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# Preliminary Phytochemical Screening and Antimicrobial Potentials of Different Extracts of *Aegiceras corniculatam* L. and *Ceriops tagal* Pers

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**Abstract**

The study was aimed to analyze the phytochemicals and antimicrobial potentials of different fractions of *Aegiceras corniculatam* and *Ceriops tagal*, two mangrove plants of Bangladesh. Leaves of *A. corniculatam* and *C. tagal* were extracted using methanol by cold extraction method followed by partitioning with *n*-hexane, chloroform and the remaining part was considered as aqueous fraction. All fractions were screened for phytochemicals and subjected to Disc diffusion method to investigate antimicrobial activity against fourteen microorganisms including gram (+ve) (*Staphylococcus aureus, Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Sarcina lutea),* gram (-ve) (*Escherichia coli, Shigella dysenteriae, Vibrio mimicus, Vibrio parahaemolyticus, Shigella boydii, Pseudomonus aeruginosa)* and fungi (*Saccharomyces cerevaceae, Candida albicans, Asperagillus niger*).The chloroform and aqueous extract of *A. corniculatam* exhibited moderate antimicrobial activity against all studied pathogenic microorganisms where n-hexane extract did not show any inhibitory activity.

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The chloroform extract of *A. corniculatam* showed maximum zone of inhibition of 13 mm against *S. dysenteriae* and *S. cervaceae*. Furthermore, the aqueous extract of *A. corniculatam* showed maximum antibacterial activity with 10 mm zone of inhibition against *P. aeruginosa & S. dysenteriae.* However, n-hexane, chloroform and aqueous leaf extracts of *C. tagal* did not exhibit promising antimicrobial activity. Phytochemical screening of both plant extracts revealed the presence of carbohydrates, glycosides, tannins and resin. The chloroform and aqueous extract of *A. corniculatam* proved the presence of reducing sugar, cardiac glycoside, anthraquinone glycoside and saponin. Most extracts of *C. tagal* contained reducing sugar, saponins and alkaloids. The results of this study support the use of mangrove plants as a source of antibiotic substance against human pathogenic organisms.

***Keywords:*** *A. corniculatam*; *C. tagal*; Phytochemical.

## 1. Introduction

When bacteria grow influential, antibiotics no longer work, that can be a matter of life and death. Resistance to antibiotics, a natural part of the evolution of bacteria threatens our ability to treat common infectious diseases, resulting in prolonged illness increasing the risk of spread to others. For this greater innovation and investment are required in research and development of new antimicrobial medicines, vaccines and diagnostic tools. Now a day, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [1]. Mangrove plants are a significant part of folk medicine, traditionally used in skin disorders, sores, leprosy, angina, asthma, backache, boils, constipation, convulsions, diarrhea, dysentery, dyspepsia, elephantiasis, eye ailments, fever, fungal infections, headaches, hemorrhage, inflammation, jaundice, kidney stones, lesions, malaria, malignancies, rheumatism, snakebites, sore throat, syphilis, toothache, tuberculosis and also reported to be an astringent, emmenagogue, expectorant, hemostat, styptic and tonic [2]. Extracts from mangroves and mangrove-dependent species have been reported to possess antimicrobial ,but only limited investigations have been carried out to identify the metabolites responsible for their bioactivities3.The present study has been focused to verify and correlate the antimicrobial potential and presence of phytochemicals of *A. corniculatam* and *C. tagal* in view of the ethnopharmacological uses.

*A. corniculatam* (Family: Myrsinaceae) is an evergreen mangrove plant, distributed over the Sundarban, a UNESCO World Heritage Site, is the largest single block of tidal halophytic mangrove forest in the world, covering parts of Bangladesh's Khulna Division and the Indian state of West Bengal [4]. Traditionally different parts of this plant are used in the treatment of asthma, rheumatism and diabetes. Previous pharmacological studies have revealed it's various biological potentials as anti inflammatory, antimicrobial, antidiabatic, antioxidant, antinociceptive, cardio protective and hepatoprotective actions [5-11]. Many chemical constituents have been isolated from *A. corniculatum* so far, including lignin, tannin, saponins, flavonoids etc. [12,13]. Seven new compounds, namely, 2-methoxy-3-nonylresorcinol, 5-O-ethylembelin, 2-O-acetyl-5-O-methylembelin, 3,7-dihydroxy-2,5-diundecylnaphthoquinone, 2,7-dihydroxy-8-methoxy-3,6-diundecyldibenzofuran-1,4-dione, 2,8-dihydroxy-7-methoxy-3,9-diundecyldibenzofuran-1,4-dione, and 10-hydroxy-4-O-methyl-2,11-diundecylgomphilactone, were isolated together with three known compounds, 5-O-methylembelin, 3-

undecylresorcinol, and 2-dehydroxy-5-O-methylembelin from this plant [14].

*C. tagal* (Family: Rhizophoraceae) distributed in the Sundarban and brackish water areas near tidal lines in coastal area of Bangladesh. The bark of *C. tagal* is used for the treatment of infected wounds, obstetric, hemorrhagic conditions, sores, malignant ulcers, malaria [15-18] Phytochemical analysis on this plant reveals the presence diterpenoids of tagalsins, triterpenoids of lupeol, betulin, betulinic acid and cereotagalols with six new dolabranes, named tagalsins P-U (1-6), seven known dolabranes, an abietane, and a pimarane, one new diterpene, methoxy-ent-8(14)-pimarenely-15-one , and three known metabolites ent-8(14)-pimarene-15R,16-diol, stigmasterol and β-sitosterol [19,20]. Pharmacologically this plant has been proved to have antitumor, antiviral, antihyperglycaemic ,antibacterial potentials [21-24].

## 2. Materials and method

### *2.1 Collection & Drying*

The fresh leaves of *A. corniculatam* and *C. tagal* were collected from the mangrove forest Sundarban, Shyamnagar, Bangladesh in July, 2015 and identified by an expert taxonomist of National Herbarium. The leaves were washed with distilled water to remove soil debris and then cut into small pieces, sun dried for 7 days. After complete drying, the entire portions were pulverized into a coarse powder with the help of a grinding machine and were stored in an airtight container for further use.

### *2.2 Extraction and Partitioning of plant material*

The dried coarse 100gm of powder of each plant leaves were extracted with 3 times ethanol of their weight in a flat bottom glass container, through occasional shaking and stirring for 10 days. The extracts were then filtered through filter paper (Double Rings filter paper 102, 11.0 cm). The filtrates were concentrated at 50 $^{o}$C under reduced pressure using vacuum pump rotary evaporator (STUART RF3022C, UK) to afford a greenish mass. The concentrated methanol extract was made slurry with water in a separating funnel and few ml of n-hexane (40 ml) was added. The funnel was shaken vigorously and allowed to stand for few minutes; the n-hexane layer (upper layer) was collected. The process was repeated two times. The n-hexane extract was concentrated. After n-hexane extraction, chloroform (40 ml) was added to the aqueous extract and shaken vigorously in a separating funnel. The funnel was allowed to stand for few minutes for the complete separation of the layers and the upper layer was separated. Finally the lower part was separated as aqueous fraction. All the extractions were evaporated in dryer.

### *2.3 Test organisms*

Antimicrobial potantial was evaluated by using fourteen microorganisms including five Gram positive bacteria (*B. cereus, B. subtilis, B. megaterium ,S. lutea and S. aureus*), six Gram negative bacteria (*E. coli, P. aeruginosa, S. dysenteriae, S. boydii, V. parahaemolyticus, V. mimicus*) and three Fungi (*C. albicans, A. niger, S. cerevisiae*). The microorganisms were obtained from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The bacterial strains were first subcultured in nutrient agar (NA) medium & incubated at

37 ºC for 18 h while the fungal strains were subcultured for 72 h at 25 ºC [25]

### *2.4 Antimicrobial Assay*

The Antimicrobial property of the crude extract was determined by disc diffusion method [26]. In this method, solutions of known concentration (500 µg /disc) of the test samples were made by dissolving measured amount of the samples (50 mg) in 1 ml of solvents. Then sterile filter paper discs (5 mm diameters) were impregnated with known test substances and dried. Dried and sterilized filter paper discs were then impregnated with known amounts of the test substances using micropipette. Discs containing the test materials were placed on nutrient agar medium uniformly seeded with the pathogenic test microorganisms. Standard discs as Kanamycin 30µg/disc, Nystatin 30µg/disc and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4oC) for 24 hrs to allow maximum diffusion. The plates were then incubated at 37C for 24 hrs and 48 hrs at 27ºC for fungi to allow maximum growth of the organisms [27]. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

### *2.5 Preliminary Phytochemical Screening*

#### *A. Tests for Carbohydrates*

2 ml of an aqueous extract of both plants were added with 2 drops of freshly prepared 10% alcoholic solution of alpha-naphthol and mixed vigorously. Then 2 ml of concentrated $H_2SO_4$ which forms a layer beneath aqueous solution and a red or reddish violet ring was formed at the junction of the two layers indicates presence of carbohydrate [28].

#### *B. Tests for Reducing Sugars*

2 ml of an aqueous extract of both plants were mixed with Fehling A and Fehling B separately; a brown color with Fehling B and a green color with Fehling A indicate the presence of reducing sugars [29].

#### *C. Tests for Saponins*

0.1g of the powdered sample from both plants were boiled in 10ml of distilled water in a water bath and filtered. To the filtered sample (10ml), distilled water (5ml) was added, shaken vigorously and observed for a stable persistent frothing that indicates the presence of saponin [29].

#### *D. Tests for Tannins*

0.5 g of both plant extracts were dissolved in 10 ml of water and filtered. a few drops of the ferric chloride (0.01 Mol/dm3) were added which shows blue, blue-black, green or blue green color or precipitation in presence of tannin [29].

### *E. Tests for Resins*

A small quantity of the extract of both plants was dissolved in 10 ml of acetic anhydride by using gentle heat. Then it was cooled and 0.05 ml of $H_2SO_4$ was added which shows a bright purplish red color rapidly changing to violet in presence of resin [29].

### *F. Tests for Glycosides*

2 ml of both plant extract were dissolved in 1ml of the water and a few drops of aqueous solution of NaOH were added. The presence of glycosides was indicated by the formation of a yellow color.

For cardiac glycoside test, 0.1 g of the alcoholic extract of the plant was dissolved in 2 ml of pyridine and 2ml of sodium nitroprusside solution and NaOH added. A pink to red color was developed which indicates the presence of cardiac glycosides.

For anthraquinone glycoside, 0.5 g of the powdered plant was shaken with 5 ml of chloroform in a test tube. The filtrate was shaken with an equal volume of the 10% ammonia solution. A rose pink to red color indicated presence of anthraquinone glycosides [28, 29].

### *H. Tests for Alkaloids*

The presence of alkaloids in extracts was tested by using Wagner reagent prepared by dissolving 2 g of iodine and 6 g of potassium iodide in 100 ml of water. Two milliliters of Wagner reagents was added to 2 ml of extracts. The formation of reddish brown precipitates indicates the presence of alkaloids [30].

## 3. Results and Discussion

### *3.1 Antimicrobial screening*

The antibacterial activity of *A. corniculatam* and *C. tagal* were assayed *in vitro* by agar disc diffusion method against fourteen human pathogenic organisms in compare to Kanamycin disc (30 µg/disc) as standard. The zone of inhibitions of n-hexane, chloroform and aqueous extracts of both plants are demonstrated in **Table-1.**

The Chloroform leaf extracts of *A. corniculatam* showed mild to moderate antimicrobial activity with 8 -12 mm zone of inhibition against the gram positive whereas aqueous extracts showed mild activity against all gram positive bacteria but n- hexane extracts showed no effect. Gram negative *V. mimicus* and *S. dysenteriae* also showed moderate sensitivity to Chloroform leaf extracts of *A. corniculatam* forming 11 and 13mm zone of inhibition respectively. Aqueous extract of this plant also showed mild activity against all gram negative bacteria and fungi with 6 – 10mm zone of inhibition as n hexane extract showed no effect. The leaf extract of *C. tagal* was also carried out in n- hexane, chloroform and aqueous solvent media but there was no evidence to have any inhibitory action against different microorganisms. It is necessary to takeout significant study protocol to evaluate antimicrobial assessment of the plant in different concentrations.

**Table 1:** Antimicrobial response of various extracts of *A. corniculatam* and *C. tagal*

| **Organisms** | | **Zone of Inhibition (mm)** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ***A. corniculatam*** | | | ***C. tagal*** | | | |
| | | ***n*-hexane** | **Chloroform** | **Aqueous** | ***n*-hexane** | **Chloroform** | **Aqueous** | **Standard Kanamycin disc (30 µg/ml)** |
| | | **Gram positive bacteria** | | | | | | |
| | ***S. aureus*** | - | 12 | 08 | - | - | - | 41 |
| | ***S. lutea*** | - | 09 | 06 | - | - | - | 44 |
| | ***B. subtilis*** | - | 08 | 08 | - | - | - | 24 |
| | ***B. megaterium*** | - | 07 | 06 | - | - | - | 40 |
| | ***B. cereus*** | - | 08 | 07 | - | - | - | 42 |
| | **Gram negative bacteria** | | | | | | | |
| | ***V. parahemolyticus*** | - | 08 | 08 | - | - | - | 44 |
| | ***E. coli*** | - | 07 | 09 | - | - | - | 38 |
| | ***P. aeruginosa*** | - | 11 | 10 | - | - | - | 39 |
| | ***S. boydii*** | - | 10 | 07 | - | - | - | 39 |
| | ***V. mimicus*** | - | 11 | 08 | - | - | - | 39 |
| | ***S. dysenteriae*** | - | 13 | 10 | - | - | - | 33 |
| | **Fungi** | | | | | | | **Nystatin(30 µg/ml)** |
| | ***A. niger*** | - | 11 | 07 | - | - | - | 39 |
| | ***S. cervaceae*** | - | 13 | 07 | - | - | - | 41 |
| | ***C. albicans*** | - | 11 | 07 | - | - | - | 40 |

### *3.2 Phytochemical Constituents Analysis*

Phytochemical constituents are secondary metabolites of plants that serve as a defense mechanism against many microorganisms, insects and other herbivores [31]. Presence of different phytochemical constituents of both plants was investigated by carrying different test methods. **(Table 2).**

The present investigation on *A. corniculatam* has revealed the presence of carbohydrate and glycosides in all extract. Reducing sugar, cardiac glycoside, anthraquinone glycoside and saponin in most of the extracts. Resins are available in aqueous extracts only whereas no alkaloids and tannins have been detected in any extract. Similarly a qualitative phytochemical analysis was performed for *C. tagal* which confirmed the existence of carbohydrates, reducing sugar, glycosides in all extract. Saponin and alkaloid were traced in most of the extracts. Anthraquinone glycosides, cardiac glycoside and resins were detected in aqueous extract and tannin in

chloroform extract only.

**Table 2:** Phytochemical constituents of *A. corniculatam* and *C. tagal* in different extraction media.

| **Phytochemical Constituent** | ***A. corniculatam*** | | | ***C. tagal*** | | |
|---|---|---|---|---|---|---|
| | *n*-hexane | Chloroform | Aqueous | *n*-hexane | Chloroform | Aqueous |
| Carbohydrates | + | + | + | + | + | + |
| Reducing Sugar | - | + | + | + | + | + |
| Glycoside | + | + | + | + | + | + |
| Cardiac Glycoside | - | + | + | - | - | + |
| Anthraquinone Glycosides | - | + | + | - | - | + |
| Saponins | - | + | + | - | + | + |
| Tannins | - | - | - | - | + | - |
| Resins | - | - | + | - | - | + |
| Alkaloids | - | - | - | - | + | + |

## 4. Discussion

Chloroform and aqueous extracts of *A. corniculatam* exhibited mild to moderate antimicrobial activity against all studied pathogenic microorganisms where n-hexane extract did not show any inhibitory activity. The phytochemical analysis of *A. corniculatam* showed that the leaf is very rich in carbohydrate and glycosides, reducing sugar, cardiac glycoside, anthraquinone glycoside and saponin which could be responsible for the observed antibacterial property. The presence of phytochemicals is the proper evidence to regard *A. corniculatam* as a medicinal plant. This explains why the leaf of *A. corniculatam* is used by traditional practitioners to minimize glucose, prevent inflammation, prevent joint pain, reduce hypertension and ulcerative diseases. Alkaloids are stimulants and act by prolonging the action of several hormones [32]. Saponins are glycosides of both triterpenes and steroids that are characterized by their bitter or astringent taste, foaming property, haemolytic effect on red blood cells, cholesterol binding properties, expectorative, anti-inflammatory, immune-stimulating activities and antimicrobial properties particularly against fungi, bacteria and protozoa [33-35]. Flavonoids have antioxidant activity and have become popular in many health-promoting activities such as anti-allergic, anti-cancer, anti-inflammatory and antiviral effects.

*C.tagal* which is rich in carbohydrates, reducing sugar, glycosides in all extract where saponin and alkaloid were traced in most of the extracts. Aqueous extract contains anthraquinone glycosides, cardiac glycoside and resins wherein chloroform extract contains only tannin. Similar results have been reported by He and his colleagues (2005) that, chemical constituents and bioactive agents of *C. tagal* include flavonoid, alkaloid, cardiac glycoside, saponin and sterol [36]. These bioactive compounds are known to act by different mechanism and exert antibacterial action in varying sensitivity [37].

## 5. Conclusion

Many researches on plants as the source of drugs and dietary supplements has been asserted to develop new potent drugs derived for treatment of infectious diseases. Mangroves have been a source of interest for their novel natural products as they contain biologically active antiviral, antibacterial and antifungal compounds. The result presented in this study claimed the revelation of antibacterial activity of chloroform and aqueous extract of *A. corniculatam* plant. On the other hand, leaf extracts of *C. tagal* did not show any inhibitory activity against the studied pathogens. Both mangrove plants are rich in several types of bioactive principles that could be promising for further isolation, identification and purification.

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