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In vitro Antibacterial Activity of the Compounds of *Trapa bispinosa* Roxb

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The research work was conducted to investigated the *in vitro* antibacterial activity of the compound isolated from *Trapa bispinosa*. Antibacterial activity of the compounds was observed against fifteen pathogenic organisms (both Gram positive and Gram negative Bacteria) by disc diffusion method. Compound MTC-4 isolated from chloroform extract showed significant *in vitro* antibacterial activity against almost all of the test organisms but maximum activity showed against *Bacillus subtilis* and *shigella dysenteriae* and produced zone of inhibition in between 12 to 15mm. While the compound MTE-1 showed moderate activity (08 to 12mm) against most of the test organisms. The other two compounds MTC-3 and MTC-5 were found comparatively less active. The MIC values of the compounds were also determined against two test organisms *Bacillus subtilis* and *Shigella dysenteriae*. The values were found to be between 8 to 128μg ml^{-1}. Thus MTC-4 possesses strong antibacterial activity and suggested its therapeutic use as an antimicrobial agent.

Key words: *In vitro*, antibacterial activity, *Trapa bispinosa*, Trapaceae

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Introduction

In recent years, attempts have been made to investigate the indigenous drugs of choice in infectious diseases for mitigating the suffering of vast masses of humanity. Scientific development in the research field of indigenous plant is a significant aspect to have a safer antimicrobial principle through isolation, characterization, identification and biological testing. Many workers (Hasan *et al.*,1989; Bhargava and Chauhan, 1968) have developed the potential drugs of plant origin with antibacterial activity.

The plant *Trapa bispinosa* Roxb is an aquatic floating herb belonging to the family Trapaceae (George and Lawrence, 1951; Kirtikar and Basu, 1987). The plant has a folkloric reputation as a cure for various diseases. The acrid juice is used for diarrhea and dysenteriae (Vhotracharcho, 1987) and fruits are used in aphrodisiac, astringent to the bowels, leprosy, inflammations, urinary discharges, fractures, sore throat, bronchitis, leucorrhoea, bad teeth and malaria (Kirtikar and Basu, 1994). It is also a drug of good repute in Yunani and Ayurvedic medicine in Indian subcontinent, still the plants is being used by the rural people of the Northern parts of Bangladesh in the treatment of diarrhea and dysenteriae.

Recently our report has shown that the crude extract of *T. bispinosa* possesses the antimicrobial and cytotoxic activity (Rahman *et al.*, 2000). As a part of our continuing search for novel antibacterial agents we have further studied the plant. In this investigation we report the isolation of few compounds from the extract of *T. bispinosa* and also discuss about the antibacterial spectra of the compounds.

Materials and Methods

Collection of the plants: The plants were collected from Amnura marshy land of Nawabgong district, Bangladesh.

Extraction and isolation of the compound: The whole plant was collected in fresh condition, dried for seven days in an oven at 45°C, crushed and then the crushed powder was extracted in a soxhlet apparatus using rectified spirit (CH_3OH) at 70°C for 96 hours. The concentrated rectified spirit extract was diluted with distilled water and solvent-solvent partitioning were successfully carried out by Kupchan method (Beckett and Stenlake, 1986) using petroleum ether ($C_2H_5OC_2H_5$) chloroform ($CHCl_3$) and ethyl acetate ($CH_3COOC_2H_5$). Each of the extract was concentrated at reduced pressure and appropriate temperature using rotary evaporator to yield the semisolid masses.

The compounds MTC-3, MTC-4 and MTC-5 were isolated from the $CH_3COOC_2H_5$ extract by column chromatography (CC) followed by Thin Layer Chromatography (TLC) and Preparative Thin Layer Chromatography (PTLC) as described in the literature (Egon and Stahl, 1969). The compound MTE-1 was isolated from $CH_3COOC_2H_5$ extract in the same way as described above. All of the isolated compounds were subjected to antibacterial screening.

Antibacterial screening: Fifteen pathogenic bacteria (five Gram positive and ten Gram negative) were selected. These organisms were available in the microbiological research laboratory of Pharmacy Department, Rajshahi University. The pure cultures of these were collected from the Microbiological Laboratory of the Institute of Nutrition and Food Science (INFS) and Department of Microbiology, University of Dhaka, Bangladesh. The compounds were dissolved separately in CH_3OH to get a concentration of 200μg 20 μl^{-1}. Then *in vitro* antibacterial activity of these compounds was carried out by standard disc diffusion method (Vander and Vlietnck, 1991) against the selected test organisms. The diameter of zone of inhibition produced by compounds, were then compared by the standard antibiotic Kanamycin 30μg $disc^{-1}$.

Minimum Inhibitory Concentration (MIC): The MIC values of the compounds were determined against one gram positive (*Bacillus subtilis*) and one gram negative (*Shigella dysenteriae*) bacteria. The test was carried out by a serial tube dilution technique (Reiner, 1982). Nutrient agar was used as bacteriological media.

Results and Discussion

In the previous investigation, we demonstrated that the $CHCl_3$ and $CH_3COOC_2H_5$ extract of *T. bispinosa* possesses significant antibacterial activity (Rahman *et al.*, 2000). But now attempts have been made to isolate the antimicrobial agents from the extracts.

The CH_3OH extract of *Trapa bispinosa* after resolution by

Table 1: *In vitro* antibacterial activity of MTC-3, MTC-4, MTC-5 and MTE-1

	Zone of Inhibition (Diameter in mm)				
Name of Bacterial Strains	MTC-3 200μg $disc^{-1}$	MTC-4 200μg $disc^{-1}$	MTC-5 200μg $disc^{-1}$	MTE-1 200μg $disc^{-}$	Kanamycin 30μg $disc^{-1}$ Standard
Gram positive					
Bacillus subtilis	08	12	08	08	15
Bacillus cereus	-	14	10	09	14
Bacillus megaterium	09	13	09	12	16
Staphylococcus aureus	09	15	10	08	15
Staphylococcus β haemoliticus	-	13	-	-	14
Gram negative					
Escherichia coli	09	13	08	12	15
Klebsiella species	08	13	09	08	16
Pseudomonas aeruginosa	-	13	08	10	15
Shigella dysenteriae	08	15	09	12	10
Shigella flexneri	08	12	09	08	15
Shigella shiga	08	13	10	11	13
Shigella sonnei	-	14	-	12	13
Shigella boydii	08	15	-	11	14
Salmonella typhi A	09	15	10	11	16
Salmonella typhi B 56	09	14	10	12	15

"-" No antibacterial activity

Table 2: The MIC values of the isolated compounds against test organisms

Sample	Minimum inhibitory concentration in μg ml^{-1}	
	Bacillus subtilis	*Shigella dysenteriae*
MTC-3	64	128
MTC-4	08	16
MTC-5	64	128
MTE-1	128	128

conventional (CC) yielded three compounds designated as MTC-3, MTC-4 and MTC-5 having R_f values 0.833, 0.619 and 0.309 respectively (silica gel GF_{254}; solvent system, n-hexane: ethyl acetate = 1:1). These compounds showed purple, black and yellowish black colored single spots respectively when sprayed with vanillin-sulphuric acid reagent followed by heating at 111°C for few minutes. Similarly the $CH_3COOC_2H_5$ extract after resolution by PTCL the compound MTE-1 having R_f value 0.522 (silica gel GF_{254}; solvent system, CH_3OH = 5 : 2). The characterization of these compounds has been conducted by Rahman(1998).

The antibacterial activity of the isolated compounds against all tested organisms is shown in Table 1. Compound MTC-4 showed strong antibacterial activity against almost all gram positive and gram negative bacteria and produced zone of inhibition between 12 to 15mm. It showed intense activity against gram positive *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus β-haemolyticus* and gram negative *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Shigella shiga*, *Shigella sonnei*, *Shigella boydii*, *Salmonella typhi-A*, *Salmonella typhi-B 56*. While the compound MTE-1 showed strong activity against *Shigella dysenteriae* and *Shigella sonnei*. It showed moderate activity against gram positive *Bacillus megaterium* and gram negative *Escherichia coli*, *Pseudomonus aeruginosa*, *Shigella shiga*, *Shigella boydii*, *Salmonella typhi-A* and *Salmonella typhi-B 56*. The compound failed to show any sensitive response over *Staphylococcus β-haemolyticus*. Less antibacterial activity was observed with MTC-3 and MTC-5 which probably indicate that the compounds could be effective at a higher concentration. The MIC values of the isolated compounds were determined against *Bacillus subtilis* and *Shigella dysenteriae* (Table 2). The values were found between 8 and 128μg ml^{-1}.

In conclusion, this investigation reports that the compound MTC-4 isolated from the CH_3Cl extracts possesses strong antibacterial activity among the compounds tested. However further work is necessary to establish its structure and to better evaluation of its therapeutic use as an antimicrobial agent.

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