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In Vitro Alpha Amylase Inhibitory Effect of Selected Medicinal Plants

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ABSTRACT

Diabetes mellitus is a complicated health disorder that pushes human life into misery. Oral hypoglycemic agents are not effective for a complete cure, and it has side effects, too. Herbal remedies have long been used to treat diabetes and are widely accepted due to reduced side effects. Aqueous extracts made from dried leaves of Azadirachta indica, whole plant parts of Biophytum sensitivum, aerial roots of Cocos nucifera, and dried leaves of Passiflora edulis were used in this work to examine the antioxidant and antidiabetic effect. The extracts underwent additional phytochemical analysis to determine their total tannin, total flavonoid, and total phenolic contents. In our study, aerial roots of Cocos nucifera extract showed maximum antioxidant power (0.37±0.005) and α-amylase inhibition (83.50±0.57). Antidiabetic activity of coconut roots was reported for the first time. Hence, it opens the door for the creation of a potential drug against hyperglycemia. However, further investigations must be carried out to determine the specific chemicals and the exact mode of action behind the antidiabetic and insulin-mimetic activity of coconut roots.

Key words: *α- amylase, Antidiabetic, Antioxidant, Diabetes mellitus, In vitro study*

INTRODUCTION

Diabetes mellitus is a major endocrine disease that causes disturbances in glucose, protein, and fat metabolisms that result from defects in the insulin hormone [1]. It causes cardiovascular illness, hypertension, blindness, renal failure, neurological damage, amputations of legs, and premature death [2]. According to the World Health Organization, kidney disease due to diabetes caused 2 million deaths in 2019 [3]. Type I, type II, and gestational diabetes are the three main forms of diabetes mellitus [4]. The most prevalent kind of diabetes is type II, and in recent years, the number of diabetics has sharply increased. India accounts for 77 million diabetics, which may reach up to 134 million in 2045 [5].

In addition to insulin intake, prevention and treatment of diabetes include many oral hypoglycemic agents along with appropriate diet and exercise. Metformin, one of the most commonly used oral hypoglycemic, decreases glucose synthesis in the liver either directly or indirectly, and it increases glucose absorption in the stomach [6]. Oral hypoglycemic agents such as Acarbose, Miglitol, and Voglibose act by inhibiting the enzymes in carbohydrate metabolism, which delays glucose uptake [7]. The enzymes that are responsible for the breakdown of starch to simple sugars are α-amylase and α-glucosidase [8]. The postprandial plasma glucose rise is hindered by inhibitors of these enzymes, which cause a substantial reduction in the rate at which simple sugars are absorbed and extend the general carbohydrate breakdown [9]. Nevertheless, a number of gastrointestinal adverse effects, including diarrhea, flatulence, and abdominal pain, are linked to these medications [10].

Here comes the importance of herbal medicines that demonstrate a potential activity against diabetes, and also they attain wide acceptability due to their fewer side effects, easy availability, and reasonable cost [11]. Worldwide, a broad spectrum of plants and their products are suggested and utilized in the control of diabetes,

with inadequate knowledge of the mechanism and mode of action of these drugs [12]. In undeveloped nations, plant therapies form the mainstay of medical care.

Azadirachta indica, commonly called neem, is well known for its medicinal action against skin disease, cancer, diabetes, liver toxicity, and malaria [13, 14]. Administration of *Azadirachta indica* aqueous leaf extract on type II diabetic male rats showed glucose tolerance [15]. *Biophytum* is one of the potential herbs in dashapushpam, and its therapeutic uses are well documented in Ayurveda and Siddha systems [16]. Ethyl acetate extract of the whole part of *Biophytum sensitivum* have shown antidiabetic activity in streptozotocin-induced diabetic rat [17]. *Cocos nucifera* holds an important position in traditional medicine and has several health benefits, like antibacterial, antifungal, antioxidant, immunostimulant, and hypoglycemic [18]. Different plant parts of *Cocos nucifera*, such as husk, flower, and endocarp, have shown hypoglycaemic activity [19-21]. *Passiflora edulis*, also known as passion fruit, has a variety of bioactivities, including antioxidant, antimicrobial, anti-inflammatory, antihypertensive, antidiabetic, and antidepressant activity [22]. The leaf extract of *Passiflora edulis* is effective in reducing blood glucose levels [23].

It is noteworthy that over 400 plants are used to cure diabetes mellitus [24]. However, very few of these have been subjected to chemical evaluation and clinical testing to determine their effectiveness [25]. In the present study, four different plants, namely *Azadirachta indica*, *Biophytum sensitivum*, *Cocos nucifera*, and *Passiflora edulis*, were screened for alpha-amylase inhibition, antioxidant potential along with the estimation of total phenols, tannins and flavonoids. Among these, aerial roots of *Cocos nucifera* were first ever studied for antidiabetic potential.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *Azadirachta indica* A. Juss., whole plant of *Biophytum sensitivum* (L.) DC., aerial roots of *Cocos nucifera* L., and leaves of *Passiflora edulis* Sims **(Figure 1)** were used for the present study. Plant materials were collected in healthy and disease-free conditions from Vettoor (9°25'N, 76°82'E), Pathanamthitta district, Kerala, India.

Preparation of plant extract

After shade drying, all the plant parts were finely powdered. From this, 10g of dried powder was boiled with 100 mL of distilled water for 30 minutes and filtered with filter paper. The filtrate was collected in a petri dish and allowed to dry in a hot air oven. After drying, the dried extract was scrapped and collected into the Eppendorf tube. 10 mL of distilled water was used to dissolve 0.1g of dry extract to prepare the aqueous extract.

Total phenols estimation

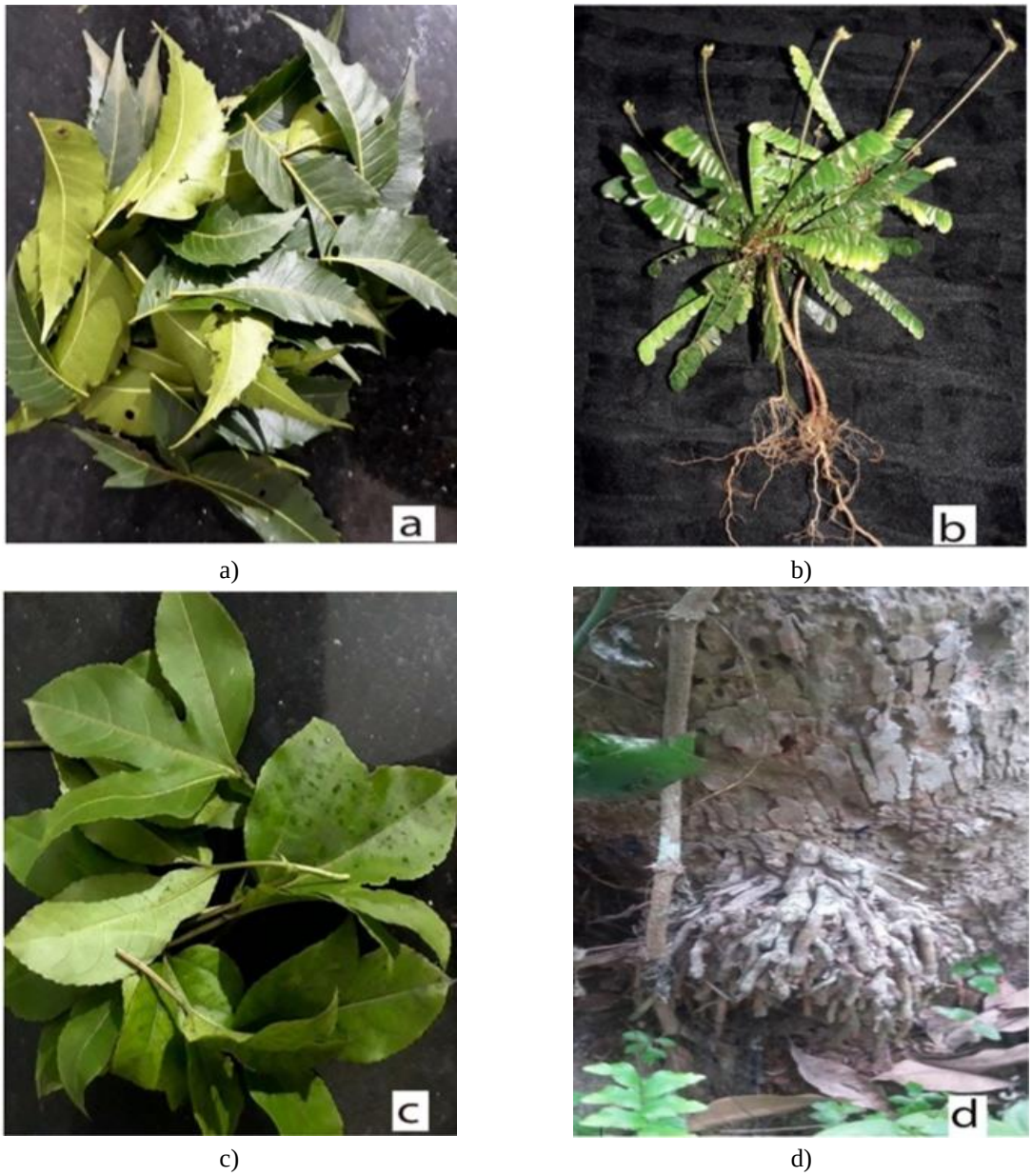
The Folin Ciocalteau technique [26] was used to calculate the total phenolics. In short, 0.5 mL of Folin Ciocalteau reagent and 5.0 mL of sodium carbonate were added to 0.1 mL of the extract. After letting the reaction mixture stand for thirty minutes, the absorbance at 640 nm was calculated. The standard was gallic acid.

Estimation of total tannins

The method of [27] was used to estimate the tannins. For this, 10mL of methanol-water (7: 3) was used to dissolve 1 mg of each extract after it had been weighed. After adding 0.5 mL of Folin's phenol reagent (1: 2) and 5 ml of 3.5% sodium carbonate, and incubated for 5 minutes. The absorbance was measured at 640 nm. Tannic acid was used as the standard.

Estimation of total flavonoids

The aluminum chloride method was used to calculate the total flavonoid content of the filtered fraction [28]. 4 mL of distilled water and 0.3 mL of 5% sodium nitrite were added to 1 mL of the extract. 2 mL of 1M sodium hydroxide and 0.3 mL of 10% aluminum chloride were added after 5 minutes. The solutions were well mixed, and the final volume was adjusted to 10 mL using distilled water. At 510 nm, the absorbance was measured. The standard was quercetin.



a) b)

c) d)

Figure 1. Different plant parts used: a) Leaves of *Azadirachta indica* b) whole plant of *Biophytum sensitivum* c) Aerial roots of *Cocos nucifera* d) *Passiflora edulis* leaves.

Antioxidant assay

Antioxidant assay of selected plants was done by reducing power method [29]. Different concentrations (20-100µg/ml) of extract were combined with 5mL each of 1% potassium ferric cyanide and 0.2M phosphate buffer (pH-6.6). Following incubation at 50˚C for 20 minutes, 5 mL of 10% trichloroacetic acid was added and centrifuged for 10 minutes. 5 mL of the top layer was thoroughly mixed with 1 mL of freshly made 0.1% ferric chloride and 5 mL of distilled water. At 700 nm, the color intensity was measured.

Assay of in vitro α-amylase inhibition

The studies on *in vitro α* -amylase inhibition were performed by the method [30]. To put it briefly, 200 µl of the enzyme α-amylase and 100 µl of 2 mM phosphate buffer (pH-6.9) were allowed to react with 100 µl of the test extract. Upon incubation for 20 minutes, 100 µl of 1% starch solution was added. The controls underwent the same procedure, with 200µl of buffer in place of the enzyme. 500 µl of the dinitro salicylic acid reagent was added to the control and test samples after they had been incubated for five minutes, and they were kept in a water bath at 100˚C. Using a spectrophotometer, the absorbance at 540 nm was recorded. The following formula was used to determine the % inhibition of the α-amylase enzyme.

$$\% \text{ α amylase inhibition} = \left\{\frac{\text{Abs of 100\% control} - \text{Abs Sample}}{\text{Abs of 100\% Contro}}\right\} \times 100 \quad (1)$$

Inhibitor controls and appropriate reagent blanks were performed concurrently. Metformin [3- (diamino methylidene)-1,1-dimethylguanidin], a known alpha-amylase inhibitor, is used as the positive control in this study.

Statistical analysis

Every treatment consisted of three duplicates, and the data were presented as mean±SE. With SPSS (free trial), statistical analysis (one-way analysis of variance -ANOVA) and comparison of mean values (Duncan's New Multiple Range Test -DNMRT) were performed.

RESULTS AND DISCUSSION

Estimation of total phenols, tannins and flavonoids

The selected plant parts are commonly used as traditional antidiabetic medicines. Secondary metabolites such as alkaloids, tannins, phenols, and flavonoids have been observed to be present in the majority of plants with hypoglycemic activities [31]. For instance, previously, it has been revealed that ferulic acid, a phenolic compound, shows inhibitory activity against α-amylase and α- -glucosidase [32]. The total phenolics, tannins, and flavonoids of plant extracts are given in **Table 1**. The total phenolics are expressed as Gallic acid equivalents in µg/ml of extract. Results revealed that total phenols were found to be significantly high ($p<0.001$) in *Biophytum sensitivum* (1620±1.15 µg/ml). The least amount of phenol was found in *Azadirachta indica* (300±2.80 µg/ml). The inhibitory activity might be due to the interaction between phenolic compounds and enzymes [33].

Table 1. Total content of phenolics, tannins, and flavonoids in selected plants

Plants	Total phenolics (µg/ml)	Total tannins (µg/ml)	Total flavonoids (µg/ml)
Azadirachta indica	300±2.80 ^{d}	14.50±1.32 ^{c}	325±2.80 ^{a}
Biophytum sensitivum	1620±1.15 ^{a}	19.25±0.43 ^{b}	150±1.15 ^{c}
Cocos nucifera	347±3.00 ^{c}	6±0.57 ^{d}	100±5.70 ^{d}
Passiflora edulis	373.30±3.33 ^{b}	34.75±0.80 ^{a}	275±4.00 ^{b}
Main effect F Df(n-1) 3	55134.92***	199.68***	744.38***

The values are mean±SE followed by the same letterers within a column and are not significantly different at ($P<0.05$) as determined by DNMRT. ***significant at $P<0.001$

In a previous study, larger and more complex tannins extracted from pomegranate and cranberry effectively inhibited α-amylase and α-glucoamylase [34]. In our study, total tannins were represented as tannic acid equivalents in µg/ml of extract. Total tannin was found to be significantly high ($p<0.001$) in *Passiflora edulis* (34.75±0.80µg/ml), and the least amount of tannin was found in *Cocos nucifera* (6±0.57 µg/ml). In another study, flavonoids isolated from the butanol extract of the *Zhumeria majdae* plant showed alpha-amylase inhibitory activity [35]. Total flavonoid was found to be significantly high ($p<0.001$) in *Azadirachta indica* (325±2.80µg/ml). The least amount was found in *Biophytum sensitivum* (150±1.15 µg/ml). Moreover, the findings of the present study are consistent with previous works on phytochemicals and α-amylase inhibitory activity.

Estimation of antioxidant property

Numerous investigations have found that diabetes causes increased reactive oxygen species generation and impaired antioxidant capability [36]. Intake of antioxidants could have a chemo-protective effect [37]. Moreover, it has been demonstrated that supplementing with vitamin C reduces the blood sugar level of diabetic patients [38]. In our study, maximum reducing power ($p<0.001$) was obtained at 20µg/ml of *Cocos nucifera* extract **(Table 2)**. In addition, all the other plant extracts show an increasing tendency to reduce power from concentration 20 - 100µg/ml. The present study proves that plants with antioxidant potential also possess alpha-amylase inhibitory activity.

Table 2. Determination of reducing the power of different plant extracts

Sl. No.	Conc. (µg/ml)	Reducing power (O.D)			
		A. indica	*B. sensitivum*	*C. nucifera*	*P. edulis*
1	20	0.01±0.003 [b]	0.03±0.005 [c]	0.37±0.005 [a]	0.01±0.003 [b]
2	40	0.02±0.008 [b]	0.04±0.005 [b]	0.09±0.005 [d]	0.02±0.005 [b]
3	60	0.03±0.005 [ab]	0.05±0.053 [b]	0.10±0.003 [d]	0.02±0.008 [b]
4	80	0.03±0.003 [ab]	0.06±0.005 [b]	0.13±0.006 [c]	0.04±0.006 [a]
5	100	0.04±0.006 [a]	0.08±0.003 [a]	0.15±0.003 [b]	0.05±0.005 [a]
Main effect F Df(n-1) 4		4.28*	15.77***	488.66***	6.86**

The values are mean±SE followed by the same letterers within a column and are not significantly different at ($p<0.05$) as determined by DNMRT. significant at ***P<0.001, **P<0.01, *P<0.05

α-Amylase inhibition activity

The aqueous extracts of leaves, whole plant parts, and aerial roots of selected plants (10, 20, 40, 60, 80, 100µg/ml) were examined individually for their ability to inhibit α-amylase activity. The percentage inhibition of each extract is shown in **Figure 2**. α-amylase inhibition studies on *Cocos nucifera* had shown a significantly ($p<0.001$) high amylase inhibition activity. It shows a maximum of 83.5±0.57% inhibition at 80µg/ml concentration. Research on the antidiabetic potential of aerial roots of *Cocos nucifera* has not been reported, but the effects of other parts like inflorescence husk have been studied, and, in all studies, it gives a positive response [39, 40]. Leaves of *Passiflora edulis*, whole plant of *Biophytum sensitivum*, and leaves of *Azadirachta indica* also showed effective results such as 62±0.28%, 57.23±0.088%, 48.07±0.36%, respectively in 100µg/ml concentration **(Table 3)**. Metformin, which was used as a standard, shows a maximum of 59.66 ±0.30% inhibition in 100µg/ml concentration. *Azadirachta indica* has been shown to possess hypoglycemic activity in previous studies, both in normal and experimental animals [41, 42]. The results of the present study using *Azadirachta indica* coincided with previous observations and supported the pharmacological activity. *Biophytum sensitivum* has traditionally been used for various treatments such as antimicrobial, anti-inflammatory, and wound healing [43]. The previous work done on *Biophytum sensitivum* leaf extract had shown a marked reduction in blood glucose levels in diabetic rats [44]. The antidiabetic activity of *Biophytum sensitivum* may be due to the increased production of insulin from the β-cells of the pancreas [44]. *Passiflora edulis* leaf and fruit extract proved to have sedating, anxiolytic, anti-inflammatory, and antibacterial properties [22, 45]. *Passiflora edulis* leaf extract has shown hypoglycemic activity in previous studies. The action could be due to the increasing ability of *P. edulis* to release insulin [46].

Table 3. Percentage inhibition of alpha-amylase in plant extracts

Sl. No.	Conc. (µg/ml)	Percentage inhibition				
		Metformin	*A. indica*	*B. sensitivum*	*C. nucifera*	*P. edulis*
1	10	26.26 ±0.24 [f]	2.56±0.73 [f]	4.44 ± 0.64 [e]	25.50±0.86 [e]	35 ±0.05 [f]
2	20	31.08 ±0.30 [e]	10.89±0.73 [e]	6.11 ± 0.00 [d]	61.50±0.28 [d]	39 ± 0.57 [e]
3	40	49.41 ±0.30 [d]	16.66±0.36 [d]	6.11 ± 0.01 [d]	73±0.57 [c]	42.50± 0.12 [d]
4	60	53.53 ±0.14 [c]	32.69±0.36 [c]	15.5 ± 0.63 [c]	79±0.28 [b]	47.50 ±.05 [c]
5	80	58.45 ±0.47 [b]	42.33±0.14 [b]	32.21± 0.32 [b]	83.50±0.57 [a]	57.50 ±0.28 [b]
6	100	59.66 ±0.30 [a]	48.07±0.36 [a]	57.23 ± 0.08 [a]	82.10±0.12 [a]	62 ±0.28 [a]
Main effect F Df(n-1) 5		2122.87***	1309.77***	2833.26***	1827.73***	793.86***

The values are mean±SE followed by the same letterers within a column are not significantly different at $p<0.05$) as determined by DNMRT
***significant at P<0.001

For the action of these plant extracts, a different mode of action has been suggested. One of the mechanisms may be due to their impact on pancreatic β cell function through an increase in insulin production [47]. There are additional mechanisms at play, including enhanced peripheral glucose utilization, elevated hepatic glycogen production or decreased glycogenolysis [48], inhibition of intestinal glucose absorption, and glucose production from hepatocytes [49].

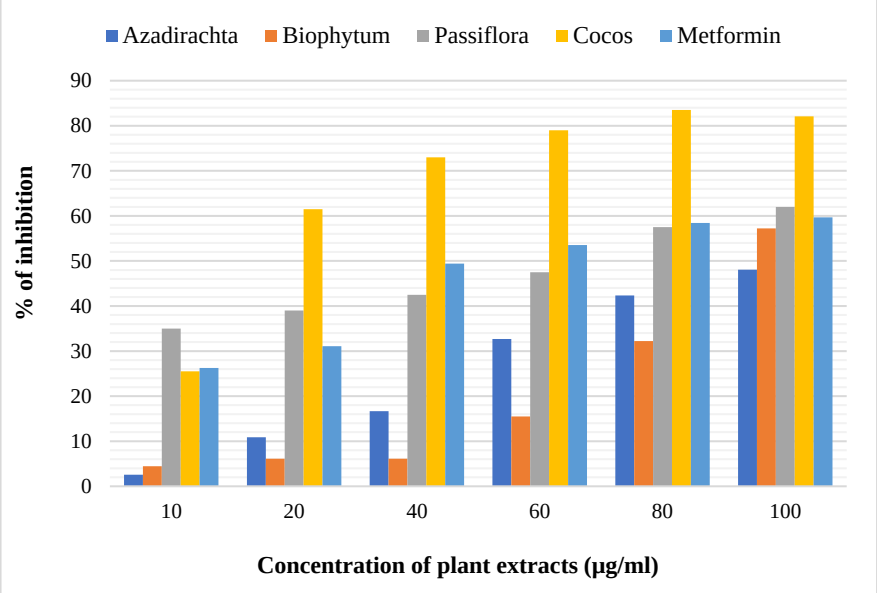


Figure 2. Alpha amylase enzyme inhibition percentage by water extract of *Azadirachta indica*, *Bioiphytum sensitivum*, *Cocos nucifera*, *Passiflora edulis*, and Metformin tablet.

CONCLUSION

The antidiabetic effect of aqueous extract prepared using dried leaves of *Azadirachta indica*, whole plant part of *Biophytum sensitivum*, aerial roots of *Cocos nucifera*, and dried leaves of *Passiflora edulis* were studied. It was demonstrated that certain plants contain glucose-lowering compounds, and these can be employed for the treatment of different types of diabetes mellitus. The use of all selected plant extracts represents a therapeutic promise for diabetes mellitus and also contributes to the reduction of free radicals. Compared to the Metformin tablet, higher alpha-amylase inhibitory activity was observed in aerial roots of *Cocos nucifera* and leaves of *Passiflora edulis*. Plants are widely used as hypoglycemic agents in traditional medicine. Although the exact mechanisms of action of these plants in the treatment of diabetes are still largely unknown, the majority of plants have been found to contain compounds like glycosides, alkaloids, terpenoids, flavonoids, etc., that are frequently associated with antidiabetic effects. The active compounds responsible for the alpha-amylase inhibitory action have not yet been identified in the chosen plants. Further research is needed to determine the precise mechanism of action of selected plants with antidiabetic and insulin mimic activities.

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ETHICS STATEMENT : None

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