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Phytochemical Composition, *in vitro* Studies on α-Amylase and α-Glucosidase Inhibitory Activity of Selected Mangrove Plants

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ABSTRACT

The purpose of this study is to show the phytochemical composition and *in vitro* antidiabetic potential in the leaves of *Ceriops tagal*, *Bruguiera cylindrica,* and *Salvadora persica* mangrove plants. The phytochemical composition was studied by qualitative analysis. To determine *in vitro* antidiabetic activity leaves were subjected to solvent extraction by the Soxhlet method using methanol, ethanol, ethyl acetate, and pet ether and α-amylase, α-glucosidase inhibition assays were performed. The findings indicates that alkaloid, steroid, flavonoid, terpenoid, glycosides, tannin, saponin, phenol, quinones and coumarin principles are present in the leaves of selected mangrove species. Among the selected mangrove species *C. tagal* leaves recorded the highest antidiabetic activity for both the assay followed by *B. cylidrica* and *S. persica.* Overall *C. tagal* was found highly potent in the antidiabetic activity.

Keywords: Mangroves, *In vitro,* Antidiabetic activity, α-glucosidase, α-amylase.

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INTRODUCTION

Diabetes mellitus, characterized by both elevated production and low utilization of glucose, could be a major endocrine disorder touching nearly 10% of the population throughout the world. [1] It is a long lasting condition that solemnly affects a person's life, characterized by metabolic disorders in carbohydrate, fat, and protein and resulting in hyperglycemia. [2] Since centuries, plants are consistently been an admirable source of medicines and drugs available today, which are derived from plants to overcome a wide variety of clinical diseases. Mangroves are woody plants growing in marshy areas in tropical and subtropical regions where they do exist in relatively high salinity, utmost tides, robust winds, high temperatures and filthy, anaerobic soils. [3-4] To thrive in these extreme conditions, they synthesize diverse classes of secondary metabolites responsible for various medicinal properties. Mangrove plants are used traditionally in folk medicines to treat various diseases such as skin disorders, sores, leprosy, angina pectoris, asthma, tuberculosis, constipation, convulsions, diarrhoea, dysentery, elephantiasis, eye ailments, fever, headaches, haemorrhage, inflammation, malaria, jaundice, fungal infections, kidney stones, lesions, malignancies, rheumatism, sore throat, and syphilis. They are also used as an astringent, emmenagogue, hemostat, styptic, expectorant, and tonic. [5] A few

species like *Acanthus ilicifolius* [6], *C. tagal* [7-8], *Rhizophora mucronata* [9-10], *Diospyros peregrine* [11] and *Heritiera fomes* [12] are used as folklore medicine to cure diabetes. Besides folklore uses, many mangrove species like *R. mucronata* [13-14], *Xylocarpus moluccensis* [15], *Sonneratia apetala* [16], *Drosera heterophylla* and *S. alba* [17], *C. decandra* [18] scientifically proved active against the hyperglycaemic activity. Mangrove plants are the major source of phytoconstituents like phenolic compounds, flavonoids, terpenoids, saponin, coumarins, glycopeptide, alkaloids, tannin, essential oils, anthraglycosides [19-22] and other constituents rich in plant products tend to show a reduction in blood glucose levels. [23] They can also maintain the function of β-cells by hampering the formation of diabetes-induced reactive oxygen species. [24] Mangrove possesses secondary metabolites in abundance such as alkaloid, saponin, flavonoid, tannin and they are proved to have antidiabetic activity. [25-28] We have selected *C. tagal*, *B. cylindrica* and *S. persica* mangrove species, thereafter studied their phytochemical compositions and *in vitro* antidiabetic potential in this study.

MATERIALS AND METHODS

Plant Material

Three mangrove plants *C. tagal*, *B.cylindrica*, and *S.persica* leaves were collected in May from the Gorai beach area located at 19°15'0'' N and 72°46'59''E Borivali, Mumbai, Maharashtra, India and recognised by a taxonomist. The leaves were washed under running water to get them free from dust and other contaminants followed by oven-drying at 40ºC to remove any moisture, ground, and the powder was sieved through a muslin cloth for further uses of the extract preparation.

Extract Preparation

Different solvent leaves extracts were prepared from the selected plants' leaves. 10 g leaf powder of all the three mangroves was added with the 200mL methanol, ethanol, ethyl acetate and pet ether (polar to non-polar) using the Soxhlet apparatus. The extracts obtained were concentrated using a rotary flash evaporator (Buchi, Japan) to get the residues and used for antidiabetic activity.

Phytochemical Analysis

The qualitative estimation of secondary metabolites in methanol leaf extract was studied as per Harborne. [29] The tests for various classes of secondary metabolites were as follows.

Alkaloids: 0.2 ml extract was taken and 0.2 ml HCl was added. 2-3 drops of Dragendorff's reagent was added to the mixture and then the orange or red precipitate and turbid solution indicate the presence of alkaloids.

Tannins: 2 ml of water was added to 0.2 ml extract followed by heating on a water bath for 10 min. The mixture was filtered; $FeCl_3$ was added to the filtrate and observed for the dark green solution which indicates the presence of tannin.

Terpenoids: In a test tube, 0.2 ml extract was mixed with 0.2 ml chloroform. Conc. H_2SO_4 was added to this cautiously to form a layer. The presence of reddish-brown colour at the interface indicated that terpenoids are present.

Glycosides: 0.2 ml extract was mixed with 0.2 ml acetic acid and 0.2 ml of chloroform and then it was chilled on ice. H_2SO_4 was added carefully and the colour change from violet to blue and then to green confirmed the existence of the steroidal nucleus (Aglycone portion of glycoside).

Steroids: 0.2 ml extract was added to 0.2 ml chloroform followed by 0.2 ml Conc. H_2SO_4. The red colour in the lower layer of chloroform indicated the presence of steroids.

Saponins: A mixture of 0.2 ml extract and 5 ml distilled water was shaken vigorously and was observed for a stable persistent froth. After adding 3 drops of olive oil, the formation of stable foam confirmed the presence of saponins.

Flavonoids: 0.2 ml plant extract was mixed with dilute NaOH solution followed by dilute HCl. The change of yellow solution into a colourless one indicates the presence of flavonoids.

Phlobatannins: The red precipitate after mixing 2 ml extract in 1% aqueous HCl, witnessed the presence of phlobatannin.

Anthocyanins: 2 ml extract was added in 2 ml 2N HCl containing ammonia. Pink-red color turns into blue-violet which shows the presence of anthocyanins.

Coumarins: The yellow colour solution after adding 2 ml extract in 10% NaOH confirms the presence of coumarins.

Phenolics: Addition of 1 ml extract in 0.5 ml 1% Lead acetate and 1% $FeCl_3$ gave a blue-black and green-brown precipitate respectively.

Quinones: 3 ml Conc. HCl was added in the 2 ml extract. The green color was obtained, indicating quinones are present in the extracts.

In vitro Antidiabetic Activity

The antidiabetic activity was estimated by α-amylase [30] and α-glucosidase inhibition assays [31] as described below:

α-amylase Inhibition Assay

A mixture of 100µL plant extract and 100µL 0.02 M sodium phosphate buffer (pH 6.9) containing *a*-amylase solution was kept for incubation at 37ºC for 30 min. In each tube, 800µL 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added and kept again for incubation at 37°C for 15 min followed by addition of 1 ml dinitrosalicylic acid (DNSA) reagent. All the test tubes were then placed in a boiling water bath for 5 min and the optical density was measured at 540 nm.

α-glucosidase Inhibition Assay

A mixture containing plant extract, 1 ml starch substrate (2% w/v maltose) and 0.2 M Tris buffer (pH 8.0) was kept for incubation at 37°C for 5 min. The addition of 1 ml of α-glucosidase enzyme (1U/ml) followed by incubation at 35°C for 40 min initiates the

reaction and further addition of 2 ml 6N HCl terminates the reaction. Thereafter absorbance of the resultant colour was measured at 540 nm.

Inhibitory Concentration (IC_{50})

Half-maximal Inhibitory concentration (IC_{50}) is the amount of a substance required to inhibit a biological process such as an enzyme, cell, cell receptor or microorganism by half. The IC_{50} value was calculated by non-linear regression analysis of % inhibition recorded for different concentrations of test substances/standard. The relative activity of the sample was determined by comparing the IC_{50} value of a sample with the standard. Higher the IC_{50} value, lower will be the relative activity in comparison to standard and vice-versa. Acarbose was used as the standard and all tests were carried out in triplicate. Percentage inhibition (I %) was calculated by:

$$\% \text{ Inhibition} = (\text{Absorbance}_{control} - \text{Absorbance}_{sample}) / (\text{Absorbance}_{control}) \times 100$$

Where $\text{Absorbance}_{control}$ = absorbance of the solution with no extract

$\text{Absorbance}_{sample}$ = absorbance of the solution containing extract and enzyme solution

Statistical analysis

All the data are means of three independently performed experiments and shown as means ± SD. Their significance was tested by one-way ANOVA (SPSS ver-21; IBM Japan Ltd., Tokyo, Japan) followed by Post hoc Duncan's test at significance level $P < 0.05$.

RESULTS

The result of phytochemical analysis has shown the presence of alkaloid, steroid, flavonoid, terpenoid, glycosides, tannin, saponin, phenol, quinones and coumarin in all the selected mangrove species (Table 1). The α-amylase inhibition assay of *C. tagal* leaves has shown a gradual increase in percent inhibition as concentration increases for all the extracts. In this plant best percent inhibition was recorded in methanol extract (73.26%) followed by ethanol (62.76%), ethyl acetate (61.83%) and pet ether (54.55%) extracts at highest concentration (5 mg/ml) (Table 2). The percent inhibition and IC_{50} value for standard drug acarbose was 89.39% and 0.095 mg/ml respectively. Similarly, α-glucosidase assay has shown the best percent inhibition (66.13%) and IC_{50} value (2.448 mg/ml) for methanol extract (Table 5).

A similar dose-dependent pattern of inhibition was seen in *B. cylindrica* leaves for all the extracts. The methanol extract registered significant percent inhibition (69.23%) and IC_{50} (3.027 mg/ml) for α-amylase inhibition assay (Table 3) The lower percent inhibition (55.19%) and higher IC_{50} value (4.266 mg/ml) were recorded in pet ether extract of this plant. The results of a similar trend were observed in this plant with α-glucosidase inhibition assay (Table 6). The slightly lower antihyperglycaemic activity was recorded in *S. persica* leaves as compared to *C. tagal* and *B. cylindrica*. In this plant best percent inhibition (59.82%) and IC_{50} value (3.637 mg/ml) was seen in methanol extract followed by ethanol, ethyl acetate, and pet ether extracts (Table 4). Similarly, the α-glucosidase assay has shown the best percent inhibition and IC_{50} value (62.41%, 3.797 mg/ml) for methanol extract (Table 7).

Table 1: Phytochemical composition of selected mangrove species.

Phytochemical constituents	*C. tagal*	*B. cylindrica*	*S. persica*
Alkaloids	+	+	-
Tannins	+	+	+
Terpenoids	+	+	+
Glycosides	+	-	+
Steroids	+	-	+
Saponins	+	+	+
Flavonoids	+	+	+
Phlobatannins	+	-	+
Anthocyanins	+	+	+
Coumarins	+	+	+
Phenolics	+	+	+
Quinones	+	+	+

DISCUSSION

In the present study selected mangrove species *C. tagal, B. cylindrica* and *S. persica* have shown the presence of alkaloid, steroid, flavonoid, terpenoid, glycosides, tannin, saponin, phenol, quinones and coumarin. This indicates selected mangroves contain diverse classes of phytochemicals. Similar classes of secondary metabolites were seen in *L. racemosa* [19], *S. alba* [20], *C. tagal* [32], *S. apetala* [22] mangrove species. These results are in agreement with the present finding, therefore it is suggested that mangroves are affluent in a diverse range of phytoconstituents. In the present study, we have made leaves extracts of methanol, ethanol, ethyl acetate and pet ether of selected mangrove species and determined its antidiabetic activity using *in vitro* antidiabetic assays i.e. α-amylase and α-glucosidase inhibition assays.

Table 2: The percent inhibition of α- amylase by methanol, ethanol, ethyl acetate and pet ether extracts of *C. tagal* leaves at varying concentrations.

Conc. (mg/ml)	Acarbose		Methanol		Ethanol		Ethyl acetate		Pet ether	
	%inhibition	IC_{50}	%inhibition	IC_{50}	%inhibition	IC_{50}	%inhibition	IC_{50}	%inhibition	IC_{50}
0.5	50.32^a ± 0.292	0.095 ± 0.007	21.06^a ± 0.106	2.576 ± 0.029	17.52^a ± 0.476	3.129 ±0.009	15.63^a ± 0.186	3.435 ± 0.002	8.383^a ± 0.673	4.284 ± 0.023
1.0	57.1^b ± 0.165		38.97^b ± 0.529		32.58^b ± 0.181		27.53^b ± 0.252		14.22^b ± 0.277	
2.0	70.25^c ± 0.16		46.84^c ± 0.274		39.04^c ± 0.474		36.42^c ± 0.040		29.48^c ± 0.296	
3.0	80.22^d ± 0.113		55.33^d ± 0.528		53.54^d ± 0.159		45.76^d ± 0.345		35.58^d ± 0.212	
4.0	88.09^e ± 0.037		65.03^e ± 0.049		62.05^e ± 0.337		60.24^e ± 0.061		50.24^e ± 0.465	
5.0	89.39^f ± 0.87		73.26^f ± 0.571		62.76^f ± 0.219		61.83^f ± 0.223		54.45^f ± 0.106	

Table 3: The percent inhibition of α-amylase by methanol, ethanol, ethyl acetate and pet ether extracts of *B. cylindrica* leaves at varying concentrations.

Conc.	Acarbose		Methanol		Ethanol		Ethyl acetate		Pet ether	
(mg/ml)	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$
0.5	50.32^{a} ± 0.292		19.34^{a} ± 0.329		15.86^{a} ± 0.048		12.53^{a} ± 0.261		3.33^{a} ± 0.177	
1.0	57.1^{b} ± 0.165		29.53^{b} ± 0.345		23.66^{b} ± 0.458		18.08^{b} ± 0.191		12.77^{b} ± 0.308	
2.0	70.25^{c} ± 0.16	0.095 ±	33.63^{c} ± 0.172	3.027 ±	31.28^{c} ± 0.243	3.455 ±	30.19^{c} ± 0.579	3.681 ±	28.17^{c} ± 0.107	4.266
3.0	80.22^{d} ± 0.113	0.007	53.90^{d} ± 0.457	0.028	50.92^{d} ± 0.442	0.013	46.59^{d} ± 1.195	0.022	35.42^{d} ± 0.089	± 0.13
4.0	88.09^{e} ± 0.037		63.98^{e} ± 0.899		59.00^{e} ± 0.458		58.04^{e} ± 0.274		49.34^{e} ± 0.237	
5.0	89.39^{f} ± 0.87		69.23^{f} ± 0.618		62.12^{f} ± 0.554		60.15^{f} ± 0.587		55.19^{f} ± 0.149	

Table 4: The percent inhibition of α-amylase by methanol, ethanol, ethyl acetate and pet ether extracts of *S. persica* leaves at varying concentrations.

Conc.	Acarbose		Methanol		Ethanol		Ethyl acetate		Pet ether	
(mg/ml)	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$
0.5	50.32^{a} ± 0.292		18.84^{a} ± 0.553		11.24^{a} ± 0.144		10.39^{a} ± 0.432		8.26^{a} ± 0.025	
1.0	57.1^{b} ± 0.165		22.51^{b} ± 0.306		18.11^{b} ± 0.031		16.43^{b} ± 0.837		10.70^{b} ± 0.488	
2.0	70.25^{c} ± 0.16	0.095 ±	33.23^{b} ± 0.553	3.637±	27.81^{c} ± 0.735	4.047±	25.21^{c} ± 0.544	4.609±	22.67^{c} ± 0.046	5.138±
3.0	80.22^{d} ± 0.113	0.007	45.71^{c} ± 0.737	0.046	34.82^{d} ± 0.392	0.044	29.87^{d} ± 0.062	0.024	26.88^{d} ± 0.141	0.048
4.0	88.09^{e} ± 0.037		57.32^{d} ± 0.484		54.99^{e} ± 0.733		49.58^{e} ± 0.125		43.28^{e} ± 0.538	
5.0	89.39^{f} ± 0.87		59.82^{e} ± 0.307		58.08^{f} ± 0.309		51.95^{f} ± 0.330		47.42^{f} ± 0.375	

Table 5: The percent inhibition of α-glucosidase by methanol, ethanol, ethyl acetate and pet ether extracts of *C. tagal* leaves at varying concentrations.

Conc.	Acarbose		Methanol		Ethanol		Ethyl acetate		Pet ether	
(mg/ml)	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$
0.5	50.61^{a} ± 0.429		31.38^{a} ± O.421		16.27^{a} ± 0.314		13.65^{a} ± 0.182		6.37^{a} ± 0.475	
1.0	56.86^{b} ± 0.494		40.31^{b} ± 0.420		29.35^{b} ± 0.103		26.06^{b} ± 0.282		14.55^{b} ± 0.579	
2.0	70.23^{c} ± 0.399	0.059 ±	49.14^{c} ± 0.359	2.448 ±	40.18^{c} ± 0.157	3.633 ±	36.61^{c} ± 0.464	3.519 ±	30.40^{c} ± 0.455	4.235 ±
3.0	82.00^{d} ± 1.525	0.035	56.33^{d} ± 0.295	0.001	46.78^{d} ± 0.441	0.018	43.49^{d} ± 0.486	0.019	36.47^{d} ± 0.331	0.029
4.0	86.67^{e} ± 01.587		62.86^{e} ± 0.461		60.87^{e} ± 0.353		57.27^{e} ± 0.324		50.21^{e} ± 0.454	
5.0	89.26^{f} ± 0.156		66.13^{f} ± 0.419		61.80^{f} ± 0.352		61.76^{f} ± 0.443		54.80^{f} ± 0.498	

Table 6: The percent inhibition of α-glucosidase by methanol, ethanol, ethyl acetate and pet ether extracts of *B. cylindrica* leaves at varying concentrations.

Conc.	Acarbose		Methanol		Ethanol		Ethyl acetate		Pet ether	
(mg/ml)	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$
0.5	50.61^{a} ± 0.429		23.75^{a} ± 0.257		14.61^{a} ± 0.815		12.54^{a} ± 0.376		8.06^{a} ± 0.252	
1.0	56.86^{b} ± 0.494		26.21^{b} ± 0.510		23.79^{b} ± 0.384		19.96^{b} ± 0.124		10.55^{b} ± 0.411	
2.0	70.23^{c} ± 0.399	0.059 ±	33.09^{c} ± 0.453	3.178 ±	30.83^{c} ± 0.241	3.612 ±	28.65^{c} ± 0.024	3.520 ±	25.62^{c} ± 0.465	4.56 ±
3.0	82.00^{d} ± 1.525	0.035	53.88^{d} ± 0.303	0.003	50.45^{d} ± 0.575	0.011	43.49^{d} ± 0.457	0.013	32.51^{d} ± 0.519	0.039
4.0	86.67^{e} ± 01.587		62.27^{e} ± 0.378		57.88^{e} ± 0.455		57.24^{e} ± 0.498		47.39^{e} ± 0.498	
5.0	89.26^{f} ± 0.156		64.27^{f} ± 0.567		59.06^{f} ± 0.432		57.77^{e} ± 0.212		52.31^{f} ± 0.412	

Table 7: The percent inhibition of α-glucosidase by methanol, ethanol, ethyl acetate and pet ether extracts of *S. persica* leaves at varying concentrations.

Conc.	Acarbose		Methanol		Ethanol		Ethyl acetaate		Pet ether	
(mg/ml)	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$	%inhibition	IC$_{50}$
0.5	50.61^{a} ± 0.429		13.87^{a} ± 0.543		10.84^{a} ± 0.441		9.09^{a} ± 0.084		7.51^{a} ± 0.199	
1.0	56.86^{b} ± 0.494		24.71^{b} ± 0.611		21.55^{b} ± 1.08		20.78^{b} ± 0.444		17.81^{b} ± 0.091	
2.0	70.23^{c} ± 0.399	0.059 ±	32.99^{c} ± 0.410	3.797 ±	29.65^{c} ± 0.645	3.941 ±	27.45^{c} ± 0.506	4.219 ±	24.43^{c} ± 0.400	5.125 ±
3.0	82.00^{d} ± 1.525	0.035	43.83^{d} ± 0.567	0.027	36.05^{d} ± 0.517	0.011	32.21^{d} ± 0.177	0.014	31.30^{d} ± 0.546	0.021
4.0	86.67^{e} ± 01.587		56.19^{e} ± 0.385		56.17^{e} ± 0.468		49.99^{e} ± 0.366		43.11^{e} ± 0.504	
5.0	89.26^{f} ± 0.156		62.41^{f} ± 0.140		59.29^{f} ± 0.413		52.19^{f} ± 0.464		47.61^{f} ± 0.527	

Overall, the best activity was recorded in *C. tagal* leaves followed by leaves of *B. cylindrica* and *S. persica*. In *C. tagal* best α-amylase inhibition was recorded in methanol extract (73.26%) followed by ethanol (62.76%), ethyl acetate (61.83%) and pet ether (54.45%) at higher concentration (Table 2). Results of similar magnitude were recorded for α-glucosidase assay. These results indicate that methanol is the best solvent for the extraction of antidiabetic principle in this plant. There are no reports on *in vitro* antidiabetic activity in this plant however; *in vivo* antidiabetic activity has been recorded in the leaves of *C. tagal* [33]. This confirms antidiabetic potential in leaves of *C. tagal*. In *B. cylindrica* also the highest α-amylase inhibition was recorded in methanol extract than other solvent extracts. Similar results were recorded for α-glucosidase assay. Like-wise lower α-amylase inhibition was recorded in methanol extract of *S. persica* than other solvents at higher concentrations. The result of the plant indicates slightly lower antidiabetic activity in the leaves of *B. cylindrica* and *S. persica* than *C. tagal*. Overall highest *in vitro* antidiabetic potential indicated in *C. tagal* followed by *B. cylindrica* and *S. persica* besides this methanol was found to be the best solvent for the extraction of antidiabetic principle.

Antidiabetic potential of the plants is related to the presence of alkaloid, flavonoid, saponin, terpenoids classes of secondary metabolites [25-28], which all are seen in *C. tagal*, *B. cylindrica* and *S. persica* in this study. Therefore, the antidiabetic potential of the selected mangroves can be related to these phytochemicals. However the quantity and quality of these phytochemicals may be responsible for the antidiabetic potency of selected mangrove species.

This study proves that *C. tagal* exhibited maximum inhibitory activity in both the assays out of the mangrove species selected, thus establish a good relationship with the antidiabetic properties. This implicates the probability of containing some herbal bioactive compounds in plant which are inhibiting the activity of the enzyme, so in future; the identification of the bioactive constituents needs isolation, structural elucidation, and characterization.

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