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*Full Length Research Paper*

# Assessment of antibacterial and cytotoxic activity of some locally used medicinal plants in Sundarban mangrove forest region

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***Polyalthia longifolia*** **(Annonaceae),** ***Elaeocarpus serratus*** **(Elaeocarpaceae) and** ***Trachyspermum ammi*** **(Umbelliferae) are traditionally employed to cure various health ailments in Bangladesh. Extracts of these medicinal plants were tested for their potential antibacterial activity and cytotoxicity using the disc diffusion method and the brine shrimp lethality tests respectively. Ethanol extracts of the barks of** ***P. longifolia*****, leaves of** ***E. serratus*** **and seeds of** ***T. ammi*** **showed significant antibacterial activity against some pathogenic bacteria and moderate to mild lethality to the brine shrimps tested. This study provides some scientific bases for the use of this plant as a remedy for stomach, skin and bacterial infections in folkloric medicine whose causative agents are some of the pathogens studies. The activities observed could be attributed to the presence of some of the phytochemicals detected which have been associated with antibacterial activity and cytotoxic property.**

**Key words**: *Polyalthia longifolia*, *Elaeocarpus serratus*, *Trachyspermum ammi*, antibacterial activity, cytotoxic activity.

## INTRODUCTION

*Polyalthia longifolia* (Annonaceae) commonly called *Asopalav* (Gujarati), false ashoka, green champa, Indian mast tree, Indian fir tree, *glodogan tiang* (Indonesian) is a widely distributed tree in the Mediterranean region, west and central Asia, South Asia, South East Asia, Africa, South East America, Austrilia, India, China, Bangladesh and Myanmar ( Langeland KA et al., 2000). It is a high evergreen tree, narrow branching, about 25 - 60 ft high with long green leaves (7-15 cm in length) and round or oval shaped fruits (Langeland KA et al., 2000).

*Elaeocarpus serratus* (Elaeocarpaceae) is a medium to big sized tree with simple leaves, small flowers in axillaries and one seeded drupes for its edible fruits and timber. Commonly called Ceylon-olive *E. serratus* is widely distributed in the Chittagong region many other areas of Bangladesh, India, Srilanka, Pakistan, Thailand, and Madagascar. The plant is also found in East Africa as well as the subtropical and tropical Asia and tropical Australia (Ghani, 2003).

*Trachyspermum ammi* (Umbelliferae) commonly called ajwain is a glabrous annual plant. Its fruits is aromatic, stems is hollow, striated, much branched. Flower is white, sepals small, acute and with petals hairy beneath. Its upper leaves are smaller, similar or, simply pinnatisect (Kiritikar and Basu, 1999).

Traditionally, these plants are employed in the cure of various diseases. *P. longifolia* is used in the treatment of colitis, diarrhea, anorexia, skin diseases, sore throat, cough and colds (Ghani, 2003). *E. serratus* is used as diuretic and as a cardiovascular stimulant. The leaves are used in the treatment of rheumatism and as antidote to poison, while the fruits are locally prescribed for the treatment of diarrhea and dysentery. The fruit juice of *E. serratus* is given for stimulating secretions from taste buds thus increasing appetite in patients (Chopra, 1956). *T. ammi* is much used as a medicinal plant in Ayurvedic medicine (India). It is mainly used against diseases of the digestive tract and as feverish. In the Bagerhat, Sathkhira, Sharankhola, Khulna of Bangladesh it is used in the treatment of cough and throat irritation (Kritikar, 1998).

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**Table 1**. Phytochemical screening of ethanol extracts of *Trachyspermum ammi* (seeds) *Polialthia longifolia* (stem bark) and *Elaeocarpus serratus* (leaves).

| Ethanol Extract | Steroid | Alkaloid | Reducing sugar | Tannins | Gums | Glycoside | Flavonoids | Saponinis |
|---|---|---|---|---|---|---|---|---|
| Leaves of *Elaeocarpus serratus* | + | + | + | + | - | + | - | - |
| Barks of *Polialthia longifolia* | + | + | + | + | + | + | + | - |
| Seeds of *Trachyspermum ammi* | - | - | + | + | - | + | + | - |

(+) indicate presence (-) indicate absence.

## MATERIALS AND METHODS

### Collection and preparation of plant samples

*P. longifolia* (collected from Kapalia, Jessore), *E. serratus* (from Khulna) were collected in November 2005, while *T. ammi* (from Satkhera), collected in July, 2006 were all identified by the taxonomists of Bangladesh National Herbarium, Mirpur, Dhaka (accession numbers DACB-30217, DACB-31155 and DACB-31381 respectively) where a voucher specimens were also deposited. Barks of *P. longifolia*, leaves of *E. serratus* and seeds of *T. ammi* were separated from undesirable materials. They were air-dried for one month. The plant parts were then ground into a coarse powder with the help of a grinder.

For extraction purposes, barks of *P. longifolia* 300 gm dried barks take in 1000ml, 90% ethanol and stay 7 days, from their 9 gm extract was found (Yield= 3%), leaves of *E. serratus* 200 gm dried leaves, 700 ml, 90% ethanol and stay 7 days from their 7 gm extract was found (Yield = 3.5%) and seeds of *T. ammi* 500 gm dried seeds, 1000 ml 95% ethanol and stay it 19 days from their 9 gm extract was found (Yield = 1.8%).

### Screening of extracts for phytochemicals

The ethanol extract was screened for phytochemical constituents using standard procedures of analysis (Evans, 1989; Harborne, 1984; Ghani, 1998).

### Test of antibacterial activity

The plate-hole diffusion assay as described by Bauer et al . (1966) and Ahmed et al. (2001) was used to determine the growth inhibition of bacteria by the plant extracts. The following bacteria obtained from Microbiology Section, Square Pharmaceuticals Bangladesh Ltd. All bacteria were maintained at 40°C on nutrient agar plates before use.

The tests were carried out by using a stock concentration of 500 mg/ml prepared by dissolving 1 g of the ethanol extract into 2 ml of distilled water. Nutrient agar was prepared and 25 ml each was poured into sterile Petri dish. This was allowed to solidify and dry. Using a sterile cock-borer of 9 mm diameter three equidistant holes per plate were made in the set agar and were inoculated with 0.5 ml over night suspension of the bacteria. There after, the wells (holes) were filled with the extract solution at varying concentrations of 400 and 600 g/ml respectively. This was done in triplicate and the plates were incubated at 37°C for 18 h. The antibacterial activities were observed and measured using a transparent meter rule and recorded.

### Cytotoxic activity of the plant extracts

The brine shrimps used were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubated ion at about 29°C for 48 h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Five dose of plant extract (20, 40, 60, 80, 100, 120 and 140 µg/ml) in 5% DMSO and/or seawater was tested. Each extract preparation was dispensed into clean test tubes in 10 µl/ml for control; same procedure was followed except test samples. After marking the test tubes properly, ten living shrimps were added to each of the eighteen vials with the help of a Pasteur pipette (Meyer et al., 1982). The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving brine shrimps counted and recorded. From this, the percentage of mortality was calculated at each concentration.

## RESULTS AND DISCUSSION

Table 1 shows the results of Phytochemical screening of the ethanol extracts of leaves of *E. serratus*, barks of *P. longifolia* and the seeds of *T. ammi.* Results showed the presence of reducing sugars, tannins, steroids and glycosides. Ethanol extracts of leaves of *E. serratus* did not show the presence flavonoid and gums, where as the bark extracts of *P. longifolia* and seed extracts of *T. ammi* showed the presence of flavonois. Saponins were not found in any part of the plants studied. The presence of reducing sugar is a qualitative measure and is not much definitive, since plant extracts contain more or less sugar. The presence of glycosides (all the three plant extract) in the extracts is also indicative due to their medicinal importance, particularly glycoside could be used as cardiac stimulant and tannin may be hydrolyzed and subsequent propylene to obtain propyl gallate, which is a powerful antioxidant (Ann et al., 1998).

Result of the study also showed that, out of the three plants investigated, only one plant exhibited antibacterial activity against the 14 test bacteria investigated (Table 2). Barks of *P. longifolia* showed significant (21 - 32 and 30 - 44 mm in diameter zone of inhibition) antibacterial activity against all the test bacteria at 400 and 600 µg/ml, leaves of *E. serratus* show moderate activity (10 - 13 and 6 - 18 mm in diameter) against three test organisms with the least activity against except *Enterococci* at 600 µg/ml. The largest zone of inhibition (44 mm in diameter) was recorded against *Staphylococci epidermidis* with the barks of *P. longifolia* at 600 µg/ml. The antibacterial activity of the stem barks extracts of *P. longifolia* were comparable to those of the standard antibiotic gentamicin. Since the extracts from *P. longifolia, E. serratus*

**Table 2.** *In vitro* antibacterial activity of ethanol extracts of *Trachyspermum ammi Polialthia longifolia* and *Elaeocarpus serratus*.

| Bacterial strains | Diameter of zone of inhibition in mm | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gentamicin (30µg/ml) | Ethanol extract (400µg/ml) | | | Ethanol extract 600µg/ml) | | |
| | | Seeds of *Trachyspermum ammi* | Barks of *Polialthia longifolia* | Leaves of *Elaeocarpus serratus* | Seeds of *Trachyspermum ammi* | Barks of *Polialthia longifolia* | Leaves of *Elaeocarpus serratus* |
| *Escherichia coli* | 41 | - | 21 | - | - | 30 | - |
| *Plesiomonas* | 25 | - | 21 | 13 | - | 33 | 17 |
| *Shigella dysenteriae* | 35 | - | 24 | - | - | 27 | - |
| *Shigella flexneri* | 34 | - | 24 | - | - | 30.2 | - |
| *Shigella boydii* | 26 | - | 24 | - | - | 39 | - |
| *Shigella sonnei* | 34 | - | 26 | - | - | 40.4 | - |
| *Salmonella typhi* | 41 | - | 19 | 12 | - | 35.6 | 15 |
| *Pseudomonas spp* | 23 | - | 21 | - | - | 35 | - |
| *Enterococci* | 43 | - | 21.8 | - | - | 31 | 6 |
| *Staphylococci saprophyticus* | 25 | - | 26 | - | - | 38 | - |
| *Staphylococci aureus* | 24 | - | 22 | - | - | 36 | - |
| *Staphylococci pyogenes* | 21 | - | 25 | - | - | 39 | - |
| *Staphylococci epidermidis* | 35 | - | 32 | - | - | 44 | - |
| *Proteus spp* | 41 | - | 23.4 | 10 | - | 37.4 | 18 |

**Table 3.** Cytotoxic activity of plant extracts against brine shrimps.

| Conc. ( g/ml) | Log (Conc.) | % of mortality | | |
|---|---|---|---|---|
| | | Seeds of *Trachyspermum ammi* | Barks of *Polialthia longifolia* | Leaves of *Elaeocarpus serratus* |
| 10 | 1 | 35 | 20 | 5 |
| 20 | 1.30 | 40 | 50 | 25 |
| 40 | 1.60 | 55 | 65 | 50 |
| 80 | 1.90 | 100 | 100 | 85 |
| 160 | 2.30 | 100 | 100 | 100 |
| $LC_{50}$( g/ml) | | 35.48 | 20 | 40 |
| $LC_{90}$( g/ml) | | 66.83 | 70.8 | 100 |

and *T. ammi* showed activity against a wide range of bacterial strains, the plants could be useful as a nonspecific therapy for antibacterial infection. Moreover ethanol is medium polar solvent and it capable to extract both polar and non-polar chemical constituent. The results therefore support the traditional use of this plant as a remedy for diarrhoeal, dysentery and systemic shigellosis infections (Table 2).

Table 3 showed the results of investigation of cytotoxic activity and % mortality of brine shrimps studied. Results showed that the stem bark extracts of *P. longifolia*, leaf extracts of *E. serratus*, seed extracts of *T. ammi* showed very low to moderate level of general toxicity in the brine shrimps lethality assay ($LC_{50}$ = 20, 40, 35.48 µg/ml and $LC_{90}$ = 70.80, 100, 66.83 µg/ml) respectively. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal and antitumor activities (Anderson et al., 1988). Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds. However further study is necessary to find out the active principles responsible for these activities.

## Conclusion

The plant extracts demonstrated cytotoxic with antibacterial activity an indication of the possession of a wide range of pharmacological activities including anticancer, antiviral and pesticidal properties. The plants may therefore be a potential source for novel drug development. However, further studies are necessary to find out the active principles responsible for these activities.

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