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Antibacterial, Cytotoxicity and Antioxidant Activity of *Trema orientalis*

Palash Kumar Sarker[1], Khan Mohammad Ahsan Uddin[2], Mahmud Morshed Sagor[2], Sarder Nasir Uddin[2], Firoz Ahmed[3]

[1]National Institute of Biotechnology, Savar, Dhaka, Bangladesh, [2]Biotechnology & Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh, [3]Pharmacy Discipline, Khulna University, Khulna 9208, Bangladesh

Abstract

Methanol and aqueous extracts of leaf of *Trema orientalis* Linn were subjected to the phytochemical and pharmacological screening. The phytochemical study showed the presence of alkaloids, tannins and steroids in both methanol and aqueous extract. In addition, the methanolic extract contains reducing sugars, and aqueous extract contains gums, flavonoids and saponins. The pharmacological interest of these compounds, coupled with the use of this plant in traditional medicine prompted us to test *Trema orientalis* for cytotoxic, antibacterial and antioxidant activities. The extract showed mild to moderate antibacterial activity against both Gram-positive and Gram-negative bacteria. The methanolic extract also possesses potent anti-oxidant activity as compared with ascorbic acid, which was used as standard. The results also showed toxicity in the brine shrimp lethality bioassay with lethal concentration 50 (LC_{50}) of 120 µg/ml and (LC_{90}) of 200 µg/ml. The present study supports the use of this plant in traditional medicine and suggests its further investigation.

Key words: Antibacterial activity, antioxidant, cytotoxicity, lethal concentration 50% (LC_{50}), inhibitory concentration 50% (IC_{50}), phytochemical, *Trema orientalis*

Introduction

Nature is and will still serve as the man's primary source for the cure of his ailments. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs.

Trema orientalis (Bengali: Jibon or Chikon) is a small tree belongs to the family Ulmaceae. The plant, reaching 10-20 m tall, is widely distributed through a range of altitudes in higher rainfall areas. It is common along the margins of lowland and upland forests, extending into riverine forests and forest gaps. It is a pioneer species and is found in clearings and on abandoned farmland[1]. The plant is distributed in almost all districts of Bangladesh. It is also found in subtropical and tropical regions of southern Asia, northern Australasia, Africa and South and Central America. The leaves are alternate, simple, 7-15 cm long, ovate-acuminate to lanceolate with a long pointed tip. The leaf base is frequently unequal. Leaves taper from the base to the apex, and vary from 60 to 150 mm long and 25 to 50 mm wide. Leaf margins are finely serrated, and the young leaves are rough and hairy, occasionally becoming smooth when old. Plant leaves are widely used in traditional medicine by the rural people and possesses various interesting pharmacological activities[2].

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

Antioxidant substances block the action of free radicals, which have been implicated in the pathogenesis of many diseases

including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process[3-5]. The therapeutic effects of several plants and vegetables, which are used in traditional medicine, are usually attributed to their antioxidant compounds. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns[6-9].

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, pesticidal etc.[10]. The aim of the present study was to investigate the scientific basis of the traditional use of this plant. The traditional use of plant leads us to determine the antibacterial, antioxidant and cytotoxicity activity of methanolic and aqueous extract of leaves *of Trema orientalis* Linn.

Materials and Methods

Plant materials: Fresh leaves of *Trema orientalis* were collected from Khulna University Campus in Bangladesh. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 31,285) and a voucher specimen was also deposited there. The fresh leaves were cleaned, dried and pulverized. About 400 g of powdered material was taken in a clean, flat bottom glass container (4-l) and soaked in 1,300 ml of 80% of methanol. The container was sealed and kept for a period of 10 days with occasional shaking and stirring. Then it was filtered and concentrated by evaporation.

Screening for phytochemical components: The method described by Odebiyi and Sofowora[11] was used to test for the presence of the bioactive constituents of the plant material.

Organisms used for antibacterial activity test: Total 12 Gram-positive and Gram-negative bacterial isolates were included. They were collected from the Microbiology Laboratory of Pharmacy Discipline, Khulna University, Khulna. The bacterial isolates used were *Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Plesiomonas shigelloides, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Shigella flexneri, Shigella boydii, Shigella sonnei* and *Vibrio cholerae*. The organisms were maintained on nutrient agar slants at 2-8°C. Purity of the organisms was checked at regular intervals by plating and staining[12].

Determination of antibacterial activity: The screening for antibacterial activity was done as described by Akoma *et al.*[13]. Briefly, 1 ml of the test culture (1.0×10^7 cell/ml) was placed into a sterile plate and 19 ml molten agar (at 45°C) was poured. The contents were mixed thoroughly and allowed to solidify. Wells of approximately 6 mm in diameter and 2.5 mm deep were made on the surface of the agar medium using a sterile cork borer. The plate was turned upside down and the wells were labelled with a marker. The wells were filled with 10 µl methanol extract (50 µg/µl), 10 µl aqueous extract (50 µg/µl), 10 µl methanol and 6 µl gentamicin (5 µg/µl). Then, the plate was incubated at 37°C for 24 h and the zone of inhibition was measured with a pair of callipers and a millimetre ruler. The experiments were repeated twice.

Brine shrimp lethality bioassay (cytotoxicity): The brine shrimp lethality bioassay was carried out on standard procedure[14]. Briefly, brine shrimp eggs were hatched in a hatching chamber filled with fresh seawater. The chamber was kept under illumination using a fluorescent bulb for 48 h for the eggs to hatch into shrimp larvae (nauplii). Then these nauplii were taken for brine shrimp lethality bioassay. Crude methanol or aqueous extract (500 mg) was dissolved in dimethyl sulfoxide in distilled water to give 50 µg/µl solutions. Thirty clean test tubes were taken, 15 for the test samples of five concentrations and 15 others as control. Seawater (5 ml) was taken to each of the test tubes and then 10, 20, 40, 80 and 160 µl of stock solutions were transferred to the test tubes to obtain the final concentrations of 50, 100, 200, 400 and 800 µg/ml respectively of the test samples. Finally, with the help of a Pasteur pipette, 10 living shrimps were kept to each of the test tubes. After 24 h the test tubes were observed and the number of survived nauplii in each test tube was counted. The percentage of lethality of brine shrimp nauplii was calculated for each concentration.

Quantitative analysis of antioxidant activity: The anti-oxidant potential of the extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 µg/ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH. After 30 min, absorbance of each test tube was determined by UV spectrophotometer at 517 nm and IC_{50} (inhibitory concentration 50%) was determined from percent inhibition *vs.* concentration graph. Ascorbic acid was taken as standard. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals[15].

Results and Discussion

Based on traditional uses and pharmacological interests of *Trema orientalis* Linn, the present study was performed to evaluate the antibacterial, cytotoxicity and antioxidant activities to substantiate the claim about the plant to be used as traditional medicine. The methanolic and aqueous extracts were tested for the presence of different chemical groups, such as alkaloids, reducing sugars, tannins, steroids, flavonoids, gums and saponins. The methanolic extract showed the presence of alkaloids, reducing sugars, tannins and steroids. On the other hand, aqueous extract showed the presence of alkaloids, tannins, gums, flavonoids, saponins and steroids (Table 1).

Table 1: ***Presence of different chemical groups methanolic and aqueous extracts of Trema orientalis leaf***

Phytochemical	Methanol extract	Aqueous extract
Alkaloids	Present	Present
Reducing sugars	Present	Absent
Tannins	Present	Present
Gums	Absent	Present
Flavonoids	Absent	Present
Saponins	Absent	Present
Steroids	Present	Present

The effect of plant extracts on bacteria growth have been studied by a large number of workers world-wide[16-18]. Much work has been done on ethnomedicinal plants in India[19-21]. Interest in a large number of traditional natural products has increased[22]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumour and antimicrobial agents[23-24].

In this study, crude methanolic extract of *Trema orientalis* showed antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis, Plesiomonas shigelloides, Shigella dysenteriae* and *Vibrio cholerae* (Table 2). On the other hand, aqueous extract showed antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis, Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Plesiomonas shigelloides, Shigella dysenteriae, Vibrio cholerae, Shigella Flexner, Shigella sonnei* and *Pseudomonas aeruginosa*. Both extract did not show any activities against *Salmonella typhi* and *Shigella boydii.*

Table 2: ***Antibacterial activity of methanol and aqueous extract of leaves of Trema orientalis***

Test organism	Diameter zone of inhibition (mm)		
	Gentamicin (30 µg/well)	Methanol extract (500 µg/well)	Aqueous extract (500 µg/well)
Gram-positive organism			
Staphylococcus aureus	23	9	11
Staphylococcus epidermidis	21	9	11
Staphylococcus saprophyticus	32	0	13
Streptococcus pyogenes	21	0	10
Gram-negative organism			
Plesiomonas shigelloides	24	8	9
Shigella dysenteriae	24	9	10
Vibrio cholerae	28	9	9
Salmonella typh	31	0	0
Shigella flexneri	21	0	10
Shigella boydii	23	0	
Shigella sonnei	24	0	9
Pseudomonas aeruginosa	27	0	10

The antibacterial activity was measured in terms of diameter of zone of inhibition. Standard antibiotic disks of gentamicin were used as standard for comparison purpose. Both extract showed antibacterial activity against both Gram-positive and Gram-negative bacteria. Aqueous extract showed higher anti microbial activities than methanol extract. The methanol extract produced average diameter of zone of inhibition of 8-9 mm against both Gram-positive and Gram-negative bacteria, whereas the aqueous extract produced 10-13 mm diameter of zone of inhibition against Gram-positive bacteria and 9-10 mm against Gram-negative bacteria. The aqueous extract produced highest zone of inhibition (13 mm) against *Staphylococcus saprophyticus*. On the other hand, control gentamicin (30 µg/ml) showed an average 21-32 mm diameter of zone of inhibition against the test organisms.

The reputation of *T. orientalis* as a remedy for different microbial diseases traditionally including diarrhoea and dysentery was supported by the antibacterial screening tests. Inference can be drawn that the antibacterial constituents are present in the extract in moderate concentration. The antibacterial activity of the n-hexane, ethyl acetate and methanol extracts of the root of *Trema orientalis* had been investigated by disk diffusion method[25]. The ethyl acetate and methanol extract of the roots showed moderate activity against Gram-positive bacteria and fungus[26].

Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus, *in vivo* lethality of a simple zoological organism (brine shrimp nauplii) can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products[27]. In this bioassay, the results of brine shrimp lethality bioassay were summarized in Table 3 and 4. Methanolic extract of leaf of *T. orientalis* showed different mortality rate at different concentrations. The mortality rate of brine shrimp was increased with the increase in concentration of the sample. In toxicity evaluation of plant extracts by brine shrimp lethality bioassay, LC50 values lower than 1,000 µg/ml are considered bioactive[13]. The methanolic extract displayed toxicity, with LC_{50} <1,000 µg/ml. It showed strong cytotoxicity (LC_{50} = 120 µg/ml and LC_{90} = 200 µg/ml), while the aqueous extract did not show any cytotoxic effect on brine shrimp.

In recent years one of the areas that attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. Several plant extracts and different classes of phytochemical have been found to have quite prominent antioxidant activity[28-30].

In the present study, methanol extracts of the leaf of *T. orientalis* showed potential free-radical scavenging activity, while the aqueous extract showed very little free-radical scavenging activity.

The free radical scavenging property may be one of the mechanisms by which this drug is effective in traditional medicine. Most of the tannins and flavonoids are phenolic compounds and may be responsible for antioxidant properties of many plants[31]. Inhibitory concentration 50 (IC_{50}) of the methanol extract was 110.25 µg/ml (Fig. 1), which indicated a strong antioxidant activity of the plant extract. The aqueous extract showed mild antioxidant activity (Table 5).

Table 3: ***Effect of methanol extract of Trema orientalis on brine shrimp***

Methanol extract (80%) concentration (µg/ml)	Log (Concentration)	Average No. of alive shrimp (Sample)	Average No. of alive shrimp (Control)	Percent mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
0	0	10	10	0	120	200
50	1.7	8	10	20		
100	2	6	10	40		
200	2.3	1	10	90		
400	2.6	0	10	100		
800	2.9	0	10	100		

LC_{50} or LC_{90} = Lethal concentration 50% or 90% based on Brine shrimp lethality bioassay.

Table 4: ***Effect of aqueous extract of Trema orientalis on brine shrimp***

Aqueous extract concentration (µg/ml)	Log (Concentration)	Average No. of alive shrimp (Sample)	Average No. of alive shrimp (Control)	Percent mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
0	0	10	10	0	No	No
50	1.7	10	10	0	effect	effect
100	2	10	10	0		
200	2.3	10	10	0		
400	2.6	10	10	0		
800	2.9	10	10	0		

LC_{50} or LC_{90} = Lethal concentration 50% or 90% based on Brine shrimp lethality bioassay.

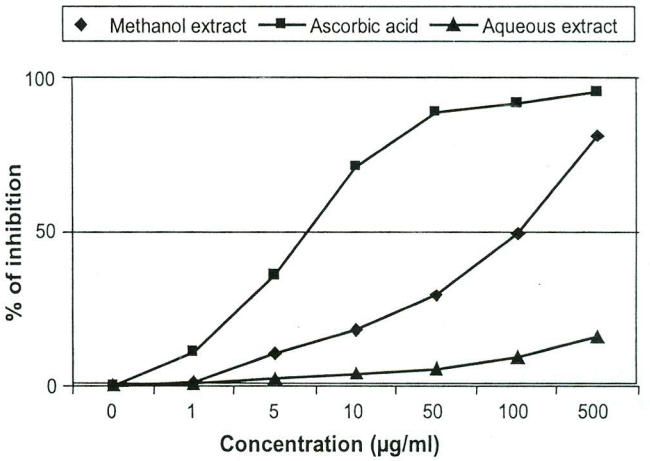

Fig. 1: *DPPH (1,1-diphenyl-2-picryl hydrazyl) scavenging assay of Trema orientalis compared with standard ascorbic acid.*

Table 5: ***Antioxidant activity of Trema orientalis***

Sample	Concentration (µg/ml)	Average absorbance at 517 nm	Percent inhibition	IC_{50} (µg/ml)
Methanol extract	0	0.978	0	110.25
	1	0.964	1.32	
	5	0.875	10.53	
	10	0.801	18.08	
	50	0.690	29.44	
	100	0.497	49.18	
	500	0.184	81.18	
Aqueous extract	0	0.978	0	Not
	1	0.971	0.71	applicable
	5	0.955	2.35	
	10	0.939	3.98	
	50	0.923	5.62	
	100	0.888	9.20	
	500	0.827	15.43	
Ascorbic acid	0	0.978	0	7.08
	1	0.872	10.83	
	5	0.631	35.48	
	10	0.286	70.75	
	50	0.110	88.87	
	100	0.084	91.41	
	500	0.048	95.19	

IC_{50} = Inhibitory concentration 50%.

Conclusion

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant products to produce drugs and medicines. This huge foreign exchange can be saved if the manufacturers utilize the indigenous medicinal plants or their semi-process products to satisfy their needs. From the above experiment, it could be suggested that both aqueous and methanol extract of leaves of *Trema orientalis* posses phytochemical, antibacterial antioxidant and cytotoxic activity. So, further pharmacological and toxicological study is required to establish the therapeutic uses of the plant.

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Correspondence: *Palash Kumar Sarker*
Scientist
Fermentation & Bioprocess Division
National Institute of Biotechnology
Savar, Dhaka, Bangladesh
Tel (Office): (02) 7701458
Cell: 01718 700078
E-mail: palashnib@yahoo.com