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Antioxidant and Antibacterial Activities of *Trema cannabina*

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Abstract: Methanol and aqueous extract of leaves of *Trema cannabina* Linn. were subjected to the potential antioxidant and antibacterial activities. The pharmacological interest of this plant coupled with traditional use (antidiarrhoeal, antiseptic, analgesic etc) prompted to test for antioxidant and antibacterial activities. The antioxidant potential of the methanolic extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. IC_{50} of the methanol extract of *T. cannabina* was 110.25 µg/ml which indicated the strong antioxidant activity of the plant. However the aqueous extract showed mild antioxidant activity. In case of antibacterial activities test, the extract was subjected for its effectiveness against both Gram-positive and Gram-negative bacteria in agar diffusion method. The zones of inhibition produced by the crude methanol and aqueous extract against few sensitive strains were measured and compared with those of standard antibiotic Gentamycin. It is evident that both extracts are active against the bacteria at low concentrations. The obtained results provide a support for the use of this plant in traditional medicine and suggest its further advance investigation.

Key words: Antioxidant · Antibacterial · *Trema cannabina* · Activities · Extract

INTRODUCTION

Bangladesh possesses rich floristic wealth and diversified genetic resources of medicinal plants. It has a widely ranging tropical and the agro climatic conditions, which are conducive for introducing and domesticating new and exotic plant varieties. The use of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds. *Trema cannabina* is one of the common medicinal plant grown in Indian subcontinent. Different parts of this plant have been used in traditional medicine.

T. cannabina is a tree and belongs to the Cannabaceae family. The plant is distributed in almost all districts of Bangladesh and is used in traditional medicine by the rural people and possesses various interesting pharmacological activities [1]. The root of the plant is used in the treatment of diarrhoea, asthma and passing of blood in urine; the bark is used as poultice in muscular pain; the roots, barks and leaves are used in epilepsy [1, 2]. In African folk medicine, it is used in many diseases including dysentery, hypertension, etc [3] Fruit, leaves, bark, stems, twigs and seeds are also used in traditional medicine. The leaves are used to treat coughs and sore throats and the bark is used to make cough syrups. Other reported uses include remedies for bronchitis, gonorrhoea, malaria, yellow fever, toothaches and intestinal worms [1, 4].

Free radicals are metastable chemical species which, after being generated in vivo as by products of various biochemical reactions, tend to rob electrons from the molecules in the immediate surrounding in order to replace their own losses. These radicals may be envisaged as molecular sharks, which if not scavenged effectively on time, are capable of damaging crucial bio-molecules including those present in cell membranes, mitochondria, DNA etc. and thus predisposing various pathophysiological states. The role of free radicals, especially of the so called 'reactive oxygen species' (ROS), has been well-established in the pathogenesis of many disease conditions such as rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, some metabolic disorders, neurodegenerative diseases (e.g. Parkinsonism, Alzheimer's disease), gastrointestinal ulcerogenesis, AIDS and even early senescence [5, 6]. ROS is a collective term, which includes not only the oxygen radicals ($O_{2^{\cdot-}}$ and $^{\cdot}OH$) but also some

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non-radical derivatives of oxygen. These include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3).

In recent years one of the areas which 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 phytochemicals have been found to have quite prominent antioxidant activity [7-10]. Methanol extract of Senna tora possess strong antioxidant activity [11]. The objective of the present study was to investigate the antioxidant and antimicrobial activity of the crude extract of *Trema cannabina.*

During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs [12].

MATERIALS AND METHODS

Plant Materials: Fresh leaves of *T. cannabina* were collected from Khulna University Campus in Bangladesh. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka 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 bottomed glass container (4 L) and soaked in 1300 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.

Determination of Antioxidant Activities: The anti-oxidant potential of the ethanolic 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 was added to 3 ml of a 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min and IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals [13].

At first 6 test tubes were taken to make aliquots of 6 conc. (1, 5, 10, 50, 100 and 500 µg/ml). Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentration by dilution technique. Here ascorbic acid was taken as standard. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used. After making the desired concentrations 3 ml of 0.004% DPPH solution was applied on each test tube by pipette. The room temperature was recorded and kept the test tubes for 30 mins in light to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. After 30 mins, absorbance of each test tubes were determined by UV spectrophotometer. IC_{50} was determined from % inhibition vs concentration graph.

Determination Antibacterial Activities: Nutrient agar media was prepared by adding water to a dehydrated product that contains all the ingredients. Practically all media are available commercially in powdered form [14].

Three Types of Discs Were Prepared for Antibacterial Screening: One gram sample extracts was dissolved in 10 ml of ethanol to prepare sample solution, 0.03 gm/10ml gentamicin standard disc used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by known antibacterial agent with that produced by test samples and third one was a blank sample (only ethanol) which was used as negative control to ensure that the residual solvents was not active. Specific organisms were inoculated into previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile petri dish in an aseptic condition. It was stored in an incubator for about 24 hours to allow the proper growth of microbes. Prepared sample solutions were applied to the corresponding cups or holes with the help of a micropipette. The plates were then allowed to stand to diffuse the sample solution into the antibiotic medium at room temperature for 2 hours. The plates were then incubated at 37°C for overnight. After proper incubation, clear zones of inhibition around the point of application of sample solution were formed. These inhibition zones were measured by slide calipers and expressed in millimeter [15] .

RESULTS

For Antioxidant Activities Test: In the present study, methanol extracts of the leaves of *T. cannabina* showed potential free-radical scavenging activity but aqueous extract showed very little free-radical scavenging activity.

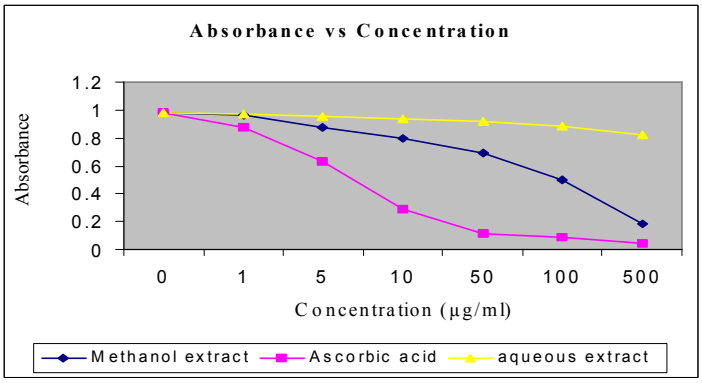

Fig. 1: DPPH Scavenging Assay of *Trema cannabina* compared with Standard ascorbic acid (absorbance vs concentration)

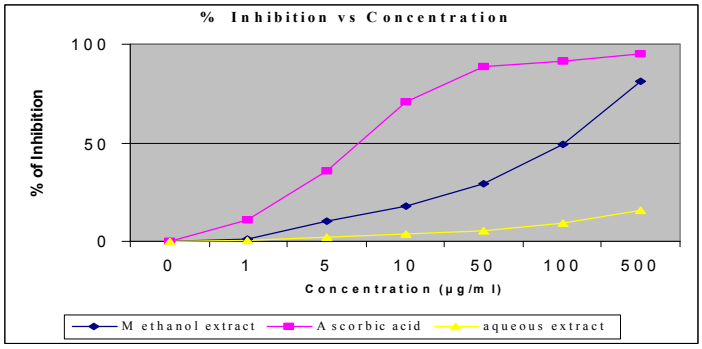

Fig. 2: DPPH Scavenging Assay of *Trema cannabina* compared with standard ascorbic acid (% of inhibition vs concentration)

Table 1: Antibacterial activity of methanol and aqueous extract of leaves of *Trema cannabina*

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

'-' No inhibition

IC_{50} of the methanol extract of *T. cannabina* was 110.25 µg/ml which indicated the strong antioxidant activity of the plant extract. However the aqueous extract showed mild antioxidant activity. DPPH Scavenging Assay of *Trema cannabina* compared with Standard ascorbic acid absorbance vs concentrations are shown in Fig. 1 & % of inhibition vs concentration are shown in Fig. 2.

For Antibacterial Activities Test: The result of the antibacterial activity measured in term of diameter of zone of inhibition in mm. Standard antibiotic discs of Gentamycin was used as standard 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. Inference can be drawn that the antibacterial constituents are present in the extract in moderate concentration. Antibacterial activity of methanol and aqueous extract of leaves of *Trema cannabina* are shown in Table 1.

DISCUSSION

DPPH is the best, easiest and widely used method for for testing preliminary free radical scavenging activity of a compound or a plant extract. In present study, methanol extracts of the leaves of *T. cannabina* possess strong antioxidant activity. However the aqueous extract showed mild antioxidant activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective as a traditional medicine. Most of the tannins and flavonoids are phenolic compounds and may be responsible for antioxidant properties of many plants [7]. So, this activity may be due to the presence of phenolic compounds (tannins and flavonoids) present in the extract [16].

Crude methanol extract of *Trema cannabina* showed antibacterial activity against *Staphylococcus aureus*, Staphylococcus epidermidis, Plesiomonas shigelloides, *Shigella dysenteriae* and *Vibrio cholerae* 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 extracts did not show any activities against Salmonella typhi and Shigella boydii. In fact, both methanol and aqueous extract of *Trema cannabina* show significant antibacterial activity against few gram positive and gram negative bacterial strains. The reputation of *T. cannabina* as a remedy for different microbial diseases traditionally including diarrhoea and dysentery was supported by the antibacterial screening tests.

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 indigenous medicinal plants or their semi-processed products are utilized by the manufacturers to satisfy their needs. So, further pharmacological and toxicological study is required to establish the therapeutic uses of the plant and particularly with its active principles.

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