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

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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.

A B

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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References:

1. Mohajan D, Mohajan HK. Basic Concepts of Diabetics Mellitus for the Welfare of General Patients. Studies in Social Science & Humanities. 2023 Jun 20;2(6):23-31.
2. Bushi G, Padhi BK, Shabil M, Satapathy P, Rustagi S, Pradhan KB, Al-Qaim ZH, Khubchandani J, Sah R, Sah S, Anand A. Cardiovascular disease outcomes associated with obstructive sleep apnea in diabetics: a systematic review and meta-analysis. Diseases. 2023 Aug 7;11(3):103.
3. Kumar A, Gangwar R, Ahmad Zargar A, Kumar R, Sharma A. Prevalence of diabetes in India: A review of IDF diabetes atlas 10th edition. Current diabetes reviews. 2024 Jan 1;20(1):105-14.
4. Aneesa NN, Anitha R, Varghese S. Antidiabetic activity of ajwain oil in different in vitro models. Journal of Pharmacy and Bioallied Sciences. 2019 Apr 1;11(2):142-7.

5. Kumar A, Gangwar R, Ahmad Zargar A, Kumar R, Sharma A. Prevalence of diabetes in India: A review of IDF diabetes atlas 10th edition. Current diabetes reviews. 2024 Jan 1;20(1):105-14.
6. Pan A, Naskar P. LITERATURE REVIEW OF KANKOLA (PIPER CUBEBA LINN. F.): A ROUTINELY USED SPICE WITH POTENTIAL MEDICINAL VALUES.
7. Loying R, Sharmah B, Barman H, Borah A, Bora HK, Kalita J, Manna P. Anti-inflammatory potential of Piper betleoides C. DC., a promising Piper species of Northeast India: in vitro and in vivo evidence and mechanistic insight. Inflammopharmacology. 2024 Aug 10:1-8.
8. Manikandan R, Anand AV, Muthumani GD. Phytochemical and in vitro anti-diabetic activity of methanolic extract of Psidium guajava leaves. International journal of current microbiology and applied sciences. 2013 Dec 18;2(2):15-9.
9. Pachpute AP, Deshmukh TA, Jagdishprasad S, Tibrewal J. Antioxidant and hepatoprotective activity of an ethanol extract of piper cubeba fruits. Int J Res Dev Pharm L Sci. 2013 Feb;2:321-9.
10. Manikandan R, Anand AV, Kumar S. Phytochemical and in vitro Antidiabetic activity of Psidium Guajava leaves. Pharmacognosy Journal. 2016;8(4).
11. Bothon FT, Debiton E, Avlessi F, Forestier C, Teulade JC, Sohounhloue DK. In vitro biological effects of two anti-diabetic medicinal plants used in Benin as folk medicine. BMC Complementary and Alternative Medicine. 2013 Dec;13:1-8.
12. Ali H, Houghton PJ, Soumyanath A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. Journal of ethnopharmacology. 2006 Oct 11;107(3):449-55.
13. Shai LJ, Masoko P, Mokgotho MP, Magano SR, Mogale AM, Boaduo N, Eloff JN. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. South African Journal of Botany. 2010 Aug 1;76(3):465-70.
14. Funke I, Melzig MF. Traditionally used plants in diabetes therapy: phytotherapeutics as inhibitors of alpha-amylase activity. Revista Brasileira de Farmacognosia. 2006;16:1-5.
15. Hasbal G, Ozden TY, Can A. In vitro antidiabetic activities of two Sorbus species. European Journal of Biology. 2017 Dec 1;76(2):57-60.
16. Grussu D, Stewart D, McDougall GJ. Berry polyphenols inhibit α-amylase in vitro: Identifying active components in rowanberry and raspberry. Journal of Agricultural and Food Chemistry. 2011 Mar 23;59(6):2324-31.