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Preliminary Phytochemical and Anti-Bacterial Sensitivity Test of Ethanolic Stem Bark Extract of *Acanthus ilicifolius* (L.)

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Abstract: This study aimed at the investigation of phytochemical and anti-bacterial activity of *Acanthus ilicifolius* (L.). For this, the qualitative identification of phytochemicals was done in the crude ethanolic stem bark extract of the plant. The anti-bacterial sensitivity test was performed by using disc diffusion and serial dilution methods against *Escherichia coli*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus entericus* and *Staphylococcus aureus*. Results suggest that the crude extract revealed the presence of alkaloids, flavonoids, terpenoids, phenols, glycosides, steroids, tannins, carbohydrates, fixed oils and fats. The extract at 500 µg/disc produced 14 and 12 mm zones of inhibition against *B. subtilis* and *B. megaterium*, respectively. The minimum inhibitory concentrations calculated for *B. subtilis* and *B. megaterium* was 46.875 µg/mL. In conclusion, the ethanolic stem bark extract of *A. ilicifolius* showed an inhibitory effect against the test bacterial strains, suggesting the plant may be a good source of anti-bacterial agents.

Keywords: *Acanthus ilicifolius*, phytochemicals, anti-bacterial effect.

1 Introduction

Acanthus ilicifolius (L.) (Sea holly) (Family: Acanthaceae) is a perennial herbaceous mangrove plant, popularly recognized as “Holy leaved acanthus" or “Kayalchulli”. Traditionally *A. ilicifolius* is known as Sahachara and used in Indian Ayurveda. *A. ilicifolius* is popularly used for its wound healing ability by the coastal inhabitants of West Bengal. The root is expectorant, and is used in coughs and asthma. The plant normally lives in areas of modest salinity, forming bush around mangrove palms. From very ancient time, the plant is applied in traditional systems of medicine, including traditional Indian medicine or Ayurveda and traditional Chinese medicine [1,2] in the intend of medicating various diseases. Different parts of the plant have been used to treat asthma, diabetes, dyspepsia, leprosy, hepatitis, paralysis, paralysis, snake bite, rheumatoid arthritis and are used as diuretics [3-5].

Phytochemical studies with the plant revealed the presence of lignans [6] and megastigmane glycosides [7]. Scientific reports suggest that the plant has antioxidant, anti-inflammatory, anti-leishmanial, osteoblastic, hepatoprotective, anticancer, antiulcer, analgesic and antimicrobial activities [1,4,5,8-10].

Drug resistance in human pathogenic microorganisms is a continuous process, which leads to search new antimicrobial drugs. These circumstances imposed scientists to search for new anti-microbial substances from various sources, including medicinal plants [1]. This study aimed to investigate anti-bacterial activity of ethanolic bark extract of *A. ilicifolius* against a number of human pathogenic bacterial strains along with the preliminary phytochemical screening.

2 Experimental Section

2.1 Collection and identification of plant material

The fresh stem bark of the *A. ilicifolius* was collected from the Adampur reserve forest, Kamalgonj, Moulvibazar, Bangladesh in the month of July, 2019 and was identified by the taxonomist at the Department of Botany, Jahangirnagar University, Bangladesh. A voucher specimen (Accession number-DACB: 48258) was deposited in the Bangladesh National Herbarium, Dhaka (Mirpur), Bangladesh.

2.2 Drying and grinding

The collected stem bark was separated from undesirable materials, washed with running tap water and shade-dried for one week. Then the plant materials were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until the test commenced.

2.3 Cold extraction (maceration)

The plant was extracted by the cold extraction or maceration method. About 250 g of powder was soaked in 900 mL of 99.9% ethanol in an amber color glass container for 9 days. Then the extract was concentrated by solvent evaporation at room tempearture and dried to a solid in an oven (temperature not exceeding 50 °C).

2.4 Phytochemical screening

In preliminary phytochemical screening test for secondary metabolites such as alkaloids [11,12], flavonoids [12,13], triterpenoids [14,15], tannins [16,17], carbohydrates [12,18], glycosides [13,19,20], phenolic compounds, amino acids and proteins [11], steroids [14], fixed oil and fats [18] were done.

2.5 Anti-bacterial sensitivity test

The agar diffusion assay was done according to Bonev *et al*. [21], while the m*inimum* inhibitory concentration test was done according to Andrews [22].

3 Results and Discussion

35.36 mg greenish color type of ethanolic crude extract was found. The yield value of the plant extract was 0.14. Phytochemicals can protect against disease and are the good sources of modern medicine [23]. In this study, the phytochemical screening report suggests that the crude extract possesses alkaloids, flavonoids, terpenoids, phenols, glycosides, steroids, tannins, carbohydrates, fixed oils and fats (**Table 1**). Generally, the secondary metabolites of medicinal plants are the promising source of many modern drugs [24]. Flavonoids and phenols are responsible for the antioxidant and hepatoprotective effects [23]. Babu *et al*. [4] also previously reported that *A. ilicifolius* has antioxidant and hepatoprotective activity.

Table 1. Phytochemical relevance of ethanolic stem bark extract of *A. ilicifolius*

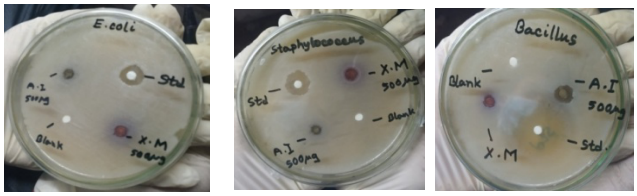
Phytochemical groups	**Tests**	**Relevance**
Alkaloids	Dragendroff's test	-
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Flavonoids	Alkaline reagent	++
	Shinoda's test	++
	Lead acetate test	+++
Terpenoids	Salkowaski test	++
Tannins	Ferric Chloride test	+++
	Lead sub acetate test	++
Carbohydrates	Molisch test	++
	Benedict's test	+++
	Fehling's test	+++
Glycosides	Legal test	++
	Keller-killinani	+++
	Borntrager's test	++
Phenols	Ferric chloride test	+++
Amino acids and proteins	Biuret test	-
	Million test	-
Steroids	Libermann-Burchard's test	++
Fixed oils and fats	Spot test	+++
Intensity: + = Trace; ++ = Moderate; +++ = Strong; - =Absence		

In a study, the alcoholic and chloroform extracts of the leaves of the plant were found to exhibit strong inhibitory activity against *B. subtilis, S. aureus, Candida albicans, Aspergillus fumigatus* and *Aspergillus niger, while m*oderate inhibitory action against *Pseudomonas aeruginosa* and *Proteus vulgaris* [25]. In another study, the chloroform extract showed maximum activity against the bacterial pathogens methicillin-resistant *S. aureus*, *Streptococcus pyogenes, Pseudomonas aeruginosa, C. albicans* and *Trichophyton rubrum*. Methanol and acetone extracts showed maximum activity against *S. epidermis* and *Lactobacillus plantarum,* respectively [26]. The plant is also evident to act against *Aspergillus fumigatus* [27]. In this study, the ethanolic stem bark extract produced highest zone of inhibition (14 mm) against *B. subtilis*, then followed by *B. megaterium*, *S. aureus*, *E. coli* and *S. entericus*, respectively. The crude extract of the plant showed lowest the MIC value (46.875 µg/mL) against *B. subtilis* and *B. megaterium*. It showed MIC value 187.5 µg/mL against *S. aureus*. Against other two strains, *E. coli* and *S. entericus*, the extract showed 750 µg/mL MIC value (**Table 2** & **Figure 1**).

Table 2. Anti-bacterial activity of ethanolic stem bark extract of *A. ilicifolius*

Bacteria	**ZI (mm)**		**MIC value**
	Azithromycin (30 µg/disc)	*Crude extract (500*	*Crude extract* (µg/mL)

		µg/disc)	
Bacillus megaterium	33	12	46.875
Bacillus subtilis	30	14	46.875
Escherichia coli	13	7	750
Staphylococcus aureus	15	9	187.5
Staphylococcus entericus	16	7	750
ZI: Zone of inhibition; MIC: Minimum inhibitory concentration			



E. coli *S. aureus* *B. megaterium*

Figure 1. Zone of inhibition of the plant extract against some test bacterial strains

4 Conclusions

The ethanolic crude extract of *A. ilicifolius* possesses many important secondary metabolites, including alkaloids, flavonoids, terpenoids, phenols, glycosides, steroids, and tannins. The extract showed moderate anti-bacterial activity against *B. subtilis* and *B. megaterium*. Scientific reports also suggest that various parts of the plant also possess broad-spectrum anti-microbial effect. Further research is required to isoate the active compounds responsible for its anti-microbial effects.

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Conflict of interest

None declared.

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