

Evaluation of *In Vitro* Antidiabetic and Antioxidant Activities and Preliminary Phytochemical Screening of Leaf Extracts of *Avicennia officinalis*

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Abstract

Avicennia officinalis is a medicinal mangrove plant and used in traditional folk medicine for treatment of various ailments. In the present study, the antidiabetic and antioxidant activities of petroleum ether and aqueous extracts of leaves of *A. officinalis* were evaluated. The antidiabetic activity was evaluated out by α -amylase and α -glucosidase inhibition study and antioxidant capacity by DPPH, superoxide and hydrogen peroxide scavenging activity of leaf extracts at various concentrations. The maximum antidiabetic activity was observed in 1.0 mg/ml of leaves extract of aqueous extracts. The maximum superoxide and hydrogen peroxide scavenging activity were exhibited by aqueous extracts with IC_{50} values of 0.27 and 0.17 mg/ml respectively and maximum DPPH scavenging activity was shown by petroleum ether extracts with IC_{50} value of 0.25 mg/ml. The qualitative and quantitative phytochemical analysis also showed presence of different phytochemicals including phenols, flavonoids and tannins which may be attributed to their antidiabetic and antioxidant activities. Thus, the present study gives a clear evidence that leaf extracts of *A. officinalis* possess both antidiabetic and antioxidant activities.

Keywords: *Avicennia officinalis*; *In vitro*; Antidiabetic; Antioxidant; Phytochemicals

Introduction

Mangroves are the halophytes plants living between interface of sea and land. The mangroves are known as the stress tolerant plant as they can thrive in ecological inhospitable conditions exemplified by high salinity, heavy metal, low oxygen, flooding, UV-B, water logging where no other plants can survive [1]. To survive under these stressful conditions, these unique plants are reported to be enriched with unique and diversified class of phytochemicals. The ethnomedicinal uses of these plants for treatment of various ailments may be attributed to the presence of these phytochemicals.

Amongst the different mangrove species, *Avicennia officinalis* L. is an important mangrove species belonging to family Acanthaceae [2]. The stem/bark of this plant is ethnomedicinally as contraceptive, astringent, diuretic, antiulcer, treatment for snake bites, rheumatism, small pox, skin diseases, hepatitis, leprosy, antitumor, bronchial asthma, antibacterial, gastroprotective, aphrodisiac, boils and abscesses [3,4]. The pharmacological studies have also reported the antimicrobial [5], antiulcer [6], anti-nociceptive [7], anti-inflammatory [3], anticancer [3] and antioxidant [8] activities of different leaf and bark extracts of *A. officinalis*. However, no studies have been done to assess the antidiabetic activities of this plant. Therefore, in the present study, the antidiabetic and antioxidant activities of leaf extracts were evaluated employing *in vitro* assay methods.

Materials and Methods

Chemicals

p-Nitrophenyl- α -D-glucopyranoside (pNPG), Folin-Ciocalteu's Phenol reagent (FCP), catechol, catechin, EDTA, Nitro Blue Tetrazolium (NBT), hydroxylamine hydrochloride, sodium potassium tartrate, 3,5-dinitrosalicylic acid (DNS), sodium hydroxide, hydrogen peroxide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Butylated Hydroxy Toluene (BHT) and α -glucosidase, α -amylase were purchased from SRL

India, Ltd. Acarbose was purchased from Sigma Aldrich India. All the chemicals and reagents used in the study were of analytical grade.

Collection of plant and preparation of extracts

The leaf sample of *Avicennia officinalis* were collected from the Mahanadi delta region of Odisha coast. The specimen was identified by Prasanna Kumar Nayak, ICZMP, Forest Department, Govt. of Odisha. The leaf samples of *A. officinalis* were air dried in shade for 10 days, cut into pieces and were grinded but not to fine powder. For extraction, 10 g of dried sample was taken and mixed with petroleum ether and distilled water and shaken at room temperature for 72 h (separately carried out) to obtain Petroleum Ether Extracts (PEL) and Aqueous Extracts (AQL). The filtered liquid obtained was concentrated under reduced pressure in rotary evaporator. The obtained crude extract was stored in airtight container in refrigerator below 4°C for further studies.

Antidiabetic assay

α -amylase inhibition assay: α -amylase inhibitory assay was carried out using the method described by Ali et al. [9]. To each of the test tube, 100 μ l crude plant extract of 1, 0.5 and 0.1 mg/ml conc. was added. Then 100 μ l distilled water followed by 100 μ l enzyme (4 units/ml α -amylase) was added and mixed. This reaction was carried out at 25°C for 5 min followed by addition of 200 μ l starch (0.5%). It was again re-incubated at 25°C for 5 min. For terminating the reaction 200 μ l DNS (Dinitro

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Salicylic Acid) was added and incubated in water bath at 85°C for 15 min. After incubation the mixture was diluted with 1800 µl distilled water. The α-amylase inhibitory activity was determined by measuring absorbance at 540 nm using UV-vis spectrophotometer.

α-glucosidase inhibition assay: α-glucosidase inhibitory assay was carried out using the method developed by Apostolidis et al. [10] with slight modification. 50 µl crude plant extract of 1, 0.5 and 0.1 mg/ml conc. was added. Then 125 µl of enzyme solution (1 units/ml α-glucosidase) and 150 µl of phosphate buffer (pH-6.9) was added and incubated at 37°C for 10 min. 100 µl PNPG (3 mM) was added to start the reaction and the reaction was re-incubated at 37°C for 20 min and stopped by adding 2 ml of Na₂CO₃. α-glucosidase activity was determined by measuring the p-nitro phenol release from PNPG at 405 nm using UV-Vis spectrophotometer.

Antioxidant assays

DPPH scavenging assay: Free radical scavenging activity was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl), as described by Brand-Williams et al. [11] with slight modification. 2 ml of DPPH solution (0.1 mM DPPH dissolved in methanol), 50 µl of the plant extract were added and 150 µl DMSO were added. The reaction mixture was shaken and incubated in the dark at 37°C for 30 min. The absorbance was read at 517 nm against methanol as a blank by using UV-VIS spectrophotometer.

Superoxide scavenging assay: The superoxide scavenging activity was assayed by following the method of Kono [12]. 50 µl of plant extract, 1 ml Na₂CO₃ (50 mM) 0.4 ml NBT and 0.2 ml of EDTA were added. The reaction was initiated by adding 0.4 ml of hydroxyl amine hydrochloride (3 mM). Zero time absorbance was taken at 560 nm using spectrophotometer followed by recording the absorbance after 15 min at 25°C. The control was simultaneous run without plant extract.

Hydrogen peroxide scavenging assay: The ability of the extracts to scavenge hydrogen peroxide (H₂O₂) was determined by the reported method Rath et al. [13]. Hydrogen peroxide (43 mM) was prepared in phosphate buffered saline (pH 7.4). Aliquots of standard or extract solutions (3.4 mL) were added to 0.6 mL of H₂O₂ solution. The reaction mixture was incubated at room temperature for 10 min, and the absorbance was determined at 230 nm.

Phytochemical analysis

Petroleum ether and aqueous extracts of *A. officinalis* leaf were subjected to preliminary qualitative phytochemical analysis using standard procedure for identification of phytochemicals like terpenoids, glycosides, tannins, saponins, flavonoids and phenols. Similarly, the quantitative phytochemical analysis was carried for total phenol, flavonoid and tannins [14].

UV-vis spectral analysis

All the extracts of *A. officinalis* plant, were scanned in the wavelength ranging from 240-700 nm by using UV-VIS spectrophotometer and the characteristic peaks were detected for presence of phenolic compounds.

Statistical analysis

All experiments were conducted in triplicate and the data obtained were expressed as mean ± SD. The IC₅₀ values were estimated by linear curve-fitting method.

Results and Discussion

As part of our continuous interest in the research of biological activities and chemical composition of different mangrove plants, this article reports for the first time the hypoglycaemic activity of leaf extracts of *Avicennia officinalis* via the inhibition of α-amylase and α-glucosidase enzymes along with its antioxidant properties.

In vitro antidiabetic activity

The present study has endeavoured to investigate the possible inhibitory effect of *A. officinalis* to modulate key enzymes involved in diabetes i.e., α-amylase and α-glucosidase. The inhibitory activity of crude petroleum ether and aqueous leaf extracts of *A. officinalis* against both the enzymes were carried out. The potential to inhibit the α-amylase and α-glucosidase enzymes of the PEL and AQL were carried out at 0.1, 0.5 and 1 mg/ml. The results showed that both PEL and AQL inhibit the enzymes in a dose dependent manner (Table 1). However, the AQL showed better α-amylase and α-glucosidase enzyme inhibition capacity in comparison to PEL. However, the IC₅₀ value of AQL for both the assays was >1 mg/ml.

In vitro antioxidant activity

It has been well established that antioxidant activity plays a major role in the treatment of diabetes mellitus [15]. Many plants have been reported to possess both antidiabetics as well as antioxidant activity. Therefore, in the present study the antioxidant activity of the *A. officinalis* was investigated using the DPPH, super oxide and hydrogen peroxide scavenging method. The antioxidant potential of PEL and AQL were evaluated at 0.05, 0.1 and 0.15 mg/ml concentrations. Decreases in absorbance of the DPPH radical caused by the antioxidant were attributable to radical scavenging by hydrogen donation. The reaction was visible as a colour change from purple to yellow. The DPPH scavenging assay showed that the PEL possess better scavenging potential than AQL. The maximum DPPH scavenging was reported as 28.7% at 0.15 mg/ml for PEL (Table 2). The IC₅₀ values for DPPH scavenging activities of PEL and AQL were reported as 0.25 and 0.43 mg/ml. The present finding also corroborated the earlier study of Ravindran et al. [16] that reported the DPPH radical scavenging potential of methanol leaf extracts of *A. officinalis* with the EC₅₀ value of 0.239 mg/ml.

The superoxide scavenging activity is based on capacity of the antioxidants to decrease the absorbance of the chromophoric substance diformazan which is formed due to reduction of Nitro Blue Tetrazolium (NBT) by superoxide radicals generated by hydroxylamine hydrochloride [12]. The superoxide scavenging assay reported that the AQL could scavenge superoxide radicals more efficiently as compared to PEL. Highest superoxide scavenging activity was recorded as 27.7% at 0.15 mg/ml concentration for AQL and the IC₅₀ values for AQL and PEL 0.27 and 0.44 mg/ml respectively.

Hydrogen peroxide is a weak oxidizing agent and can inactivates a number of enzymes by oxidation of thiol (-SH) groups, and also by forming hydroxyl radicals [17]. Hydrogen peroxide scavenging capacity of an extract is directly related to its antioxidant activity. The present study indicated maximum effect of *A. officinalis* in AQL extract and minimum in PEL, and the concentration of 0.15 mg/ml showed best result compared to other concentrations. Maximum hydrogen peroxide was observed as 39.9% in AQL at 0.15 mg/ml. The IC₅₀ value for AQL and PEL were recorded as 0.17 and 0.48 mg/ml respectively. Earlier study also showed that the methanol leaf extracts of *A. officinalis* exhibited hydrogen peroxide scavenging activity with IC₅₀ value of 0.378 mg/ml [16].

Phytochemical analysis

Phenolic compounds, such as flavonoids, phenolic acid and tannins possess diverse biological activities, such as anti-inflammatory, anticarcinogenic, antidiabetic and anti-atherosclerotic activities which might be related to their antioxidant activity [18]. Phytochemicals from plants are known to be good natural antioxidants and therefore in the present study, different phytochemicals from PEL and AQL in *A. officinalis* were analyzed by both qualitative and quantitative phytochemical assays. The qualitative phytochemical analysis showed the presence of steroids, saponins, phenols, terpenoids, tannins and flavonoids in both PEL and AQL extracts of *A. officinalis*. The quantitative phytochemical analysis showed presence of phenol, flavonoid and tannin in different quantities in PEL and AQL. The AQL recorded higher amount of total phenol content (12.8 mg Gallic Acid Equivalent (GAE)/g of dry sample), total tannin (3.31 mg Gallic Acid Equivalent (GAE)/g of dry sample), but higher amount of flavonoid

was recorded in PEL (3.92 µg Quercetin Equivalent (QE)/g of dry sample). The present study also corroborated with earlier findings of where crude ethanol and aqueous leaf extracts of *A. officinalis* showed presence of higher amount of phenols (Tables 3 and 4) [4].

The UV-Visible profile of the PEL and AQL of *A. officinalis* was studied at a wave length range of 200-700 nm (Figures 1 and 2). The profile showed the peaks at 270 nm confirming the presence of phenolic derivatives in the leaf extracts.

Conclusion

In this study, using various *in vitro* assay methods, the antidiabetic potential of petroleum ether and aqueous extracts of *A. officinalis* were evaluated based on the basis of their capacity to inhibit carbohydrate metabolizing enzymes like α -amylase and α -glucosidase. The antioxidant potential was also evaluated by studying DPPH, superoxide, and hydrogen peroxide scavenging activities. In addition, we further

Concentration	% of α -Amylase inhibition		% of α -Glucosidase inhibition	
	PEL	AQL	PEL	AQL
0.1 mg/ml	10.5± 0.02	23.2 ±0.01	10.0 ± 0.01	15.7 ± 0.01
0.5 mg/ml	13.0 ± 0.03	27.7 ±0.01	13.2 ± 0.01	22.1± 0.01
1.0 mg/ml	17.4 ± 0.01	32.0 ± 0.01	17.4± 0.01	27.4± 0.01
IC ₅₀ mg/ml	>1.0 mg/ml	>1.0 mg/ml	>1.0 mg/ml	>1.0 mg/ml

Table 1: Antidiabetic activities of leaf extracts of *Avicennia officinalis*. Data are expressed as mean ± SD (n=3).

Concentration	% of DPPH scavenging		% of Superoxide scavenging		% of H ₂ O ₂ scavenging	
	PEL	AQL	PEL	AQL	PEL	AQL
0.05 mg/ml	18.9 ± 0.71	12.7 ± 0.50	7.6	16.7 ± 0.02	7.9 ± 0.02	30.9 ± 0.02
0.1 mg/ml	23.7 ± 1.81	15.2 ± 0.40	12.1± 0.01	21.2 ± 0.01	10.6 ± 0.38	34.6 ± 0.09
0.15 mg/ml	28.7 ± 1.62	17.5 ± 1.11	16.9 ± 0.01	27.7	16.2 ± 1.06	39.9 ± 0.86
IC ₅₀ mg/ml	0.25	0.43	0.44	0.27	0.48	0.17

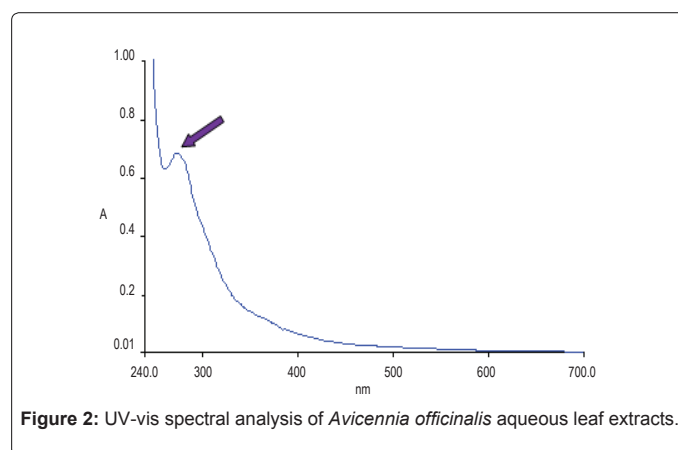
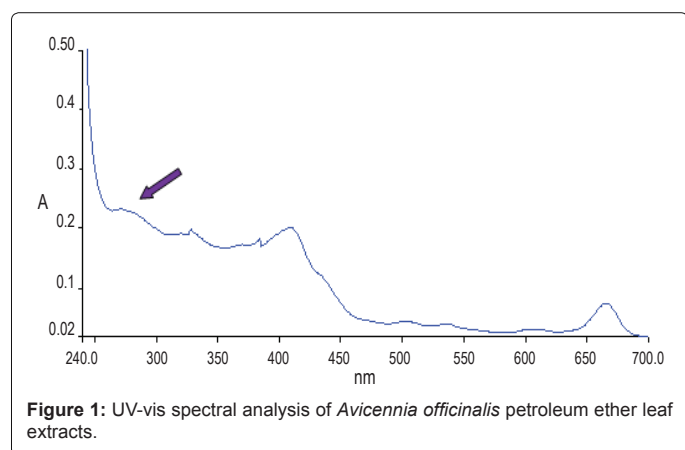
Table 2: Antioxidant activities of leaf extracts of *Avicennia officinalis*. Data are expressed as mean ± SD (n=3).

Sample	Steroids	Saponins	Phenols	Terpenoids	Flavonoids	Tannins
PEL	+	+	+	+	+	+
AQL	+	+	+	+	+	+

Table 3: Preliminary phytochemical screening of all the extracts of *Avicennia officinalis*. Data are expressed as mean ± SD (n=3).

Sample	Total Phenol (mg/g)	Total Flavonoid (µg/g)	Total tannin (mg/g)
PEL	10.46	3.92	2.17
AQL	12.80	3.49	3.31

Table 4: Quantitative phytochemical assay of all the extracts of *Avicennia officinalis*.



evaluated the presence of different phytochemicals both qualitatively and quantitatively. The results clearly confirmed the antidiabetic and radical scavenging effect of the *A. officinalis* leaves extracts. Therefore, this mangrove plant merits further exploration both chemically and biologically to exploit its relevant therapeutic effect.

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Authors' Contributions

S. K. Das, D. Samantaray have conducted all the experiments. H. N. Thatoi has edited the manuscript.

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