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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *ACANTHUS ILICIFOLIUS*

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ABSTRACT

Acanthus ilicifolius is very potential mangrove plant species. Traditionally used in treatment of several diseases (Hepatoprotective, Anticancer and antimicrobial). The present study was carried out to investigate qualitative and quantitative phytochemical profile of leaves of *A. ilicifolius.* The leaves powder was successively extracted with hexane, ethyl acetate and ethanol solvents. Results preliminary phytochemical analysis for carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, terpenoids, coumarins, steroids and phytosteroids, phylobatannins, anthraquinones and phenols both solvent extracts. Whereas, carbohydrates, tannins, saponins, alkaloids, quinones, glycosides, terpenoids, steroids and phytosteroids, phylobatannins and anthraquinones were deficient in *A. ilicifolius*. Also, cardiac glycosides, were absent in only ethyl acetate extract. In the case of quantitative phytochemical analysis studies highly observed in total phenolics and followed by total flavonoids, total tannins, and total antioxidants. It can be concluded that the species is effective in anti-oxidant anticancer and anti-microbial potential effects.

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INTRODUCTION

Acanthus ilicifolius is a coastal plant and commercially used as fuel and timber in coastal areas for a long period of time and are lesser known for therapeutic usage (1). They are a rich source of medicines and produce a wide range of phytochemicals because of their ecosystem which consist of heterogeneous habitats (2). Based on World Health Organization data, more than 80% of world inhabitants depend on plant for their medicine and mangroves have been widely used for that purpose (3,4). *Acanthus ilicifolius* is an important medicinal plant from mangroves but its intact medicinal value has not been fully explored as yet. *Acanthus ilicifolius* belongs to Acanthaceae family and is known as Kayzimulli, Attumulli and Kazhuthai mulli in vernacular name. It is widely distributed in India (Pitchavaram, Tamilnadu), Malaysia and South Asian countries and used in traditional Indian medicinal system to treat dyspepsia, paralysis, and skin disease and wound healing (5). Scientifically explored for their biological activities of *Acanthus ilicifolius* like hepato protective (6), anti osteoporotic activity (7), antimicrobial (8), anticancer (9), analgesic (10), anti inflammatory (11), anti diabetic (12), antiulcer (13), anti nociceptive (14) and lesmanicidal activity (15). According to a good deal of literature survey most of the adverse reactions and diseases are associated with oxidative stress which is produced by free radicals. Free radicals are generated due to metabolic and physiological reactions. The free radicals are highly reactive which can damage normal cells and that leads to a variety of diseases. Free radicals can be deactivated and neutralized by the novel compounds as known as antioxidants (16). Medicinal plants are a rich source of antioxidants and the search for natural effective and non-toxic antioxidants with lesser side effects is a still challenge for researchers. The search for a new therapeutic agent with anti-oxidant is an interesting part of research. Recent years, there is a great attention towards antioxidants from plants which is rapidly increased. The aim of this study was to quantify the phyto-chemicals with antioxidant activity present in the extract of hexane, ethyl acetate and ethanol from mangrove plant, *Acanthus ilicifolius*. The present investigation is undertaken to determine the phytoconstituents present in the extracts by qualitative and quantitative analysis in the extracts of hexane, ethyl acetate and ethanol from mangrove plant, *Acanthus illicifolius.*

MATERIALS AND METHODS

The leaves of *Acanthus ilicifolius* L. (Acanthaceae) were collected freshly from Alapakkam area, Cuddalore District, Tamilnadu during the month of January 2018. Plant specimens were authenticated by Dr. T. Manikandan, Assistant Professor, Department of Botany, Arignar Anna Government Arts College, Villupuram, Tamilnadu. The fresh leaves of *Acanthus ilicifolius* were washed thoroughly cut into small pieces, dried under shade completely at room temperature. Dried materials were ground into coarse powder and stored in airtight for further works (Fig. 1).

Preparation of extracts: The powdered leaf samples (100g each) were immersed separately in different solvents including hexane, ethyl acetate and ethanol. The cold percolation was carried out for three times in solvents (300 ml each) with increasing polanty to ensure exhaustive extraction. After 72hrs, the extracts were filtered through whatman filter paper No-1 and were concentrated under

reduced pressure at 40 using rotary vaccum evaporator. This was stored in cold condition from 2 to 8 for further use in subsequent experiments.

Fig. 1. Habit of *Acanthus ilicifolius*

Qualitative screening of phytochemicals

The hexane, ethyl acetate and ethanol extracts of *A. ill icifolius* were screened for the presence of phytochemicals such as carbohydrates,tannins,saponins, flavonoids, alkaloids, quinines, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, steroidsandphytosteroids, phlobatannins, anthra quinones, total flavonoids, total tannins, total phenolic and antioxidant activities by following the standred methods.

Test for carbohydrates: To 2ml of A. ilicifolius plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the carbohydrates. 1ml of Fehling's A and 1 of Fehling's b. Boil for 10 min. Reddish brown color indicates presence (17).

Test for tannins: To 1ml of plant extract and 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins (18).

Test for saponins: To be added 2ml of extract, 2ml of distilled water and shaken in a 15 minutes length wise. Formation of 1 cm layer of foam indicates the presence of saponins (19).

Test for flavonoids: Plant extract 2 ml, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids (20).

Test for alkaloids: Plant extract 2 ml and 2ml of concentrated hydrochloric acid was added. Next, a few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids (21).

Test for quinones: Plant extract 2 ml, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones (22).

Test for glycosides: To added 2ml of plant extract and 3ml of chloroform then 10% ammonia solution. Formation of pink color indicates of glycosides (23).

Test for cardiac glycosides: To 0.5ml of extract, 2ml of glacial acetic acid and to add few drops of 5% ferric chloride were added. Formation of brown ring at the interface indicates presence of cardiac glycosides (20).

Test for terpenoids: To 0.5ml of plant extract and 2ml of chloroform then carefully added concentrated sulphuric acid. Formation of red brown color at the presence of terpenoids (20).

Test for phenols: To 1ml of the extract, a few drops of Folin-Ciocalteau reagent was added followed by few drops of 15% Sodium carbonate solution. Formation of blue or green color indicates presence of phenols (22).

Test for coumarins: To 1 ml of extract and1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins (22).

***Steroids and phytosteroids* :** To 1ml of plant extract and 1 ml of chloroform is added and few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids (24).

Test for phlobatannins (HCl test): Two millilitres (2 mL) of the aqueous solution of the plant extract were added into dilute HCl and observed for red precipitate that was indicative the presence of phlobatannins (20).

Anthraquinones: To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones (20).

Quantitative screening of phytochemicls

Determination of flavonoids content: Total Flavanoid content in the extracts (Ethyl acetate) was determined using the method described by Sankanaka *et al.*, 2005. The flavonoid content was determined by aluminium chloride method using Quercetin as standard. Extracts and Quercetin were prepared in Ethyl acetate (10 mg/ mL). 0.1 mL of extract was mixed with 0.9 mL of distilled water in test tubes, followed by addition of 75 µL of 5% sodium nitrate solution. After 6 minutes, 10 µL of 10% aluminium chloride solution was added and the mixture was allowed to stand for further 5 minutes after which 0.5 mL of 1M Sodium hydroxide was added to the reaction mixture. The reaction mixture was brought to 2.5 mL wit distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. A calibration curve was generated using various concentrations of Quercetin and the Quercetin equivalence (QE) of the sample was expressed in µg/mg of the extract.

Determination of tannin content: The tannin content in the extracts were determined by Folin - Ciocalteu method. 0.1 ml of the sample extracts containing 1 mg were added to test tubes containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35 % Na2CO3. It was made up to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80 and 100 µg/ml) were also used. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of Tannic acid equivalence (TE) µg/mg of extract.

Determination of total phenolic content: The amount of phenolic compounds in the extracts was determined by the Folin Ciocalteu colorimetric method and calculated from a calibration curve obtained with Gallic Acid as standard (10mg/10ml).

From the standard solution 20 to 100 µl was taken and added to different test tubes. Extract was added in a separate test tube at a concentration of 10 mg/ml and 5ml of folins – ciocalteu (1:10 dilution) was added and the contents were mixed thoroughly. 4ml of 0.7 M sodium carbonate was added and the mixture was incubated for 30 minutes. The absorbance was measured at 765nm in a UV-Visible Spectrophotometer. The results were expressed in Gallic acid equivalence of the samples (GE) µg/mg of the extract.

Antioxidant activity (DPPH)

DPPH free radical scavenging assay: The ability of the samples to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by (Blois 1958). Stock solution of compound was prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 400, 600, 800, 1000 µg) of sample were added, at an equal volume to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. The annihilation activity of free radicals was calculated in percentage inhibition according to the following formula

% of Inhibition = (A of control – A of Test)/A of control * 100

RESULTS

Phytochemical analysis plays a major resource for information on analytical and instrumental methodology in plant sciences. A preliminary study was done to identify the active constituents from *Acanthus illicifolius.*

The phytochemical characteristics of hexane, ethanol and ethyl acetate extracts of *Acanthus ill icifolius* tested and summarized in Table 1. From the Table 1, it could be observed that Flavonoids, Coumarins and Phenols were presence in three solvent extracts but Cardiac glycosides presence in only two solvent extracts (Hexane and ethanol). Also cardiac glycosides were absent in only ethyl acetate extract only. Whereas, the Carbohydrates, Tannins, Saponins, Alkaloids, Quinones, Glycosides, Terpenoids, Steroids, Phyto steroids, Phlobatannins and Anthraquinones were deficient in *A.illucifolius.*

Total phenolic content (TPC): Total phenolic content of *A.ilicifolius* ethanol extract were varying widely between 17mg GAE/10g extract and in the case of hexane 0.72mg GAE/10g and ethyl acetate 0.92mg GAE/10g extracts are exhibited.

Total flavonoids content (TFC): The total flavanoids content were observed in ethanol extract of *A.ilicifolius* 8.4 mg GAE/10g extract and in the case of hexane -1.45 mg GAE/10g and ethyl acetate 2.68mg GAE/10g.

Total tannin content (TTC): Total content were observed in ethanol extract of *A.ilicifolius* 2.58 mg GAE/10g extract and in the case of hexane 0.19 mg GAE/10g and ethyl acetate 3.20 mg GAE/10g.

Antioxidant activity

The total antioxidant capacity observed in ethanolic of A. ilicifolius at range of 476mg/ µ mol Trolox equivalent/g extract. The hexane extract exhibited total antioxidant capacity observed in 410mg/ µ mol Trolox equivalent/g extract respectively.

Table1. Hexane, Ethyl acetate, Ethanol of *A. ilicifolius L.* in different extracts

S.No	Phytochemical Tests	Leaf extract		
		Hexane Extract	Ethyl acetate Extract	Ethanol Extract
1	Carbohydrates	-	-	-
2	Tannins	-	-	-
3	Saponins	-	-	-
4	Flavonoids	+	+	+
5	Alkaloids	-	-	-
6	Quinones	-	-	-
7	Glycosides	-	-	-
8	Cardiac glycosides	+	-	+
9	Terpenoids	-	-	-
10	Coumarins	+	+	+
11	Steroids & Phytosteroids	-	-	-
12	Phlobatannins	-	-	-
13	Anthraquinones	-	-	-
14	Phenols	+	+	+

Table 2. Quantitative analysis of *A. ilicifolius*

S.No	Phytochemical Assay	Hexane extract mg/10ml of extract	Ethyl acetate mg/10ml of extract	Ethanol mg/10ml of extract
1.	Total Phenolics	0.72	0.92	17.22
2.	Total Flavonoids	-1.45	2.68	8.4
3.	Total Tannins	0.19	3.20	2.58

Table 3. Percentage Inhibition of Hexane, Ethyl acetate and Ethanol

Concentration (µg)	Percentage inhibition					
	Hexane	Positive control	Ethyl acetate	Positive control	ethanol	Positive control
200	36.24	89.29	29.59	93.79	20.59	91.18
400	42.62	89.93	36.76	94.16	42.86	91.70
600	52.82	93.37	45.49	96.15	46.22	94.54
800	68.12	94.39	48.82	96.75	53.99	95.38
1000	73.22	95.66	58.28	97.49	76.79	96.43

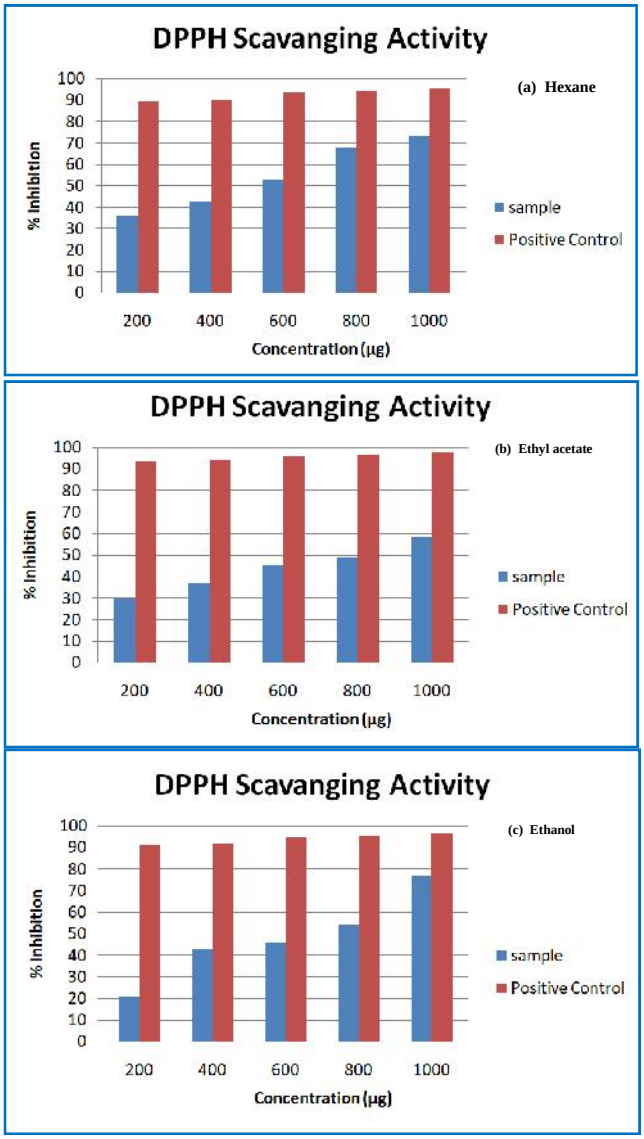

Figure 1. Percentage inhibition of (a) hexane, (b) ethyl acetate and (c) ethanol

DISCUSSION

Preliminary qualitative phytochemical analysis made for the leaf of *A.ilicifolius* revealed the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, terpenoids, coumarins, steroids and phytosteroids, phyloba tannins, anthraquinones and phenols were presence in the three different solvent extracts. Whereas, the carbohydrates, tannins, saponins, alkaloids, quinones, glycosides, terpenoids, steroids and phytosteroids, phylobatannins and anthraquinones were deficient in *A.ilicifolius*. cardoac glycosides were also absent in only ethyl acetate extract. These secondary metabolities are reported to have many biological and the rapeutic properties (29,30) so this species is expected to have many medicinal uses. The extraction yield calculated both hexane, ethyl acetate and ethnol extract showed that ethanol extract registered higher percentage of yield. It may be due to high polarity of ethanol solvent which can draw high variety of plant constituents than the other solvents (31). Generally, majority of the secondary metanolites studied and ascorbic acid in leaf of *A.ilicifolius* have present with higher amount in ethanolic extract than that of the other extract hexane and ethyl acetate. The biological property, antioxidant activity was determined to be effective through various as says for the leaf of *A.ilicifolius*. The presence of phenolic compounds (Total phenolic, flavonoids, tannin and antioxidents) provides pharmacologucal activities like anti-cancer (32, 33) anti-oxidant (33, 34) antimicrobial (35, 36) wound healing (37) and anti-inflammatory (38, 39) that may suggest an association to the species here investigated.

The healing properties of medicinal plants are possible due to the presence of various phytochemical constituents such as phenolics, flavonoids and tannins etc. phytochemical analysis of plant extracts revealed the presence of constituents which are known to show medicinal properties in addition to physiological activity (40, 41). Several classes of polyphenolic compounds such as phenolics, flavonoids and tannins contribute to plant defence mechanism in resisting pathogenic microorganisms (42). In the present study, phytochemical analysis showed that extracts both hexane, ethyl acetate and ethanol from *A.ilicifolius* leaves contain most of principles. Such phytochemical are an indicative for the antimicrobial and antioxidant activities. Therefore, the beneficial medicinal effects of plant materials may result from the combinations of antimicrobial, antioxidant and other secondary products present in plant, as well as phytochemical (43). Such secondary products play and important role in a plants defense through cytotoxicity towards microbial pathogen and this could prove the usefulness of these secondary products as antimicrobial medicines for humans.

Mangrove plants are biochemically unique, producing a wide array of novel natural products. Mangrove possesses novel agrochemical products, compounds of medicinal values and biologically products, compounds. For a long period of time in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs (44). Mangrove and mangroves associates contain biologically active antiviral, antibacterial and antifungal compounds. Extracts from different mangrove plants are active against human and plant pathogens. Similar result of phytochemical screening of flower extract of this species were obtained by shanmoga priya (45).

Conclusion

The work is a successful attempt of phytochemical characterization and antimicrobial efficiency of mangrove plant *A.ilicifolius*. In recent years screening of mangrove plants for a variety of biological activities, further attention should be paid to develop the novel drugs from natural product.

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