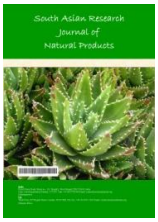

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Antibacterial Efficacy of the Methanol Extract of Fruits of *Trewia nudiflora* Linn. (*Euphorbiaceae*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author ASC designed the study, wrote the protocol and the first draft of the manuscript. Authors ASC, IJKC and SRS did the experimental works of the study. Author M. F. Hasan managed the literature searches. Author M. F. Haque generated the graph from data, edited the manuscript and finalized it. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Trewia nudiflora* Linn. (Euphorbiaceae) which is a soft wooded tropical dioecious tree is used as traditional medicinal tree in many countries. Hence, this study was designed to assess the antibacterial activity of fruit extract of *Trewia nudiflora* against twelve pathogenic bacteria.
Methodology: Twelve pathogenic bacterial strains already available in the lab were used in this study. The four of the collected bacteria were Gram positive *viz. Staphylococcus gallinarum*, *S. sciuri, Streptococcus iniae* and *S. constellatus* and remaining eight were Gram negative *viz. Xanthomonas axonopodies, X. campestris, Edwardsiella anguillarum, Siccibacter colletis, Aeromonas cavernicala, A. diversa, Vibro rotiferianus* and *Enterobacter xiangfangensis*. Antibacterial efficacy of the methanol extract of fruits of *Trewia nudiflora* was evaluated by disc diffusion test and minimum inhibitory concentration (MIC) test.
Results: The result of disc diffusion test showed the significant antibacterial efficacy as indicated by the generation of zone of inhibition ranging from 15-20 mm around the disc soaked with fruit

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extract (15 µl/disc) against tested bacteria. The largest zone of inhibition (20 mm) was produced against *E. xiangfangensis, A. cavernicala* and *V. rotiferianus* while the smallest zone (15 mm) was produced against *X. axonopodies, X. campestris, S. iniae* and *S. constellatus*. The MIC value for fruit extract of *Trewia nudiflora* varied with bacterial species ranging from 100-200 µg/ml.
Conclusion: Therefore, it can be concluded that methanol extracts of the fruits of *Trewia nudiflora* may be a good agent for further research to confirm its use as a natural antibacterial compound against pathogenic bacteria in economic animals and human.

Keywords: Trewia nudiflora; fruit; methanol extract; bacteria; antimicrobial.

1. INTRODUCTION

Trewia nudiflora Linn. (Euphorbiaceae) is a rapidly growing, soft wooded versatile dioecious tree which grows within the semi-evergreen and moist tropical forests as well as different non-forest areas of tropical districts of Bangladesh, India, Malaysia and China. *T. nudiflora* is a branchless tree and leaves long pointed, reverse, hairy underneath when young, ovate 11-20 cm by 7-12 cm, stalks 2-7.5 cm long. Male and female flowers appear on separate trees, males are yellow in long lax drooping inflorescences while females are green, solitary or 2-3 together in the leaf Axis [1]. Fruits are fleshy, grayish green, depressed globose and with diameter 3.5 cm by 3 cm [1]. Natural restoration of *T. nudiflora* is relatively common in the moist and coarse textured soil [2]. The seedlings of the species are usually sensitive to drought and get killed at the exposed upland sites. The light and moisture regimes are the common factors which affects the relative abundance and distribution of male and female individuals [2]. In a study, antifeedant effect of ethanol extract of seed of *T. nudiflora* was evaluated against several insects which were economically important [3]. It was reported that this plant extract acts as an antifeedant for two of tested insect *viz*. the European corn borer and the spotted cucumber beetle, but not effective for the other tested insects [3]. In addition, the toxic effect of the extract was observed for the striped cucumber beetle and gave 100% control of the chicken body louse from 5 to 28 days [3]. All parts of *T. nudiflora* were used as traditional medicine in India for the removal of bile and phlegm. The leaves of *T. nudiflora* were used for treatment of several diseases including neuronal and blood disorders [4]. Bark of that tree is used for preparation of traditional medicine for the treatment of enlarged thyroid. Decoction of the root is good for increasing appetite and digestion as well as used in rheumatism, flatulence and gout [5]. Ethanol leaves extract of *T. nudiflora* showed cerebro-protective effects, such as protection from neuronal damage and hyper locomotion [1]. In addition to uses the parts of *T. nudiflora* as traditional medicine, it might be a good sources of biologically active chemical constituents. For example, the fruit extract of *T. nudiflora* also showed very good activity against *Shigella boydii* with 22.5 mm zone of inhibition [4]. Moreover, fruit extracts of other plants such as *Pandanus tectorius* [6], *Vaccinium macrocarponc* [7], *Prunus domestica* [8], *Eugenia uniflora* [9] and *Prunus cerasifera* [10] were reported for potential antibacterial efficacy against different pathogens indicating that fruit extract of *T. nudiflora* could be potential source of novel antibacterial compounds against different pathogens of economic animals and human. But, information on efficacy of fruit extract of *T. nudiflora* is scarce especially against large number of pathogenic bacteria. On the other hand, emergence of multidrug resistant pathogens is increasing gradually which is one of major threats to the human health in near future [11-14]. In this circumstance, researches on isolation of novel antimicrobial compounds from diverse sources as well as development of alternative approaches to control the multidrug resistant pathogens are increasing day by day [15-18]. Hence, this study was designed to assess antibacterial efficacy of fruit extract of *T. nudiflora* against 4 Gram-positive and 8 Gram-negative bacterial species to explore its potentiality as a novel source of antibacterial compounds.

2. MATERIALS AND METHODS

2.1 Fruits Collection and Authentication

The fresh fruits of *Trewia nudiflora* were obtained from several areas of the Campus of Rajshahi University, Rajshahi, Bangladesh. The fruits were authenticated by the botanist of the Department of Botany, University of Rajshahi, Bangladesh.

2.2 Extraction of Fruit Material

The collected fruits of *Trewia nudiflora* were peeled. Peels of fruits were sterilized with 70%

alcohol, which rinsed with sterile distilled water. Then the peels were dried and made powder form with the help of grinding machine. Fruits extract were collected from 100 gram of dried powder by extraction with methanol using conical flask, through shaking and stirring for 14 days. For obtaining the large quantity of extracts the content was pressed through the sterilized marking cloth and the whole mixture was then filtered using Whatman filter paper after that the remaining filtrate were dehydrated in vacuo to afford a blackish mass. Finally remaining output extracts and fraction were collected in vials and stored in a refrigerator at 4°C carefully.

2.3 Antibacterial Activity of Fruit Extracts

2.3.1 Collection of bacteria

Twelve pathogenic bacterial strains which were previously isolated and identified by others in the Microbiology laboratory, Department of Genetic Engineering and Biotechnology, Rajshahi University, Bangladesh were used in this study. Among twelve bacteria, four were Gram positive bacteria *viz*. *Staphylococcus gallinarum, S. sciuri*, *Streptococcus iniae* and *S. constellatus* and remaining eight were Gram negative *viz*. *Xanthomonas axonopodies, X. campestris, Edwardsiella anguillarum, Siccibacter colletis, Aeromonas cavernicala, A. diversa, Vibro rotiferianus* and *Enterobacter xiangfangensis*.

2.3.2 Determination of antibacterial activity of fruit extracts

The antibacterial activity of the fruit extract were determined by disk diffusion method [14,19]. For doing the test, 250 µl of fresh nutrient broth (HiMedia, India) culture of each isolated bacteria was poured on a nutrient agar (HiMedia, India) plate and was spread evenly with a sterilized glass spreader. Discs soaked with 15 µl of fruit extract were placed on cultured plates of studied bacteria. Kanamycin (5 µg/disc, Thermo Scientific™) was used as a positive control. All the discs were incubated at 37°C for 14 hrs. Finally, diameters of zone of inhibition resulted from fruit extracts were measured by mm scale.

2.4 Determination of Minimum Inhibitory Concentration (MIC) of Fruit Extracts

The level of minimum inhibitory concentration (MIC) was measured according to the method described by Owoseni and Ajayi (2010) in which different concentrations of collected methanol extract of fruits of *Trewia nudiflora* were used [20]. Briefly, a pure culture of a single bacterial strain was grown in nutrient broth (HiMedia, India). Then, the culture was made homogeneous using standard protocol to get the culture with $1x10^6$ cells/ml. The methanol extract of fruits of *T. nudiflora* and the control antibiotic Kanamycin were diluted a number of times at 1:1 ratio using a sterile nutrient broth. Then, 2.5 ml of the homogeneous inoculum was added to 2.5 ml of the diluted methanol extract of fruits of *T. nudiflora* or Kanamycin in each dilution tubes resulting in $5x10^5$ cells/ml in tubes. Then, the inoculated tubes were incubated for 48 hrs at 37°C.

2.5 Statistical Analysis

The data was used to generate the graph with Prism software (GraphPad, La Jolla, CA, USA).

3. RESULTS

The results of study showed that the disc impregnated with 15 µl of the methanol extract of the *Trewia nudiflora* fruit produced a zone of inhibition with varied sizes against the 12 bacterial strains (Table 1). It was found that the biggest zone of inhibition (20 mm) was produced against *Edwardsiella anguillarum, Aeromonas cavermicala,* and *Vibro rotiferianus* while the smallest zone was produced against *X. axonopodies, X. campestris, S. iniae* and *S. constellatus*. The control disc, Kanamycin (5 µg/disc) produced a zone of inhibition of 7-18 mm against the tested bacteria (Table 1). It was found that the larger zones were produced by the disc of methanol extract as compared with the zones produced by Kanamycin against the most bacterial strains which might be resulted from higher amount of methanol extract per disc (15 µl/disc) than Kanamycin (5 µg/disc).

The twelve bacterial species were also used to measure the MIC value for *Trewia nudiflora* fruit extract. The values of MIC were ranged from 100-200 µg/ml depending on species of tested bacteria (Fig. 1). Negative controls showed no inhibition against all the tested bacteria. Kanamycin, a standard antibiotic, was used as a Control which showed MIC value varying from 10-30 µg/ml for it against the tested bacteria (Fig. 1).

Table 1. Antibacterial activities of methanol extract of the *Trewia nudiflora* fruit

	Name of bacteria	Zone of inhibition (mm)	
		Plant extract	Kanamycin
Gram Negative	*Aeromonas diversa*	19	7
	Xanthomonas campestris	15	10
	Xanthomonas axonopodies	15	10
	Siccibacter colletis	17	10
	Edwardsiella anguillarum	17	10
	Aeromonas cavernicala	20	10
	Enterobacter xiangfangensis	20	10
	Vibro notiferianus	20	11
Gram positive	*Streptococcus constellatus*	15	12
	Steaphylococcus gallinarum	16	18
	Steaphylococcus sciuri	19	18
	Strephylococcus iniae	15	10

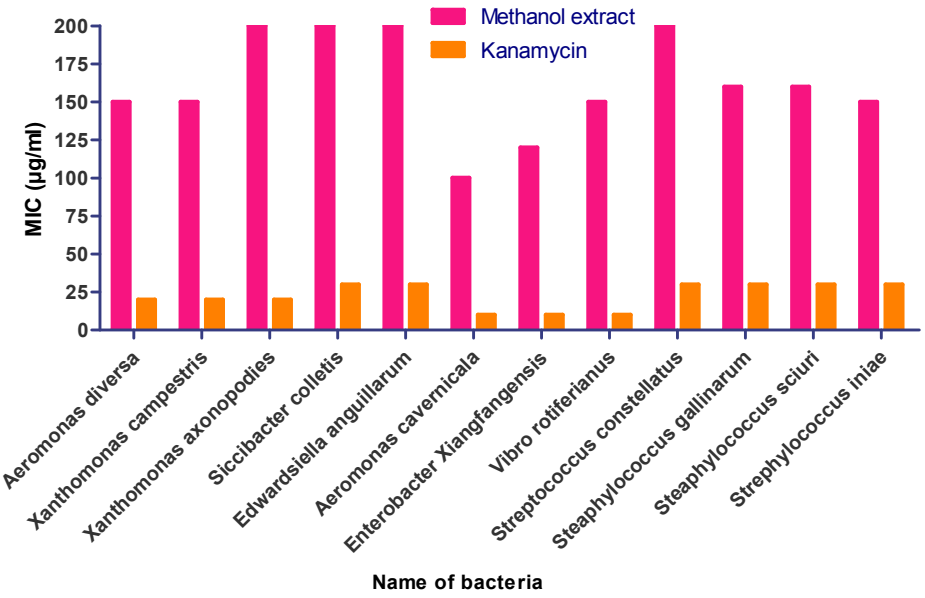

Fig. 1. Minimum inhibitory concentration (MIC) values of *Trewia nudiflora* fruit extract against the tested bacteria

4. DISCUSSION

In this study it was found that the methanol extract of the *Trewia nudiflora* fruit produced a zone of inhibition with varied sizes against the 12 bacterial strains when tested with disc diffusion method. Moreover, the fruit extract of *T. nudiflora* showed the Minimum inhibitory concentration (MIC) ranging from 100-200 µg/ml for tested bacteria. The comparable result was reported by Guo-Hong et al., 2004 as they revealed that the fruit extract of *T. nudiflora* showed significant antimicrobial activity against *Mycobacterium tuberculosis* [21]. They also reported that compounds 9"-butyl americanol A and americanin showed antimicrobial activities against Gram-negative bacterium *Mycobacterium tuberculosis* and Gram-positive bacterium *Staphylococcus aureus* at the minimum inhibitory concentrations (MIC) of 100 mg/mL and 50 mg/mL, respectively, but 3,4-diacetyl americanin and 3,4,9-triacetyl americanin did not exhibit antibacterial activity at 200 mg/disk [21]. The seeds of *T. nudiflora* contain the different types of fatty acids such as elaeostearic acid 38.50%, oleic acid 34.35%, linoleic acid 26.15% and small amount of arachidic acid 1% [22]. In another study it was reported that the ethanolic

extract of this plant possessed antimicrobial activity against crown gall tumors on KB cell Culture, potato discs, and P388 leukemia in mice [23]. Likewise, antibacterial activity of the ethanolic extracts of different parts of *T. nudiflora* was done by disc diffusion method in another study [4]. The finding of the study revealed that the leaf extract of *T. nudiflora* exhibited effective antibacterial activity against *Shigella dysenteriae* with production of a large zone of inhibition (37.5 mm) and moderate antibacterial activity against *Pseudomonas aeroginosa* with 16.5 mm zone of inhibition. Likewise, the extract of fruit of *T. nudiflora* exhibited potential antibacterial activity against *Shigella boydii* as indicated by production of a large zone of inhibition (22.5 mm) [4]. Moderate activity was shown by the seed extract of *T. nudiflora* against *Pseudomonas aeroginosa* while very potential antibacterial activity was exhibited by the extract of the twig as indicated by the generation of 20 mm zone of inhibition against the same bacteria [2]. In addition to antibacterial effect the ethanolic extract of *T. nudiflora* exhibited substantial anti-ulcer activity against peptic ulcers prompted by Indomethacin in a dose dependent manner [24].

5. CONCLUSION

It can concluded that the methanol extract of the fruits of *Trewia nudiflora* exhibited antibacterial activity against 12 different bacteria species indicating that this extract might be a new choice to control antibiotic resistant bacterial infections. However, further studies are needed to find out their efficacy in animal model as well as to purify the active compounds from extract and explore their molecular mechanism for antibacterial activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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