a hormone that aids in blood sugar regulation. Type 2 Diabetes: A more

prevalent condition when the body either grows sensitive to insulin or produces insufficient amounts of it, frequently due to lifestyle reasons(2).

Zingiber officinale, commonly known as ginger, is a flowering plant whose rhizome (underground stem) is widely used as a spice and for its medicinal properties(3). Reactivity of oxygen species (ROS), one type of free radical that is overproduced, have been linked to the onset of numerous chronic illnesses(4). Many natural goods, including fruits, vegetables, cereals, edible flowers, medicinal herbs, and herbal infusions, have been shown to have antioxidant potential. Here's an overview(5)

Biological Properties:

1. Family- Zingiberaceae, 2. Common Name- Ginger, Ginger Root, 3. Parts Used- Rhizome

***Chemical contituents of zingiber officinale:***


**Uses:** For millennia, ginger has been utilized in conventional medicine. Here are a few possible health advantages of it. It can be consumed in the form of juice or oil, dried, powdered, or fresh. The medicinal properties are digestive aid, anti inflammatory, blood sugar regulation, menstrual pain relief(6).

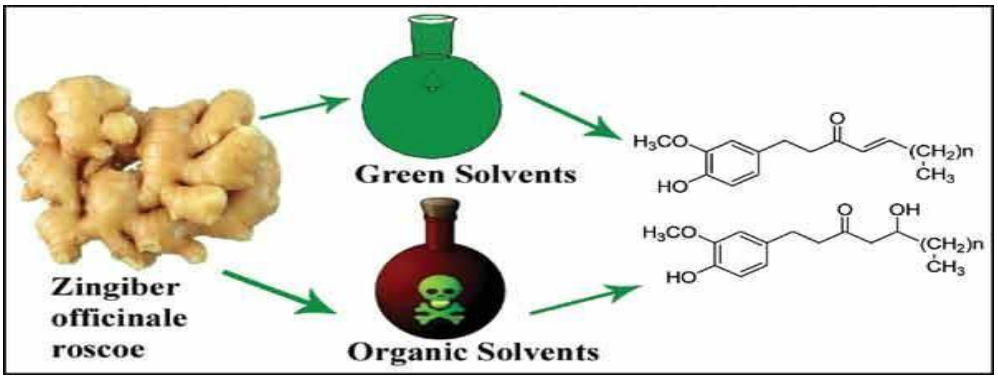
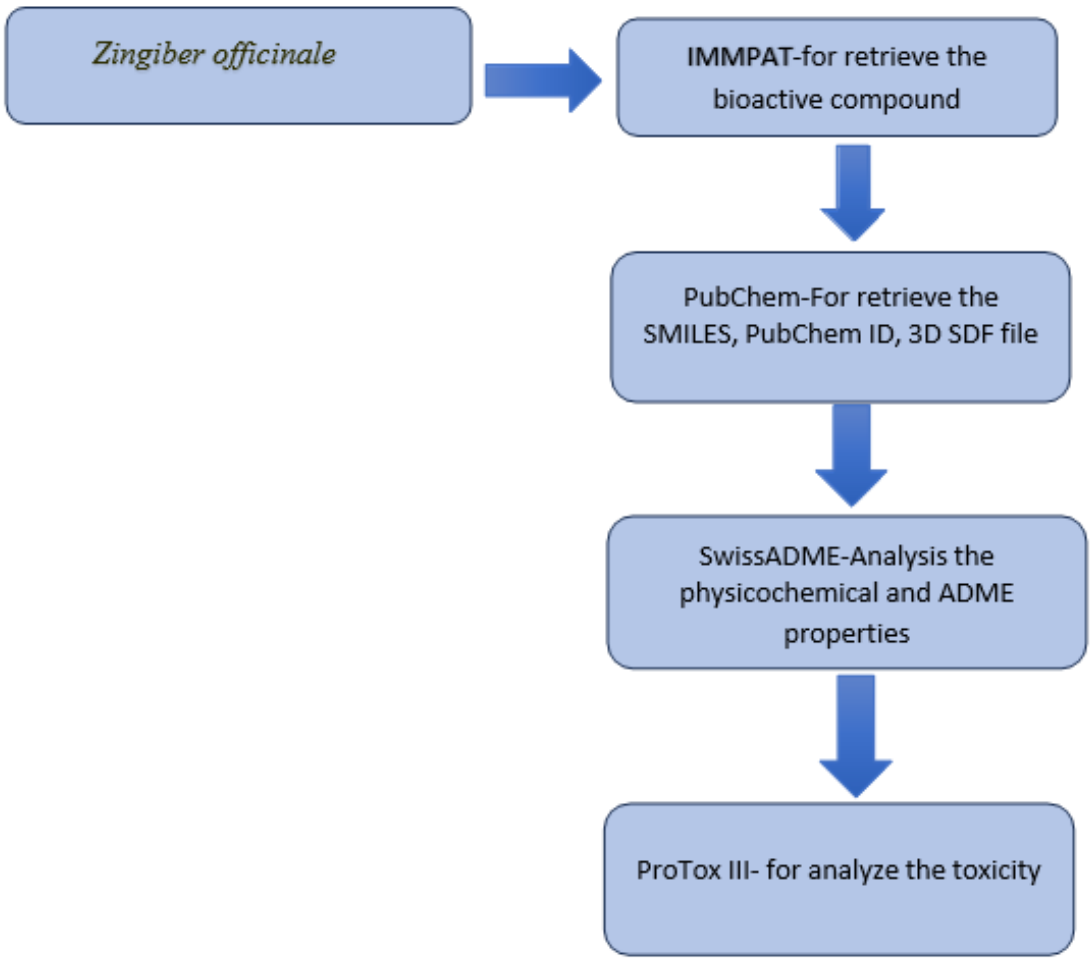



**Fig.1. Pant and chemical composition of ginger**

The terms Absorption, Distribution, Metabolism, Excretion, and Toxicity are combined to form ADMET(7). It provides a crucial framework for pharmacology and drug development, helping scientists assess a drug's possible safety profile and how it acts in the body(8). Overall, ADMET is crucial for forecasting how a medication will work in people, helping to enhance drug design and limit dangers in clinical use(9).

## 2. METHODOLOGY

### 2.1. A flow chart of physiochemical properties and pharmacokinetic activity:




### 2.2. To determine the bioactive compound by using the IMMPAT webtools:

The immpat wed tools was used for the determination of bioactive compound and its IMPPAT (2.0) is a hand curated database that includes 1742 Indian plants for medicinal purposes, 9596 phytochemicals,

which and 1124 therapeutic uses. It spans 27074 plant- phytochemical connections and 11514 plant-therapeutic linkages. This is the first step towards achieving our goals. And more data are collected from the Pubchem webtools. In Pubchem webtools we retrieved the SMILES, molecular formula and pubchem ID also. The retrieved data are mention in table.1.

**Table .1- To retrieved the data by using the IMMPAT webtools.**

| S. No. | Bioactive compounds | Pubchem ID | Molecular formula | SMILE |
|---|---|---|---|---|
| 1 | 3-Methylbutanal | 11552 | C5H10O | CC(C)CC=O |
| 2 | Zingerone | 31to211 | C11H14O3 | CC(=O)CCC1=CC(=C(C=C1)O)OC |
| 3 | Zingiberene | 92776 | C15H24 | CC1=CC[C@@H](C=C1)[C@@H](C)CCC=C(C)C |
| 4 | Citral | 638011 | C10H16O | CC(=CCC/C(=C/C=O)/C)C |
| 5 | Benzaldehyde | 240 | C7H6O | C1=CC=C(C=C1)C=O |
| 6 | Eucalyptol | 2758 | C10H18O | CC1(C2CCC(O1)(CC2)C)C |
| 7 | Camphor | 2537 | C10H16O | CC1(C2CCC1(C(=O)C2)C)C |
| 8 | Menthone | 26447 | C10H18O | C[C@@H]1CC[C@H](C(=O)C1)C(C)C |
| 9 | Menthol | 1254 | C10H20O | CC1CCC(C(C1)O)C(C)C |
| 10 | Octanal | 454 | C8H16O | CCCCCCCC=O |
| 11 | alpha-Santalol | 5281531 | C15H24O | C/C(=C/CCC1(C2CC3C1(C3C2)C)C)/CO |
| 12 | 2-Heptanone | 8051 | C7H14O | CCCCCC(=O)C |

2.3. 2D Molecular structure: The 2D molecular structure are draw by using the Chem sketch webtools with the help of SMILES. The all 2D structure is draw in below:

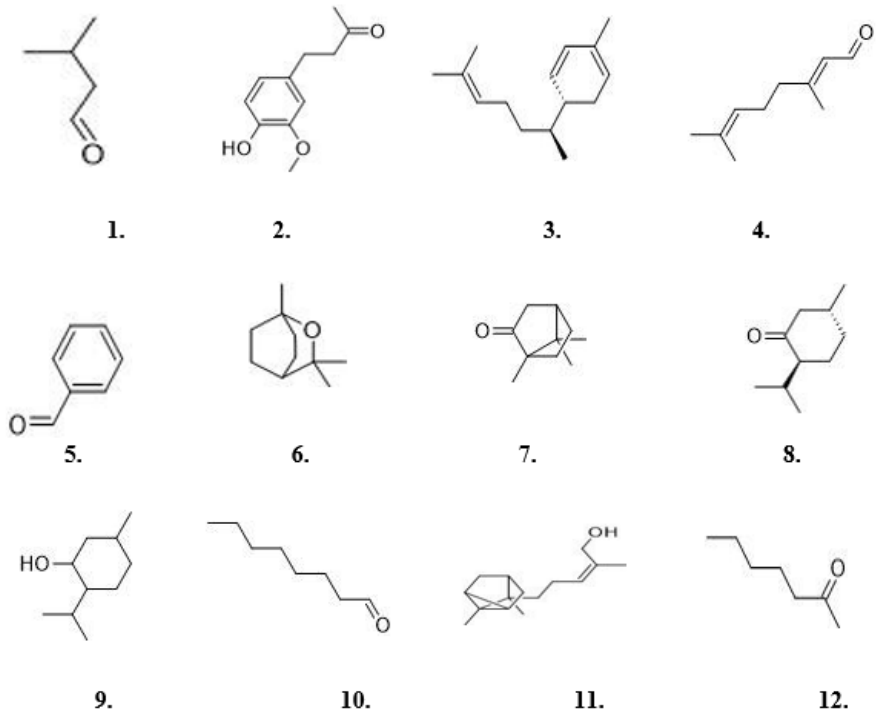



**Fig. 2. The 2D structure of zingiber officinale: 1) 3-Methylbutanal, [2] Zingerone, [3] Zingiberene, [4] Citral, [5] Benzaldehyde, [6] Eucalyptol, [7] Camphor, [8] Menthone, [9] Menthol, [10] Octanal, [11] alpha-Santalol, [12] 2-Heptanone.**

## 3. RESULT AND DISCUSSION

### 3.1. To analysis the physical and chemical properties of bioactive compound:

**Table-2. To analysis the physicochemical properties:**

| S. NO. | Phytochemical compound | Molecular weight | Num. rotatable bonds | Num. H-bond acceptors | Num. H-bond donors | TPSA | %Abs. | Log Po/w (iLOGP) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3-Methylbutanal | 86.13 g/mol | 2 | 1 | 0 | 17.07 Å² | 103.11 | 1.49 |
| 2 | Zingerone | 194.23 g/mol | 4 | 3 | 1 | 46.53 Å² | 92.94 | 2.09 |
| 3 | Zingiberene | 204.35 g/mol | 4 | 0 | 0 | 0.00 Å² | 109 | 3.63 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | Citral | 152.23 g/mol | 4 | 1 | 0 | 17.07 Å² | 103.11 | 2.51 |
| 5 | Benzaldehyde | 106.12 g/mol | 1 | 1 | 0 | 17.07 Å² | 103.11 | 1.36 |
| 6 | Eucalyptol | 154.25 g/mol | 0 | 1 | 0 | 9.23 Å² | 105.81 | 2.58 |
| 7 | Camphor | 152.23 g/mol | 0 | 1 | 0 | 17.07 Å² | 103.11 | 2.12 |
| 8 | Menthone | 154.25 g/mol | 1 | 1 | 0 | 17.07 Å² | 103.11 | 2.4 |
| 9 | Menthol | 156.27 g/mol | 1 | 1 | | 20.23 Å² | 102.02 | 2.55 |
| 10 | Octanal | 128.21 g/mol | 6 | 1 | 0 | 17.07 Å² | 103.11 | 2.29 |
| 11 | alpha-Santalol | 220.35 g/mol | 4 | 1 | 1 | 20.23 Å² | 102.02 | 2.93 |
| 12 | 2-Heptanone | 114.19 g/mol | 4 | 1 | 0 | 17.07 Å² | 103.11 | 2.09 |

- When we analysis the physicochemical properties by using the SwissADME webtools then they find the some datas like molecular weight, no. of hydrogen donor, no. of hydrogen acceptor , TPSA, and log Po/w.
- After that we analysis all the data and find the best percentage of abs. is Zingiberene(3), Eucalyptol(6) and ehw the %of abs. is high the the TPSA value is low , the Standard TPSA value is 140 A2 AND above the % of abs. very poor and the TPSA value is 90 A2 and less the the % of abs. is best. In this all compound are follow RO5 rules.
- For optimal oral and intestinal absorption, an oral medication ought to have a LogP value <5, preferably between 1.35 and 1.8, in accordance with Lipinski's Rule of 5. According to this the best hydrophilic compound is Benzaldehyde(1.36), 3-Methylbutanal(1.49) and more lipophilic properties is Zingiberene(3.63) and alpha-Santalol(2.93) , all data are meantion in below table -2.

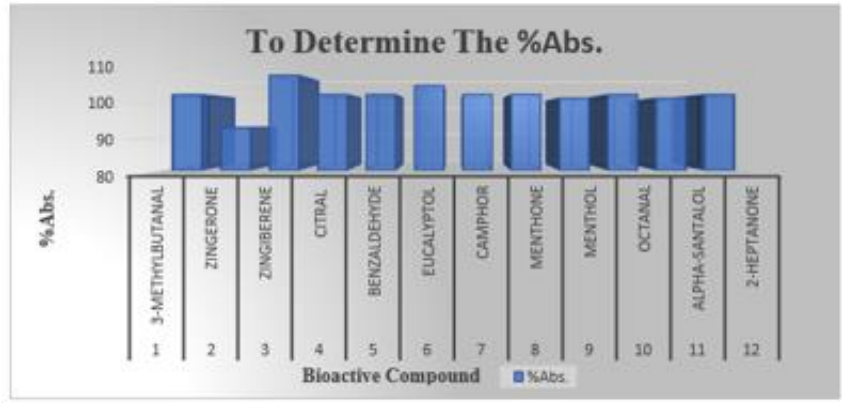



**Fig. 3- To represent the % of abs. by using graph**

### 3.2. To the analysis of pharmacokinetic properties:

**Table-3. To the predication of pharmacokinetic properties:**

| S. No. | Bioactive Compounds | GI Absorption | BBB Permeant | P-Gp Substrate | CYP1A2 Inhibitor | CYP2C19 Inhibitor | CYP2C9 Inhibitor | CYP2D6 Inhibitor | CYP3A4 Inhibitor | Log Kp(Skin Permeation) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3-Methylbutanal | High | Yes | No | No | No | No | No | No | -6.12 Cm/S |
| 2 | Zingerone | High | Yes | No | Yes | No | No | No | No | -6.70 Cm/S |
| 3 | Zingiberene | Low | No | No | No | Yes | Yes | No | No | -3.88 Cm/S |
| 4 | Citral | High | Yes | No | No | No | No | No | No | -5.08 Cm/S |
| 5 | Benzaldehyde | High | Yes | No | Yes | No | No | No | No | -5.90 Cm/S |
| 6 | Eucalyptol | High | Yes | No | No | No | No | No | No | -5.30 Cm/S |
| 7 | Camphor | High | Yes | No | No | No | No | No | No | -5.67 Cm/S |
| 8 | Menthone | High | Yes | No | No | No | No | No | No | -5.08 Cm/S |
| 9 | Menthol | High | Yes | No | No | No | No | No | No | -4.84 Cm/S |
| 10 | Octanal | High | Yes | No | No | No | No | No | No | -5.15 Cm/S |
| 11 | Alpha-Santalol | High | Yes | No | No | Yes | Yes | No | No | -4.83 Cm/S |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 2-Heptanone | High | Yes | No | No | No | No | No | No | -5.59 Cm/S |

- In this evalution we find the pharmacokinetic properties like- GI Absorption, BBB Permeant, P-Gp Substrate, CYP1A2 Inhibitor , CYP2C19 Inhibitor, CYP2C9 Inhibitor, CYP2D6 Inhibitor, CYP3A4 Inhibitor and Log Kp(Skin Permeation) by using the swiss ADME webtools with the help of SMILES.
- We find different parameter and different best bioactive compounds. In the GI absorptionwe analysis that only one compound is show the poor absorption is Zingiberene(3) and restall the compound show the high GI absorption. Mentain in table-3.
- In the distribution, we retrieved the bioactive compound by using the swissADME . Thanthey found the only one compound Zingiberene(3) should not be cross the BBB Permeantand rest of all the compound are cross the BBB Permeant for the drug distribution. Mentionin table-3.
- At last we evalute the metabolism and excretion we found the some of compound they donot show any Inhibitor like 3-Methylbutanal(3), Citral(4), Eucalyptol(6), Camphor(7), Menthone(8), Menthol(9), Octanal(10), 2-Heptanone(12).
- In CYP1A2 Inhibitor we found only two inhibitor first is Zingerone(2) and second is Benzaldehyde and rest of all the compound are not show any inhibitor. And the next we determine the CYP2C19 Inhibitor and CYP2C9 Inhibitor both are inhibit the same compounds is Zingiberene(3) and Alpha-Santalol(11) and remaning of all compound are not show any inhibition. Mention in table-3.
- In the final we determine the Log Kp(Skin Permeation) also by using the swissADME. Inthis properties the normal range from -6.10 to -0.76 cm/s. In this retrieval we found the highest skin permeation is Zingiberene(-3.88 cm/s) and the lowest skin permeation is Zingerone(-6.70 cm/s) mention in table -3

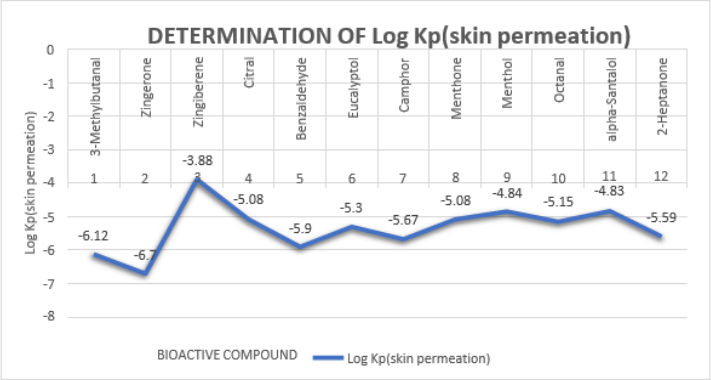



**Fig. 4-** To represent the Log Kp(skin permeation) by using graph

| S.No. | Bioactive compo | Hepatotoxicity | Neurotoxicity | Nephrotoxicity | Respiratory toxicity | Cardiotoxicity | Carcinogenicity | Immunotoxicity | Mutagenicity | Cytotoxicity | LD50(mg/kg) | Toxicity Class |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3-Methylbut | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 2490mg/kg | 5 |
| 2 | Zingerone | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 2380mg/kg | 5 |
| 3 | Zingiberene | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 1680mg/kg | 4 |
| 4 | Citral | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 500mg/kg | 4 |
| 5 | Benzaldehyde | inactive | active | inactive | inactive | active | active | inactive | inactive | inactive | 28mg/kg | 2 |
| 6 | Eucalyptol | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 2480mg/kg | 5 |
| 7 | Camphor | inactive | active | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 775mg/kg | 4 |
| 8 | Menthone | inactive | active | inactive | active | inactive | inactive | active | inactive | inactive | 1190mg/kg | |
| 9 | Menthol | inactive | inactive | inactive | active | inactive | inactive | active | inactive | inactive | 940mg/kg | 4 |
| 10 | Octanal | inactive | active | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 5000mg/kg | 5 |
| 11 | alpha-Santalol | inactive | inactive | inactive | active | inactive | inactive | inactive | inactive | inactive | 3800mg/kg | 5 |
| 12 | 2-Heptanone | inactive | active | inactive | inactive | inactive | inactive | inactive | inactive | inactive | 5000mg/kg | 5 |

- The ProTox 3.0 webtools are used for the determination of toxicity end point, toxicity class and LD 50 value by the help of SMILES.
- They have various types of toxicity end point like Hepatotoxicity, Neurotoxicity, Nephrotoxicity, Respiratory toxicity, Cardiotoxicity, Carcinogenicity, Immunotoxicity,

Mutagenicity and Cytotoxicity.

- Firstly we retrieved all the compound than they found some compound like 3-Methylbutanal(1), Zingerone(2), Zingiberene(3), Citral(4), and Eucalyptol(6) are inactive for all the toxicity end point.

- In Neurotoxicity, there are two compound Benzaldehyde(5) and Menthone (8) are highly toxic and two more compound are toxic like Camphor(7) and Octanal(10) and remaining compound are showed non toxic in nature.

- In Respiratory toxicity, there are three compound are highly toxic Menthone(8), Menthol(9), alpha-Santalol(11), and remaning are non toxic in nature.

- In Cardiotoxicity and Carcinogenicity only one compound is highly Benzaldehyde(5) and remaning all the compound are show non toxic in nature.

- At last we retrieved the last toxicity endpoint is Immunotoxicity, two compound show the highly toxic Menthone(8) and Menthol(9) and remaning all the compound are non toxic mention in table -4.

- The more toxic of all bioative is Benzaldehyde(5). It is highly toxic in nature. And also we retrieved the LD 50 dose than they found the best LD 50 values is 2-Heptanone(12), Octanal(10) mentain in table -4

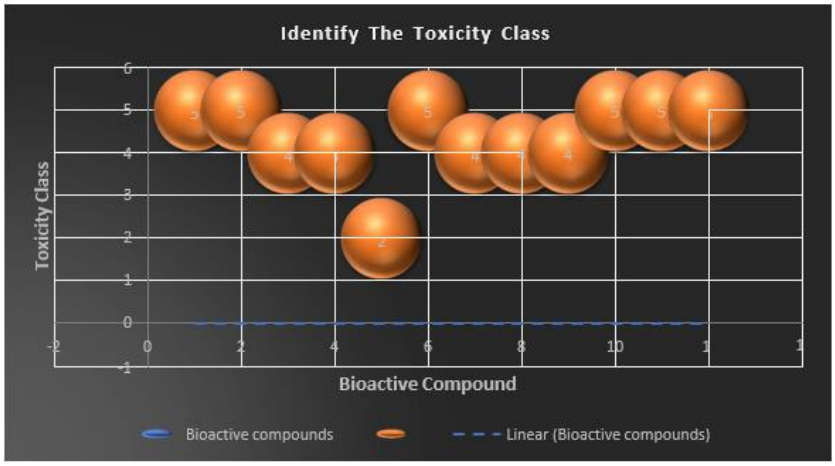



Fig.5- to represent the toxicity class by using graph

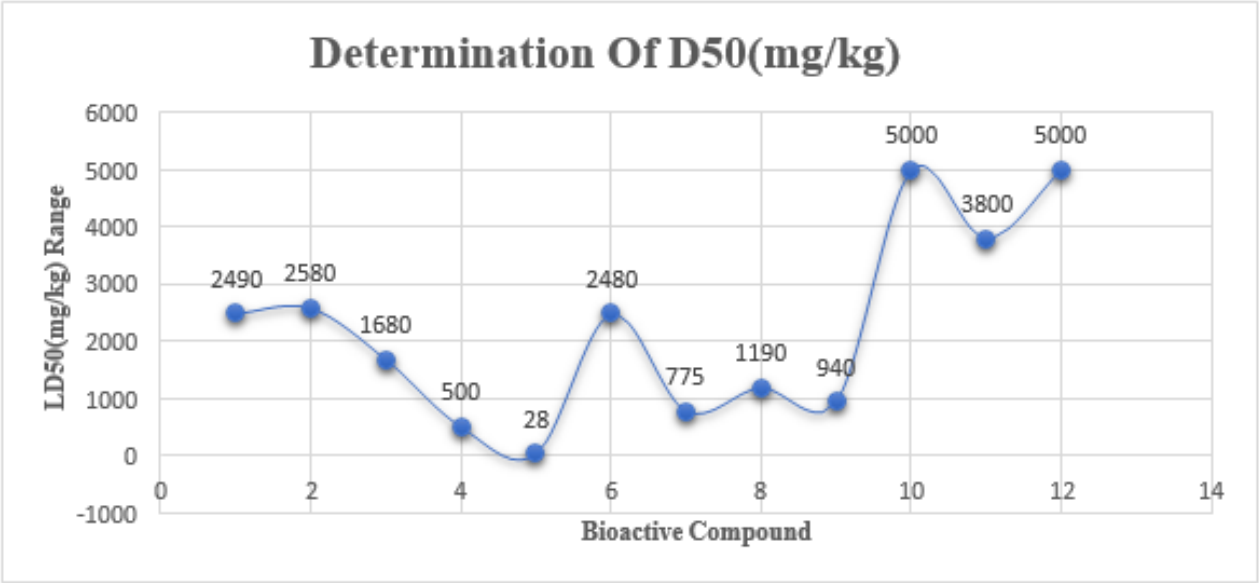



Fig.-5. To represent the LD50 value by using graph

## 4. CONCLUSION

- In the final when we retrieved all the data like physicochemical, pharmacokinetic and toxicity than they found the some best compound out of twelve. The all bioactive compound are followed RO5 rules. And the high GI absorption of all compound except Zingiberene. Zingiberene is a low GI absorption.
- In pharmacokinetic study we find the best Log kp value is Zingiberene(-3.88 cm/s) and the toxicity class is 3-Methylbutanal, Zingerone, Camphor, Menthone are 5,5,4 and 4 respectively.
- Overall research that indicated the best potential for the Anti Diabetic are 3-Methylbutanal, Zingiberene and Benzaldehyde. In future we perfome the molecular docking of these compounds.

**ACKNOWLEDGEMENT**

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frustration. For that, I am grateful.

Conflict of Interest

All the author declare that there is no conflict of inerest.

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# Pigment Variations in Soil-Isolated Cyanobacteria: A Case Study of the Meerut Region

**Anjali singh[1], Kamakshi[1*], Riya Ahlawat[2] Kumud gaur[2] and Devendra Kumar[3]**

*[1]Department Biology, SRM Institute of Science and Technology (DELHI NCR Campus) Modinagar, Ghaziabad, 201204.*

*[1*]Department of Biology, SRM Institute of Science and Technology (DELHI NCR Campus) Modinagar, Ghaziabad, 201204.*

*[2]Department of Biotechnology, SRM Institute of Science and Technology (DELHI NCR Campus) Modinagar, Ghaziabad, 201204.*

*[3]Department of Microbiology, C.C.S University Meerut 250004.*

## ABSTRACT

Cyanobacteria, a varied array of photosynthetic microorganisms, play a crucial role in soil ecosystems by participating in biogeochemical processes and maintaining ecosystem stability. This study examines the pigment content of cyanobacteria strains obtained from soils, from different locations of the Meerut region. The objective is to understand the range of pigments present and their ecological importance. Cyanobacterial strains were obtained from various soil environments, cultivated under controlled conditions, and analyzed for pigments using the spectrophotometric technique. The investigation primarily examined prominent pigments such as chlorophylls, carotenoids, and phycobiliproteins, in order to distinguish changes within and across species. The results demonstrated significant variation in pigment profiles among the cyanobacteria strains, indicating their adaptability to specific environmental habitats. In addition, the study investigated the connections between pigment composition and environmental parameters like light availability, soil pH, and nutrient levels, which shed light on the ecological significance of pigment variety. This study improves our comprehension of cyanobacterial ecology in soil ecosystems, which has consequences for the functioning of ecosystems, the cycling of biogeochemical elements, and the development of strategies for environmental management.

**KEYWORDS**: Physiological parameters, pigments, Cyanobacterial strains, Pigments composition, Chlorophyll, Carotenoids, Phycobiliproteins

## INTRODUCTION

Pigments are compounds with specific chemical properties that can absorb light within the visible spectrum. Cyanobacteria are a diverse group of photosynthetic prokaryotes known for their ability to perform oxygenic photosynthesis, making them crucial contributors to Earth's oxygen-rich atmosphere

and primary production in aquatic ecosystems (Vidal L, Ballot A, Azevedo SM, Padisák J, Welker M 2021). Their physiological parameters, including growth characteristics, nutrient requirements, and adaptations to environmental conditions, are key factors influencing their ecological roles and potential applications (Abed RM, Dobretsov S, Sudesh K. 2009.). Understanding the physiological characteristics of cyanobacteria, such as the levels of chlorophyll, carotenoids, and phycobiliproteins, offers valuable knowledge about their methods of adaptability and ecological importance (Jaiswal A, Koli DK, Kumar A, Kumar S, Sagar S. 2018). Chlorophyll is the main pigment that captures light energy during the process of photosynthesis (Zavřel T, Sinetova MA and Červený J. 2015). Cyanobacteria mostly contain chlorophyll-a as their primary photosynthetic pigment, in addition to accessory pigments including carotenoids and phycobiliproteins (Trees CC, Clark DK, Bidigare RR, Ondrusek ME, Mueller JL. 2000) Carotenoids have crucial functions in protecting against damage from excessive light energy and removing reactive oxygen species (Hirschberg J and Chamovitz D.1994). Phycobiliproteins, like phycocyanin and phycoerythrin, serve as pigments that capture light, allowing photosynthesis to occur across a wider range of wavelengths (Pagels F, Guedes AC, Amaro HM, Kijjoa A, Vasconcelos V. 2019). The physiological characteristics of cyanobacteria, including their composition and abundance, can undergo substantial variations Regarding environmental issues, such as light intensity, nutrition availability, temperature, and pH (Alghanmi HA and Jawad HM.2019). Under conditions where nutrients are limited, cyanobacteria can modify their pigment composition to maximize light absorption and optimize energy use (Sukharevich VI, Polyak YM. 2020). The objective of this study is to examine the fluctuation of physiological parameters in cyanobacteria. By analyzing the properties and characteristics of chlorophyll, carotenoids, and phycobiliproteins, we can obtain valuable information about how cyanobacteria adapt to their environment and how they respond to changes in their surroundings.

The present investigations aimed to obtain cyanobacterial isolates from different fields of western Uttar Pradesh (Meerut), which can produce metabolites such as pigments and phycobiliproteins, these chemicals are highly valuable and have the potential to be used in the food, pharmaceutical, and cosmetics industries.

## METHOD AND MATERIALS

### ISOLATION OF CYANOBACTERIAL STRAINS FROM SOIL SAMPLES

The soil samples were gathered from the inhabited areas of the Meerut region in western Uttar Pradesh, India. 10 grams of soil samples were thoroughly shifted to the flask containing 90ml sterile distilled water and homogenized for 30 minutes at 120 rpm following the serial dilution up to $10^{-4}$. 1ml aliquots were spread and grown in a BG-11 medium at 28±2°C under Light:Dark cycle of 16:8 h; light intensity 3-4 Klux for one weak. identified using the keys given by Desikachary 1959 for microscopic parameters. These isolates were examined under controlled conditions for pigment analysis at the exponential growth stage.

List of cyanobacteria isolated from soil from 3 porch areas of Meerut region of Uttar Pradesh.

| S. No. | Generic description | Origin/site |
|---|---|---|
| As1 | *Anabaena* | KANKER KHERA MEERUT |
| As2 | *Nostoc* | KANKER KHERA MEERUT |
| As3 | *Phormidium* | KANKER KHERA MEERUT |
| As4 | *Anabaena* | KANKER KHERA MEERUT |
| As5 | *Nostoc* | BANK ROAD, MEERUT CANTT |
| As6 | *Calothrix* | BANK ROAD, MEERUT CANTT |
| As7 | *Plectonema* | BANK ROAD, MEERUT CANTT |
| As8 | *Aulosira* | BANK ROAD, MEERUT CANTT |
| As9 | *Anabaena* | BANK ROAD, MEERUT CANTT |
| As10 | *Oscillatoria* | GANGA NAGAR MEERUT |
| As11 | *Calothrix* | GANGA NAGAR MEERUT |
| As12 | *Anabaena* | GANGA NAGAR MEERUT |
| As13 | *Westielopsis* | GANGA NAGAR MEERUT |
| As14 | *Chrococcus* | GANGA NAGAR MEERUT |

**ESTIMATION OF PIGMENTS**

**CHLOROPHYLL**

Chlorophyll estimation was done, and centrifugation was done for a homogenized suspension for 5 mins at 4000 g. At 60 °C for 30 minutes chlorophyll was extracted from the pellet with 95% methanol. Subsequently, the last volume was prepared and the amount of chlorophyll was determined by measuring

the optical density at wavelengths of 650 and 665 nm. In 1941, McKinney calculated the total amount of chlorophyll.

**CAROTENOIDS**

At 4000 g for about 10 minutes, the algal suspension was centrifuged. The adhering salt traces were removed by washing them off with distilled water. Subsequently, 2-3 ml of 85% acetone was introduced and exposed to a series of freezing and thawing cycles. Until acetone became colorless continuous extraction was performed. A pooled acetone fraction was obtained and total volume was recorded. The total carotenoid concentration was determined by measuring the maximum absorbance at 450 nm using 85% acetone as a blank, according to Jensen (1978).

**PHYCOBILLIPROTEINS**

The amount of phycobilins (Phycocyanin, Phycoerythrin, Allophycocyanin) was extracted by following the process of repeated freezing and thawing at a pH of 7.5 until the particle lost its color as the pigments seeped out into a supernatant.

**RESULT AND DISCUSSION**

Cyanobacterial strains from different locations of Meerut were isolated and grown in BG11 medium for estimation of pigments (dry weight). The strains were observed on the $14^{th}$ day of the incubation period. Chlorophyll is the main photosynthetic pigment found in cyanobacteria. The various aspects of physiological parameters in cyanobacteria has been studied by the members of IARI. Little attention has been given to the physiological parameters as compared to the nitrogen fixation (Tabassum R, Kumar R, Yadav R, Dhar DW, Bhatnagar SK. 2012). Total chlorophyll content was recorded highest of 0.96 $\mu g/mg^{-1}$ dry weight in (As5) *Nostoc sp.* followed by Anabaena sp. and Calothrix sp. ranging between 0.06 to 0.77$\mu g/mg^{-1}$. Cyanobacteria include carotenoids, a distinct pigment category (Kumar R, Bhowmick A, Chakdar H, Elumalai S and Pabbi S. 2015). The highest among all carotenoids was recorded in *Anabaena sp.* (As1) at 0.79 $\mu g/mg^{-1}$ dry weight and lowest in (As7) *Plectonema sp.* 0.08 $\mu g/mg^{-1}$. In addition to chlorophyll, Nitrogen-fixing heterocystous species of filamentous cyanobacteria are highly valued for their ability to produce phycobiliproteins and carotenoids, and other significant compounds that act as accessory pigments in photosynthesis (Deepika C, Wolf J, Roles J, Ross I, Hankamer B. 2022).

Carotenoids safeguard cyanobacterial cells from photooxidative harm and are commonly linked with proteins (Kirilovsky D, Kerfeld CA. 2016). Phycocyanin varied from the highest of 4.5µg/mg$^{-1}$ in *Nostoc sp.* (As2) to the lowest of 0.94 µg/mg$^{-1}$ dry weight in (As7) *Phormidium sp*. Phycoerythrin was highest at 3.34µg/mg$^{-1}$ dry weight in *Westielopsis sp*. (As13) and minimum in *Phormidium sp.* (As3) 0.63µg/mg$^{-1}$ dry weight. Allophycocyanin was highest in (As5) *Nostoc sp*. 20.05µg/mg$^{-1}$ followed by *Nostoc sp.* 15.02µg/mg$^{-1}$ dry weight and lowest in *Phormidium* sp. and *Aulosira sp.* Phycobiliproteins play a major role in the photosynthetic process of cyanobacteria by facilitating the effective absorption and utilization of light energy (Li W, Su HN, Pu Y, Chen J, Liu LN, Liu Q, Qin S. 2019). The continuous research emphasizes the significance of these organisms not only in ecological settings but also in biotechnological advancements. Due to their distinct characteristics, they possess significant value in a diverse array of applications, spanning from scientific investigations to commercial merchandise (Bryant, D. A. 1982) examines the evolutionary importance of several phycobiliproteins, specifically phycoerythrocyanin, in cyanobacteria. An extensive examination of the chemical characteristics of phycobiliproteins and their many uses in biotechnology, including their utilization as organic pigments and in photodynamic therapy by (Pagels, F., Guedes, A. C., Amaro, H. M., & Malcata, F. X. 2019).

## CONCLUSION

The present study concludes that the Porsche areas of Meerut, U.P are rich in useful blue-green algae and further investigation and advancement in these crucial domains will not only unleash the complete capabilities of cyanobacteria in transforming agriculture but also guarantee their secure and sustainable application, which can be harnessed for commercial purposes.

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# DEVELOPMENT AND EVALUATION OF NATURAL SKIN WHITENING FOMULATION USING CLOVE EXTRACT

**Archana Gautam***
Associate Professor,
**Amit Kumar**
Assistant Professor
MIT College of Pharmacy, Moradabad
Email- archna.gautam909@gmail.com

**Abstract-** The aim of this study was to investigate the antioxidant and antityrosinase properties of clove extract and its components, and to assess the correlation between their antioxidant and antityrosinase activities. The standard solution showed an inhibition of 84.21% in the DPPH method at a concentration of 500µg/mL, whereas the test solution exhibited a 61% inhibition at the same concentration. The methanolic extract of clove was also assessed for antioxidant activity using ferric reducing antioxidant power. The control exhibited a percentage inhibition of 78.28% at a concentration of 500µg/mL, while the test solution showed 25.47% inhibition at the same concentration. The methanolic extract of clove displayed antityrosinase properties. The standard showed an antityrosinase activity result of 86.28% at a concentration of 100µg/mL, while the test sample showed a result of 52.65% at the same concentration. The current study sought to create a natural cream that nourishes, moisturizes, and treats different skin issues. The developed product underwent testing for various attributes including color, appearance, viscosity, acidity, durability assessments, and consumer satisfaction.

**Keywords:** Melanocyte, Melanogenesis, keratinocytes, antioxidant, Herbal Cosmetic.

**Introduction -** The amount of Asian women desiring lighter skin tones has significantly risen in the past few years. This is partly due to the fact that many powerful skin-lightening substances have been found, especially those derived from plants. Skin color is mostly determined by the level of melanin, a pigment found in the epidermis. It is secreted by melanocyte cells located in the basal layer of the epidermis. (1) Excessive melanin production can result from reactive oxygen species (free radicals), melasma, prolonged sun exposure, and other hyperpigmentation disorders. Excessive melanin production is not preferred as it could lead to an uneven or darker complexion. Changes in color are also desired for aesthetic reasons. The

initial stages of melanin production, known as melanogenesis, are triggered by the enzyme activity of tyrosinase.

Herbal products assert that they do not have the usual negative impacts associated with artificial ingredients. The popularity of herbal remedies has resulted in an excessive supply in the Indian market due to advancements in technology and social factors. Hence, we aimed to select plants known for their anti-tyrosinase properties to create a botanical cream. Plants used in cosmetics have multiple properties like anti-tyrosinase, antioxidant, anti-inflammatory, antiseptic, and antibacterial properties. Please provide the text you would like me to paraphrase.

The aromatic dried flower buds of the clove tree (Syzygium aromaticum) are part of the Myrtaceae plant family. Clove essential oil is used for its qualities of being anti-inflammatory, anti-mutagenic, and antioxidant (5). The main goals of this study were to examine the antioxidative and tyrosinase inhibiting properties of clove oil and its components in vitro, as well as their effects on dermal fibroblasts. (6, 7, 8)

**THE BIOSYNTHESIS OF MELANIN**

Gertrude (2000) stated that Melanin biosynthesis is a multipart pathway that found in highly specialized cells, called melanocytes, within membrane-bound organelles referred to as melanosomes. Melanosomes are transferred by dendrites to surrounding keratinocytes, where they play a critical role in photo protection. The anatomical relationship between keratinocytes and melanocytes is known as “the epidermal melanin unit” and it has been estimated that each melanocyte is in contact with 40 keratinocytes in the basal and supra basal layers. Several important steps must occur for the proper synthesis and distribution of melanin, as follows. (5, 6)

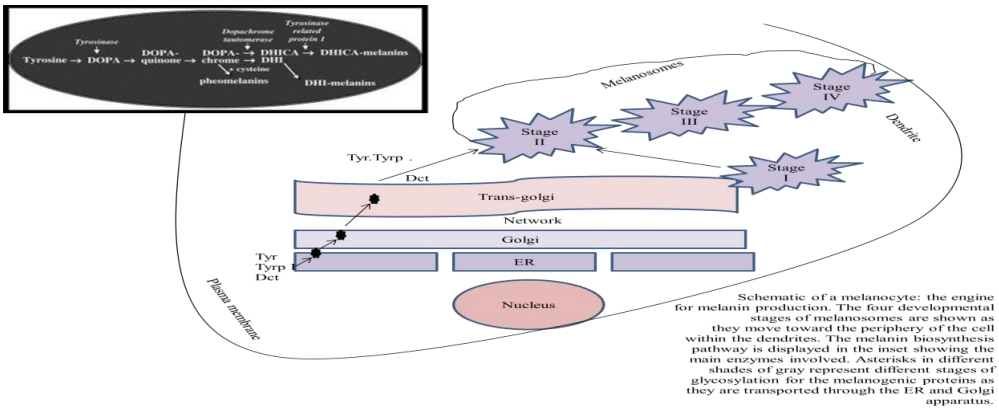



**Figure 1.1 – Bio-synthesis of Melanin**

**Materials and methods:**

Clove buds were collected from local market of Meerut and the plant material authenticated by the Natural Bureau of plant genetic resource (NBPGR), Delhi.

***Extraction process of Syzygium aromaticum (clove)-***

The clove buds was weight (15gm) and powered with mortar pestle. Powder was transferred into conical flasks and extracted with 90 ml of methanol. The conical flask was kept over rotatory shaker on 240-340 rpm for two days at room temperature. The extract was filtered and concentrated by rotatory evaporator. The concentrated solution was collected and stored in deep freezer until further use.

**EVALUATION PARAMETERS OF METHANOLIC EXTRACT OF CLOVE**

The naturally obtain extract was evaluated for its antioxidant and anti-tyrosinase activity.

a) Antioxidant activity

The methanolic extract of clove has antioxidant activity; this activity was determined using two different types of method.

**1. DPPH Radical Scavenging Activity**

The DPPH free radical method was use for antioxidant activity of extracts. The lower absorbance of the reaction mixture was indicating higher free radical scavenging activity.

**Preparation of solutions for DPPH method**

**Preparation of standard solution-**100mg of Ascorbic acid was dissolved in 100 mLof methanol and drawn 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 mL of this solution volume make up

10mL to give the concentration of 50, 100, 150, 200, 250, 300, 350 and 400µg/mL.

**Preparation of test sample-**Stock solutions of samples were prepared by dissolving 20 mL of methanolic extract in 20 mL of methanol and drawn 0.5, 1, 1.5, 2, 2.5.3, 3.5 and 4 mL of this

solution volume make up 10mL to give the concentration of 50, 100, 150, 200, 250, 300, 350 and s400µg/mL.

**Preparation of DPPH solution-**10mg of DPPH was dissolved in 10 mL methanol: it was protected from light by covering the test tubes with aluminum foil.1mL was drawn and again dissolved up to 100 mL of solvent to give 10µg / mL of DPPH solution.

**Protocol for DPPH free radical scavenging activity**

- 10µg/mL solution of DPPH, absorbance was taken immediately at 516 nm for control reading.
- Different dilutions of test and standard solution were taken into volumetric flask and 1ml of each concentration taken in to volumetric flasks.
- Same volume of DPPH solution was added to each test tube.
- Absorbance was taken at 516 nm in UV spectrophotometer (on visual UV spectroscopy Pharmaspec-1700, Shimadzu, Japan). Methanol used as a blank.

The percentage reducing power of DPPH free radical method was calculated by using equation no.1-

$$\% \text{ Reducing power} = \frac{\text{Control absorbance - Sample absorbance} \times 100}{\text{Control absorbance}}$$

**2. Ferric Reducing antioxidant power**

The ferric reducing antioxidant power of extract was determined according to the method of Oyaizu (1986).

**Preparation of solutions for ferric reducing antioxidant power method**

**Preparation of standard solution-** 100mg of ascorbic acid dissolved in 100 mL of distilled water. Dilutions of this solution with 10mL distilled water were prepared to give the concentration of 50, 100, 200, 300, 400 and 500µg/mL.

**Preparation of test sample-** 20mL of methanolic extract was dissolve in 20 mL methanol. Dilutions of this solution with 10mL methanol were prepared to give the concentration of 50, 100, 200, 300, 400 and 500µg/ml.

**Preparation of reagents**

- Phosphate buffer: 0.2M phosphate buffer of pH 6.8 was prepared according to I.P.

- 1% Potassium ferricyanide solution: 1 gm of potassium ferricyanide was dissolved in 100mL of distilled water.
- 10% Trichloro acetic acid: 10 gm of Trichloro acetic acid was dissolved in 100mL of distilled water.
- 0.1% ferric chloride solution: 0.1 gm of ferric chloride was dissolved in 100mL of distilled water.

**Protocol of ferric reducing antioxidant power method-**

- Ascorbic acid was used as standard and used different concentration (50,100,200,300,400 and 500µg/mL) of standard and test solution.
- To each of the volumetric flask 2.5mL of 0.2M phosphate buffer (pH 6.8) and 2.5mL of potassium ferricyanide was added.
- The mixture was incubated for 20min at 50C in oven.
- To incubate solutions 2.5 mL of 10% w/v trichloroacetic acid was added and centrifuged for 10min at 3000rpm, 2.5 mL of the solution was taken without disturbing.
- In above 2.5mL solution added 2.5mL of distilled water and 0.5mL of 1% freshly prepared ferric chloride solution was added.
- Absorbance was recorded at 697nm.

The percentage reducing power of ferric reducing antioxidant power method was calculated by using equation no.2-

$$\%\ reducing\ power = \frac{Control\ absorbance - Sample\ absorbance \times 100}{Control\ absorbance}$$

**b. Skin-whitening activity-**

The method of Tomita et al (1990) was slightly modified.

**Preparation of Standard solution-** 100mg of ascorbic acid was dissolved in 100 mL of methanol. Dilutions of this solution with 10mL methanol were prepared to give the concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100µg/mL.

**Preparation of test sample-** 30mL of methanolic extract was dissolve in 30 mL methanol. Dilutions of this solution with 10mL methanol were prepared to give the concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100µg/mL.

**Preparation of reagents**

- Phosphate buffer: 0.2M phosphate buffer of pH 6.8 was prepared according to I.P.
- 23.5mg of L-Tyrosine was dissolve in 25mL of double distilled water.
- 33.1mg of L-Dopa was dissolve in 25mL of double distilled water.
- 0.1ml of H2O2 was dissolved in 10mL of double distilled water.

**Protocol of skin-whitening activity**

- Different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100µg/mL) of standard and test solution was used in this method.
- To each of the volumetric flask 1.8mL of 0.2M phosphate buffer (pH 6.8) and 0.6mL of H2O2, 0.1mL of test and standard solution in each concentration and 0.1mL of aqueous solution of L-Tyrosine.
- The mixture was incubated for 5 minutes at 200C in oven.
- In above solution added 0.4mL of 6.3mM L-Dopa.
- Absorbance was recorded at 476nm.

The percentage inhibition of anti-tyrosinase method was calculated by using equation no.3-

$$\% \text{ inhibition} = \frac{(A - B)}{A} \times 100$$

Where

A = absorbance at 476 nm without the test sample, B=absorbance at 476nm with the test sample.

**FORMULATION DEVELOPMENT OF METHANOLIC EXTRACT OF CLOVE-**

1. Preparation of oil Phase- Cetyl alcohol and steric acid were added together in chine dish and liquefied on hot plate. The methanolic extract was added in this phase after liquefaction of oily phase.
2. Preparation of aqueous Phase-Distilled water, Potassium hydroxide, Methyl paraben, and propyle paraben were mixed together.
3. Oil phase was added in aqueous phase with continues starring until homogenous of formulation and packed.

**Table1 – Composition of cream**

| S.no | Ingredients | Formula w/w % | | | | |
|---|---|---|---|---|---|---|
| 1 | Cetyl alcohol | F1 | F2 | F3 | F4 | F5 |
| | | 1 | 2 | 3 | 4 | 5 |
| 2 | Steric acid | 15 | 14 | 13 | 12 | 11 |
| 3 | Methyl paraben | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 4 | Propyl paraben | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 5 | Glycerin | 10 | 10 | 10 | 10 | 10 |
| 6 | Potassium hydroxide | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| 7 | Methanolic Extract | 5 | 5 | 5 | 5 | 5 |
| 8 | Distilled water | q.s | q.s | q.s | q.s | q.s |

## EVALUATION PARAMETERS OF FORMULATIONS CONTAINED METHANOLIC EXTRACT OF CLOVE

### a. Physical Evaluations Homogeneity

Homogeneity of the preparation was measured by visually inspecting the formulations for uniformity and by checking the presence of aggregates and uniformity of colour. The homogeneity of semisolids formulation is a primary need.

### b. Measurement of pH

The pH of the formulations was measured in order to determine that whether its pH value lies in the pH range of skin or not. For this prepared formulations were taken and the pH was measured using pH meter. The adjustment in pH was done Triethanolamine.

### c. Spreadebility

The efficacy of topical therapy depends on the patient spreading the formulation in an even layer to deliver a standard dose. The optimum consistency of such a formulation helps ensure that a suitable dose is applied or delivered to the target site. This is particularly important with formulations of potent drugs. A reduced

dose would not deliver the desired effect, and an excessive dose may lead to undesirable side effects Garg et al (2002).

The Spreadebility was calculated by using the equation no.4. in gm.cm/sec

$$S= m\times L\ T$$

Where,

S, is the Spreadebility of cream formulations M, is the weight (g) tied on the upper plate, L, is the length cm) of the glass plates, and T is the time taken for plates to slide the entire length.

The parallel plate method is the most widely used method for determining and quantifying the Spreadebility of semisolid preparations. The advantages of the method are simplicity and relative lack of expense.

**d. Viscosity**

Viscosity is defined as an index of resistance of a liquid to flow. The higher the viscosity of the liquid, the greater is the resistance to flow. Viscosities of the formulations were measuring the yield value using Brookfield viscometer.

**e. In vitro permeation test of formulations**

Methanolic extract of clove release was determined through rat skin by invert test tube method, and the receptor medium was phosphate buffer with methanol (1:1) in a beaker Nikunjana (2009). The amount of the formulation was taken 1gm in a test tube and medium was 100mL in a beaker. The temperature of the medium was maintained 37±1 0C and samples were collected in a 30min of interval for 5 h. The absorbance of samples was taken by UV spectroscopy and all experiments were repeated 3 times. The percentage of cumulative drug release was determined.

**f. Permeation test**

The whole project was approved with institutional animal ethical committee. In the skin irritation method rats were used as animal modules for skin irritation test. Rats of weight between200-300gm were used for testing of skin irritation. The animals were divided into two groups, one group was control and another

was test. The animals were maintained on standard animal feed and allow free access to water. The animals were kept under standard conditions. Hairs were removed from back of rats and area 2cm2 was marker on both sides. One side served as control while the other side was test. On the test side final formulation containing combination of extracts from clove whereas without extract use control. Emulsion was applied (500mg/rats) twice a day for 7days and the site was observed for any sensitivity and the reaction if, any was graded as 0, 1, 2, 3 for no reaction.

**g. Stability**

It was recommended that samples for the evaluation of stability be placed in neutral, transparent glass flasks with a lid that assured good closing, avoiding gas or vapor losses to the environment. The quantity of product was must be sufficient for the necessary appraisals. In the case of a known incompatibility between the components of the formulations and glass, must choose another containing material. The incorporation of air in the product must be avoided during placement in the test recipient. It was important not to fill the total volume of the package, allowed a head space of approximately one third of the capacity of the flask for possible gaseous exchanges. The final containing material was used parallel to the neutral glass thus anticipating the appraisal of compatibility between the formulation and the packaging material. The duration of the study was generally fifteen days and helps in the screening of the formulations. Stability of the formulations was assessed by using humidity chamber on condition temperature 400C /75RH.

**RESULTS**

**EVALUATION PARAMETERS OF METHANOLIC EXTRACT OF CLOVE**

**a. Antioxidant activity-**

**1. DPPH free radical method-**

The DPPH free radical method was used for antioxidant activity of methanolic extract of clove. The percentage inhibition of standard solution on 500µg/mL give 84.21% while test solution given 61% on same concentration. The Table no.3.2 show the percentage inhibition between standard and test solution and the figure no.3.1 shown the graph of percentage inhibition between standard and test solution.

**Table 1.1- Percentage inhibition of DPPH method between standard and test solutions**

| S.NO. | CON (µg/mL) | % INHIBITION OF STANDARD | % INHIBITION OF TEST |
|---|---|---|---|
| 1 | 50 | 29.26 | 10.7 |
| 2 | 100 | 34.62 | 16.79 |
| 3 | 150 | 41.84 | 22.38 |
| 4 | 200 | 48.02 | 29.6 |
| 5 | 250 | 54.62 | 35.09 |
| 6 | 300 | 61.45 | 42.18 |
| 7 | 350 | 69.91 | 48.1 |
| 8 | 400 | 76.41 | 53.07 |
| 9 | 500 | 84.21 | 61 |

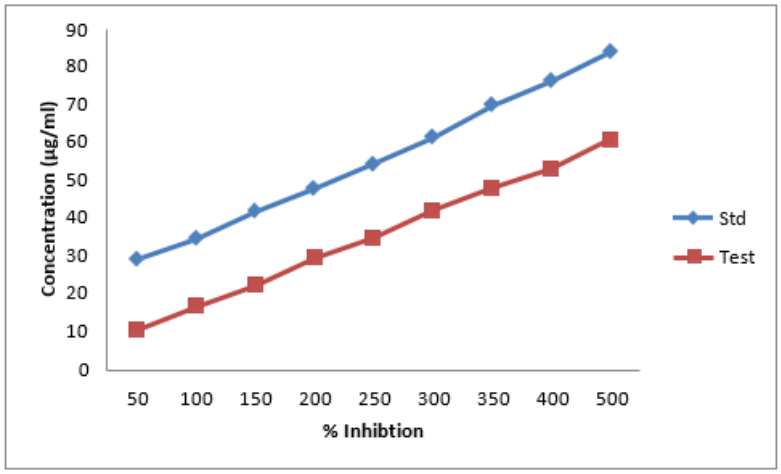



**Figure 2.1- Graph of Percentage inhibition of DPPH method between standard and test solution**

## 2. Ferric reducing antioxidant power (FRAP) method

The ferric reducing antioxidant power was also used for antioxidant activity of methanolic extract of clove. The result was come out of percentage Inhibition between standard and test solution shown on table no.3.3 and comparative graph of percentage inhibition between standard and test shown in figure no.3.2. The standard was given percentage inhibition on 500µg/mL 78.28% and test solution given 25.47% on same concentration.

**Table 2.2 - Percentage inhibition of ferric reducing antioxident power method between standard and test solution**

| S.NO. | CONCENTRATION (µg/mL) | %INHIBITION OF STANDARD | % INHIBITION OF TEST |
|---|---|---|---|
| 1 | 50 | 70.77 | 2.14 |
| 2 | 100 | 71.04 | 4.76 |
| 3 | 200 | 72.65 | 9.52 |
| 4 | 300 | 73.99 | 13.15 |
| 5 | 400 | 78.28 | 19.7 |
| 6 | 500 | 78.28 | 25.47 |

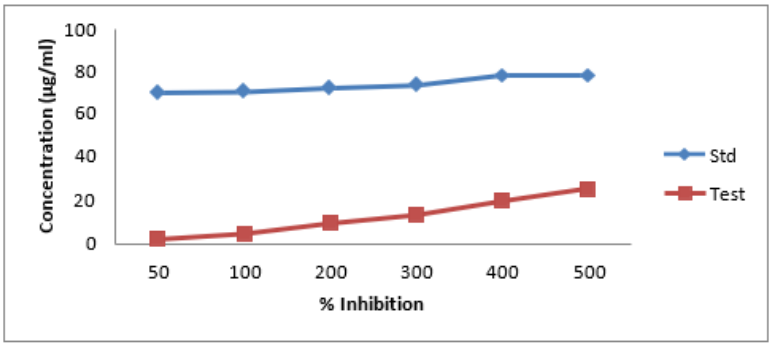



**Figure 2.2 - Percentage inhibition graph between standard and test by ferric reducing antioxidant power method**

**b. Skin-whitening activity of methanolic extract of clove**

The methanolic extract of clove was show antityrosinase activity. The result of comparatively study of percentage inhibition between standard and test solution was shown on table no.3.4 and comparatively graph of percentage inhibition between in figure no.3.3.The result of antityrosinase activity of standard on 100µg/mL given 86.28% and test sample given 52.65% on same concentration.

**Table 2.3- Antityrosinase activity of standard and test solution**

| S.NO | CONCENTRATION (µg/mL) | % INHIBITION OF STANDARD | % INHIBITION OF TEST |
|---|---|---|---|
| 1 | 10 | 40.24 | 1.38 |
| 2 | 20 | 45.86 | 5.79 |
| 3 | 30 | 51.27 | 11.23 |
| 4 | 40 | 57.62 | 16.55 |
| 5 | 50 | 61.23 | 22.13 |
| 6 | 60 | 65.24 | 27.85 |
| 7 | 70 | 70.12 | 35.65 |
| 8 | 80 | 75.27 | 42.19 |
| 9 | 90 | 81.11 | 47.34 |
| 10 | 100 | 86.28 | 52.65 |

## EVALUATION OF FORMULATION

### A. Physical evaluation

### Determination of Physical appearance

The color was observed visually. The average of three reading was recorded. The physical appearance of the formulations is shown in table no.3.6-

**Table 3.1- Physical appearance of formulations**

| **F1** | **F2** | **F3** | **F4** | **F5** |
|---|---|---|---|---|
| Off white | Off white | Off white | Yellowish | White |

**B. pH of formulations-**

pH of the formulations was determine as there is an essential need to prepared a formulations which is compatible to be used on a skin without any adverse effect. So to check the compatibility of the formulations with the skin it was assessed that whether the pH of the formulations come in range of skin (4.5-7) or not by using digital pH meter. The result is shown in table no.3.7 from which it can be concluded that all formulations have pH to be used on skin. The pH of the formulations, were found immersing pH meter to a depth 0.5 cm in a beaker containing formulations. The determinations were carried out in triplicate and the average of three reading was recorded.

**Table 3.2- pH of the formulations**

| **F1** | **F2** | **F3** | **F4** | **F5** |
|---|---|---|---|---|
| 6.4±0.3 | 6.8±0.3 | 6±0.2 | 6.6±0.21 | 5.5±0.1 |

**c. Determination of Spreadability :**

The Spreadability of the formulations was determining by parallel plate method. The result of Spreadability is shown in table no.3.8-

**Table 3.3-Spreadebility (gm.cm/sec) of formulations**

| **F1** | **F2** | **F3** | **F4** | **F5** |
|---|---|---|---|---|
| 1.77±0.88 | 0.74±0.11 | 0.48±0 | 0.88±0.08 | 0.38±0.05 |

**d. Viscosity**

The viscosity of formulated semisolids bases was determined. The viscosity determinations were carried out on Brook-field viscometer using spindle number S-06 and the average of nine reading is recorded and given in table no.3.9-

**Table 3.4- Viscosity (cps) of formulations**

| F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|
| 1155±1.2 | 1729±0.57 | 4386±0.58 | 5088±0.55 | 4766±0.33 |

**e. In vitro Permeation study-**

Methanolic extract of clove release was determined through rat skin by invert test tube method, and the receptor medium was phosphate buffer with methanol (1:1) in a beaker. The amount of the formulation was taken 5gm in a test tube and medium was 100 mL in a beaker. The temperature of the medium was maintained 37±1 0C and sample collected in a 30min of interval. The absorbance was taken by UV spectroscopy. The percentage of cumulative drug release was found out and given in followings tables no

**Figure 1.- Invert test tube method for In-Vitro release of formulations**

**Table 3.5- In-vitro drug release of formulations**

| | **% Cumulative drug release** |
|---|---|

| TIME (min) | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 3.11 | 2.46 | 0.16 | 5.9 | 9.35 |
| 60 | 4.23 | 3.36 | 1.216 | 11.73 | 11 |
| 90 | 10.2 | 4.82 | 3.25 | 14.33 | 15.22 |
| 120 | 17.4 | 11.97 | 6.67 | 16.9 | 18.19 |
| 150 | 26.54 | 21.92 | 15.4 | 18.62 | 21.98 |
| 180 | 31.01 | 26.19 | 26.28 | 29.06 | 27.98 |
| 210 | 34.39 | 37.26 | 34 | 33.85 | 34.13 |
| 240 | 50.14 | 42.33 | 42.8 | 45.93 | 44.72 |
| 270 | 58.52 | 59.55 | 51.6 | 62.57 | 52 |
| 300 | 68.32 | 68 | 60.7 | 71 | 81.6 |

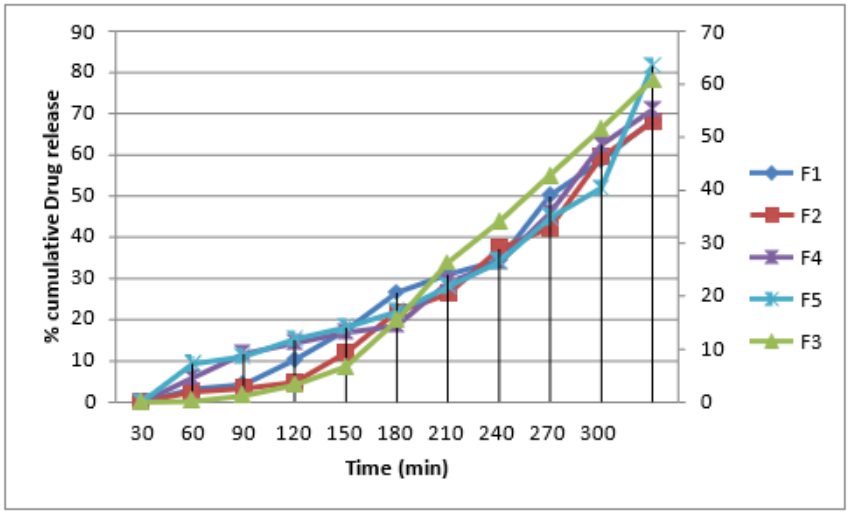



**Fig. 2.4 Graph of cream formulation drug release**

Comparison of the in-vitro drug release of the formulations demonstrated that the rank order was approximately the same, F5 > F4 > F2 > F1 > F3, for all combinations. The results of the formulations F1and F2 were found similar. The lowest release was showed F3 and the maximum release was showed in F5 formulation. The highest release profile was showed by F5.

**f. Stability test of formulations**

The stability studies of formulations were carried out under the following condition 40/75(0C/RH) for one month. The effects of temperature, humidity and time on the physical characteristics of the creams were for assessing the stability of the prepared formulations.

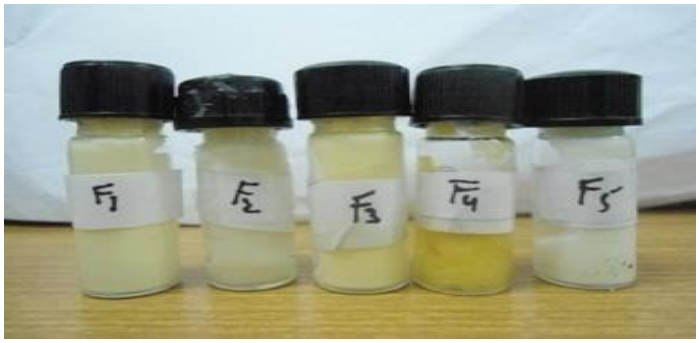
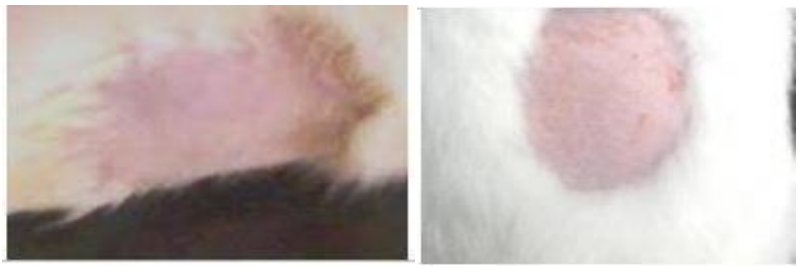



**Figure 3.10- Formulations for skin whetining**

**EVALUATION PARAMETER FOR FINAL FORMULATION**

**a. Skin irritation testing on Rat**

The skin irritation test of the final formulation was performed on rat. The animals were divided into two groups control and test. After the application of final formulation on rat skin was not found any type of erythema or any other skin sensitivity reaction. The result was found that the application of formulation completely safe on skin



Control Test

**Figure 3.12- Skin-irritation test on rat skin**

| PARAMETER | INITIAL | AFTER ONE MONTH<br>$^{0}$<br>40 C 75 RH |
|---|---|---|

| | | |
|---|---|---|
| Appearance | White | White |
| Feel on application | smooth | Smooth |
| pH | 5.5 ±0.1 | 5.7 ±0.3 |
| Viscosity | 47676 Cm Poise | 47679 Cm Poise |
| Spreadebility | 0.38±0.5 | 0.38±0.5 |
| Skin-irritation | Non-irritant | Non-irritant |
| Homogeneity | Homogeneous | Homogeneous |
| Stability | Stable | Stable |

The selection of best formulation was based on in-vitro release profile, non-irritant property and stability on 450C.

**SUMMARY AND CONCLUSION**

The present study was designed in order to formulate a herbal formulation of methanolic extract of clove for skin whitening activity. The plant Syzygium aromaticum was used in the formulation of cream.

The methanolic extract of clove was used as active ingredient for development of formulations. Different types of formulation (Vanishing cream, o/w emulsion and multiple emulsions w/o/w) were prepared by using 5% of methanolic extract of Syzygium aromaticum as skin whiting ingredient. The various parameters (pH, viscosity, homogeneity, Spreadebility, skin-irritation, in-vitro release and stability) of formulation were evaluated.

Comparison of the in-vitro of the formulations demonstrated that the rank order was approximately the same, F5 > F4 > F2 > F1 > F3, for all combinations. The results of the formulations F1and F2 were found similar. The lowest release was showed F3 and the maximum release was showed in F5 formulation. The highest release profile was showed by F5. The parameters of formulation were evaluated and on basis of that best formulation was selected. The formulation was found non-irritant to the skin and showed satisfactory result for the physico-chemical parameters such as pH, spreadability, homogeneity and viscosity. The formulation was also found to be stable on 450C temperature.

Thus from the above study we can establish that the formulation containing herbal ingredients can be used effectively to protect against the harmful effect of skin pigmentation as wall as inhibition of tyrosinase

which provided skin-whitening effect. Further studies can be done in order to isolate the active constituent of the plant which is responsible for showing skin- whitening activity.

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# Determination of antioxidant and anti-inflammatory activity of ethanolic extract of Ailanthus excelsa on SHSY-5Y cells

**Bharti Verma1, Nidhi Tyagi1***
1. SRM Modinagar College of Pharmacy, SRM Institute of Science and Technology (Deemed to be University), Delhi-NCR Campus, Modinagar, Ghaziabad, Uttar Pradesh, 201204, India
**Corresponding Author* Dr. Nidhi Tyagi Assistant Professor**
Department of Pharmacology,
SRM Institute of Science and Technology
Mail: nidhityagi029@gmail.com, nidhit@srmist.edu.in
ORCID: https://orcid.org/0000-0001-7162-4601

**Abstract**

Ailanthus excelsa plant belonging to the Simaroubaceae family has been used for various biological activities due to the presence of various sterols, phenols, saponins, flavonoids, glycosides and proteins which were also confirmed by performing preliminary studies. It is hepatoprotective, hypoglycemic, anti-fungal, anti-bacterial, anti-oxidant and anti-inflammatory in nature. Using SHSY-FY human neuroblastoma cell lines, the antioxidant, cytotoxic, and anti- inflammatory properties of A. excelsa ethanolic extract (AEEE) were investigated in this present work. Firstly, stem bark of this plant were collected and progressively extracted using ethanol as a solvent with the help of cold maceration method. The percentage yield was then computed. The concentration of phenols and flavonoids was evaluated to assess the anti-oxidant potential which was calculated as 39.51±0.167 mg GAE/gm and 24.92±0.261 mg QE/gm respectively. DPPH (2,2-diphenyl-1-picrylhydrazyl) method was also used to evaluate the antioxidant profile in which percentage inhibition was observed as 78.751±0.005 % which was compared with the standard i.e. ascorbic acid (89.165±0.233 %). Moreover, MTT assay was also performed which has showed a significant decline in the cell viability at a concentration of 200-1200 µg/ml. Along with this, it also consists anti-inflammatory activity and antioxidant activity. The cells were exposed with the concentrations of 100 and 200 µg/ml of ethanolic extract of A. excelsa in which it decreased the level of IL-6, MDA and increased the level of GSH (glutathione). Therefore, all the results suggested that A. excelsa could be a potential therapeutic approach for the treatment of neurodegenerative diseases which are related with oxidation and inflammation.

**Keywords:** Ailanthus excelsa, DPPH, Antioxidant, IL-6, and ethanolic extract, SHSY-5Y cell line.

## Introduction

Many disorders can now be treated with therapeutic medicines derived from natural resources.

Ailanthus plants belonging to the Simaroubaceae family are of extremely valuable because of their numerous therapeutic benefits [1]. In traditional medicine, several portions of this plant are used to treat a wide range of diseases. It has been observed that the root bark has cytotoxic and anticancer properties in both animals and cell cultures [2]. Extracts from stem bark shown strong antifungal and antibacterial properties [3, 4]. The alcohol extract derived from the bark of leaves and stems demonstrates exceptionally strong anti-implantation and early abortifacient properties [5]. A. excelsa is filled with diverse chemical compounds with a wide range of possible biological uses such as sterols, alkaloids, phenols, terpenoids, flavonoids, and quassinoides [6]. Since free radicals can trigger inflammatory reactions, antioxidants play a crucial role in reducing inflammation. Inflammatory diseases like arthritis, heart problems, and neurological disorders have been related to chronic oxidative stress. By lowering oxidative damage, plant species containing antioxidant property help to promote cellular health and modulate inflammatory pathways [7]. In this study, SHSY-5Y cell lines were used which are neuroblastoma cells to check the anti-oxidant and anti-inflammatory potential due to which this plant extract can be used as a therapeutic approach for the treatment of neurodegenerative diseases.

## Material and methods

### Preparation of Extract

Plant material (stem bark) was purchased from Khari Baoli, Delhi, India, and authenticated at CCS University, Meerut, Uttar Pradesh, India. Firstly, the stem bark was properly washed, shade dried, grinded in powdered form, and finally extracted by cold maceration method using ethanol as a solvent. The obtained extract was reduced with a rotary evaporator, weighed, collected in a air tight container, and preserved in a refrigerator for further work.

### Evaluation of total phenol and flavonoid content

In this plant extract, the concentration of phenol was demonstrated by the Folin Ciocalteu method used by Singleton et al., 1999 and total flavonoid by AlCl3 method described by Ebrahimzadeh et al., 2008 [8, 9].

**Culture of cells**

The SHSY-5Y cells were cultured at 37°C in a humidified atmosphere with 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), growth factors, and antibiotics [10].

**DPPH assay**

The free radical scavenging activity of AEEE was evaluated by DPPH method used by Munda et. al., 2019 [11].

**Cell viability Assay**

SH-SY5Y cells were cultured in high-glucose DMEM with 10% fetal bovine serum. The cytotoxicity of extract doses was evaluated using the MTT test. After 24 hours, cells were treated with various extract doses. After a 24-hour break, MTT was applied to each well, and the iMarkTM Microplate Absorbance Reader determined the absorbance [12].

**Determination of IL-6**

The concentration of interleukin-6 was determined by the method described by Dietrich et. al., 2020 [13].

**Determination of GSH**

The study used SH-SY5Y cells to detect GSH levels, which were then stimulated with LPS for

23 hours. The DetectX Glutathione Fluorescence Detection Kit and iMARkTM microplate absorbance reader were used to detect total and free GSH from cell lysate, with excitation and emission wavelengths set to 370-410 nm and 450 nm, respectively [14].

**Determination of MDA**

Enzyme-Linked Immunosorbent Assay was used to quantify the cell-permeable concentrations of MDA. A twelve-well plate with 125 SH-SY5Y cells was pre-treated with NAC and LPS for 23 hours. Cell pelletization was done, and liquid was collected. The iMARkTM microplate absorbance reader was used to read the samples at 450 nm [15].

**Statistical analysis**

The study used Graph Pad Prismsoftware, Version 5.01, to interpret results using T-test, One- way ANOVA, and Tukey post hoc test, with $p<0.05$ probability value.

**Results**

**Total phenol and flavonoid concentration**

The anti-oxidant potential which was observed as 39.51±0.167 mg GAE/gm and 24.92±0.261 mg QE/gm respectively

**Inhibitory activity of AEEE on DPPH**

Significant percentage inhibition was observed by AEEE (78.751±0.005 %) which was compared with ascorbic acid ((89.165±0.233 %). (Figure-1)

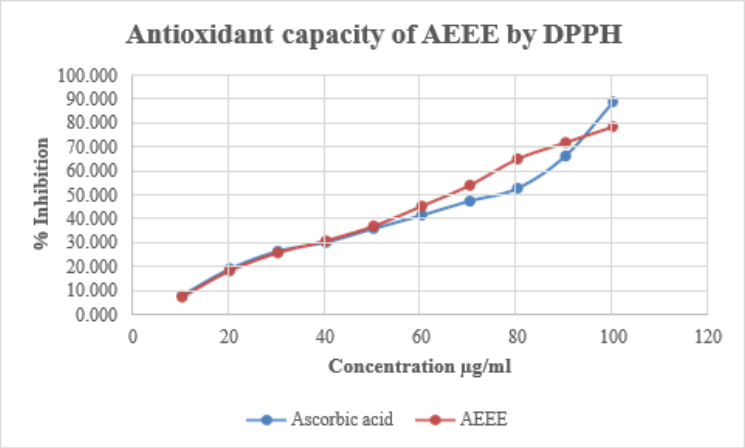


**Figure:1 Antioxidant potential of AEEE.**

The % inhibition of the test sample was compared with Ascorbic acid at different concentrations.

**Inhibitory activity of AEEE on cell viability**

The plant extract AEEE decreased the concentration the percentage of cell viability when treated with different doses (200-1200 µg/ml) ($p<0.0001$).(Figure-2)

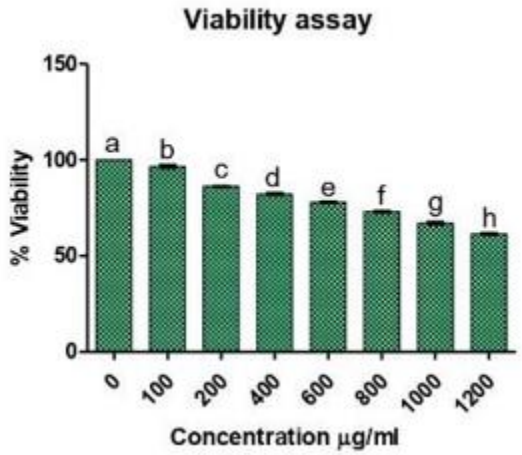


Figure:2 Cytotoxic effect of AEEE.

After treatment of the cells, cell viability was measured according to the concentration of formazan produced by mitochondrial dehydrogenase enzymes in the cells and compared to the untreated cells. Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. $p<0.05$.b, c, d, e, f, g, h versus a.

**Inhibitory activity of AEEE on IL-6**

The plant extract AEEE at a dose 200 µg/ml decreased the concentration of IL-6 more significantly than the dose 100 µg/ml as compared to LPS treated cells ($p<0.001$).(Figure-3).

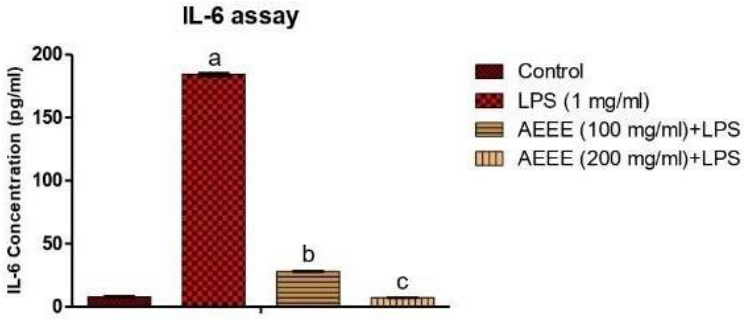


**Figure:3 Inhibitory activity of AEEE on IL-6 concentration.**

Group-1 normal control, and the negative control group (Group-2) was given LPS (1 µg/ml), and both test groups (Group-3 & 4) were treated with AEEE (100 and 200 µg/ml). Data were measured by One-way

ANOVA followed by Tukey post hoc test and represented as mean±SEM. a versus Normal control; b versus LPS (1 µg/ml); c versus AEEE (100 µg/ml).

**Inhibitory activity of AEEE on GSH**

In comparison to the normal control group, the LPS-treated cells showed a significant decline in the level of GSH ($p<0.0001$). Whereas, AEEE at a dose 100µg/ml treated cells significantly increased the level of GSH as compared to the LPS-treated group. Moreover, the AEEE at a dose 200µg/ml group demonstrated a significant elevation in the level of GSH than AEEE (100µg/ml) ($p<0.001$).(Figure-4).

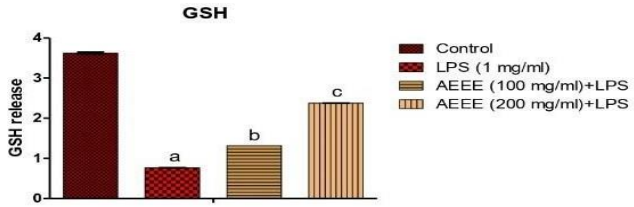


**Figure:4 Protective action of AEEE on GSH.**

Group-1 was normal control, and the negative control group (Group-2) was given LPS (1 µg/ml), and both test groups (Group-3 & 4) were treated with AEEE (100 and 200 µg/ml). Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. a versus Normal control; b versus LPS (1 µg/ml); c versus AEEE (100 µg/ml).

**Inhibitory activity of AEEE on MDA**

In comparison to the normal control group, the LPS-treated cells showed a significant increase in the level of MDA ($p<0.0001$). Whereas, AEEE (100µg/ml) treated cells significantly decreased the level of MDA as compared to the LPS-treated group. Moreover, the AEEE (200µg/ml) group demonstrated an effective decline in the level of MDA than AEEE (100µg/ml) ($p<0.0001$).(Figure-5).

**Figure:5 Inhibitory activity of AEEE on MDA.**

Group-1 was normal control, and the negative control group (Group-2) was given LPS (1 µg/ml), and both test groups (Group-3 & 4) were treated with AEEE (100 and 200 µg/ml). Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. a versus Normal control; b versus LPS (1 µg/ml); c versus AEEE (100 µg/ml).

## Conclusion

The ethanolic extract of A. excelsa has demonstrated antioxidant and anti-inflammatory properties due to its high concentration of phenols and flavonoids, demonstrating notable radical scavenging activity in the phytochemical study. The stem ark of this plant has also showed anti- inflammatory activity, reducing IL-6 concentration, MDA and increasing GSH. Further studies are needed to identify compounds responsible for these effects. The anti-oxidant and anti-inflammatory activity of this plant suggested that it could be a therapeutic potential for the treatment of various neurological diseases.

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## Conflict of Interest

The authors declare no conflict of interest.

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# Computational Strategies for the Rational Design of Tetrahydroisoquinoline-3-Carboxylic Acid Derivatives as Novel Antibacterial Agents

Avani Sharma[1], Jayendra Kumar[1*]

1. SRM Modinagar College of Pharmacy, SRM Institute of Science and Technology (Deemed to be University), Delhi-NCR Campus, Modinagar, Ghaziabad, Uttar Pradesh, 201204

*Corresponding author Email-ID jayendrk@srmist.edu.in

**Abstract**

The rising incidence of antibiotic resistance is one of the most urgent issues in the treatment of bacterial diseases. Furthermore, certain bacterial strains have developed resistance to a number of medications. As a result, an effective treatment option for bacterial infections needs to have higher bioavailability, good therapeutic effects, and minimal side effects. In order to combat this threat, the current study employed computational techniques like docking and in silico ADMET analysis to develop a new Tetrahydroisoquinoline-3-carboxylic acid-based derivative. After a review of the literature, molecules were designed. All designed compounds underwent molecular docking, and ADMET analysis. Protox II and pkCSM toxicity estimates showed that these compounds were non-toxic and had negligible to no negative effects. Molecular docking studies carried out with THIQ-3CA derivatives (referred AS-1 -AS-5) docked against selected target proteins (**PDB ID: 4DUH**) of *DNA Gyrase enzyme* demonstrated ideal binding energies ranging from -8.7 to -7.8 kcal/mol. **AS-3 and AS-4** were identified to exhibit better binding affinity compared to the marketed drug ciprofloxacin, showing their potential as anti-bacterial medicines. Among all 5 compounds, **AS-3** showed the most promising ADME profiles as confirmed by pkCSM, demonstrating good pharmacokinetic properties. Moreover, **AS-5** has the least toxicity, with no AMES toxicity and a high maximum tolerated dose, making it the safest among the AS compounds. This research work will provide ample opportunities to explore medicinal and computational research areas. It will facilitate the development of novel antibacterial agents in future experimental studies. Numerous opportunities to investigate computational and medical research fields will arise from this research work. It will facilitate the development of novel antibacterial agents in future experimental studies.

**Keywords:** *DNA Gyrase enzyme*, Tetrahydroisoquinoline-3-carboxylic acid derivatives, docking studies, ADME, toxicity

## 1. INTRODUCTION

Microbial infections are associated with higher rates of morbidity and mortality [1]. Common bacterial resistance to current antibiotic treatments is quickly growing into a significant global public health issue. The occurrence of multi-drug resistant Gram-positive and Gram-negative bacteria is increasing and diseases caused by them are becoming problematic now-a-days [2], and multiple studies have been claimed to increase and improve the present antibacterial compounds. More than 70% of bacteria have been shown to be resistant to one or more of the antibiotics that are typically used to treat the infection [3]. Although the emergence of multidrug resistance in *Bacillus subtilis* is not a recent development, research on *B. subtilis* inhibition profiling is currently being conducted to find ways to counteract this resistance [4–7].

As a result, development of new antimicrobial drugs is a topic for current research that seeks to control resistant bacteria strains worldwide [8]. We may highlight Tetrahydroisoquinolines-3-carboxylic acid hydrazides as one of the chemical compounds with biological potential that could be new potential antibacterial agents [9].

Nitrogen heterocycles are essential components of many compounds with potential applications in medicine and act as basic building blocks for the discovery of new medications [10]. The chemical and biological significance of the isoquinoline skeleton cannot be overstated. Medications containing this skeleton, whether natural or synthetic, can be used for a variety of therapeutic purposes [11]. One of the most advantageous heterocyclic scaffolds is the tetrahydroisoquinoline moiety, which is widely found in a variety of plants, soils, and marine microbes. Tetrahydroisoquinolines have a wide range of applications in medicine, including analgesic, antibacterial, antifungal, anticancer, anti-inflammatory, anticonvulsant, antileukemic, anti-HIV, and antithrombotic [12].

Moreover, THIQ-containing isolated alkaloids from natural sources are found in large quantities in a number of pharmaceuticals. Based on the 1,2,3,4-tetrahydroisoquinoline molecule, the most well-known medicinal drugs are quinapril, noscapine, and praziquantel [13].

The vast diversity of biological importance of hydrazide-hydrazones is potentially due to azomethine group in their structure. Mainly antibacterial, antitubercular, antifungal, anti-inflammatory, and antioxidant activities are among their biological effects [14]. Their activity was occasionally significantly higher than that of the reference drugs (ampicillin, nitrofurantoin, cefuroxime, or ciprofloxacin) [15–18].

Because of this, the current study focuses on designing novel derivatives of Tetrahyroisoquinoline-3-carboxylic acid and investigating these derivatives against the DNA Gyrase enzyme in silico. The purpose of the study is to screen the hit compounds for pharmacokinetic and toxicological profiles and to analyze the binding affinities of the newly design derivatives against the active pockets of the DNA Gyrase enzyme.

## 2. MATERIALS AND METHODS

### 2.1 Protein Preparation

Using the accession code 4DUH, the target protein structure was obtained from the Protein Data Bank (PDB). The protein structure was then transformed with AutoDock Vina version 1.5.6 into PDBQT format. The protein was then placed into AutoDock in order to extract the protein and ligand independently. This software was used to evaluate the ligands' binding affinities and choose the optimal lead molecule for antibacterial drugs [20].

### 2.2 Molecular Docking Program

By locating its binding site, the chosen protein 4DUH has been prepared. The ligand (RL1301) was first removed in order to validate the protein, and the altered structure was stored in PDB format. The protein in PDB format was then loaded into AutoDock Vina for docking studies after water and other redundant structures were removed, missing atoms were fixed, polar hydrogen atoms were added, and Kollman charges were applied. To develop the ligand, it was extracted from the protein, polar hydrogen atoms were added, the root was found, torsions were chosen (which were left flexible to explore various ligand conformations), and the ligand was eventually converted into a PDBQT file extension [20–22]. Finally, the ligand was created as the center of a grid box. Below is the configuration for docking:

center_x = 3.024

center_y = 2.008

center_z = 36.902

size_x = 40

size_y = 40

size_z = 40

### 3. 2-D Interaction Studies

Using the Discovery Studio molecular docking was performed, where the prepared protein and ligands were subjected to multiple docking runs. This procedure located the protein's binding site and evaluated the ligand-protein interaction patterns and binding affinities. [23, 24].

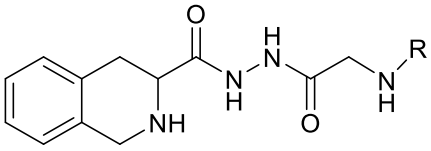
### 4. ADME and Toxicity Prediction

Using the pkCSM online platform, ADME (Absorption, Distribution, Metabolism, and Excretion) predictions were used to assess the pharmacokinetic features of each of these hit substances. ChemDraw 2D was initially used to convert the compounds to their corresponding SMILES IDs, which were subsequently input into the pkCSM portal. This study offered a thorough assessment of the ligands' potential as therapeutic candidates by shedding light on their drug-likeness, solubility, permeability, and metabolic stability [25]. Furthermore, the compounds underwent toxicity prediction with the use of web portals, namely protox-II and pkCSM [26-27].

## 5 Results and Discussion

AutoDock Vina 1.5.6 software was then used to perform molecular docking in order for assessing the binding affinity in the active site of the DNA Gyrase enzyme protein (PDB ID: 4DUH).. After the compounds were screened, their kcal/mol free binding energies were calculated. The compounds' binding affinities ranged from -8.7 to -7.8 kcal/mol, according to the molecular docking studies.

**Table 1: Docking Scores of designed compounds in comparison to standard drug ciprofloxacin.**

| Docking Score | | | |
|---|---|---|---|
|  | | | |
| **S.No** | **Compound** | **R** | **Binding Affinity (kcal/mol)** |
| **1** | **AS-1** | $-C_6H_4NO_2$ | -7.8 |
| **2** | **AS-2** | $-C_6H_3ClNO_2$ | -8.0 |
| **3** | **AS-3** | $-C_6H_4Br$ | -8.7 |
| **4** | **AS-4** | $-C_6H_3(CH_3)_2$ | -8.5 |

| | | | |
|---|---|---|---|
| **5** | **AS-5** | $-C_6H_4CH_3NH_2$ | -8.1 |
| **6** | Co-Crystallized Ligand | - | -7.4 |
| **7** | Ciprofloxacin | - | -8.2 |

**Table 2: Types of Interactions for Compounds**

| S. No. | Compound | Binding Affinity (kcal/mol) | Hydrogen Bond Interactions | Carbon Hydrogen Bond Interactions | π-σ Interactions | π-alkyl Interactions | Pi-Cation | Halogen (Fluorine) Interaction | Amide-Pi Stacked | Pi-sulphur Interactions |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | AS-1 | **-7.8** | VAL120 HIS99 LYS103 ASP49 | GLY117 | ILE48 | LYS103 | - | - | - | |
| 2. | AS-2 | **-8.0** | ARG136 ARG76 | HIS83 | - | ALA90 PRO79 ILE78 | - | - | GLY77 | |
| 3. | AS-3 | **-8.7** | VAL120 ASN46 | GLY119 GLY101 | - | ILE78 LYS103 | GLY117 GLU50 | - | ASN46 | |
| 4. | AS-4 | **-8.5** | GLY117 PHE104 LYS103 | -- | -- | ILE78 | GLU50 GLY117 LYS103 | - | | |
| 5. | AS-5 | **-8.1** | GLY101 LYS103 | -- | ASN46 ILE78 | PRO79 ALA90 | -- | -- | VAL120 | |
| 6. | Co-Crystallized Ligand | **-7.4** | ALA100 ASP45 ASP49 | PRO79 | ILE94 | LYS103 | LYS103 GLY117 | -- | -- | PHE104 |
| 7. | Ciprofloxacin | **-8.2** | GLY77 ALA100 | ILE94 GLY119 PRO79 | -- | ILE78 VAL120 ILE94 LYS103 | -LYS103 GLU50 | GLY101 ALA100 | - | |

Furthermore, compounds with either strong or equipotent activity in relation to the ligand and the reference medication ciprofloxacin (binding affinity = - 8.2 kcal/mol) were found by analysis. With a docking score of -8.7 kcal/mol, compound AS-3 demonstrated the most favorable results, suggesting a substantial binding affinity to the target protein. (Reference Table 1). To determine how these five chemicals interacted with the protein's amino acids (given in Table 2), a thorough interaction study was conducted. The results, which are shown in Table 2, provide further information about the binding mechanisms of each chemical by highlighting several interaction types such as π-π interactions, π-alkyl interactions, hydrogen bonds (H-bonds), and π-cation interactions.

As depicted in Figs. (**1**), the interaction analysis of **AS-3** with the highest binding affinity (-8.7 kcal/mol), reveals that the compound exhibits significant hydrogen bond interactions with VAL120 and ASN46, carbon

hydrogen bonding with GLY119 and GLY101 and π-alkyl interactions with GLU50 and GLY117. A special type of interaction, namely amide-pi Sacked and pi cation was also found between enzyme protein and compound **AS-3** involving the amino acid ASN46. and GLY117, GLU 50 respectively.

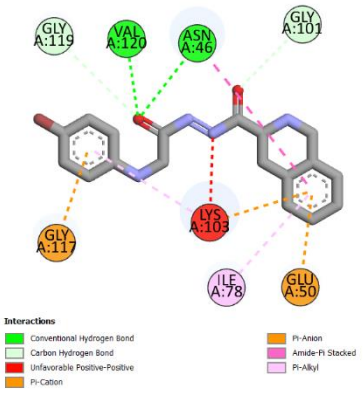



**Figure-1 Interaction of AS-3 & DNA gyrase protein (4DUH)**

The interactions of compounds **AS-4 are** presented in Figs. (**2**), compound was found to have **three** H-bond interaction with GLY117, PHE104 and LYS103, and also showed a π-alkyl interaction with ILE78. Furthermore, this compound engaged in π-alkyl interactions with residues such as PHE764, LEU, and MET895.

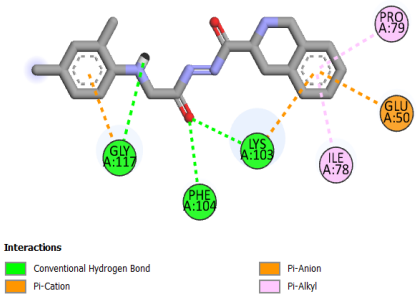



**Figure-2. Interaction of AS-4 & DNA gyrase protein (4DUH)**

**AS-5 (Fig-3)** also demonstrate the H-bond interaction with residue GLY101 and LYS103, π-sigma interactions with residues ASN46 and ILE78. Along with that this compound also show a π-alkyl interaction with PRO79 and ALA90. Furthermore, this compound engaged in amide π-sacked interactions with residues such as VAL120.

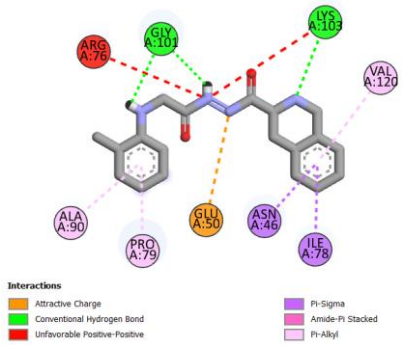



**Figure-3. Interaction of AS-5 & DNA gyrase protein (4DUH)**

Figures (4 and 5) depict the interactions of compounds AS-1 and AS-2, respectively. It was discovered that both compounds exhibited hydrogen bond interactions; AS-1 interacted with residues VAL120, HIS99, LYS103, and ASP49, while AS-2 interacted with ARG136 and ARG76 in the DNA gyrase enzyme's active site. Other than that, C-H interactions were shown by both compounds with ILE48 and HIS83, respectively. Whereas AS-2 has three π-alkyl interactions with ALA90, PRO79, and ILE78, AS-1 only exhibits one π-alkyl contact with LYS103.

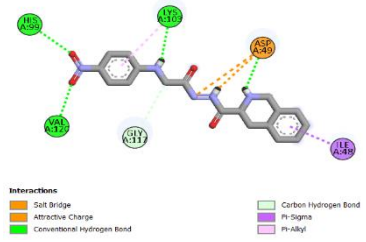



**Figure-4. Interaction of AS-1 & DNA gyrase protein (4DUH)**

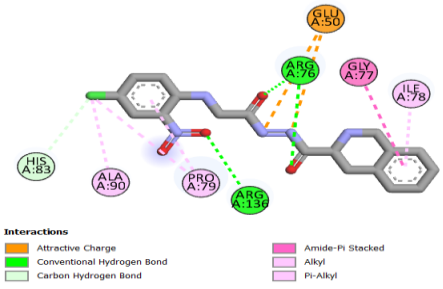



**Figure-5. Interaction of AS-2 & DNA gyrase protein (4DUH)**

The co-crystallized ligand is presented in **Fig. (6)** have H-bond interaction with ALA100, ASP45 and ASP49, C-H Interaction with PRO79, also have one π-alkyl and π -sigma interactions with ILE94 and LYS103 respectively. Along with this it displayed pi-cation interaction with LYS103 and GLY117, and have one Pi-sulphur interaction with PHE104.

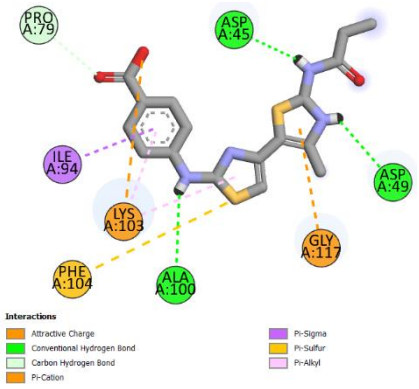



**Figure-6. Interaction of Ligand & DNA gyrase protein (4DUH)**

Additionally, Fig. (7) showed the interactions of the standard drug ciprofloxacin. Two H-bond interactions between ciprofloxacin and distinct amino acid residues, GLY77 and ALA100, were observed. Apart from that, it displayed, C-H interaction with ILE94. GLY119 and PRO79, several π-alkyl interactions with ILE78, VAL120, ILE94, and LYS103, and pi-cation interaction with LYS103 and GLU50. Notably, it showed Halogen interaction with GLY101 and ALA100. The interaction patterns showed that several residues were common between Ciprofloxacin and the designed ligands.

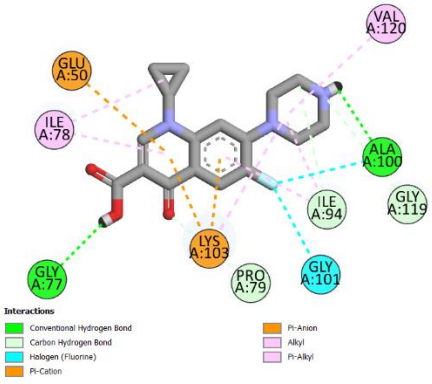



**Figure-7. Interaction of Ciprofloxacin & DNA gyrase protein (4DUH)**

Based on the interaction patterns that the majority of ligands and ciprofloxacin displayed with VAL120 and LYS103, it can be deduced that the designed ligands display interactions that are comparable to those of the standard drug ciprofloxacin, which makes them promising compounds for the treatment of bacterial infection.

### 5.1 ADME Analysis

ADME analysis was carried out to evaluate their potential even further, since these physicochemical characteristics are critical in defining a compound's outcome. Table 3 displays the physicochemical parameters, such as molecular weight, Log P values and water solubility, for the hit compounds. A range of molecular weights, from 338.411 to 639.381 g/mol, is revealed by the ADME analysis of five new substances (AS-1 to AS-5) and a reference medication (ciprofloxacin), with AS-1 having the highest value. The range of lipophilicity (LogP) is 0.8686 (AS-1) to 1.7229 (AS-3), suggesting a range of hydrophobicity that might affect the permeability and solubility of membranes. Each compound exhibits intestinal absorption ranging from 38.87% (AS-5) to 97.31% (Ciprofloxacin), and moderate to high water solubility (log mol/L between -3.249 and -3.338). With distribution values (VDss) close to neutral, they are all P-glycoprotein (P-gp) substrates with restricted tissue distribution. Permeability of the blood-brain barrier (BBB) ranges from -1.347 to -0.534, and the CNS is likewise limited in permeability for all substances (log PS values < -2.5). With the exception of ciprofloxacin, all substances are substrates of CYP2D6 and CYP3A4. The compounds' total clearance values differ, with AS-1 exhibiting the maximum clearance (0.874 log ml/min/kg).

**Table 3. Drug likeness property of hit compounds using pkCSM.**

| S. No | Code | Molecular Weight (g/mol) | LogP | Water Solubility (log mol/L) | Intestinal Absorption (human %) | P-gp Substrate | VDss (human, log L/kg) | BBB Permeability (log BB) | CNS Permeability (log PS) | CYP2D6 Substrate | CYP3A4 Substrate | Total Clearance (log ml/min/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | **AS-1** | 639.381 | 0.8686 | -3.307 | 68.591 | Yes | -0.068 | -1.169 | -2.946 | Yes | Yes | 0.874 |
| 2. | **AS-2** | 403.026 | 1.522 | -3.317 | 69.729 | Yes | -0.016 | -1.347 | -2.818 | Yes | Yes | 0.77 |
| 3. | **AS-3** | 403.28 | 1.7229 | -3.338 | 94.816 | Yes | -0.024 | -1.153 | -2.592 | Yes | Yes | 1.131 |
| 4. | **AS-4** | 352.438 | 1.57724 | -3.292 | 96.139 | Yes | 0.046 | -0.99 | -2.581 | Yes | Yes | 1.112 |
| 5. | **AS-5** | 338.411 | 1.26882 | -3.249 | 38.872 | Yes | 0.027 | -0.967 | -2.649 | Yes | Yes | 1.154 |
| 6. | **Ciprofloxacin** | 331.347 | 1.5833 | -3.172 | 97.306 | Yes | 0.042 | -0.534 | -3.03 | Yes | No | 0.545 |

### 5.2 Toxicity Analysis

Protox-II and pkCSM, which are accessible online at https://tox-new.charit e.de/protox_II/ and https://biosig.lab.uq.edu.au/pkcsm//, respectively, were used to predict the toxicity of these chemicals. The highest tolerated dose (HTD), oral rat acute toxicity (LD50), AMES toxicity, hERG I and II inhibitor, organ toxicity (particularly hepatotoxicity), skin sensitization, T. pyriformis toxicity, and minnow toxicity are all included in Table 4's toxicity predictions for these three drugs. With the exception of AS-1 and AS-5, all drugs exhibit active neurotoxicity but lack hepatotoxicity. In general, nephrotoxicity is inert; however, AS-2 and Ciprofloxacin exhibit efficacy. With the exception of AS-1, most chemicals are respiratoryly poisonous. T. pyriformis toxicity varies from 0.291 to 0.318 log µg/L, and none of the chemicals show signs of skin sensitization. Each of the compounds are AMES hazardous, except AS-5, which is non-AMES toxic like Ciprofloxacin. All substances, with the exception of ciprofloxacin, inhibit hERG II but not hERG I. Minnow toxicity values range from 1.129 log mM for ciprofloxacin to 4.152 log mM for AS-1.

**Table 4. Predicted toxicity for hits compounds.**

| S. No | Code | AMES Toxicity | Max Tolerated Dose (log mg/kg/day) | hERG I Inhibitor | hERG II Inhibitor | ORAT (LD50, mol/kg) | ORCT (LOAEL) | Hepato-toxicity | Neuro-toxicity | Nephro-toxicity | Respiratory toxicity | Cardio Toxicity | Skin Sensitisation | T. Pyriformis Toxicity (log µg/L) | Minnow Toxicity (log mM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | AS-1 | Yes | -0.079 | No | Yes | 2.47 | 2.686 | Inactive | Inactive | Inactive | Active | Inactive | No | 0.294 | 4.152 |
| 2. | AS-2 | Yes | -0.097 | No | Yes | 2.407 | 2.54 | Inactive | Active | Active | Active | Inactive | No | 0.291 | 3.569 |
| 3. | AS-3 | Yes | -0.03 | No | Yes | 2.549 | 2.109 | Inactive | Active | Inactive | Active | Inactive | No | 0.318 | 2.359 |
| 4. | AS-4 | Yes | -0.045 | No | Yes | 2.565 | 1.951 | Inactive | Active | Inactive | Active | Inactive | No | 0.316 | 2.88 |
| 5. | AS-5 | No | -0.035 | No | Yes | 2.504 | 2.042 | Inactive | Active | Inactive | Active | Inactive | No | 0.315 | 2.917 |
| 6. | Ciprofloxacin | No | 0.274 | No | No | 2.2 | 0.994 | Inactive | Active | Active | Active | Inactive | No | 0.295 | 1.129 |

## CONCLUSION

Our goal in this work was to use computational techniques to create and find effective antibacterial chemicals. Novel compounds were first created by a survey of the literature. These compounds were then docked using AutoDock Vina 1.5.6. The docking analysis revealed that two compounds, AS-3 and AS-4, had better binding affinities than the commercial medication ciprofloxacin, suggesting that they may be used as antibacterial agents. As validated by pkCSM, AS-3 showed encouraging ADME profiles among these drugs, suggesting good pharmacokinetic qualities. Furthermore, AS-5 is the least hazardous of the

AS compounds due to its lack of hERG I inhibition, high maximum tolerated dose, and lack of AMES toxicity.

This work introduces new opportunities to investigate computational and therapeutic studies to create new antibacterial agents. In addition, further research in the synthesis and biological assessment of these strong leads might yield new and less toxic DNA gyrase inhibitors for the treatment of bacterial infections.

## COMPETING INTERESTS

The authors declare no conflict of interest, financial or otherwise.

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NA

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# Formulation And Characterization of a Pulsatile Zafirlukast Delivery System For Enhanced Asthma Therapy

**[1]P.Bikash Kumar Patra[*],** [2]Ritesh Kumar Dash, [3]Sunita Patnaik, [4]Aiswarya Patnaik, [5]Bimala Tripathi, [6]Karishma Behera, [7]Goutami Jani, [8]Abhipsa Mohapatra
Roland Institute of Pharmaceutical Sciences, Brahmapur, Odisha

## ABSTRACT

The objective of this study was to develop and optimize a pH-sensitive, sustained-release formulation of Zafirlukast using core and coated tablet designs for targeted, controlled drug release. Initial formulation involved preparing core tablets with various super disintegrants, where croscarmellose sodium (F3) was identified as the optimal agent based on disintegration and dissolution profiles. The core was then coated with Eudragit-RS100 and Eudragit-L30 polymers at varying concentrations. Among these, formulation F9, coated with 9.75% Eudragit-L30, exhibited an effective enteric coating with a lag time of 4.6 hours, followed by rapid drug release. FTIR studies confirmed no interactions between drug and excipients, while scanning electron microscopy verified the coating thickness. In vitro release studies indicated that F9 followed a zero-order kinetic release, demonstrating controlled and sustained drug delivery. This formulation approach for Zafirlukast offers potential for enhanced therapeutic outcomes and patient adherence.

**Keywords**: Zafirlukast, sustained-release, enteric coating, Eudragit, controlled release

## I. INTRODUCTION

Pulsatile drug delivery systems (PDDS) are designed to release drugs in a controlled, time-dependent manner to align with the body's circadian rhythms and site-specific therapeutic needs. Unlike traditional sustained drug delivery, which offers a steady release of medication over time, PDDS release drugs in response to a programmed schedule. This allows for optimal release at specific times or targeted sites, improving therapeutic efficacy and reducing side effects. With chronic diseases and disorders often displaying time-dependent symptoms or worsening at certain times, PDDS offer a promising approach for enhanced patient outcomes.

PDDS release drugs in two or more distinct phases following an initial lag period, allowing release to be controlled by time, site, or both factors. Time-based systems delay drug release until specific periods, catering to conditions that require night-time or early-morning dosing, such as asthma or hypertension. Alternatively, site-specific PDDS target particular gastrointestinal regions, such as the colon, which is beneficial for treating localized diseases like inflammatory bowel disease and can improve bioavailability for drugs sensitive to the upper digestive tract environment.

In PDDS, drug release is governed by formulation and the patient's gastrointestinal environment. Time-controlled systems use polymer barriers that degrade or dissolve to trigger release after a set period, while site-specific systems are influenced by GI factors. Through such mechanisms, PDDS enable delivery tailored to disease states and minimize continuous drug presence in the bloodstream, which can lead to tolerance and reduced therapeutic effect. Conditions benefitting from PDDS include those with circadian rhythms, where symptoms fluctuate, as in asthma, cardiovascular disease, and arthritis.

The circadian rhythm, an intrinsic biological clock, affects physiological processes over a 24-hour cycle. Many diseases exhibit symptoms linked to these rhythms, such as bronchial asthma and myocardial infarction, which often peak at night or early morning. PDDS are therefore ideal for delivering drugs in sync with these fluctuations, allowing for a more natural and effective treatment approach. Current PDDS research aims to develop systems that match these rhythms, which can lead to improved patient compliance, reduced side effects, and enhanced therapeutic effectiveness.

Chronopharmaceutics, a field focused on creating delivery systems that align with the body's natural rhythms, is crucial to PDDS. By leveraging circadian rhythms, chronopharmaceutic systems allow the release of medications at the most opportune times, based on the biological needs of specific diseases. The goal is to maximize efficacy while minimizing adverse effects, especially for drugs that interact with sensitive body systems, such as those that cause gastric irritation or nausea.

With advancements in PDDS technology, a more dynamic approach to medication is emerging, shifting from continuous, zero-order release to adaptive, time-based strategies. This evolution in drug delivery is essential for managing diseases where conventional methods fall short.

## II. LITERATURE REVIEW

Sachin et al. (2019) demonstrated the effectiveness of multiparticulate drug delivery systems for achieving sustained or delayed oral release with low risk of dose dumping and short gastric residence time. Their study highlights the flexibility of multiparticulates to adapt to different release profiles and emphasizes their benefits over single-unit dosage forms. Specifically, they explored the use of floating multiparticulates formulated via solvent evaporation techniques to increase gastric retention time, showcasing this approach as a significant advancement in pulsatile drug delivery.

Ahmed et al. (2020) developed a multifunctional system incorporating a “tablets-in-capsule” design for the programmable release of ketorolac and famotidine. Their formulation involved mini-tablets providing rapid and delayed release of famotidine to ensure maximum gastric protection, while ketorolac was designed for timed release. This dual drug delivery approach ensured a pulse of famotidine after a lag time of 6 hours, demonstrating the versatility of this capsule-in-capsule technique for treating conditions requiring staggered release to improve therapeutic efficacy.

MD. Sarfaraz, Prasad Y, et al. (2021) formulated press-coated time-release tablets of nifedipine aimed at managing hypertension chronotherapeutically. Using a compression coating technique, nifedipine was embedded within a polymer matrix of PEG 6000 and HPMC K 100M. The lag time achieved ranged between 2 and 6 hours, showing the system's adaptability to patient-specific circadian requirements in hypertension management, further substantiating the utility of polymeric coatings for achieving desired release patterns.

Efentakis et al. (2022) examined an innovative oral pulsatile drug delivery system using a core-in-cup tablet design, where the active ingredient was encapsulated within a cellulose acetate propionate outer shell. A soluble polymer top layer, composed of hydrophilic materials like polyethylene oxide and sodium alginate, controlled the drug release of caffeine or theophylline. This three-part configuration allowed for timed and efficient drug release, suggesting that such designs could be highly effective in regulating drug exposure to synchronize with circadian rhythms.

Kausalya et al. (2023) developed a multiparticulate chronotherapeutic delivery system for flurbiprofen using cellulose acetate cores within Eudragit S-100 microspheres. The system was formulated through

emulsion solvent evaporation at different drug-to-polymer ratios and demonstrated a pH-dependent release profile. The release was influenced by the cellulose acetate content, achieving a 12-hour lag time in pH 7.4, indicating the potential of this formulation in achieving targeted, delayed release tailored for specific GI conditions.

Jalap R. Patel et al. (2023) formulated and evaluated an oral controlled drug delivery system for metformin, a drug with inherently poor water solubility and bioavailability constraints. By incorporating polymers like HPMC, PVP, ethylcellulose, and carbopol-934, they enhanced the solubility and sustained the release of metformin, with direct compression used as the preparation technique. Their results underscore the effectiveness of HPMC and ethylcellulose in enhancing metformin's bioavailability, presenting promising polymers for controlled release systems designed to improve therapeutic outcomes in diabetes management.

## III. RESEARCH METHODOLOGY

This study outlines a detailed procedure for the formulation and evaluation of Zafirlukast tablets designed for controlled drug release. The methodology encompasses the preparation of standard solutions, construction of a calibration curve, drug-excipient compatibility studies, tablet formulation through direct compression, coating with specific polymers, and in-vitro release studies. Core tablets containing Zafirlukast were formulated using superdisintegrants such as sodium starch glycolate, croscarmellose sodium, and crospovidone. Additional excipients included polyvinyl pyrrolidone-K30 as a binder, microcrystalline cellulose (MCC) as a diluent, and magnesium stearate and talc as lubricants. The ingredients were precisely weighed and mixed, sieved through a 60-mesh screen, and compressed into tablets using a rotary compression tablet machine (Rimek mini press I) with an 8-mm concave punch. The compressed Zafirlukast tablets were coated using Eudragit-L 30 and Eudragit-RS 100 polymers to control drug release. The coating solution was prepared using a solvent mixture of isopropyl alcohol (IPA), acetone, and water. Triethyl citrate was incorporated as a plasticizer, titanium dioxide as an opacifier, and talc as an anti-tacking agent. Eudragit powder was initially dissolved in 50% of the solvent mixture, followed by the addition of talc and triethyl citrate in the remaining diluent. The complete suspension was sieved through a 0.5 mm mesh to remove any undissolved particles, ensuring a smooth and uniform coating.The release of Zafirlukast from the coated tablets was tested in-vitro using a modified USP XXIII

dissolution apparatus (Lab India, DS-800). The test conditions included a dissolution medium of 900 ml phosphate buffer at pH 6.8, with a rotational speed of 50 rpm at 37°C, replicating human physiological conditions. At fixed intervals, 5 ml samples were withdrawn, measured for absorbance at 240 nm, and replaced with fresh buffer to maintain the volume constant. These values provided a release profile for Zafirlukast, helping evaluate the effectiveness of the controlled release formulation in achieving the intended lag and release times.

## IV. RESULT AND DISCUSSIONS

In the present study, a standard calibration curve for Zafirlukast was established in 0.1 N HCl media. Concentrations of 5, 10, 15, 20, and 25 µg/ml were prepared through serial dilutions, and the absorbance at 242 nm was measured for each. A linear relationship between concentration and absorbance was observed, yielding a straight line on the calibration graph with a coefficient of determination R2R^2R2 of 0.9987. The calibration constants obtained were K1=0.0392K_1 = 0.0392K1=0.0392 and K0=0.012K_0 = 0.012K0=0.012, indicating a high degree of accuracy and reliability for concentration determination in subsequent dissolution studies. Fourier Transform Infrared (FTIR) analysis confirmed that the Zafirlukast pure drug and the optimized formulation exhibited absorption peaks within the expected wavenumber range, corresponding to the nature of chemical bonds present. No significant differences in absorption peaks between the pure drug and the formulation indicated the absence of any interaction between Zafirlukast and the excipients used in the tablet formulation. This compatibility is crucial for maintaining the stability and efficacy of the drug within the formulated product. Physical properties of the powder blends used to formulate the rapid-release core tablets were evaluated as part of preformulation studies. Parameters such as angle of repose, bulk density, tapped density, Carr's consolidation index, and Hausner's ratio were assessed, showing that the powders had good flow properties as well as favorable volume and density characteristics. These properties are essential for ensuring uniformity and consistency during tablet compression. In-vitro drug release studies demonstrated the efficacy of the sustained-release mechanism for all tested formulations over a 5-hour period. The enteric coating applied to the formulations remained intact for the first 2 hours in a gastric pH of 1.2, successfully resisting the acidic environment, and then gradually dissolved in the intestinal pH. Among the formulations, F7 and F8 released the drug within 262 minutes due to a lower percentage of coating, while F9 and F10, with a slightly higher coating percentage, extended the release up to 320 minutes. Specifically, formulation F9 achieved an optimal

balance, releasing 96.6% of the drug within 16 minutes following a lag time of 4.6 hours. Formulation F9, coated with 9.35% Eudragit L30, exhibited the most favorable release profile among the tested formulations. This coating composition provided both adequate gastric resistance and a controlled release in the intestinal environment, aligning with the objectives of sustained and delayed release. Overall, F9 was identified as the most promising formulation, providing an efficient release mechanism while protecting the drug from premature dissolution in gastric conditions. This controlled-release characteristic could significantly enhance the therapeutic effectiveness of Zafirlukast.

**Table 1 Dissolution profile of formulations**

| T (min) | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 36.62 | 38.06 | 44.15 | 30.42 | 37.26 | 44.41 |
| 4 | 54.02 | 55.49 | 53.26 | 49.44 | 49.66 | 52.44 |
| 6 | 62.37 | 69.61 | 72.16 | 61.55 | 60.16 | 70.62 |
| 8 | 77.23 | 74.69 | 82.11 | 73.23 | 72.98 | 79.83 |
| 10 | 80.55 | 85.56 | 88.01 | 79.06 | 79.77 | 86.11 |
| 12 | 83.41 | 89.54 | 91.77 | 84.60 | 85.62 | 88.62 |
| 14 | 88.39 | 91.69 | 98.48 | 92.15 | 88.49 | 93.13 |
| 16 | 92.46 | 93.80 | 98.74 | 94.76 | 94.01 | 96.34 |
| 18 | 94.27 | 95.16 | 98.06 | 94.95 | 95.22 | 96.66 |
| 20 | 95.25 | 96.93 | 98.40 | 94.23 | 95.32 | 96.85 |

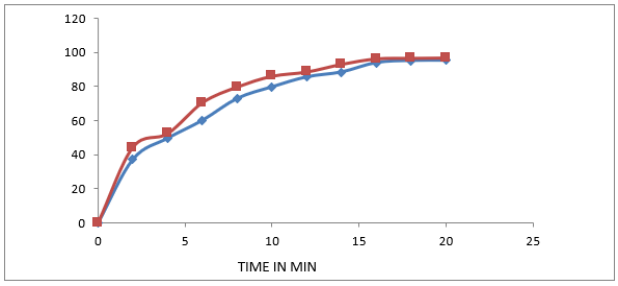


Figure 1 : Dissolution of optimized formulation

## V. CONCLUSION

This study successfully developed and optimized a Zafirlukast sustained-release tablet formulation with enteric coating for controlled drug release. FTIR analysis confirmed compatibility between the drug and excipients, ensuring formulation stability. Among the core formulations, F3, containing croscarmellose sodium, was optimized based on favorable disintegration and dissolution times. This core was then coated with pH-sensitive polymers, specifically Eudragit-RS100 and Eudragit-L30, to achieve delayed release.The final optimized formulation, F9, coated with 9.75% Eudragit-L30, demonstrated a desirable release profile with an initial lag time of 4.6 hours, followed by rapid drug release. Scanning electron microscopy confirmed an average coating thickness of 33.28 µm, indicative of effective enteric protection. In vitro release studies showed that F9 followed a zero-order release mechanism, providing sustained drug delivery aligned with therapeutic objectives. This formulation strategy offers a promising approach for the controlled delivery of Zafirlukast, enhancing its therapeutic efficacy and patient compliance.

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# Exploring Albumin Microspheres for Enhanced Delivery of Ketoprofen: Formulation And Assessment

[1]Bikram Kumar Sahu*, [2]Gourav Sahu, [3]Kalpana Mahapatra, [4]Sarmistha Das, [5]Rashika Shrivastav, [6]Sarthak Behera, [7]Dibyajyoti Pani
Roland Institute of Pharmaceutical Sciences, Brahmapur, Odisha

**ABSTRACT**

The aim of this study was to develop and evaluate albumin-based microspheres loaded with ketoprofen (KP), a nonsteroidal anti-inflammatory drug (NSAID), to achieve sustained drug release and reduce dosing frequency. KP, an NSAID with a short half-life, requires frequent administration, which can be addressed by formulating a sustained-release delivery system. Albumin microspheres of KP were prepared using the solvent evaporation method with bovine serum albumin (BSA) as the polymer. Six formulations were developed with varying BSA proportions and evaluated for multiple parameters, including FTIR, SEM, particle size, yield, drug content, entrapment efficiency, in vitro dissolution, release kinetics, DSC, and XRD. FTIR analysis confirmed no interaction between KP and BSA, while SEM imaging verified the spherical shape of the KP microspheres with a normal size distribution. The optimized formulation achieved a maximum drug entrapment efficiency of 96.50%. In vitro dissolution tests demonstrated sustained KP release, influenced by BSA concentration. Release kinetics followed zero-order kinetics, with diffusion-controlled release as per the Higuchi model, while Korsmeyer-Peppas model indicated a non-Fickian release mechanism. The DSC and XRD analysis showed a reduction in KP crystallinity within the microspheres, suggesting effective drug encapsulation. This study highlights the potential of albumin microspheres in achieving a sustained release of NSAIDs, with applications for enhanced therapeutic efficacy and patient compliance.

**Keywords:** Sustained release, albumin microspheres, ketoprofen, drug encapsulation.

## I. INTRODUCTION

Microspheres are defined as small, solid, spherical particles ranging from 1 to 1000 μm in diameter, made from a variety of polymeric, waxy, or protective materials. These materials may include synthetic biodegradable polymers, such as polylactic acid and polyglycolic acid, and modified natural products, including starches, gums, proteins, fats, and waxes. Natural polymers, like albumin and gelatin, offer advantages as they are derived from living organisms, are readily available, cost-effective, eco-friendly,

and can undergo various chemical modifications. Microspheres possess a high surface-to-volume ratio, making them ideal for applications in drug delivery systems due to their colloidal properties and enhanced interfacial activity. In recent years, microspheres have gained significant attention for their potential to improve drug stability, enhance handling, and deliver drugs in a controlled, sustained-release manner.

The pharmaceutical industry has explored microspheres for diverse applications such as converting oils and liquids into solids, taste masking, improving drug stability, and enhancing flow properties of powders. Moreover, microspheres allow for safe handling of toxic substances and offer improved solubility for water-insoluble drugs. Specifically, sustained-release microsphere formulations reduce dose dumping compared to traditional drug delivery systems, making them highly suitable for delivering nonsteroidal anti-inflammatory drugs (NSAIDs) such as ketoprofen (KP). KP, known for its anti-inflammatory properties, has a short half-life, necessitating frequent administration, which can lead to gastrointestinal (GI) side effects. A sustained-release form, particularly using albumin microspheres, has the potential to alleviate these issues, improve patient compliance, and reduce adverse effects.

Natural polymers, especially albumin, are widely researched for drug delivery due to their non-toxic, biodegradable, and non-antigenic properties. Albumin, which constitutes a significant portion of plasma proteins, has shown promising results in sustained-release systems and as a carrier for tumor-targeted therapies due to its selective accumulation in solid tumors. Research has highlighted its utility in targeting drugs to inflamed joints in rheumatoid arthritis, as albumin binds to the synovium similarly to tumor cells, thereby increasing drug circulation half-life and minimizing distribution to non-target tissues. This specificity reduces adverse effects and improves treatment efficiency, demonstrating albumin’s versatility in biomedical applications.

Various techniques for preparing albumin microspheres include solvent evaporation, spray drying, wax coating, coacervation, and chemical cross-linking. Each technique offers distinct benefits based on parameters such as particle size, drug encapsulation efficiency, and desired release profile. For example, solvent evaporation is suitable for both aqueous and non-aqueous systems, making it a flexible option, while spray drying provides a rapid and single-step approach for heat-sensitive materials. Each method is adaptable based on the required particle size, drug loading, and release duration, making albumin microspheres an attractive option for sustained and controlled drug release.

Despite their advantages, there are some limitations associated with NSAID-loaded albumin microspheres. For instance, modified release can vary based on gut transit time, food intake, and the integrity of the dosage form. Additionally, the larger size of sustained-release formulations may pose ingestion difficulties, and prolonged release may lead to GI toxicity. Nevertheless, the therapeutic benefits of albumin microspheres in delivering NSAIDs outweigh these challenges, positioning them as a promising technology for sustained drug delivery with potential for enhanced patient outcomes and compliance.

## II. LITRETURE REVIEW

Murali Mohan Babu GV et al. formulated a controlled-release version of Diclofenac Sodium using a gum karaya-chitosan complex via the coacervation method. In vivo studies revealed a sustained blood level pattern for the microcapsules, comparable to that of a commercial controlled-release formulation, highlighting the potential for extended drug action through natural polymer complexes.

Thakkar H et al. prepared Celecoxib-loaded albumin microspheres using BSA through an emulsification chemical cross-linking method. In vitro release studies indicated that the microspheres could sustain drug release for approximately six days. Blood kinetic studies further revealed that Celecoxib-loaded albumin microspheres provided prolonged circulation compared to a standard Celecoxib solution.

Tabassi SAS et al. developed albumin microspheres encapsulating Propranolol Hydrochloride using an emulsion-internal phase stabilization technique. The resulting BSA microspheres had diameters ranging from 1 to 25 μm, and drug release studies indicated that approximately 70% of Propranolol Hydrochloride was released after 12 hours, suggesting a controlled release mechanism.

Rajamanickam D et al. formulated albumin microspheres containing Aceclofenac aimed at providing sustained release. Egg albumin was employed as a release-retarding agent, resulting in discrete, spherical microspheres with an average particle size of 99.6 μm. The optimized formulation exhibited significant analgesic and anti-inflammatory activity, underlining the utility of albumin microspheres as effective release-retarding agents.

Deore BV et al. designed sustained-release microspheres of Ketoprofen (KP) via a quasi-emulsion solvent diffusion method. The KP microspheres incorporated aerosol as an inert dispersing carrier to enhance dissolution rate and Eudragit RS as a retarding agent to control release rate. The particle size was within the 104-108 μm range, and drug content was between 62-96%. The study concluded that an increase in Eudragit concentration led to a decreased release rate of KP, affirming its effectiveness in sustaining drug release.

## III. RESEARCH METHODOLOGY

Bovine serum albumin microspheres were prepared using a solvent evaporation method, adapted from Tabassi et al. with slight modifications. A 1% w/v solution of BSA was prepared in distilled water, and Ketoprofen (KP) was dispersed into this solution. This mixture was then dispersed in 100 ml of sunflower oil containing 0.5 ml of Tween 80 in a 200 ml beaker, and stirred at 600 rpm for 30 minutes. After stirring, the microspheres were centrifuged, washed several times with petroleum ether and acetone, then dried at 50°C, and stored in a desiccator.

**Evaluation of Ketoprofen-Loaded Albumin Microspheres**

**1. Drug-Polymer Interaction (FTIR) Study**

FTIR spectroscopy was performed on a Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan). Drug and potassium bromide pellets were prepared by compressing the powders at 20 psi for 10 minutes on a KBr press, and spectra were scanned in the wave number range of 4000-600 $cm^{-1}$. FTIR analysis was conducted on KP, a physical mixture of KP and polymer, KP microspheres, and blank microspheres.

**2. Surface Morphology (SEM)**

Scanning electron microscopy (SEM) was used to determine particle size distribution, surface topography, and morphology. SEM analysis was conducted using a JEOL JSM T-330A scanning microscope (Japan). Dry KP microspheres were placed on a brass stub, coated with an ion sputter, and imaged by random scanning.

**3. Frequency Distribution Analysis**

The average particle size of KP microspheres was measured using optical microscopy with a stage micrometer. A small amount of KP microspheres was spread on a clean glass slide, and the size of 300

microspheres was recorded for each batch. A histogram was created to present the particle size distribution.

**4. Percentage Yield**

The percentage yield was calculated to evaluate the efficiency of the production method, using the formula:

Percentage Yield=(Practical YieldTheoretical Yield)×100\text{Percentage Yield} = \left( \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \right) \times 100Percentage Yield=(Theoretical YieldPractical Yield)×100

**5. Percentage Drug Entrapment Efficiency (PDE)**

The drug entrapment efficiency was calculated using the formula:

PDE=(Practical Drug ContentTheoretical Drug Content)×100\text{PDE} = \left( \frac{\text{Practical Drug Content}}{\text{Theoretical Drug Content}} \right) \times 100PDE=(Theoretical Drug ContentPractical Drug Content)×100

**6. Calibration Curve of Ketoprofen in Phosphate Buffer (pH 7.0)**

A standard stock solution of Ketoprofen was prepared by dissolving 10 mg of KP in phosphate buffer (pH 7.0) in a 100 ml volumetric flask. Aliquots were taken to create drug concentrations ranging from 2.0 to 10.0 µg/ml, and absorbances were measured at 258 nm to validate the calibration curve.

**7. Practical Drug Content Analysis**

KP microspheres equivalent to 100 mg of KP were dissolved in 100 ml of phosphate buffer (pH 7.0) and allowed to dissolve completely overnight. After filtering and diluting to a concentration of 10 µg/ml, absorbance was measured at 258 nm, and the percentage of KP was calculated.

**In Vitro Dissolution Studies**

**Calibration Curve of Ketoprofen**

The calibration curve of Ketoprofen in phosphate buffer (pH 7.0) was constructed as previously described.

**Dissolution Studies**

Dissolution studies were conducted using a USP XXIII dissolution apparatus in phosphate buffer (pH 7) for 12 hours, with a maintained temperature of 37 ± 0.5°C and a basket rotation speed of 50 rpm. Samples were withdrawn at 1-hour intervals and replaced with fresh dissolution media. The amount of KP released was measured by UV absorption at 258 nm.

**Kinetics of Drug Release**

The in vitro release data were fitted to various kinetics models:

- Cumulative Percentage Drug Release vs. Time (Zero Order)
- Log Cumulative Percentage Drug Retained vs. Time (First Order)
- Cumulative Percentage Drug Release vs. √T (Higuchi's Model)
- Log of Cumulative Percentage Drug Release vs. Log Time (Peppas Model)

**Differential Scanning Calorimetry (DSC)**

The physical state of KP in the microspheres was analyzed by Differential Scanning Calorimetry (Mettler-Toledo star 822e system, Switzerland), with thermograms recorded at a scanning rate of 10°C/min over a temperature range of 25–300°C.

**X-Ray Power Diffractometry (XRD) Study**

XRD analysis of KP, the KP-polymer physical mixture, and KP microspheres was performed using a Joel JDX-8030 diffractometer with graphite crystal monochromator (Cu-Kα) radiation to determine the physical state of KP in the microspheres.

## IV. RESULT AND DISCUSSIONS

The present study reports a novel attempt to prepare microspheres of the non-steroidal anti-inflammatory drug (NSAID) Ketoprofen (KP) using a natural polymer, Bovine Serum Albumin (BSA), as a carrier for improved treatment of rheumatoid arthritis, pain, inflammation, and related conditions. The microspheres of KP were prepared by the solvent evaporation method utilizing BSA. Various evaluation parameters were assessed with the aim of obtaining a sustained release of KP.

In this work, a total of six formulations were prepared, with detailed compositions shown in Table 4.3. The prepared KP microspheres were subjected to Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), particle size and size distribution analysis, percentage yield, drug content, drug entrapment efficiency, in vitro dissolution studies, release kinetics analysis, Differential Scanning Calorimetry (DSC), and X-Ray Powder Diffractometry (XRD).

**Preformulation Studies**

The solubility of KP in 10 mg/10 ml of solvent was assessed, revealing that KP is freely soluble in ethanol, chloroform, acetone, and ether, and soluble in benzene and strong alkali, but practically insoluble in water

at 20°C. The melting point of KP was found to be 94°C, complying with IP standards, thus indicating the purity of the obtained drug sample. A solution of KP with a concentration of 10 µg/ml was prepared in ethanol, and the UV spectrum was taken using a Shimadzu (UV-1800) double beam spectrophotometer, scanning between 200 to 400 nm. The maxima obtained in the graph were considered as λmax for the drug KP.

**Evaluation of Ketoprofen Microspheres**

**Drug-Polymer Interaction (FTIR) Study**

FTIR spectra were obtained for KP, the physical mixture of KP and polymer, KP microspheres, and blank microspheres (Fig. 5.1 to 5.4). The characteristic peaks of KP were compared with those of the physical mixture of KP and polymer. The findings are summarized in Table 5.1. The characteristic peaks found in KP, the physical mixture, and the formulations suggest that there was no chemical interaction between KP and the polymer, indicating that the characteristic bands of KP were not affected after successful loading.

**Surface Morphology of Ketoprofen Microspheres (SEM)**

The surface morphology of the KP microspheres was studied using SEM. The SEM photographs of various formulations are shown in Fig. 5.5. The surface smoothness of the KP microspheres increased with higher polymer concentration, confirmed by SEM. At lower polymer concentration (1:1), a rough, wrinkled surface of KP microspheres was observed (Fig. 5.5 KP1), whereas at a higher polymer concentration (1:6), smooth KP microspheres were obtained (Fig. 5.5 KP6).

**Frequency Distribution Analysis**

As the KP-to-polymer ratio increased, the mean particle size of KP microspheres also increased (Table 5.2 and Fig. 5.7). This significant increase may be attributed to the higher viscosity of the droplets, which could be due to the increased concentration of the polymer solution. The KP microspheres exhibited a size range of 10 to 240 µm (Table 5.3 and Fig. 5.8) with a normal frequency distribution.

**Percentage Yield**

The percentage yield for KP microspheres was calculated as follows:

- **Formulation KP1:** 50.91%
- **Formulation KP2:** 66.46%
- **Formulation KP3:** 79.10%
- **Formulation KP4:** 83.86%
- **Formulation KP5:** 90.40%

- **Formulation KP6:** 96.99%

These results are presented in Table 5.5.

**Percentage Drug Entrapment Efficiency**

Entrapment efficiency was observed to increase with higher polymer concentration. The results indicate a proper distribution of KP in the microspheres, with deviations within acceptable limits. The percentage of drug content in the formulations ranged from 12.32% to 20.42%, while the percentage entrapment efficiency ranged from 26.00% to 96.50%. The results are detailed in Table 5.5 and represented in histograms in Fig. 5.9. A maximum entrapment efficiency of 96.50% was achieved in KP microspheres prepared using BSA, further confirming that drug entrapment was proportional to the KP: polymer ratio and the size of the microspheres.

**In Vitro Dissolution Studies**

The in vitro performance of KP microspheres demonstrated prolonged and sustained release of KP. The results of the dissolution studies for formulations KP1 to KP6 are shown in Table 5.6 and Fig. 5.10. The findings indicated that the amount of drug released decreased with an increase in polymer concentration. Formulation KP1 showed a maximum cumulative drug release of 90.54%, while formulation KP6 exhibited a minimum release of 59.18%.

**Release Kinetics of Ketoprofen Microspheres**

The plots of cumulative percentage drug release versus time, cumulative percent drug retained versus root time, log cumulative percent drug retained versus time, and log cumulative percent drug release versus log time were drawn and are graphically represented in Fig. 5.11 to 5.14 and Tables 5.7 to 5.10. The slopes and the regression coefficients of determination ($r^2$) are listed in Table 5.11. The coefficients of determination indicated that the release data were best fitted with zero-order kinetics. The Higuchi equation explains the diffusion-controlled release mechanism. The diffusion exponent ‘n’ values of the Korsemeyer-Peppas model were found to be in the range of 0.5 to 1 for the KP microspheres prepared with BSA, indicating a non-Fickian drug release mechanism.

**Differential Scanning Calorimetry (DSC)**

To confirm the physical state of KP in the microspheres, DSC of KP, the physical mixture of KP and polymer, KP microspheres, and blank microspheres were performed (Fig. 5.15 to 5.18). The DSC trace of KP showed a sharp endothermic peak at 94.96°C, its melting point. The physical mixture of KP and polymer, as well as blank microspheres, displayed similar thermal behavior at 93.04°C, indicating no

interaction between KP and the polymer in the solid state. The absence of the endothermic peak of KP at 94.96°C in the DSC of KP microspheres suggests that KP exists in an amorphous or disordered crystalline phase as a molecular dispersion in the polymeric matrix.

**X-Ray Powder Diffractometry (XRD)**

To confirm the physical state of KP in the microspheres, powder X-ray diffraction studies of KP, the physical mixture of KP and polymer, and KP microspheres were performed. The X-ray diffractograms (Fig. 5.19 to 5.21) indicated that KP is still present in its lattice structure in the physical mixture, whereas it appears completely amorphous within the KP microspheres. This may be due to the conditions used to prepare the KP microspheres, which led to the complete drug amorphization.

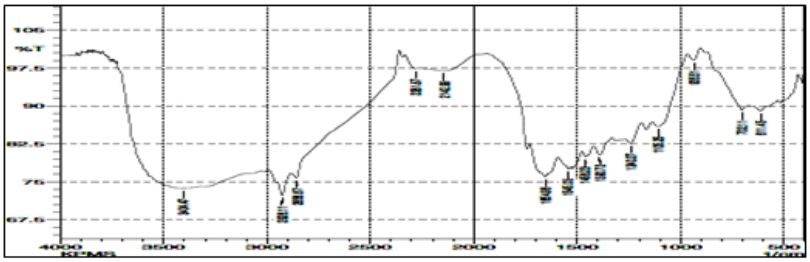

Figure 1: FTIR Spectrum of Ketoprofen microspheres using Bovine serum albumin of the optimized formulation

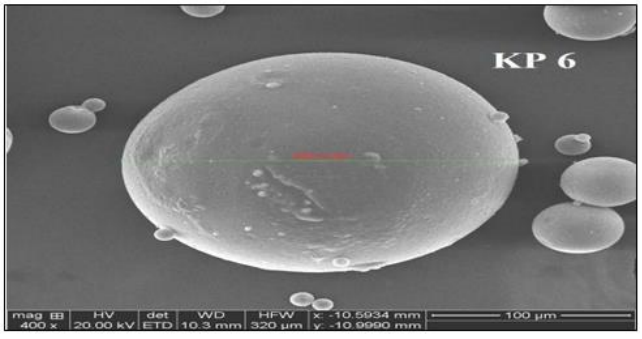


Figure 2: SEM of Ketoprofen microspheres using Bovine serum albumin of the optimized formulation

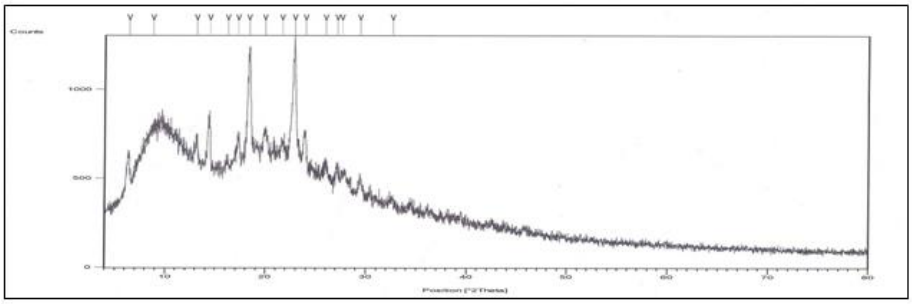

Figure 3: XRD of Ketoprofen microspheres using Bovine serum albumin of the optimized formulation

| Formulation | Zeroorder | Firstorder | HiguchiMatrix | Peppasplot | |
|---|---|---|---|---|---|
| | | | | $r^2$value | 'n'value |
| KP1 | 0.9927 | 0.9227 | 0.9758 | 0.9881 | 0.7082 |
| KP2 | 0.9930 | 0.9512 | 0.9741 | 0.9868 | 0.7084 |
| KP3 | 0.9959 | 0.9671 | 0.9764 | 0.9928 | 0.7634 |
| KP4 | 0.9971 | 0.9773 | 0.9764 | 0.9937 | 0.7952 |
| KP5 | 0.9990 | 0.9820 | 0.9761 | 0.9978 | 0.8705 |
| KP6 | 0.9472 | 0.8867 | 0.8587 | 0.9162 | 0.8992 |

Table 1 : Regression co-efficient ($r^2$) values of different kinetic models and diffusion exponent(n)of Peppas model for Ketoprofen microspheres

## V. CONCLUSION

Preformulation studies, including solubility, melting point, and UV analysis of ketoprofen (KP), complied with IP standards. The FTIR spectra revealed no interaction between the polymers and KP, indicating compatibility of all the polymers used with KP. Surface smoothness of the KP microspheres increased with higher polymer concentration, as confirmed by SEM analysis. Additionally, as the drug-to-polymer ratio increased, the mean particle size of the KP microspheres also increased, resulting in a normal frequency distribution. Entrapment efficiency improved with an increase in polymer concentration. The results suggest a proper distribution of KP within the microspheres, with deviations falling within acceptable limits. The study further indicated that the amount of drug release decreased with increasing polymer concentration, demonstrating that the in vitro performance of KP microspheres showed prolonged and sustained release of the drug. The coefficient of determination indicated that the release data were best fitted with zero-order kinetics. The Higuchi equation explains the diffusion-controlled release mechanism, while the diffusion exponent 'n' values from the Korsmeyer-Peppas model ranged from 0.5 to 1, indicating Non-Fickian drug transport through the KP microspheres prepared with BSA. DSC and

XRD data indicated that KP remained in its lattice structure in the physical mixture, while it was completely amorphous inside the KP microspheres. This amorphization may be attributed to the conditions used in preparing the KP microspheres. The melting points of KP, estimated by open capillaries, were consistent with the DSC data. Overall, this study demonstrates that promising sustained-release microspheres of KP can be developed using the solvent evaporation technique with natural polymers such as BSA.

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# In-Vivo Analysis and Optimization Of Dual-Release Minitablets With Prolonged Bosentan And Immediate Sildenafil Citrate For Enhanced Therapeutic Outcome

Deepak Kumar Sarangi[1*], Chandra Sekhar Patro[2], Ch. Niranjan Patra[3]
Nalini Kanta Sahoo[4],
[1,2]Centurion University of Technology & Management, Bhubaneswar, Odisha, India
[3]Roland Institute of Pharmaceutical Sciences, Brahmapur, Odisha, India
[4]MIT College of Pharmacy, Moradabad, Uttar Pradesh, India

**ABSTRACT**

The purpose of this study was to prepare and evaluate multiple unit mini tablets (MTs) of prolonged released(PR)bosentan(BSN) and fast release (FR)sildenafil citrate (SDC) on the basis of encapsulation method for the effective management of pulmonary artery hypertension. The system consists of MTs are in the form of uncoated tablets containing the superdisintegrating agents where as the PRMTs contains the polymer with film coating. All the units are prepared by direct compression method and encapsulated using size 1 hard gelatin capsule shells. The formulations were evaluated fordifferential scanningcalorimetry, dissolution test and post compression studies for their quality attributes and it was found that all the parameters were in the acceptable limits. The optimized formulation for BSN PRMTs i.e BS3 which contains 5% HPMC K15M shows a promising sustained release profile of 82.31% in 24 hours similarly FRMTs of SDC of formulation SD6 that having 3% of magnesium aluminium silicate shows about 90% of drug release within 15 minutes. The in-vivo pharmacokinetic characterization of the system was carried out using wister rats where theAUC value for SDCFRMTs andBSNPRMTswas found to be 94256.88625±123.65ng h/mL and 142438.084±324.11ng h/mL respectively. The other pharmacokinetics are also determined and found to be satisfactory.The six months stability samples showed no significant change in the drug content, hardness and uniformity of the content of the optimized formulations.

**Keywords:**bosentan, sildenafil citrate, mini tablets, encapsulation, prolong release, fast release, pulmonary artery hypertension.

## I. INTRODUCTION

Pulmonary arterial hypertension (PAH) represents a condition seldom encountered, marked by unfavourable alterations in the arterial network, resulting in heightened vascular opposition followed by

a rise in right ventricular burden and eventual onset of cardiac insufficiency. Initial manifestations often lack specificity, commonly manifesting as exertionaldyspnea and fatigue.The present endorsed treatment for PAH encompasses medications augmenting the nitric oxide-cyclic guanosinemonophosphatebiological pathway which agonists targeting the prostacyclin pathway and antagonists of the endothelin pathway including BSN. Modern therapy consists of a combination of medication regimens that target several biological pathways, including the oxide cyclic guanosine mono phosphate and endothelin and pathways. This strategy has demonstrated observable improvements in health outcomes and mortality when compared to conventional single-pathway targeted medication.

Three crucial mechanistic pathways the prostacyclin, endothelin, and nitric oxide pathways are recognised for their essential role in the advancement of PAH.Therapies focusing on these pathways are readily accessible. Combination therapies addressing multiple pathways offer an enticing approach to PAH treatment, potentially yielding superior long-term outcomes compared to monotherapy. BSN is a groundbreaking accomplishment as the first non-peptide ETA and ETB endothelin receptor antagonist approved for PAH treatment in this particular regimen. With a half-life of 5 hours and an oral bioavailability of 50%, a controlled-release formulation of BSNwould be preferable. This approach would help sustain therapeutic plasma concentrations and, consequently, mitigate potential side effects. SDC, a potent and selective orally active phosphodiesterase type 5 (PDE5) inhibitor, is widely distributed throughout the body, with notably high concentrations observed in the lungs. Due to its inhibition of phosphodiesterase type 5 (PDE5), SDCprevents the degradation of cGMP, thereby promoting vascular smooth muscle relaxation and enhancing flow of the blood. This mechanism augments the vasodilatory effects of nitric oxide, leading to improved pulmonary hypertension outcomes.

The objective of the current investigation is to integrate BSN and SDC into a MT encapsulation system for the management of PAH. Utilizing combination therapy would aid in reducing pulmonary hypertension by antagonizing dual receptors and enhancing vasodilation of the pulmonary arteries. Such therapy is beneficial for patients who have not responded adequately to mono therapy. This therapeutic approach not only decreases dosing frequency but also mitigates side effects, thereby improving patient compliance.

## II. LITRETURE REVIEW

Naeije et al., 2022, Pulmonary arterial hypertension (PAH) is a rare yet severe condition marked by adverse remodeling of the pulmonary arterial tree, leading to elevated vascular resistance and increased right ventricular afterload, ultimately progressing to heart failure. The disease's nonspecific symptoms and limited awareness of its pathology contribute to delayed diagnosis and treatment, which worsen the prognosis. Recent guidelines emphasize the importance of timely differential diagnosis to distinguish PAH from other types of pulmonary hypertension and advocate treatment tailored to the patient's mortality risk. Advances in diagnostic methods, the development of novel treatments, and the establishment of specialized PAH referral centers have collectively enhanced both the prognosis and quality of life for PAH patients.

Fatima et. al 2018 Pulmonary arterial hypertension (PAH) is a rare and life-threatening disease characterized by adverse remodeling of the pulmonary arterial tree, which increases vascular resistance and right ventricular afterload, ultimately leading to heart failure. Diagnosis is often delayed due to nonspecific symptoms and limited awareness of the disease, resulting in poor prognosis. Current guidelines emphasize early differential diagnosis from other forms of pulmonary hypertension and recommend treatments based on mortality risk assessment. Advances in diagnostic techniques, along with new targeted therapies and the establishment of specialized referral centers, have significantly improved both prognosis and quality of life in patients with PAH. The aim of this study is to assess the impact of early diagnosis and tailored therapeutic approaches on survival and clinical outcomes in PAH patients.

Xu, C., et al. 2022 Gut microbes play a critical role in human health, with orally ingested probiotics effectively enhancing intestinal microbial balance. However, the harsh environment of the digestive tract poses challenges for probiotic viability. Probiotic encapsulation technology offers a promising solution, although traditional methods face limitations, including sensitivity to extreme temperatures and difficulty in achieving optimal microcapsule sizes. Advances in encapsulation technology now range from bulk probiotic encapsulation using nanofibers and nanoparticles to innovative nano “armor” coatings that protect individual probiotics through biofilm and nanocoating techniques. This review explores the materials and systems used in encapsulated probiotic carriers and examines current encapsulation methods for probiotic nanoagents. It further highlights the advantages and limitations of existing systems and discusses the future development and challenges in the field of probiotic encapsulation.

## III. RESEARCH METHODOLOGY

BSN and SDC underwent analysis via FT-IR (IR Affinity-1, Shimadzu, Japan) both individually and in conjunction with their respective polymers and also with their respective super disintegrating agent. The MTs prepared underwent various physicochemical tests to assess their pharmaceutical quality, including measurements of weight, thickness, hardness, and friability. Initially, a sample of 20 MTs was randomly selected for weight measurement using an analytical balance (Wensar, High Precision Balance PGB -200). Subsequently, the diameter, thickness, and hardness variations were determined using Pharmatron M50 multi-test instrument. All the MTs are evaluated according to the specified evaluation parameters. The prepared SDC FRMTs and BSN PRMTs underwent an in vitro dissolution study using USP Type 2 equipment. The time intervals for sampling were set differently for SDC FRMTs at 5, 10, 15, 30,45 and 60 minutes and for BSN PRMTs at 1, 3, 6, 9, 12, 15, 18, and 24 hours. The medium of the dissolution was taken as simulated gastric fluid having pH 1.2 (without enzyme). The dissolution jar was refilled with 5 mL of new media after testing samples were removed in 5 mL aliquots to maintain the sink condition. The Institutional Animal Ethical Committee sanctioned the research on assessing SDC and BSN in wister rat blood serum, utilizing UFLC method. For analysis, a system was employed employing a mixture of HPLC grade methanol and phosphate buffer (20mM, pH 4.4) in a ratio of 75:25, which underwent filtration and degassing using a 0.22-µm nylon membrane filter prior to use

## IV. RESULT AND DISCUSSIONS

FTIR study confirms the purity of the sildenafil citrate and bosentan. By using the pure drug total 18 numbers of MT formulations has been prepared. The FT-IR for the final formulation of SDC and BSN did not exhibit any major deviation in the absorption bands of pure drugs. Hence it confirms that the drug and excipients are compatible.For the preparation of sildenafil citrate FRMTsdifferent types of superdisintegrants are used. For SD1 to SD3 formulation 1% to 5% of sodium starch glycollate has been used, for SD4 to SD6 and SD7 to SD9 same percentage of magnesium silicate andcrosslinked PVP are used respectively.The use of superdisintegrants in the concentration range of 1 to 5% in the formulation of MTs is justified by several factors that contribute to the effectiveness and efficiency of MTs.Superdisintegrants speed accelerate the breakdown of tablets into smaller pieces, which is necessary for the drugs to dissolve and absorb more quickly. These are cost-effective while retaining their efficacy

since they are very effective even at low concentrations. The range offers flexibility in attaining the intended disintegration time and drug release profile by allowing modifications based on the particular drug and formulation requirements.The concentration range of 1 to 5% for superdisintegrants in MTs is a balance between efficacy, cost, and compatibility, which is crucial for the successful development of MTs that meet therapeutic needs and enhance patient adherence to medication regimens. The rats were administered MTs orally, with each group receiving specific doses of SDC and BSN. Blood samples were collected at predetermined time intervals post-administration to assess drug concentration levels in the serum. Blood samples were collected via the tail vein and processed meticulously to obtain plasma samples for analysis. Proper handling procedures, including centrifugation and storage at appropriate temperatures, were followed to maintain sample integrity[43]. The collected samples were analyzed using validated methods to determine the concentration of SDC and BSN in the serum over time. The obtained data are shows maximum concentration will be 15602±212.12 and 14323±321.29 for both SDC FRMTs and BSN PRMTs respectively. Similarly the value of maximum time of serum concentration are 1.01±0.02 and 4.03±0.03 hours, area under curve values are 94256.88625±123.65ng h/mL and 142438.084±324.11ng h/mL, volume of distribution($V_d$) for both the MTs are 17260.51769±23.43 and 83927.75502±12.43 ml respectively. Clearance value for both MTs are 38364.74682 and 24492.72949ml/hr. The mean residence time is 2.12±0.23 and 5.32±0.34 hours for both SDC FRMTs and BSN PRMTs respectively.

**Table 1**. Formulation of Bosentan PRMTs

| **Formulation Code** | **Quantities (mg/minitablet)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **BS1** | **BS2** | **BS3** | **BS4** | **BS5** | **BS6** | **BS7** | **BS8** | **BS9** |
| Bosentan | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| Starch | 1.7 | 1.5 | 1.1 | 1.7 | 1.5 | 1.1 | 1.7 | 1.5 | 1.1 |
| MCC | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 |
| Lactose | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 |
| HPMC K15M | 0.2 | 0.6 | 1 | | | | | | |
| Ethyl Cellulose | | | | 0.2 | 0.6 | 1 | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sodium CMC | | | | | | | 0.2 | 0.6 | 1 |
| Magnesium stearate | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Purified Talc | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total weight (mg) | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

**Table 2**: In-vivo pharmacokinetic parameters of prepared MTs

| **Pharmacokinetic Parameters** | **Sildenafil Citrate FRMTs** | **Bosentan PRMTs** |
|---|---|---|
| $C_{max}$ (ng/ml) | 15602±212.12 | 14323±321.29 |
| $T_{max}$ (h) | 1.01±0.02 | 4.03±0.03 |
| AUC (ng hr/ml) | 94256.88625±123.65 | 142438.084±324.11 |
| $V_d$ (ml) | 17260.51769±23.43 | 83927.75502±12.43 |
| Clearance CL (ml/hr) | 38364.74682±31.33 | 24492.72949±25.54 |
| MRT (hr) | 2.12±0.23 | 5.32±0.34 |

Mean±SD, n=5

## V. CONCLUSION

In conclusion, this study aimed to develop and assess the effectiveness of multiple unit mini tablets (MTs) containing bosentan and sildenafil citrate for the management of pulmonary artery hypertension.This combination of BSN and SDC as MTs encapsulated into capsules is a novel concept. As this approach is particularly innovative because it combines two different pharmacological actions in one capsule shell, potentially simplifying the treatment regimen and improving patient adherence to therapy. Additionally, the use of MTsin the form of encapsulation is a novel delivery system that may offer advantages over traditional tablet or liquid forms, such as ease of swallowing and the ability to combine different release profiles within a single dosage form.The formulations were prepared using direct compression method and encapsulation with size 1 hard gelatin capsule shells. The MTs were designed to have prolonged release properties for BSN and fast release properties for SDC.The formulations underwent thorough evaluation, including differential scanning calorimetry, dissolution testing, and post compression studies.

Results indicated that all parameters met acceptable limits, demonstrating the quality and efficacy of the formulations. The optimized PRMTs formulation for BSN (BS3) showed a promising sustained release profile, while the FRMTs formulation for SDC (SD6) exhibited rapid drug release within 15 minutes.Furthermore, pharmacokinetic characterization conducted in wistar rats revealed favourable results, with significant AUC values for both SDC and BSN formulations. The stability testing conducted over six months demonstrated no significant changes in drug content, hardness, or uniformity of the optimized formulations.Overall, the findings of this study suggest that the developed microencapsulated MTs have the potential to effectively manage pulmonary artery hypertension. Further research and clinical trials may be warranted to validate these findings and explore their therapeutic benefits in human subjects.

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# Enhancing Quercetin's Neuroprotective Effects in Alzheimer's Disease Through Advanced Nano formulations

DEEPIKA SINGH, PRASHANT KUMAR
RESEARCH SCHOLAR
PHARMACY
**Affiliation:** SRM Modinagar College of Pharmacy, SRM Institute of Science and Technology (Deemed to be University), Delhi-NCR Campus, Modinagar, Ghaziabad, Uttar Pradesh 201204, India.

## ABSTRACT

Alzheimer's disease (AD) is a crippling neurological illness characterized by a steady loss of memory and cognitive impairment. Strong antioxidant and anti-inflammatory qualities make Quercetin a flavonoid that has gained resistant as a potential neuroprotective agent for AD. Unfortunately, its poor bioavailability, fast metabolism, and restricted solubility limit its therapeutic potential by preventing it from reaching the brain at levels that are useful. Nano-formulation methods such nanoparticles, liposomes, and nanoemulsions have been used to improve quercetin delivery in order to overcome these obstacles. By enhancing quercetin's stability and facilitating its targeted release in the brain, these cutting-edge delivery methods boost the drug's effectiveness in treating AD-related diseases such oxidative stress and amyloid-beta aggregation. This abstract provides a prospective approach for more successful management of Alzheimer's disease by examining the role of quercetin in modifying neurodegenerative pathways in AD and highlighting how nano-formulations can maximize its protective benefits.

**Keywords**: Quercetin, Alzheimer's Disease, Nano-formulations, Neuroprotection, Targeted Drug Delivery, Antioxidant, Cognitive Health.

## Introduction

Different neurodegenerative disorders have different etiologies and clinical consequences. Numerous risk factors, including age, genetic defects, excitotoxicity, oxidative stress, abnormalities of antioxidant enzymes, deficiencies in neurotransmitters, metabolic toxicity, autoimmunity, and hypertension, have been linked to neurodegenerative disorders in epidemiological and experimental studies (1). Aloes Alzheimer, a German physician, is credited with first describing Alzheimer's disease (AD) in 1906. An estimated 50–60% of dementia cases are thought to be caused by AD, a progressive neurological disease that usually affects those over 65 (2). According to estimates, there are currently 35.6 million dementia sufferers globally, and by 2030, that number will rise to 65.7 million, with developing nations accounting

for the majority of the growth (3). Clinically, it manifests as cognitive deficiencies, progressive memory impairments, and reduced learning capacity. AD may arise as a result of a number of conditions, including a decrease in physical activity, infection, smoking, and the prevalence of disorders like obesity and diabetes ***(4)***.

Extracellular neuritic plaque aggregates and intracellular hyper-phosphorylated microtubule-associated Tau protein accumulations, or neurofibrillary tangles, are the histopathologic hallmarks of AD. Proteolytic cleavage of the amyloid precursor protein, a large type I integral membrane protein with 695–770 amino acids expressed in many organs but mostly concentrated at the synapse of neurons, results in the formation of neuritic plaques, which are primarily composed of Aβ peptides (5).

The greatest recognized effect of phytochemicals is their ability to lower the risk of chronic illnesses like cancer, diabetes, hypertension, and cardiovascular disorders. The most varied class of phytochemicals are flavonoids, which are found in many higher plants and have exceptional medicinal potential. Based on their chemical structure, flavonoids are further classified into six classes: isoflavonoids, anthocyanidins, flavanols, flavanones, and flavones. They have been shown to be helpful in preventing neurodegenerative illnesses and may slow the progression of neurodegeneration even though they target several targets at once. Since flavonoids have anti-inflammatory and antioxidant properties that are key in initiating the pathophysiology of AD, they have been the subject of much research. Research has indicated that flavonoids possess the ability to penetrate the blood–brain barrier (BBB), indicating their potential as agents in the prevention of neurodegenerative illnesses. Nevertheless, the degree to which flavonoid subclasses are able to traverse the BBB varies. Their effectiveness in the case of AD is linked to a decrease in oxidative stress and a reduction in Aβ toxicity (6).

Szent-Gyorgyi discovered and recognized quercetin (3,30,40,5,7-pentahydroxyflavone) as a flavonol for the first time in 1936. Fruits and vegetables include a flavonoid called quercetin, which has special biological qualities that may enhance mental and physical function and lower the risk of illness. These characteristics serve as the foundation for potential advantages to general health and resistance to disease, such as antiviral, anti-inflammatory, anticarcinogenic, antioxidant, and psychostimulant effects; they can also be used to suppress platelet aggregation, lipid peroxidation, and capillary permeability, as well as to promote mitochondrial biogenesis (6). One of the most powerful antioxidants derived from plants, quercetin is a major flavonoid that is more frequently present in edible plants. It is a member of the flavonol class of flavonoids, which is a significant class of polyphenols (7).

Quercetin reverses the neurotoxicity caused by Aβ by interfering with the development of neurotoxic oligomeric Aβ species and exhibiting fibril destabilizing effects on preformed fibrillary Aβ. A minimum of three hydroxyl groups are present in aromatic rings, and these groups are crucial in inhibiting fibrils because they create hydrogen bonds with β-sheet structures through hydrophobic contact. The aromatic rings' electron density is raised by the phenolic hydroxyls, potentially improving quercetin's ability to attach to the aromatic amino acids in peptide beta-sheet structures. Because quercetin meets these structural requirements and has hydrophobic moieties, it prevents the development of fibrils. The modulation of BACE-1 Expression, the reduction of tau protein phosphorylation, and the suppression of NFT formation are all impacted by quercetin-induced NF-kB inhibition. In HT22 cells, quercetin reverses the hyperphosphorylation of tau proteins through the PI3K/Akt/GSK3β and MAPK signaling pathways (8).

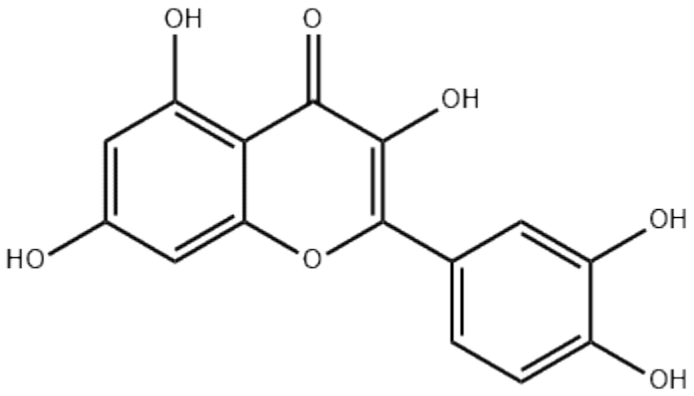


**Figure 1: Quercetin chemical structure**

**Literature review regarding Quercetin's Nano-formulations used in AD**

**Qi, Yujie, et al. (2020)** synthesize nanocomposites of P80-Que@Se. The two pathological hallmarks of Alzheimer's disease (AD) are abnormal aggregation of amyloid-β (Aβ) particles and oxidative stress. A popular flavonoid antioxidant called quercetin (Que) has the ability to reduce oxidative stress and has been shown to prevent the production of Aβ fibrils. Its therapeutic use is, however, restricted by its poor water solubility, high first-pass metabolism, and limited blood-brain barrier (BBB) permeability. In order to create nanocomposites (NC) for drug delivery, we present a straightforward method here. In order to create selenium nanoparticles, this approach combines Que with Na2SeO3. Polysorbate 80 (P80-Que@Se NC) and acacia are then used to modify these nanoparticles. The blood-brain barrier (BBB) may be more effectively crossed by this newly created nanocomposite, and polysorbate 80 functions as a

pharmaceutical excipient to make Que more soluble in water. The in vitro results showed that P80-Que@Se could successfully prevent Aβ fibrillation and had a high aqueous solubility when compared to individual Que. According to an examination using the In vitro Cell Counting Kit (CCK)-8, P80-Que@Se nanocomposites might shield PC12 cells from H2O2-induced cell death. Furthermore, P80-Que@Se demonstrated strong antioxidant activity and significant 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (9).

The efficacy of quercetin-loaded nanoparticles to enhance neuroprotective effects was explored in this **Pinheiro, R. G. R., et al. (2019)** study employing an in vitro model of Alzheimer's disease with Aβ (1-42) peptide. When compared to the control sample with the Aβ (1-42) sample alone, it was shown that quercetin-loaded nanoparticles, particularly transferrin-functionalized NLC, were able to inhibit fibril formation, reverse the aggregation effect of unloaded nanoparticles, and also decrease peptide aggregation. Consequently, because of their greater ability to deliver quercetin to specific brain sites and their enhanced ability to inhibit amyloid-beta aggregation, the developed nanosystems functionalized with transferrin and loaded with quercetin appear promising for the treatment of neurological diseases, including Alzheimer's disease, primarily NLC. These nanosystems may offer a fresh approach to enhancing the current treatment, providing significant data and raising new expectations for Alzheimer's patients (10).

**Rifaai, Rehab Ahmed, et al. (2020)** explored the quercetin nanoparticles (QNPs) neuroprotective role in Alzheimer’s. AD hippocampi displayed a significant amount of extraneuronal and neuronal structural and ultrastructural abnormalities. comprising astrogliosis, downregulation of tyrosine hydroxylase (TH), neuronal degeneration, AP and NFT formation, and suppression of proliferative activity (all $P \leq 0.05$). Using electron microscopy, neuronal degeneration was observed together with astrocyte and microglia activation, myelination disruption, and disruption of the blood-brain barrier (BBB). Interestingly, the treatment of QNPs significantly decreased the production of APs, NFTs, and neuronal degenerative alterations (all $P \leq 0.05$). Moreover, there were indications of regeneration (all $P < 0.05$) and increased TH expression. In the group receiving preventative treatment, the impact was significant. Therefore, QNPs mitigated the deleterious impact of AlCl3 on hippocampus neurons on a molecular, cellular, and subcellular level and an adjuvant therapy for AD (11).

**Priprem, Aroonsri, et al. (2008)** Quercetin liposomes demonstrated anxiolytic and cognitive-enhancing effects and could be reliably synthesized from EPC/chol at a 2:1 ratio. The anxiolytic and cognitive effects

of quercetin, a potent flavonol utilized as an antioxidant, were studied in male Wistar rats. Oral and intranasal quercetin liposomes (20 μg/day) were compared with oral quercetin (300 mg/kg body weight/day). When quercetin liposomes were dissolved in 50% polyethylene glycol in water and mixed with egg phosphatidylcholine, cholesterol, and quercetin (2:1:1), they had a mean particle diameter of about 200 nm, a negative surface charge, and an encapsulation effectiveness that ranged from 60% to 80%. Morris water maze and elevated plus maze tests were used to assess the cognitive-improving and anxiolytic effects of conventional and liposomal quercetin, respectively. Anxiolytic and cognitive-enhancing effects were demonstrated by conventional and quercetin liposomes. Quercetin can be effectively delivered to the central nervous system using intranasal quercetin liposomes and it can be a potentially novel strategy for AD (12).

According to **Pinheiro, R. G. R., et al. (2020)** RVG29-nanoparticles below 250 nm had a spherical morphology and size, making them suitable for use in brain applications. The values of the zeta potential ranged from -20 to -25 mV. In general, quercetin entrapment efficiency was greater than 80%, while NLC nanoparticles could encapsulate up to 90% of the drug. The LDH experiment demonstrated that the hCMEC/D3 cell line is not cytotoxic, and after 4 hours of incubation, RVG29-functionalized nanoparticles significantly increased the permeability across the in vitro blood-brain barrier by 1.5 times when compared to non-functionalized nanoparticles. In conclusion, the thioflavin T binding experiment demonstrated that our nanosystem may suppress amyloid beta aggregation, indicating a significant potential for neuroprotection. Delivering quercetin effectively and offering hope for future therapies to Alzheimer's disease are RVG29-nanoparticles, which simultaneously target the blood-brain barrier and stimulate neuronal protection against amyloid-beta fibrillation and provide better protection in AD (13).

**Table 1: Quercetin based drug delivery system and their application in Alzheimer's**

| **Formulation** | **Model** | **Route of administration** | **Pharmacological benefit** | **Outcome** | **Reference** |
|---|---|---|---|---|---|
| Liposomes | Rats | Intranasal | Increased solubility | Anxiolytic and cognitive benefits | (12) |
| | | Oral | Enhanced plasma concentration | QT-SPIONs prevent neural cell | (14) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | apoptosis and improves learning and memory. | |
| SLNs | Rats | Intravenous | Drug entrapment increased | Behavioural improvement in memory retention | (15) |
| NPQ | SAMP8 mice | Oral | Enhanced bioavailability | Increased memory and cognition | (16) |
| Silica nanoparticles | Hippocampal cells | Peripheral | Retain physiochemical integrity | Improves antioxidant activity potential | (17) |
| Polymeric NPs | SH-SY5Y cells | | sustainable release | disassembles Aβ fibrils | (18) |

**Conclusion**

Quercetin, a flavonoid known for its antioxidant and neuroprotective properties, has shown promising therapeutic potential in managing Alzheimer's disease when delivered through advanced nanotechnology-based formulations. The efficacy of numerous quercetin formulations, including silica nanoparticles, polymeric nanoparticles, liposomes, solid lipid nanoparticles (SLNs), and nano-encapsulated quercetin (NPQ), in diverse animals and administration routes, is demonstrated by this. Utilizing these nanocarriers improves the pharmacological advantages of quercetin by addressing issues such as its low bioavailability and poor solubility. Increased drug entrapment through intravenous SLNs led to greater memory retention, while liposome-based intranasal and oral delivery markedly enhanced cognitive outcomes. An important pathology in Alzheimer's disease, amyloid-beta fibrils, was successfully disassembled and quercetin was stabilized and released over time with the use of polymeric and silica nanoparticles. The above study highlights quercetin's potential for better administration and efficacy as a neuroprotective drug in Alzheimer's therapy.

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# Extraction and Phytochemical Screening of Kankola (Piper Cubeba) for in-Vitro Antidiabetic Potential

**Gaurav Kumar[1*] and Jayendra Kumar[1]**
[1]SRM Modinagar College of Pharmacy, SRM Institute of Science and Technology (Deemed to be Univeristy), Delhi-NCR Campus, Modinagar, Ghaziabad, Uttar Pradesh, 201204
*Corresponding Author Email Id- gauravkardam67@gmail.com

**Abstract:**

**Introduction-** Diabetes mellitus is a common long-term condition. A lack of insulin or a malfunction in insulin action is the fundamental cause of the condition. Rich historical evidence supports the use of plant-based remedies to treat diabetes. **Objective-** in our study we were investigated and phytochemically screening P. cubeba for his antidiabetic potential. **Material and method-** To extract the plant for its in vitro antidiabetic activity, we soak it in shade and use a Soxhlet apparatus with hexane, ethanol, methanol, and an aqueous solvent. **Result-** P. cubeba contains several phytoconstituents, such as alkaloids, flavonoids, tannins, and phenol. In vitro studies have demonstrated the good antidiabetic potential of various extracts of P. cubeba seeds by blocking both enzymes. P. cubeba ethanolic extract inhibited α-amlyase and α-glucodsidase activity by 92.1% and 84.7%, methanolic extract by 76.6% and 71.2, and aqueous extract by 70.1% and 69.6%, respectively. **Conclusion-** According to the findings of the current studies, P. cubeba seeds exhibit a significant antidiabetic potential in an in vitro model. This study suggested undertaking in vivo studies on this herb to validate its antidiabetic mechanism.

**Keywords:** Diabetes, α-amylase, α-glucosidase, phytoconstituents, Anti-oxidants.

**Introduction:** Diabetes mellitus (DM) refers to a group of metabolic illnesses characterized by increased blood sugar levels (hyperglycaemia) caused by either insufficient insulin synthesis, poor cellular responsiveness to insulin, or a combination of the two.[1] Diabetes is becoming more common over the world, with approximately 637 million people currently affected.[2] According to projections, this number might rise to 643 million by 2030 and 783 million by 2045. According to the IDF's tenth edition, the prevalence of diabetes in South-East Asia has been rising for at least two decades, with current levels exceeding previous projections. The illness is caused by a variety of risk factors, most notably lifestyle choices. However, early detection can help reduce or eliminate the major long-term problems associated with this chronic condition.[3] Effective glycemic control, beginning with monotherapy and escalating to combination medication in conjunction with diet and exercise, can considerably minimize the long-term

microvascular and macrovascular consequences of type 2 diabetes.[4] The American Diabetes Association recommends metformin as the most widely administered oral hypoglycemic medication. Type 1 diabetes is associated with higher microvascular problems, such as retinopathy, neuropathy, and nephropathy, whereas macrovascular complications include heart attacks, strokes, and peripheral vascular disorders.[5]

Kankola, or cubeba, is a medicinal plant from the Piperaceae family, predominantly located in Java and Sumatra. The dried berries, known as "Tailed pepper," were transported to Europe via Indian-Arab trade and introduced to China from Srivijaya, where they were termed "Kabab chini" in India. Kankola possesses a sharp, mildly bitter flavor akin to black pepper and is frequently utilized, especially in Indian cuisine, for its flavor-enhancing, digestive, and therapeutic properties.[6] The Piperaceae family comprises more than 2,000 species, including Piper nigrum and Piper betle, which have been employed in traditional medicine for millennia.


Figure 1: Plant material of raw seeds (A) and powder form(B)

These herbs serve as spices and may alleviate diseases such as fever, headaches, and respiratory disorders, while also offering antibacterial, antioxidant, and anti-inflammatory benefits. Piper betle leaves are utilized to address inflammation, ulcers, arthritis, asthma, and to enhance immunity.[7] In the present study, we performed various phytochemical studies of P. cubeba, and their antidiabetic effect was determined via in vitro studies, including alpha-amylase and alpha-glucosidase.

**Materials and Methods:**

**Plant Materials and Extraction-** Locally acquired fresh Piper cubeba seeds were authenticated by the Botany department at CCS University, Meerut, India. The seeds were dried in the shade, pulverized with an electric grinder, and stored in an airtight container. Extraction was conducted via the hot percolation method employing a Soxhlet apparatus, with a sequence of solvents—hexane, ethanol, methanol, and

water—arranged by ascending polarity. Approximately 100 grams of powdered seeds were extracted using 300 ml of solvent.[8] The solvent from the extract was further evaporated utilizing a vacuum rotary evaporator under reduced pressure, yielding a semi-solid residue with percentages of 8.7%, 14.22%, 13.22%, and 6.54% w/w relative to the dry material. The final extract was preserved in vacuum desiccators.[9]

**Phytochemical Screening-** Standard phytochemical techniques were applied to the freshly obtained crude extracts of P. cubeba seeds in order to determine whether or not different phytoconstituents, such as reducing sugars, tannins, flavonoids, steroids, and alkaloids, were present. The objective of this investigation was to conduct a preliminary phytochemical assessment.[10]

Table1: Phytochemical analysis of various extract of P. cubeba.

| **Phytochemical vconstitutent** | **Test performed** | **Ethanol** | **Methanol** | **Water** | **n-Hexane** |
|---|---|---|---|---|---|
| Alkaloids | Dragandroff's test | +++ | ++ | + | - |
| Carbohydrate | Molish's test | + | ++ | + | + |
| Tannins and phenol | Lead acetate test | +++ | + | + | - |
| Steroidal glycosides | Salkowaski test | + | + | - | - |
| Anthraquinone glycosides | Borntrager's test | + | - | - | - |
| Flavonoids | Schinoda's test | +++ | ++ | ++ | + |
| Terpenoids | | + | - | - | - |
| Cynogenetic glycoside | Sodium picrate paper test | ++ | + | + | - |
| Protein | biuret test | + | + | + | + |

**Determination of α-Glucosidase Inhibitory Activity-** The α-glucosidase inhibitory effects of P. cubeba extracts were evaluated using the methodology outlined by Bothon et al. [11]. In the α-glucosidase experiment, 25 µL of the P. cubeba extract was mixed with 75 µL of 0.1 M sodium phosphate buffer (pH 6.8) and 50 µL of α-glucosidase solution (1 U/mL), followed by preincubation at 37°C for 10 minutes. Subsequent to incubation, 50 µL of substrate solution (5 mM PNPG) was introduced to the reaction mixture, and the absorbance variation at 405 nm was recorded at 37°C over a duration of 10 minutes utilizing a microplate reader.

**Determination of α-Amylase Inhibitory Activity-** The inhibitory impact of P. cubeba on α-amylase was assessed utilizing the DNS technique [12]. In summary, 10 µL of each extract was preincubated with 50 µL of α-amylase solution (3 U/mL) and 40 µL of 0.1 M sodium phosphate buffer (pH 6.8) at 25°C for a duration of 10 minutes. The reaction commenced with the addition of 50 µL of a 0.75% starch solution. After 5 minutes, the reaction was halted by the addition of 75 µL of DNS colour reagent, which consists of 96 mM DNS and 5.31 M potassium sodium tartrate in 2 M NaOH. The combinations were thereafter heated to 85°C for a duration of 15 minutes. Subsequent to cooling, the liquid was diluted four times with distilled water, and the absorbance was measured at 540 nm.

**Calculation of the 50% Inhibitory Concentration (IC50)-** The concentration of plant extracts required to scavenge 50% of the radicals (IC50) was established by evaluating the percentage scavenging activity at five distinct extract concentrations. Percentage inhibition (I%) was determined using the formula: I%=(Ac-As)/Ac X 100.

where Ac represents the absorbance of the control and As denotes the absorbance of the sample.[13]

**Results:**

The results of the different phytoconstituents discovered in the various P. guajava leaf extracts are shown in Table 1. Different methods were used for phytochemical screening, which identified the presence of alkaloids, carbohydrates, tannins, terpenoids, flavonoids, phenols, and total proteins among other substances in aqueous, ethanol, methanol, and hexane extracts. The findings showed that the methanol, aqueous, and ethanolic extracts had the maximum concentration of phytoconstituents.

**Alpha glucosidase inhibition** this method was used to measure the in vitro antidiabetic activity of P. cubeba seed extracts. The results are shown in Table 2. The alpha-glucosidase enzyme was significantly inhibited by these extracts in a dose-dependent manner. The extracts displayed varying percentages of inhibition: the aqueous extract displayed an inhibition range of 25.4% to 69.6%, methanol ranged from 24.8% to 71.2%, and ethanol showed an inhibition range of 32.6% to 84.7%. It was discovered that the ethanol, methanol, and aqueous extracts had 50% inhibitory doses of 0.58, 0.63, and 0.57 mg/mL, respectively.

**Table 2:** in vitro antidiabetic potential of ethanolic extract, methanolic extract and aqueous extract pf P. Cubeba by α-glucosidase inhibition assay method.

| Conc. Of sample (mg/ml) | Ethanolic extract | Methanolic extract | Aqueous extract |
|---|---|---|---|
| 0.2 | 32.6 | 24.8 | 25.4 |
| 0.4 | 48.3 | 41.3 | 35.6 |
| 0.6 | 57.6 | 51.5 | 49.8 |
| 0.8 | 68.8 | 62.5 | 59.4 |
| 1 | 84.7 | 71,2 | 69.6 |
| **IC50** | **0.44** | **0.58** | **0.61** |

**α-amylase inhibition** in vitro investigations revealed that different Piper cubeba extracts had α-amylase inhibitory action. A concentration-dependent rise in inhibition was seen in the percentage inhibition at concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/mL (Table 3). Interestingly, the ethanolic extract at its greatest dosage (1 mg/mL) had a maximal inhibition of over 88.7%. It was discovered that the ethanol, methanol, and aqueous extracts had 50% inhibitory doses of 0.43, 0.62, and 0.71 mg/mL, respectively. According to these findings, P. cubeba's ethanolic extract has strong in vitro antidiabetic action. P. cubeba may work by interacting with endoglucanases, which catalyze the hydrolysis of internal α-1,4 glycosidic linkages in starch and similar polysaccharides, as well as the carbohydrate-binding domains of α-glucosidase and α-amylase. Since α-amylase breaks down dietary starch into maltose, which is then further transformed to glucose before absorption, this action aims to suppress postprandial hyperglycaemia.

**Table 3:** in vitro antidiabetic potential of ethanolic extract, methanolic extract and aqueous extract pf P. Cubeba by α-amylase inhibition assay method.

| Conc. of sample (mg/ml) | Ethanolic extract | Methanolic extract | Aqueous extract |
|---|---|---|---|
| 0.2 | 28.6 | 22.3 | 15.1 |
| 0.4 | 48.2 | 33.2 | 29.4 |
| 0.6 | 64.3 | 49.4 | 46.2 |
| 0.8 | 86.4 | 58.3 | 57.2 |
| 1 | 92.1 | 76.6 | 70.1 |
| **IC50** | **0.43** | **0.62** | **0.71** |

The presence of such inhibitors in food can be advantageous because α-amylases are essential for the digestion of starch in both humans and animals.

**Discussion:** Carbohydrate metabolic abnormalities have the potential to cause a number of global health problems, such as obesity, diabetes, and dental illnesses. The main cause of diabetes mellitus is inadequate insulin secretion or action. Inhibiting the breakdown of polysaccharides and disaccharides and increasing insulin production are common treatments for type II diabetes.[8,14]

In the management of diabetes, lowering postprandial hyperglycemia is a crucial therapeutic approach.[15] The pace of starch digestion has a major impact on blood glucose levels. Because α-glucosidase and α-amylase are crucial for the breakdown of carbohydrates, blocking these enzymes is an important part of treating diabetes.

In this investigation, we evaluated the α-glucosidase and α-amylase inhibitory properties of Piper cubeba extracts. Our results showed that several Piper cubeba extracts efficiently reduced the activity of these enzymes, pointing to possible antidiabetic benefits.[16]

A phytochemical examination of the various P. cubeba seed extracts showed that they include a range of phytoconstituents, especially the ethanolic, methanolic, and aqueous extracts that contain alkaloids, tannins, and carbohydrates. α-amylase and α-glucosidase tests were used in this work to assess and compare the extracts' in vitro antidiabetic efficacy. All of the extracts had anti-diabetic properties, but the ethanolic extract had the strongest inhibitory effect on the two enzymes. To pinpoint the precise substances causing P. cubeba's antidiabetic effects, more study is required.

**Conclusion:** Within the finding of an in vitro model, the current investigation unequivocally demonstrated that the extract of P. cubeba seeds has an anti-diabetic effect. Therefore, there is a need for additional in vivo animal model investigation as well as compound isolation studies.

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# Multidisciplinary science for drug development

**Iram**
M. pharma ( pharmacology)
Six Sigma Institute, Rudarpur

## Abstract

This Article Provide a brief overview of the processes of drug discovery and development. Our aim is to help scientists whose research may be relevant to drug discovery and development to frame their research report in a way that appropriately places their finding within the discovery and development process and thereby support effective translation of preclinical research to humans. One overall theme of our article is that the process is sufficiently long, complex, and expensive so that many biological targets must be considered for every new medicine eventually approved for clinical use and new research tools may be needed to investigate each new target. Studies that contribute to solving any of the many scientific and operational issues involved in the development process can improve the efficiency of the process. An awareness of these issues allows the early implementation of measures to increase the opportunity for success. As editors of the journal, we encourage submission of research reports that provide data relevant to the issues presented. New drugs are continually required by the healthcare system to address unmet medical needs across diverse therapeutic areas, and pharmaceutical industries primarily strive to deliver new drugs to the market through the complex activities of drug discovery and development. This involves identifying and validating lead compounds that can bind to a target.

## Introduction

Drug discovery has a long history and dates back to the early days of human civilization. In those ancient times, treatments were often discovered by chance or resulted from observation of nature, typically but not exclusively, using ingredients extracted from plants/ animals , and not just used for physical remedy but also for spiritual healing. Modern drug discovery research started to being performed around the early 1900s. Nowadays, the development of a new medicine usually starts when basic research, often performed in academia, identifies a macromolecule (i.e. a molecule with a large molecular weight lie genes/proteins), or a dysfunctional signaling pathway or a molecular mechanism apparently linked to a disease condition (pre-discovery stage). In general, at this stage, research teams attempt to identify the so-called therapeutic targets (often a protein) that are linked to the disease state. To be nominated therapeutic target, scientists

will also have to find therapeutic agents that modify the function of the perturbed target and restore health or alleviate symptoms. Finding the right target is however extremely challenging. Further, drugs are efficient in humans because of specific actions on the intended therapeutic target but also due to interactions with other, unintended (often unknown) targets! The process continues with the search of therapeutic agents followed by a preclinical phase, during which potential drugs are tested in a battery of animal models, to demonstrate safety and select drug candidates novel strategies to avoid animal testing are being developed, see below). Clinical studies in humans can then get started to establish safety and efficacy of the drugs in patients with the highest benefit- to-risk ratio). The studies are then submitted to regulatory agencies, which review the documents and decide about market approval. If the review is positive, the drug can then be released to the market and be administrated to patients. Once a drug has been approved, investigations continue to monitor putative side effects that could be caused, over time, by the new treatment. This last step is often referred to as pharmacovigilance studies (or real-world evidence), generally dubbed "phase 4" clinical trial. The entire drug discovery and development process involves many disciplines, years of efforts and is very expensive. It also implies the generation and use of vast amount of data usually obtained via different types of high-throughput technologies. Many of these experiments and the analysis of the results can be automated via computer-assisted methods to speed-up some steps of the process, gain knowledge and reduce mistakes.

Drug discovery consists of 5 major steps, including a few subdivisions in each of them:

1. Pre discovery

2. Pre-clinical research

3. Clinical research

4. Post-marketing surveillance

Today Indian pharmaceutical industry is ranked 3rd in terms of generic discovery of drugs in a large volume due to development in the field of chemistry and collaborative research and development with other drug agencies for multi- disciplinary outcomes. The following phases of drug discovery need to be understood in detail in order to know the drug discovery and development process.

**Pre-clinical research**

When a suitable drug candidate has been found, the next step is to carry out in vivo testing of the drug candidates to ensure its safety and efficacy using pre-clinical studies. This particularly involves testing on laboratory animal species like rats, mice, rabbits, monkeys, and guinea pigs to test the appropriate benefits and mechanism of action, routes of administration, dosage, adverse drug events, non- targeted interactions, comparison of efficacy, etc [26]. It ensures that the drug is sufficiently safe to be tested on humans; it enlightens the clarity if there is any effect of the selected drug on gender, particular age group, race, or other ethnic groups. Thus, pre-clinical research can be done to know the toxicity, pharmacokinetics and efficacy of a new drug entity before experimenting on humans [26, 27]. It gives a preliminary idea regarding the behavior of drug.

**Clinical research**

Before a drug is approved by the regulatory authority, it has to undergo extensive clinical trials that have been divided into few phases where each phase has its own relevance. Clinical trial is basically done to know whether a new drug in the verge of getting developed actually works or is it safe for the people. This research can be helpful in estimating the disease diagnosis, extent of a disease, detection, safety and presence of any side effects related to the drug. For this purpose, healthy volunteers from various regions are selected and trial is conducted on them to answer the questions regarding the disease and the drug profile. Before the clinical study is actually started, an application to conduct the research on a particular drug needs to be submitted to the Central Drugs Standard Control Organization (CDSCO) for approval. This application is known as Investigational New Drug (IND) application which contains results from pre-clinical studies, drug information, outline or study protocol to be carried out, and details about the research team who will be responsible for carrying out the trials.

**Phase-0**

This is done to know whether the drug does what it is expected to do. This also helps to save a huge amount of time and money. Here, micro-dosing of a drug is given, due to which risk factors are not there, and as a result, a drug can be tracked if it is reaching the site of action where it is desired, whether it is acting in a positive way and how the body reacts to it. Not every drug undergoes this trial, and it is conducted with a very limited no. of people for a short period of time.

**Phase-I**

This is the first step in clinical trial where less than 100 volunteers are involved, may be around 20-80 people. Drug is given to check the safety dose and the maximum tolerable dose of a drug up to which it does not show any considerable side effect, here safety is the main concern and studying the response of a disease is not the main motive.

**Phase-II**

Here the disease response is studied in around 25-100 volunteers and a comparative high dose is given as compared to the previous phase of trial. Efficacy is the main concern of this study, if majority of the patients are showing response with minimal
side effects, then the drug may proceed towards the phase-III clinical trial.

**Phase-III**

In this phase, comparative evaluation is done with the already established drug of similar category to assess the safety and efficacy of the developed drug. Here the volunteers are assigned to random groups and they himself including the physician are not aware of the specific group in which they are placed (double-blinded) . It involves a large no. of people, among thousands from different geographical regions or countries and the tests are conducted for a long time period. Placebo groups are also included in this study to compare the standard and the test drug. If a patient experiences serious side effects which are less likely to be manageable, the treatment is stopped immediately and care as given . All the parameters for sample collection, treatment and particulars should be stringent and followed with proper skill and knowledge

**Post marketing surveillance-Phase-IV**

Is there still something remaining to be known about the drug? The answer to this question is yes. Whenever a new drug candidate is introduced into the market, it contains a lot of questions that needs to be addressed. Whether it shows any rare side effect that was not observed before, does it improves the quality of life when taken for a long period of time, can all groups of people regardless of being wealthy or poor can have an access to this drug . All this things needs to be questioned and answered at the same time which is only possible if the drug is reaching to the population affected with a cause. It can help future patients to increase the reliability on a drug. Thus, it is a post marketing surveillance study and marks the final process of clinical research after which the drug is free to be used without any hindrance.

**Objectives and Purpose of Drug Discovery**

The purpose of drug discovery and development research is to identify and characterize molecules with the potential to safely treat diseases by developing new drugs that are both effective and well-tolerated, ultimately aiming to improve patient lives by providing new therapeutic options for unmet medical needs; this involves a multi-step process including target identification, lead compound discovery, optimization, preclinical testing, and clinical trials to bring a safe and efficacious drug to market.

Key objectives of drug discovery and development research include:

1. **Identifying a disease target:** Understanding the molecular mechanisms underlying a disease to pinpoint specific proteins or pathways that can be targeted by a drug.
2. **Lead compound identification:** Screening large libraries of chemical compounds to find molecules that interact with the chosen target and exhibit desired biological activity.
3. **Lead optimization:** Modifying the chemical structure of a lead compound to improve its potency, selectivity, and pharmacokinetic properties (absorption, distribution, metabolism, and excretion).
4. **Preclinical testing:** Evaluating the safety and efficacy of potential drug candidates in animal models to assess their therapeutic potential and identify potential toxicities.
5. **Clinical trials:** Conducting human studies in different phases to evaluate the safety and efficacy of the drug in various patient populations, determining the appropriate dosage and treatment regimen.
6. **Regulatory approval:** Submitting comprehensive data to regulatory agencies to gain approval for marketing and distribution of the new drug.

**Important aspects of drug discovery and development research:**

**Target validation:** Confirming that the chosen molecular target is directly involved in disease pathogenesis.

**Structure-activity relationship (SAR):** Studying how changes in the chemical structure of a compound affect its biological activity.

**High-throughput screening (HTS):** Utilizing automated systems to rapidly screen large compound libraries against a target

**Drug delivery systems:** Designing methods to efficiently deliver drugs to the desired site of action in the body.

**Pharmacokinetics (PK) and pharmacodynamics (PD):** Studying how a drug is absorbed, distributed, metabolized, and excreted within the body, and how it interacts with its ta

**Strategies for improved success in the drug discovery and development process**

**Key approaches**

Several strategic approaches to enhance efficiency in the drug discovery and development process have been proposed, adopted, and exploited to varied extent in the pharmaceutical research and development (R&D) projects. They include exploitation of genomics and proteomics, the complementarity of phenotypic and target-based screening platforms, expanding the use of existing drug molecules through repurposing and repositioning, use of collaborative research, exploring under-served therapeutic areas, outsourcing approach, and pharmaceutical modeling and artificial intelligence.

**Exploitation of genomics and proteomics**

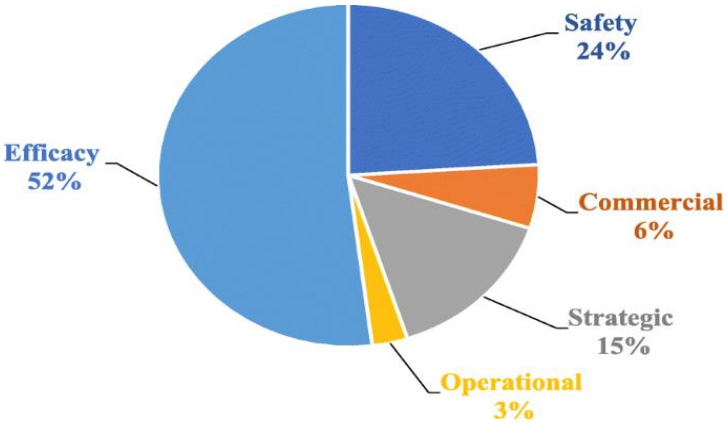
It is an established fact that majority of diseases have a molecular or genetic etiology [12, 13]. Some conditions including sickle cell disease, cystic fibrosis, muscular dystrophy, and Huntington disease are caused by single gene mutations [14]. Syndromic conditions such as diabetes and cardiovascular diseases have multifactorial causes including multiple gene mutations confounded by environmental and lifestyle factors [12]. In the concept of drug discovery, genes have therefore been classified as disease genes, disease-modifying genes, and druggable genes [15]. Disease genes are those whose mutations cause or predispose a person to the development of a given disease [16]. Disease-modifying genes encode functional proteins whose altered expression is directly linked to the etiology and progression of a given disease. Druggable genes encode proteins that possess recognition domains capable of interacting with drug molecules eliciting a pharmacological response [17].

In the current era of target-based drug discovery, it is imperative that the target is scrupulously identified and validated to establish its essentiality in the disease phenotype. This prevents downstream attrition with available data indicating that a significant proportion (52%) of drug failure in clinical trials is due to poor efficacy. Figure 2 depicts the various causes of attrition [18, 19]. Classical cases of the drugs imatinib and

trastuzumab exemplifies the value of careful target identification and validation in enhancing the success of the drug discovery process [20,21,22]. While the above were new molecules carefully designed with the knowledge of the underlying genetic mutation, existing drugs may find new applications through repositioning from their approved indications based on information obtained through genomics [23]. Genomics can be used to identify and validate druggable genes thus expanding the number of targets available for exploration in drug discovery [17, 24]. The use of genomics in target validation has expansively widened through advancement in antisense technology, small interfering RNA (siRNA) that mimic the natural RNA interference (RNAi) and transgenic animal models [25].

Exploitation of genomics is not restricted to target identification and validation. Rather, recent trends in pharma R&D show that genomics may be employed in the recruitment of study participants for clinical trials with the selection favoring those subjects more likely to benefit from the intervention being trialed. This ensures that the effect of the drug will be evident if the drug is indeed effective against the target disease and absent if ineffective.

The outcome so observed would therefore be attributable to the therapeutic intervention and shielded from other confounders. Genomics can also be used as a predictive tool to forecast potential toxicities emanating from a specific molecule [22]. Not surprising, the discipline of pharmacogenomics where drugs are adapted to meet individual profiles is fast gaining traction among researchers and medical practitioners, and has positively impacted the process of drug discovery and development [22].



The human genome was fully described in the year 2002, uncovering a vast treasure trove from which a wide array of novel drug targets could be discovered. Nonetheless, the scientific hype that was associated with the genome project has not been followed with solid benefits as less than 500 of the potential 10,000 targets have been utilized according to the repertoire of drugs registered by the United States Food and Drug Administration (US-FDA) [1, 26]. These targets are protein molecules including DNA, RNA, G protein-coupled receptors (GPCRs), enzymes, and ion channels. The GPCRs constitute the largest proportion of targets for currently registered molecules [27]. It is however expected that the genomic revolution will enhance the drug discovery process significantly given the intensive research currently being done in this field [28].

Proteomics which is a subset of genomics has been widely explored as an avenue of drug discovery [29]. Proteomics entails identification, characterization, and quantification of cellular proteins with the aim of establishing their role in the disease progression and the underlying potential for chemotherapeutic manipulation [25]. Proteomics has been applied widely in drug discovery projects for antineoplastics, neurological, cardiovascular, and rare diseases [30]. Technologies used in proteomics include gel electrophoresis for protein separation and characterization, mass spectrometry (MS) for identification, and yeast hybrid systems to study protein-protein interactions [31]. These approaches have the potential to identify novel drug targets and their corresponding genes.

**Complementarity of phenotypic and target-based screening platforms**

Two distinct screening approaches are routinely employed in the efficacy studies, namely phenotypic (whole-cell) screening and target-based (biochemical) screening. Phenotypic screening evaluates the effects of potential drugs on cultured cell lines (in vitro), isolated tissues/organs (ex-vivo), or in whole animals (in vivo) while target-based screening involves testing the molecules on purified target proteins in vitro [32]. In the first instance, phenotypic screens are primarily aimed at identifying molecules capable of eliciting the desired pharmacological effect without necessarily elucidating the underlying mechanism of action at the molecular level. They are therefore empirically driven as they focus on phenotypic endpoints. Phenotypic drug screening is information-rich, and the therapeutic relevance of the drug is established much earlier in the drug discovery process. The approach is more physiologically relevant as it is conducted in biological systems that simulate the real physiological environment where cognizance

that pharmacological effects result from an interplay of many factors is well appreciated [33, 34]. It also provides a huge biological space for serendipitous drug discoveries [32, 35]. On the contrary, target-based screening is hypothesis-driven, systematic, and rational. Of essence, it requires identification and isolation of a biochemical target whose modulation leads to a desired pharmacological effect. It employs advanced molecular technologies and biological methods that are facilitative of high throughput screening (HTS) platforms [36].

Whereas phenotypic screening predominated in the decades before 1980, it has largely been de-emphasized as advances in molecular biology, and genomics took root and favored the target-based screening [37]. The significant decline in the discovery of first-in-class molecules has in part been attributed to an increasing emphasis on the target-based drug discovery approach [34]. Analysis of data of the drugs registered by the US-FDA reveals that phenotypic drug discovery has yielded more first-in-class molecules than target-based screening [38]. These findings have been challenged by a study that established that 78 of 113 first-in-class molecules registered between years 1999 and 2013 were discovered using target-based screening approaches [39]. Target-based drug discovery has been the predominant approach of screening putative molecules in the last three decades [33, 42]. This has majorly been due to advances in cloning technologies that allow isolation of pure proteins that are then used to screen a large library of compounds using HTS. The high screening capacity afforded by this approach has cemented target-based platform as the default drug discovery approach as companies seek a competitive edge to deliver novel molecules to the market [36]. Target-based drug discovery begins with understanding the pathophysiological basis of the disease and subsequent identification of the errant biochemical pathway that leads to the disease phenotype. The specific protein that is aberrantly expressed is identified, isolated and its role in the disease phenotype validated by modulation using genomic or pharmacological approaches.

Target-based drug discovery, therefore, elucidates the specific mechanism through which potential drugs produce a pharmacological response. While it lags behind the phenotypic drug discovery approach in yielding first-in-class molecules, target-based drug discovery is unrivalled in producing the best-in-class follower molecules [38]. This is due in part to the rational, hypothesis and systematic approach employed leading to highly selective, potent molecules with better pharmacokinetic and toxicological profiles. Target based-drug discovery has the advantages of being simpler to undertake, enable faster development,

and it enables elucidation of the underlying mechanism of action. It also enables the utilization of modern technological advances including computational modeling, molecular biology, combinatorial chemistry, proteomics, and genomics. Conversely, since the approach is based on the modulation of isolated protein targets, the observed effect may have little physiological relevance as there is oversimplification of the physiological environment in which the drug molecules are evaluated [43].

**Collaborative research**

By its nature, the corporate pharmaceutical industry is highly competitive with each company aspiring to dominate the race to launch new blockbuster molecules. It is an established industry fact that early market entrants reap more than those who launch follower molecules. Pioneer companies are able to establish strong brand recognition as well as patient and physician loyalty before competition enter the market [55]. Further, early entrants have sufficient time to perfect their product and set the market price. At any given time, the pharma companies are working to discover and develop molecules addressing similar or very closely related drug targets. Given the astronomical funding channeled into pharmaceutical R&D, these duplicated research efforts collectively end up utilizing resources that could better be invested in the R&D of other disease areas with unmet medical needs. A number of collaborative arrangements have been proposed and utilized for greater success of the pharma R&D. These include precompetitive research, pharma-academia collaboration, and public-private partnerships (PPP) models [56].

The precompetitive research entails collaboration among pharmaceutical companies, biotechnology companies, and the academic drug discovery units that would otherwise compete but are brought together by a common desire to conduct fundamental research that is facilitative of subsequent drug discovery and innovation. In essence, precompetitive research establishes scientific viability of pursuing a given therapeutic pathway prior to initiation of full-throttle drug discovery and development campaign. Some of the areas in which precompetitive research may be practiced include target identification and validation, sharing of compound libraries, and biomarker and assay development. There are numerous benefits deriving from precompetitive collaboration including reduced costs of research as companies share their resources and expertise, greater efficiency as companies focus on their core competencies thus furthering their excellence, and cross-fertilization of scientific ideas [57]. Precompetitive collaborations are modeled as virtual institutions with scheduled video conferences to monitor and evaluate the progress made. Once

the objectives set upon are attained, companies can then venture into separate drug discovery projects [58]. Renown precompetitive collaborations include the Biomarkers Consortium, Innovative Medicine Initiative and TranSMART [59]. TransMART is an inter-organizational collaboration including government agencies, academia, and patient advocacy groups that serves as an open data warehouse arising from clinical trials and basic research [60, 61]. In recognition of the potential gains that could accrue from precompetitive collaborations, the US-FDA developed guidelines for registration of drugs discovered through collaborative strategies in 2011 [62].

**Under-served therapeutic fields**

Strategic considerations are vital before a company commits to a drug discovery project. Among the key considerations is the economic viability of a potential drug molecule upon market entry. For sustainable pharma R&D, any drug development candidate must have an acceptable return on investment to ensure the discovery company remains a viable going concern and is able to fund other drugs in the research pipeline. As such majority of the pharmaceutical R&D efforts are inclined to the therapeutic areas with vast economic potential such as oncology, immunotherapy, endocrinology, neurology, and cardiovascular fields where the probability of recouping the huge capital investment is more certain [41]. Therapeutic areas that offer negligible financial benefits such NTDs and rare diseases do not attract much attention and therefore the opportunities for novel discoveries largely remain unexplored [70]. Rare diseases are genetic disorders that afflict a small patient population and thus offer little economic promise. The NTDs, on the other hand, are vector-borne diseases that afflict billions of people in resource-poor countries. However, these populations have low purchasing power and as such, the pharma companies may not recoup their investments let alone enjoy profitability [71].

**Pharmaceutical modeling and artificial intelligence**

Modeling entails the use of in silico simulations to predict diverse attributes of a drug molecule including pharmacokinetics and pharmacodynamics profiles [80]. Advances in computing power have enabled development of software that allows simulation of the drug-receptor binding processes, a subset of computer-aided drug design (CADD) also referred to as virtual screening, with tremendous benefits to drug discovery efficiency. First, CADD facilitates generation of focused screens that are then validated in

vitro. Second, the CADD is well positioned to guide the lead optimization process thus providing valuable information to the medicinal chemistry team aspiring to enhance the lead molecules receptor affinity or optimize drug metabolism and pharmacokinetics (DMPK) properties including absorption, distribution, metabolism, excretion, and the potential for toxicity (ADMET). Third, the CADD facilitates rational drug design either by "growing" starting molecules one functional group at a time (de novo drug design) on the target site or by piecing together fragments into novel molecules (fragment-based drug design) [81]. Two screening approaches, namely ligand-based virtual screening and target-based virtual screening, have been used in CADD to filter out the compounds that are unlikely to be successful in the development pipeline due to poor physicochemical properties and/or intolerable toxicological profile while identifying those likely to have the activity of interest.

In ligand-based virtual screening, structural features of known compounds are used to construct computer models that are used to predict the properties of other compounds not included in the training data set. The data sets are then used to generate quantitative-structure activity relationship (QSAR) models correlating structural features and the physicochemical properties of a homologous series to the observed biological activity. The chemical structure of known compounds is reduced to a set of molecular descriptors that are used to generate a mathematical model that is used to predict the properties of the test compounds. Molecular descriptors with the highest activity are chosen for the model [82]. Target-based virtual screening entails computer models that test the docking properties of test compounds against the three-dimensional structure of the target (X-ray crystal structure or homology model) [83,84,85]. Each of the test compounds is optimally positioned on the binding site and assigned a score based on the binding affinity. Top scoring compounds are synthesized and tested in vitro [86]. Application of these models can enhance the efficiency of drug discovery projects by providing focused screens that can have better chances of succeeding downstream. Problematic molecules are also identified earlier in the drug discovery process thus avoiding expensive late-stage failures. Integration of ligand-based and target-based virtual screening yields better results [32, 87].

**Conclusion**

The ever-increasing costs of drug discovery projects have not translated into increased efficiency in delivering new medicines. On the contrary, fewer drugs are transiting through the drug development

pipeline than ever before. The observed productivity decline is majorly attributable to the overreliance of the industry on high technology platforms, stringent drug registration and approval requirements for new medicines, and the exhaustion of the obvious and easy-to-reach drug targets necessitating exploration of more complex biological systems.

Scientific advancements allow the application of advanced molecular techniques that include genomics and lately proteomics in identification and validation of drug targets. Carefully executed target identification and validation will reduce the attrition rates attributable to poor efficacy that currently accounts for more than 50% of drug failures. The complementarity of phenotypic and target-based drug discovery approaches would enable discovery of first-in-class molecules while also delivering safer, more efficacious and potent best-in-class follower molecules.

Collaborative strategies, such as precompetitive research and public-private partnerships, have positively impacted efficiency in drug discovery. Expansion of research activities into the underserved therapeutic areas covering rare and neglected diseases would offer a safeguard for companies whose blockbuster drugs are teetering on the patent cliff. Advances in computing technologies will also facilitate selection of focused screens with better success rates downstream. Pharmaceutical modeling and AI are expected to continue contributing significantly to improved efficiency in drug discovery and development in the years to come. Carefully executed outsourcing strategies allow companies to focus on their core competencies while delegating other development activities to expertise offered by the CROs, a strategy that accelerates the discovery process while reducing overhead costs.

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# DEVELOPMENT AND IN-VITRO CHARACTERIZATION OF SUSTAINED RELEASE LINEZOLID TABLETS: A NOVEL DRUG DELIVERY APPROACH

[1]Itishree Maharana[*], [2]Biswajit Sahoo, [3]Shuvendu Sekhar Dinda, [4]C Mena Kumari, [5]Ashish Kumar Behera, [6]Supriyo Satapathy , [7]Sanjiv Kumar Panda [8]Sabyasachi Pradhan, [9]Subham Kar
[1,2,3,4,5,6,7]Roland Institute of Pharmaceutical Sciences, Brahmapur, Odisha
[8,9]School of Pharmaceutical Science, Siksha O Anusandhan University, Bhubaneswar, Odisha

**Abstract**

This study focuses on the development and evaluation of sustained-release tablets of linezolid to achieve the desired bioavailability and in-vivo release pattern. Preformulation studies examined the active pharmaceutical ingredient (API) for physical properties and solubility, along with weekly drug-excipient compatibility checks under specified temperature and humidity conditions, which confirmed stability with no changes in color or physical integrity. Linezolid tablets (300 mg) were prepared using fluidized bed processing, followed by formulation trials with varied ratios of ethyl cellulose (EC), PEG 6000, and magnesium stearate to optimize tablet characteristics and drug release profiles. The formulations were analyzed for particle size, bulk and tap density, moisture content, and weight variation, all of which were within acceptable ranges. Optimization through Trial 6 matched the release profile of the reference product, achieving a similarity factor of F2 = 0.828. Stability studies of this optimized formulation (Trial 6) conducted under different storage conditions (25°C/60% RH and 40°C/75% RH for 90 days) demonstrated stability in terms of physical appearance, moisture, drug content, and drug release. Kinetic analysis indicated a first-order release with Higuchi diffusion ($R^2 = 0.9734$), aligning the bioequivalence of the test formulation with the reference product. This validated approach supports the use of Trial 6 as a stable and bioequivalent sustained-release formulation for linezolid.

Keywords: Linezolid, sustained-release tablets, bioavailability, in-vivo release pattern preformulation studies, drug-excipient compatibility.

## I. INTRODUCTION

Drug delivery systems have long played a critical role in treating acute and chronic illnesses, traditionally utilizing dosage forms such as tablets, injectables, capsules, and creams. These systems aim to deliver therapeutic agents to the human body effectively and safely, with modern advances allowing for the enhancement of drug bioavailability and stability. An ideal drug delivery system is characterized by two primary requisites: it releases the drug at a rate matching the therapeutic needs of the body and targets specific areas within the body. The growing demand for precision in drug delivery has paved the way for modified release technologies, which provide increased efficacy, reduced toxicity, and fewer required doses. Conventional drug delivery methods, while effective for many drugs, pose limitations when dealing with agents that are unstable, toxic, or possess narrow therapeutic windows. For these cases, continuous or modified drug administration is essential to maintain consistent plasma levels. Modified release dosage forms, including delayed and extended-release types, address these challenges. Designed to release the drug either over an extended duration or at a specific location, these systems aim to improve therapeutic outcomes while enhancing patient compliance.

In delayed-release formulations, such as enteric-coated tablets, are structured to release the drug at a predetermined location, typically beyond the stomach. This approach not only protects the active ingredient from stomach acid but also mitigates gastric irritation. These systems utilize pH-sensitive polymers, which dissolve once the dosage form passes from the acidic stomach environment to the more neutral small intestine. Intestinal and colonic release systems are typical delayed-release types used in treating localized conditions like ulcerative colitis or for systemic absorption of specific drugs.

In contrast, extended-release forms are engineered for a prolonged therapeutic effect, gradually releasing the drug to reduce dosing frequency. By minimizing the peaks and troughs in plasma drug levels, extended-release formulations improve patient convenience and safety. They also allow a reduction in dosage frequency, making them more favorable for medications requiring consistent therapeutic levels over time, such as sustained-release formulations designed for conditions requiring extended drug action.

Controlled-release drug delivery systems (CRDDS) precisely regulate the rate of drug release to maintain steady therapeutic levels, minimize side effects, and enhance drug targeting through spatial and temporal

control. This controlled release can be adapted to target specific disease sites, improve bioavailability, and reduce systemic side effects. The most advanced systems even adjust to physiological needs, ensuring that drug release aligns with therapeutic requirements.

Sustained-release dosage forms are integral to controlled release, extending the therapeutic effect by gradually releasing medication over an extended period. This reduces the frequency of dosing while achieving prolonged drug action, making them ideal for medications with shorter half-lives. The key factors affecting sustained release include drug stability, solubility, and biological half-life, with innovations continuously optimizing the efficacy and safety of these dosage forms.

In recent years, sustained and controlled-release systems have garnered research attention due to their potential for improved patient compliance and minimized adverse effects. These systems, such as polymer-based drug delivery, offer significant advantages over conventional methods by enhancing drug efficacy, reducing toxicity, and providing sustained therapeutic action.

## II. LITRETURE REVIEW

Basling et al. (2022) developed and evaluated an immediate-release tablet of linezolid with effective taste-masking properties. The study involved optimizing several parameters, including swelling time, resin activation, drug-resin ratio, and stirring duration, to maximize taste-masking and drug-loading efficiency. The resultant Drug-Resin Complex (DRC) was thoroughly characterized through infrared spectroscopy, thermal analysis, and X-ray diffraction. Tablets were subsequently prepared via wet granulation, incorporating PVP K-30 as a binding agent, while alginic acid NF and crospovidone were tested as superdisintegrants. The optimal disintegration time was established at 55 seconds. Notably, tablets containing alginic acid exhibited a marginally longer disintegration time than those with crospovidone, which emerged as the most effective superdisintegrant for the DRC. Crospovidone-fortified tablets demonstrated rapid disintegration, short wetting time, and favourable friability profiles (Basling et al., 2022).

Jani and Patel et al. (2023) developed a sustained-release tablet formulation that combined linezolid with Aegle marmelos, a naturally occurring antibacterial. The gum derived from Aegle marmelos, valued in pharmaceutical formulations, was used as a plant-based excipient due to its biocompatibility, biodegradability, minimal side effects, and cost-effectiveness. The formulation incorporated Aegle

marmelos fruit gum with HPMC K100M to create a matrix for controlled drug release, prepared using the wet granulation technique. These tablets underwent evaluation for weight variation, hardness, diameter, physical appearance, friability, thickness, and in vitro drug release, meeting all required physical standards. Dissolution testing confirmed sustained drug release over 10–12 hours. Additionally, various polymer combinations and fillers were assessed to fine-tune drug release profiles using a 3^2 factorial design. The final formulation, combining Aegle marmelos gum with HPMC K100M, successfully controlled drug delivery and exhibited effective antibacterial activity, positioning it as a promising option in sustained-release linezolid formulations (Jani & Patel, 2023).

## III. RESEARCH METHODOLOGY

In this study, the research methodology involves a series of steps that are carefully designed to prepare, evaluate, and optimize a multiple unit mini-tablet (MT) formulation for prolonged-release and fast-release drug delivery. The following details break down each step of the process:

The formulation is made using a variety of pharmaceutical excipients including:

Microcrystalline Cellulose (MCC): A binder that ensures the adhesion of powder particles.

Sodium Alginate, Ethyl Cellulose, and HPMC K15M: Polymers used to control drug release rates.

Starch: A disintegrant, facilitating fast drug release for some mini-tablets.

Magnesium Stearate and Talc: Lubricant and glidant, respectively, to improve the compressibility and flow of the mixture.

1. Weighing and Dispensing

All excipients and active pharmaceutical ingredients (API) are precisely weighed and dispensed according to the formulation design, ensuring accuracy in drug dosage.

2. Dry Mixing

The dry mixing process ensures that the API (Linezolid in this case) and other powdered excipients are uniformly distributed in the blend. A dry blender or mixer is used to create a homogenous mixture. This step is crucial for consistency in the tablet formulation.

3. Binder Solution Preparation

A binder solution is prepared by dissolving MCC in purified water or an appropriate solvent. The concentration and viscosity of the binder solution are carefully controlled to ensure optimal granulation in the following step.

4. Wet Granulation

In this step, the dry powder mixture is granulated by adding the binder solution while mixing. The gradual addition ensures the formation of uniform granules. The granules provide improved flow properties and help in achieving better tablet compressibility.

5. Drying of Wet Granules

The wet granules are dried using either a fluid bed dryer or tray dryer. The moisture content is reduced to a specified level (typically 1-3% loss on drying) to ensure stability and prevent degradation of the formulation during storage.

6. Sizing and Milling (if needed)

Post-drying, the granules are sized and milled to ensure uniform particle size distribution. This is an important step to achieve consistent tablet weights and avoid variability in tablet properties.

7. Blend Preparation

The dried granules are blended with additional excipients such as disintegrants and lubricants. This ensures the uniformity of the final tablet blend before compression.

8. Compression

The final blend is compressed into tablets using a tablet press machine. The pressure is controlled to form tablets of the desired size, shape, and hardness.

Pre-Compression Parameters

The research also focuses on analyzing several pre-compression parameters, including:

Angle of Repose: Measured to assess powder flowability. A lower angle indicates better flow properties.

Bulk Density and Tapped Density: These parameters reflect how tightly the powder particles pack together and are used to optimize compression processes.

Carr’s Index: Calculated to assess the compressibility of the powder blend. It helps evaluate flow properties and predict the tablet's uniformity.

Hausner Ratio: A ratio of tapped density to bulk density, which further helps in understanding the flowability of the powdered material.

## IV. RESULT AND DISCUSSIONS

The sustained-release Linezolid tablets (300 mg) were successfully formulated and characterized to meet the required bioavailability and in-vivo release patterns. The preformulation studies included evaluating the active pharmaceutical ingredient (API) for its physical properties and solubility, with no significant changes observed in the drug-excipient compatibility studies, confirming the stability of the combinations under the given storage conditions. Particle size distribution, bulk, and tapped density assessments for all formulations showed values within the expected ranges (0.64-0.67 gm/ml for bulk density and 0.68-0.71 gm/ml for tapped density). The moisture content was also found to be within acceptable limits at around 1.04%. By modifying the quantities and ratios of ethyl cellulose (EC), PEG 6000, and magnesium stearate in various formulations, differences in the drug release profiles were observed. Formulation 6 (Trail 6) exhibited the closest drug release profile to the reference standard, with a similarity factor (F2) of 0.828. Stability studies conducted over 90 days at two storage conditions (25°C ± 2°C / 60%RH ± 5% and 40°C ± 2°C / 75%RH ± 5%) showed that the tablets remained stable with respect to physical appearance, moisture content, drug content, and drug release.

**Table 1 : Formulation Table**

| **S.No.** | **Ingredient** | **F1 (mg)** | **F2 (mg)** | **F3 (mg)** | **F4 (mg)** | **F5 (mg)** | **F6 (mg)** | **F7 (mg)** | **F8 (mg)** | **F9 (mg)** |
|---|---|---|---|---|---|---|---|---|---|---|
| **1.** | Linezolid | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 |
| **2.** | MCC | 15 | 14 | 15 | 15 | 14 | 15 | 15 | 14 | 15 |
| **3.** | Starch | 16 | 10 | 2 | 16 | 10 | 2 | 16 | 10 | 2 |
| **4.** | HPMC.K15M | 7 | 14 | 21 | - | - | - | - | - | - |
| **5.** | Sodium Alginate | - | - | - | 7 | 14 | 21 | - | - | - |
| **6.** | Ethylcellulose | - | - | - | - | - | - | 7 | 14 | 21 |
| **7.** | Magnetic stearate | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **8.** | Talc | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **9.** | Purifiedwater | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| **10** | Totalwt. | 340 | 340 | 340 | 340 | 340 | 340 | 340 | 340 | 340 |

**Table 2: Post-compression parameter:**

| Formulation No. | Wt. variatio | Thicknes s(mm) | Hardness ($kg/Cm^2$) | Disintegration Time (min) | %Friability |
|---|---|---|---|---|---|
| **F1** | 340±0.23 | 3.1±0.12 | 5.0±1.2 | 8.31±1.2 | 0.17±0.009 |
| **F2** | 342±0.31 | 3.2±0.2 | 6.5±1.3 | 7.42±1 | 0.24±0.004 |
| **F3** | 341±0.22 | 3.3±0.22 | 6.4±1.28 | 8.22±1.19 | 0.26±0.005 |
| **F4** | 343±0.35 | 3.2±0.2 | 6.3±1.27 | 7.53±1.0 | 0.17±0.007 |
| **F5** | 344±0.25 | 3.4±0.34 | 5.3±1.21 | 7.61±1.1 | 0.22±0.009 |
| **F6** | 346±0.33 | 3.1±0.12 | 6.2±1.23 | 8.32±1.21 | 0.17±0.009 |
| **F7** | 342±0.24 | 3.3±0.23 | 5.4±1.22 | 8.21±1.18 | 0.2±0.013 |
| **F8** | 345±0.36 | 3.1±0.12 | 5.5±1.25 | 7.12±1 | 0.25±0.046 |
| **F9** | 341±0.32 | 3.3±0.22 | 6.0±1.29 | 8.11±1.12 | 0.18±0.004 |

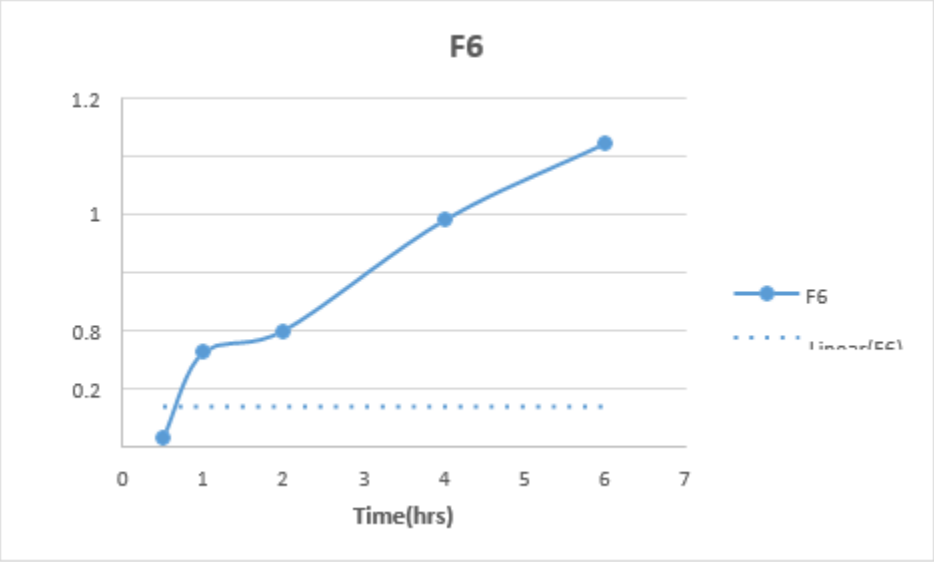


**Figure: 1 Dissolution graph profile of optimized formulation F6**

## V. CONCLUSION

The optimized formulation F6 of sustained-release Linezolid tablets was developed using fluidized bed processing and exhibited favorable in-vitro and in-vivo release profiles. The drug release followed first-order kinetics with Higuchi diffusion ($R^2 = 0.9734$), making the formulation bioequivalent to the reference product. Stability studies confirmed the formulation's robustness, maintaining its quality over time under controlled environmental conditions. The development of these tablets demonstrates the potential for improved therapeutic efficacy and patient compliance through controlled drug release over a sustained period.

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# RHODIOLA ROSEA: A PHYTOCHEMICAL POWERHOUSE FOR COSMECEUTICAL AND THERAPEUTIC USES

**D Jayesh Kumar**

## ABSTRACT

*Rhodiola rosea*, a well-known adaptogen traditionally used in various cultures, has gathered significant attention over the years for its potential as a cosmeceutical ingredient due to its multi-therapeutic properties. In the current review, we have carried out a comprehensive analysis of *R. rosea's* pharmacological activities, such as antioxidant, anti-inflammatory, antistress, and immunomodulatory, highlighting its relevance in cosmetic applications. In addition, this present review also covers the microscopic characters, cultivation, and collection of the plant. We have also summarized the primary bioactive constituents, including salidroside, rosavin, tyrosol, and various flavonoids, which are known for their potent medicinal and cosmetic applications. Despite the various medicinal and cosmetic applications, plants also exhibit some minor adverse effects, which have been highlighted in the article. In summary, *Rhodiola rosea* presents a valuable opportunity for developing advanced cosmeceutical products, offering natural solutions for enhancing skin health and appearance. Continued exploration and clinical validation will be essential in realizing its full potential in the cosmeceutical industry.

## INTRODUCTION

Ayurveda, the ancient system of medicine in India, underlines several approaches to maintaining the health of mankind. Thousands of medicinal plants, their benefits, phytochemicals and formulations have been listed in the Ayurveda. This traditional system of medicine, which is officially recognized in India, documents the uses of various plants and plant parts for the treatment of various ailments, as well as for their cosmetic value, such as enhancing skin health, promoting hair growth, and maintaining overall beauty [1]. It describes the specific herbs, their preparation methods, and application techniques for different cosmetic purposes such as complexion improvement, wrinkles reduction, managing acne, and hair strengthening. These formulations often involve the combination of multiple plants, each chosen for its unique properties, to provide a natural and sustainable solution for beauty and skincare needs [2].

Plant *Rhodiola rosea* is one of the well-acknowledged plants of Ayurveda studied for various cosmetic and medicinal purposes. It is a perennial herbaceous plant that belongs to family Crassulaceae. Plant is also known as "golden root", "roseroot" or "arctic root" with synonyms *Sedum rhodiola* DC., *Sedum roseum* (L.) Scop). The plant is distributed throughout the arctic regions of Asia and Europe and coastal areas of North America [3,4]. Plant thrives at clefts of Rocky Mountains, and sandy soil of coastal regions. The common name of the plant roseroot is based on the yellow flowers that bear the essence of a rose. Roots of the plant exhibit huge medicinal properties and are hence exploited in many countries due to high demand. Industrial supply of the plant material is majorly met by harvesting wildly grown plant. Therefore, increased scarcity and lack of regulation has led to the adulteration, illegal marketing and habitat destruction of the species [4].

*Rhodiola rosea* exhibits distinctive anatomical features throughout its various parts, owing to its adaptation in the harsh environment. Roots and rhizomes exhibit a typical dicotyledonous structure, with an outer cork layer composed of 6-7 layers of thick-walled, rectangular cells that are suberized, providing protection against external stressors. Cortex comprises thin-walled parenchymatous cells filled with starch grains with calcium oxalate crystals frequently observed as prismatic crystals. The plant's stem consists of a single-layered epidermis covered by a thick cuticle with unicellular or multicellular non-glandular trichomes that provide a protective function. The cortex beneath consists of collenchymatous cells that offer mechanical support and parenchymatous cells containing chloroplasts, facilitating photosynthesis [5]. The leaf anatomy is marked by a single-layered epidermis covered with a thin cuticle, with stomata of the anomocytic type present on both surfaces, but more abundant on the lower side. The mesophyll is differentiated into a palisade layer of elongated chloroplast-rich cells, promoting efficient photosynthesis, and a spongy parenchyma with intercellular air spaces facilitating gas exchange. Altogether, these microscopic characteristics show *Rhodiola rosea's* anatomical adaptations, which are helpful to plants for survival in extreme conditions, supporting its pharmacological and medicinal properties [6].

Among the various species of the *Rhodiola, R. rosea* is massively studied for its medicinal uses. The earliest record of the *Rhodiola rosea* for medicinal purposes dates back to Dioscorides in 77 AD, where it was acknowledged for antifatigue, and antidepressant activity. Thereafter from 1969 to 1985 plant was listed as adaptogen by Health Ministry and medicinal agency of Russia, Sweden and Europe for its antistress properties. Apart from this *Rhodiola* has history to alleviate the mountain sickness in Tibetan

traditional system of medicine since ancient times. Some other species of *Rhodiola* such as *R. crenulata* and *R. kirilowii* can be also traced in the Chinese pharmacopeia dated 1977 to 1985 [4].

*R. rosea* has a long-standing traditional significance in various cultures, particularly in cold and mountainous regions such as Russia, Europe, and Tibet. Root of the plant has been prized as an adaptogen, which is believed to help the body adapt to the stress, enhance resilience and restore balance. Traditionally, people used *R. rosea* to combat fatigue, increase physical endurance, and improve mental clarity and concentration. It is also used to reduce anxiety and depression, elevate the mood, and promote the overall well-being of the person. The literature on the plant indicates that the root of the *R. rosea* is the most used part for its medicinal purposes and is reported to contain phenylpropanoids, flavonoids and their glycosides, lignans, terpenoids, coumarins, gallic acid and its derivatives. In addition to its folk medicine, recent and advanced research on *Rhodiola rosea* depicts its multiple pharmacological activities such as antioxidant, neuroprotective, antihypertensive, antidiabetic, antiinflammatory, and immunomodulatory [7].

*Rhodiola rosea* is valued in cosmetics for its rich content of bioactive compounds, with various parts of the plant offering unique benefits. The roots are the most commonly used part in cosmetic formulations, primarily due to their high concentration of phenolic compounds like rosavin, salidroside, and tyrosol. These metabolites exhibit potent antioxidant properties, which help neutralize free radicals, reduce oxidative stress, and protect the skin from premature aging and environmental damage[8]. Root extracts are often incorporated into anti-aging creams, serums, and moisturizers to enhance skin elasticity, improve tone, and reduce the appearance of fine lines and wrinkles. The leaves of *R. rosea* are known to contain flavonoids and tannins, which have antiinflammatory and astringent properties. Leaf extracts can be used in formulations to soothing irritated skin, reducing redness, and improving skin texture. The stems also contain bioactive compounds that contribute to skin hydration and can be used in products targeting dry or sensitive skin types [9].

Despite its recognized benefits, *Rhodiola rosea* is often subject to adulteration due to high demand and limited supply. Common substitutes or adulterants include other *Rhodiola* species, such as *Rhodiola crenulata*, *Rhodiola quadrifida*, and *Rhodiola sachalinensis*. These species may resemble *R. rosea* but differ significantly in their phytochemical profiles, often lacking the characteristic compounds like rosavin

and salidroside responsible for *R. rosea's* specific cosmetic benefits. Such adulteration can diminish the efficacy of cosmetic products, misleading consumers and potentially causing unwanted side effects. Therefore, accurate identification and standardization of *R. rosea* extracts are crucial to ensuring product quality and maintaining consumer trust in cosmetic applications [10].

**GEOGRAPHIC DISTRIBUTION, COLLECTION & CULTIVATION:**

As discussed in the previous section, this herbaceous plant grows in cold mountain regions throughout the Northern hemisphere. It is distributed along the various continents, including Europe, Asia, and North America.

In Europe, *R. rosea* is found across the Arctic and mountainous regions of Scandinavia, Iceland, the British Isles, and the Alps. In Russia, the plant is particularly present in the Ural Mountains, Siberia, and the Altai Mountains, where it thrives in the harsh, cold climates of high altitudes. In Asia, *R. rosea* is widely distributed across the Himalayas, including regions of Bhutan, Nepal, and parts of northern India, where it grows at an altitude of 3,000 to 5,000 meters from sea level. In North America, *R. rosea* is native to Alaska and the mountainous areas of Canada [11].

*Rhodiola rosea* is well grown in sandy or rocky soils, and is commonly found in alpine meadows, slopes, and along the riverbanks. Its ability to thrive in these extreme conditions is attributed to its extensive root system, which is the integral part of plant support and allows it to absorb nutrients efficiently from the scarce soil. The plant's distribution is influenced by its preference for cool temperatures, high altitudes, and areas with sufficient sunlight, which is on the main region why this plant is predominantly found in regions with long, harsh winters and short, cool summers [12].

The plant reaches full bloom in the summer from June to August, which is the peak time for its highest bioactive compound. Most plant is harvested for their roots, which are the primary source of their therapeutic and cosmetic benefits. The plant collection is mostly carried out manually, as the plant grows in remote and unreachable locations, which require careful extraction to avoid and minimize disturbing the fragile alpine ecosystem. In traditional practices, the roots are dug up using simple tools, and only mature plants that are several years old are harvested to ensure sustainability. Further, the roots or required part is washed, sliced, and dried at low temperatures to preserve their active phytometabolites [11,13] **Fig. 1** Shows the different parts of the plant [40].


**Fig 1**: Images of different parts of *Rhodiola rosea* plant

Due to the massive medicinal and cosmetic applications, global demand for *Rhodiola rosea* has increased over the years, resulting in overharvesting, which has become a significant concern, particularly in areas where the plant is collected in the wild. In regions such as the Altai Mountains, the Himalayas, and parts of Russia, unregulated harvesting practices have led to a decline in wild populations, threatening the plant's natural habitats and biodiversity. Several countries have initiated significant conservation efforts to promote sustainable collection practices to avoid damaging the plant's natural habitat. These efforts include cultivating *R. rosea* under controlled conditions, implementing stricter regulations on wild harvesting, and developing guidelines for the fair and sustainable use of natural resources [13,14].

Cultivation of *Rhodiola rosea* is gaining importance as an alternative to wild collection, especially in countries like China, Russia, and Canada, where research is focused on developing high-yield, high-quality plant varieties. Cultivation practices typically involve selecting suitable altitudes and climatic conditions that mimic the plant’s natural habitat, using organic farming techniques, and ensuring soil quality is optimized to maintain the concentration of active compounds in the roots. This controlled cultivation helps meet global demand and contributes to the conservation of wild populations [10].

While sustainable practices are necessary to prevent overexploitation, continued research and conservation efforts are essential to protect this valuable plant and ensure its availability for future generations.

**PHYTOCONSTITUENTS:**

*R. rosea* exhibits complex and rich phytochemistry that have been reported by researchers all over the globe. Till date around 120 compounds have been reported from the different parts of plant. These

phytoconstituents belongs to different classes of compounds such as phenylpropanoids, lignans, terpenoids, flavonoids and their glycosides and gallic acid derivatives. Compounds such as salidroside, rosavin, rosin and rhodiolosides are the common biomarkers found in *R. rosea* that are reported for many pharmacological activities. **Fig. 2** depicts the phytochemical profile of the *R. rosea.* In this review, we have discussed different classes of compounds present in the plant reported till date [15].

**Flavonoids and flavonoids glycosides**

Flavonoids are a large class of polyphenolic compounds which are characterized by their basic structural skeleton, the flavone nucleus. The basic skeleton of flavonoids consists of 15 carbon units arranged in a C6-C3C6 configuration, forming two aromatic rings (A and B) connected by a three-carbon bridge that usually forms a heterocyclic ring (C). Numbers of flavonoids have been reported form the *R. rosea.* These are the crucial class of secondary metabolites widely accepted for their several pharmacological activities. These secondary metabolites are further divided into subclasses, based on additional functional groups on the basic skeleton [16]. For example compounds of subclass flavonols (hydroxyl group at 3-C position) such as Kaempferol **1**, Herbacetin **2**, Kaempferol-3-*O*-α-L-rhammopyranoside **3**, Astragalin **4**, Kaempferol 7-*O*-α-L- rhamnopyranoside **5**, Rhodionin **6**, Rhodalin **7**, Herbacetin-8-glucoside **8**, Leucoside **9**,

Kaempferol-3-xylosylglucoside **10**, Quercetin **11** and Quercitrin **12** are reported from the different parts of *R. rosea* plant [16–18]. Another important class of flavonoids are flavones (3-hydroxy is absent), which also contribute significant number of molecules in *R. rosea*. Compounds such as Tricin **13,** tricin 5-*O*-glucoside **14** and tricin-7-*O*-β-D-glucoside **15** are the flavones reported from the *R. rosea*. Apart from this, Flavanones (saturated C ring) and flavanonoles like 5,7,3′,5′-tetrahydroxy-flavanone **16,** dihydrokaempferol **17** have been reported till date [4]. Further catachins like Epicatechin, epigallocatechin and epicatechin-3-*O*-gallate are also reported from the roots of *R. rosea* [19].

**Coumarins and their derivatives**

Coumarins are the benzopyrone structure, specifically a 1,2-benzopyrone. The basic building blocks of these compounds are a benzene ring (ring A) fused with a pyrone ring (a six-membered heterocyclic ring containing one oxygen atom and a carbonyl group at the 2-position). The lactone ring present in the structure is considered critical for chemical reactivity as well as for the biological activity. In our literature

studies we found only one coumarin reported from the *R. rosea*, that is crenulatin **18.** It is derivative of umbelliferon having substitution at position C-6 and C-7 [20].

**Cinnamaldehyde and Lignin derivatives**

Cinnamaldehyde consist of an aromatic ring and a propenal side chain that is made up of *α, β*-unsaturated aldehyde. Based upon the presence of oxygen at the terminal carbon these compounds are further divided into cinnamyl alcohol-type and cinnamic acid-type. Compounds like cinnamyl alcohol **19** and trans-cinnamic alcohol **20,** and their glycoside derivatives such as rosavin **21**, rosin **22** and rosarin **23** are reported from the plant [21]. Addition to this, cinnamyl alcohol such as 3-phenyl-2-propenyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside **24,** and (2E)-3-phenyl-2-propen-1-yl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside **25** are also found in the plant**.** Apart from this**,** triandrin **26**, vimalin **27,** sachaliside 1 **28**, (2E)-3-(4-methoxyphenyl)-2-propen-1-yl β-D-glucopyranoside **29**, and (2E)-3-(4-methoxyphenyl)-2-propen-1-yl
6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside **30.** *p*-Coumaric acid **31**, trans-*p*-hydroxycinnamic acid **32**, caffeic acid **33** and trans-caffeic acid **34,** are the derivatives of cinnamic acid found in the plant. Lignins are the complex, high-molecular-weight polymers of Cinnamaldehyde found in the cell walls of plants. The basic skelton of tses compounds are made up of three primary monolignols which are *p*-coumaryl alcohol (no methoxy group), coniferyl alcohol (one methoxy) and sinapyl alcohol (two methoxy). These compound provides rigidity and strength to the plant cell wall and protect against the pathogen. Where different lignin are found in the other species of Rhodiola, till date no lignin has been reported from the *R. rosea* [22].

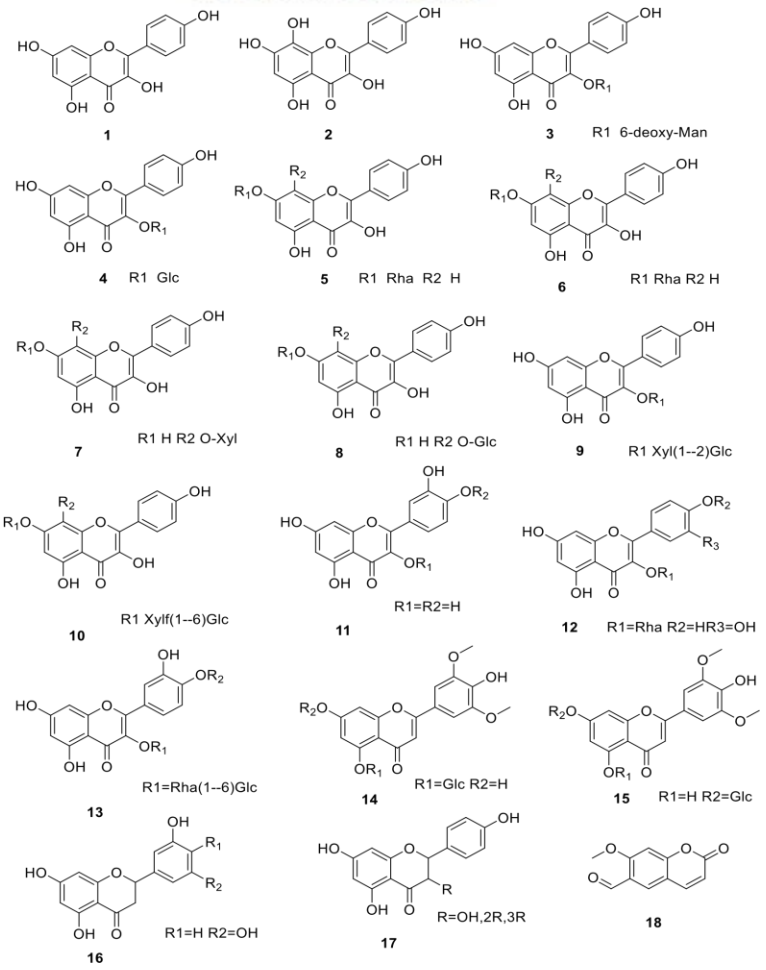


**Fig. 2:** Chemical structures of the phytoconstituents present in *Rhodiola rosea* Cont.

**Gallic acid derivatives**

Gallic acid derivatives are known for their strong antioxidant activity. Gallic acid **35** is the most abundant form of this class found in *R. rosea* plant. Till date five gallic acid derivative were reported form this plant which includes methyl gallate **36**, 1,2,6-tri-*O*-galloyl-β-D- glucoside **37**, 1,2,3,6-tetra-*O*-galloyl-β-D-glucopyranose **38**,

1,2,3,6-tetra-*O*-galloyl- 4-*O*-p-hydroxybenzoyl-β-D-glucopyranoside **39,** and 6-*O*-galloylsalidroside **40.** These naturally occurring polyphenol compounds, has a basic skeleton consisting of a six-membered benzene ring decorated with three hydroxyl groups at the 3, 4, and 5 positions, and a carboxylic acid group at the 1 position. A large variety of gallic acid derivatives are formed by modifying their hydroxyl,

carboxylic and even aromatic ring resulting in the wide and potent chemical properties and biological activities [23].

**Phenyl ethane derivatives**

This is one of the chief class of secondary metabolites of *R. rosea.* Phenylethylglycosides named Phenethanol βvicianoside **41** and 2-phenylethyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside **42** have been isolated from the *R. rosea.* Two of the major studied compounds of this class are tyrosol **43** and salidroside **44.** These both compounds have been studied for various pharmacological activity and can be marked as biomarker of this plant. These compounds exhibit a pleasant fragrance of flowers. 4-hydroxypheny-2-ethyl β-D-glucopyranoside2-(4-methoxyphenyl)-1-ethanol **45**, icariside D2 **46**, viridoside **47**, and mongrhoside **48** are other tyrosol derivative isolated from the plant *R. rosea.* Apart from these, several other phytoconstituents have also been reported from the plant, which can be found in the comprehensive literature [4,16,20]. **Fig. 2** shows the chemical structure of major compounds found in the *Rhodiola rosea*.

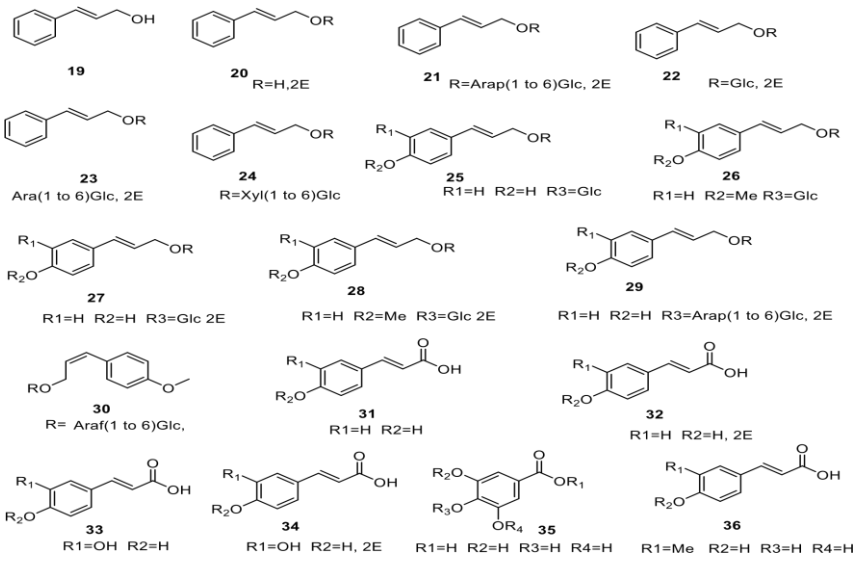


**Fig. 2:** Chemical structures of the phytoconstituents present in *Rhodiola rosea* Cont.

**COSMETIC USES AND PHARMACOLOGICAL ACTIVITIES:**

The rich phytochemical profile, particularly its roots, leaves, and stems, offers a wide range of benefits in skin health and cosmetic applications. Various parts of the *Rhodiola rosea* plant are utilized in cosmeceutical formulations due to their unique bioactive compounds that provide protective, rejuvenating, and healing effects to the skin [7].

**Roots: Antioxidants and skin protectants**

The roots of *Rhodiola rosea* are the most widely used part of the plant in cosmetics, owing to their high concentration of phenolic compounds such as rosavin, salidroside, and tyrosol. These compounds are wellknown for their potent antioxidant properties, which help neutralize free radicals and protect the skin from oxidative stress caused by environmental factors like UV radiation and pollution. This antioxidant activity reduces the damage to collagen and elastin fibers, thereby preventing premature aging and maintaining skin elasticity and firmness [24].

1. **Anti-aging formulations:** Root extracts can be utilized in anti-aging creams, serums, and lotions. The presence of rosavin and salidroside helps to enhance skin elasticity, reduce the appearance of fine lines and wrinkles, and promote a youthful glow. These compounds stimulate the production of fibroblasts, which are responsible for collagen synthesis, thereby improving skin structure and reducing signs of aging [25].

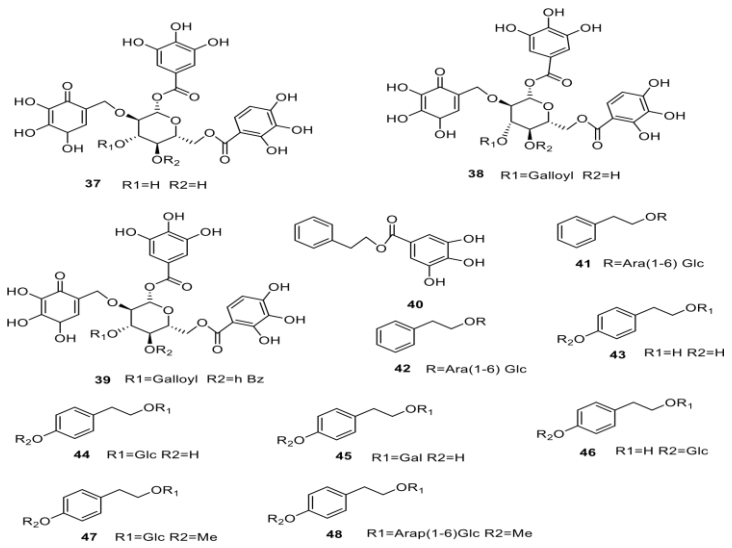


**Fig. 2:** Chemical structures of the phytoconstituents present in *Rhodiola rosea* cont.

2. **Moisturizers and hydrating products:** the root extracts also contain gallic acid derivatives and flavonoids that exhibit astringent properties, helping to tighten pores and improve skin texture. Additionally, these compounds' moisturizing effects help retain skin hydration, making *Rhodiola rosea* a valuable ingredient in hydrating products designed for dry and sensitive skin types [26].

3. **Sun protection products:** *Rhodiola rosea* root is a well-accepted adaptogen described in the many traditional systems of medicines. It has the ability to reduce stress, and protect the skin against harsh conditions. Plant extract has been studied for anti UV properties. It provides an additional layer of protection against UVinduced damage, reducing erythema (skin redness) and photodamage [27].

4. **Brightening and skin tone enhancement:** The root extracts are also known to help in skin brightening by reducing melanin production and fading hyperpigmentation, by fighting the reactive oxygen species (ROS). This makes them ideal for use in products targeting pigmentation disorders or to achieve a more even complexion [28].

**Leaves: soothing, anti-inflammatory, and healing properties**

As mentioned in the previous sections, the leaves of *Rhodiola rosea* are rich in flavonoids, gallic acid derivates, and various polyphenols that provide significant benefits for skin health. These bioactive compounds exhibit strong anti-inflammatory and soothing properties, which are crucial in managing skin conditions characterized by redness, irritation, and inflammation [29].

1. **Soothing creams and lotions:** leaf extracts are frequently used in formulations aimed at calming irritated skin, reducing redness, and soothing inflammation. Their anti-inflammatory action helps alleviate symptoms of conditions like eczema, rosacea, and dermatitis, making them suitable for use in creams and lotions designed for sensitive or reactive skin [23].

2. **Acne treatment products:** The leaves also contain compounds with mild antibacterial properties that can help manage acne. They reduce inflammation around acne lesions and prevent bacterial colonization on the skin, reducing the frequency and severity of breakouts [29].

3. **Wound healing and repair creams:** Due to bioactive compounds that promote tissue regeneration, leaf extracts are used in wound healing and skin repair products. They help accelerate the skin's natural healing process, making them effective in formulations for post-surgery or post-treatment recovery creams, particularly after dermatological procedures like chemical peels, microdermabrasion, or laser therapy [30]. **Fig. 3** shows the cosmetic benefits of the phytochemicals and extract of the plant.

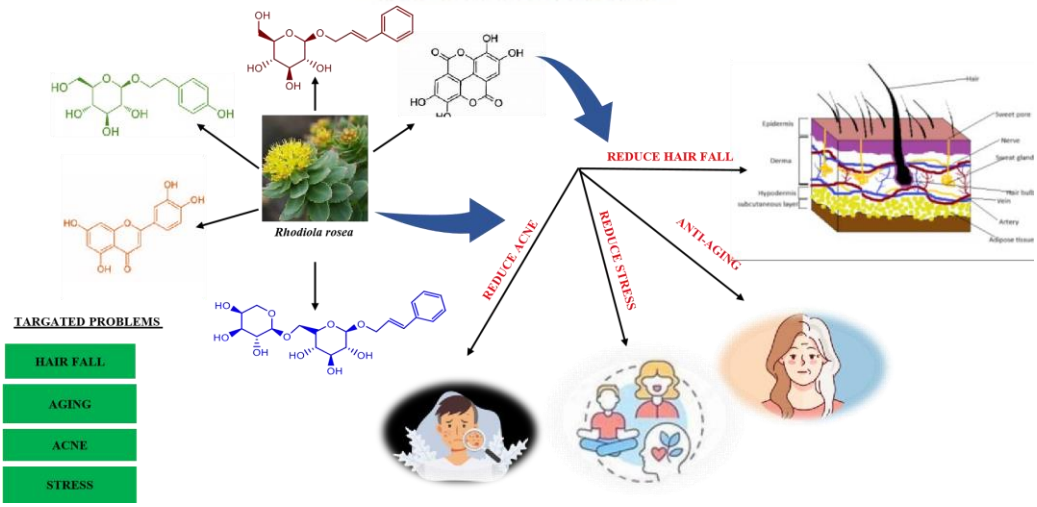


**Fig. 3**: Cosmetic uses of *Rhodiola rosea* plant and its chemical compounds

**Flowers: Aromatic and antimicrobial applications**

Although the flowers of *Rhodiola rosea* are less commonly used in cosmetics, they do have potential applications due to their aromatic properties and mild antimicrobial effects. The flowers contain volatile compounds that can be used to impart a natural fragrance to cosmetic products. Additionally, the antimicrobial properties of these compounds can contribute to formulations designed to maintain skin hygiene and prevent infections [31].

**Antioxidant activity**

Compounds such as tyrosol, salidroside, gallic acid, and a range of flavonoids serve as the key components responsible for the antioxidant properties of *Rhodiola rosea* plant. Water extract of the plant have been studied for its antioxidant activity in various cell lines such as NCTC 2544 cells, IMR-32 cells, and mouse C2C12 myotubes. Results of the study indicated that protective effects of extract against oxidative stress could be due to a pro-oxidant hormetic mechanism. In C2C12 myotubes, the antioxidant effect of the *R. rosea* extract was associated with the modulation of the molecular chaperone HSP70. The extract showed no potential activity in the rest of the cell lines [32].

**Antidiabetic activity**

Different ethanol extracts (12%) in water were evaluated for their α-amylase, α-glucosidase, and ACE inhibitory activities. Extract with the highest tyrosol content inhibited the α-glucosidase and ACE at maximum percentage. This indicates the dose-dependent inhibition of tyrosol against these two enzymes.

The study further reported that extracts containing the highest percentage of gallic acid and coumaric acid exhibit higher inhibition against the α-amylase enzyme. The author reports that these studies indicate that inhibition of different therapeutic targets depends upon the presence of phytochemical compounds in an extract. Another study showed that 95% ethanol extract can lessen the glucose generation in HepG2 cell lines. Another research reported that in diabetic rats induced by streptozotocin (STZ), a 70% plant ethanol extract could reduce formalin-induced hyperalgesia, indicating its potential use in treating diabetic hyperalgesia. It is evident from the several animal-based studies that *R. rosea* bears the potential antidiabetic activity [33,34].

**Immunomodulatory activity**

*R. rosea* has been widely explored for its immunity-enhancing properties. As a natural adaptogen, the plant is well-acknowledged as an immunomodulator. According to a study, 50 % ethanol extract stimulated the production of splenic lymphocytes in mice. Another study showed that a 70% ethanol extract from the plant could inhibit apoptosis of thymus T-lymphocytes and elevate Th1 cytokine levels by downregulating TIPE2. Extract of the *R. rosea* and one of its major components, salidroside, has also been reported to increase the section of interleukin-2 (Th-1), interferon γ, and Th-2 in mice models in a dose-dependent manner [35].

**Antifatigue activity**

Scientific studies have provided robust evidence for these antifatigue properties. Research has shown that extracts of *R. rosea* can significantly increase physical performance and reduce mental fatigue by modulating key pathways associated with energy metabolism and oxidative stress. For example, salidroside, one of the major active constituents, has been shown to enhance mitochondrial ATP production, thereby increasing cellular energy availability. Studies conducted on animal models have demonstrated that administration of *R. rosea* extract leads to increased endurance capacity, as evidenced by prolonged swimming times in mice subjected to forced swimming tests. This endurance enhancement is believed to be due to the plant's ability to reduce lactate levels and serum urea nitrogen, markers commonly associated with muscle fatigue. Moreover, the antioxidant properties of *R. rosea* play a crucial role in its antifatigue activity[36].

**Anti-inflammatory activity**

Inflammation is a complex biological response involving multiple signaling pathways and mediators such as cytokines, chemokines, and inflammatory enzymes. *R. rosea* extracts have been shown to exert antiinflammatory effects by modulating these key pathways. Salidroside, in particular, has been identified as a potent anti-inflammatory agent. It has been shown to inhibit the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, which are involved in the pathogenesis of various inflammatory diseases. Studies on macrophages have demonstrated that salidroside suppresses the activation of NF-κB, a critical transcription factor that regulates the expression of inflammatory genes. Furthermore, it has been observed that *R. rosea* extracts can downregulate the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), which are key enzymes in the inflammatory response. Additionally, rosavins have been reported to exhibit significant anti-inflammatory effects by attenuating oxidative stress and reducing the activation of inflammatory pathways. Experimental studies have shown that rosavin can inhibit the activation of MAPK (mitogen-activated protein kinase) signalling pathways, which are crucial in the inflammatory response cascade[37].

**ADVERSE EFFECTS**:

While *Rhodiola rosea* is widely recognized for its beneficial properties, the literature also indicates that its consumption may be associated with certain adverse effects, particularly when taken in high doses or over extended periods. Although *R. rosea* is generally considered safe for most users, a few studies have reported mild to moderate side effects linked to its use. Some of the most commonly reported adverse effects of *R. rosea* and its compounds are gastrointestinal-related adverse effects. These effects include symptoms like nausea, dry mouth, and stomach upset. In clinical trials, these gastrointestinal disturbances were generally mild and selflimiting, occurring more frequently at higher doses of the plant extract. The symptoms resolve quickly upon discontinuation or reduction of the dose, indicating a dose-dependent response [38]. Adverse effects related to the central nervous system have also been noted, though they are relatively rare. Some users have reported symptoms such as dizziness, restlessness, agitation, or difficulty sleeping, which may be attributed to the stimulating properties of *R. rosea*. These effects are believed to arise from the modulation of neurotransmitter systems, particularly dopamine and serotonin, which play a role in mood regulation and alertness. However, these symptoms are typically mild and diminish with continued use or dose adjustment [37].

Although uncommon, allergic reactions to *R. rosea* have been reported in some cases. These may present as skin rashes, itching, or swelling, and are thought to result from hypersensitivity to one or more of the plant's components. There is limited evidence in the literature on the prevalence or severity of such allergic reactions, but individuals with known allergies to plants in the Crassulaceae family should exercise caution when using *R. rosea* extracts [40]. Evidence suggests that *R. rosea* may influence hormonal balance, particularly concerning the thyroid gland. Animal studies have indicated that high doses of *R. rosea* extracts may affect thyroid hormone levels, potentially leading to hyperthyroid-like symptoms such as palpitations, anxiety, and weight loss. While these effects have not been widely documented in human studies, individuals with thyroid disorders should be cautious and consult a healthcare provider before using *R. rosea* supplements [39].

Overall, while *Rhodiola rosea* is generally considered safe for most individuals, it is not without potential adverse effects. These effects are typically mild and dose-dependent, but they warrant attention, especially for individuals with pre-existing health conditions, those taking medications, or those using the herb in high doses over long periods.

**MARKETED FORMULATION**:

A number of *Rhodiola rosea* root extracts have been marked mostly for immunomodulatory and adaptogenic benefits. Cosmetic formulations available in the market are given in the **fig. 4.**

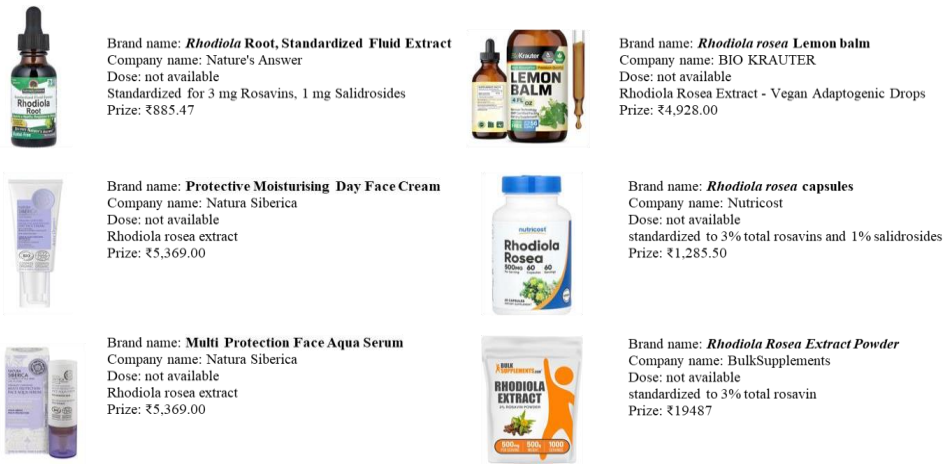


**Fig. 4:** Marketed formulations of the plant *Rhodiola rosea*

## CONCLUSION:

*Rhodiola rosea*, a renowned adaptogen, has emerged as a promising candidate for cosmeceutical applications, owing to its multi-therapeutic properties and rich phytochemical profile. This review has explored the extensive scientific literature on *R. rosea*, highlighting its antifatigue, anti-inflammatory, and antioxidant activities, particularly relevant for cosmetic and dermatological use. The plant's bioactive constituents, including salidroside, rosavin, tyrosol, and various flavonoids, play a crucial role in its efficacy. Furthermore, *R. rosea* exhibits notable antioxidant, anti-inflammatory, antistress, and adaptogenic properties, which can be harnessed in cosmetic formulations aimed at revitalizing and rejuvenating the skin. The plant's ability to enhance cellular energy production and reduce mental and physical exhaustion complements its use in products that promote a more vibrant youthfulness.

Altogether, *Rhodiola rosea* holds considerable promise as a cosmeceutical ingredient due to its extensive range of therapeutic properties. Its ability to combat oxidative stress, enhance energy levels, and reduce inflammation positions it as a valuable asset in skincare and cosmetic formulations. Continued research and clinical trials will be crucial in optimizing its use and ensuring its safety and efficacy in cosmeceutical applications. As the demand for natural and effective skincare solutions grows, *R. rosea* offers a compelling option for developing advanced and beneficial cosmeceutical products.

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# Moth Bean: An Untapped Potential Medico-Therapeutic Plant in Need of Policy Support

**Kumud gaur and Kamakshi**
[1]*Department of Biotechnology, Science and Humanities, SRM Institute of Science and Technology Delhi NCR Campus Modinagar Ghaziabad*
[1*]*Department of Biology, Science and Humanities, Faculty of Engineering and Technology, SRM Institute of Science and Technology Delhi NCR Campus Modinagar Ghaziabad*
*E-mail: Kg8057@srmist.edu.in,[1*] kamakshr@srmist.edu.in*
***Corresponding Author:** kamakshr@srmist.edu.in*

## Abstract

The moth bean (Vigna aconitifolia (Jacq.) Marechal), a neglected and underutilised legume crop (NUCL), has significant therapeutic potential due to its high protein content, amino acids, unsaturated fats, minerals, and vitamins. Legumes are used not just as food but also as fodder. Because of its many variants, India is a valuable plant genetic resource (PGR) for traditional remedies and a promising candidate for new and modern pharmaceutical applications like the antibacterial drug Vicilin. In preparation for potential larger applications, this abstract investigates the morphology of moth beans under saline stress and their diverse therapeutic qualities. As the plant endures environmental dangers and climate change, the moth's high genetic diversity and exceptional thermo-drought resistance are highly beneficial for the production of phytochemicals.

Additionally them from, converting NULC to agro-beneficial crops will support local farmers, researchers, and legislators across the globe. Therefore, there is a great need to involve community awareness, legislative support and research support for phytochemical screening and validation. Crops with inherent adaptive characteristics are more tolerant to biotic and abiotic stresses (salinity stresses).

**Keywords:** Abiotic Stress, Salinity, Moth bean, Neglected and underutilized crops, Therapeutics.

## 1. INTRODUCTION

There are many legumes native to arid and semi-arid regions of India and Pakistan, including moth beans (*Vigna aconitifolia* (Jacq.) Marechal) (Gayacharan et al., 2023; Vijendra et al., 2016). Despite its resilience to harsh environmental conditions and nutritional and therapeutic potential, moth bean remains a neglected and underutilized legume crop (NUCL). The recognition of its nutritional and medicinal properties highlights the need for renewed focus and policy support to enhance its cultivation and utilization(Singh et al., 2022). In addition to being an excellent source of protein, essential amino acids, unsaturated fats, vitamins, and minerals, moth beans are also a good source of fibre. It is known for its high nutritional value and is utilized as Food and fodder. Its potential medicinal properties, including antibacterial effects

attributed to compounds like Vicilin, make it a promising candidate for pharmaceutical applications (Tiwari, Kalim, Bangar, et al., 2018).

Moreover, the moth bean's genetic diversity and adaptability to adverse environmental conditions, such as salinity stress, underscore its importance as a resilient crop in climate change. Moth bean is celebrated for its high protein content, making it a vital food source in regions where protein deficiency is common. It also contains essential amino acids for human health (Tiwari, Kalim, Bangar, et al., 2018; Tiwari, Kalim, Tyagi, et al., 2018). This Food's nutritional value is enhanced by the presence of unsaturated fats, which are good for cardiovascular health, as well as a variety of vitamins (including B-complex vitamins) and minerals (including iron, calcium, and phosphorus).

### 1.1 Effect of Salinity Stress on Morphology

**Morphological Changes:** Abiotic stresses such as salinity severely affect plant growth and productivity. Moth bean, however, exhibits remarkable tolerance to salinity stress. In salinity stress conditions, plant height is reduced, leaf size is reduced, and root growth is stunted, as shown in Figure 1. Despite these changes, the moth bean maintains its overall growth and productivity better than many other crops.

**Adaptive Mechanisms:** The adaptive mechanisms of moth beans to salinity stress include osmotic adjustment, ion homeostasis, and the activation of antioxidant defence systems. These mechanisms enable the moth bean to survive and produce under adverse conditions, making it a resilient crop suitable for cultivation in saline and arid regions.

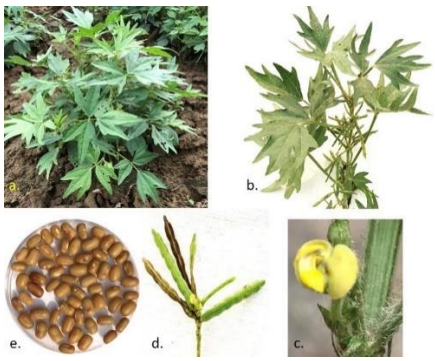


Figure 1: Morphological Key Features of Moth Bean.

### 1.2 Need of Policy Support for Moth Bean

Moth bean (*Vigna aconitifolia*) holds promise as a plant species with significant medico-therapeutic potential, yet it remains largely unexplored in mainstream healthcare and pharmaceutical research. This

underutilized legume, traditionally cultivated in arid and semi-arid regions, possesses bioactive compounds and nutritional attributes that could be harnessed for various health benefits. However, realizing its full potential requires dedicated policy support and strategic initiatives. Firstly, policy support is crucial for promoting research and development into the medicinal properties of moth bean. Government funding and incentives can encourage scientists and researchers to conduct comprehensive studies on its bioactive components, pharmacological activities, and potential therapeutic applications. This support would facilitate the exploration of moth bean's efficacy in treating specific diseases and health conditions, thereby contributing to the diversification of therapeutic options in healthcare. Secondly, policy frameworks are needed to facilitate the integration of moth bean into mainstream medicine and healthcare systems. It includes establishing regulatory guidelines for producing, processing, and marketing moth bean-based products, ensuring their safety, efficacy, and quality standards. By creating a conducive regulatory environment, policymakers can promote the commercialization of moth bean-derived pharmaceuticals, nutraceuticals, and functional foods, thereby enhancing consumer access to its health benefits.

Moreover, policy support can drive initiatives to promote sustainable cultivation practices of moth beans. It includes incentivizing farmers to adopt organic farming methods, conservation practices, and biodiversity-friendly approaches. Such initiatives support environmental sustainability and ensure a consistent and high-quality supply of moth beans for medicinal and therapeutic purposes.

## 2. LITERATURE REVIEW

A member of the Fabaceae family, the moth bean (*Vigna aconitifolia* L.) is also known as mat bean, matki bean, mout bean, dew gram, or Turkish gram. In India, it is grown for its pods and seeds, both of which are immature. Moth beans are an important food source, especially in developing nations, because of their nutritional and high protein content (Adsule, 1996; Bravo et al., 1998; Kadam et al., 1985).

There are several species of moth bean in the Fabaceae family and the genus Vigna, such as mung beans, black grams, cowpeas, and moth beans. Despite its nutritional benefits, the edible species *Vigna aconitifolia* is underutilized (Takahashi et al., 2016). Due to its favourable climate, it thrives in tropical climates and can be cultivated in infertile soil, making it a staple in Southeast Asia, particularly in Rajasthan, India. Predominantly a Kharif crop, moth bean is grown during the monsoon season from June

to November. The plant is known as dew beans, haricot mats, mat beans, moth grams, matkis, and Turkish grams. Food, fodder, feed, and green manure are all offered by moth bean, which is also an economical protein source (Sedani et al., 2021).

Many abiotic stresses such as heat, cold, moisture, and salinity severely affect crop production worldwide. Plant productivity and yield are negatively affected by morphological, physiological, biochemical, and molecular changes caused by abiotic stresses (Naya et al., 2007). Global warming predictions predict temperatures will rise 2–6°C by 2100 (Peck & Teisberg, 1992). Heat stress damages plant cell membranes through reactive oxygen species and oxidative stress. Growing, developing, and yielding of plants are all affected by temperature ((Mittler et al., 2012). Plants must, therefore, be able to adapt to extreme conditions in order to survive. The agricultural industry should select stress-tolerant varieties (Mahajan & Tuteja, 2005).

## 3. METHODOLOGY

**Sample Collection:** This study used Agro-climatic regions in India to collect different moth bean genotypes. These genotypes were cultivated under controlled conditions to ensure uniformity in growth parameters.

**Nutritional Analysis:** Nutritional analysis of moth bean seeds was conducted using standard procedures. The Kjeldahl method was used to measure protein content, and HPLC was used to measure amino acid composition. A GC-MS analysis was performed to determine the composition of lipids, including unsaturated fats. Besides spectrophotometric methods, atomic absorption spectroscopy (AAS) was used to quantify vitamin and mineral contents.

**Phytochemical Screening:** Phytochemical screening was done to identify bioactive compounds in moth bean seeds. Methanolic extracts of the seeds were prepared and subjected to qualitative analysis for alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Quantitative analysis was performed using HPLC and GC-MS.

**Salinity Stress Experiment:** The effect of salinity stress on moth bean morphology was examined by growing plants under controlled conditions under various salinity levels (0, 50, 100, 150 mM NaCl). Morphological parameters such as plant height, leaf size, root length, and biomass were measured. Physiological responses, including osmotic adjustment and ion homeostasis, were also assessed.

**Statistical Analysis:** Data collected from nutritional and phytochemical analyses and salinity stress experiments were subjected to statistical analysis using SPSS software. Statistical analysis, an analysis of variance, and post hoc tests were performed to determine whether differences between treatments were significant.

## 4. RESULTS AND DISCUSSION

**Nutritional Analysis:** In analyzing the nutritional content of moth bean seeds, essential amino acids, unsaturated fats, vitamins, and minerals were found to be high. Table 1 shows the summary of the nutritional composition: According to the nutritional analysis, moth bean seeds contain a significant amount of protein (24-26%), essential amino acids (lysine, leucine, and valine), as well as unsaturated fats (linoleic and oleic acids). Vitamins B1, B2, B3, and B6 were also significant in the seeds, confirming their high nutritional value. The comparative analysis of nutritional factors and vitamins is shown in Table 2-3.

Table 1: Nutritional Composition of Moth Bean Seeds

| Nutrient | Amount per 100g |
|---|---|
| Protein | 24-26 g |
| Essential Amino Acids | (Lysine, Leucine, Valine) |
| Unsaturated Fats | (Linoleic Acid, Oleic Acid) |
| Vitamins (B1, B2, B3, B6) | Significant amounts |
| Minerals (Iron, Calcium, Phosphorus) | Significant amounts |

Table 2: Analysis of nutritional factors in moth bean seeds

| Component | (Tresina et al., 2017) | (Opara et al., 2017) | (Bhadkaria et al., 2021) |
|---|---|---|---|
| Saponin (mg 100g-1) | - | 0.65 | - |
| Total free phenolics (g 100g-1) | 1.46 | 0.15 | 0.05-1.03 |
| Flavonoid (g 100 g-1) | - | 0.13 | 0.25 |
| Tannins (g 100 g-1) | 0.65 | 2.89 | 0.13-0.30 |
| Phytic acid (g 100 g-1) | 0.42 | - | 1.74 |
| Trypsin inhibitor activity (TIU mg-1) | 28.30 | - | - |
| Oligosaccharides (g 100 g-1) | - | - | - |
| Raffinose | 0.54 | - | - |
| Stachyose | 1.68 | - | - |
| Verbascose | 1.26 | - | - |
| Phyto haemagglutinating activity* (HU mg-1 protein | - | - | 1.60-18.48 |
| Blood Group (A) | 32 | - | - |
| Blood Group (B) | 133 | - | - |
| Blood Group (o) | 18 | - | - |

Table 3: An analysis of the vitamin content of moth bean seeds

| Element (mg 100 g-1) | (Usda, 2019) | (Opara et al., 2017) | (Tresina et al., 2017) |
|---|---|---|---|
| Vitamin A | 32(IU) | 14.65 (IU) | - |
| Thiamin | 0.562 | 0.23 | - |
| Riboflavin | 0.091 | 0.45 | - |
| Niacin | 2.8 | 0.47 | 28.08 |
| Ascorbic | 4 | 42.25 | 59.10 |
| Vitamin E | - | 0.25 | - |
| Pantothenic acid | 1.54 | - | - |

**Phytochemical Screening:** Phytochemical screening identified several bioactive compounds in moth bean seeds. Phytochemical screening identified several bioactive compounds with medicinal properties. Vicilin exhibited antibacterial activity, and other compounds demonstrated antioxidant properties.

Vicilin, a major storage protein, strongly inhibits Gram-positive and Gram-negative bacteria. Other identified compounds, such as flavonoids and phenolic acids, demonstrated antioxidant properties, supporting the potential use of moth bean in managing oxidative stress-related conditions, as shown in Figure 4-5.

Table 4: Phytochemical Composition of Moth Bean Seeds

| Compound | Detected Presence | Activity |
|---|---|---|
| Vicilin | Present | Antibacterial |
| Flavonoids | Present | Antioxidant |
| Phenolic Acids | Present | Antioxidant |
| Saponins | Present | Antimicrobial, Anti-inflammatory |
| Tannins | Present | Antioxidant, Antimicrobial |

Table 5: Analyzing the fatty acid composition of moth bean seeds

| Fatty Acid (%) | (Tresina et al., 2017) | (Kamani et al., 2020) |
|---|---|---|
| Myristic acid (C14:0) | 02 | 0.21 |
| Palmitic acid (C16:0) | 16 | 23 |
| Palmitoleic acid (C16:1) | 09 | - |
| Stearic acid (C18:0) | 07 | - |
| Oleic acid (C18:1) | 18 | - |
| Linoleic acid (C18:2) | 22 | 31 |
| Linolenic acid (C18:3) | 20 | 19 |
| Eicosenoic acid (C20:1) | 04 | - |
| Saturated fatty acid | 26 | 27 |
| Polyunsaturated fatty acid | 74 | 50 |

**Salinity Stress Experiment:** Moth bean plants showed significant tolerance to salinity stress. Morphological changes were observed at higher salinity levels, such as reduced plant height, smaller leaf size, and stunted root growth, as shown in Table 6. However, the plants maintained overall growth and productivity. Physiological responses included increased osmotic adjustment by accumulating compatible solutes and efficient ion homeostasis, contributing to the plant's resilience under salinity stress. Moth beans were studied under different salinity levels to see how their morphology changed due to salinity stress. The results showed significant tolerance to salinity stress with observable morphological changes.

Table 6: Morphological Changes under Salinity Stress

| Salinity Level (mM NaCl) | Plant Height (cm) | Leaf Size ($cm^2$) | Root Length (cm) | Biomass (g) |
|---|---|---|---|---|
| 0 | 45 | 20 | 15 | 50 |
| 50 | 40 | 18 | 13 | 45 |
| 100 | 35 | 15 | 10 | 40 |
| 150 | 30 | 12 | 8 | 35 |

**Genetic Diversity and Resilience:** The genetic diversity among the collected moth bean genotypes was evident from the morphological and physiological variations observed, as shown in Table 7. Genotypes with superior thermo-drought tolerance and salinity resistance were identified, highlighting the potential for breeding programs to enhance these traits. The resilience of the moth bean to environmental stresses positions it as a viable crop for cultivation in regions affected by climate change. Genetic diversity was assessed by evaluating various moth bean genotypes' morphological and physiological traits. The results highlighted significant variability and resilience among genotypes, particularly in thermo-drought tolerance and salinity resistance.

Table 7: Genetic Diversity and Stress Tolerance

| Genotype | Thermo-Drought Tolerance | Salinity Resistance | Key Traits |
|---|---|---|---|
| Genotype 1 | High | High | Deep root system, efficient water use |
| Genotype 2 | Moderate | High | Heat-shock protein expression |
| Genotype 3 | High | Moderate | Osmotic adjustment |
| Genotype 4 | Low | Moderate | Antioxidant defense activation |

## 5. CONCLUSION

The moth bean, or *Vigna aconitifolia*, is a legume that remains underutilized despite its high nutritional and medicinal potential. Its high protein content, rich nutritional profile, and resilience to environmental

stresses make it a valuable crop for future agricultural and pharmaceutical applications. There is a pressing need for policy support, community awareness, and focused research efforts to unlock its full potential. In addition to enhancing food security and supporting sustainable agriculture, we can discover new therapeutic agents by cultivating and utilizing moth beans. Legislative and research initiatives will be critical for the moth bean to become a cornerstone of modern medicine and agro-beneficial practices.

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# SMART APPROACH FOR TRANSDERMAL DRUG DELIVERY SYSTEM LOADED WITH HYALURONIC ACID

**Kaushalesh Kumar Sahu1*, Mr. Pankaj Kumar1 and Dr Deepesh Lall2**
**School of Pharmacy, Chouksey Engineering Collage Bilaspur (C.G.)**

**Abstract**

It was discovered that puncturing human skin with micron-sized needles increased the permeability of the skin to a model drug, calcein. Microneedles are hollow or solid cannulae used to deliver drugs. Microneedle are strong and long enough to break through the barrier, but they are also short enough to not stimulate the nerves. These are painless and irritation free delivery. It is provided direct entry of drug through the skin layers. Accurate dosing, complex release pattern, local delivery, and improved biological drug stability. TDDS patch no fear of needle and ease of administration. Dissolving microneedle made of biodegradable materials including various polymers and sugars that have medicinal properties. Weigh all polymer as per requirement, that is HPMC: CMC (4:3) and dissolved in 5ml of distilled water. Add slowly powdered polymer in ascending order, apply continuous stirring. Apply heat to make homogenous mixture at temperature 60oC for 4 minutes with continuous stirring. Transdermal patches, which provide a reduced dose of the medicine at a predefined rate, have been created to increase clinical efficacy of the drug and patient compliance.

**Keyword: -** PVP, PVA, HPMC, CMC, Hyaluronic acid, TDDS Patches

## Introduction

A transdermal patch, also known as a skin patch, is an adhesive patch applied to the skin that contains medication that is intended to be absorbed into the bloodstream through the skin. This frequently encourages the healing of a body part that has been hurt. The regulated release of the medication into the patient is a benefit of transdermal drug administration over other forms, such as oral, topical, etc. However, the fact that the skin is a very effective barrier poses a challenge to development. Transdermal patches can administer a wide range of medications.

## Transdermal Drug Delivery Systems' Basic Components

1. **Polymer: -** The drug's release from the device is managed by the polymer. The following polymers could be advantageous for transdermal devices:

2. **Natural Polymer: -** For instance, gelatin, shellac, zein, proteins, gums and their derivatives, natural rubber, starch, and waxes are all examples of cellulose derivatives.
3. **synthetic Elastomers: -** Polybutadieine, Hydrin rubber, Polysiloxane, Silicone rubber, Nitrile, Acrylonitrile, Butyl rubber, Styrenebutadieine rubber, Neoprene etc.
4. **Synthetic Polymers:** - PA, PC, Polyethylene, Polypropylene, Polyacrylate, Polyamide, Polyurea, PVP, Polymethyl methacrylate, Epoxy etc.

i. **Drug: -** Drug The drug should be carefully chosen in order to construct a transdermal drug delivery system properly. The following are some characteristics of a medicine that are ideal for transdermal distribution.

**ii.** **Permeation Enhancer: -** These chemicals increase skin permeability by altering the skin's capacity to act as a barrier to the flux of a desired penetrant.

**TYPES OF TRANSDERMAL PATCHES**

**One layer medication in adhesive: -** In this form, the medicine is included in the sticky layer. The adhesive layer is in charge of delivering the medicine onto the skin in addition to holding the other layers together. There is a backer and a temporary liner around the adhesive layer.

**Multilayer drug in adhesives: -** This type is also similar to the single layer but it contains a immediate drug release layer and other layer will be a controlled release along with the adhesive layer. The adhesive layer is responsible for the releasing of the drug. This patch also has a temporary liner-layer and a permanent backing.

**Drug in adhesive reservoir: -** This technique involves sandwiching a membrane that regulates flow rate between an impervious backing layer and a drug reservoir. The medicine is only released through the rate-regulating membrane, which may or may not be microporous. The medication may be in a solid polymer matrix, a solution, suspension, gel, or another form in the drug reservoir compartment. It is possible to use a polymeric membrane with a hypoallergenic and drug-compatible outside surface.

**Drug in adhesives matrix: -** The addition of a semisolid matrix containing a medication solution or suspension that is in direct contact with the release liner characterizes the Matrix system design. The element causing skin adherence is built into an overlay and arranges itself in a concentric pattern all around the semisolid matrix.

## Methodology or Materials and Methods

### Materials

Ethyl cellulose, Polyvinyl pyrrolidone (PVP K-30), Polyvinyl alcohol (PVP), Dibutyl phthalate (DBP) and dibutyl sebacate (DBS), Linseed oil, L-menthol, Resin, hydrates, Polylactic acid, HPMC, PEGDMA and all the other chemical of analytical reagent grade.

### Method of Preparation

#### a) Preparation of master mold

MN's mold was fabricated containing 340um x 340um x 300um (LxWxH) wider, length and height and had 640 um center-to-center spacing the mold cavities (MNs holes) were prepared by mechanical method using needle top were pork into the mould surface. Standard mould was fabricated using materials (resin and hydrate) by hand rolling method. Prepared mould was placed for 24 hours under room temperature.

#### b) Preparation of polymeric microneedle arrays (MN's)

The natural and biodegrable polymeric solution used to fabricate the microneedles transdermal patch. The polymer solution of polyvinyl alcohol (PVA) about 20% w/v was dissolved in purified water at 90°C at a ratio of 0.80gm PVA per 1ml of purified water, stirred using magnetic stirrer for 20 minutes. Once the smooth consistency of polymer solution was prepared, allow the solution to pour in to the microneedles standard mould. Then standard mould containing polymer solutions were attached under centrifuges apparatus and allow immediately centrifuged at 1500 rpm for 15 minutes for even and uniform distribution of the solution in to the mould. After the centrifugation mould should be removed carefully and allow it to stand for 24-48 hours for complete drying of the microneedles. To take out microneedles from the mould, it kept under the freezer for 30 minutes at 4-5°C for easy removal of microneedle (MNs).

#### c) Fabrication of Transdermal Patches

Transdermal patches were fabricated using established fabrication technique with some modifications. Prepared 10% polymer solution, 0.75gm PVP and PVA dissolved per 1ml of purified water. 1gm of dextran per 1ml of PVP/PVA solution was mixed. This Prepared polymer solution were poured in

microneedle mould arrays [20-26]. It kept for 12 hours for completely dried and removed microneedle (MN's) arrays with supported backing layer of 10% polymer backing layer.

**Characterization of Microneedle Transdermal Patch**

**a) Scanning electron microscopic (SEM) analysis**

Prepared microneedle array was investigated for magnification, tilt degree, width, spots and other imaging characteristics on SEM images. Microneedle arrays were mounted on the disc and morphological characteristic feature scanned in scanning electron microscope (SEM) in highvacuum mode, attached ETD detector at 10-5 Torr and 15 kV, model (FEI Quanta TM ESEM, QUanta 200 FEG; FEI, Oregon).

**b) Differential scanning calorimetry (DSC) analysis**

The sample of microneedles were investigater under differential scanning calorimetry (DSC) system model (Netzsch 204 F1 Phoenix®; Geratebau GmbH, Bavaria). Microneedles sample were heated at a lenear heating rate of about 10ºC/minutes from 25 ºC to 250 ºC, generated graph and report were analysed with Netzsch-compatible software, parameter such as, melting peak, delta Cp of microneedle arrays.

**c) Measurement of mechanical strength and swelling index**

Quantitative and qualitative assessment of microneedle was observed included the experimental variability artificial skin were designed related to anatomical variation as the human skin structure, using polymer (CMC/dextran) thin film fabricated using 40:2 ratio. In contrast microneedle arrays with different geometrics delivered. On the other hand, insertion force was approximately constant, enabling deeper and more reproducible insertion with greater proportion pores.

**d) Measurement of dissolution efficient of microneedle arrays**

In this measurement, the application of microneedle were investigated, including the type of bioactive cargo to be dissolution time release, dissolution pH specific, dissolution by-products and estimated mechanical strength. In additions, we demonstrated six type of microneedle from different type of polymer together. We created microneedle from, (i) Carboxymethyl cellulose (CMC); (ii) Polyvinyl pyrrolidone (PVP); (iii) CMC/PVP at 60:40 dry weight ratio; (iv) PVP/PVA 20:10 at dry weight ratio; (v) CMC/dextran at 50/50 dry weight ratio; (vi) PVP/HPMC at 60:40 dry weight ratio. Microneedles from all

the seven geometric polymers were fabricated and sample successfully dissolves in particular solvent for specific time and 5.4-7.2 pH buffers to demonstrate the dissolution efficient.

**Result & Discussion**

TDDS patch is a new modern drug delivery technique through skin. These drug administration techniques cause no discomfort. Microneedle are strong and long enough to break through the barrier, but they are also short enough to not stimulate the nerves. These are painless and irritation free delivery. It is provided direct entry of drug through the skin layers. Accurate dosing, complex release pattern, local delivery, and improved biological drug stability. TDDS patch no fear of needle and ease of administration. Dissolving microneedle made of biodegradable materials including various polymers and sugars that have medicinal properties. The transdermal patch, also known as a skin patch, is an adhesive applied to the skin that contains medication that is intended to be absorbed into the bloodstream through the skin.

**Requirement: -**

- **Chemical: -** Hydroxypropyl methylcellulose, Carboxymethyl cellulose, Distilled water (PEG – 400) Elastomers.
- **Drug** – Hyaluronic Acid
- **Apparatus** – Beaker 250ml, Spatula, Glass rod, Glass Petridis, Electric weighing balance.

**Procedure: -**

- Preparation of Polymer Solution

i. Weigh all polymer as per requirement, that is HPMC: CMC (4:3) and dissolved in 5ml of distilled water.
ii. Add slowly powdered polymer in ascending order, apply continuous stirring.
iii. Apply heat to make homogenous mixture at temperature 60oC for 4 minutes with continuous stirring.

- **Preparation of Microneedle Mold**

i. Mix hydrate mixture (pistil) in appropriate quantity and make soluble as per requirement.
ii. With the help of needle puncture as (4x6) area.
iii. Keep it on place (dry place) for 24 to 48 hours.

- **Preparation of Microneedle and Loaded Hyaluronic Acid**

i. Clean the mold, with the help of H2O2 and cotton body.
ii. Add slowly the polymer solution on surface of mold.
iii. After dropping on the surface, hammer continuously to help easily penetration in the holes of molds.
iv. Once a layer formed on mold slop dropping and cleans the surface.
v. Place this mold at safe and open environment for drying the microneedles.
vi. Remove slowly microneedle from the mold.

**Evolution of Transdermal patches**

Transdermal dosage form development is a challenging process that requires substantial investigation. Transdermal patches, which provide a reduced dose of the medicine at a predefined rate, have been created to increase clinical efficacy of the drug and patient compliance. In order to guarantee their expected performance and reproducibility under the stipulated environmental circumstances, evaluation studies are now even more crucial. These researches, which can be categorized under the following groups, are prescient of transdermal dosing forms:

**Physiochemical Evolution**

- **Thickness: -** At various spots along the transdermal film, the thickness is measured using a travelling microscope, dial gauge, screw gauge, or micrometer.
- **Uniformity of Weight: -**By individually weighing 10 randomly chosen patches and figuring out the average weight, weight variation is explored. The weight of a person shouldn't differ noticeably from the average weight.
- **Drug Content Determination: -** In a shaker incubator, a precisely measured quantity of film (about 100 mg) is dissolved in 100 mL of a suitable solvent in which the medication is soluble. The solution is then agitated continuously for 24 hours. The entire solution is then sonicated after that. Following sonication and filtering, the amount of medication in the solution is determined spectrophotometrically by the proper dilution.

- **Moisture Content: -** The produced films are weighed separately and maintained at room temperature in desiccators with calcium chloride for 24 hours. The films are weighed once more after a certain period of time until they display a steady weight.
- **Interaction Studies: -**The compatibility of the medicine with the excipients is one of the elements that affect a formulation's stability. To create a stable product, the drug and excipients must be compatible with one another. As a result, it is essential to identify any potential physical or chemical interactions because they may impair the bioavailability and stability of the medication. The compatibility studies are crucial for formulating new excipients that have never been used in formulations containing the active ingredient. By contrasting their physicochemical properties, such as assay, melting endotherms, distinctive wave numbers, absorption maxima, etc., interaction studies are frequently conducted in thermal analysis, FT-IR, UV, and chromatographic procedures.
- **Shear Adhesion Test: -**This test determines the cohesive strength of an adhesive polymer. The level of cross-linking, the molecular weight, the make-up of the polymer, and the quantity of tackifiers used can all have an impact on the strength value. A stainless-steel plate is used to stack an adhesive-coated patch, with a specific weight suspended from the patch parallel to the plate. The cohesive strength is determined by how long it takes to remove the patch from the plate. The shear strength increases as the amount of time increases.
- **Peel adhesion test: -**Adhesion is the measurement of the patch strength between an adhesive and a substrate. The amount of force necessary to remove the adhesive coating from the steel test substrate. The composition of polymers as well as the kind and quantity of polymer molecular weight determine the adhesive capabilities. The one patch adheres to the test substrate (steel), and it is being dragged away from the substrate at an angle of 1800 degrees. Absence of residue on the test substrate suggests that the adhesive failed.
- **Tack properties: -** Tack, which is a polymer's ability to stick to a surface with little force, is crucial in transdermal systems that require little force to apply. The molecular weight, content, and testifying resins used in the polymer all affect the tack.
- **Probe tack test: -**In this, an adhesive is brought into touch with a probe tip with a specified level of surface roughness. Once a bond is formed between the adhesive and probe, the probe is removed at a set rate away from the glue, breaking the bond. Tack, which is measured in grammes, is the amount of force needed to separate the bond.

- **Thumb tack test: -**By pressing the thumb into the adhesive, the results of this subjective test are evaluated. Using the test requires prior testing experience.
- **Rolling ball tack test: -** To conduct this test, a stainless steel is moved along the adhesive's upper face, and the distance it travels is measured. Ball is launched on inclined track at an angle of 22.50 and has a 7/16" diameter. Less sticky polymer is seen at longer distances. The tackiness of the polymer is determined by measuring the ball's travel distance in inches. The sticky polymer's softness is determined by it.

**In Vitro Test**

You can evaluate the drug release from the produced patches using the paddle over disc method (USP equipment V). A glass plate must be covered with dry films of defined thickness that have been cut into a specific form, weighed, and fastened with an adhesive. The device was then brought to an equilibrium temperature of 32 0.5 °C before the glass plate was submerged in 500 mL of the dissolving liquid or phosphate buffer (pH 7.4). The paddle was then turned on at a speed of 50 rpm while being placed 2.5 cm away from the glass plate. At suitable intervals up to 24 hours, samples (5-mL aliquots) can be taken out and examined using a UV spectrophotometer or HPLC. The test must be carried out in duplicate and to describe the drug dissolution profile from a controlled release dosage form and therefore there in vivo performance, it is vital to understand the drug release mechanisms and kinetics of the dosage form. There are different techniques available for determining the drug release rate of TDDS, and numerous mathematical models have been created to characterize the drug dissolution kinetics from controlled release drug delivery systems.

**Conclusion**

One of the most innovative drug delivery methods with good safety and efficacy is transdermal route. Were demonstrated to speed up passive diffusion and allow significant volumes of both chemical and biological medications to penetrate the skin at higher depths. It has more patient acceptance due to low price, excellent pharmacological action in low doses with significant reduction in GI adverse events. Transdermal patches have the potential to become the preferred method of drug delivery in the near future with some improvements to their production and drug delivery systems. They can be used safely and comfortably in elderly, intellectually challenged, and pediatric age groups without running the risk of

overdosing or negative side effects. When compared to the skin damage brought on by hypodermic needle skin puncture, it has been observed that microorganisms' ability to pass through microneedle-induced skin pores within the skin is minimal and has a lower incidence for occurrence. It is intended that improvements in microneedle-based technology would improve illness prevention, diagnosis, and control, as well as the quality of life for patients around the world.

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# Recent Advances in Nanotechnology for Cartilage Regeneration in Osteoarthritis

**Mahak Singh Kasana**
Research Scholar
SRM Modinagar College of Pharmacy, SRM IST, Modinagar, Ghaziabad, Uttar Pradesh
**Alok Pratap Singh**
Guide
SRM Modinagar College of Pharmacy, SRM IST, Modinagar, Ghaziabad, Uttar Pradesh

**Abstract**

Osteoarthritis (OA) is a progressive joint disorder that leads to cartilage degeneration, pain, and impaired mobility. Traditional treatments offer limited efficacy, often failing to provide sustained relief or reverse cartilage damage. In recent years, nanotechnology has emerged as a promising approach to improving OA management. This review focuses on various nanofomulations developed by researchers, such as liposomes, polymeric nanoparticles, solid lipid nanoparticles (SLN), nanoemulsion and nano structured lipid carriers (NLC). These nanocarriers offer significant advantages, including enhanced drug delivery, improved bioavailability, and targeted release of therapeutic agents to the affected tissues. By enabling controlled and sustained release, these nanosystems can potentially improve cartilage repair and reduce inflammation, offering more effective treatment outcomes with fewer systemic side effects. Despite these advancements, key challenges remain, particularly in ensuring the long- term safety, biocompatibility, and scalability of nanomaterials for clinical use. Additionally, the complexity of OA calls for more personalized treatment approaches, combining nanotechnology with other therapeutic strategies. Although many nanofomulations have demonstrated promising preclinical results, extensive clinical trials are required to translate these innovations into approved therapies.

**Keywords:** Osteoarthritis; Nanoparticle; Nanotechnology; Cartilage repair.

## 1. Introduction

The most common chronic joint disease causing disability in adults is osteoarthritis (OA), which is characterized by stiffness, discomfort, and restricted movement in the joints [1]. Age, gender, obesity, joint damage, and other factors are associated with the incidence of OA [2]. People and society at large bear a significant financial burden from OA because of its high incidence [1]. The pathophysiological aspects of OA include the formation of osteophytes, inflammation of the

synovium, remodeling of the subchondral bone, sclerosis, and progressive and degenerative loss of articular cartilage. This illness is thought to be complicated, involving several different tissues and systems. The reasons are still not entirely known [3]. Many variables, including genetic predisposition, the biochemistry and biomechanics of the diseased joint, and the degree of inflammatory response, are linked to OA in contemporary views [4]. As such, pinpointing precise targets for treatment has proven challenging. Symptomatic therapy for pain relief, functional enhancement, and even mechanical joint replacement constitute the majority of current therapeutic practice; the underlying molecular causes of OA are not addressed [5].

Diagnostic imaging procedures like magnetic resonance imaging (MRI), computed tomography (CT), and plane radiographs are used to confirm the diagnosis of OA, which is based on clinical complaints such as pain, swelling, and reduced function. Sadly, imaging is skewed toward displaying the pathoanatomy of late-stage OA (i.e., loss of cartilage volume, edema in the bone marrow, thickening of the subchondral bone, development of cysts, and marginal osteophyttes) [6]. Hyaline cartilage has a limited capacity for self-healing, and permanent deterioration can place even before symptoms and radiographic indicators become apparent. Prognosis worsening and limited treatment choices result from late-stage diagnosis of OA, which occurs after macroscopic and microscopic alterations in tissue structure have occurred. OA is tough to treat and incurable. Reducing OA requires a comprehensive plan.

Three methods can be employed to address the mechanical causes that lead to chondrocyte injury and hyaline cartilage wear:

1. One possible approach is to stop the inflammatory cascade and neutralize the catabolic enzymes that degrade hyaline cartilage.
2. Replace end-stage chondral lesions or enhance component tissue elements.
3. A multidisciplinary field including physics, chemistry, biology, electronics, and engineering is nanotechnology.

Globally, there is a thriving nanotechnology research and development community. The scientific study and manipulation of atomic, molecular, or macromolecular particles—typically ranging in size from 1 to 100 nm—is known as nanotechnology [7]. Due to the particularity of the scale structure,

which gives birth to their distinctive features including size effects, interfacial phenomena, and quantum effects, among others, nanoparticles (NPs) exhibit many remarkable characteristics and novel capabilities [7]. The behavior of NPs is less predictable than that of microparticles. Therefore, by manipulating and modifying nanostructures, it may be possible to utilize the distinct chemical, physical, and biological characteristics of NPs in the future.

The structures of molecules at the microscopic level in the body, their large surface area relative to volume ratio, and the ideal size for catalysis have made nanotechnology increasingly significant [7]. Top-down and bottom-up approaches are commonly used in the fabrication of NPs using nanotechnology. Using methods for nanofabrication to reduce macro-sized structures to nanoscale particles is the top-down approach. However, the bottom-up strategy integrates atomic or molecular components into larger nanoscale particles by means of physical and chemical processes [8,9]. Numerous facets of our everyday life, such as sunscreen, cosmetics, textiles, and sporting goods, have demonstrated the outstanding application value of nanotechnology.

However, there isn't a clinical use of nanotechnology for OA treatment at this time. Nanotechnology has several benefits for the administration of OA therapies.

(1) More effective medication distribution and targeting;

(2) Increased drug solubility and consistency;

(3) Preventing and prolonging drug dispersion and breakdown in body fluids and increasing drug distribution and resorption time in the body;

(4) Enhanced therapeutic effectiveness and reduced adverse drug responses [10].

The recent fast advancements in pharmaceutical delivery methods facilitated by nanotechnology have opened up new treatment concepts and avenues for OA. In this review, the most recent developments and innovative applications of OA-related NP-based drug delivery, including as exosomes, polymers of nanoparticles (PNPs), liposomes, micelles, the dendrimers and inorganic NPs has been discussed.

## 2. LITERATURE REVIEW REGARDING NANOFORMULATIONS USED IN MANAGEMENT OF OA

**Chang, et al. (2021)** developed Hyaluronic acid (HA)-Liposomal (Lipo)-DIC/DEX formulation to

treat OA and relieve joint discomfort. HA was combined with diclofenac (DIC) and dexamethasone (DEX) to create the formulation for long-term OA use. These medications were subsequently put into and placed onto nanostructured lipid carriers (Lipo-DIC/DEX). The NPs showed an average size of 103.6 ± 0.3 nm, a zeta potential of −22.3 ± 4.6 mV, and an entrapment effectiveness of 90.5 ± 5.6%. The results showed that the DIC and DEX content were, respectively, 22.5 ± 4.1% and 2.5 ± 0.6%. HA-Lipo-DIC/DEX was shown to attain maximum efficacy in 4 hours and to continue the release of drugs for at least 168 hours. Increased cell counts were seen in co-culture with articular chondrocyte cells without appreciable toxicity. The reduction of knee joint inflammation in OA mice was validated by *in-vivo* imaging (IVIS) after four weeks of intra-articular injection with HA-Lipo-DIC/DEX. A single injection decreased the inflammation volume to 77.5 ± 5.1% of the starting level. These results point to the safety and efficacy of this innovative drug-releasing device, providing a viable means of managing OA pain [11].

**Maestrelli F, et al. (2020)** developed drug-in-cyclodextrin–double-loaded liposomes (DCL– DL). Curcumin (Cur) has anti-inflammatory and anti-osteoarthritic effects but suffers from low solubility. This issue was addressed with the development of drug-in-cyclodextrin–double- loaded liposomes (DCL–DL). The water compartment of these liposomes contains a drug– cyclodextrin combination, whereas the lipid bilayer contains free drug. Cur–DCL–DL formulations were evaluated for their effectiveness in treating OA using the monoiodoacetate (MIA) model for OA pain in rats. Three types of liposomes were injected intraarticularly: empty liposomes, ordinary liposomes, and Cur as DCL–DL. Assessments were conducted at 7 and 14 days for pain, balance, and gait; DCL–DL had significantly better results than SL. The ankle- joint tissue's histological examination showed that DCL–DL had protective effects against some forms of OA [12].

**Jyothi VG, et al. (2022)** aimed to assess how lipid characteristics such as chain length and functional groups affect meloxicam (MLX)-loaded SLNs (MLX-SLNs) in-vitro permeability. The manufactured MLX-SLNs had an entrapment effectiveness of between 89.13 ± 0.1% and 97.81 ± 0.01%, and their sizes varied from 152 ± 17 nm to 246 ± 5 nm with a polydispersity coefficient below 0.3. A parabolic connection between lipid chain width and permeation was found when these MLX-SLNs were incorporated into a chitin gel (MLX-SLNs-Gel) and tested for flow in ex vivo skin permeation assays. After being examined in a Wistar rat model of OA caused by monosodium

iodoacetate, stearylamine SLNs (MLX-SAM-SLNs-Gel) exhibited the greatest flow of 0.76 ± 0.03 μg/cm²/h across all the formulations. The findings demonstrated that the MLX-SAM-SLNs-Gel group's IL-1β and TNF-α levels were comparable to those of the oral MLX group and were considerably lower than those of the control groups. MLX-SAM-SLNs- Gel exhibited notably different TNF-α levels but similar IL-1β levels when compared to the MLX i.v. the solution group. Furthermore, the OA Research Society International, histology, and X-ray scores were all higher for the MLX-SAM-SLNs-Gel group. In general, MLX-SAM-SLNs- Gel seems to be a promising option for more clinical research [13].

**González-Rodríguez ML, et al. (2017)** employed cationic carriers to target the anionic cartilage matrix, establishing a reservoir of Rhein (RH), a dihydroxy-anthraquinone acid, which exhibits potential chondroprotective effects but suffers from poor oral bioavailability and gastrointestinal side effects within the tissue to enhance its therapeutic efficacy while minimizing adverse effects. The lipophilic properties of RH were improved using hydrophobic ion pairing (HIP), allowing for efficient loading into lipid NPs designed for slow release. The resultant RH-HIP solid lipid nanoparticles (RH-SLNs) were found in the joints of both healthy and arthritic rats, and they rapidly entered cartilage tissue. They also persisted in the joints for up to three weeks. Additionally, in rats with arthritis caused by MIA, RH-SLNs greatly reduced oxidative stress, inflammatory reactions, and cartilage deterioration. All things considered, intra-articular cationic RH-SLNs represent a potentially beneficial development in the management of OA [14].

**Nagalakshmi S, et al. (2017)** formulated sustained-release aceclofenac niosome formulation to improve its topical effectiveness and lessen gastrointestinal adverse effects. Various surfactant ratios were used to generate niosomes using the modified ether injection method. The niosomes were then adjusted based on in-vitro release and entrapment efficiency. The batch that was optimized was added to a niosomal gel and its vesicle size, shape, stability, and drug content were assessed. The entrapment efficiency of 88.68 ± 0.64% and 40% sustained drug release over 8 hours were demonstrated by the results, which outperformed commercial formulations in terms of effectiveness, bioavailability, and penetration [15].

**Peng Y, et. al., (2024)** investigated OA etiology and acknowledged it to be complex and closely

related to joint tissue's oxidative stress response. In addition to producing reactive oxygen species, or ROS, and other oxidizing agents, oxidative stress (OS) in OA leads to decreased cartilage flexibility and strength as well as detrimental effects on chondrocytes. All of these factors hasten joint degradation. Antioxidant effects are important and should be considered while treating OA. Because of stability problems, several Traditional Chinese Medicine (TCM) components have not been widely applied despite having their antioxidant and anti-inflammatory qualities scientifically proven. The use of nanotechnology in conjunction with TCM components has improved treatment efficacy and overcome these issues [16].

**Lee CK, et al. (2022)** delivered berberine, a naturally occurring anti-inflammatory chemical, using proniosome gels to demonstrate the viability of the therapy of OA. The main component of proniosome gel is non-ionic surfactant; this was created so that berberine could be released without the need for mechanical effort. *Ex vivo* studies on skin penetration revealed that the utilization of sorbitan stearate (S60), sorbitan oleate (S80), and polyethylene glycol sorbitan monolaurate (T20) together ensured efficient skin delivery. Experiments carried out in-vitro on OA models demonstrated that chondrocytes could once more synthesize sulphated glycosaminoglycan (sGAG) at modest concentrations (1 μg/mL) without damaging keratinocytes. The mixture reduced cartilage deterioration, pain, and inflammation in a mouse model of OA. This information supports the use of proniosome gels for the administration of active medications in the management of OA [17].

**Corciulo, et al. (2020)** fabricated nanostructured lipid carriers (NLC) of lornoxicam (LRX) to overcome is gastrointestinal adverse effects, short half-life, and low solubility when taken orally for OA. LRX-NLCs were optimized and produced via hot homogenization, yielding particles with the following characteristics: size of 172.1 ± 2.0 nm, a polydispersity coefficient of 0.293 ± 0.01, zeta potential of −15.5 ± 1.21 mV, and entrapment efficiency of 92.85 ± 0.25%. Throughout the course of 24 hours, a consistent drug release pattern was seen. pH, spreadability, drug content, skin penetration, and retention were among the characteristics assessed when LRX- NLCs were added to a gel based on carbopol (LRX-NLCs-Gel). When monosodium iodoacetate was used to generate OA in rats, pharmacodynamic investigations showed a considerable decrease in pain and inflammation as well as better radiographic and histological characteristics. COX-2, TNF-α, and IL-1β levels of cytokines that promote inflammation decreased substantially, and cartilage structure

was improved by the gel. Emu oil, which is supplemented with omega-3, omega-6, and omega-9 fatty acids, also gave LRX synergistic effects by inhibiting the proteolytic functions of MMP-2 and MMP-9. Therapeutically viable dose forms such as LRX-NLCs-Gel appear promising in treating OA [18].

**Table 1: Application of Nanoparticles for intra-articular medication delivery in OA treatment**

| Study/NP Type | Model | Treatment | Key Findings | Reference |
|---|---|---|---|---|
| Liposomes | Obesity-induced (mice) | Adenosine, CGS21680 | Favorable histology; prevention of OA progression | [19] |
| Liposomes | Post-traumatic (rats) | Rapamycin | Decreased IL-6, MMP-13; increased collagen II; lower OARSI scores; improved histology | [20] |
| Fish oil protein + GNPs + DPPC | Collagenase-induced (rats) | Intra-articular injection | Increased GSH, SOD, catalase; decreased pro-inflammatory cytokines; increased anti-apoptotic activity; reduced NFκB levels | [21] |
| Clodronate | Post-traumatic (rats) | Intra-articular injection | Decreased M1 macrophages; lowered collagen X levels; positive histology | [22] |
| Micelles (MRC-PPL/Psoralidin) | Papain-induced (mice) | Intra-articular treatment | Lowered TNF-α, NFκB, MMP- 13; positive histology | [23] |
| Micelles (Curcumin + PAE) | MIA-induced (mice) | Intra-articular treatment | Decreased TNF-α, IL-1β; improved histology | [24] |

| | | | | |
|---|---|---|---|---|
| Dendrimers | Post-traumatic (rats) | PAMAM/IGF-1 | Decreased synovial inflammation and deteriorated cartilage; improved histology and micro-CT results | [25] |
| Dendritic polyglycerol sulfates | Post-traumatic (rats) | dPGS | Decreased Mankin and Glasson scores; enhanced cartilage integrity | [26] |
| Polymeric NPs | MIA-induced (rats) | p66shc si-PLGA | Improved histology; reduced TNF-α, IL-1β, COX2 levels | [27] |
| Polymeric NPs | MIA-induced (rats) | p47phox si-PLGA | Enhanced histology; reduced ROS | [28] |
| PLGA NPs | Post-traumatic (rats) | Etoricoxib/PLGA-PEG-PLGA | Improved OARSI scores; positive histology; decreased COX2, iNOS, MMP-13, ADAMTS-5 | [29] |
| PLGA NPs | Post-traumatic (rats) | PLA-PEG-adenosine | Decreased NFκB levels and OARSI scores; positive histology results | [30] |

**Abbreviations:** HA: Hyaluronic acid; i.a.: intra-articular; PEG: poly (ethylene glycol); KGN: Kartogenin; BDMC: bisdemethoxycurcumin; CNPs: Chitosan nanoparticles; COLBP: Collagen binding peptide; dPGS: Dendritic polyglycerol sulfates; DPPC: dipalmitoyl phosphatidylcholine; BBR: Berberine chloride; SOD: Superoxide dismutase; FP: Fish oil protein; GSH: Glutathione reductase; HABP: Hyaluronic acid-binding peptide; IGF-1: Insulin-like growth factor 1; MIA: Monoidoacetic acid; OARSI: Osteoarthritis Research Society International; PAMAM: polyamidoamine; PNPs: Polymeric nanoparticles; PPL: Poly (2-ethyl-2-oxazoline)-poly (ε-caprolactone); s.c.: subcutaneous; HA/CS-CrmA: Hyaluronic acid-chitosan nanoparticles containing plasmid DNA encoding CrmA; MRC: MMP-13 responsive/Coll-II α1 chain-binding peptide–CollB; PAE: Poly(β-amino ester); GNPs: Gold nanoparticles; In a post-traumatic OA rat

model, polyurethane-KGN decreased OARSI scores and produced favorable histology outcomes (31). Reductions in OARSI scores and positive histology were also observed in another investigation that used KGN-PLA in post-traumatic OA animals [32].

Novel pairings between biomolecules and carriers have also been investigated. Collagen-binding peptide HABP-PEG-COLBP decreased MMP-13, IL-1β, and IL-6 in a post-traumatic OA model in rats, but improved histology and OARSI scores (33). When injected intraperitoneally (i.p.) to post-traumatic OA rats, berberine chloride nanoparticles (BBR-CNPs) exhibited good histology, decreased expression of bax and caspase-3, and enhanced Bcl-2 levels [34].

Curcumin-based NPs, namely curcuminoid-hyaluronic acid (HA)-CNPs, showed anti- inflammatory benefits in a post-traumatic OA rat model. They increased collagen II expression and showed good histology while reducing NFκB, MMP-1, and MMP-13 levels [35]. In addition to better OARSI scores and histological results, a different research using CrmA-HA-CNPs in post-traumatic OA rats also revealed decreased levels of IL-1β, MMP-3, and MMP-13 [36].

These investigations demonstrate the potential of NPs for intra-articular medication administration, exhibiting encouraging outcomes in lowering inflammation, cartilage deterioration, and the overall course of OA in a range of animal models.

## 3. Conclusion

In conclusion, the exploration of various nanofomulations for the management of OA has underscored the significant potential of nanotechnology in enhancing therapeutic outcomes. Through the innovative work of numerous scientists, nanocarriers such as liposomes, SLNs, polymeric NPs, and show on have been developed, each offering distinct advantages in drug delivery, bioavailability, and targeting of osteoarthritic tissues. These formulations provide more controlled and sustained release of therapeutic agents, minimize systemic side effects, and enhance cartilage repair processes.

Despite the encouraging progress, certain challenges remain, including optimizing the biocompatibility, long-term safety, and large-scale production of these nanocarriers. Variability in patient response and the complexity of OA also call for more personalized and multi-targeted treatment strategies. Furthermore, while preclinical studies have demonstrated promising results, translating these nanofomulations into clinically approved therapies will require extensive trials to ensure their efficacy and safety in humans. Overall, the advances in nanotechnology-based

formulations offer a promising future for the effective management of OA, potentially transforming current therapeutic approaches and improving patient quality of life. As research progresses, these innovative strategies may become integral components of OA treatment, providing more efficient, targeted, and patient-centered solutions.

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# PHYTOCHEMICAL PROFILE OF LEAVES OF *IPOMOEA OBSCURA*

**Neethu Varghese[*1], Mythreyi R[1], Arun Rasheed[2]**
[1]Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Tamilnadu 603103.
[2] Department of pharmaceutical chemistry, Al Shifa College of Pharmacy, Perinthalmanna, Malappuram
**[*1]Presenting author: neethu.va@gmail.com**

**Abstract**

This research study discusses the process of extracting plant compounds, from the leaves of *Ipomoea obscura* using water and alcohol as solvents. Initial analysis of the plant compounds revealed that the ethanol and water extracts were rich in phenols, flavonoids, tannins, alkaloids and terpenoids. The identification of these compounds was carried out using UV spectroscopy. Ethanolic extract of leaves contains more phenolic compounds and flavonoids compared to alcohol extract. The ethanolic leaf extract, from this plant displayed the concentration of compounds at 82.03± 0.088 (GAE/gDM). Flavonoids were found to be the prevalent, with a concentration of 123,83 ± 0.120 (QE mg/g DM). The LCMS method was employed to ascertain the metabolites, in both extracts and a total of 16 compounds were identified as positive.

## 1. Introduction

Ipomoea is a diverse genus within the family Convolvulaceae, consisting of approximately 600 to 800 species that are primarily distributed across tropical and subtropical regions worldwide. This genus includes a variety of growth forms such as herbs, shrubs, lianas, and small trees, with many species characterized by their twining or climbing habits. Commonly known as morning glories, Ipomoea species are admired for their striking funnel-shaped flowers, which display a variety of colors, including violet, blue, pink, and red. Notable members include economically important crops such as sweet potato (*Ipomoea batatas*) and water spinach (*Ipomoea aquatica*), both of which have significant culinary uses worldwide. Furthermore, numerous Ipomoea species are grown as ornamental plants for their appealing blooms.

However, some species have become invasive in various regions, posing ecological threats. The genus also holds potential for medicinal applications, with various species traditionally used in herbal remedies for ailments ranging from diabetes to inflammation.[1] *Ipomoea obscura* is a small climbing vine,

characterized by its small cordate (heart-shaped) leaves with an acuminate apex. The plant produces a corolla composed of five fully fused petals, contributing to its distinct funnel-shaped flowers. It typically grows on fences or as low ground cover in disturbed areas, where it thrives in substrates such as soil or along structures. This plant is often found in tropical and subtropical environments, where its twining or climbing growth habit allows it to spread easily across surfaces.[2] *Ipomoea obscura* has been recognized in Ayurveda for its various medicinal properties. It is effectively used to treat dysentery, and its application extends to open sores and pustules. A paste made from the leaves is applied to ulcers, haemorrhoids, and swellings to provide relief. The seeds and fruits are known to act as cleansing agents, improving difficult breathing, alleviating pain, and potentially enhancing vision. In addition to its medicinal uses, the plant holds ornamental value as a climber with attractive flowers. Furthermore, *Ipomoea obscura* is also included in lists of plants that affect the central nervous system and is actively utilized for its antioxidant properties.[3]

## 2. Materials and methods

### 2.1. Reagents and solution preparations

All chemicals used were of analytical grade reagents. Ethanol, , Gallic acid, 7% Sodium carbonate, Folin-Ciocalteu reagent (FCR), Quercetin, ferric chloride ($FeCl_3$), Potassium Chloride, Ammonium molybdate Fehling solution A Fehling solution B Magnesium turnings Methanol Sodium nitrite Aluminium Chloride Sodium hydroxide Sodium carbonate Mayer's reagent Dragendorff's reagent Wagner's reagent Acetyl chloride nitric acid Sulphuric acid Copper Sulphate Benedict's reagent Barfoed's reagent Hydrochloric acid Ammonia were obtained from Nice Chemicals pvt. Ltd

### 2.2. Collection of plant samples

The leaves of *Ipomoea obscura* were harvested from sites, in Malappuram, Kerala. The collected plants were authenticated by the Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala. After collection, the leaves were carefully cleaned thoroughly with tap water and allowed to naturally dry under ambient room temperature. Once dried, the leaves were ground into a fine powder using a mixer. The powdered samples were securely stored in an airtight glass bottle to maintain their quality for further analysis.

### 2.3. Instrumentation

**UV–Vis spectrophotometer (Schimadzu, Japan)** have a wavelength range from 190 nm to 1100 nm, allowing for the detection of both the phenolic and flavonoid compounds, which typically absorb in the UV-visible range (200-600 nm).

### 2.4 Extraction

Soxhlet extraction using 200 g of *Ipomoea obscura* leaf powder was done with 95% ethanol as the solvent for 24 hours. The solvent was subsequently removed and evaporated to dryness at 42°C under reduced pressure using a rotary evaporator. The extraction process yielded an extract with a 22% (w/w) yield.[4]

### 2.5. Procedure for phytochemicals screening

The initial phytochemical screening of the extracted plant samples was carried out using various chromophoric reagents. The crude extract was treated with the chemical reagents in test tubes, to identify the chemical constituents present.[5] The detailed procedure for the qualitative analysis of phytochemicals in the plant samples is presented in Table 1.

| Sl no | Name of phytochemical | Method of detection | Observation |
|---|---|---|---|
| 1 | Alkaloid | Dragendorf's Test | orange-red colour |
| | | Mayer's Test | creamy-white precipitation |
| 2 | Flavonoids | Shinoda Test | yellow coloured precipitate |
| | | Ethyl Acetate Test | yellow colour |
| 3 | Phenols | Ferric Chloride Test | greenish-black color |
| 4 | Terpenoids | Liebermann-Burchard's Test | violet coloured ring at the junction |
| 5 | Steroids | Salkowski Test | green fluorescence |
| 6 | Glycosides | Baljet Test | yellow to orange colour |
| 7 | Saponins | Foam Test | persistent frothing |
| 8 | Carbohydrates | Molisch's Test | Violet ring at the junction of two liquids |

**Table 1: Method for preliminary phytochemical screening using different chemical reagents.[5]**

### 2.6. Estimation of total phenolic contents

The Folin-Ciocalteu (F.C.) method is used to quantify total phenols, also known as the GAE method. This method involves reacting the sample with a reagent that produces a blue color, the intensity of which is proportional to the phenolic content. The procedure measures the quantity of phenolics required to prevent oxidation of the reagent. 0.1 g plant sample was taken into a conical flask containing 10 mL of ethanol and then ultra-sonicated for 15 min of extraction time. One ml of the sample solutions of the ethanol extract and aqueous extract was blended 1 mL of two-fold diluted FCR and after 5 min, 2 mL of 7.5% $Na_2CO_3$ was added and the total volume of solution mixture was made with 10 mL DW. It was kept under cover of darkness for 60 min and analyzed for absorbance at 765 nm. A standard curve based on gallic acid solutions was utilized to assess the total phenolic content. The phenolic content in plant samples was quantified using a calibration curve generated from the relationship between gallic acid concentration and its absorbance. The phenolic content was reported as milligrams of gallic acid equivalent per gram of dry weight of the powdered plant sample (mg GAE/g sample), along with the ± SD (standard deviation) for three replicate measurements.[6]

### 2.7. Estimation of total flavonoid contents

The total flavonoid content was determined according to the aluminum chloride-colorimetric method. The $AlCl_3$ colorimetric assay functions on the principle that aluminum chloride forms stable complexes with flavonoids. This reaction occurs through the interaction between the aluminum chloride and the carbonyl group at the fourth carbon position, as well as the hydroxyl groups at either the third or fifth carbon positions of flavones and flavonols. This complexation results in a color change, which can be measured spectrophotometrically to estimate the flavonoid content.[7]

0.1 g aliquots of each plant sample were extracted following the procedure outlined in Section 2.6. 1 mL of the sample solutions of the ethanol extract and aqueous extract was mixed with, 0.3 mL of 5 percent $NaNO_2$. After a five-minute interval, 0.3 ml of 10 percent $AlCl_3$ solution was introduced, subsequently, 2 ml of 1M NaOH was added. The final volume of the solution was made upto 10 mL with distilled water. The solution mixture was then left at room temperature for 30 minutes before measuring the absorbance at 415 nm using a spectrophotometer. A standard calibration curve was similarly constructed by plotting the absorbance values against the concentrations of the quercetin standard. The results were expressed as

milligrams of quercetin equivalents per gram of dry weight of the powdered plant samples (mg QE/g sample), along with the ± SD (standard deviation) for three replicate analyses.[6,8,9,11]

**2.8 LC MS analysis**

LC-MS is a sophisticated technique commonly used for screening bioactive secondary metabolites. It is a simple, fast, and widely accepted method for identifying bioactive molecules from crude plant extracts, requiring only a small amount of the sample. In this investigation, LC-MS was employed to detect and identify the bioactive compounds in the leave extracts of *Ipomoea obscura*. This technique allows for efficient and precise analysis, contributing significantly to the understanding and exploration of plant-based bioactive compounds**.** The chemical constituents of the ethanolic and aqueous extracts of *Ipomoea obscura* were determined using LC-MS. LC-MS analysis was performed using Mariner Bio spectrometry equipped with a binary pump. The HPLC was interfaced with a Q-TOF mass spectrometer equipped with an ESI source. Full-scan mode was conducted over an m/z range of 100 to 1200, with a source temperature set at 140°C. HPLC column Phenomenex 5µ C8, (150 × 2 mm i.d.) was used for the analysis. Solvent was methanol with 0.3% formic acid. The solvents were delivered at a flow rate of 0.1 mL/min using isocratic elution. MS spectra were obtained in positive ion mode.[11,12]

**3. Results and discussion**

**3.1 Extraction yield**

The choice of organic solvent plays an important role in the separation of specific chemicals from plant samples. The extraction yield depends on the chosen solvent as it targets different phytochemicals. It depends on their polarity, which affects the efficiency of extraction. Water and alcohol were selected as solvents for *Ipomoea obscura* leaf extraction because of their polarity to efficiently extract a variety of phytochemicals. Alcohol is particularly effective in extracting phenolic compounds, flavonoids, and other bioactive compounds, while water helps to extract more polar compounds, including certain glycosides and tannins. The present study demonstrated that the ethanolic extract of *Ipomoea obscura* had a yield of 2.05%, while the aqueous extract yielded 3.07%.

**3.2. Screening of phytochemicals using reagents**

Preliminary phytochemical examination of the alcohol and aqueous extracts of *Ipomoea obscura* leaves revealed the presence of alkaloids, flavonoids, phenolic acids, terpenoids, tannins, saponins, carbohydrates,

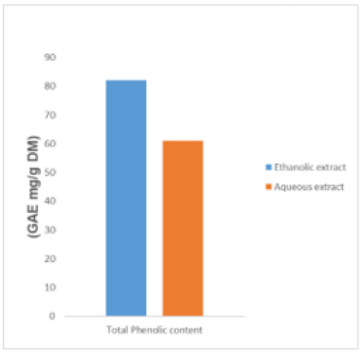
proteins and quinones. The results are summarized in Table 2. Alkaloids, glycosides and terpenoids were detected in the ethanol extract. Flavonoids, phenolics, and carbohydrates were found in both ethanol and aqueous extracts. Saponins were present in the aqueous extract. Proteins, quinones, and oxalates were absent in both extracts.

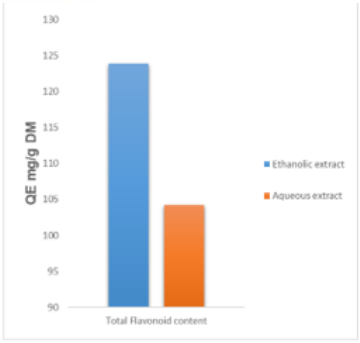
| Sl. no | Chemical Test | Ethanolic extract | Aqueous extract |
|---|---|---|---|
| 1 | Alkaloids | + | - |
| 2 | Flavonoids | + | + |
| 3 | Phenolic compounds | + | + |
| 4 | Terpenoids | + | - |
| 5 | Steroids | + | - |
| 6 | Glycosides | + | - |
| 7 | Saponins | - | + |
| 8 | Carbohydrates | + | + |

**Table 2: Preliminary phytochemical screening of all extracts (+ indicates presence and - indicates absence of phytoconstituents)**

### 3.3. Determination of total phenolic and flavonoid contents in the plant extracts

Phenolic compounds and flavonoids are natural substances known to have strong antioxidant properties. These compounds are found in different parts of plant which contributes to the health benefits of plants. Total phenolic and flavonoid contents in the leaf extracts of *Ipomoea obscura* are given in Table 2. Total phenolic contents were expressed as GAE using the standard curve equation ($y = 0.0028x + 0.0166$), where the correlation coefficient ($R^2$) was 0.9956. Ethanolic leaf extract of the plant, had the highest amount of phenolic compounds with a concentration of 82.03 ± 0.088 (GAE/gDM). The total flavonoid content of the extract was expressed as QE mg/g dry weight (DM) using the standard curve equation ($y = 0.0037x - 0.0331$), with the correlation coefficient ($R^2$) 0.9943. The highest flavonoid content was observed in ethanolic extract with a concentration of 123.83 ± 0.120 ( QE mg/g DM) compared with aqueous extract.




### 3.4 LC MS

The preliminary identification of compounds in the ethanolic and aqueous extracts was conducted through LC-MS analysis. Compounds were detected and identified based on their fragmentation patterns and by analysing their peak areas and retention times. Nine compounds were identified in the ethanolic extract of *Ipomoea obscura* leaves. The main compounds detected were 2,2-dimethyl-1,3-butanediol, bergamotol, Z-α-trans, β-sitosterol, quinic acid, 3-methoxy-2,2-dimethyloxirane, heptadecane, 2-cholestanone, 3-phenyl-, caffeic acid, quercetin, and 3,5-di-O-caffeoylquinic acid methyl ester.

| Sl.No | Phytochemicals | Molecular formula | m/z (g/mol) |
|---|---|---|---|
| 1 | 2,2-dimethyl-1,3-butanediol | $C_6H_{14}O_2$ | 118 |
| 2 | Bergamotol, Z-α-trans- | $C_{15}H_{24}O$ | 220 |
| 3 | Quinic acid | $C_7H_{12}O_6$ | 192 |
| 4 | 3-methoxy-2,2-dimethy loxirane | $C_5H_{10}O_2$ | 102 |
| 5 | Heptadecane | $C_{17}H_{36}$ | 240 |
| 6 | 2-Cholestanone, 3-phenyl- | $C_{33}H_{50}O$ | 462 |
| 7 | Caffeic acid | $C_9H_8O_4$ | 180 |
| 8 | Quercetin | $C_{15}H_{10}O_7$ | 302 |
| 9 | 3,5-di-O-caffeoylquinic acid methyl ester | $C_{26}H_{26}O_{12}$ | 530 |

Seven compounds were identified in aqueous extract of *Ipomoea obscura* leaves. The prevailing compounds were 2,2-dimethyl-1,3-butanediol, 3-methoxy-2,2-dimethyloxirane, 3,7-dimethyl-7-octen-1-ol, Butane-1,2,3,4-tetraol, Butanoic acid, 2-oxo, cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, methyl 6-deoxy-alpha-L-galactopyranoside. The identified compounds are presented in Tables 3 and 4 along with their experimental m/z values and molecular formulas.

**Table 3: Compounds identified from the ethanolic extract of *Ipomoea obscura* leaves by LC-MS**

| Sl.NO | Phytochemicals | Molecular formula | m/z (g/mol) |
|---|---|---|---|
| 1 | 2,2-dimethyl-1,3-butanediol | $C_6H_{14}O_2$ | 118 |
| 2 | 3-methoxy-2,2-dimethyloxirane | $C_5H_{10}O_2$ | 102 |
| 3 | 3,7-dimethyl-7-octen-1-ol | $C_{10}H_{20}O$ | 156 |
| 4 | Butane-1,2,3,4-tetraol | $C_4H_{10}O_4$ | 122 |
| 5 | Butanoic acid, 2-oxo | $C_4H_6O_3$ | 102 |
| 6 | cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)- | $C_{15}H_{26}O$ | 222 |
| 7 | methyl 6-deoxy-alpha-L-galactopyranoside | $C_7H_{14}O_5$ | 178 |

Table 4: Compounds identified from the aqueous extract of *Ipomoea obscura* leaves by LC-MS

## 4. Conclusions

In summary, *Ipomoea obscura* leaf extract contains many phytochemicals, makes it a promising candidate for use as a phytotherapy with their further study. This extract is rich in phenolic compounds and flavonoids, known to have important antioxidant properties, suggests potential therapeutic benefits, particularly in the management of oxidative stress-related diseases. However, more research is needed to fully explore the medicinal properties of *Ipomoea obscura* and to establish its efficacy and safety for clinical use.

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# FORMULATION AND EVALUATION OF HERBAL ANTI-FUNGAL GEL FOR DANDRUFF

Neha Singh, Dr. ivaniPreeti Garg, Sangeet Sharma
Hindu College of Pharmacy, Sonipat, Haryana, India-131001
e-mail-preeti_garg20@yahoo.com

## ABSTRACT

Fungal diseases become a major medical problem. Fungal diseases are difficult to manage because they tend to be chronic, and hard to diagnose. The fungal infection is a common condition caused by fungi. The herbal antifungal gel was formulated using neem and aloe vera. Herbal medicine is one of the oldest and most universal healthcare systems. The herbal antifungal gel is very helpful and it has fewer side effects. All herbal ingredients are easily available in the market. The herbal antifungal gel is used to treat fungal infections which most commonly affect our skin, hair, and scalp. Herbal antifungal gels are used to treat fungal skin infections such as dandruff, and seborrheic dermatitis. The herbal antifungal gel is natural and safe to use, and the herbal antifungal gel is beneficial in the reduction of fungal infection.

Keywords: Herbal Anti-fungal gel; Dandruff; Herbs; Herbal ingredients; Fungal -infection.

## 1. INTRODUCTION

### 1.1 HERBAL COSMETICS

The word “cosmetics” is derived from the Greek word “*kosmtikos*”, which means “power, arrangement, and ability in beautifying. According to the Drug and Cosmetic Act 1940, any article intended to be rubbed, poured, sprinkled or sprayed on or introduced to or applied to any part of the human body for cleansing, beautifying, promoting, attractiveness, or altering the appearance and includes any article intended for use as a component of cosmetics (1-5). Herbal cosmetics utilize natural ingredients derived from plants, herbs, and minerals to nourish and enhance the skin and hair. These products often exclude synthetic chemicals, making them popular for those seeking gentler alternatives. Ingredients like aloe vera, tea tree oil, lavender, and rosemary are common in herbal cosmetics for their soothing, moisturizing, and antioxidant properties. Many herbal cosmetics also incorporate traditional remedies from various cultures, such as Ayurveda and traditional Chinese medicine (6-10). These products are believed to be safer and less likely to cause adverse reactions or skin irritations compared to their synthetic counterparts.

Additionally, they're often environmentally friendly, as they're derived from renewable resources and may be biodegradable (11, 12). However, it's important to note that while herbal cosmetics can offer benefits, individual reactions may vary, and it's crucial to choose products carefully and consult with a dermatologist if you have specific skin concerns. Herbal cosmetics can be of various types based on their application site- Skin, Hair, Eyes, Tooth, and Nails (13-16).

- **Herbal ingredients for various types of skin preparation include-**

a) For Dry Skin type- Manjista, Avacado, Honey, Aloe vera, Banana, Tulsi, Liquorice, Shea butter, Vitamin E, Sesame oil, etc. (17).

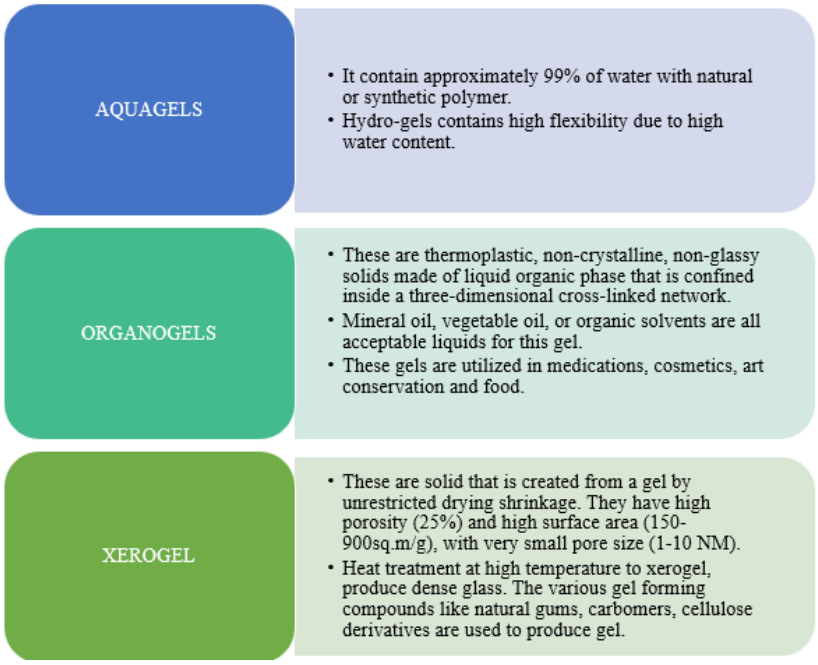
b) For Sensitive Skin types- Tulsi, Neem, Carrot, Apricot, Eucalyptus, Cucumber, Turmeric, Banana, Pineapple, Coconut oil, Triphala, etc. (18).

c) For Oily Skin type- Tulsi, Triphala, Arjuna, Milk, Sour milk, Mustard oil, Cream, Buttermilk, Strawberry, Papaya, etc. (19).

- **Herbal ingredients for hair cosmetics include**- Brahmi, Amla, Mint, Sandalwood, Shikakai, Heena, Aloe vera, Rosemary, Dill, Reetha, Bhringraj, Haritaki, Jatamasi, Shatravari, Oregano, etc. (20).
- **Herbal ingredients for dental preparations include**- Clove, Mint, Peppermint, Ginger, Banyan, Ginger, Neem, Acacia, Black pepper, Amla, etc. (21).
- **Herbal ingredients for nail preparation include**- Thyme, Garlic, Onion, Methi, Rose oil, Aloe vera, etc. (22).
- **Herbal ingredients for eye preparation include**- Parsely, Vegetable oil, etc. (23-25).

**1.2 GEL**

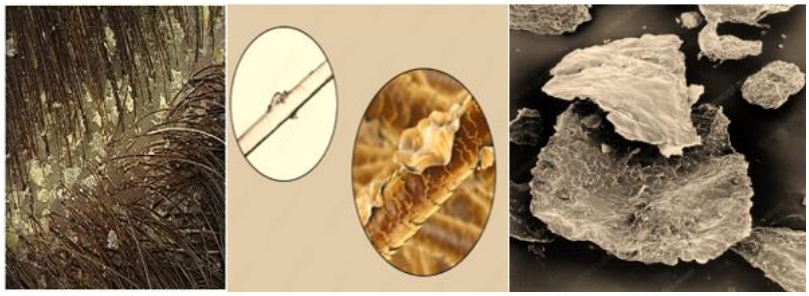
The gel is a solid, jelly-like material with a three-dimensional cross-linked network in the liquid. The physical connectors and crystalline or other junctions that are still present in an extended liquid can provide the internal network structure (26). Usually, the "solid" phase forms an interconnected network through which the "liquid" phase lies. The gels may be of any type i.e.; Hydro-gels, Organ o-gels, or Xero-gels.




Gel denotes a semi-solid mixture with the following properties- does not flow under the stress of gravity, i.e., at low shear, does flow under higher imposed shear, frequently it is clear, i.e. not an opaque oil-in-water suspension (ointment) nor an opaque water-in-oil suspension (cream), Swelling, Syneresis, Hydration, Clarity, Shear reversibility, Ageing, and Rheology modification (27). Advantages of gel formulation- easy to formulate, elegant and non-greasy formulation, have good adherence properties, non-toxic and non-irritant, softens and moisturizes the skin, shear reversibility, good stability. Risk factors- may dry out, so far increasing the viscosity and stability of gels the glycerol (10%), polyethylene glycol is added, may cause local irritation, so far reducing the size (<500 Dalton) to diffuse through the stratum corneum (28).

### 1.3 DANDRUFF

Dandruff is a general scalp condition that affects most of the population at teen-age. The word dandruff is of Anglo-Saxon origin which is a combination of ‘tan’ meaning ‘teeter and ‘druff’ meaning ‘dirty’ (29). The dandruff is distributed most commonly in the hairy part of the head, forehead, external ear canals, eyebrows eyelashes, etc. Dandruff is a group of cells (corneocytes) that have a strong degree of cohesiveness and separate from the surface of the stratum corneum surface (30).



The most prevalent cause of dandruff is the fungus *Malassezia fur-fur*. This fungus is dependent on lipids. It is responsible for many cutaneous diseases like dandruff, seborrheic dermatitis, etc. (31). During dandruff, there is an increased level of *Malassezia fur-fur* by 1.5-2 times its normal level. Dandruff may also be caused by frequent exposure to extreme heat and cold; family history; food allergies; excessive perspiration; use of alkaline soaps; and stress contribute to the dandruff production (32). Mainly there are three types of dandruff- Oily scalp dandruff; Dry skin dandruff and Fungal-dandruff (33).


For treatment of dandruff-

a) **Follow a healthy diet-** Food rich in omega-3 fatty acids, B vitamins, and zinc. The dietary sources for these vitamins and minerals include salmon fish, broccoli, walnuts, spinach, egg, cabbage, whole grain, cereals, dairy products, nuts, red meat, etc. (34).

b) **Avoid stress-** Invest in stress management techniques like yoga, meditation, and breathing exercises to keep your stress away (35).

c) **Shampoos with a combination of various active ingredients against dandruff-** Ketoconazole, Selenium sulfide, Coal tar, Salicylic acid, Ciclopirox, Zinc pyrithione, etc. (36).

d) **Anti-fungal properties carrying herbal ingredients-** Egg oil, Olive oil, Fenugreek seed, Apple cider vinegar, Neem, Rosemary, Hibiscus, Lime juice, Onion, Aloe vera, Amla, Coconut oil, etc. (37).

## 1.4 ANTIFUNGAL ACTIVITY

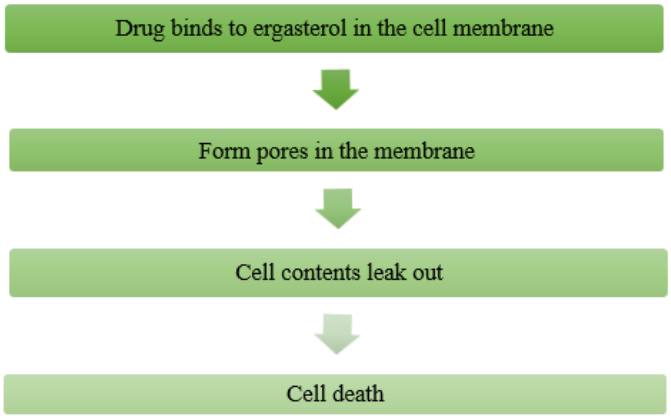
Antifungal gels are topical formulations intended to treat fungal infections of the skin, such as dandruff, seborrheic dermatitis, and hair fall. These gels typically contain active ingredients like clotrimazole, miconazole, terbinafine, or ketoconazole, which work by inhibiting the growth of fungi and eliminating the infection (38). Antifungal gels are applied directly to the affected area and are usually used for a specified duration as directed by a healthcare professional (39). They provide relief from symptoms such as itching, skin flakes, and irritation, and can effectively clear up fungal infections when used consistently and as prescribed (40). It's essential to follow the instructions on the packaging or provided by a healthcare provider for safe and effective use (41-43). Antifungal gel contains agents that either kill or stop the fungal growth and are used to treat or prevent the fungal infection. However, the mechanism of action of most antifungal gels is of two types-

**1.3.1 Fungistatic agent**- The agent that inhibits fungi's growth and reproduction but does not necessarily kill them is called a fungistatic agent. The fungal growth resumes when such an agent is removed from the environment.

**1.3.2 Fungicidal agent**- The agent that kills fungi is called a fungicide agent.

Mainly fungicidal mechanism of action is used to treat various types of fungal infections for long-term relief and prevention. It includes various mechanisms of action-

a) Fungal cell wall synthesis inhibitor-




b) Inhibition of ergasterol and lanosterol synthesis-

Ergasterol is the major fungal cell component, important for cell membrane rigidity and stability. By inhibiting ergasterol synthesis, initiate the cell membrane loosening and hence results into cell lysis (the process is represented below in the fig 1).

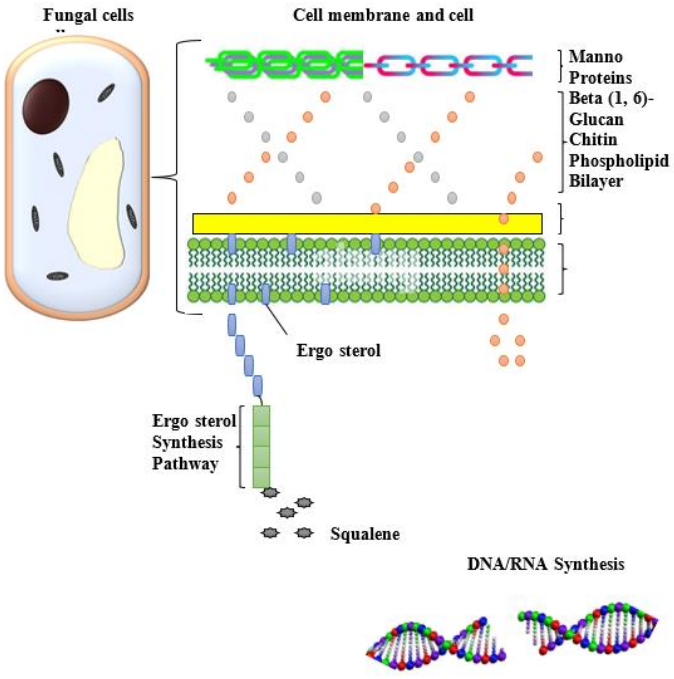
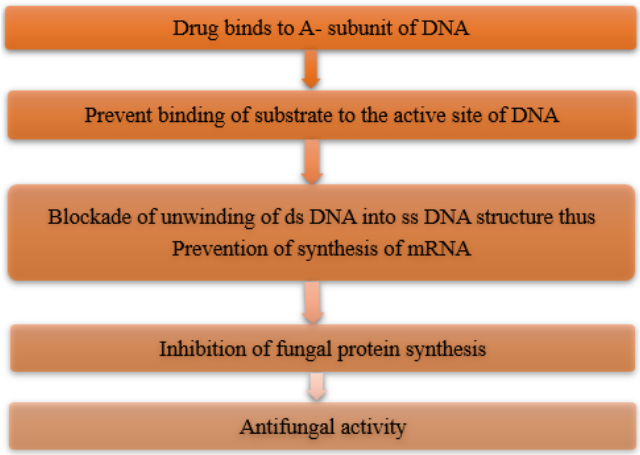
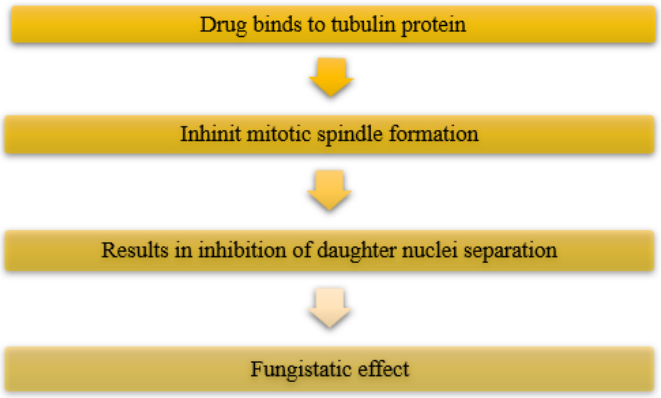



Fig. No. 1 Ergasterol synthesis inhibition

c) Inhibition of nucleic acid synthesis-




d) Disruption of mitotic spindle and inhibition of fungal mitosis-




## 1.5 HERBAL INGREDIENTS

Herbal ingredients contain active ingredients. This importance lies in their chemical substances that produce a definite physiological action on the human body (44). The most important of these bioactive compounds include alkaloids, tannins, flavonoids, and phenolic compounds. The health effects of flavonoids include antioxidant, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anti-carcinogenic. Alkaloids show many useful effects like antihypertensive and anti-tumor. Alkaloid-based drugs include caffeine, quinine, nicotine, artemisinin, colchicine, and amblyopia. Tannins include corilagin and geraniin, which show anti-human immunodeficiency syndrome activity by inhibiting reverse transcriptase. Herbal ingredients are safe because they are natural. These are the ingredients with

medical properties that manifest beneficial topical actions and provide protection against degenerative skin conditions. Advantages of herbal ingredients- they do not provoke allergic conditions, they do not have negative side effect, they are easily incorporated with cosmetic preparations, with small quantities, they are very effective, easily available and found in large variety and quantity, provide additional skin benefits (45). Disadvantages of herbal ingredients- have a slower effect as compared to the allopathic dosage form, it requires long-term therapy, they are difficult to hide taste and door, the manufacturing process is time-consuming and complicated, no pharmacopeia defines any specific procedure or ingredients to be used in any herbal cosmetics. Storage of herbal products- herbs should be stored in a cool and dry place, avoid moisture around the herbs because it can promote bacterial and fungal growth, dried herbs can be stored in an airtight container, and the herb should be stored in direct sunlight (46). From the use of these herbal ingredients, various types of hair care preparations are formulated. These herbal hair care preparations carry numerous amounts of scalp nourishing properties as well as hair care properties. The herbal ingredients carry a large amount of active constituents that are beneficial in various kinds of skin or scalp-related problems with very less negative side effects. For these herbal hair care preparations, various parts of the plant or whole plant are used based on their active constituent. For nourishing action Aloe vera, Amla, and essential oils are used. For antimicrobial action Neem, Guava, Garlic, Onion, Thyme, and Lime are used. For hair loss prevention Amla, Shikakai, Reetha, and Heena are used (mentioned in Table 1)

**Table 1: Herbal plants used in antifungal preparations**

| Sr. No | Plant | Biological Source | Chemical Constitu-Ents | Part Used | Marketed Products | Reference |
|---|---|---|---|---|---|---|
| 1 | Neem | *Azadiracta indica* (Meliaceae) | Nimbosterol, nimbin, quercetin, nimbidin, nombosterol, mergosine, azadiractin, azadiradione | Leaf, Flower, Seed, Bark | Greenleaf anti-hair fall serum; Khadi Natural Neem sat hair conditioner; Nimbin 2-in-1 shampoo with conditioner | 47-49 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | Garlic | *Allium sativum* (Alliaceae) | Allin, allyl methyl sulfide, diallyl sulfide, allicin, z-ajoene, e-zone | Bulb | Cosmo hair treatment cream; Hollywood Secrets garlic oil; Ayubal Wellness garlic soft gel capsule | 50-51 |
| 3 | Cumin | *Cuminum cyminum* (Umbelliferae) | Cumin aldehyde, p-cymene, neo-piene, limonene, perilla aldehyde | Seed, Flowering part | Alvia black cumin seed oil; Avimee herbal Radha moisturizing hair conditioner; Dr. Jain cumin seed oil | 52 |
| 4 | Ginger | *Zingiber officinale* (Zingiberaceae) | Gingerol, shagol, zingiberine, zingiberol, bisabilone, trace amount of Ca, Mn, F**e**, Cu, K | Rhizome | Lotus botanicals ginger root dandruff control shampoo; Lanthome hair growth essence; Sandarbh hair nutrient solution oil, shampoo, and conditioner | 53-54 |
| 5 | Clove | *Syzygium aromaticum* | Thymol, eugenol, eugenyl acetate, quercetin, caffeic acid | Dried flower bud | Wellmee natural clove oil; Mesmara clove bud essential oil; | 55-56 |

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|---|---|---|---|---|---|---|
| | | (Myrtaceae) | | | Seyal clove essential oil | |
| 6 | Basil | *Ocimum sanctum* (Lamiaceae) | Eugenol, carvacrol, estragole | Leaf | Hollywood Secrets tulsi oil; Adven biotech anti-dandruf shampoo; Tulsi adivasi neelambari hair oil | 57-60 |
| 7 | Oregano | *Origanum vulgare* (Lamiaceae) | Carvacerol, tymol, linalool, p-cymene, terpiene | Leaf | Pavak herbals oregano essential oil; Muckery oregano essential oil; Alvia oregano oil | 61-62 |
| 8 | Lemon | *Citrus limon* (Rutaceae) | Citric acid, ascorbic acid, flavonoids | Fruit, Seed | Kisra lemon anti-dandruff hair care combo; Mamaearth lemon anti-dandruff shampoo; Soulflower lemon essential oil | 63-64 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | Dill | *Anethum graveolens* (Apiaceae) | Phellandrone, eugenol, anethole, flavonoids, coumarins, triterpenes, phenolic acids, umbelliferones | Seed | Radiv anti-fungal hair oil; Glowwelle dandruff control hair serum; Bioayurveda anti-fungal and anti-allergy creme | 65-66 |
| 10 | Olive | *Olea europaea* (Oleaceae) | Triglycerides, phenols, flavonoids, oleocanthal, tyrosol | Fruit, Seed, Leaf | Khadi organique olive oil; Palmers olive oil formula deep conditioner pack; Mamaearth Coco soft massage oil for babies | 67-68 |
| 11 | Guava | *Psidium guajava* (Myrtaceae) | Vitamin A, C, Fe, P, Ca, saponin, oleanolic acid, lyxopyranoside, asabopyraneside, guaijavarin | Leaf | Vriddhi guava leaf hair oil; Foressence apple-guava hair cleanser for dry hair; Oziva plant-based collagen builder | 69-70 |
| 12 | Rosemary | *Salvia rasmerinus* | Cineole, piene, camphor, camphene, borneol | Leaf, Twig | Bubblefarm rosemary | 71-72 |

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|---|---|---|---|---|---|---|
| | | (Lamiaceae) | | | hydrosol and hair oil; Avimee herbal rosemary hair oil; Orbellaa rosemary water hair spray | |
| 13 | Tea tree | *Malaleuca alternifolia* (Myrtaceae) | Terpinen-4-ol, terpinene, cymene, terpineol, limonene | Leaf | Newish tea tree essential oil; Mamaearth tea tree shampoo, hair mask, and conditioner; Body Gold Herbal Essentials anti-dandruff shampoo and conditioner | 73-74 |
| 14 | Aloe vera | *Aloe barbadensis miller* (Liliaceae) | Aloetin A, B, phytosterol, saponins, vitamins, lignins, amino acids, proteins | Leaf, Rind | Pilgrim's ultimate healthy and happy hair kit; Aloe global aloe vera shampoo; Bio-organic pure aloe vera gel; Himalayan Organics aloe | 75 |

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|---|---|---|---|---|---|---|
| | | | | | vera shampoo and conditioner | |
| 15 | Jojoba | *Simmondsia Chinensis* (Simmondsiaceae) | Sterols, vitamins, trigylcerides | Seed, Root | Organic netra jojona oil; Dr Batra's hair oil; Cliganic jojoba hair oil | 76-77 |
| 16 | Onion | *Allium cepa* (Amaryllidaceae) | Allicin, quercetin, fisetin, diallyl disulfide, diallyl trisulphide | Root, Peel, Bulb | Ayur ever red onion hair oil; Lotus botanicals red onion hair care range; Sesa onion hair growth and damage repair kit; Mamaearth onion hair care kit | 78-79 |
| 17 | Pumpkin | *Cucurbita maxima* (Cucurbitaceae) | Na, Ca, K, P | Seed, Leaf, Pulp, Peel, Rind | Herbins pumpkin seed oil; Young chemist pumpkin seed oil; Cosmo organic neem butter, pumpkin, and ginger hair mask | 80-81 |

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|---|---|---|---|---|---|---|
| 18 | Thyme | *Thymus vulgaris* (Lamiaceae) | Minerals-K, Ca, Mg, Se, Fe, Mn, Vitamins-A, C, B, K | Flower, Leaf | Gowoo thyme essential oil; Jovees herbal thyme and Tee Tree anti-dandruff shampoo and conditioner; BRM herbals thyme essential oil | 82-83 |
| 19 | Turmeric | *Curcuma longa* (Zingiberaceae) | Curcumanoids, sesqueterpienes, ketones, alcohol | Dried rhizome | Healthgenix pure turmeric oil; Trichoturm dietary supplement; Volamena organics turmeric hair growth scalp scrub | 84-86 |
| 20 | Honey | *Apis mellifera* (Apidae) | Glucose, fructose, formic acid, dextrin, proteins, vitamins, enzymes | Liquid fluid | Herbal khadi sandalwood honey natural herbal shampoo and conditioner; RHRI psoriasis, eczema, Seborrheic | 87-88 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | dermatitis shampoo; Bioorganic hair regrowth pack | |
| 21 | Amla | *Phyllanthus Emblica* (Phyllanthaceae) | Emblicanin, A and B, pungigluconin, chebulagic acid, phyllantine, phyllembein, gallic acid, glutamic acid, cysteine, lysiene, pectin, ascorbic acid, citric acid | Fruit, Seed, Bark, Leaf, Root, Flower | Earth organic Ayurvedic and organic Amla hair oil; Nutriol Amla extract for skin and hair care; Adivasi herbal hair oil; Vedix Ayurvedic hair care regimen | 89-90 |
| 22 | Cinnamon | *Cinnamomum verum* (Lauraceae) | Cinnamaldehyde, cinnamic acid, eugenol, cinnamate, cinnamyl alcohol, volatile oils, monoterpenes, sesquiterpenes, phenyl propenes | Bark, Leaf | Vedsun cinnamon oil; J. K. Darchini oil; Healthgenix cinnamon essential oil | 91-92 |
| 23 | Grapefruit | *Citrus xparadise* (Rutaceae) | Lycopene, cryptoxanthin, lutein, zeaxanthin, naringenin, narirutin, naringin, | Peel, Seed | Actizeet grapefruit essential oil; Laritelle organic shampoo; | 93 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | hesperidin, didymium, poncirin | | Head and shoulder deep cleanse anti-dandruff shampoo | |
| 24 | Goldenseal | *Hydrastis Canadensis* (Ranunculaceae) | Berberastine, hydrastinine, canadine | Root, Rhizome | Botanica goldenseal liquid herb; Nature's Answer alcohol-free goldenseal root extract; Oneka goldenseal and citrus shampoo | 94 |
| 25 | Echinacea | *Echinacea purpurea* (Asteraceae) | Glycosides, achinacosides, echinacin, polysaccharides, caffeic acid, essential oils, flavonoids, volatile oils, vitamins, minerals | Leaf, Flower, Root | Bio India echinacea plus tonic; Biotic Echinacea capsules; Alvia echinaceae oil; Nature's Way echinacea and vitamin C vegan capsule | 95 |
| 26 | Geranium | *Geranium* | Citronellol, linalool, citronellyl formate, p-menthone, | Leaf, Stalk, Flower | Divine aroma geranium essential oil; | 96 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *graveolens* (Geraniaceae) | tannins, flavones, organic acids, lignans, steroids, triterpenoids | | Lifemantraa essential oil; Keya Seth aromatherapy geranium therapeutic essential oil | |
| 27 | Eucalyptus | *Eucalyptus globulus* (Myrtaceae) | Cineole, piene, eucalyptol, hydrocyanic acid, cymene, terpinene, methyl amyl acetate, aromadendrene, viridiflorol | Leaf | Camveda pure eucalyptus essential oil; Froot root flake fixer; Vaadi herbals eucalyptus essential oil | 97 |
| 28 | Calendula | *Calendula officinalis* (Composite) | Flavonoids, carotenoids, coumarins, triterpenoids, glycosides, saponins, volatile oils, amino acids, steroids, sterols, quinine | Flower petals | Nature 4 Nature growth potion hair oil; California baby calendula shampoo and body wash with hair conditioner; Lemora Cosmetics calendula oil | 98 |
| 29 | Fenugreek | *Trigonella foenum-gracum* | Flavonoids, amino acids, saponins, | Seed, Leaf | MeriBana Khadi and Silky anti- | 99 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | (Fabaceae) | alkaloids, soluble fibers | | dandruff shampoo; Khadi Organique Fenugreek hair cleanser; Khadi Natural onion and fenugreek shampoo | |

**1. FORMULATION OF HERBAL GEL**

## 2.1 MATERIALS AND METHOD

### 2.1.1 Collection of plant material

The plant *Psidium guajava, Azadiracta indica, Emblica officinalis, and Aloe barbadensis* was collected from the HCP, campus, Sonipat. The fresh leaves and seeds were separated from the plant and used for the extraction.

### 2.1.2 Preparation of leaf extract of *Psidium guajava and Azadiracta indica*

The collected fresh leaves of *Psidium guajava* were washed with water and dried in shade (100). After drying plant leaves were coarsely powdered and kept in well closed container. 20gm of each plant's coarse powder leaf was weighed and soaked in 200ml of water and left for maceration for about 4-5 days. After maceration the extract was concentrated and used for further formulations (101).

### 2.1.3 Preparation of fruit extract of *Emblica officinalis*

The collected fresh fruit of *Emblica officinalis* were washed with and dried. After drying the fruits after removal of seeds were blended in a mixer or juicer (102). After proper blending the extract was strained and used for further formulation.

### 2.1.4 Preparation of leaf extract of *Aloe barbadensis*

The collected fresh leaves of *Aloe barbadensis* were washed and dried. After drying the leaves were peeled and the fresh gel is extracted. The gel extract was used for further formulation (103).

### 2.1.5 Formulation of Herbal anti-fungal gel formulation for dandruff

The herbal anti-fungal gel formulations for dandruff were prepared by simple gel formulation preparation method with carbopol gel base (104-107). The gel formula contains phenoxyethanol, polyethylene glycol (PEG), carbopol 940, and triethanolamine. Carbopol 940 four grams and measured quantity of extracts was dispersed in 80ml of distilled water and mixed by stirring continuously in magnetic stirrer at 800rpm for 1h or by manually. The mixture was neutralized by drop wise addition of triethanolamine. Mixing was continued until a transparent gel was formed. The concentrations as shown in Table 2 were incorporated in carbopol base gel and prepared herbal anti-fungal gel formulations for dandruff.

**Table 2. General formula for Herbal Anti-fungal Gel Formulation for Dandruff**

| Ingredients | Quantity taken |
|---|---|
| Carbopol 940 | 4.0gm |
| Propylene glycol | 10.4ml |
| Phenoxyethanol | 0.1% |
| Triethanolamine | 1-2 % |
| Distilled water | Q.S. |

**Table 3. Development of Herbal Anti-fungal Gel Formulation for Dandruff**

| Ingredients | Quantity taken (100ml) |
|---|---|
| *Azadiracta indica* extract | 2.5ml |
| Psidium guajava extract | 2.5ml |
| *Emblica officinalis* extract | 1ml |
| *Aloe barbadensis* extract | 1ml |
| Carbopol 940 | 4.0gm |
| Propylene glycol | 10.4ml |
| Phenoxyethanol | 0.1% |
| Triethanolamine | 1-2 % |
| Distilled water | Q.S. to 100% |

**2.2 Evaluation of Herbal Anti-fungal Gel Formulation for Dandruff**

2.2.1 **Physical Examination -** All the formulated herbal gels were checked for colour, texture, lumps formation, smoothness and homogeneity by visual observation (108-111).

**2.2.2 PH-** The pH of all the formulated herbal gels was measured by using digital pH meter or pH strips (112-115).

**2.2.3 Viscosity-** Viscosity of herbal gels was determined by using Brookfield rotational viscometer at 100rpm using spindle no. 64 (116-118).

**2.2.4 Spreadability-** The spreadability of gel formulation was determined by measuring the spreading diameter of 1gm of gel between two horizontal plates (119-125).

**Procedure**

For the determination of spreadability excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5 min.

Weight (240gm) was added to the pan.

The time required separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability (s).

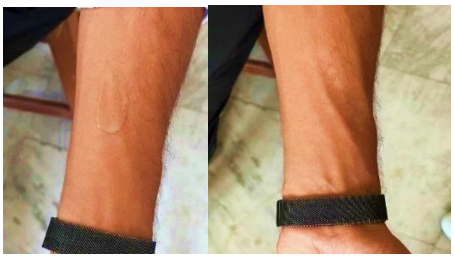
$S = m * l / t$

Where m = weight tide to upper slide

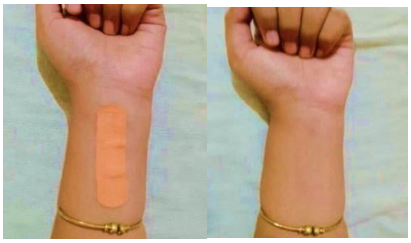
l = length moved on the glass slide

t = time taken.

**2.2.5 Skin irritation test -** Apply a small amount of gel to the skin of volunteers or animal models. Observe for signs of redness, irritation, or allergic reactions over 24 to 48 hours (126-128).



**Patch test-** Apply the gel to a small patch of skin and cover with a bandage. Observe for any adverse reactions over 48 hours (129-131).



## 2. RESULTS AND DISCUSSION

All the formulations of herbal hair gels were studied for colour, homogeneity, pH, viscosity, color, texture, lumps formation, skin irritation, patch test, and spreadability (132). The results are given in Table 4.

**Table 4. Evaluation parameters and their results**

| Parameters | Observations |
|---|---|

| | |
|---|---|
| Appearance | Gel |
| Color | Olive Green |
| Texture | Soft and Uniform |
| Smoothness | Smooth |
| Lumps formation | No lumps formation |
| Homogeneity | Homogeneous |
| pH | 5.7 |
| Viscosity | 2500cps |
| Spread-ability | Good spread-ability |
| Skin irritation | No skin irritation |
| Patch | No skin irritation |

The antifungal activity of herbal gels was done by cup plate method. The gels were tested against *Candida albinos*. A loopful of the pure fungal culture was suspended in nutrient broth and incubated for 24 hours. Nutrient agar media was sterilized, inoculated with *C. albicans* and poured into petri plates. After solidification, bore was made with the help of borer and different concentrations of herbal gels were added. A marketed preparation acts as control. Later, the zone of inhibition around the bore was measured and recorded (133).

## 3. CONCLUSION

On the basis of prepared herbal anti-fungal gel for dandruff (prepared with *Psidium guajava, Azadirachta indica, Emblica officinalis, and Aloe barbadensis)* and their evaluation studies, it is concluded that: the gel gives no local irritation, the gel is stable with the pH of scalp, the gel is highly viscous, the gel is maintaining the homogeneity, the gel smoothens the texture of scalp and provide moisture, the gel can be used for any type of scalp condition. Hair gels containing natural ingredients and herbal extracts have potential effects in controlling hair fall and removing dandruff. Herbal hair gels help to overcome the various damages caused by chemical agents in various marketed products. The prepared product was

tested for its antifungal activity using test organism, which causes dandruff and various scalp problems. Herbal hair gel containing herbal plant extract is a solution for nourishing hair, treating dandruff and other scalp problems. The products can be manufactured in large scale and commercialized as an alternative for chemical hair gels.

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# Studies of a Potential Drug Target for the Hypertension Agent Arjuna indica: Physicochemical and Pharmacokinetic Analysis

**Pawan Verma1*,Garima Awasthi2**
1*Department of Pharmaceutical Chemistry, Goel institute of Pharmacy & Sciences, Faizabad Road , Near Indira Canal, Luck now, U.P., India, Pin- 226028
2Department of Pharmaceutical Chemistry, Goel institute of Pharmacy & Sciences, Faizabad Road , Near Indira Canal, Luck now, U.P., India, Pin- 226028
***Corresponnding Author** Email Id: pawanvermap54@gmail.com

**ABSTRACT**

The well-known medicinal plant arjuna indica has been the subject of much research due to its antihypertensive qualities. In order to improve Arjuna indica's therapeutic efficacy in the management of hypertension, this study explores the physicochemical and physiological properties of putative pharmacological targets. We assessed the solubility, security, and permeability of the active chemicals extracted from the plant using a number of assays. In vitro models were utilized to evaluate the pharmacokinetic profiles in order to ascertain the characteristics related to absorption, distribution, metabolism, and elimination (ADME). According to our research, several substances have good physicochemical characteristics that point to their potential as therapeutic agents and high bioavailability. The analysis also identifies certain processes by which these chemicals function to lower blood pressure, indicating potential directions for future clinical research. With implications for the creation of fresh pharmaceutical treatments, this study advances our knowledge of Arjuna indica as a potential natural medicinal agent in the management of hypertension.

**Keywords:** Hypertennsion, Arjuna indica, ADMET

## INTRODUCTION

The plant Terminalia arjuna or arjuna, is a member of the Combretaceae family. Based on the observations of ancient physicians dating back centuries, its bark decoction is used on the Indian subcontinent for anginal discomfort, hypertension, congestive heart failure, and dyslipidemia[1]. More research is required to determine the effectiveness of arjuna in treating different cardiovascular conditions [2]. Consequently, the goal of this review is to provide a thorough overview of the literature that summarizes the clinical and experimental research relevant to arjuna in cardiovascular problems, especially that conducted in the past

ten years. β-sitosterol, flavonoids, glycosides, and triterpenoids are among its beneficial phytoconstituents [3]. Its favorable antioxidant cardiovascular activities are thought to be attributed to flavonoids and triterpenoids. The medication's impact on ischemic cardiomyopathy has been encouraging. Arjuna treatment has not yet been associated with any significant negative effects [4]. Nevertheless, more research is needed to determine its long- term safety. The precise role it plays in primary/secondary coronary prevention remains unknown, despite its demonstrated efficacy in treating angina pectoris, moderate hypertension, and dyslipidemia. The bark is useful in treating fractures, ulcers, leukorrhea, diabetes, anemia, cardiopathy, and cirrhosis [5]. It has been classified as an astringent, demulcent, expectorant, cardiotonic, styptic, antidysenteric, and urinary astringent. It has been demonstrated that arjuna lowers blood pressure in both people and animals. One of the main risk factors for kidney disease, cardiovascular disease, and other chronic illnesses is hypertension, or high blood pressure. Many bioactive substances found in T. arjuna bark extract, such as triterpenoids, tannins, and flavonoids, are thought to be involved in the plant's ability to decrease blood pressure[6].

## AIMS & OBJECTIVES

- To perform in silico screening of natural bioactive compounds of Terminalia arjuna against Hypertension.
- To draw the 2D chemical structure of bioactive compound of Terminalia arjuna using chemSketch tool.
- To calculate ADME properties of natural bioactive compounds of Terminalia arjuna using SwissADME webtool.
- To calculate toxicity profile of natural bioactive compounds of Terminalia arjuna using ProTox III tool.

## MATERIALS & METHODS

### Retrieval of Phytochemical Compounds

The selection may be based on an examination of the literature. IMPPAT (Indian Medicinal Plants, Phytochemicals, and Therapeutics) provided the phytochemical bioactive components of Terminalia arjuna along with their molecular formula, PubChem ID, and SMILE (Simplified Molecular Input Line Entry System).

**2D Molecular Structure**

The 2D structure of bioactive compounds is drawn by using Chemsketch tool with help of SMILE. The 2D structure of Terminalia arjuna which are represented in Figure 1.

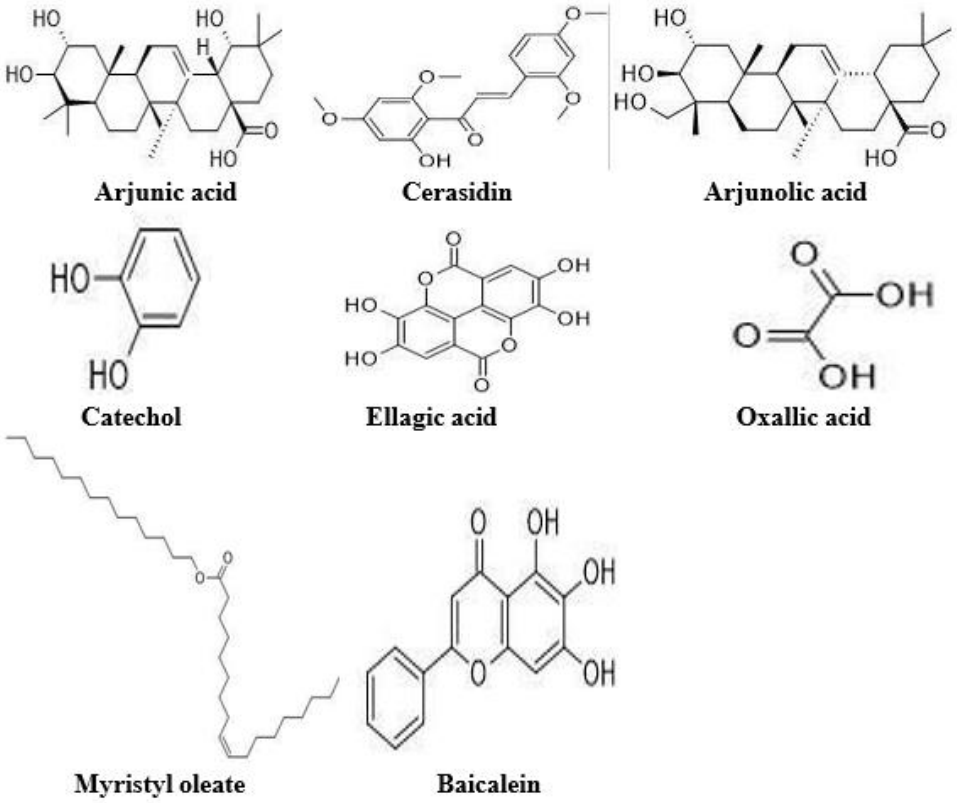


**Figure 1 The 2D Structure of bioactive compounds Physiochemical Properties**

SwissADME predicted physicochemical characteristics such as molecular weight, topological polar surface area, lipophilicity (logPO/w), % absorption, number of hydrogen bond donors/number of hydrogen bond acceptors, and drug similarity. The percent absorption (% Abs) of the ligands was calculated using the formula. Abs. % = 109-(0.345*TPSA)

Pharmacokinnetic Properties

Pharmacokinetics includes the absorption, distribution, metabolism, and excretion (ADME) of drugs in the body. Understanding these properties is necessary to evaluate the potential safety and efficacy of therapeutic compounds derived from Terminalia arjuna against breast cancer. SwissADME assessed the pharmacokinetic characteristics, including GI absorption, BBB penetration, P-gp substrate, cytochrome-P enzyme inhibition, and skin penetration (log Kp).

**Toxicity Prediction**

The ProTox 3.0 server was used to assess the phytochemicals of Terminalia arjuna for toxicity class and level of toxicity (LD50, mg/kg) as well as toxicological endpoints like hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutationagenicity, cytotoxicity, ecotoxicity, clinical toxicity, and nutritional toxicity.

**RESULTS AND DISCUSSION**

**Analysis of physiochemical properties**

Physiochemical characteristics play a major impact on drug likeness and oral bioavailability. Using the SwissADME webtool and SMILE, the physiochemical characteristics such as molecular weight, number of hydrogen bond donors and acceptors, log P, number of rotable bonds, TPSA (Topological Polar Surface Area), and % absorption are obtained. All phytochemical substances adhere to the RO5 rule (Lipinski Rule of Five) for excellent oral bioavailability and drug similarity. LogP is less than five, the number of hydrogen bond donors is fewer than five, and the number of hydrogen bond acceptors is less than ten, all of which are determined by RO5. All of the bioactive compounds follow the rule of five except Arjunin and Arjunetin which are summarized in table 1.

The TPSA value establishes a good intestinal absorption and good blood-brain barrier penetration are indicated by TPSA values less than 140 $Å^2$ and 60 $Å^2$, respectively. In this study we find that Arjunin, Arjunetin, Ellagic acid have high TPSA value and low percentage absorption and Catechol, Myristyl oleate, have low TPSA value and high percentage absorption. The log Kp value determined the compound is hydrophilic and lipophilic.All of the bioactive compounds are lipophilic except Oxalic acid. The number of rotable bond represents the flexibility of compounds. All of the bioactive compounds have rotable bond except Catechol, Ellagic acid.

**Table - 1 The analysis of physiochemical properties**

| S.NO. | Bioactive compounds | Molecular weight | Num. rotatable bonds | Num. H-bond acceptors | Num. H-bond donors | TPSA | Log Po/w (iLOGP) | Percentage of absorption |
|---|---|---|---|---|---|---|---|---|
| 1 | Arjunin | 934.63 g/mol | 3 | 26 | 15 | 452.00 Å² | 0.94 | -46.94 |
| 2 | Arjunic acid | 488.70 g/mol | 1 | 5 | 4 | 97.99 Å² | 3.31 | 75.19 |
| 3 | Cerasidin | 344.36 g/mol | 7 | 6 | 1 | 74.22 Å² | 3.34 | 83.39 |
| 4 | Catechol | 110.11 g/mol | 0 | 2 | 2 | 40.46 Å² | 1.13 | 95.04 |
| 5 | Arjunetin | 650.84 g/mol | 4 | 10 | 7 | 177.14 Å² | 2.57 | 47.88 |
| 6 | Ellagic acid | 302.19 g/mol | 0 | 8 | 4 | 141.34 Å² | 0.79 | 60.23 |
| 7 | Baicalein | 270.24 g/mol | 1 | 5 | 3 | 90.90 Å² | 2.43 | 77.63 |
| 8 | Myristyl oleate | 478.83 g/mol | 29 | 2 | 0 | 26.30 Å² | 7.79 | 99.92 |
| 9 | Oxalic acid | 90.03 g/mol | 1 | 4 | 2 | 74.60 Å² | -0.35 | 83.26 |
| 10 | Arjunolic acid | 488.70 g/mol | 2 | 5 | 4 | 97.99 Å² | 3.11 | 75.19 |

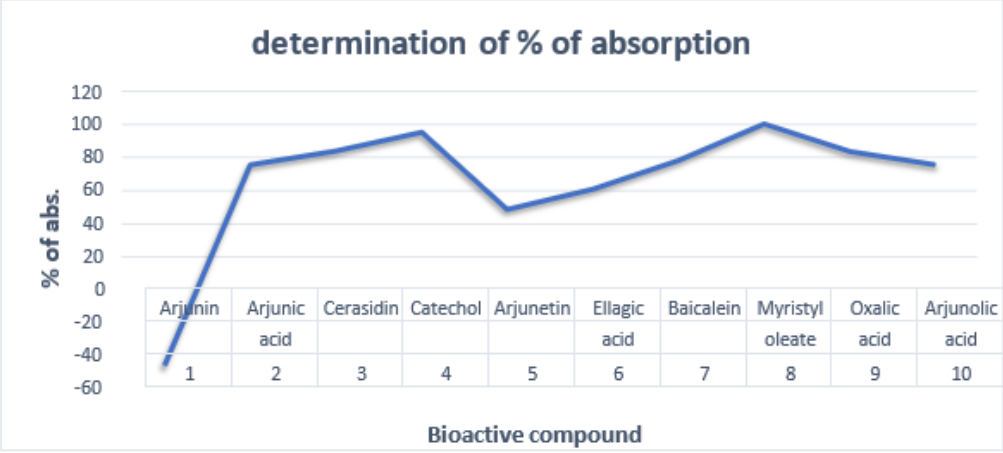


**Figure 2 Graphical representation of % absorption**

**Analysis of Pharmacokinetics Properties**

Using SMILE, the Swiss ADME webtool was used to assess the pharmacokinetic characteristics of phytochemical substances, including GI absorption, BBB penetration, P-gp substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, and LogKp (cm/s).All of the bioactive compounds shows high GI absorption except Arjunin, Arjunetin, Myristyl oleate.

Cerasidin, Catechol is permeating to BBB and rest of the bioactive compounds do not permeate the BBB. The result showed that all of the bioactive compounds showed Arjunin, Arjunic acid, Arjunetin, Myristyl oleate, and Arjunolic acid are P-gp transporter and rest of the bioactive compounds do not P-gp transporter. Ellagic acid, Baicalein were found inhibitors for CYP1A2 inhibitor and rest of the compounds were not inhibitors for CYP1A2.All of the bioactive compounds were not found inhibitors for CYP2C19 inhibitor and CYP2C9 inhibitor except Cerasidin. In this study we find that Baicalein were found inhibitors for CYP2D6 inhibitor and rest of the bioactive compounds were not found inhibitors for CYP2D6. Cerasidin, Catechol, Baicalein were found inhibitors for CYP3A4 inhibitor and rest of the compounds were not found inhibitors for CYP3A4. In this study we analyzed that Arjunin shows highest skin permeation and Myristyl oleate shows lowest skin permeation.

**Table-2 The analysis of Phramcokinetics properties**

| S. No. | Bioactive compounds | GI bsorption | BBB permeant | P-gp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | Log Kp(skin permeati |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Arjunic acid | high | no | yes | no | no | no | no | no | -5.61 |
| 2 | Cerasidin | high | yes | no | no | yes | yes | no | yes | -5.72 |
| 3 | Catechol | high | yes | no | no | no | no | no | yes | -6.35 |
| 4 | Ellagic acid | high | no | no | yes | no | no | no | no | -7.36 |
| 5 | Baicalein | high | no | no | yes | no | no | yes | yes | -5.7 |
| 6 | Myristyl oleate | low | no | yes | no | no | no | no | no | 0.89 |
| 7 | Oxalic acid | high | no | no | no | no | no | no | no | -7.03 |
| 8 | Arjunolic acid | high | no | yes | no | no | no | no | no | -5.13 |

**Prediction of toxicity**

The assessment of phytochemical substances' toxicity profiles with the aid of SMILE, ProTox 3.0 assessed the toxicity of phytochemicals. Hepatotoxicity, neurotoxicity, respiratory toxicity, cardiotoxicity, nephrotoxicity, carcinogenicity, mutagenicity, cytotoxicity, immunotoxicity, clinical toxicity, nutritional toxicity, toxicity class, and LD50 value are some of the several kinds of toxicity endpoints.

In this study we found that all of the bioactive compounds are inactive for Hepatotoxicity and Neurotoxicity. Cerasidin, Ellagic acid, Baicalein, Oxalic acid are active for nnnephrotoxicity.

**Table-3 The analysis of Toxicity Profile**

| S. No. | Bioactive compounds | Hepatotoxicity | Neurotoxicity | Nephrotoxicity | Respiratory toxicity | Cardiotoxicity | Carcinogenicity | Immunotoxicity | BBB-barrier | LD50(mg/kg) | Toxicity Class |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Arjunic acid | inactive | inactive | inactive | active | active | active | active | active | 2000mg/kg | 4 |
| 2 | Cerasidin | inactive | inactive | active | inactive | inactive | inactive | active | active | 3000mg/kg | 5 |
| 3 | Catechol | inactive | inactive | inactive | inactive | active | active | inactive | inactive | 100mg/kg | 3 |
| 4 | Ellagic acid | inactive | inactive | active | active | inactive | active | inactive | active | 2991mg/kg | 4 |
| 5 | Baicalein | inactive | inactive | active | active | inactive | active | inactive | active | 3919mg/kg | 5 |
| 6 | Myristyl oleate | inactive | inactive | inactive | inactive | inactive | active | inactive | active | 339mg/kg | 4 |
| 7 | Oxalic acid | inactive | inactive | active | inactive | inactive | inactive | inactive | active | 660mg/kg | 4 |
| 8 | Arjunolic acid | inactive | inactive | inactive | active | active | inactive | inactive | active | 2000mg/kg | 4 |

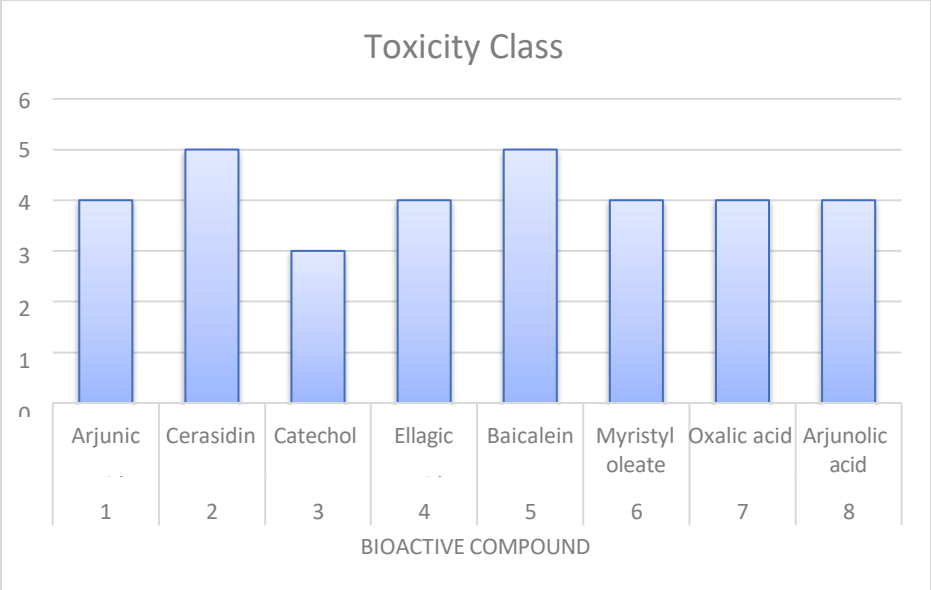


**Figure 3 Graphical representation of toxicity class**

## CONCLUSION

The primary duty of the drug design and development process is the evaluation of pharmacokinetic and physicochemical characteristics. Ten phytochemicals were assessed in this study using in silico screening techniques based on ADMET characteristics. According to evaluations of in silico computer studies, such as ADME, all phytochemicals have the highest GI absorption and follow the RO5 rule for oral medication bioavailability. Ellagic acid and Arjunic acid have a toxicity class of 4, which is innocuous, and they exhibit good intestinal absorption. According to our research, Ellagic acid and Arjunic acid may be potential agent for hypertension. We will molecularly dock these molecules in the future to see which ligand-protein interactions and binding energies work best.

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**Conflict of interest**

Every author affirms that there isn't a conflict of interest.

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# A Computational Approach to Medication Design: Illustrating The Physiochemical and Pharmacokinetic Characteristics of Linum Usitatissimum Against Breast Cancer"

**Prakriti Maurya1*Salil Tiwari2**
1*Department of Pharmaceutical Chemistry, Goel institute of Pharmacy & Sciences, Faizabad Road , Near Indira Canal, Luck now, U.P., India, Pin- 226028
2Department of Pharmaceutical Chemistry, Goel institute of Pharmacy & Sciences, Faizabad Road , Near Indira Canal, Luck now, U.P., India, Pin- 226028Corresponding
*Corresponnding Author Email Id: pkt99maurya@gmail.com

## ABSTRACT

This study explores a computational approach to the design of a novel medication derived from Linum usitatissimum (flaxseed) for the treatment of breast cancer. L. usitatissimum is commonly referred to as flax and linseed and its Linaceae family. We examine the physiochemical properties and pharmacokinetic characteristics of bioactive compounds extracted from flaxseed. We employ ADMET analysis to predict the absorption, distribution, metabolism, excretion, and toxicity of these compounds with key cancer-related targets. Our findings indicate that certain lignans and fatty acids in Linum usitatissimum exhibit promising anti-cancer activities. We are using the SwissADME webtool for the ADME study and ProTox 3.0 for the toxicity profile. Over the past fifty years, ADME has played a significant part in the drug design process. Time and money could be saved by evaluating these qualities early in the process.Furthermore, we analyze their absorption, distribution, metabolism, and excretion (ADME) properties, confirming that lotaustralin and pantothenic acid are potential agents for effective systemic delivery. This research underscores the therapeutic potential of Linum usitatissimum in breast cancer treatment, paving the way for future molecular docking studies to validate binding energy and ligand-protein interactions. Ultimately, this approach may facilitate the development of more targeted and effective therapies for breast cancer, leveraging the rich pharmacological potential of natural compounds.

**Keywords:** Breast Cancer, Linum usitatissimum, ADMET

## INTRODUCTION

The most frequent cause of cancer in women and the second leading cause of cancer- related deaths among women in the United States is breast cancer. Breast cancer is the term used to describe malignancies that start in breast tissue, usually the lobules that provide milk to the ducts or the inner lining of the milk ducts [1]. One of the most prevalent malignancies in women globally, breast cancer claimed around 570,000

lives in 2015. Every year, more than 1.5 million women worldwide receive a breast cancer diagnosis, accounting for 25% of all cancer-stricken women [2]. The two primary tissue types that make up the breast are glandular tissues and stromal (promoting) tissues. The ducts (the milk channels) and the lobules (the milk-producing glands) are located in glandular tissues, whereas the stromal tissues comprise the breast's fatty and fibrous connective tissues. Additionally, the immune system's lymphatic tissue, which eliminates waste products and cellular fluids, makes up the breast [3]. When DNA and/or RNA are altered or mutated, cancer cells are created from healthy cells [4]. A lump in the breast or armpit is the classic indication of breast cancer. Breast cancer can be identified by its general warning signs, which include breast swelling or lump (mass), swelling in the armpit (lymph nodes), clear or bloody nipple discharge, nipple pain, inverted (retracted) nipple, scaly or pitted skin on the nipple, persistent breast tenderness, and unusual breast pain or discomfort [5].

One of the oldest crop plants is flax, sometimes known as linseed. It is part of the Linaceae family and genus Linum. In his book "Species Plantarum," Linnaeus provided the botanical name Linum usitatissimum [6]. In North America and Asia, L. usitatissimum is commonly referred to as flax and linseed, respectively. This species has developed specific oilseed and fiber variants [7]. In addition to their various nutritional benefits, flaxseeds are rich in short-chain omega-3 fatty acids. Flax is grown mostly for its fiber, but its edible seeds can also help prevent diabetes, cancer, heart disease, and strokes. [8]. Flaxseed has 40–45% fatty acids and 20–25% protein. Since flaxseed also yields vegetable oils, it is also referred to as "linseed oil." Linseed oil is edible oil that is used in medicine and is regarded as one of the first commercial oils [9]. It has been demonstrated that α-linolenic acid possesses anti-inflammatory properties and antiproliferative effects in animal models of premenopausal (high estrogen) breast cancer [10]. In the Women’s Healthy Eating and Living Study, a large intervention study, a dietary pattern high in fiber was found to lower the risk of breast cancer recurrence, most likely through effects on hormone metabolism and disposal [11]. According to recent research, flaxseed may have anti-inflammatory and hormone-activity-modulating properties, among other protective factors, against breast cancer [12]. The purpose of this work is to assess the physiochemical and pharmacokinetic properties of bioactive substances in Linum usitatissimum by utilizing computational methodologies. We want to clarify the potential of these compounds as therapeutic agents against breast cancer by ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling.

**AIMS & OBJECTIVE**

- To perform in silico screening of natural bioactive compounds of Linum usitatissimum against breast canncer.
- To draw the 2D chemical structure of bioactive compound of Linum usitatissimum using chemSketch tool.
- To calculate ADME properties of natural bioactive compounds of Linum usitatissimum using SwissADME webtool.
- To calculate toxicity profile of natural bioactive compounds of Linum usitatissimum using ProTox III tool.

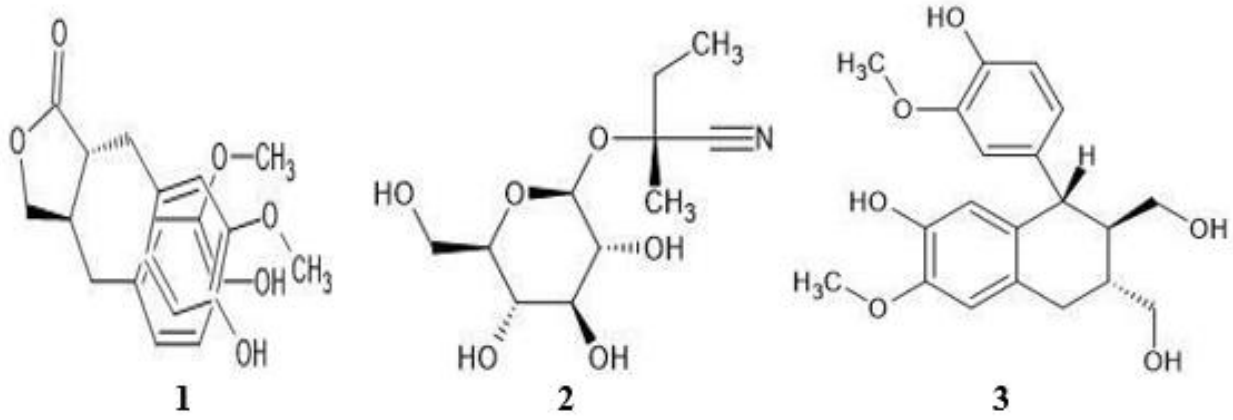
## MATERIALS & METHODS

### Restitution of Phytochemical Compounds

Determine and choose the phytochemicals in Linum usitatissimum that may be used to treat breast cancer. A literature review may serve as the basis for this choice. Linum usitatissimum phytochemical bioactive substances were obtained from IMPPAT (Indian Medicinal Plants, Phytochemicals, and Therapeutics) along with their molecular formula, PubChem ID, and SMILE (Simplified Molecular Input Line Entry System).

### The 2D Molecular Structures of Phytochemical Compounds

Linum usitatissimum Phytochemicals are examined for their molecular structures, which are depicted in Figure 1. The two-dimensional structure of the phytochemicals of Linum usitatissimum was drawn using the chemsketch tool.

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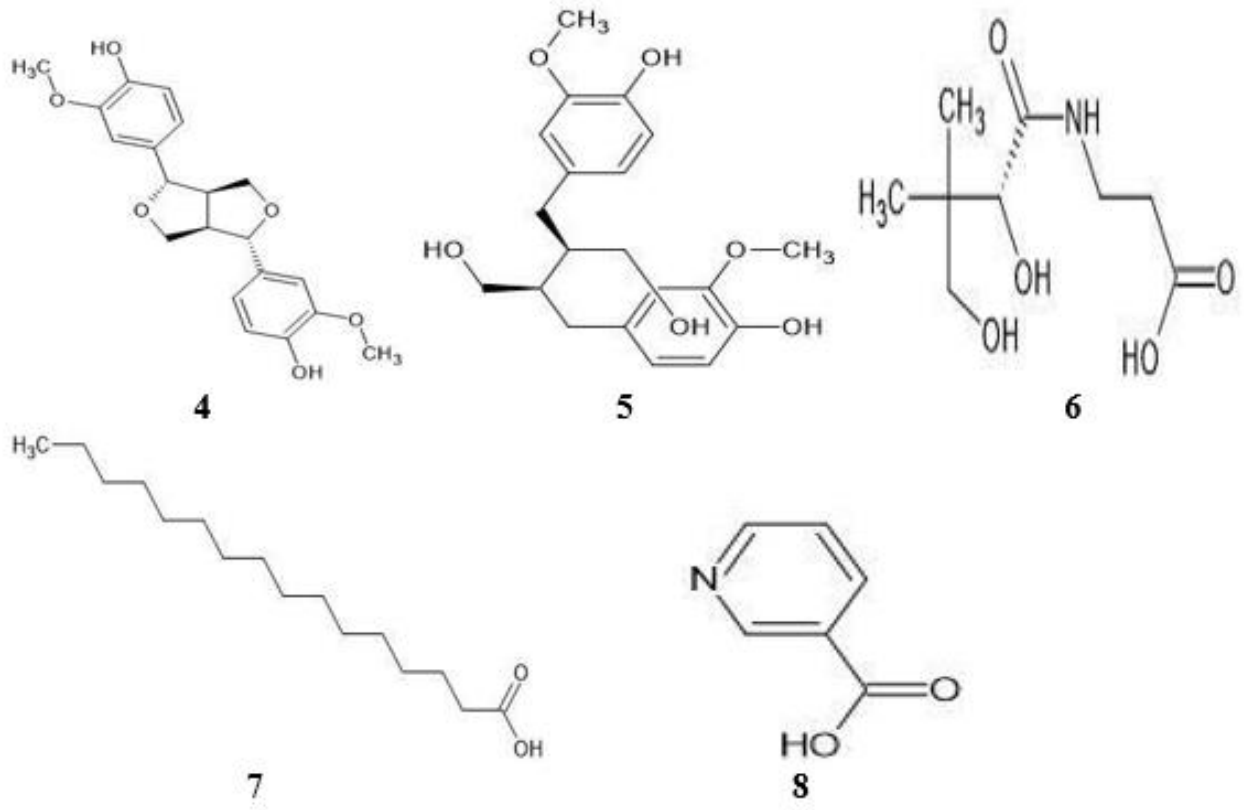


**Figure1 The 2D structure of phytochemical compounds**

The number 1 to 8 stands for 1= Matairesinol, 2=Lotaustralin, 3=Isolariciresinol, 4=Pinoresinol, 5=Secoisolariciresinol, 6=Pantothenic acid, 7=Palmitic acid, 8=Palmitic acid.

The evaluation of Physiochemical Characteristics

The physicochemical properties like molecular weight, topological polar surface area, number of hydrogen bond donors/number of hydrogen bond acceptors, lipophilicity (logPO/w), percentage absorption, and drug likeness were predicted by SwissADME. The formula was used to determine the ligands' percent absorption (% Abs). [% Abs = 109-(0.345*TPSA)].

**The evaluation of Pharmacokinetic Characteristics**

The absorption, distribution, metabolism, and excretion (ADME) of substances within the body are all included in pharmacokinetics. Assessing the possible safety and effectiveness of therapeutic compounds made from Linum usitatissimum against breast cancer requires an understanding of these traits. The pharmacokinetic properties like GI absorption, BBB permeation, P-gp substrate, cytochrome-P enzyme inhibition, and skin permeation (log Kp) were evaluated by SwissADME.

**The evaluation of Toxicity**

It is essential to comprehend toxicity while assessing the potential of Linum usitatissimum (flaxseed) and its bioactive components as a treatment for breast cancer. The toxicity class and level of toxicity (LD50, mg/kg) as well as the toxicological endpoints such as hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutationagenicity, cytotoxicity, ecotoxicity, clinical toxicity, and nutritional toxicity of the phytochemicals of Linum usitatissimum were evaluated using the ProTox 3.0 server.

**RESULTS AND DISCUSSION**

The Evaluation of physicochemical characteristics and drug-likeness of phytochemical compounds

The ADME-related physicochemical characteristics of phytochemical compounds like molecular weight, number of hydrogen bond donors, and number of hydrogen bond acceptors, log P, number of rotable bonds, TPSA (Topological Polar Surface Area), and percentage absorption are obtained via the SwissADME webtool with the help of SMILE. For the drug likeness and good oral bioavailability, all phytochemical compounds follow the ROF rule (Lipinski Rule of Five). According to ROF, the molecular weight of the compound less than 500 daltons, the number of hydrogen bond donors is less than 5, the number of hydrogen bond acceptors is less than 10, and logP is less than 5. We find that all of the phytochemical compounds follow the ROF rule.

TPSA value determines the drug polarity and lipid solubility. The ideal range of TPSA value for absorption is 60-140 $Å^2$. TPSA value <140 $Å^2$ indicates good intestinal absorption and <60 $Å^2$ indicates good blood-brain barrier penetration. The TPSA values of Lotaustralin, Pantothenic acid are 123.17 $Å^2$, 106.86 $Å^2$, respectively, which indicates good intestinal absorption, and Palmitic acid and Nicotinic acid are 37.30 $Å^2$, 50.19 $Å^2$, respectively, which are summarized in Table 1. The LogP value determines whether the compound is hydrophilic or hydrophobic. The negative value of log P indicates the compound is hydrophilic; a positive value indicates the compound is lipophilic; and a zero value indicates the compound is partitioned equally. In this study, we find that all of the phytochemical compounds are hydrophobic. The number of rotable bonds determines the flexibility that allows free rotation around them. In our study, the number of rotable bonds ranges from 1 to 14. The highest% absorption of phytochemicals like palmitic acid and nicotinic acid and the lowest% absorption like lotaustralin.

**Table-1 The evaluation of physicochemical characteristics**

| S NO. | Phytochemical Compound | Molecular Weight (g/mol) | No. of H-bond donor | No. of H-bond acceptor | log P | No. of rotable bond | TPSA (Å²) | % Absorption |
|---|---|---|---|---|---|---|---|---|
| 1. | Matairesinol | 358.39 | 2 | 6 | 2.47 | 6 | 85.22 | 89 |
| 2. | Lotaustralin | 261.27 | 4 | 7 | 1.40 | 4 | 123.17 | 66.5 |
| 3. | Isolariciresinol | 360.40 | 4 | 6 | 2.37 | 5 | 99.38 | 74.7 |
| 4. | Pinoresinol | 358.39 | 2 | 6 | 2.67 | 4 | 77.38 | 82.3 |
| 5. | Secoisolariciresinol | 362.42 | 4 | 6 | 2.77 | 9 | 99.38 | 74.7 |
| 6. | Pantothenic acid | 219.23 | 4 | 5 | 0.95 | 7 | 106.86 | 72.1 |
| 7. | Palmitic acid | 256.42 | 1 | 2 | 3.85 | 14 | 37.30 | 96.1 |
| 8. | Nicotinic acid | 123.11 | 1 | 3 | 0.86 | 1 | 50.19 | 91.6 |

**The Evaluation of Pharmacokinetics characteristics of phytochemical compounds**

The pharmacokinetic properties like GI absorption, BBB permeant, P-gp substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, and LogKp (cm/s) of phytochemical compounds were determined by the SwissADME webtool with the help of SMILE. In this study, we analyzed that all of the phytochemical compounds show the highest GI absorption, which is represented in Table

For the distribution of drugs, we determined the BBB permeant in which all of the phytochemicals do not permeate BBB except pinoresinol, palmitic acid, and nicotinic acid. The P-gp substrate was determined for the drug absorption and excretion.

In this study, we find that all of the phytochemicals are P-gp transporters except Matairesinol, pantothenic acid, palmitic acid, and nicotinic acid. Cytochrome P450 isoforms are very important parmeters for the metabolism of drugs. All of the phytochemicals were not found inhibitors for CYP1A2 inhibitor and CYP2C9 inhibitor except palmitic acid. There is no inhibitor for CYP2C19. Matairesinol, isolariciresinol,

pinoresinol, and secoisolariciresinol were found to inhibitors for CYP2D6 and the rest of all phytochemicals are not inhibitors for CYP2D6. Matairesinol, and pinoresinol were found to inhibitors for CYP3A4, and the rest of all phytochemicals are not inhibitors for CYP3A4.

The LogKp value represents the skin permeability and its range from -8.0 to -1.0 cm/s. A lower log Kp value indicates the lower permeability through the skin. In this study, we found that palmitic acid (92.77 cm/s) shows the highest skin permeation and lotaustralin (9.13 cm/s) shows the lowest skin permeation.

**Table -2 The evaluation of Pharmacokinetics characteristics**

| S. No. | Phytochemical compounds | GI Absorption | BBB permeant | P-gp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | LogKp (cm/s) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Matairesinol | High | No | No | No | No | No | Yes | Yes | -6.17 |
| 2. | Lotaustralin | High | No | Yes | No | No | No | No | No | -9.13 |
| 3. | Isolariciresinol | High | No | Yes | No | No | No | Yes | No | -7.04 |
| 4. | Pinoresinol | High | Yes | Yes | No | No | No | Yes | Yes | -6.87 |
| 5. | Secoisolariciresinol | High | No | Yes | No | No | No | Yes | No | -6.72 |
| 6. | Pantothenic acid | High | No | No | No | No | No | No | No | -8.40 |
| 7. | Palmitic acid | High | Yes | No | Yes | No | Yes | No | No | 2.77 |
| 8. | Nicotinic acid | High | Yes | No | No | No | No | No | No | -6.80 |

**The Evaluation of Toxicity Profile of phytochemical compounds**

The toxicity of phytochemicals was determined by ProTox 3.0 with the help of SMILE. There are various types of toxicity endpoints like hepatotoxicity, neurotoxicity, respiratory toxicity, cardiotoxicity, nephrotoxicity, carcinogenicity, mutagenicity, cytotoxicity, immunotoxicity, clinical toxicity, nutritional toxicity, toxicity class, and LD50 value. All of the phytochemicals are inactive for hepatotoxicity and neurotoxicity except nicotinic acid. In nephrotoxicity, all of the phytochemicals are active except palmitic acid. Isolariciresinol, pinoresinol, and nicotinic acid are active for respiratory toxicity, and the rest of the phytochemicals are inactive for respiratory toxicity.

The result showed that all of the phytochemicals are inactive of cardiotoxicity except pinoresinol. Matairesinol, isolariciresinol, and pinoresinol are active for immunotoxicity, and the rest of the

phytochemicals are inactive for immunotoxicity. In the ecotoxicity, all of the phytochemicals are inactive except palmitic acid. Pantothenic acid is active for clinical toxicity, and the rest of the phytochemicals are inactive for clinical toxicity. In this study, we found that all of the phytochemicals are inactive for carcinogenicity, mutagennicity, cytotoxicity, and nutritional toxicity.

The result showed that LD50 (median lethal dose) ranges from 900 to 29700 mg/kg, which are represented in Table 3. In this study, toxicity class 4 indicates that matairesinol, pinoresinol, secoisolariciresinol, and palmitic acid were harmful, and toxicity class 5 is isolariciresinol. Nicotinic acid may be harmful. Lotaustralin and Pantothenic acid belong to toxicity classes 6, which are nontoxic.

**Table-3 The evaluation of Toxicity Profile**

| S. No. | Phytoconstituents | Hepatotoxicity | Neurotoxicity | Nephrotoxicity | Respiratory | Cardiotoxicity | Carcinogenicity | Immunotoxicity | Mutagenicity | Cytotoxicity | Ecotoxicity | Clinical Toxicity | Nutritional Toxc. | LD 50 (mg/kg) | Toxicity Class |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Matairesinol | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1500 | 4 |
| 2. | Lotaustralin | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 29700 | 6 |
| 3. | Isolariciresinol | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 5000 | 5 |
| 4. | Pinoresinol | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1500 | 4 |
| 5. | Secoisolariciresinol | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2000 | 4 |
| 6. | Pantothenic acid | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 10000 | 6 |
| 7. | Palmitic acid | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 900 | 4 |
| 8. | Nicotinic acid | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3720 | 5 |

Whereas, Inactive=0, active=1

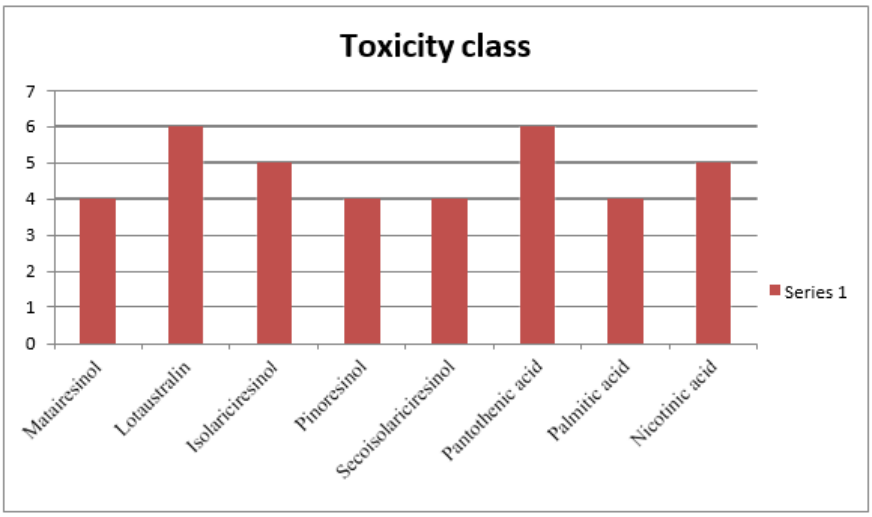


**Figure 2 Graphical representation of toxicity class**

## CONCLUSION

The assessment of pharmacokinetic and physicochemical properties is the main responsibility for the drug design and development process. In this study, 8 phytochemicals were evaluated with in silico screening procedures based on ADMET parameters. The assessment of in silico computer research, such as ADME, showed that all of the phytochemicals have the highest GI absorption and obey the ROF rule for oral bioavailability of drugs. Lotaustralin and Pantothenic acid show good intestinal absorption and their toxicity class is 6, which is nontoxic. Our findings indicate that lotaustralin and pantothenic acid are potential agents for breast cancer. In the future, we will perform molecular docking of these compounds and observe the best binding energy and ligand-protein interactions.

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## Conflict of interest

Every author affirms that there isn't a conflict of interest.

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# In-vitro evaluation of cytotoxic, antioxidant, and anti-inflammatory potential of methanolic extract of *Artemisia vulgaris* on SHSY-5Y cells

**Rahul Kumar[1], Nidhi Tyagi[1*]**

1. SRM Modinagar College of Pharmacy, SRM Institute of Science and Technology (Deemed to be University), Delhi-NCR Campus, Modinagar, Ghaziabad, Uttar Pradesh, 201204, India

**Corresponding Author***

Dr. Nidhi Tyagi
Assistant Professor
Department of Pharmacology,
SRM Institute of Science and Technology
Mail: nidhityagi029@gmail.com, nidhit@srmist.edu.in

**Abstract**

*Artemisia vulgaris* plant has been used as a folk medicine for a long time. Therefore, being rich in flavonoids, terpenoids, and Phyto steroids, it is reported to have various crucial activities, such as antioxidant, anti-inflammatory, analgesic, etc. In the present study, the antioxidant, cytotoxic, and anti-inflammatory ability of the methanolic extract of *Artemisia vulgaris* (AVME) was carried out using SHSY-FY human neuroblastoma cell lines. Following the operation, the aerial parts of *Artemisia vulgaris* were taken and successively extracted by the Soxhlet apparatus using methanol as solvent, and the percentage yield was calculated. A preliminary and phytochemical study was also performed which exhibited the presence of some compounds namely- carbohydrates, proteins, tannins, steroids, glycosides, phenols, and flavonoids. To evaluate the antioxidant capacity, the concentration of phenols and flavonoids was calculated and found to be 179.406 mg GA/gm 136.138 mg QE/gm. DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to determine the antioxidant capacity and the percentage inhibition was noted as 77.83±0.099 % in comparison to ascorbic acid (85.32±0.051 %). Furthermore, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay has revealed a significant reduction in the viability of cells from 200-1200 µg/ml concentration. Besides this, AVME manifested a potent anti-inflammatory activity and antioxidant activity by reducing the IL-6 concentration to 44.10 and 9.87 pg/ml at the dose of 100 and 200 µg/ml and increasing the % SOD (Superoxide dismutase) release at both doses (65.77 and 71.52 %) respectively. Eventually, all these findings suggested that *Artemisia vulgaris* could be a potential aid in the treatment of diseases linked with oxidation and inflammation.

**Keywords:** Artemisia vulgaris; DPPH; Antioxidant; IL-6; and methanolic extract.

## 1. Introduction

Artemisia is a shrub or little plant that thrives in arid and semi-arid environments. This genus is mostly found in the northern hemisphere and less often in the southern hemisphere. The genus Artemisia is a member of the Anthemideae tribe of the Asteraceae family. This genus comprises over 500 species of plants and shrubs (1). Artemisia vulgaris, often known as mugwort, is a perennial plant found in Europe, Asia, and North America. It has long been utilized in herbal medicine for its medicinal characteristics, which include treating digestive issues, and menstrual irregularities, and acting as an anti-inflammatory agent. The plant includes a variety of bioactive chemicals, including flavonoids, terpenoids, and essential oils, which contribute to its therapeutic qualities, including antioxidant, antibacterial, and antispasmodic activity. Artemisia vulgaris is an excellent topic for pharmacological study due to its rich phytochemical profile (2). Antioxidants are essential for controlling inflammation because they neutralize free radicals, which may cause inflammatory reactions. Chronic oxidative stress has been linked to the development of inflammatory illnesses such as arthritis, cardiovascular ailments, and neurological conditions. Antioxidants aid in reducing oxidative damage, which in turn helps to moderate inflammatory pathways and improve cellular health (3). The SH-SY5Y cell line, generated from human neuroblastoma, is often utilized in neurological research because of its propensity to develop into neuron-like cells. It is a useful model for investigating neurodegenerative disorders, neurotoxicity, and neuronal function. SH-SY5Y cells are especially useful for studying pathways behind Parkinson's and Alzheimer's illnesses (4).

## 2. Material and methods

### 2.1.Extract

Plant material (Aerial parts) was procured from Khari Baoli, Delhi, India, and validated at CCS University, Meerut, Uttar Pradesh. The material was washed, shade dried, powdered, and finally extracted by Soxhlet apparatus with methanol. The obtained extract was reduced with a rotary evaporator, weighed, collected in a container, and preserved in a refrigerator for further analysis.

### 2.2.Total phenol and flavonoid content

In plant extract, the concentration of phenols was analysed by the Folin Ciocalteu method used by Singleton et al., 1999 and total flavonoid concentration by the $AlCl_3$ method described by Ebrahimzadeh et al., 2008 (5,6).

### 2.3.Culture of cells

The SHSY-5Y cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), antibiotics, and growth factors at 37°C in a humidified environment with 5% CO2 (7)(8).

### 2.4.DPPH assay

The radical scavenging activity of AVME was assessed by the DPPH method used by Munda et. al., 2019 (9).

### 2.5.MTT Assay

SH-SY5Y cells were cultured at 37°C with 5% $CO_2$ in high-glucose DMEM supplemented with 10% fetal bovine serum. The cytotoxic effects of varying extract concentrations on SH-SY5Y cells were evaluated using the MTT assay reported by Shivani et. al., 2021 (8)(10).

### 2.6.IL-6 measurement

The concentration of interleukin-6 was determined by the method described by Shivani et. al., 2021 (8).

### 2.7.Measurement of SOD-2

The percentage of SOD release was determined with the help of the instructions given by the manufacturer in the kit (11)(10).

### 2.8.Statistical analysis

All the results were interpreted with the help of Graph Pad Prism software. Version 5.01 applying the T-test and One-way ANOVA method accompanied by the Tukey post hoc test. Results are expressed in mean ± SEM with $p<0.05$ probability value.

## 3. Results

### 3.1.Total phenol and flavonoid concentration in AVME

In both estimations, it was found that AVME contained 179.406 mg GAE/g phenols and 136.138 mg QE/g of flavonoids.

### 3.2.Inhibitory actions of AVME on DPPH

Significant percentage inhibition was manifested by AVME (77.84%) in comparison to ascorbic acid (85.32%) ($p<0.001$). (Figure-1)

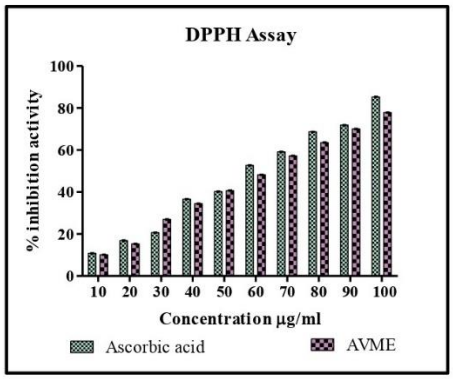


**Figure: 1** Antioxidant activity of AVME.

The sample cuvette contained DPPH solution with AVME in the U.V spectrophotometer and the % inhibition of the test sample was compared by Ascorbic acid at different concentrations. Data were measured by T-test and represented as mean±SEM, $p<0.05$.

### 3.3.Inhibitory actions of AVME on cell viability

The remarkable reduction was exhibited in the percentage of cell viability when treated with AVME at the dose of 200-1200 µg/ml ($p<0.00001$). (Figure-2)

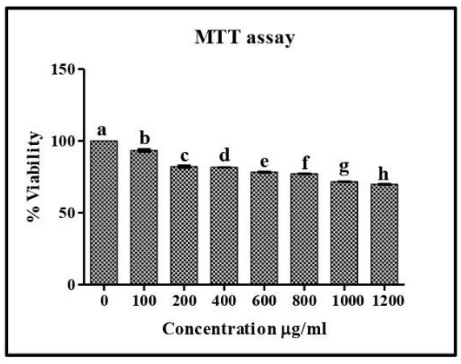


**Figure:2** Cytotoxic effect of AVME on cell viability.

After the treatment of the cells, viability was measured on the basis of the concentration of formazan produced by mitochondrial dehydrogenase enzymes in the cells and compared to the untreated cells. Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. $p<0.05$. *b, c, d, e, f, g, h versus a.*

### 3.4.Inhibitory actions of AVME on IL-6

A notable reduction was observed in IL-6 concentration when cells were treated with AVME at the dose of 100 µg/ml as compared to LPS-treated ones ($p<0.0001$). The AVME **(**200 µg/ml) has also reduced the concentration of IL-6 compared to AVME (100 µg/ml) ($p<0.0001$). (Figure-3)

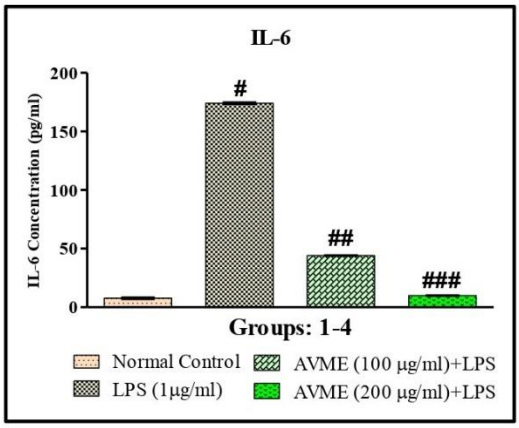


**Figure:3** Inhibitory action of AVME on IL-6 concentration.

Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. *# versus Normal control; ## versus LPS (1 µg/ml); ### versus AVME (100 µg/ml).*

### 3.5.Excitatory actions of AVME on SOD-2 release

In comparison to the normal control group, the LPS-treated cells showed a heavy reduction in the release of SOD ($p<0.0001$). On the other hand, AVME-100µg/ml treated cells significantly increased the % release as compared to the treated group. Along with it, the AVME-200µg/ml group manifested elevation in % release more significantly than AVME-100µg/ml ($p<0.001$). (Figure-4)

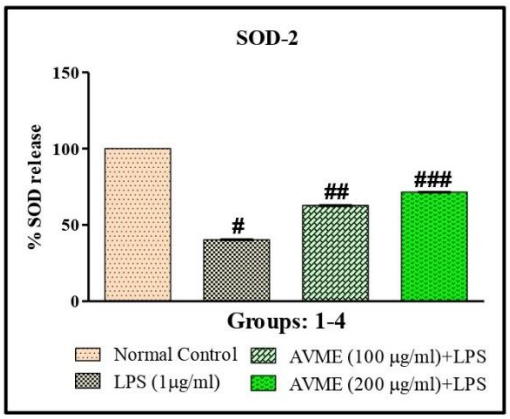


**Figure:4** Protective action of AVME on SOD release.

Group-1 was normal control, and the toxin group (Group-2) was given LPS (1 µg/ml), and both test groups (Group-3 & 4) were treated with AVME (100 and 200 µg/ml). Data were measured by One-way ANOVA followed by Tukey post hoc test and represented as mean±SEM. *# versus Normal control; ## versus LPS (1 µg/ml); ### versus AVME (100 µg/ml).*

## 4. Conclusion

The antioxidant and anti-inflammatory activity of methanolic extract of *Artemisia vulgaris* was evaluated. In the preliminary study, because of the non-aqueous nature of the solvent, the extract showed a good concentration of phenols and flavonoids which are known for their antioxidant and anti-inflammatory actions. Therefore, in the DPPH assay, AVME revealed a notable percentage of radical scavenging activity. Additionally, the extract considerably reduced the concentration of IL-6 and enhanced the % release of SOD at both doses which proclaimed its anti-inflammatory activity. Supplementary studies are needed to identify and find out the concentration of compounds responsible for these actions. All the interpretation has signalized that the aerial parts of *Artemisia vulgaris* could be a better alternative for the treatment of conditions associated with oxidation and inflammation.

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# The Silent Crisis: Understanding Suicide Risk Factors Among College Students in Metropolitan Cities

**S. Vaibhav[*1] & R. Mythreyi[2]**

[*1:] School of Medicine, University of Perpetual Help System Dalta, Jonelta Foundation School of Medicine, Las Piñas, Philippines

[2:] Chettinad School of Pharmaceutical Sciences, Chettinad Hospitals and Research Institute, Chettinad Academy of Research Education, kelambakkam, Tamil Nadu 603 103.

*1: Presenting Author: vaibhav2499jiju@gmail.com

## Abstract

Rates of suicide among college students in urban areas/metropolitan cities has emerged as a significant concern of public health. This review indulges into various factors contributing to this alarming trend, encompassing academic pressures, mental health challenges, social isolation, financial constraints, and failure to meet societal-expectations. The impact of social media, inadequate coping mechanisms, substance abuse, relationship problems, incompetence and limited emotional support are also explored. By understanding the underlying causes, we can develop effective strategies to prevent suicide and promote mental well-being among college students in urban environments.

## Introduction

In this article, we shall discuss about the rising suicide-rates among college students located in urban/metropolitan cities. Three out of four suicides occur in low and middle-income countries, which is a topic of concern. The confluence of academic demands, social pressures, and mental health challenges can create a perfect storm for vulnerable individuals. This review aims to identify and analyze the primary factors contributing to suicide risk among college students in urban settings.

## Purpose/Objectives

To identify the reason for a rise in suicide-rates among college-students, particularly in urban areas.

## Methodology/Approach

Literature review

**1. Academic Pressure and Performance Anxiety**

- Rigorous academic schedules and high expectations
- Fear of failure and parental pressure
- Intense competition for academic honours and job opportunities

**2. Mental Health Issues and Mental Turbulence**

- Prevalence of depression, anxiety, and substance abuse
- Stigma associated with mental illness
- Limited access to mental health services
- Impact of sedentary lifestyle on mental health

**3. Social Isolation and Loneliness**

- Difficulty in forming social connections in urban environments
- Impact of social media on social interactions and self-esteem
- Cultural and societal barriers to seeking help

**4. Financial Constraints and Uncertainty**

- High cost of living in metropolitan areas
- Financial stress and academic performance
- Job insecurity and future uncertainty

**5. Parental and Societal Expectations**

- Pressure to meet unrealistic expectations
- Cultural norms and family dynamics
- Fear of disappointing family and society

**6. Impact of Social Media**

- Social comparison and negative self-perception
- Cyberbullying and online harassment
- Addiction to social media and decreased face-to-face interaction

**7. Inadequate Coping Mechanisms and Life Skills**

- Lack of emotional intelligence and stress management skills

- Difficulty in seeking help and support

**8. Substance Abuse and Risky Behaviour**

- Use of substances as a coping mechanism
- Increased risk-taking behaviour and impulsive decisions

**9. Relationship Problems and Identity Struggles**

- Romantic relationship difficulties
- Family conflicts
- Sexual orientation and gender identity issues

**10. Limited Institutional Support and Awareness**

- Inadequate mental health services on campus
- Lack of awareness and training among faculty and staff
- Stigma associated with seeking help

**Discussion**

The complex interplay of these factors highlights the need for a multi-faceted approach to address suicide risk among college students. Interventions should focus on reducing academic pressure, promoting mental health awareness, enhancing social support networks, addressing financial concerns, and providing adequate institutional support.

**Conclusion/Implications/Recommendations**

Suicide among college students in metropolitan cities is a serious public health issue with far-reaching consequences. By understanding the underlying causes and implementing effective prevention strategies, we can work towards creating a more supportive and compassionate environment for young people.

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# FORMULATION AND IN VITRO EVALUATION OF IMMEDIATE RELEASE SOFTGEL OF NAPROXEN SODIUM

**[1]Rabinarayan Rana, [2]Sujit Kumar Patro, [3]Snehasish Nayak**

[,2,3]Roland Institute of Pharmaceutical Sciences, Brahmapur, Odisha, INDIA

**ABSTRACT:** Oral drug delivery has been known for decades as the most widely utilized route of administered among all the routes that have been employed for the systemic delivery of drug via various pharmaceutical products of different dosage forms. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration and the belief that oral administration of the drug is well absorbed. One such area of research is design of Softgel technology. Softgel technology is one of the most attractive and promising approach for increasing oral bioavailability by means of increasing solubility of the poorly soluble drug. Naproxen Sodium is one of the most important Non-steroidal anti-inflammatory agents used in the treatment of acute to chronic pains, inflammation and it belongs to BCS class-II drug so as to increase its aqueous solubility for enhancing the bioavailability, it is formulated as a liquid filled soft gelatin capsules.

**Keywords :** Softgel, Naproxen Sodium, bioavailability, capsules

## INTRODUCTION

Soft gelatin capsules or softgels are a single-unit solid dosage form, consisting of a liquid or semi-solid fill enveloped by a one-piece sealed elastic outer shell. The amount of drug or extract together with adjuvant is enclosed within a globular, oval or other shape of a soft shell. Soft gelatin capsules offer the possibility of delivering a liquid in a solid oral dosage form. The softgel can contain the active ingredient in solution, suspension or emulsion which will inherently lead to better absorption of the active ingredient as compared with delivery in a tablet or as a powder. Liquid filled softgel have beneficial to oxidative or hydrolytic degradable drugs. The liquid is prepared and encapsulated under a protective nitrogen atmosphere and the subsequently dried shell has very low oxygen permeability. The shell may be transparent and opaque. Opacity provides protection for photosensitive substances. Softgel capsules are also protected against UV radiation and light, which provides stability to the supplement and minimizes the formation of free radicals, and prevents especially rancidity.Soft gelatin capsules offer many advantages in comparison with other delivery systems. They are easy to swallow, have no taste (unless gelatin is intentionally flavored) odors and provides an elegant look.

## RESEARCH METHODOLOGY

Gift sample of Naproxen sodium is received from M/s Merck Pharma ltd. Mumbai and the soft gelatin capsule shells are also received from the same factory .The remaining ingredients like propylene glycol, povidone, lactic acid etc are used from RIPS laboratory raw materials. Pre-formulation testing is an investigation of physical and chemical properties of a drug substance alone and combined with excipients. It is the first step in the rationale development of the dosage forms. Pre-formulation studies yield necessary knowledge to develop suitable formulations. It gives information about the nature of the drug substance. Hence, the following pre-formulation studies were performed for the obtained sample of drug.

## RESULTS & DISCUSSIONS

The basic goal of formulation is to achieve an enhanced bioavailability that is therapeutically effective and non-toxic, when compared to other oral solid dosage forms. The design of proper dosage form is an important element to accomplish this goal. One such area of research is design of Softgel technology. Softgel technology is one of the most attractive and promising approach for increasing oral bioavailability by means of increasing solubility of the poorly soluble drug. Naproxen Sodium is one of the most important Non-steroidal anti-inflammatory agents used in the treatment of acute to chronic pains, inflammation and it belongs to BCS class-II drug so as to increase its aqueous solubility for enhancing the bioavailability, it is formulated as a liquid filled soft gelatin capsules.. Preformulation study was performed by formulating binary mixtures of drug with selected excipients. Binary mixtures were screened for physical appearance at initial and 40°C 2°C / 75% ± 5% RH, 4 weeks in close condition. Physical observations of binary mixtures and FTIR study revealed that there is no incompatibility between Naproxen Sodium and selected excipients in the formulation, when exposed to accelerated stability condition of 40˚C/75%RH for 1 month. UV spectrophotometric analytical method was developed for the model drug in pH 7.4 Phosphate buffer. Absorption maxima were found to be at 272 nm and the linearity was fixed between the ranges of 10 to 50 µg/ml. Various physical properties of like hardness, surface characteristics, practical size, pH weight variation and rupture time can significantly affect the rate of dissolution of drugs contained in a formulation. Various formulation trials of Naproxen Sodium Soft gelatin capsules were developed using various excipients for aqueous based fill formulation and gelatin shell formulation. Results of evaluation parameters like hardness, weight variation, pH of fill medicament, assay, disintegration test and encapsulation parameters were evaluated. Observations of all formulations for physical

characterization had shown that, all of them comply with the specifications of official pharmacopoeias and/or standard references. The formulations was optimized for binder, re-crystallization inhibitor, solubilizer, pH modifier for fill formulation and plasticizer, different bloom strength of gelatine for gelatine shell formulation and evaluating different trials (F1-F8). Formulation F8 had showed better release profile.

## CONCLUSIONS

The in vitro drug release data obtained were extrapolated by zero order, First order to know the mechanism of drug release from the formulations. The release kinetics shows that the release of drug followed first order release in all the formulations. As the drug release was best fitted in First order kinetics, indicating that the rate of drug release is dependent on concentration. From the said observations it can be concluded that combination of lactic acid, povidone, propylene glycol, water, PEG 400, glycerin, sorbitol special and gelatin has shown effective release of Naproxen Sodium by increasing solubility and enhancing bioavailability. Hence it can be evident that by formulating the Naproxen Sodium soft gelatin capsules by softgel technology which results in more effective release of drug, increased solubility and oral bioavailability may also be enhanced.

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# "Enhancing Resveratrol Delivery for Neuroprotection in Parkinson's Disease by Nano-formulations"

**Shivani Tyagi**
Research Scholar (Pharmacy)
SRM Modinagar College of Pharmacy, SRM IST, Modinagar, Ghaziabad, Uttar Pradesh
**Jayendra Kumar**
Guide
SRM Modinagar College of Pharmacy, SRM IST, Modinagar, Ghaziabad, Uttar Pradesh

**ABSTRACT**

Resveratrol (RV), a polyphenol found in grapes, berries, and peanuts, has garnered attention for its neuroprotective properties and minimal side effects, making it an attractive candidate for the treatment of neurodegenerative diseases like Parkinson's disease (PD). Despite its therapeutic potential, RV faces limitations such as low bioavailability, poor stability, and difficulty crossing the blood-brain barrier (BBB), which hinder its effectiveness in clinical applications. Nanotechnology offers a solution to these challenges by enhancing the delivery and functionality of RV. Through advanced nano formulation techniques, RV can be encapsulated into nanocarriers that provide targeted brain delivery, controlled release, and improved cellular uptake, all while reducing the required dosage and minimizing systemic side effects. By leveraging nanotechnology, the therapeutic efficacy of RV can be significantly enhanced, positioning it as a promising approach for neuroprotection in PD and other neurodegenerative conditions. This review explores the synergistic potential of nanotechnology in optimizing RV-based therapies, offering new hope for safer and more effective treatments in the management of PD.

**KEYWORDS:** Nanotechnology, Parkinson's disease, Polyphenols, Resveratrol.

## 1. INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative illness brought on by a deficiency of dopamine-producing neurons in the brain region responsible for motor coordination, the basal ganglia. Due to dopaminergic neuron degeneration, PD affects millions of people worldwide and is expected to affect 10 million by 2030. PD causes a wide range of motor and non-motor symptoms. It is a long-term, progressive neurological condition characterized by a high level of the intracellular protein alpha-synuclein and an early loss of dopaminergic neurons in the substantia nigra pars compacta. The ensuing dopamine imbalance in the basal ganglia, which causes bradykinesia and other Parkinsonian motor symptoms as

stiffness, tremor, and postural instability. Triggering variables in the pathological cascade of PD include neuroinflammation, oxidative stress, and the excitotoxicity of the N-methyl-D-aspartate (NMDA) receptor (1).

Despite a great deal of research, there are few pharmaceutical alternatives for the condition that can stop the disease from progressing beyond treating its symptoms. As a result, scientists are searching for potentially synthetic or natural substances to use as PD treatments. Due to required continuous consumption of anti-PD medicine to the PD patients, the natural substances are attracting attention recently. Many research has demonstrated the neuroprotective benefits of natural components against dopaminergic neuronal death, with generally harmless side effects that are infrequent, mild, or temporary (2).

A group of secondary metabolites found in plants called polyphenols have become more and more popular because of their many uses as food additives, medicines, and preservatives. They are frequently added to a wide range of foods to improve their flavour, texture, shelf life, and general quality. Effective antioxidants and radical scavengers, polyphenols provide numerous health advantages, including anti-inflammatory and antibacterial properties. Numerous studies have shown that eating more foods high in polyphenols may help lower the risk of cancer and metabolic diseases. However, because of their low solubility in water, instability at low pH levels, and challenges with absorption in the small intestine, their bioavailability is restricted after intake (3).

3,5,4′-trihydroxy-trans-stilbene, (RV), is a polyphenol and phytoalexin which occurs naturally and has drawn a lot of interest due to its neuroprotective and antioxidative qualities in neurodegenerative disease like Parkinsonism. Their enormous potential for therapeutic application was hampered by their low solubility, photostability, and decreased bioavailability. The need for new potent medications is highlighted by the fact that current drugs effectively control PD symptoms but are ineffective in halting the disease's development. A formulation based on convention does not provide the best therapeutic results. In order to overcome these obstacles, new technological procedures as well as the use of polyphenol-loaded nanoparticles and nanotechnology are needed to maintain the biological activities of polyphenols and increase their bioavailability, making them more useful as functional food ingredients and drug delivery systems (3).

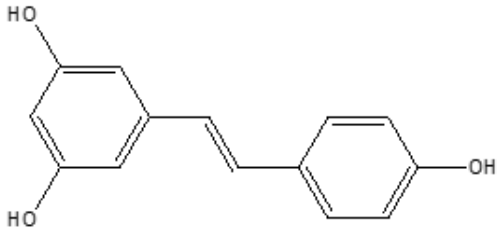


**Figure 1: RV chemical structure**

## 2. LITERATURE REVIEW REGARDING RESVERATROL'S NANO-FORMULATIONS USED IN PD

### 2.1 Solid lipid nanoparticles (SLNs)

**Bandiwadekar A et al. (2024)** explored intradermal solid lipid nanoparticles (SLNs) to enhance RV stability. In this study, an RV-loaded SLNs (RV-SLNs) microneedle patch was designed for transdermal delivery of RV to improve stability and enhance patient compliance. Characterization studies confirmed favourable sustained drug release profile ($78.36 \pm 0.74\%$) and physical properties. Microneedles demonstrated effective skin penetration. *Ex-vivo* permeation studies showed significant drug permeation of $68.39 \pm 1.4\%$. *In-vivo* pharmacokinetic analysis indicated a notable increase in $AUC_{0-t,}$ $T_{max,}$ and $C_{max}$, values, along with an inferior elimination rate when compared to pure RV delivered *via* microneedles. Treated animals exhibited improvements in behaviour along with elevated antioxidant levels in the brain. Additionally, *in-vivo* skin irritation tests indicated no signs of irritation for up to 24 hours, suggesting the suitability of extended microneedle application Histo-pathological findings revealed changes in the substantia nigra and striatum of the brain, post-treatment. These findings suggest that the RV-SLN loaded microneedle patch (RVSNLMP) offers a novel and promising approach to improving drug efficacy, patient compliance, and therapeutic outcomes in PD treatment (4).

### 2.2 Nanocrystals

**Xiong S et al. (2020)** developed a nanocrystal (NCs) formulation of RV to boost its brain delivery and oral bioavailability for the management of PD. RV-NCs were prepared using hydroxypropyl methylcellulose (HPMC) as a stabilizing agent through an antisolvent precipitation technique. The RV-

NCs achieved a particle size of 222.54 ± 1.66 nm, a PDI of 0.125 ± 0.035, and a zeta potential of −9.41 ± 0.37 mV, with a fast in-vitro dissolution rate. Molecular dynamics simulations indicated an interaction energy of −68.09 kJ/mol and a binding energy of −30.98 ± 0.388 kJ/mol between RV and HPMC, suggesting spontaneous binding via van der Waals interactions. The RV-NCs showed enhanced cellular uptake and superior permeability compared to unformulated RV. Additionally, they provided neuroprotection against $MPP^+$-induced cytotoxicity without exhibiting significant toxic effects on zebrafish embryos or larvae, preserving their survival and hatching rates. Pharmacokinetic studies in rats showed that orally administered RV-NCs achieved higher plasma and brain concentrations than pure RV. In MPTP-induced PD mice, RV-NCs resulted in notable behavioral improvements, alleviated dopamine deficiency, and elevated levels of dopamine along with its metabolites Overall, RV-NCs present a promising strategy for improving RV's oral bioavailability and brain accumulation, making them a potential treatment modality for PD (5).

**2.3 Polymeric nanoparticles**

**Rahman M et al. (2019)** conducted a study to assess the neuroprotective effects of RV delivered *via* chitosan glutamate nanoparticles (RV-CG-NPs) in a PD mouse model induced by MPTP. The nanoparticles were produced using the ionic gelation method with chitosan and tripolyphosphate, and the optimized formulation was administered intranasally. These nanoparticles had a polydispersity index (PDI) of 0.21, good entrapment efficiency (89%), and a particle size of 131 nm. Pharmacodynamic evaluations showed that MPTP significantly, elevated oxidative stress in the striatum, decreased tyrosine hydroxylase expression, and impaired social recognition memory. Biodistribution studies indicated that the nanoparticle formulation achieved significantly higher brain concentrations of RV compared to plain RV solution. The $C_{max}$ (890.12 ng/ml) and AUC (1986.51 ng·h/ml) of the optimized formulation (administered intranasally) were notably higher at all time points compared to the RV solution (intranasally) and the chitosan glutamate-RV-NPs (administered intravenously). Overall, the study demonstrated that nose to brain delivery of RV-loaded nanoparticles enhanced brain targeting and neuroprotection in PD (6).

**da Rocha Lindner G et al. (2015)** examined the neuroprotective properties of bulk RV with those of RV-loaded polysorbate 80 (PS80)-coated poly(lactide) nanoparticles in a rat model of PD. After receiving intraperitoneal treatment with RV (nanoparticulate or non-nanoparticulate) for 15 days, MPTP was

administered once intraperitoneally to C57BL/6 mice, a neurotoxin that induces PD-like symptoms by damaging dopaminergic neurons. MPTP caused significant impairments in olfactory discrimination, social recognition memory, and oxidative stress in the striatum, along with a reduction in tyrosine hydroxylase expression. Bulk RV did not exhibit the same level of neuroprotection against the behavioural and neurochemical alterations caused by MPTP as did RV-loaded nanoparticles. These results imply that PS80-coated RV-loaded poly(lactide) nanoparticles offer a viable new avenue for PD adjuvant therapy and nanomedical instrumentation (7).

**2.4 Liposomes**

**Wang M et al. (2018)** designed Fe3O4-modified RV liposomes (RV-lips@Fe3O4) as a magnetic targeting drug nanocarrier. T2 relaxation times and Factional anisotropy (FA) values were measured using magnetic resonance imaging in RV-lips@Fe3O4-treated rats. The RV-lips@Fe3O4 formulation demonstrated high drug loading capacity, stability, and strong magnetic responsiveness, with slow and sustained drug release *in-vitro*. RV-lips@Fe3O4 efficiently penetrated the blood-brain barrier and raised medication concentration at the target region when exposed to an external magnetic field, according to *in-vivo* tests. Increased T2 relaxation periods and FA values supported the improved therapeutic efficacy of magnetic RV-liposomes. According to these results, RV-lips@Fe3O4 presents a viable platform for effectively overcoming the blood-brain barrier and treating neurological conditions like Parkinson's disease when combined with an external magnetic field (8).

**2.5 Nanoemulsion**

**Pangeni R et al. (2014)** formulated a kinetically stable oil-in-water nanoemulsion of RV by employing vitamin E:sefsol (1:1), Tween 80, and Transcutol P as oil, surfactant and co-surfactant respectively for managing PD efficiently. Despite RV's potent antioxidant and pharmacological properties, its low oral bioavailability, attributed to extensive hepatic and presystemic metabolism, was addressed through this nanoemulsion approach. Spontaneous emulsification followed by high-pressure homogenization was used for preparing nanoemulsion, demonstrated favorable characteristics, including a polydispersity index of 0.158 ± 0.02, a zeta potential of −35 ± 0.02, and a globule size of 102 ± 1.46 nm. Drug release studies showed significantly enhanced cumulative release in both *in-vitro* and *ex-vivo* conditions, with 88.57 ± 1.92% and 85.48 ± 1.34% release after 24 hours, respectively. The optimized formulation exhibited strong

antioxidant activity, and pharmacokinetic studies revealed a high brain concentration following intranasal administration. Histopathological analyses revealed less degenerative alterations, while GSH and SOD levels were significantly increased and MDA levels decreased, confirming the nanoemulsion's neuroprotective potential in PD (9).

**Table 1: Application of few drug delivery carriers containing RV to the brain targeting.**

| Nano-formulations | Animal | Route | Outcome | Reference |
|---|---|---|---|---|
| NLCs | Rats | Intra-nasal | NLC-loaded in situ gel results in higher drug distribution in the brain. | (10) |
| Liposomes | | Intra-venous | The formulation outperformed free RV in terms of AUC, $t_{1/2}$, and MRT. | (11) |
| | | | Formulations increased the brain distribution by nine times and the plasma half-life by up to eighteen times. | (12) |
| Nanosuspension | | Intra-nasal | Additional distribution and localization revealed a direct brain-to-nose transport pathway. | (13) |
| Nanoemulsion | | Intra-nasal | Increase in Brain AUC after Intranasal administration in Rats. | (14) |

## 3. Conclusion

RV stands out as a potent polyphenol with remarkable neuroprotective properties, making it a promising candidate in the fight against PD. However, its clinical application is hampered by inherent challenges such as poor bioavailability, low stability, and limited ability to cross the BBB. The integration of nanotechnology offers a powerful solution to overcome these barriers. Nanoformulation strategies, including the encapsulation of RV in nanocarriers, enable targeted brain delivery, controlled release, and enhanced cellular uptake, while minimizing side effects and reducing the required dosage. By optimizing RV's therapeutic potential through nanotechnology, we open the door to more effective and safer

treatments for PD and other neurodegenerative disorders. This convergence of nanotechnology and RV-based therapy holds significant promise for advancing neuroprotective strategies in clinical practice.

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# IN SILICO APPROACH TO DETERMINE TARGET SPECIFICITY OF SEVERAL PHYTOCONSTITUENTS AGAINST HUMAN ALPHA-DYSTROGLYCAN RECEPTOR: CHALLENGING LASSA VIRUS INFECTIVITY, VIRULENCE, AND PATHOGENICITY

**Dr Uma Nath. U,**
**Professor**
**Department of Pharmaceutical Analysis**
Sree Krishna College of Pharmacy and Research Centre,
Thiruvananthapuram, Kerala, India

**ABSTRACT**

For the discovery of agents from natural sources for effectively managing the numerous challenges (Infectivity, Virulence, and Pathogenicity) imposed by Lassa virus, the present study involved screening of few natural bioactives; (1) Peonidin; (2) β-Cryptoxanthin; 3) Zeaxanthin; (4) Hydroxy cinnamic acid; (5) Myricetin; (6) Quercetin; (7) Kaemferol; (8) Luteolin; (9) Apigenin; (10) Chlorogenic acid; (11) 3,4-di-*O*-caffeoylquinic acid; (12) 4,5-di-*O*-caffeoylquinic acid; (13) 3,4,5-tri-*O*-caffeoylquinic acid; (14) 1-Ipomeanol; (15) 4-Ipomeanol; (16) 1,4-Ipomeadiol; (17) Ipomeanine; (18) Cyanidin; (19) Delphinidin; and (20) Malvidin via *in silico* structure-based drug design (SBDD) technique by utilizing the Schrodinger Maestro 9.1 software against the crystal structure of human alpha-dystroglycan (PDB ID: 5LLK), a target known for the entrance of virus into host cells by interfering with the development of the glycoprotein. Also, drug-likeliness studies, bioavailability studies, and pharmacokinetic studies have been performed for the most effective compound, Malvidin using SwissADME online tool. The study will provide clues, motivate, and throw light on imperative aspects for the globally working medicinal chemists in the rational designing of anti-infective drugs in the near future.

**Keywords:** Lassa virus, α-dystroglycan, Molecular docking, Pharmacokinetics, Phytoconstituents, *In silico*

## 1. INTRODUCTION

Among the people that live in Western Africa, Lassa fever (LF) is endemic [1]. The rat *Mastomys natalensis* is the primary reservoir and transmitter of the *Lassa mammarenavirus* (LASV) that causes the sickness in mammals [2]. The most lethal arenavirus, Lassa virus, is a member of the Old World (OW) virus family [3]. In West Africa, LASV causes between 300,000 and 500,000 annual infections and 5,000 annual fatalities [4]. Biological fluids (urine, blood, saliva, feces) from infected hosts may transfer the LASV both zoonotically and between humans [5]. The primary symptoms of LF take from 3 days to 21 days to appear in human [6]. These include fever, nausea, and bleeding, as well as neurological, pulmonary, and gastrointestinal problems.

When it comes to gaining access to the host cell, the virion glycoprotein (GP) is where it is at for LASV. The SSP-GP1-GP2 trimer's GP1 binds to the host cell's α-dystroglycan (α-DG) receptor [7]. α-Dystroglycan is highly expressed in all human tissues and acts as a connection between the extracellular matrix and the cytoskeleton [8]. Post-translational glycosylation of α-DG by glycosyltransferase is required for normal biological activity and is

also necessary for the recognition and binding of the LASV GP1 to α-DG [9]. Attachment of virion glycoproteins to extracellular α-DG triggers phosphorylation of α-DG at its tyrosine residues, paving the way for late endosomal transit of the viral particle [10]. After entering the cell, GP is processed to release a stable signal peptide (SSP) and an early GP1/GP2 complex. Cleavage of the GP1/GP2 complex into N-terminus GP1 and C-terminus GP2 subunit occurs after translocation to the Golgi apparatus [11]. The GP1 then undergoes a conformational change in a low acidic environment (pH 5), where it supports the receptor transition from α-DG to lysosome-associated membrane protein 1 (LAMP1), leading to viral genome membrane fusion, replication, transcription, and translation [7]. Thus, preventing LASV entrance into host cells by interfering with the development of the GP1 and α-DG receptor complex may disrupt the whole infection process.

Obtaining crystals of antigen-antibody complexes, determining their 3D structures via X-ray crystallography, etc. are just some of the time-consuming and money-consuming steps involved in experimentally identifying a random viral epitope [12]. Therefore, finding and using antiviral medications may be a more practical method than developing new vaccines. The antimicrobial, anti-inflammatory, and analgesic properties of naturally occurring chemical components originating from plants have all been extensively researched [13]. Many different phytochemicals have been shown to be effective against many viruses, including herpes simplex virus type 1 (HSV-1), human cytomegalovirus (HCV), poliovirus, avian influenza virus (IAV), dengue virus (DENV), and others. By interfering with the LASV replication cycle, tangeretin (pentamethoxyflavone) is able to halt the spread of Lassa virus [14]. Therefore, it is a potential technique to test naturally occurring chemicals for anti-infectious efficacy against LASV.

The main objective of this study was to explore the potential of 20 bioactive compounds [(1) Peonidin; (2) β-Cryptoxanthin; 3) Zeaxanthin; (4) Hydroxy cinnamic acid; (5) Myricetin; (6) Quercetin; (7) Kaemferol; (8) Luteolin; (9) Apigenin; (10) Chlorogenic acid; (11) 3,4-di-*O*-caffeoylquinic acid; (12) 4,5-di-*O*-caffeoylquinic acid; (13) 3,4,5-tri-*O*-caffeoylquinic acid; (14) 1-Ipomeanol; (15) 4-Ipomeanol; (16) 1,4-Ipomeadiol; (17) Ipomeanine; (18) Cyanidin; (19) Delphinidin; and (20) Malvidin] as anti-LASV agent by using Induced-Fit Molecular Docking approach. Also, drug-likeliness studies, bioavailability studies, and pharmacokinetic studies have been performed for the most effective compound, Malvidin using SwissADME online tool.

## 2. MATERIALS AND METHODS

### 2.1.1. Preparation of Ligand

The structures were created using the Schrodinger Software suite 2021-2's 2D-sketcher module. For docking analysis, the Maestro environment version 12.8 was employed. The stereoisomers of these ligands were created using the LigPrep programme. Using the Epik ionizer, a maximum of four poses with correct protonation states were created for each ligand at a target pH of 7.0. The OPLS 2005 force field was utilized to construct tautomerized,

desalted ligands while keeping the input files' requisite chiralities, resulting in an optimized low energy 3D ligand [15].

### 2.1.2. Preparation of Protein

The crystal structure of human alpha-dystroglycan (PDB ID: 5LLK) was selected as the prime targets to be studied and was obtained exclusively from the RCSB Protein Data Bank (PDB). The protein structures were created using Maestro 9.1's Protein Preparation Wizard. The pre-processed and inspected structures were taken when developing the biological target. To get the right shape, the disulfide bonds, bond ordering, and formal charges were assigned using the Protein Preparation Wizard module of the Schrodinger Maestro 9.1. Co-factors, metal ions, water molecules in crystal formations beyond a distance of 5A°, and the hetero group were all removed. The "impref utility" tool was used to optimize hydrogen atoms by keeping all heavy atoms in their original positions, while the "H-bond assignment" tool was used to optimize the hydrogen-bonding network. Molecular docking was used to define the receptor (x, y, z) grids (-30 × -20 × 15) for the protein structure, allowing a variety of ligand poses to bind at the anticipated active site. Grids were built and placed at the ligand's centroid in such a way that they covered the whole ligand in a cubic box of defined measurement with the following characteristics: 1.00 Van der Waals scale factor and 0.25 charge cut off. The docking was done in XP mode, and only the energy-minimized postures were scored, which was expressed as a Glide score. The best-docked posture with the lowest Glide score value for each ligand was considered after the highest-scoring ligands were docked [16].

### 2.1.3. Induced-Fit Molecular Docking

The structure-based drug design process is restarted after the target protein's structure is understood. The stiff receptor was docked with the low-energy ligands, and the fit into the active site was evaluated, as well as the predicted binding mechanism. In receptor-based computational techniques, the ligand interacting with the macromolecule protein (receptor) was represented using a molecular docking methodology. With low energy levels, IFD predicted that the ligand would have a good contact with the target. The method facilitates in the finding of low-free-energy conformations as well as the complete removal of steric conflicts. With 0.7 Van der Waals scaling for the receptor and 0.5 Van der Waals scaling for the ligand, side chains were eliminated, and a 0.18 RMSD value cut off, the maximum number of poses for each ligand remained at 20. The chemicals were ranked based on the information obtained, and a selection was tested for biological activity experimentally. The Glide Score was determined for each ligand [17].

## 2.2. Pharmacokinetics, Bioavailability, and Drug-likeliness studies

The SwissADME online tool was used to conduct a prediction research of pharmacokinetics, namely ADME, bioavailability, and drug-likeness of ligands. To identify drug-likeness, the technology estimates bioavailability

radar based on six physicochemical properties: lipophilicity, size, polarity, insolubility, flexibility, and insaturation. The ADME properties, such as passive human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeation, as well as substrate or non-substrate of the permeability glycoprotein (P-gp) was detected positive or negative in the BOILED-Egg model within the tool. The lipophilicity estimation (Log p/w) parameters such as iLOGP on free energies of solvation in n-octanol and water calculated by the generalized-born and solvent accessible surface area (GB/SA) model, XLOGP3 is an atomistic method with corrective factors and a knowledge-based library, WLOGP is an implementation of a purely atomistic method, and MLOGP is an archetype of topological method rely The Lipinski (Pfizer) filter, which was the first rule-of-five to be implemented in a tool, was used to predict drug-likeness. The bioavailability radar was used to predict oral bioavailability based on several physicochemical characteristics. The ranges of each parameter was mentioned as LIPO = lipophilicity as -0.7 < XLOGP3 < +5.0; SIZE = size as molecular weight 150gm/mol < MV < 500gm/mol; POLAR = polarity as 20Å$^2$ < TPSA (topological polar surface area) < 130Å$^2$; INSOLU = insoluble in water by log S scale 0 < Logs (ESOL) < 6; INSATU = insaturation or saturation as per fraction of carbons in the $sp^3$ hybridization 0.3 < Fraction Csp3 < 1 and FLEX = flexibility as per rotatable bonds 0 < Number of rotable bonds < 9 [18].

## 3. RESULTS AND DISCUSSION

### 3.1. Molecular docking

The most promising inhibitor was found to be Malvidin with Glide Score of –5.264 Kcal/mol by interacting with amino acid residues ARG76 [through –OH (hydroxyl), -O atom of $OCH_3$ as well as THR192 through –OH (hydroxyl)] along with π-cation interaction via amino acid residues LYS226 and LYS302 through aromatic component in the structure. The natural compound with lowest Glide Score (-2.195 Kcal/mol) was identified as 3,4,5-tri-*O*-caffeoylquinic acid. The interaction of several bioactive compounds against human α-dystroglycan is described in **Table 1**.

**Table 1.** Interaction of several bioactive compounds against human α-dystroglycan to challenge Lassa virus infectivity.

| S. No. | Compounds | Binding Energy (Kcal/mol) | No. of H Bonds | Interacting residues | Van der Waals & other interactions |
|---|---|---|---|---|---|
| • | Peonidin | -5.122 | 2 | THR192 (-OH), PHE236 (-OH) | LYS226, LYS302 (π-cation-aromatic) |
| • | Cryptoxanthin | -3.917 | 0 | - | - |
| • | Zeaxanthin | -3.744 | 1 | ASN267 (-OH) | - |
| • | Hydroxy cinnamic acid | -5.242 | 4 | THR192 (-OH), ARG234 (=O), PHE236 (-OH), HIE298 (-OH) | - |

| | | | | | |
|---|---|---|---|---|---|
| • | Myricetin | -4.944 | 2 | ARG76 (-OH), THR192 (-OH) | LYS226 (π-cation-aromatic) |
| • | Quercetin | -4.967 | 2 | ARG76 (-OH), THR192 (-OH) | LYS226 (π-cation-aromatic) |
| • | Kaemferol | -4.271 | 0 | - | LYS302 (π-cation-aromatic) |
| • | Luteolin | -4.785 | 1 | THR192 (-OH) | LYS302 (π-cation-aromatic) |
| • | Apigenin | -4.444 | 0 | - | LYS226 (π-cation-aromatic) |
| • | Chlorogenic acid | -4.261 | 3 | THR192 (-OH), ASN224 (-OH), ASP237 (-OH), | - |
| • | 3,4-di-*O*-caffeoylquinic acid | -4.148 | 4 | GLU159 (-OH), ARG234 (=O), PHE236 (-OH), HIE298 (-OH) | LYS226 (π-cation-aromatic) |
| • | 4,5-di-*O*-caffeoylquinic acid | -4.075 | 3 | GLU159 (-OH), ARG234 (2× -OH) | ARG234 (π-cation-aromatic) |
| • | 3,4,5-tri-*O*-caffeoylquinic acid | -2.195 | 5 | ASP160 (-OH), SER162 (-OH), LYS226 (-OH), ARG234 (=O), ASP237 (-OH) | - |
| • | 1-Ipomeanol | -4.212 | 1 | ASP237 (-OH) | ARG234 (π-cation-aromatic) |
| • | 4-Ipomeanol | -3.686 | 3 | LYS226 (-OH), THR192 (-OH), ARG234 (-O of Furan) | HIE298 (π- π stacking-aromatic) |
| • | 1,4-Ipomeadiol | -4.327 | 3 | LYS226 (-OH), SER259 (-OH), ARG234 (-O of Furan) | HIE298 (π- π stacking-aromatic) |
| • | Ipomeanine | -3.918 | 1 | ARG234 (-OH) | - |
| • | Cyanidin | -4.481 | 1 | ARG234 (-OH) | LYS226, LYS302 (π-cation-aromatic) |
| • | Delphinidin | -4.032 | 4 | THR192 (2× -OH), PRO229 (-OH) | LYS226 (π-cation-aromatic) |
| • | Malvidin | -5.264 | 3 | ARG76 (-OH, -O of $OCH_3$) THR192 (-OH) | LYS226 (π-cation-aromatic) |

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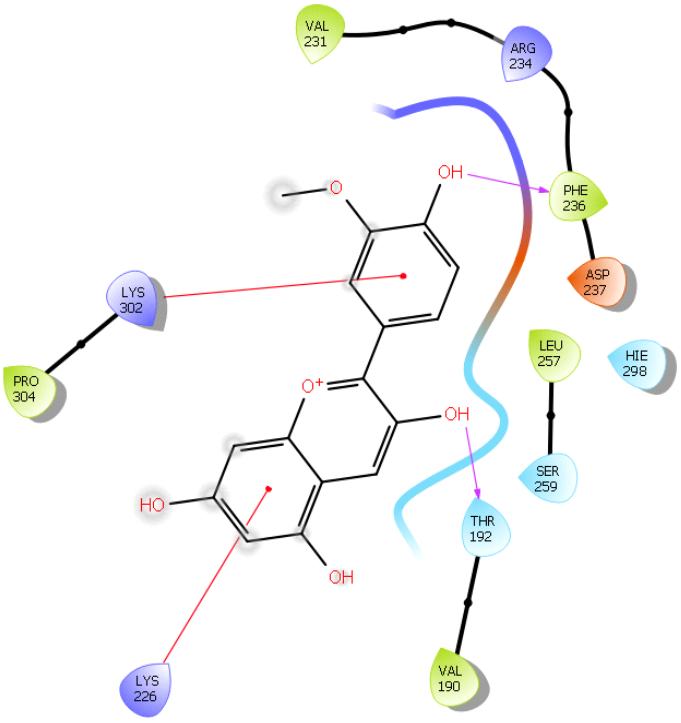
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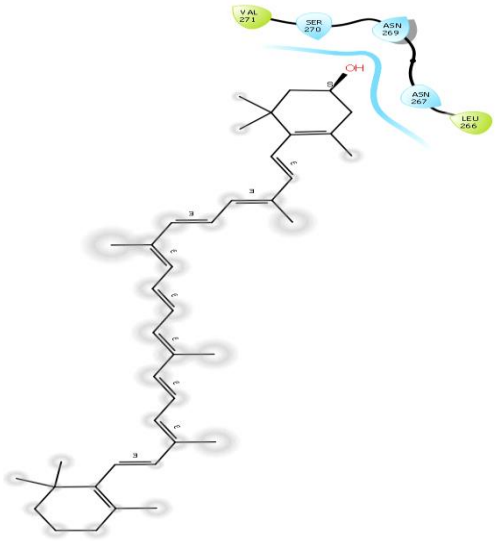
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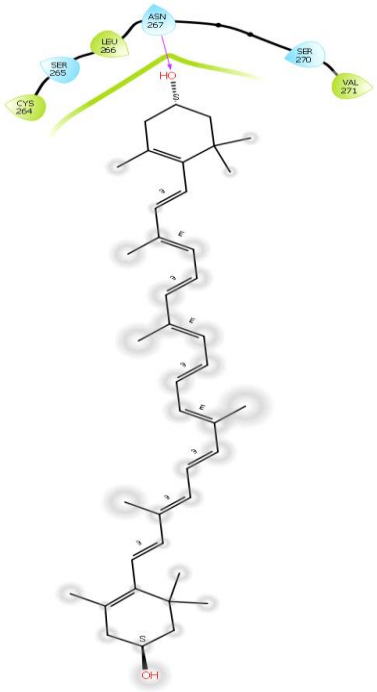
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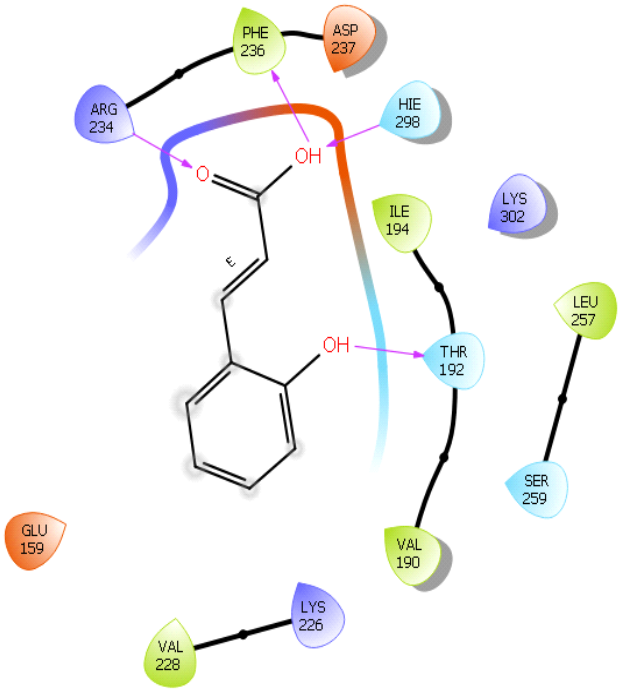
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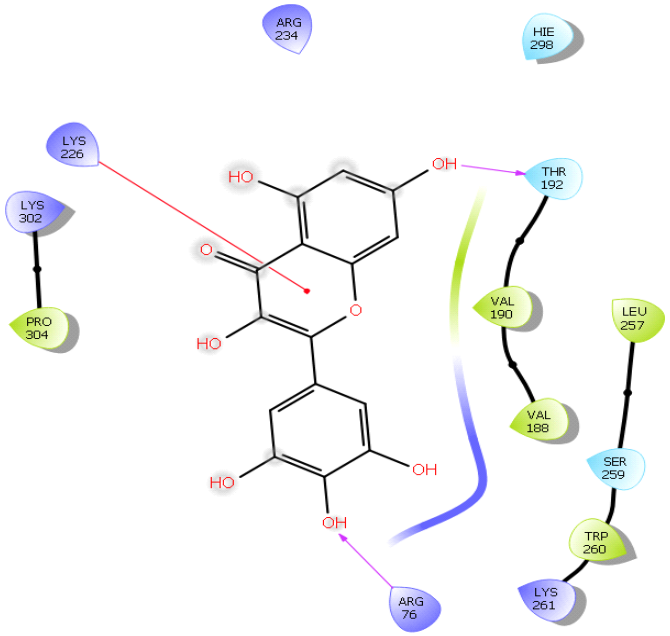
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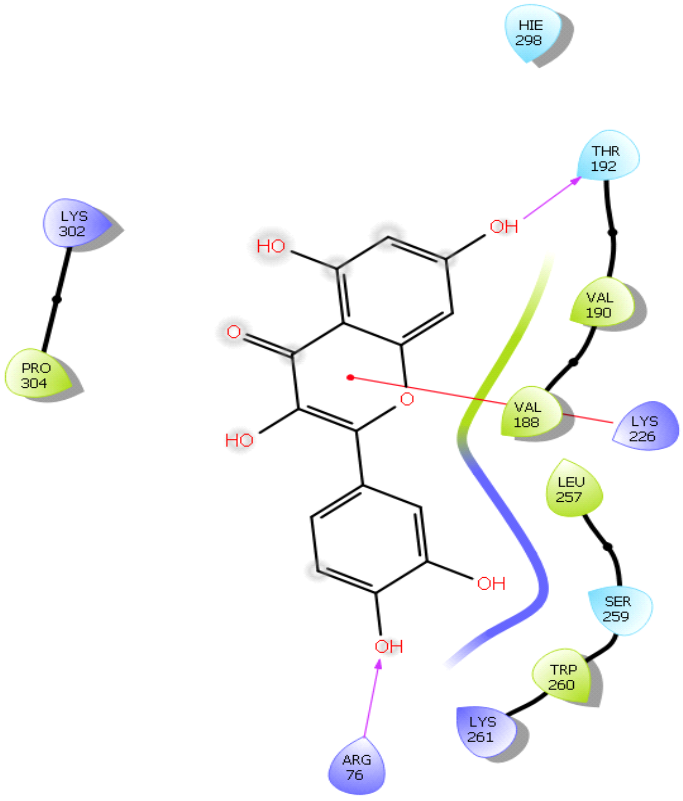
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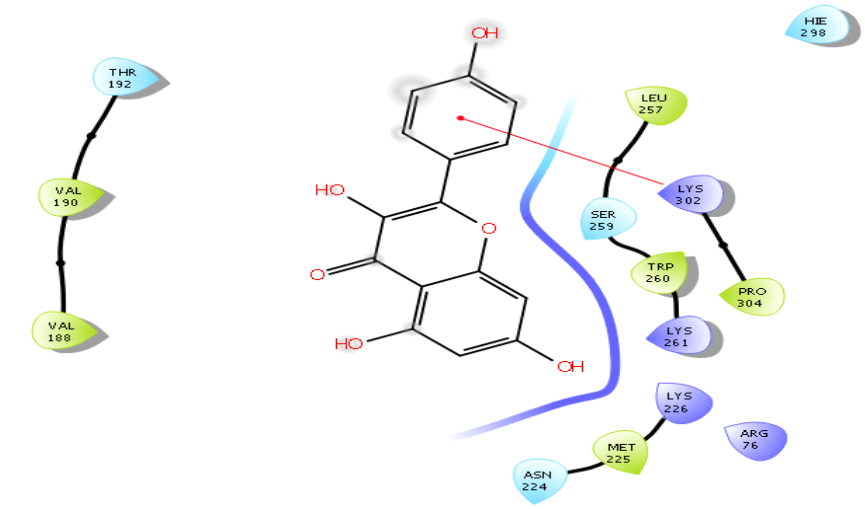
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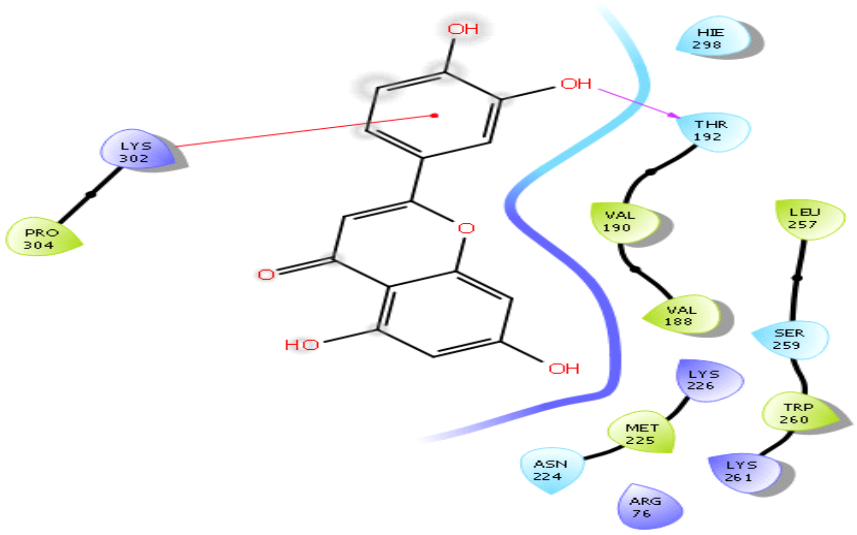
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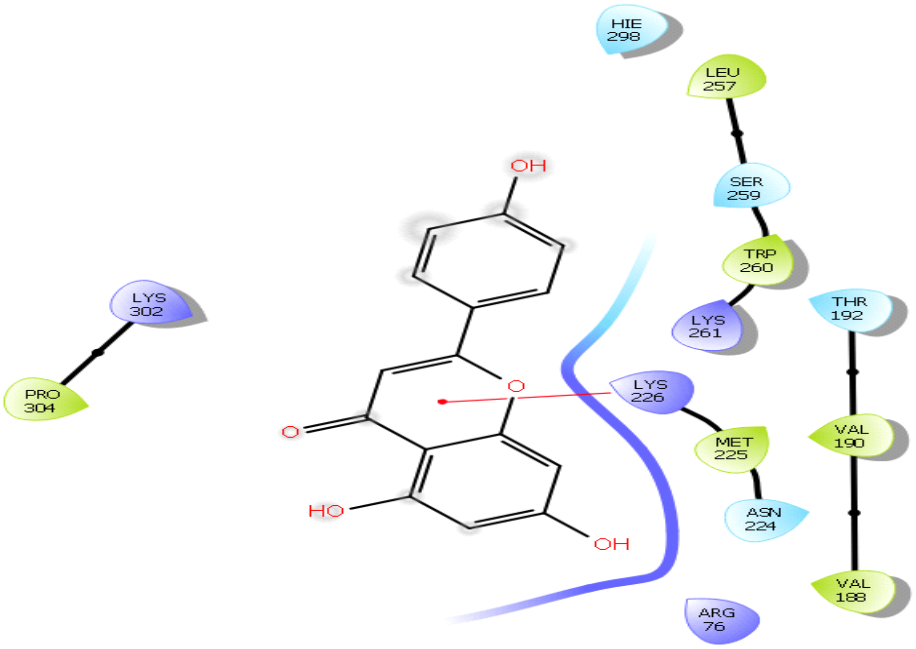
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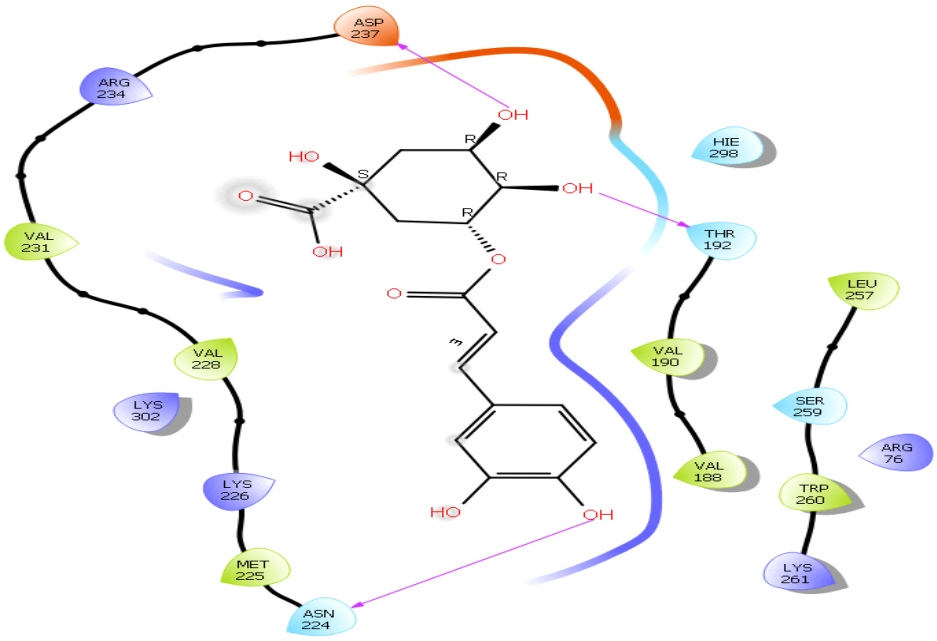
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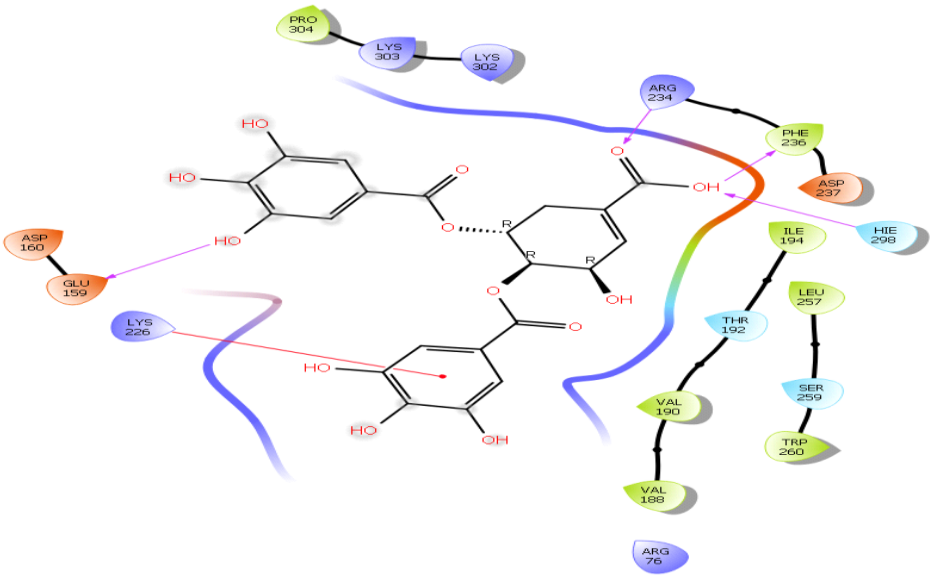
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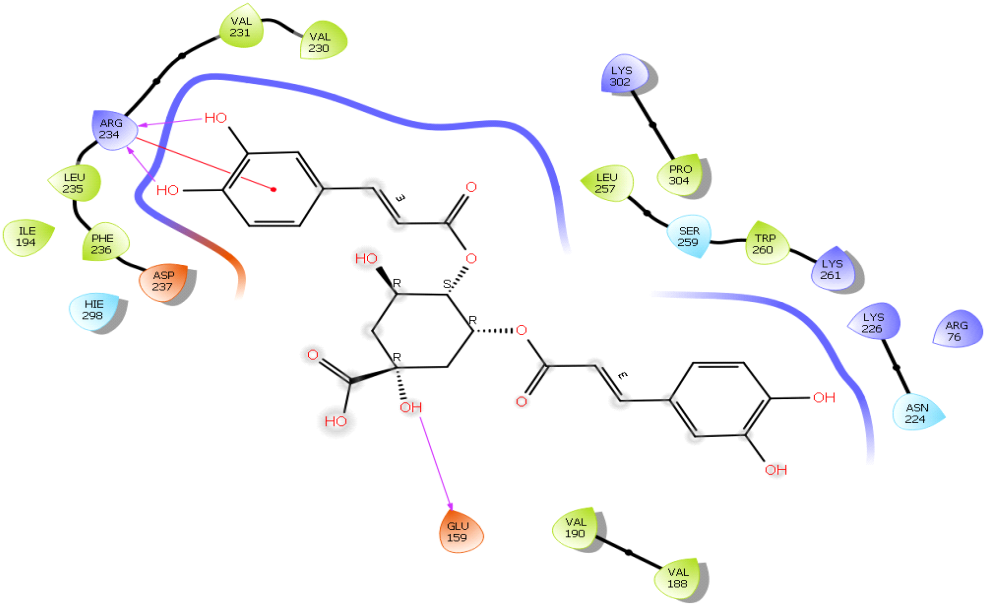
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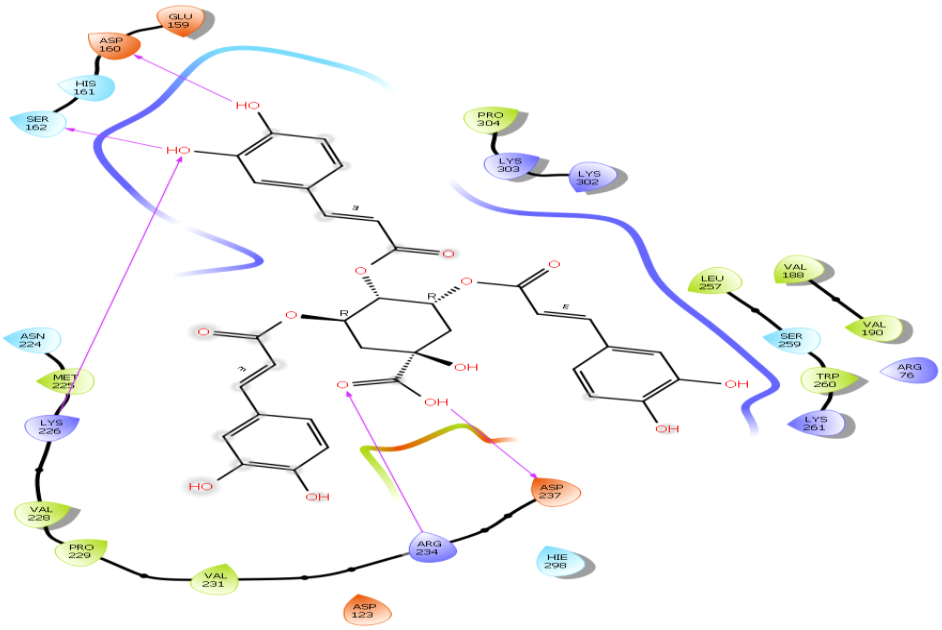
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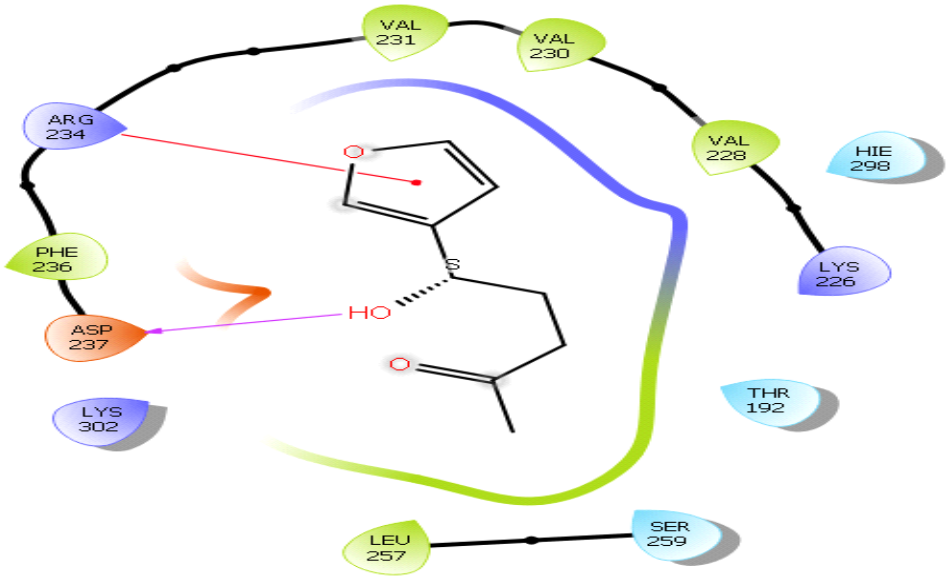
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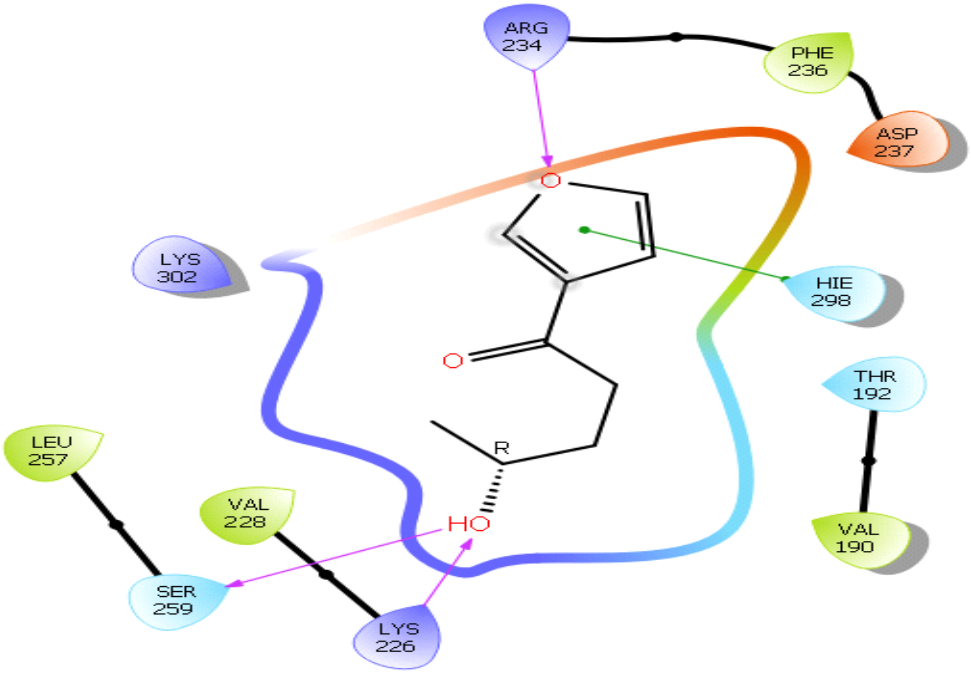
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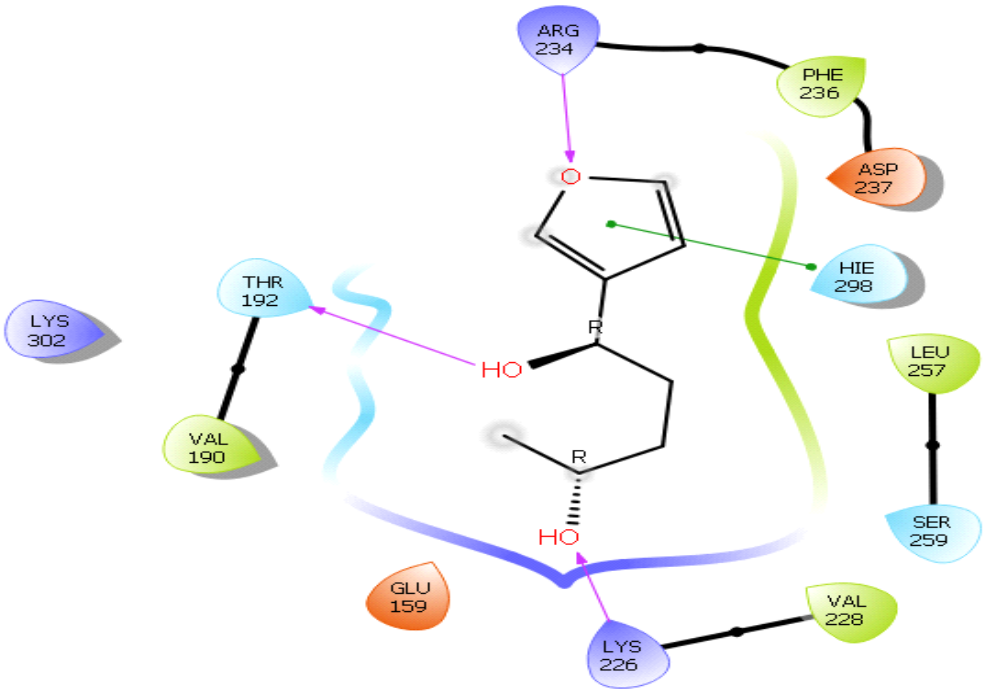
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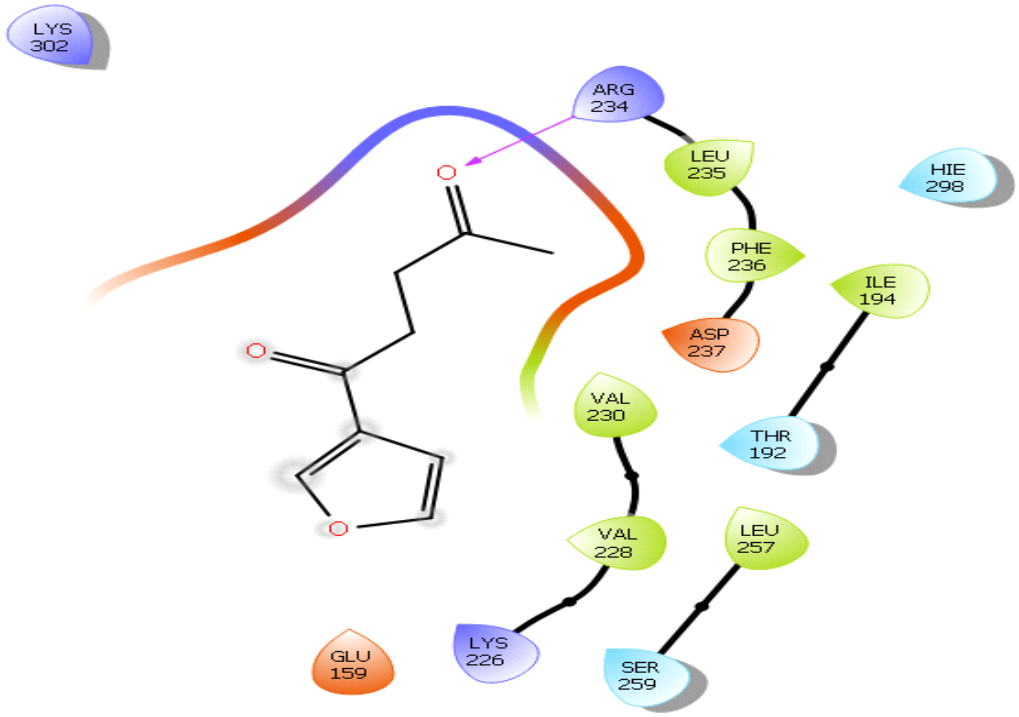
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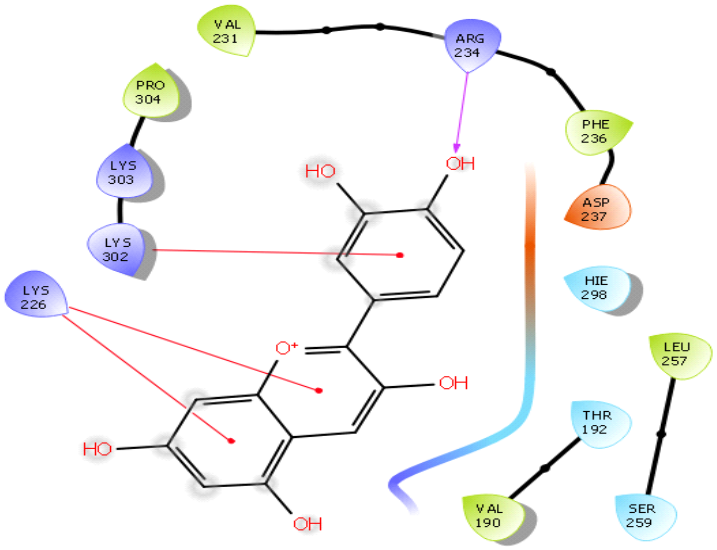
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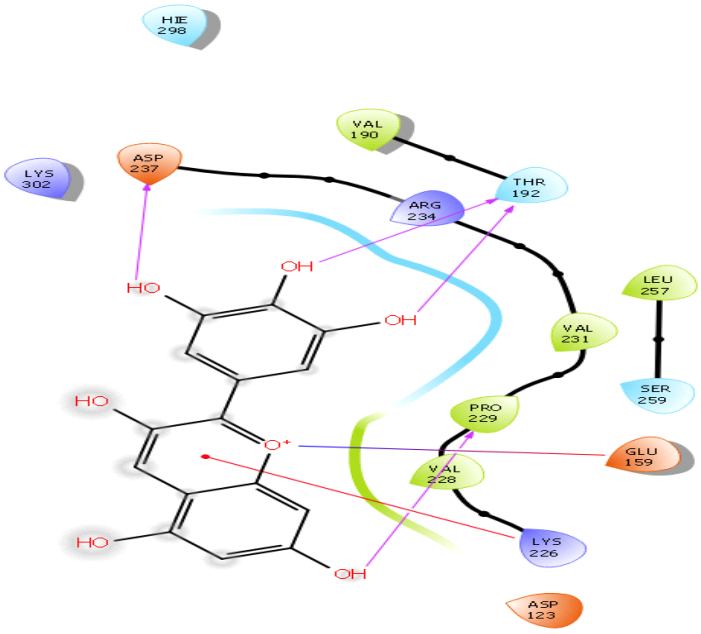


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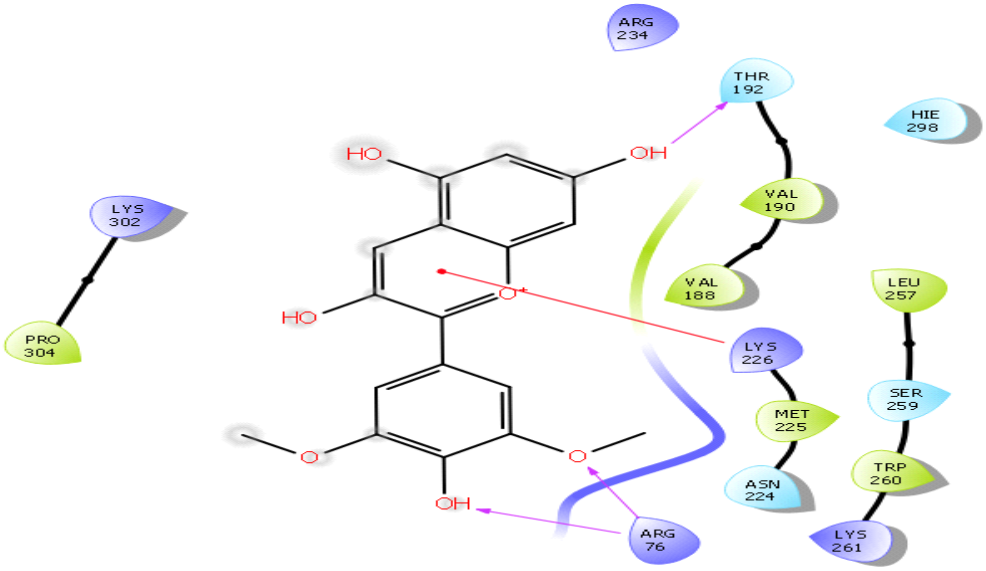


**Figure 1.** Docking poses of (1) Peonidin; (2) β-Cryptoxanthin; 3) Zeaxanthin; (4) Hydroxy cinnamic acid; (5) Myricetin; (6) Quercetin; (7) Kaemferol; (8) Luteolin; (9) Apigenin; (10) Chlorogenic acid; (11) 3,4-di-*O*-caffeoylquinic acid; (12) 4,5-di-*O*-caffeoylquinic acid; (13) 3,4,5-tri-*O*-caffeoylquinic acid; (14) 1-Ipomeanol; (15)

4-Ipomeanol; (16) 1,4-Ipomeadiol; (17) Ipomeanine; (18) Cyanidin; (19) Delphinidin; and (20) Malvidin against human α-dystroglycan.

**2.2. Pharmacokinetics, Bioavailability, and Drug-likeliness studies**

**Table 2** describes the predictive values for pharmacokinetics, bioavailability and drug-likeness data on Malvidin. The molecule showed average oral absorption rate, high gastrointestinal absorption, no blood-brain permeability, and very lower skin permeation. In case of metabolism, the molecule was found to be a p-glycoprotein positive substrate, however, the molecule did not served as a substrate for CYP2C19, CYP2C9, CYP2D6 and CYP3A4. The drug-likeness study showed no violation of Lipinski's rule of 5 as well as other rules which indicated perfection in terms of physicochemical and pharmacological perspectives.

For the prediction of bioavailability, an average bioavailability score was obtained, although high water solubility was predicted for Malvidin. The bioavailability radar for oral bioavailability prediction showed desired INSATU = insaturation as per Csp3 as 0.12, FLEX as per number of rotable bond 3, INSOLU Logs (ESOL) as -3.60, SIZE as molecular weight (g/mol) of 331.30 (**Figure 2A**), POLAR as TPSA (Å²) 112.52, and LIPO as XLOGP3 value of 2.24 (**Figure 2B**).

In case of BOILED-Egg model (**Figure 2C**), it was obtained that Malvidin has low capability of blood-brain barrier penetration as well as it also showed low penetration power of gastrointestinal absorption. The molecule was neither PGP negative nor PGP positive as non-substrate in predictive model. Interestingly, the Brain Or IntestinaL EstimateD permeation method (BOILED-Egg) has already been proposed as an accurate predictive model, which helps by computational prediction of the lipophilicity and polarity of small molecules. In overall predictive results, Malvidin can be suitable candidate as per bioavailability radar and BOILED-Egg representation. Furthermore, these predictive results should be validated by *in vitro* and *in vivo* functional and pharmacological assay for the management of various diseases originated through Lassa virus.

**Table 2.** Pharmacokinetics and physicochemical properties of Malvidin.

| PROPERTIES | DATA |
|---|---|
| **Physicochemical Properties** | |
| Formula | $C_{17}H_{15}O_7$ |
| Molecular weight | 331.30 g/mol |
| Number of heavy atoms | 24 |
| Number of aromatic heavy atoms | 16 |
| Fraction Csp3 | 0.12 |
| Number of rotatable bonds | 3 |
| Number of H-bond acceptors | 7 |
| Number of H-bond donors | 4 |
| Molar Refractivity | 87.13 |
| TPSA ($A^2$) | 112.52 |

| | |
|---|---|
| **Lipophilicity** | |
| Log Po/w (iLOGP) | -1.96 |
| Log Po/w (XLOGP3) | 2.24 |
| Log Po/w (WLOGP) | 3.22 |
| Log Po/w (MLOGP) | 0.28 |
| Log Po/w (SILICOS-IT) | 0.80 |
| Consensus Log Po/w | 0.92 |
| **Water Solubility** | |
| Log S (ESOL) | -3.60 |
| Solubility | 8.31e-02 mg/ml ; 2.51e-04 mol/l |
| Class | Soluble |
| Log S (Ali) | -4.24 |
| Solubility | 1.91e-02 mg/ml ; 5.77e-05 mol/l |
| Class | Moderately soluble |
| Log S (SILICOS-IT) | -3.46 |
| Solubility | 1.14e-01 mg/ml ; 3.44e-04 mol/l |
| Class | Soluble |
| **Pharmacokinetics** | |
| GI absorption | High |
| BBB permeant | No |
| P-gp substrate | Yes |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | No |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| Log Kp (skin permeation) | -6.73 cm/s |
| **Drug-likeness** | |
| Lipinski | Yes; 0 violation |
| Ghose | Yes |
| Veber | Yes |
| Egan | Yes |
| Muegge | Yes |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 1 alert; charged_oxygen_sulfur |
| Lead-likeness | Yes |
| Synthetic accessibility | 3.33 |

**B**

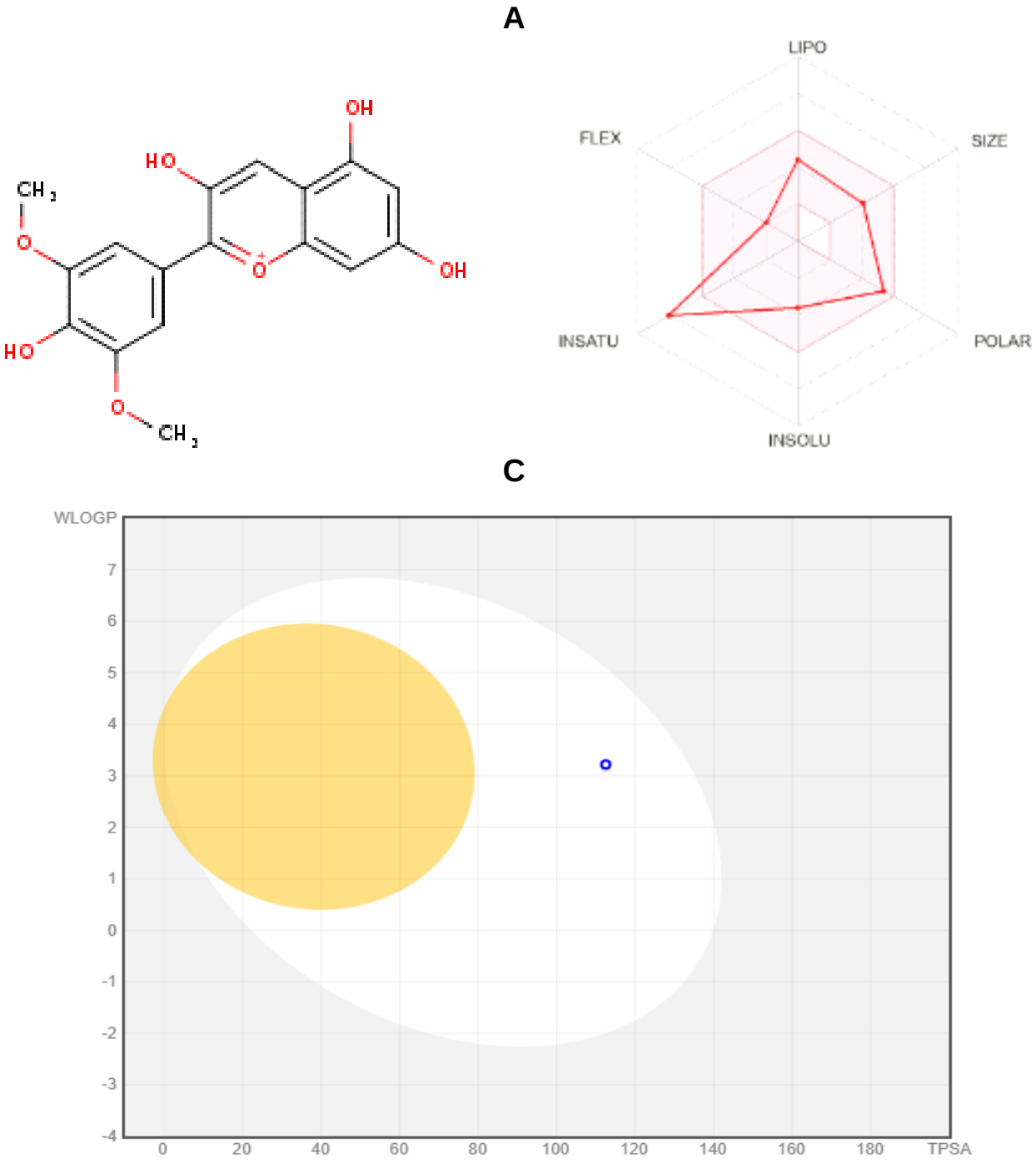


**Figure 2.** Pharmacokinetic predictions for Malvidin: (A) Chemical Structure, (B) Bioavailability radar plot, and (C) BOILED Egg Model.

## 4. CONCLUSION

The present study involved identification of novel bioactive compounds as potent anti-Lassa virus agents by inhibiting the target α-dystroglycan. It was clear from this research that all the compounds perfectly bound with the

active site cavity of the biological target with varied levels. The most promising inhibitor was found to be Malvidin with Glide Score of –5.264 Kcal/mol whereas the natural compound with lowest Glide Score was identified as 3,4,5-tri-*O*-caffeoylquinic acid. The inhibitors demonstrated hydrogen bonding interactions directly as wells as through water molecules with the amino acid residues, and thus they could penetrate deeper into the active site cavity. These may provide the compounds a better orientation and can inhibit them complementary to the active site. Therefore, the research will undeniably motivate the modern day (medicinal) chemists and biologists to further explore and study the better halves of applications.

**CONFLICT OF INTEREST**

No conflict of interest is declared.

**FUNDING INFORMATION**

No agency provided any funds.

**ACKNOWLEDGEMENT**

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# A REVIEW ON THE THIAZOLE DERIVATIVES: SYNTHESIS,CHARACTERIZATION AND BIOLOGICAL ACTIVITIES

**Vikas Kumar[1], Dr.K.Saravanan[2]**
[1]PhD Scholar, Pharmaceutical Science, Bhagwant Global University, Kotdwar
[2]Principal of Faculty of Pharmacy, Bhagwant Global University, Kotdwar
Corresponding author's E-mail: v.kumarvikas032@gmail.com

**ABSTRACT-**

The continuous review gives a blueprint of the natural activities of thiazole during the earlier years. It is heterocyclic blends, Thiazole, conceivably of the most generally perceived five-membered heterocyclic compound, has a nitrogen particle at position 3 and a sulfur bit at position 1. It is a critical arrangement of a gigantic number of made compounds. Its different pharmacological development is reflected in many clinically upheld thiazole-containing particles with, broad assortment of normal activities, similar to antibacterial, antifungal, antiviral, anthelmintic, antitumor, and quieting influences.

***Keywords:-*** *Thiazole, synthesis, Heterocyclic Compound, derivatives, biological activities.*

## Introduction-

Nitrogen-containing heterocyclic blends expect a critical part in the medicine revelation process, as practically 75% of FDA (Food and Association) upheld little iota drugs contain no less than one nitrogen-based heterocycles (1).

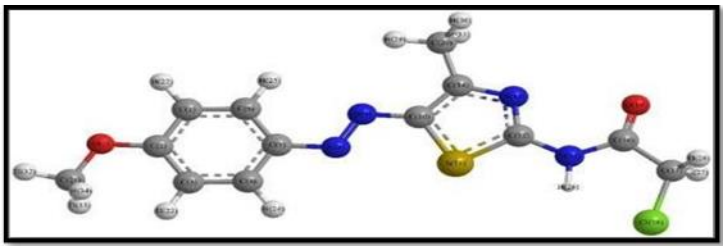


**Figure A:** 3D Structure of Thiazole

Thiazole, generally called 1, 3 - thiazole, has a spot with the get-together of combinations known as azoles and has sulfur and nitrogen particles in places 1 and 3, independently. The thiazole center is an astoundingly fundamental heterocycles in various naturally unique blends that makes it one of the extensively considered heterocycles1-3. Thiazole accepts urgent parts in numerous medicine structures. Tiazofurin and dasatinib (Antineoplastic trained professionals), ritonavir (unfriendly to HIV drug),

ravuconazole (antifungal subject matter expert) nitazoxanide (antiparasitic trained professional), fanetizole, meloxicam and fentiazac (moderating subject matter experts), nizatidine (antiulcer trained professional), and thiamethoxam (bug shower) are a couple of models for thiazole bearing things (2).

More than 18 FDA-embraced drugs contain the thiazole stage. Whenever no other decision is free, this thiazole subordinate was seen as effective against a grouping of multi-drug safe Gram-negative microorganisms, including Pseudomonas aeruginosa (P. aeruginosa). It is used to Treat tangled urinary parcel contaminations. Yet again alpelisib, sold under the brand name Pigray, is a substitute thiazole-based solution that was endorsed in 2019 for the treatment of express kinds of chest illness. Chest threatening development is potentially of the most unavoidable troublesome disorder on earth and the resulting driving justification behind illness passing, essentially in less advanced nations (3).

**Structural Characteristics-**

With the sub-nuclear condition C3H3NS, thiazole, generally called 1, 3-thiazole, is a sensible to light yellow ignitable liquid with a pyridine-like fragrance. It is a five-membered ring with two nitrogen-and sulfur-containing vertices and three carbon-containing people. The numeration scheme for naming thiazole subordinates is displayed underneath (4).

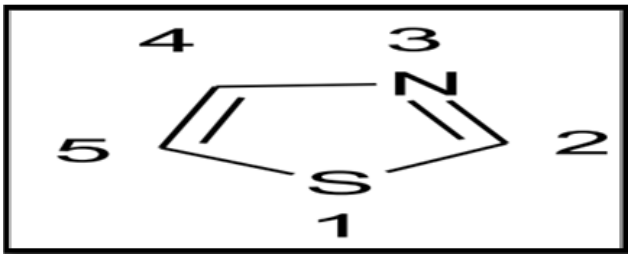



**Figure B:** The Structure of thiazole

Thiazoles are a class of regular combinations associated with azoles with a run of the mill thiazole moiety is a critical piece of vitamin B1 and epothilone. It is a sweet-smelling compound, satisfies Huckel's norm. Delocalizations of singular arrangements of electrons from the sulfur particle complete the 6 π electrons. The resonation structures are Fig-C (5).

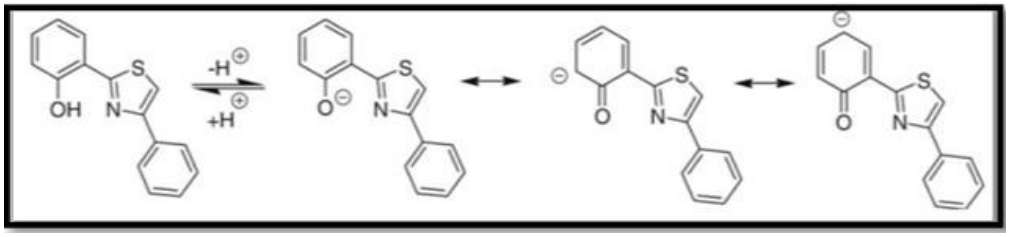



**Figure C:** Resonance forms of Thiazole

**Properties-**

**Physical Properties**

I. Thiazole generally pale-yellow flammable liquid.

II. It has pyridine like odour.

III. It is fairly soluble in ether and alcohol but sparinglysoluble in water.

IV. It has a boiling point of 116-118˚c and pka of 2.5 (conjugated acid).

V. Its density is 1.2 gm/cm3 and its ionization potentialis 9.50 Ev.

VI. It has a dipole moment of 1.61D.

**Chemistry of thiazole**

A consistent heterocyclic compound is conveyed by thiazole by using both an electron-giving get-together (- S-) and an electron-enduring bundle (C=N). The critical class of heterocycles known as thiazoles and their analogs, including oxazole, are made sure to have different normal properties. The azole compound isothiazole, which contains comparable particles (nitrogen and sulfur) but in a substitute position, is isomeric with the thiazole compound. Thiazole is dissolvable in alcohol and ether yet sensibly dissolvable in water. It has an edge of bubbling over some place in the scope of 116 and 118 oC and is a sensible, light yellow liquid. Thiazole is a heterocyclic ring that has six delocalized electrons from the sulfur particle's lone arrangements of electrons, according to Huckel's norm (6, 7).

As a result of their planar and sweet-smelling structure, which shows more unmistakable - electron delocalization than oxazole, thiazole subordinates are gainful model combinations for science research. By recognizing the engineered shift of the protons some place in the scope of 7.27 and 8.77

ppm in 1H NMR spectroscopy, the fragrant approach to acting of the thiazole ring was asserted. On account of the extension of various substituents at the C-2, C-4, and C-5 positions, the reactivity of the thiazole subordinates ring was anxious, which could require further essential idea. For instance, the methyl pack (electron giving social event) substituent noticeably impacted the thiazole ring's basicity and nucleophilicity when it was arranged at any circumstance on the ring (8, 9).

Regardless, when a solid electron-taking out pack, like a nitro bundle, was incorporated into the molecule, the basicity and nucleophilicity diminishes happen. Thiazole's adaptable construction blocks as bioactive substances lead to a capricious sub-nuclear plan. According to different assessments, the thiazole ring can be found in the vast majority of designed and typical things with a wide combination of regular properties. One of the models is vitamin B1, generally called thiamine, which has an effect in the mix of acetylcholine and in this way typically maintains the tangible framework. The way that thiazole auxiliaries contained both hydrophobic (lipophilic) and hydrophilic (lipophobic) parts gave them an amphiphilic quality too (10).

This quality extends its ability to diffuse into the bacterial cell film for limitation development easily. Blends can effectively subdue Gram-negative and Gram-positive microorganisms with both hydrophilic and lipophilic sections by entering the organisms' telephone film. This penetration causes cytoplasmic material spillage, unsettling influence of the cell's physiology, and apoptosis (11).

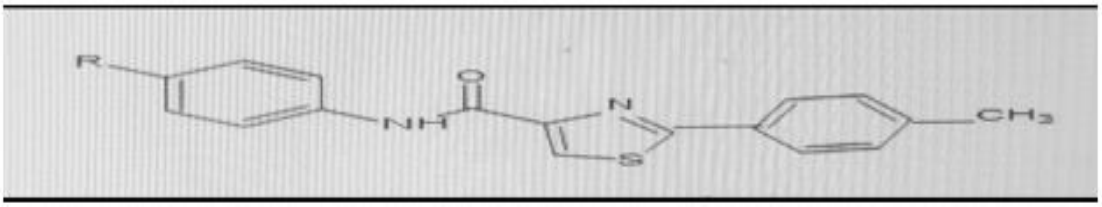
**Biological Activities of thiazole derivatives:-**

**Antifungal and Antibacterial Agents-**

The resistance of developments and microorganisms towards the antimicrobial drugs is extending rapidly a direct result of the nonselective antimicrobial activities and a foreordained number of meds. To overcome what is happening, various thiazole containing particles are coordinated to fix bacterial and infectious illnesses.

Bera et al. organized pyridinyl thiazole ligand having hydrazone moiety by combining 2-bromo-4-methoxy acetophenone with 2-acetylpyridine thiosemicarbazone. They in like manner coordinated cobalt complex by treating this ligand with cobalt forerunner. Both the ligand and its marvelous were gone after for against bacterial properties towards gram positive infinitesimal organic entities including Bacillus subtilis, Streptococcus fecalis, Staphylococcus aureus and gram-negative

microorganisms including Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Klebsiella pneumonia and Proteus vulgaris (12)




R = o-NO2, m-NO2, p-NO2, m-Cl, p-Cl, p-F

**Figure D:** 2-Phenylthiazole-4-carboxamides havinganticancer activities.

**Antidiabetic Activity-**

Bhagdev K. et al. coordinated and attempted a combination of subbed benzo-thiazole auxiliaries for hypoglycemic (antihyperglycemic) development. The ethoxybenzothiazole moiety in 2-(benzo[d]thiazol-2-ylmethylthio)- 6-ethoxybenzothiazole was seen as fundamental for redesigning glucose transport and AMPK order in L6 myotubes. 2-(benzo[d]thiazol-2-ylmethylthio)- ethoxy benzo[d]thiazole basically worked on the speed of glucose digestion in L6 myotubesat pharmacologically appropriate core interests. The effect of 2-(benzo[d]thiazol-2-ylmethylthio)- 6-ethoxybenzo[d] thiazole in on blood glucose levels in diabetic KKAy mice showed decrease in blood glucose level (13)

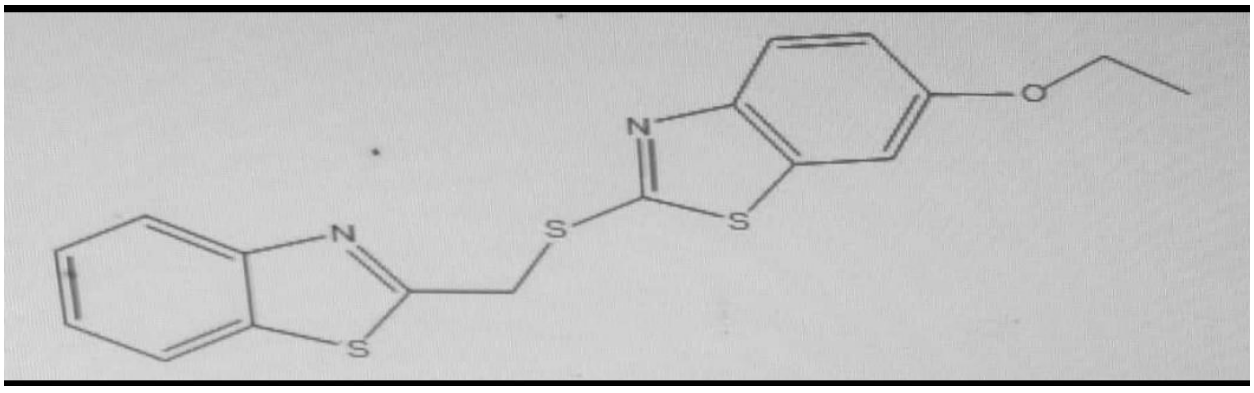



**Figure E**

**Anti-TB Activity-**

Arshad M. et al. mixed a new bisthiazolyl auxiliary and attempted it for unfriendly to TB activity.

These shrewd analogs were attempted against the Mycobacterium smegmatis MC2 155 strain. The basic displayed strong foe of tubercular feasibility at a piece of 30 mM. The SAR focuses on revealed that the presence of a fluoro-subbed phenyl ring is essential for lessening the M. smegmatis flood (14).

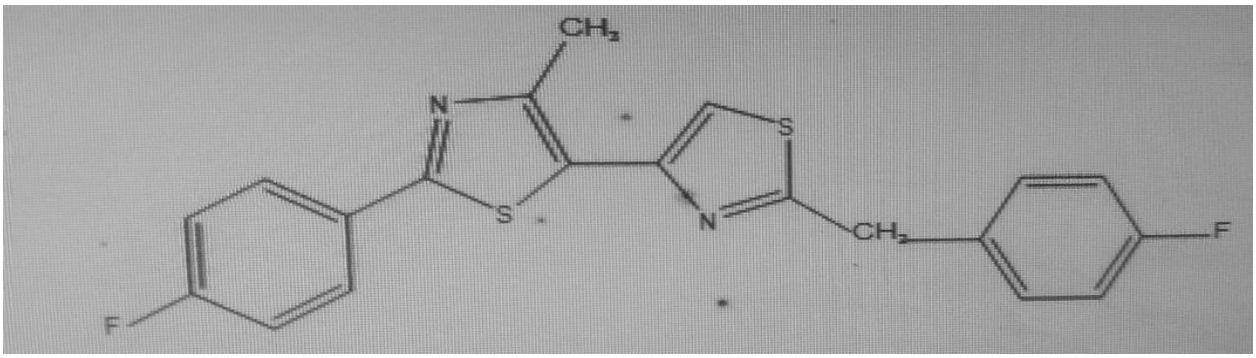



**Figure F**

**Anti-Cancer Activity-**

Kolageri S. et al. depicted the mix of fluorinated 2-aryl benzo-thiazole subordinates and their evaluation for antagonistic to development activity against illness cell lines MDA-MB-468 (mammary organ/chest tissues rising up out of metastatic site) and MCF-7 (human chest adenocarcinoma).The benzo-thiazole auxiliaries 4-(5-fluoro-1,3-benzothiazol-2-yl) phenol) and 3-(5-fluoro-1,3-benzothiazol-2-yl)phenol) with hydroxyl substituents on the third and fourth positions were all the more impressive to those containing alkoxy, methyl sulphonyl, and ethyl substituents on the Benzothiazole (15)

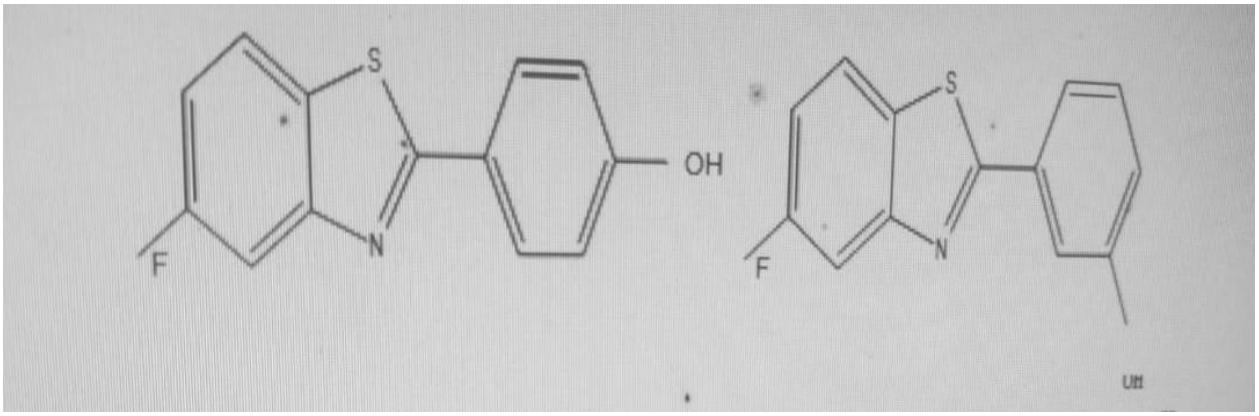



**Figure G:**

**Anti-Inflammatory Activity-**

Krishnan G. et al. made and joined a movement of 2,4-disubstituted thiazole auxiliaries, which were pursued for moderating development in vitro using the egg whites denaturation system and diverged from the standard medication diclofenac sodium (16).

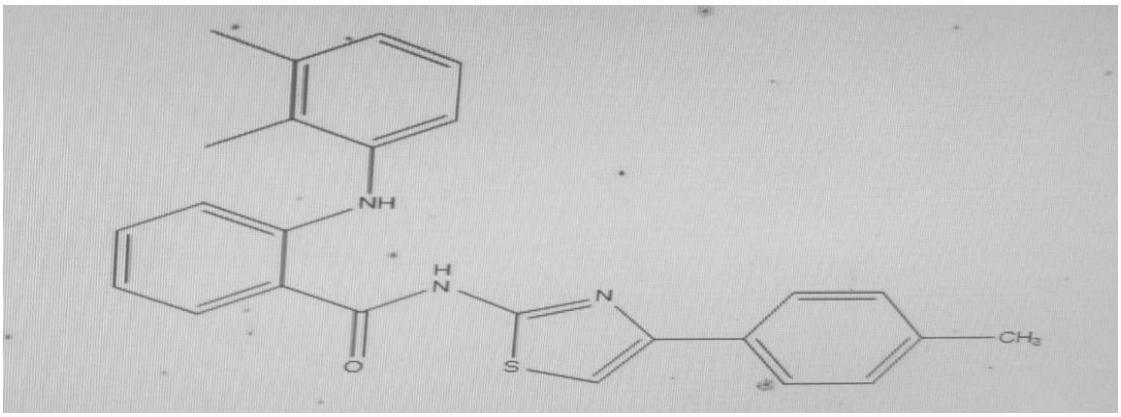



**Figure H:**

**Anti-Tumor Activity-**

Kaur H. et al. consolidated 2-(4-(pyrazol-4-yl) thiazol-2-ylimino)- 1,3,4-thiadiazole auxiliaries, which have been perused up for anticancer suitability against human hepatocellular carcinoma cells (HepG2), human chest dangerous development cells (MCF-7) and human cell breakdown in the lungs cells (A549) in vitro (17).

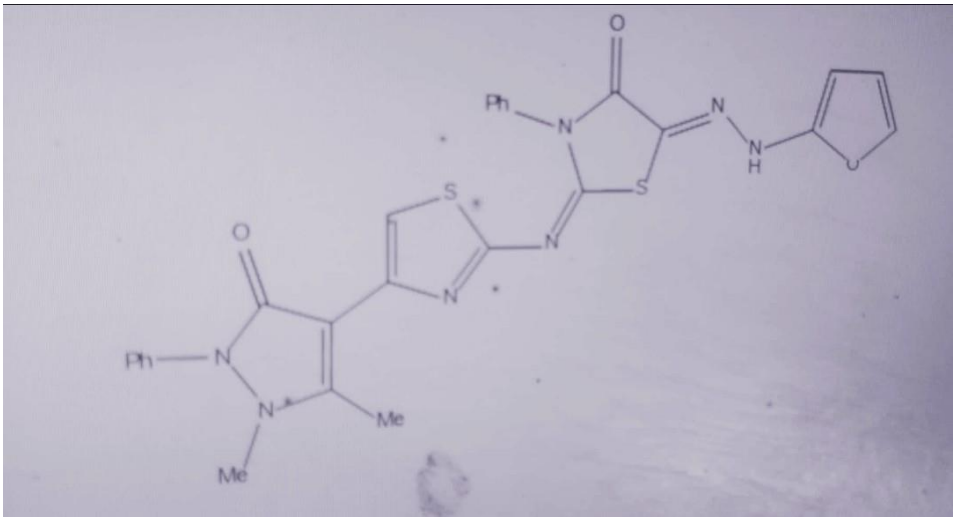



**Figure I:**

**Antimalarial Activity-**

Yadav A. et al. arranged and organized thiazole compounds and attempted them for antimalarial development. The results have shown that an electron-taking out pack at the fourth spot of the associated phenyl ring of thiazole auxiliaries is normal for dealt with foe of malarial activity and a decent drug like profile, which can provoke the ascent of an expected supportive molecule in extra development (18).

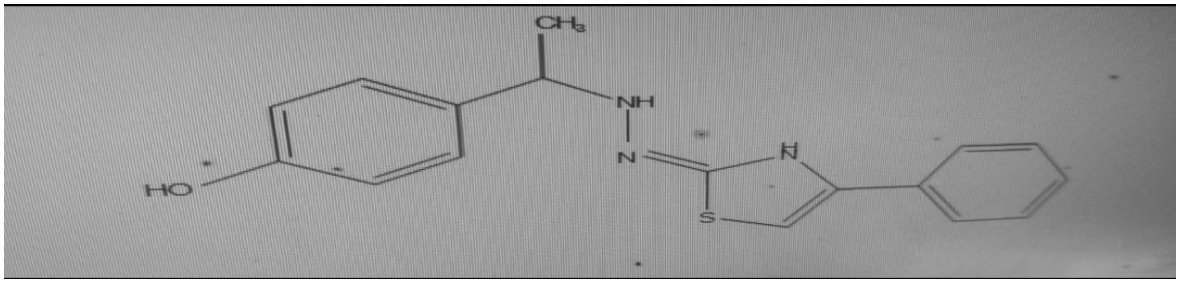



**Figure J:**

**Antioxidant Activity-**

Naminath H. et al. consolidated one more series of thiazole compounds and surveyed their malignant growth counteraction specialist works out. When diverged from customary ascorbic destructive, the molecule showed out and out higher feasibility against erythrocyte haemolysis (0.85%)(19). $CH_3$

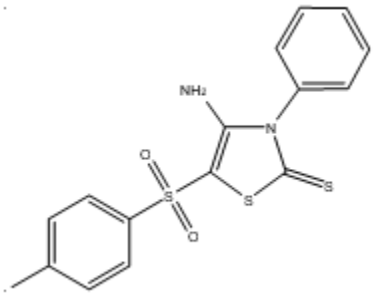



**Figure H**

**CONCLUSION-**

Thiazole center has involved a basic circumstance in the state of the art normal and supportive science due to its wide reach generally; Thiazole compounds are seen to have captivating natural components like

anticancer and antimicrobial activities.

The presence of thiazole ring in numerous prescriptions such febuxostat, dasatinib and ravuconazole convince the logical specialists to design new thiazole systems.

It has been seen that changes to the thiazole moiety shown profitable natural capacities. More assessment is supposed to think about thiazole's reasonability rather than various issues.

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# Retrospective Studies on Phytochemistry and Therapeutic Application of Pueraria tuberosa

Author:z **Ms. Sakshi N. Pandharkar**
Co-Author:
Dr. Avinash S. Dhake
Ms. Sujata Lambe
(Assistant Prof., S.M.B.T College of Pharmacy)

**Abstract:**

*Pueraria tuberosa* is widely accepted as Vidarikand and Indian kudzu. It is a valuable herb in Ayurvedic medicine. Its tubers are rich in phytochemicals like flavonoids, isoflavones, and pterocarpanoids, contributing to its diverse therapeutic properties. It has extensive pharmacological actions including antioxidant, anti-inflammatory, immunomodulatory and anticancer activities. *Pueraria tuberosa* holds promise in controlling various disorders like diabetes, inflammation and cancer. Further investigation is required to explore its mechanisms of action, pharmacological action and therapeutic applications, particularly in areas like osteoporosis and diabetes management.

**Keywords***: Pueraria tuberosa*, Phytochemistry, Osteoporosis, Antioxidant, Anti-Inflammatory.

**List of Abbreviation:**

WHO - World Health Organization

EEPT - Ethanolic Extract of *Pueraria tuberosa*

PTTE - *Pueraria tuberosa* tuber extract

ALEPT - Alcoholic Extract of Tubers of *Pueraria tuberosa*

FRAC - Fraction rich in antioxidant compounds

**1.Introduction:**Traditional medicine practices such as Ayurveda, Siddha, Homeopathy, Unani and Folk medicine are commonly utilized in India to treat various diseases and disorders. According to the World Health Organization (WHO) study about 65–80% of people worldwide go for herbal remedies to treat common health issues [1]. Ayurveda encompasses numerous herbs that not only promote skin wellness but also possess properties that contribute to delaying the aging process[1]. In the contemporary era, there is a

growing exploration of medicinal plants as traditional therapies to address various physiological difficulties [2].

The perennial climber *Pueraria tuberosa* (Roxb. ex Willd. DC) (family: Fabaceae) is found throughout the Indian subcontinent in moist, wet places. This plant's tuberous roots, which are used in Indian Ayurvedic medicine, are becoming even more significant and are being subject of studies for their potential remedies for a various health Issues[3]. It is widely utilized herb in herbal medicine [1].The species belonging to the Pueraria genus, particularly Pueraria thomsonii and Pueraria lobata are also find frequent use in Traditional Chinese Medicine [2].

*Pueraria tuberosa* is one of the most significant medications extensively detailed in all the traditional (Ayurveda) texts including Nighantus, Laghuttrayi, and Brihattrayi. Vidarikand, scientifically known as *Pueraria tuberosa (*Willd.) DC., is frequently referred to as Indian kudzu [4]. It is also known as 'Vidari' according to the Ayurvedic Pharmacopoeia of India [2]. Vidarikand is mentioned in Guduchyadi varga of Bhavaprakash and its synonyms are listed as Krostri, Sita, Ikshugandha, Kshirvalli, Swdukanda, Kshirshukla, Ikshugandha and Payasvani [4].

Vidarikanda synonymously referred to as Hedysarum tuberosum Willd, is extensively utilized in Ayurveda's 'Rasayana' category of drugs. In Ayurvedic practices, the tuber is commonly employed for its aphrodisiac qualities, as well as its reputed benefits in promoting longevity and rejuvenation [2]. The term "Rasayana" is derived from Sanskrit, where "Rasa" signifies plasma and "Ayana" denotes path. Therefore, "Rasayana" refers to the path that "Rasa" takes. Drugs categorized as "Rasayana" are abundant in antioxidants and are recognized for their hepatoprotective and immunomodulating properties [5]. In Indian traditional medicine (Ayurveda), the plant's tuberous roots find application in treating conditions such as general debility, burning sensation, intrinsic hemorrhage, nervous breakdown, spermatorrhoea, heart diseases, and tuberculosis. [3] This plant tubers are sweet in taste [6]. The tubers of the *Pueraria tuberosa* are traditionally employed for the treatment of various conditions like inflammation, sexual weakness, as well as for its contraceptive, cardiotonic, diuretic and galactagogue, refrigerant, aphrodisiac properties[2]. Additionally, it is used as food for both human and animal consumption[2].

The various formulations of this substance find application as nutritive, diuretic, and expectorants, and also in management of fever, rheumatism, and bronchitis. Some its notable biological properties include

antihyperlipidaemic, antihyperglycaemic, antifertility in male rats, and hepato-protective effects[7]. The clinical application of PT root-tuber powder includes its use as a tonic, anti-aging agent, purgative, aphrodisiac, lactagogue, demulcent, and in the treatment of scorpion stings. It is also beneficial for addressing conditions such as emaciation in children, debility, and poor digestion. Some researchers have also highlighted its effectiveness in skin care and its potential as an anti-fertility agent. Purarian have anti-diabetic property[8]. Numerous pharmacologically potent constituents, including puerarin, genistein, lupinoside, daidzein, tuberosin, have been extracted from the Vidarikanda. In recent in vitro and in vivo studies have identified wide range of biological activities of *Pueraria Tuberosa* tuber extract and its isoflavonoids[2]. The tuber has a various types of phyto-chemical constituents including tannins, glycosides, steroids, flavonoids, terpenoids and anthocyanidins, coumarins, alkaloids, carbohydrates[9]. The chemical constituents have been found as puerarin, daidzein, daidzin, alpha-sitosterol and stigmasterol.[3]

Its primary chemical constituents are flavones, including c-glycoside, isoflavones like puerarone, coumstan which include tuberostan and puerarostan, epoxychalcanol also called as puetuberosanol, and pterocarpanoids such as hydroxytuberosin, anhydroxytuberosin (3-O methylanhydrotuberosin), and tuberosin[8].

It is significant ingredient in various Ayurvedic formulations, involving the health tonic Chyawanprash[2,10].

**2.Taxonomical Classification**: Taxonomic classification of Pueraria tuberosa mentioned below in Table No.-1

**Table No 1: Taxonomical Classification**

| Kingdom | Plantae | References |
|---|---|---|
| Sub-kingdom | Trachebionta | 1 |
| Superdivision | Spermatophyta | |
| Division | Magnoliophyta | |
| Subclass | rosidae | |
| Order | Fabales | |
| Family | Fabaceae | |

| | | |
|---|---|---|
| Genus | Pueraria DC | |
| Species | *Pueraria tuberosa* | |

**3.Vernacular Names**: Pueraria tuberosa is well known by different names in different language in India as mentioned below in Table No.-2

**Table No.2: Vernacular Names**

| Language | Name | References |
|---|---|---|
| Marathi | Bhuikohala, Ghodvel | 11 |
| Hindi | Vidarikanda, Bankumara | |
| Sanskrit | Bhumikusmanda, Gajavajipriya, Kandapalash. | |
| English | Indian kudzu. | |
| Tamil | Nilapoosani | |
| Kannada | Gumadi belli, Gumadigida, Nelagumbala Gudde. | |

**4.Physical Characteristics:**

Vidari comprises sliced and dried pieces of the tuberous root, which can grow up to 60 cm in length and 30 cm in width, derived from *Pueraria tuberosa* DC (Family: Fabaceae). This perennial climber is characterized by its extensive tuberous root and is found across the country, excluding extremely humid or arid regions, with an altitude range extending up to 1200 m[11].

The whole root tuber's surface is slightly undulating, with an indistinct epidermis. It includes 3 to 4 layers of cork cells followed by 5 to 7 layers of parenchymatous cells. A fibrous pericycle and couple of layers of stone cells loaded with sand crystals follow the fully developed endodermis, and the brown cork cambium measures two or three cells thick. companion cells, bast fibres, sieve tubes and phloem parenchyma all are found in phloem. The tracheid's, fibres, parenchyma, and vessels with scalariform pores form the xylem, which was previously pentarch. Granules of starch are present in both the phloem cells and the wide parenchymatous medullary rays on which they are formed.

These grains are polygonal in shape ranging from 2 to 5 μm in diameter are frequently complex with a central cleft and undefined hilum, lamellae are thin. In macerated samples, crystal fibres are multicellular and articulated, where each cell contains a calcium oxalate crystal. Some of these fibres are swollen at the centre resembling a bulb pipette[12].

1

**Figure 1: Powder of vidari kand collected from SMBT Ayurved College and Hospital,Dhamangaon,Nashik.**

**5.Phytochemical Composition:**

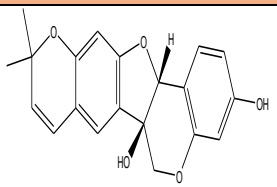
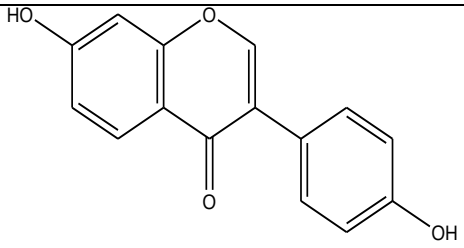
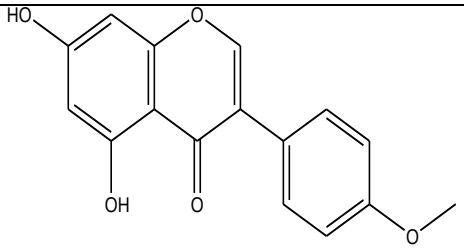
Phytochemical screening has revealed a variety of bioactive compounds such as carbohydrates, alkaloids, coumarins, tannins, flavonoids, steroids, terpenoids, anthocyanidins and glycosides in the tuber extract. According to recent RP-HPLC examination of the tuber extract, flavonoids including genistein (1.37%), diazene (1.70%), and puerarin (8.31%) are detected. Additional flavonoid chemicals found in the tuber include quercetin, irisolidon, tectoridin, robinin, daidzin, genistein, puerarone, tuberosin, hydroxytuberosone, 4-methoxypuerarin, biochanin A, biochanin B, quercetin, and glycoside (6-diacetyl

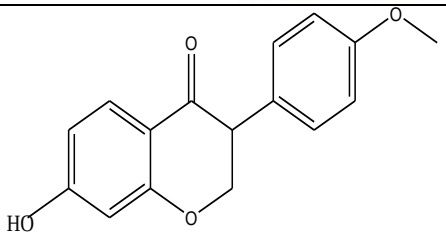
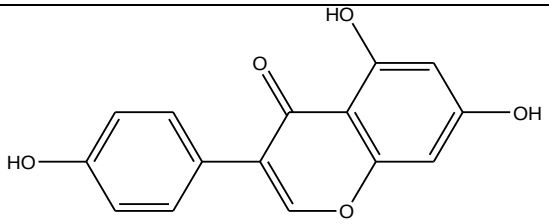
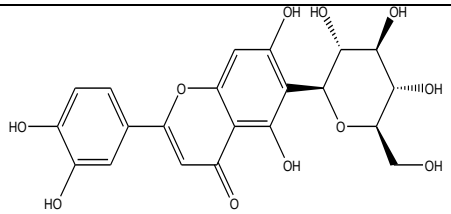
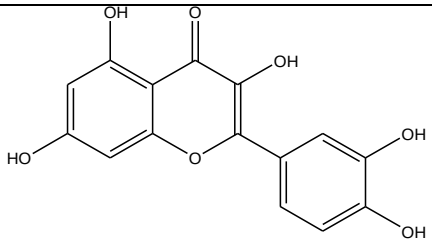
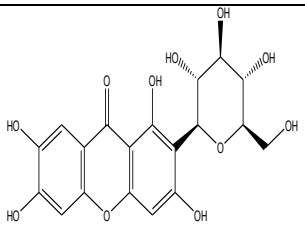
Puerarin, C-glycoside 40). It also contains pterocarpanoids (anhydroxytuberosin, 3-O-methylanhydrotuberosin), epoxychalcanol (puetuberosanol), and Coumstan (tuberostan, puerarostan). Likewise, the tuber contains organic acids (eicosanoic acid, arachidonic acid, hexadecanoic acid, tetracosanoic acid, and p-coumaric acid), phytosterols (b-sitosterol), and stigmasterol. Low amounts of anthocyanins, pterocarpintuberosin, and lupinoside (lupinoside PA4) are also present in the tuber[2].

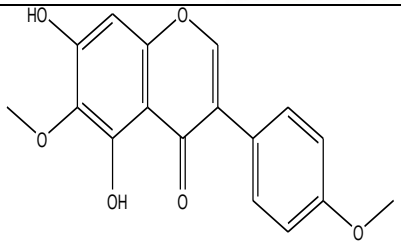
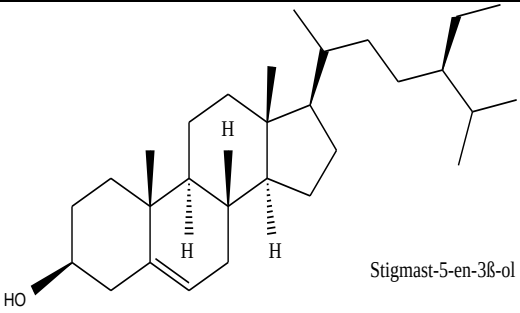
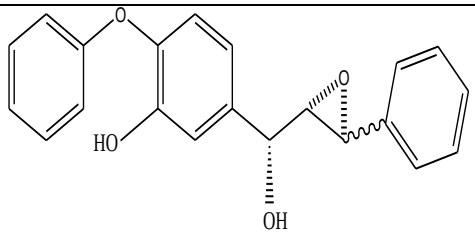
It has been revealed that the roots of Vidari contain phytoestrogen called puerarin. Its tubers contain 10.9% protein and 64.6% carbohydrates[1314].

Some of the phytoconstituent and their activity are enlisted in table no. 3

**Table 3: Phytoconstituent of Pueraria tuberosa and its pharmacological activity**

| Chemical name | Structure | Activity | References |
|---|---|---|---|
| Tuberosin |  (1R,13R)-7,7-Dimethyl-8,12,20-trioxapentacyclo[11.8.0.02,11.04,9.014,19]henicosa-2(11),3,5,9,14(19),15,17-heptaene-1,17-diol | Antioxidant, | [15] |
| Daidzein |  7,4'-dihydroxyisoflavone | Anticancer, Antidiabetic, Neuroprotective | [6,9] |
| Biochanin A |  5,7-Dihydroxy-4'-methoxyisoflavone | Anti-inflammatory, Antimicrobial | [1,6,16] |

| | | | |
|---|---|---|---|
| Biochanin B |  7-hydroxy-3-(4-methoxyphenyl)chromen- 4-one | Antimicrobial | 6,17 |
| Genistein |  5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one | Antioxidant, Anti-inflammatory | 6 |
| Isoorientin |  2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-6-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-4H-1-benzopyran-4-one | Anti-Inflammatory | 1819 |
| quercetin |  2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one | Nootropic | 17 |
| Mangiferin |  1,3,6,7-Tetrahydroxy-2-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-9H-xanthen-9-one | Anti-Inflammatory, Antioxidant | 20 2122 |

| | | | |
|---|---|---|---|
| Irisolidone |  5,7-dihydroxy-6-methoxy-3-(4-methoxyphenyl)chromen-4-one | Anxiolytic | 9 |
| Beta- Sitosterol |  Stigmast-5-en-3ß-ol | Antimicrobial | 6,17 |
| puetuberosanol |  | anti-implantation, Antimicrobial | 6,2324 25 |

## 6. Pharmacological Actions:[9]

Pharmacological action reported are anxiolytic action[3], immunostimulatory action[5], antiapoptotic action, antioxidant activity[26,27], antifertility activity[28], antimicrobial action[29], antidiabetic activity[14], anti-implantation activity[25], androgenic activity[16], anticonvulsant activity, antihypertensive action[21], hypolipidemic action, anti-inflammatory action[30,19], anticancer activity[17], hepatoprotective activity[31], fibrinolytic action, wound-healing action, fibrinolytic activity, cardiovascular activity[32], neuroprotective action, nephroprotective activity[33], immunomodulatory action[34], nootropic action[35].

### 6.1. Antidiabetic activity:

The study suggests that water base extract of vidari has the potential to modulate DPP-IV activity, both in non-diabetic and diabetic conditions. By reducing DPP-IV activity, *Pueraria tuberosa* water extract (PTWE) may enhance the levels of incretin hormones, which could contribute to improved glucose metabolism and a better hypoglycemic state. These results indicate the potential therapeutic value of

water-based extract of vidari as a natural inhibitor of DPP-IV activity, signalling further investigation into its mechanisms and clinical applications in the management of diabetes[36]. flavonoids and polyphenols exhibit dual effects on glucose uptake, mediated partly through SGLT1 modulation, suggesting their potential as therapeutic agents for diabetes management[14].

**6.2. Anti-inflammatory activity:**

The experimental results suggest that isoorientin, administered at doses ranging from10mg/kg and 20mg/kg body weight, possess anti-inflammatory properties. The reduced inflammation seen in mice treated with isoorientin in contrast to those only exposed to carrageenan recommend that isoorientin could be a potential application for inflammatory conditions. Additional investigation is necessary to understand how isoorientin exerts its anti-inflammatory effects and to uncover its possible uses in treating different inflammatory ailments[18].

**6.3. Antioxidant activity:**

The ethanolic extract of *Pueraria tuberosa* (EEPT) demonstrated significant antioxidant activity in both the reducing power assay and the hydrogen peroxide radical scavenging assay. The $IC_{50}$ values obtained for EEPT were comparable to those of standard ascorbic acid, indicating that the extract has potent antioxidant properties similar to or slightly lower than antioxidant ascorbic acid. The EEPT fraction indicated significant amount of flavonoid, phenolic content, and antioxidant action. These results suggest the potential of *Pueraria tuberosa* extract as an herbal source of antioxidants with possible health benefits[37,17,38].

**6.4. Immunomodulatory activity:**

The ethanolic extract of *Pueraria tuberosa* possesses immunosuppressant activity, as evidenced by its ability to reduce antibody titer values and mitigate drug-induced myelosuppression. The extract exhibited dose-dependent effects, with the higher dose (500 mg/kg) showing better activity compared to the lower dose (250 mg/kg) and being comparable to the standard immunosuppressant, azathioprine[37].

The ethanolic extract of P. tuberosa demonstrates immunomodulatory properties by enhancing macrophage activity and protecting against myelosuppression caused by cyclophosphamide. It appears to selectively reduce adaptive immunity while inhibiting humoral and cell-mediated immunity, leaving the

innate immune system and bone marrow cell proliferation unaffected.[34] These findings support the potential use of *Pueraria tuberosa* extract in conditions where immunosuppression is desired, although future investigation is recommended to find out its mechanism of action and evaluate its safety and usefulness in healthcare environments[37].

P. tuberosa tuber extract (PTTE) enhances innate immune functions and humoral immunity in rats, suggesting its potential therapeutic use in modulating immune responses in humans. Particularly, the isoflavones PR and DZ augment various immune parameters, while GS exhibits suppressive effects. PTTE overall stimulates immune function dose-dependently. To fully understand the mechanics behind these impacts, more research is required. particularly regarding the unidentified compounds responsible for neutrophil adhesion stimulation[39].

**6.5. Anticonvulsant activity:**

The Occurrence of flavonoids, triterpenoids, glycosides, and other chemical substance in alcoholic extract of tubers of *Pueraria tuberosa (*ALEPT) suggests that these constituents may be responsible for the detected pharmacological effects of the ALEPT in experimental animal models. The study`s finding suggest that plant extract exhibits anticonvulsant properties, confirming its traditional use to cure epilepsy. This provides pharmacological evidence for the efficacy of ALEPT in managing epilepsy, as per traditional knowledge[40].

**6.6. Antimicrobial activity:**

Extracts of P. tuberosa, specifically those from ethyl acetate, acetone and hexane, shown broad-spectrum inhibitory response against tests of antimicrobial susceptibility. Bacterial strains have higher susceptibility in comparison to fungi. The best solvent for removing antibacterial chemicals from P. tuberosa was found to be ethyl acetate. P. tuberosa is known to contain biologically active substances like phenols, polyphenols, alkaloids, tannins, terpenoids and flavonoids[29].

**6.7. Anticancer activity:[17]**

Fraction rich in antioxidant compounds (FRAC) demonstrated promising anticancer activity specifically in ovarian and breast cancer cells. This recommends that it may have ability as a therapeutic agent in the

treatment of cancer therapy. Further research and clinical trials would be essential to fully understand its effectiveness, safety profile, and potential mechanisms of action[9,41].

**6.8. Antifertility activity:**

Based on the functional toxicological experiments carried out on boar spermatozoa, Kudzu may alter cAMP, Ca2+ signalling, and mitochondrial function, which might have an impact on sperm motility. Kudzu includes substances that could influence ionophore-induced acrosome response (AR) and change Ca2+ signalling pathways. While puerarin doesn't affect motility, it inhibits other sperm functions crucial for fertilization, reducing spontaneous AR. Both compounds interact with P4 similarly to reducing agents, causing sperm decondensation. However, their mechanisms differ, possibly due to the presence of other phytoestrogenic components in kudzu[42,28].

**7.Therapeutic Applications:**

*Pueraria tuberosa* recognized as a 'Rasayana' herb in Ayurveda, exhibits a diverse array of therapeutic properties including refrigerant, aphrodisiac, emetic, galactagogue, emollient, laxative, diuretic, cardiotonic, rejuvenating, and expectorant effects. Its versatile nature allows it to be incorporated into various Ayurvedic formulations, where it functions as anti-aging agent, vital energy booster, restorative tonic, spermatogenic, immune booster, demulcent, cholagogue, purgative, and nutritive. With its wide-ranging applications, *Pueraria tuberosa* holds significant promise in traditional and folk medicine for addressing a multitude of health concerns and promoting overall well-being.

Ayurvedic practitioners frequently prescribe the tuber to address general weakness, fertility disorders, and alleviate symptoms related to dysmenorrhoea, menopausal syndrome, uterine bleeding.

In traditional medicine, the tuber is employed to address various health issues such as fever, chest pain, diarrhoea, abdominal pain and rheumatism. In the realm of folk medicine, the tuber is commonly utilized as a remedy for nerve-related conditions, a promoter of lactation, an anti-inflammatory agent, a tonic for the brain, a blood purifier, and to enhance sperm production. Some indigenous communities in India incorporate the tuber into their diet for nutritional supplementation, birth control, and the management of diabetes[23,7].

*Pueraria tuberosa* has been suggested to cure three doshas (diseases caused by the three distinct energies that is Vata, Pitta, and Kapha) in the human body. *Pueraria tuberosa* tubers powder is commonly utilized with cow's milk to stimulate milk production post-childbirth and as a growth-promoting agent when combined in Piper longum L. powder to promote growth in malnourished children's. To relieve excessive menstruation, it is mixed with honey. A mixture of milk, fried wheat or barley in ghee, and powdered vidarikanda is recommended to improve sexual endurance and weakness. Furthermore, for treating spermatorrhoea, a therapeutic mixture of fresh Vidarikanda juice, cumin seeds, and sugar is employed [6].

Its uses are mentioned in Ayurveda as Sula (Calming down pain), Kasa (useful in cough), Sula (settling down burn), mutrakricchra (helpful in dysuria), visarpa (pacifying skin disorder), visamjawar (fever), and Sukra kshya (increasing semen)[13].

The tuberous roots are brown in colour, clinically prescribed for anti-aging, rejuvenation, as tonic, demulcent, lactagogue, aphrodisiac, purgative, cholagogue, in scorpion sting, emaciation of children, poor digestion and debility.

Vidari is recorded for its activity like antihypoglycemic, diuretic, expectorant, nutritive, glowing skin to improve complexion. and good cardio protection. It also utilised in case of fever, poor indigestion, bronchitis.

According to the reports *Pueraria tuberosa* has been proven to be effective fibrinolytic agent as it reduces fibrinogen, an individual risk factor for stroke and coronary artery disease[1].

Several important Ayurvedic formulations using Indian kudzu comprises the "Maha visagarbha taila" a traditional cure for joint diseases and sciatica, "Ashwagandharishta", an ancient treatment of epilepsy. Other product use is "Sarasvatarista", "Satavaryadi ghrta", "Nityananda rasa", "Vidaryadi ghrita", "Marma gutika"[6].

*Pueraria tuberosa* is one of the frequently utilised plants of Tdraditonal (Ayurveda) medicine. Vidari is a part of numerous highly prized Ayurvedic mixtures, including the traditional Indian food supplement Chyavanaprash. The yearly trade volume of *Pueraria tuberosa* falls between 500–1000 Metric Tonnes[43]. Vidarikandadi Churna is crucial herbal medicine used to treat childhood malnutrition. Main ingredient of Vidarikandadi Churna is Vidarikand (*Pueraria tuberosa),* Yava (Hordeum vulgare) and Godhum (Triticum sativum)[13].

## 8. **Conclusion:**

*Pueraria tuberosa* emerges as a valuable natural resource with promising healing properties, offering avenues for further investigation and potential integration into mainstream healthcare practices. Its long-standing use in traditional medicine systems, coupled with modern scientific validation, positions it as a promising candidate for addressing various health conditions and promoting overall well-being. Future studies are required to further explore the mode of action of *Pueraria tuberosa* and its clinical applications particularly in areas like cancer treatment, microbial growth inhibition, epilepsy treatment, immunostimulatory, osteoporosis and diabetes management. Additionally, safety and efficacy evaluations in clinical settings are essential to fully understand the therapeutic application of this valuable herb. Overall, *Pueraria tuberosa* holds significant promise as a natural remedy for various health conditions, highlighting its importance in traditional and folk medicine practices.

## 9. Future study:

further studies are needed to evaluate its safety and efficacy in clinical settings to understand the synergistic effects of its bioactive compounds. Significant antiosteoporosis medication produced by ovariectomy was created by the FRAC of *Pueraria tuberosa*. Further it demonstrated anticancer activity in ovarian and breast cancer cells, highlighting its possible application in treatment of postmenopausal osteoporosis, osteoporotic bone degeneration and bone fractures. However, to properly assess the potential use of FRAC of *Pueraria tuberosa* in osteoporosis and bone fracture therapy more mechanistic research is required. These include research of the redox status in bone, HDL & LDL levels in serum.

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