

Research Article

Medicinal Activity of *Avicennia officinalis*: Evaluation of Phytochemical and Pharmacological Properties

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Abstract: Antioxidant activity and total phenolic content of EtOH extract of *Avicennia officinalis* leaves were determined by DPPH free radical scavenging assay and Folin-Ciocalteu assay, respectively. IC₅₀ value was 160.92 µg/ml in DPPH assay and total phenolic content was 208.57mg GAE/100 g of dry powder. The sample produced 18.75% and 51.88% (P<0.01) writhing inhibition at the doses of 250 and 500 mg/kg body weight, respectively, in acetic acid-induced writhing model using Swiss-albino mice. It showed accountable antibacterial activity against *Escherichia coli* and *Salmonella typhi* in disc diffusion assay. MIC was found to be as 62.5 µg/ml against *E. coli* and 125 µg/ml against *S. typhi*. In brine shrimp lethality bioassay LC₅₀ value was found 131.203 µg/ml. Preliminary phytochemical screening confirmed the presence of important phytochemicals like carbohydrate, reducing sugar, combined reducing sugar, glycosides, tannins, alkaloids, proteins, terpenoids and flavonoids which may be responsible for antioxidant, analgesic, cytotoxic and accountable antibacterial activity.

Keywords: Antioxidant; Total phenolic content; Analgesic; Antibacterial; Cytotoxic activity

INTRODUCTION

In recent trend of medicine, extensive research work is performing worldwide on plants to find bioactive compounds which may serve as raw materials for new drug discovery [1]. Sundarbans, the largest continuous mangrove forest in the world, situated in Bangladesh, have plenty of different plants which may have medicinal values. Some of these mangrove plants are already well-known for their medicinal values and are used extensively but most of them are still unexplored. So appropriate scientific screening can explore these plants and find out proper medicinal values.

Alkaloids, tannins, glycosides, phenolics, volatile oils, etc., obtained from medicinal plants contain a great potential for pharmacological activity and therapeutic effects [2]. The plant phenolic compounds exhibit antioxidant properties due to their high redox potential [3]. They also exhibit a wide range of biological activities as, analgesic, antimicrobial, antiproliferative and anticancer activities, and many of these biological activities can be attributed to their antioxidant properties [4]. This is because a number of human diseases such as cancer, cardiovascular disease, neurodegenerative disease, diabetes, rheumatism, etc. are related to oxidative stress [5, 6].

Avicennia officinalis L. is an evergreen species of mangrove which is mainly found in Bangladesh, India, Indonesia, Malaysia, Brunei, Myanmar, Viet-Nam and southern Papua New Guinea but not widely introduced elsewhere[7]. It is locally known as 'Dhola Baen' in Bangladesh. It is used as a folk remedy for boils and tumors [8]. Unripe seeds are poulticed onto abscesses, boils, and smallpox sores. The bark is used for skin afflictions, especially scabies. Literature on GC-MS analysis, *A. officinalis* L. leaves indicates the presence of many important phytochemicals [9]. Because of the presence of such phytochemicals *A. officinalis* L. may be a source of medicinal properties like analgesic, antioxidant, antimicrobial or anticancer activities.

The aim of present study was to screen phytochemicals from this plant and to assess pharmacological activities such as analgesic, antioxidant, antibacterial and cytotoxicity.

MATERIALS AND METHODS

Plant material collection

The leaves of *A. officinalis* were collected from the Sundarbans Mangrove forest, Bangladesh in January, 2011. The plants were mounted on paper and the sample was identified by the experts of Bangladesh

National Herbarium, Dhaka, Bangladesh (Accession No-35541 DACB).

Preparation of the plant extract

The leaves were shade-dried to ensure the active constituents remain free from decomposition and to avoid any photochemical degradation. The leaves were ground into a coarse powder and extracted by cold extraction method by 96% of ethanol. The deep green paste type concentrate was designated as crude EtOH extract of leaves of *A. officinalis* (EEAO).

Reference drug

Diclofenac sodium and Ceftriaxone were collected from Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh. Kanamycin 30 µg/disc and Vincristine sulfate were collected from Oxoid Ltd. UK and Gedeon Richter, Hungary, respectively.

Chemicals

The chemicals used in this study were 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and gallic acid (Merck, Germany); Tween-80 (Loba Chemie Pvt Ltd., India); dimethyl sulfoxide (DMSO; Gaylord Chemical Company, USA), Resazurin (Cayman Chemical) and acetic acid (Merck, Germany).

Experimental animals

Young Swiss-albino mice aged four to five weeks, average weight 28-35 g were used for the experiment. The mice were collected from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B formulated). The animals were kept under standard laboratory conditions (room temperature of 25 ± 1 °C, and relative humidity of 56% – 60 %).

Preliminary phytochemical screening

EEAO was subjected to preliminary phytochemical studies for the detection of major phytochemicals. In each test 5% (w/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test [10, 11].

Evaluation of antioxidant activity

In qualitative antioxidant test a suitably diluted stock solution of EEAO was spotted on TLC plates and the plates were developed in three solvent systems of different polarities (polar, medium polar and non-polar) by using 0.02% DPPH in ethanol [12]. The quantitative antioxidant analysis of EEAO was determined by their scavenging activity of stable DPPH free radical [12]. IC₅₀ values were determined (Figure 1) using a free software 'Graph' (version 4.4.2, copyright Ivan Johansen). The formula used for determination of % inhibition ratio is- Percent scavenging activity = $\{(1 - A_1) / A_0\} \times 100\%$, where A₀ is the absorbance of control, and A₁ is the absorbance of sample or standard.

Total phenolic content assay

Total phenolic content was measured by the Folin-Ciocalteu assay [13].

Evaluation of analgesic activity

The acetic acid induced writhing method was used with slight modification [14, 15].

Evaluation of antimicrobial activity

Antimicrobial activity was determined using disc diffusion method with slight modification [16, 17]. Kanamycin (30 µg/disc) antibiotic discs were used as positive control and EEAO was taken 250 and 500 µg/disc.

Determination of minimum inhibitory concentration (MIC)

The minimal inhibitory concentrations (MIC) were determined by a broth macro-dilution method [18].

Evaluation of cytotoxic activity

Brine shrimp lethality Bio-assay was carried out for testing the general toxicity of the DMSO solution of the extract against *Artemia salina* [19] and Vincristine sulphate was used as positive control. LC₅₀ was calculated using LdP line probit analysis software, USA.

Statistical analysis

All experimental data were expressed as mean ± standard error of mean. To assess statistical significance by one-way analysis of variance Dunnett's t test was used. Statistical analysis was performed in Prism 6.0 (Graph Pad Software Inc., San Diego, CA, USA). Results were considered as statistically significant as P<0.05.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

To get preliminary idea about the active constituents present in the leaves extract phytochemical screening was performed which revealed the presence of several important phytochemical constituents, as reducing sugar, combined reducing sugar, tannins, flavonoids, gums, alkaloids, glycoside, terpenoids and proteins. Ganesh and Vennila [9] also showed the presence of protein, resin, steroids, tannins, glycosides, reducing sugar, carbohydrates, saponins, sterols, terpenoids, phenol, cardioglycosides and catechol in *A. officinalis* leaves in GC-MS analysis. It has been reported that different phytochemicals are responsible for the antioxidant, anti-inflammatory, antinociceptive, antibacterial and cytotoxic activities [20-23]. As EEAO indicated the presence of several phytochemicals in preliminary screening, so different pharmacological tests were conducted in the present study to identify the logic for its traditional use and explore it as a source of medicine in future.

Antioxidant activity and total phenolics

Antioxidants remove free radical intermediates from the body which are responsible for some life-threatening diseases like cancer, diabetes, stroke, etc. TLC and DPPH free radical assay was conducted for the assessment of antioxidant potential of EEAO. In the TLC extract showed positive result and in DPPH free radical assay IC₅₀ value was found 160.9 µg/ml (Table 1). The total content of phenolics for EEAO (Figure 2) was found to be 208.57 mg GAE/100 gm dry powder (Table 2). Different studies suggest that the high level of antioxidant activity in plants is due to the presence of phenolic components [24, 15].

Analgesic activity

Acetic acid induces writhing by causing algia by releasing endogenous substances, which then excite nerve endings [26]. This is a procedure used to test effectiveness of peripheral analgesic agents. In this method EEAO produced 18.75 % and 51.88 % (P<0.01) writhing inhibition at the doses of 250 mg/kg and 500 mg/kg body weight respectively while the standard drug diclofenac Na inhibition was found to be 69.38 % (P<0.01) at a dose of 25 mg/kg body weight (Table 3). The active principles responsible for the analgesic

activity of different plants are terpenoids, reducing sugar, gums, xanthoprotein, flavonoids and tannins [27-29].

Antibacterial activity

One of the most popular, inexpensive and easy methods for determining antibacterial activity against pathogenic bacterial strains is disk diffusion assay. In this assay the extract showed accountable antibacterial activity against the bacterial strains *E. coli* and *S. typhi* (Table 4). Among the chemical constituents like tannins, terpenoids, alkaloids and flavonoids show antimicrobial properties [30-32]. The results of the MIC (Table 4) indicate that this plant has a scientific basis in traditional use and can be further investigated as antibacterial source.

Cytotoxic activity

In the present evaluation, the LC₅₀ for EEAO was found to be 131.2 µg/ml (Figure 3) and 1.054 µg/ml for standard anticancer drug vincristine sulfate. The cytotoxic effect of plants is principally contributed by the presence of secondary metabolites like alkaloid, glycoside, steroid, tannin, terpenoid and flavonoid which is also consistent with present study [33].

Table-1: % DPPH free radical inhibition of ascorbic acid and EEAO

conc. (µg/ml)	Ascorbic acid		EEAO	
	Avg. abs	% inhibition	Avg. abs	% inhibition
0 (Blank)	0.814 ± 0.001	0	0.814 ± 0.002	0
1	0.731 ± 0.001	10.2	0.742 ± 0.002	8.81
5	0.656 ± 0.002	19.41	0.714 ± 0.001	12.29
10	0.503 ± 0.001	38.21	0.681 ± 0.001	16.34
50	0.062 ± 0.002	92.38	0.548 ± 0.001	32.68
100	0.033 ± 0.002	95.95	0.296 ± 0.002	63.64
200	0.028 ± 0.001	96.56	0.110 ± 0.001	86.49
500	0.025 ± 0.002	96.93	0.098 ± 0.002	87.96

Average absorbance is expressed as mean ± standard deviation of four values

Table-2: Absorbance of Gallic acid

Conc. of Gallic acid (mg/L)	Avg. abs of Gallic acid
5	0.012 ± 0.002
10	0.023 ± 0.001
20	0.040 ± 0.002
40	0.060 ± 0.002
60	0.080 ± 0.001
80	0.090 ± 0.001
100	0.102 ± 0.002
200	0.145 ± 0.002

Average absorbance is expressed as mean ± standard deviation of four values

Table-3: Effects of EEAO on acetic acid induced writhing of mice

Group	No. of writhing	% Inhibition
Control (1% Tween-80 solution in water, 10 mL/kg, p.o.)	32 ± 1	—
Positive control (diclofenac sodium 25 mg/kg, p.o.)	9.8 ± 0.96*	69.38
Test group I (EEAO 250 mg/kg, p.o.)	26 ± 0.79*	18.75
Test group II (EEAO 500 mg/kg, p.o.)	15.4 ± 1.07*	51.88

Numbers of writhing are expressed as mean ± standard error of mean; *P < 0.05 vs control group (n=5); EEAO: ethanol extract of *A. officinalis*; p.o.: per oral.

Table-4: Zone of inhibition and MIC of EEAO

Bacterial strain	Disc diffusion assay (zone of inhibition in mm)			MIC (µg/ml)	
	Kanamycin 30 µg/disc	EEAO 250µg/disc	EEAO 500µg/disc	Ceftriaxone	EEAO
<i>E. coli</i>	23	7.5	11	2	62.5
<i>S. typhi</i>	25	7	10	2	125

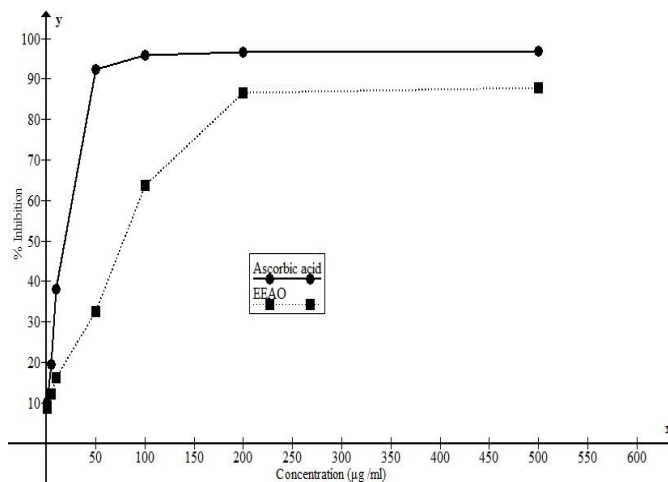


Fig-1: DPPH-scavenging activity of EEAO

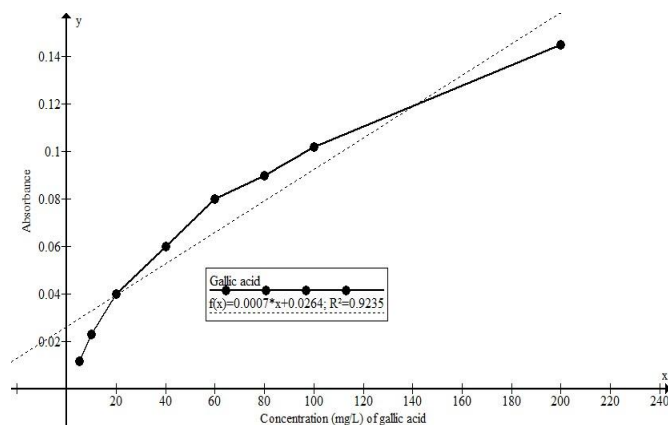


Fig-2: Absorbance VS concentration graph of Gallic acid

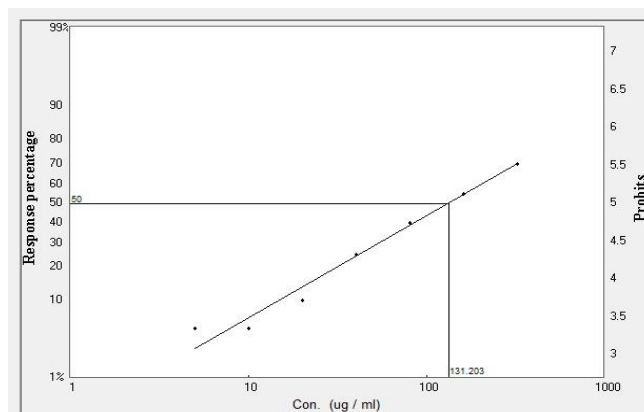


Fig-3: LC₅₀ for EEAO in brine shrimp lethality bioassay

CONCLUSION

Phytochemical screening and pharmacological evaluation of *Avicennia officinalis* leaves extract provides the evidence of the existence of antibacterial, cytotoxic and analgesic activities. These pharmacologic actions are dose dependent. These potentials may be due to the polyphenols present in the extract. It can be said that the plant extract is very useful and effective and may be potential sources of novel bioactive compounds. Further, pharmacological investigation and bioactivity guided studies are required to isolate and purify the active principle(s) responsible for these activities.

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