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Antibacterial performance of fruit extracts of wild variety of *Trapa quadrispinosa* Roxb. found in Bangladesh

Md. Anowar Razvy[1*], Shaikh Mizanur Rahman[2], Md. Motiur Rahman[3], Md. Aminul Hoque[1]

[1]*Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi- 6205, Bangladesh*

[2]*Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Jessore, Bangladesh*

[3]*Plant Breeding and Gene Engineering Lab., Department of Botany, University of Rajshahi, Rajshahi- 6205, Bangladesh*

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Abstract

Water chestnut (*Trapa quadrispinosa* Roxb.) is an annual aquatic herb fruit plant grown in fresh water basin of tropical, sub-tropical and some temperate part of the world. It is called "Singhara" in Bangladesh. The methanol extract of wild variety of water chestnut showed the highest antibacterial performance (zone of inhibition 34mm) against *Bacillus megaterium* with the doze of 200µg/disc. Whereas, the lowest antibacterial activities (10mm) were recorded for aqua's extract and petroleum ether extract in comparison to standard antibiotics, *kanamycin*. Also all gram negative bacterial strains were recorded as resistance in 100µg/disc dose of aqua's extract. In this disc diffusion assay, the methanol extract of wild variety was found to have a significant antibacterial effect than the other extracts which is in fact put forward the potency to develop a Phyto-medicine against microbes.

*Corresponding Author: Md. Anowar Razvy ✉ trapa_bdsp@yahoo.com

Introduction

Water chestnut (*Trapa quadrispinosa* Roxb.) is an annual aquatic herb fruit plant belonging to the Trapaceae family comprising about 30 species which are distributed in tropical, subtropical and temperate part of the world (Daniel *et al.*, 1983.; Kumar *et al.*, 1985; Kusum and Chandra, 1980;; Mazumdar, 1985; Srivastva and Tandon, 1951). It has four sharp blended spines called 'Singhara' in wild variety. The plant has a folkloric reputation as a cure for various diseases 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, 1987) as well. It has also good reputation as drug in Yunani and Ayurvedic medicine in Indian subcontinent (Sashi *et al.*, 2003). Still the plant is being used by the rural people of the northern part of Bangladesh for the treatment of diarrhea and dysentery.

There are hundreds of medicinal plants having long history of therapeutic properties aligned with various diseases. Higher plants have the competence to produce a large number of organic phyto-chemicals with complex structural diversity, so as to be known as secondary metabolites. Some of these secondary metabolites are produced in favor of self defense (Evans *et al.*, 1986). Secondary metabolites from different plant species have been evaluated for their antimicrobial activity. Microorganisms have developed resistance to many antibiotics and this has created vast clinical inconvenience in the treatment of infectious diseases (Davis, 1994). Since long, being in charge of manipulation, we are using the medicinal properties of plants for disease treatment. At present, there are several plant products in pre-clinical evaluation while others showed promising antimicrobial activities both in *in vitro* and *in vivo* assays. Over 50% of all modern clinical drugs are of natural origin (Stuffness and Douros, 1982; Madulid and Tyler, 1995) and natural products plays an important role in drug development in the pharmaceutical industry (Baker *et al.*, 1995; Cordell, 1995). However, there is an urgent need to identify new and newer novel antimicrobial molecules from plants as go in front for effective drug development. The present study was aimed to find out antimicrobial activities of fruit extracts of water chestnut.

Materials and methods

Plant materials

Plant materials were used from mature fresh fruit of wild water chestnut (*T. quadrispinosa*) collected from the experimental field of Botanical garden at Rajshahi University campus, Rajshahi- 6205, Bangladesh during September 2010 which was originally collected from Naogaon, Bangladesh.

Microbial strain

Eight pathogenic bacteria where five strains of gram-positive such as *Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Sarcina lutea and Bacillus cereus* and three strains of gram-negative for instance, *Escherichia coli, Salmonella typhi* and *Shigella sonnei* were used for bioassay study. The pure strains were identified and obtained from pathology laboratory, Department of Botany, University of Rajshahi, Rajshahi- 6205, Bangladesh.

Microbial culture media

To evaluate antibacterial activity and sub culturing of test organisms, nutrient agar media having 0.5gm peptone, 1gm yeast extract, 0.5gm sodium chloride and 2gm agar were dissolved in distilled water. This composition of the nutrient was maintained throughout the experiment.

Preparation of fresh culture

The nutrient agar medium was prepared and dispersed (5ml in each test tube) in clean test tubes to get ready slants. The test tubes were sterilized for 30 minutes and kept in an inclined position (450C) for solidification. The test organisms were transferred to the agar slants from the supplied pure cultures with the help of an inoculating loop in an aseptic condition. The inoculated slants were then

incubated at 37.50C for 24 hours to assure the growth of test organisms. These fresh cultures were used for the sensitivity test.

Preparation of the test plates

Nutrient agar media were poured approximately 4mm in each petridish in aseptic area and petridishes were rotated several times, first clockwise and then anticlockwise. Then 200µl of cultured test organism in nutrient broth media was spreaded on the surface of solid nutrient agar media, and preserved for inoculation of sample and standard discs.

Preparation of test sample

Different amount of extracts such as 5mg, 10mg and 20mg of aqua's extract (AE), methanol extracts (ME) and petroleum extract (PE) of water chestnut sample (wild variety) were dissolved in 0.5ml water in separate glass vial. Thus, for each extract the concentrations were 100µg/µl, 150µg/µl and 200µg/µl, respectively.

Preparation of discs

Three types of discs were prepared for antibacterial screening.

Sample discs

Sterilized (BBL) filter paper discs (5mm in diameter) were prepared with the help of punch machine and were taken in an empty petridish. Sample solution of desired concentration (10µl/disc) was applied on the discs with the help of a micropipette in an aseptic condition.

Standard discs

Standard discs were used to compare the antibacterial activity of test material. In our investigation, *kanamycin* (100µg/disc) was used as standard disc.

Solvent control discs

Solvent control discs were prepared using filter paper (5mm) and same volume of residual solvent without sample and these were then used as negative control to ensure the residual solvent.

Placement of the discs and incubation

The dried crude extract discs and standard discs were placed gently as 20mm apart from each other and 15mm outlying the edge of the plate to avoid overlapping the zone of inhibition on the solidified agar plates seeded with the test organisms. The plates were kept in a refrigerator at 40C for 24 hours in order to provide sufficient time to diffuse the antibiotics into the medium. Then the plates were incubated at 37.50C for 24 hours in an incubator.

Measurement of the zone of inhibition

After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of inhibitory zones in term of millimeter (mm) with a transparent scale.

Results and discussion

The antimicrobial activity of the Aqua's extract (AE), methanol extract (ME), petroleum extract were tested against eight bacteria at the concentration of 100µg/disc, 150µg/disc and 200µg/disc. Standard antibiotic disc, *kanamycin* (100µg/disc) was used for the comparison of the bioassay.

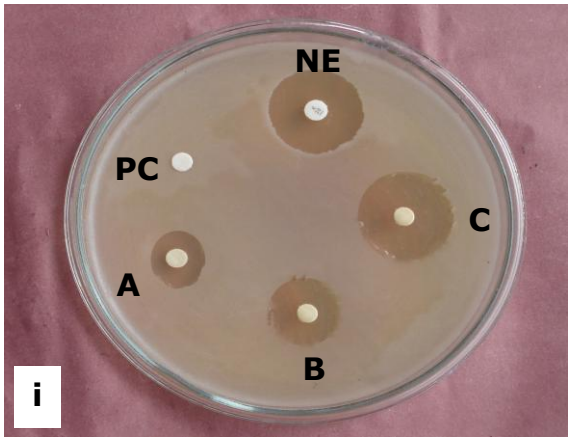
The produced zone of inhibition for isolated aqua's extract (AE) of wild water chestnut against gram positive bacteria showed that 200µg/disc dose was most effective because there was no resistant bacteria found and maximum 21mm inhibition zone produced. Whereas, except *Shigella sonnei* and *Salmonella typhi* in 200µg/disc dose, gram negative bacterial strains in all other treatments showed resistant against aqua's extract. Furthermore, in case of methanol extract (ME) of wild water chestnut showed average activities (Range 11-34mm) the tested organisms where maximum inhibition zones were produced in 200µg/disc dose by all bacterial strains. The highest 34mm zone of inhibition was produced by *Bacillus*

megaterium in gram positive and maximum 31mm zone of inhibition was formed by *Shigella sonnei* in gram negative bacteria [table 1, Fig. 1(i)]. Also among three extracts studied, the highest diameter of inhibitory zone was found in methanol extract (34mm) and the lowest was recorded in both aqua's and petroleum ether extracts (10mm). This consequence was comparable to the previous findings (Razvy *et al.,* 2011) where methanol extract of green variety of water

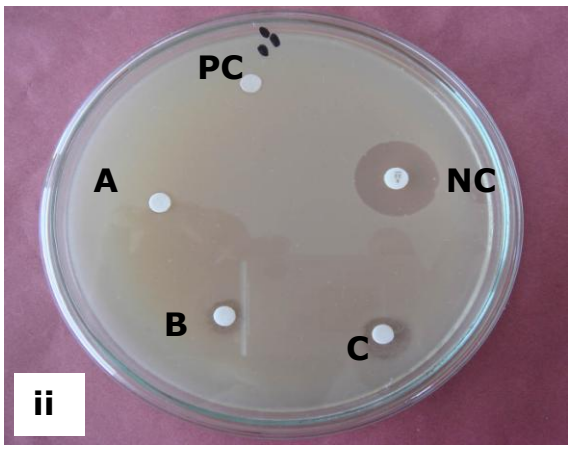
Table 1. *In vitro* antibacterial activity of different extracts of wild water chestnut.

Bacterial Strain (Pathogen)		AE			ME			PE			PC	NC		
		Inhibition zon (µg/disc)			Inhibition zon (µg/disc)			Inhibition zon (µg/disc)			Iz	AE	NE	PE
		100	150	200	100	150	200	100	150	200				
Gram positive (+ve)	*Staphylococcus aureus*	R	R	09	18	23	20	14	14	19	33	-	-	-
	Bacillus subtilis	R	13	21	11	11	18	21	22	29	31	-	-	-
	Bacillus megaterium	R	12	13	27	29	34	21	22	26	32	-	-	-
	Sarcina lutea	10	12	12	15	18	21	17	24	26	28	-	-	-
	Bacillus Cereus	08	10	10	18	23	26	10	18	23	27	-	-	-
Gram negative (-ve)	*Escherichia colli*	R	R	R	12	21	29	14	20	22	25	-	-	-
	Shigella sonnei	R	R	07	19	27	31	20	22	22	28	-	-	-
	Salmonella typhi	R	R	10	22	25	27	14	19	27	28	-	-	-

*** AQ=Aqua"s extract, ME =Methanol extract, PE= Petroleum ether extract, PC= Positive control, NC= Negative Control (Kanamycin), Iz = Inhibition zone and R = Resistance



Highest Performance of methanol extracts



Lowest Performance of aqua's extracts

Fig. 1. Antibacterial activity of fruit extracts of *T. quadrispinosa* against bacteria. (i) Highest and (ii) lowest performances. A, B, C & NC are the produced zone of inhibition at 100µg/disc, 150µg/disc, 200µg/disc and 100µg/disc, respectively.

Chestnut showed maximum inhibitory zone against gram positive bacteria and remarkable antibacterial efficiency (9-12mm) were recorded against most of the tested organisms. Also extracts of some other plants have been used for the antimicrobial activity such as antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* where they have found significant potency of aqueous and methanol extracts (Karaman *et al.*, 2003). No resistant bacteria strain was found in petroleum ether extract experiment and in case of all strains, maximum inhibition zone was recorded in 200µg/disc dose. Therefore antibacterial activities of three different extracts were preeminent in 200µg/disc dose (table 1). It was also perceptible that in low concentration of aqua's extract, all gram negative bacteria were found as resistant [Fig. 1(ii)]. In positive control, significant inhibitory zone was formed such as maximum 33mm and minimum

25mm zone of inhibition was produced, in contrary, not a single inhibitory zone was formed in negative control. Previous study (Parekh and Chanda, 2007) also documented antibacterial activity of *T. natans* fruit extracts against different gram positive and gram negative bacteria.

The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the exploration for new, efficient and cost effective way for the control of infectious diseases. The result of different studies provides that some medicinal plants might be potential sources of new antibacterial agents (Kone *et al.*, 2004.). From this study, we can conclude that, this medicinal plant has a wide range of antibacterial activity and supports the traditional use of these plants as medicine.

This study demonstrated that herbal medicine could be as effective as modern medicine to combat pathogenic microorganisms. This work has highlighted the antimicrobial effects of fruit of *T. bispinosa* on some of the medically important pathogens. This is in fact a promising result, with the comparable standard antibiotics and suggests the potency of these extracts. Hence, *T. bispinosa* fruit could be used as a guide in our continuing search for being used in the field of biopharmaceutical industry or in ethno-medicine.

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