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Research Article

IN VITRO α-AMYLASE AND A-GLUCOSIDASE INHIBITOR ACTIVITIES OF *ALBIZIA PROCERA* STEM BARK

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ABSTRACT

Objective: This current study was carried out to determine the *in vitro* α-amylase and α-glucosidase inhibitory activity of extract and fractions of *Albizia procera.*

Methods: The α-amylase and α-glucosidase inhibition assay was carried out at concentrations 50–2000 µg/ml and acarbose used as standard. The absorbance was measured at 540 nm and recorded by spectrophotometer. Percentage inhibition was calculated for both the assays. Preliminary phytochemical screening was also evaluated using standard procedures.

Results: There was a dose-dependent percentage inhibition of extracts (petroleum ether and ethanol) and fractions (chloroform, ethyl acetate, and n-butanol). The ethanol extract and n-butanol fraction show good inhibitory activity against both α-amylase and α-glucosidase with the percentage inhibition of 86.20% and 88.20% and 83.13% and 87.10%, respectively. The preliminary phytochemical screening shows that ethanol extract consists of active constituents such as flavonoids and phenolic compounds and tannins.

Conclusion: This finding suggests that the ethanol extract and n-butanol fraction show good inhibitory activity against both α-amylase and α-glucosidase and show good antidiabetic activity.

Key words: *Albizia procera*, *in vitro* antidiabetic, α-amylase, α-glucosidase.

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INTRODUCTION

Diabetes mellitus is a very complicated disease, which can lead to nerve damage and kidney failure, atherosclerosis, blindness, stroke, and heart diseases. There are about 8.3 million diabetics in the United States, of which about 600,000 suffer from type 1 (insulin-dependent) diabetes [1]. The incidence is far higher among some ethnic groups, in which diet play a major role. 50% of group of native Americans (Pima) over 40 years of age have diabetes. Diabetes is the fifth leading cause of death in the U.S. as well as the leading cause of adult blindness due to diabetic retinopathy and is responsible for 50% of heart attacks, 75% of strokes, and 85% of gangrenous leg amputation due to diabetic peripheral neuropathy [2,3].

Developing countries are the most affected because of expensive and inadequate treatment associated with the synthetic oral hypoglycemic agents. The treatment from medicinal plants exhibits potential agonistic/antagonistic pharmacological agents within themselves due to incorporation of bioactive constituents with variety of pharmacological actions with minimal side effects. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy. There are wide varieties of traditional medicines used for the treatment of diabetes and can combat the threat of diabetic epidemic [4,5].

This plant is used traditionally for the treatment of cancer, convulsions, delirium, and septicemia. The decoction of bark is used traditionally for rheumatism and hemorrhage and is considered useful in treating pregnancy problems, stomach ache, and sinus [6,7]. They were reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, and hypoglycemic activity. The leaves of *Albizia procera* were used for the treatment of variety of wounds and also to suppress skin diseases [8,9]. Seeds are powdered and used in amoebiasis. It cures urinary tract infections including glycosuria, hemorrhoids, fistula, and worm infestation. Fruits of *A. procera* act as astringent and diminish Kapha and Sukra. In India, leaves are used for the treatment of ulcers [10,11].

METHODS

Collection of plant

The bark of *A. procera* was collected from Chennai (near Melur, Madurai), Tamil Nadu, India. The plant material was identified and authenticated by Dr. K.N. Sunil Kumar R.O. and HOD Pharmacognosy, Central Siddha Research Institute, Government of India, Arumbakkam, Chennai-106, authentication code (A17062401P). The bark was dried under shade, segregated, pulverized by a mechanical grinder, and passed through a 40-mesh sieve.

Preparation of plant extracts

The dried powder of bark was extracted sequentially by hot continuous percolation method by Soxhlet apparatus, using petroleum ether and ethanol as solvent. The extracts were concentrated using a rotary evaporator.

Phytochemical screening of extracts from *A. procera*

The extract was subjected to preliminary qualitative test for the extracts to identify the phytoconstituents present in the bark [12,13].

Fractionation of active extract

Ethanol extract comprises of flavonoids, tannins, and phenolic compounds, and hence, ethanol extract has been selected for further fractionation using solvents, chloroform, ethyl acetate, and n-butanol. Ethanol extract was filtered through a Buchner funnel fitted with a vacuum pump with a thin layer of activated charcoal and then

concentrated using a rotary evaporator. The solvent is recovered and the residue is dried. The crude dried ethanol extract was partitioned between equal volumes (250 ml each) of distilled water and chloroform. It was shaken for some time and the chloroform layer was separated and concentrated to get a residue, which is considered as chloroform fraction. The aqueous layer which is present was again fractionated by the solvent ethyl acetate. It will be shaken for some time, and ethyl acetate layer was separated and concentrated to get a residue which is considered as ethyl acetate fraction. The aqueous layer was again fractionated by the solvent n-butanol. It was shaken for sometimes and n-butanol layer was separated, concentrated to get a residue, and considered as n-butanol fraction. The aqueous fraction which was remained finally was not used for further study.

***In vitro* antidiabetic activity**

α-amylase assay

α-amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of extracts and fractions of 50 µg/ml–2000 µg/ml (0.25 mL) were mixed with α-amylase solution (0.25 mL) and incubated at 37°C for 5 min. Then, the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37°C for 3 min, the reaction was stopped by adding 0.5 mL DNS reagent (1% dinitrosalicylic acid, 0.05% Na_2SO_3, and 1% NaOH solution) to the reaction mixture, and it was boiled at 100°C for 5 min. After cooling to room temperature, the absorbance (Abs) was measured at 540 nm and recorded by a spectrophotometer [14,15]. The inhibition percentage was calculated by the following equation:

Inhibition (%) = [(Abs1 − Abs2)/Abs1]×100

where Abs1=sample and Abs2 = control

α-glucosidase assay

α-glucosidase was dissolved in PBS (0.02 mol/L, pH 6.8 at a concentration of 0.1 mg/mL [16]. Various concentrations of extracts and fractions of 50 µg/ml–2000 µg/ml (0.25 mL) were mixed with α-glucosidase solution (0.25 mL) and incubated at 37°C for 5 min. Then, the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37°C for 3 min, the reaction was stopped by adding 0.5 mL DNS reagent (1% dinitrosalicylic acid, 0.05% Na_2SO_3, and 1% NaOH solution) to the reaction mixture, and it was boiled at 100°C for 5 min. After cooling to room temperature, the Abs was measured at 540 nm and recorded by a spectrophotometer [17]. The inhibition percentage was calculated by the following equation:

Inhibition (%) = [(Abs1 − Abs2)/Abs1]×100

Where Abs1=sample and Abs2 = control.

RESULTS

From the preliminary phytochemical screening, it was observed that ethanol extract consists of active constituents such as flavonoids and phenolic compounds and tannins. While petroleum ether extract shows the presence of phytosterols, triterpenoids, and fats (Table 1). Hence, ethanol extract was chosen for *in vitro* antidiabetic study since flavonoids and phenolic compounds play a major role in diabetic research.

In alpha-amylase inhibition assay, as illustrated in Table 2, reveals that ethanol extract has greater alpha amylase inhibition with the percentage inhibition of 86.20% at 2000 µg/ml. The petroleum ether extract shows weak percentage inhibition of 57.11% at 2000 µg/ml.

In alpha-glucosidase inhibition assay, as illustrated in Table 3, it reveals that ethanol extract has greater alpha-glucosidase inhibition with the percentage inhibition of 88.2027 at 2000 µg/ml. Petroleum ether extract shows the percentage inhibition of 59.90% at 2000 µg/ml. The ethanol extract shows greater α-amylase and α-glucosidase inhibition than standard acarbose.

Table 1: Preliminary phytochemical analysis on bark extracts of *A. procera*

S.NO	Test	Petroleum ether	Ethanol
1	Alkaloids	-	_
2	Carbohydrates and glycosides	-	+
3	Phytosterols	+	+
4	Triterpenoids	+	+
5	Flavonoids	-	+
6	Phenolic compounds and tannins	-	+
7	Protein and amino acid	-	_
8	Saponins	-	+
9	Fixed oils and fats	+	_

+: Positive, -: Negative. *A. procera: Albizia procera*

Table 2: Effect of extracts on percentage inhibition of α-amylase

Concentration µg/ml	Petroleum ether extract	Ethanol extract
50	4.23729±0.04	22.8669±0.04
100	11.0236±0.06	31.7221±0.07
250	20.1413±0.03	50.655±0.06
500	33.3333±0.05	66.8622±0.04
1000	54.251±0.02	81.3839±0.03
2000	57.1157±0.04	86.2027±0.05

Each value in the table was obtained by calculating the average of three experiments and data are presented as mean±SEM

Table 3: Effect of extracts on percentage inhibition of α-glucosidase

Concentration µg/ml	Petroleum Ether extract	Ethanol extract
50	5.11029±0.09	27.8069±0.14
100	11.8036±0.12	38.7021±0.09
250	21.1413±0.08	56.655±0.17
500	34.3333±0.11	69.8622±0.08
1000	53.201±0.06	83.3039±0.09
2000	59.9057±0.05	88.2027±0.07

Each value in the table was obtained by calculating the average of three experiments, and data are presented as Mean±SEM

As described in Table 4, the fractions, chloroform, ethyl acetate, and n-butanol also have greater alpha-amylase inhibition than standard acarbose. n-butanol fraction has greater alpha-amylase inhibition than chloroform and ethyl acetate fraction. The percentage inhibition of n-butanol fraction against alpha-amylase was found to be 83.104%. Interestingly, it was observed that n-butanol fraction has greater alpha-amylase inhibition than standard acarbose with 79.37% inhibition. The results of extracts and fractions against alpha-amylase are also depicted in Figs. 1 and 2.

As described in Table 5, the fractions, chloroform, ethyl acetate, and n-butanol also have greater alpha-glucosidase inhibition than standard acarbose. The n-butanol fraction has greater alpha glucosidase inhibition than chloroform and ethyl acetate fraction. The percentage inhibition of n-butanol fraction against alpha-glucosidase is found to be 87.100%. The n-butanol fraction has greater alpha-glucosidase inhibition than standard acarbose with 74.37% inhibition. The results of extracts and fractions against alpha-amylase are also depicted in Figs. 3 and 4.

DISCUSSION

The antioxidants (tannins, flavonoids, Vitamins C and E, etc.) have the capacity to prevent the destruction of β-cells by inhibiting the peroxidation chain reaction, by which they may provide protection against the development of diabetes [18]. The mechanism of tannins as antidiabetic activity is by Mitogen-Activated Protein Kinase activation and GLUT-4 translocation [19,20]. The protective effects of flavonoids

Table 4: Effect of fractions of *A. procera* on percentage inhibition of α-amylase

Concentration μg/ml	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction	Acarbose (standard)
50	1.31004±0.04	0.87719±0.07	14.41860±0.04	5.04202±0.06
100	28.7066±0.06	23.1293±0.05	24.27983±0.07	21.7993±0.05
250	43.7811±0.03	48.2838±0.03	46.51162±0.05	34.1108±0.04
500	64.5768±0.09	73.2861±0.06	66.6061±0.09	42.9293±0.07
1000	79.5104±0.07	84.5417±0.02	81.6410±0.03	53.4979±0.06
2000	81.6467±0.02	80.822±0.04	83.1304±0.02	79.3764±0.04

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean±SEM. *A. procera: Albizia procera*

Table 5: Effect of fractions of *A. procera* on percentage inhibition of α- glucosidase

Concentration (μg/ml level)	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction	Acarbose Standard
50	2.30004±0.04	1.90719±0.05	16.40860±0.05	5.04202±0.06
100	29.7066±0.06	25.1093±0.07	27.27983±0.08	21.7993±0.08
250	51.7811±0.06	50.2838±0.11	49.51162±0.12	34.1108±0.07
500	68.5768±0.08	76.2861±0.15	70.6061±0.10	42.9293±0.05
1000	80.5014±0.07	82.0017±0.17	81.6010±0.05	53.4979±0.03
2000	83.6067±0.04	82.902±0.06	87.1004±0.04	74.3764±0.04

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean±SEM

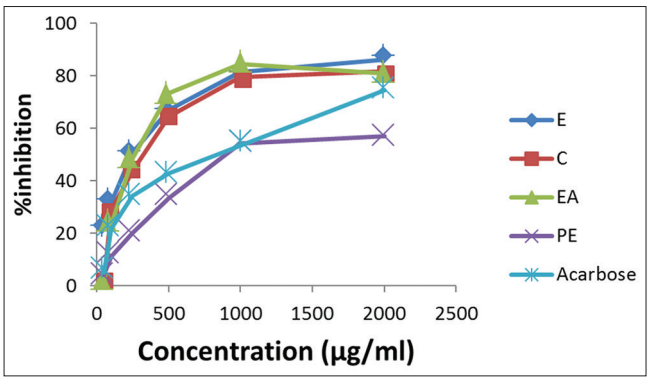

Fig. 1: α-amylase inhibition assay of extract and fractions from *Albizia procera.* E - Ethanolextract, C - Chloroform extract, EA - Ethyl acetate fraction, PE - Petroleum ether fraction, Acarbose (Standard

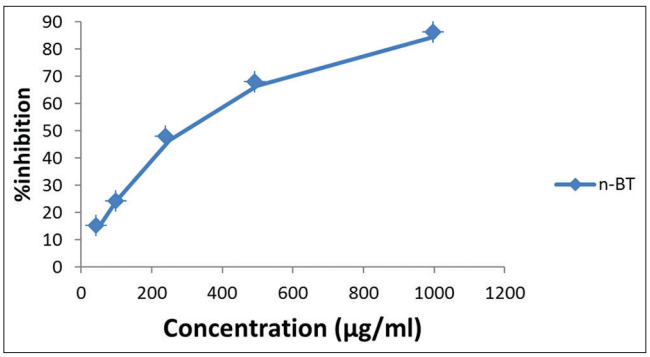

Fig. 2: α-amylase inhibition assay of n-butanol (n-BT) fraction of *Albizia procera*

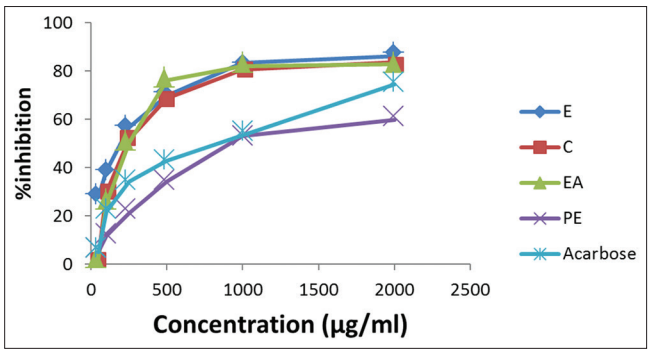

Fig. 3: α-glucosidase inhibition assay of extract and fractions from *Albizia procera*. E - Ethanol extract, C - Chloroform extract, EA - Ethyl acetate fraction, PE - Petroleum ether fraction, Acarbose (Standard)

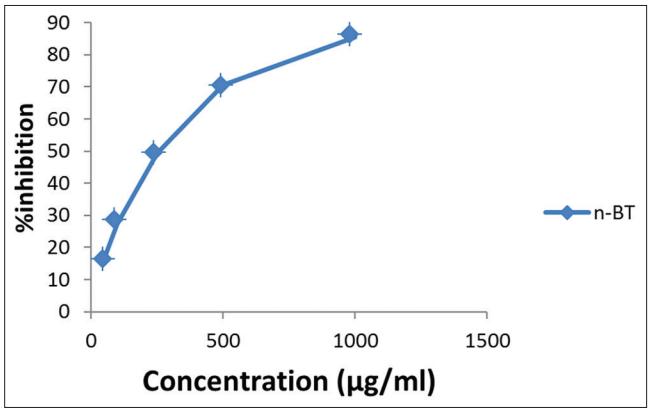

Fig. 4: α-glucosidase inhibition assay of n-butanol (n –BT) fraction from *Albizia procera*

against diabetes are by (a) acting as free radical scavengers and (b) activating antioxidant enzymes which may be suppressed by the oxidative stress induced by hypoglycemia [21]. Moreover, fibers of plants may also interfere in the absorption of carbohydrates and thus may have an effect on blood glucose [22].

The extracts and fractions of *A. procera* showed significant α-amylase inhibitory activity. α-amylases catalyze the hydrolysis of α-1, 4-glucosidic linkage of starch to initiate starch digestion and promote glucose absorption. α-amylase inhibitors were considered to be effective in diabetes control [23]. The extracts and fractions of *A. procera* also show significant α-glucosidase inhibitory activity. α-glucosidase constitutes a family of enzymes hydrolase, located in the brush-border surface membrane of small intestinal cells, and it is the key enzyme by which the final step of digestion is catalyzed [24]. Hence, glucosidase inhibitors can stop the liberation of D-glucose from complex dietary carbohydrates and can delay the glucose absorption which in turn

reduce blood glucose level and decrease hyperglycemia [25,26]. In this present study, *in vitro* α-amylase and α-glucosidase inhibitor activity of extracts and fractions of *A. procera* stem bark were evaluated. The retardation and delay of carbohydrate absorption with a plant-based α-amylase and α-glucosidase inhibitor offer a prospective therapeutic approach for the management of type 2 diabetes mellitus.

CONCLUSION

From above these observations, it is concluded that the ethanol extract and n-butanol fraction of *A. procera* have good *in vitro* antidiabetic activity by evaluating against alpha-amylase and alpha-glucosidase inhibition assay. The flavonoids and phenolic compounds present in ethanol extract may be responsible for the *in vitro* antidiabetic activity. However, the active principles responsible for inhibitory action of α-amylase and α-glucosidase need to be identified and characterized for the development of indigenous botanical resources for novel hypoglycemic drug development.

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CONFLICTS OF INTEREST

All authors have none to declare.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to this work. D. Anand and Dr. M. Sathish, carried out the extraction, fractionation, and preliminary phytochemical screening of extracts. D. Anand, Dr. M.Sathish, and L.S Dhivya equally contributed the *in vitro* α-amylase and α-glucosidase inhibitory activities of stem bark of *A. procera*. Dr. M.Sathish wrote the main paper, and all the authors discuss the results and implications and commented on the manuscript at all stages. All authors contributed extensively to the work presented in paper.

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