Antibacterial, Antioxidant and Cytotoxic activities of Trewia nudiflora

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ABSTRACT

The aim of the study is to verify the traditional uses of different parts of *Trewia nudiflora.* Leaves, fruits, twigs & seeds of *T. nudiflora* were extracted with ethanol to evaluate antibacterial, antioxidant and cytotoxic activities. Antibacterial activity of the ethanolic extracts of different parts of *T. nudiflora* was revealed by disc diffusion method using kanamycin as standard. The leaf extract showed very good activity against *Shigella dysenteriae* with 37.5 mm zone of inhibition and moderate activity against *Pseudomonas aeroginosa* with 16.5 mm. The fruit extract also showed very good activity against *Shigella boydii with 22.5* mm zone of inhibition. The twig extract also showed very good activity with 20mm zone of inhibition against P*seudomonas aeroginosa* and the seed extract showed moderate antibacterial activity. The antioxidant activity of the extracts was determined by using DPPH spectrophotometrically and ascorbic acid was used as standard. Among all the parts, the twig extract showed highest antioxidant activity having an IC_{50} value of 35.51µg/ml. The leaf, twig and fruit extracts showed significant cytotoxicity with LC_{50} value of 9.17 µg/ml, 10 µg/ml and 10.53 µg/ml, respectively evaluated by brine shrimp lethality bioassay using vincristine sulfate as standard.

Key words: *Trewia nudiflora,* Antibacterial, Antioxidant and Cytotoxic Activity.

INTRODUCTION

Trewia nudiflora (Family: Euphorbiaceae) is tall arbor and distributes in the tropical districts of India, Malaysia and China. All parts of this plant are used as traditional medicine in India for the removal of bile and phlegm. The leaves of *T. nudiflora* are used for various diseases including blood and neuronal disorders. Leaves and its decoction are also applied to swellings and in healing of wounds and injuries. Bark is used for the treatment of enlarged thyroid. Decoction of the root is stomachic and alterative and used in flatulence, gout and rheumatism [1]. Apart from the uses in traditional medicine, It was reported in various published articles that *T. nudiflora* to possesses various biologically active chemical constituents. Ethanol extract of the leaves of *T. nudiflora* showed cerebroprotective effects, hyper locomotion and neuronal damage[2]. Some maytansinoids such as trewiasine and treflorine isolated from the *T. nudiflora* seeds are tumor inhibitors and may be responsible for the resistance of the seeds to fungal degradation[3,4]. (+)-Dihydrodehydrodiconiferyl alcohol 4-O-β-(6''-O-galloyl)-glucopyranoside; 4,4'-O-dimethylellagic acid 3-(2''-acetyl)- α-rhamnopyranoside and ethyl -β - (6'-galloyl)-glucopyranoside were isolated from the stem bark of *T. nudiflora* having significant antioxidant activity[5] . Four new lignans were isolated from the seed endotheliums of the plant with antimicrobial activity[6] .Two new cardenolides as trewianin and trewioside were also isolated from the stem bark[7] .

MATERIALS AND METHODS

Drugs and chemicals

DPPH (1, 1-diphenyl - 2-picryl hydrazyl) was obtained from Sigma Aldrich USA. Ascorbic acid was obtained from SD Fine Chem. Ltd, Biosar, India. DMSO (dimethylsulfoxide) was purchased from Merck, Germany. Kanamycin was collected from Square Pharmaceuticals Ltd., Bangladesh. Vincristine sulfate was collected from Alfa Asear Ltd. USA.

Instrumentation

The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts using UV-

spectrophotometer (Model NO. 1501PC Shimadzu, Japan) at 517 nm.

Collection and identification of the plant

The fresh leaves, seeds, twigs and fruits of *T. nudiflora* were collected in June 2013 from Brahmanbaria district, Bangladesh and authenticated at Bangladesh National Herbarium, where a voucher specimen No. DACB 35852 has been deposited.

Extraction of the plant material

The collected fresh leaves, seeds, twigs and fruits were sun dried for seven to twelve days. The dried plant parts were ground into small powder by a grinder machine. Then 50 gm of powder of leaves, fruits, seeds and twigs were extracted separately by cold extraction process using ethanol (300 ml) with daily shaking and stirring for 7 days at room temperature. After 7 days the extracts were filtered through cotton followed by filter paper (Double filter paper 102, 11.0 cm). Then the liquid extracts were dried at room temperature (37 °C) to obtain a greenish mass.

Microbial strains and culture media

Antimicrobial activity was carried out against seven Gram negative bacteria such as Vibrio parahemolyticus, Vibrio mimicus, Shigella dysenteriae, Pseudomonas aeruginosa, Shigella boydii, Escherichia coli, Salmonella paratyphi and five Gram positive bacteria such as Staphylococcus aureus, Sarcina lutea, Bacillus megaterium, Bacillus cereus and Bacillus subtilis,. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. For bacteria, the culture media was prepared by nutrient agar, reconstituting with distilled water according to specification (2.8% w/v).

Antibacterial screening by disc diffusion method

Antibacterial activities of various extracts of *T. nudiflora* were carried out *in vitro* by the standard disc diffusion method [8]. 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 methanol. Then sterile filter paper discs (5 mm diameters) were impregnated with known test substances and dried. The dried discs were placed on plates (Petri dishes, 120 mm diameters) containing a suitable medium (nutrient agar) seeded with the test organisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control. These plates were kept at low temperature (4 ºC) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37 º C) for 24 hours to allow the growth of microorganisms. Antibacterial activity of the test samples was observed by growth inhibition of organisms forming clear, distinct zone surrounding the discs. The antibacterial activity was expressed in terms of millimeter by measuring the diameter of the zone of inhibition. The greater zone of inhibition indicates the greater activity of the test material against the test organism.

Cytotoxicity screening by brine shrimp lethality bioassay:

The brine shrimp lethality bioassay was used to predict the cytotoxic activity [9,10] of the various extracts of *T. nudiflora*. The eggs of brine shrimp (*Artemia salina*) were hatched in a tank in artificial seawater (3.8% NaCl solution) at a temperature around 37 ^{0} C with constant air supply. For the experiment, the samples are prepared by dissolving the extracts in dimethylsulfoxide (DMSO) not more than 50 µl in 5 ml solution and solutions of varying concentrations (20, 40, 60, 80 and 100 µg/ ml) were prepared by the serial dilution process using simulated seawater and a vial containing 50 µl DMSO diluted to 5ml was used as a control. Then 10 live brine shrimp nauplii were added to each of the experimental vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. Vincristine sulfate was used as positive control. From this data, the percent of lethality of the brine shrimp nauplii for each concentration and control was calculated.

Antioxidant activity by DPPH radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the plant extracts on the stable radical

1,1-diphenyl-2- picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams [11]. During this experiment the test samples of the extracts of *T. nudiflora* at different concentrations were mixed with 3.0 ml of DPPH methanol solution. The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts by UV-spectrophotometer at 517 nm. Ascorbic acid was used as a positive control. Percent scavenging of the DPPH free radical was measured using the following equation-

% DPPH radical scavenging = [1 – (As/Ac)] × 100

Here, Ac = absorbance of control, As = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated. The lower IC_{50} indicates higher radical scavenging activity and vice versa.

RESULTS AND DISCUSSION

The ethanolic extracts of different parts of *T. nudiflora* were screened against Gram positive and Gram negative bacteria. Most organisms were found to be sensitive to the extracts which were shown in (Table 1).

Among the all extracts leaf extract showed very good antibacterial activity against *S. dysenteriae* with 37.5 zone of inhibition. *P. aeruginosa* and *V. mimicus* were also sensitive to the leaf extract with zone of inhibition of 16.5 mm and 11.5 mm, respectively. The fruit extract showed poor to moderate activity against gram positive bacteria with average zone of inhibition of 5-12 mm and maximum zone of inhibition against Gram negative *S. boydii* with 22.5 mm. The twig extract showed good activity against *P. aeruginosa* (20 mm), *E. coli* (12 mm). Seeds of the plant showed mild to moderate antibacterial activity.

According to the procedure of Meyer, the lethality of the extracts of *T. nudiflora* to brine shrimp was determined and the results (% mortality at different concentrations and LC_{50} values) were shown in Fig. 1. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality [12] was plotted on the graph paper and the values of LC_{50} were calculated using Microsoft Excel 2003. The percent mortality increased with an increase in concentration. Leaves, twigs, fruits and seeds extracts showed significant cytotoxic activity with LC_{50} values at 9.17 µg/ml, 10 µg/ml, 10.53 µg/ml and 18.81 µg/ml, respectively in comparison with standard vincristine sulfate (LC_{50} at 6.11 µg/ml).

The antioxidant activity of the different extracts of *T. nudiflora* was evaluated by using DPPH. The ethanolic extracts of leaves, fruit, seed, twigs showed antioxidant activity with the IC_{50} value of 67.23 µg/ml, 49.29 µg/ml, 39.93 µg/ml and 35.51 µg/ml respectively compared with the standard ascorbic acid at 45.78 µg/ml.

All the tested parts of *T. nudiflora* showed significant antibacterial, antioxidant and cytotoxic activities. Leaf extract possesses potential antibacterial activity and cytotoxicity.

CONCLUSION

The various traditional uses, phytochemical investigations and pharmacological studies of the plant correlate well with our findings. Chemical and biological studies on the plant have established many important active constituents which are already investigated for the treatment cancer and tumor. The results of this preliminary evaluation give evidence that the different parts of *T. nudiflora* can be regarded as promising resources for antibacterial, antioxidant and cytotoxic drugs. It seems that further investigations are necessary in order to isolate the active constituents to evaluate the cytotoxic activity in human cell line.

Table 1: Antibacterial Screening of different parts of T. nudiflora.

Group	Test Organisms	Diameter of Zone of Inhibition (mm)				
		Leaves 500µg/ disc	Fruits 500µg/ disc	Twigs 500µg/ disc	Seeds 500µg/ disc	Kanamycin 30µg/ disc
Gram positive bacteria	*B. subtilis*	8.5	10	13	10	34
	S. lutea	9	5	10	9	29
	B. cereus	9	9	13	5	28
	S. aureus	9.5	8.5	6	11	32

	B. megaterium	9	5	11	-	31.2
Gram negative Bacteria	*S. paratyphi*	-	7	-	-	30
	V. parahaemplyticus	10	6	10	9	35
	V. mimicus	11.5	6	9	-	36
	S. dysenteriae	37.5	12	12	6	34
	E. coli	-	-	8	14	35
	P. aeruginosa	16.5	9	13	20	28
	S. boydii	10	22.5	11	-	26.7

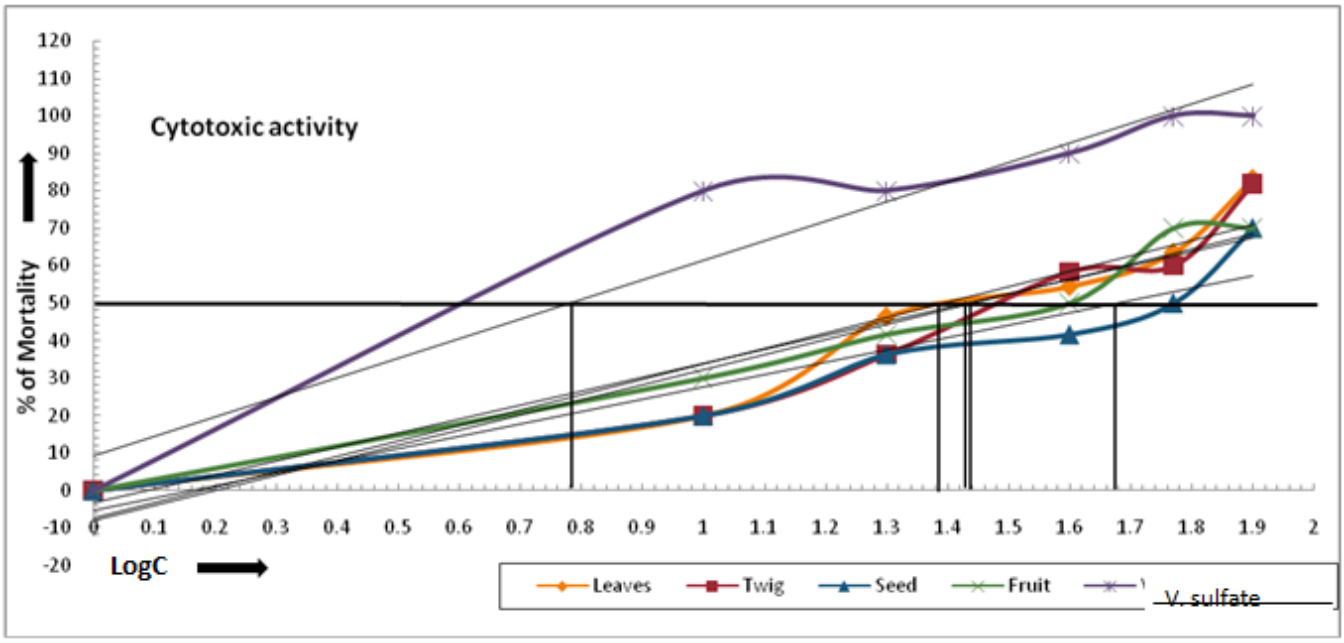

Figure 1: Determination of LC_{50} values for standard and ethanolic extracts of leaves, fruits, twigs and seeds of *Trewia nudiflora* from linear correlation between logarithms of concentration versus percentage of mortality.

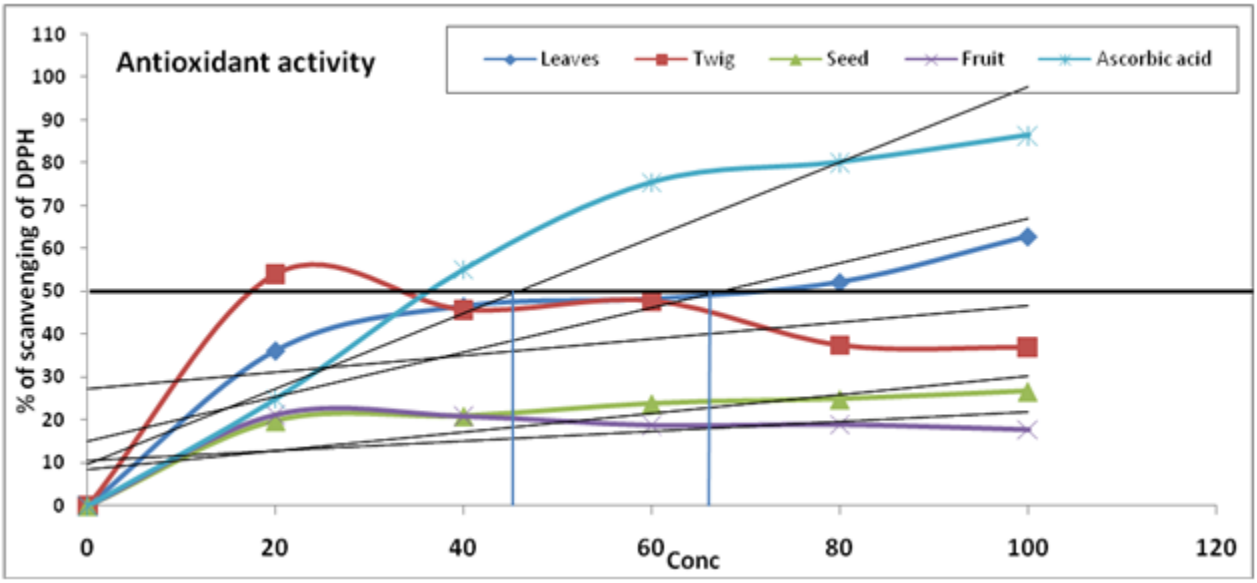

Figure 2: Determination of IC_{50} values for standard and ethanolic extract of leaves, fruits, twigs and seeds of *Trewia nudiflora* from linear correlation between of concentration versus percentage of scavenging of DPPH

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