



## ANTIBACTERIAL ACTIVITY OF TWO VARIETIES OF WATER CHESTNUTS (*TRAPA* SP.)

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### Abstract

**Context:** Water chest nut (*Trapa* sp.), an aquatic fruit plant has been used in some area of the rural people of Bangladesh for the treatment of some chronic diseases.

**Objective:** The main objective of this study is to investigate the antibacterial activities of the two varieties (red and green) of water chest nuts.

**Material and Methods:** The antibacterial activities of the methanol extracts of two varieties of water chestnut fruits were studied against a number of human pathogenic bacterial species by using the disc diffusion method. The antibiotic Kanamycin (30 µg) was used as standard.

**Results:** The methanol extract of red variety of water chestnut showed high antibacterial potential (31 mm) against *Bacillus subtilis* at concentration of 600 µg. On the other hand, green variety showed highest antibacterial activities (12 mm) against *Staphylococcus aureus* at concentration of 600 µg. In disc diffusion assay, the methanol extract of red variety was found to have a significant antibacterial efficiency as compared to the methanol extract of green variety of water chestnut.

**Conclusion:** These findings pinpoint the efficiency of these extracts to inhibit microbial growth. It may lead to the development of a new phyto-medicine.

**Key words:** *Trapa* fruit, methanol extract, antibacterial activities, *Bacillus subtilis*, disc diffusion assay.

### Introduction

Water chestnut is an annual aquatic dicotyledonous plant commonly known as "Singhara" or "Paniphol" in Bangladesh. Several varieties are grown in Bangladesh among which *Trapa bispinosa* Roxb. and *Trapa natans* L. are important. It grows well in shallow fresh water basin in the tropical, sub-tropical and temperate zone of the world (Daniel *et al* 1983, Kumar *et. al.* 1985). *Trapa bispinosa* has two varieties, one is red (Leaf, petiole and fruit) and the other is green (Leaf, petiole and fruit). Both of the fruit is large in size having four dull spines. It has been commercially cultivated in India, Japan, China, Pakistan, Srilanka and different parts of Southeast Asia (Kumar *et. al.* 1985, Mazumdar 1985).

Water chestnut is a seasonal fruit that grows well everywhere in Bangladesh. Now-a-days a large number of people have been eating this delicious fruit for its rich nutrient contents (Pandit and Quadri 1986) as well as low price. For human consumption water chestnut is eaten in many countries as raw, boiled or roasted and its stem or leaves as vegetables or flavor, or is added in other dishes.

The fruit has also been used in medicinal purposes e.g. in rheumatism and sunburn (Kosuge *et al.* 1985). The plant has a folkloric reputation as a cure for various diseases. The acrid juice is used for diarrhea, dysentery (Vhotracharcho 1987) and the fruits are also used in aphrodisiac, astringent to the bowels, leprosy, inflammations, urinary discharges, fractures, sore throat, bronchitis, leucorrhoea, bad teeth and malaria (Kirtikar and Basu 1994).

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It is well established that natural products play an important role for drug development programs in pharmaceutical industry (Baker *et al.* 1995, Cordell 1995). The demand on plant-based therapeutics is increasing in both developing and developed countries due to their recognition as natural products, non-narcotic, readily biodegradable, has no adverse side-effects and availability at affordable prices. Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit and bark of plant are the constituents of herbal medicines.

Secondary metabolites (photochemical) of plants are extensively found at different levels in various medicinal plants and used in herbal medicine to treat diverse ailments such as cough, malaria, wounds, toothache and rheumatism diseases (Exarchou *et al.* 2002) and protection of crops. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. Species used in traditional medicine continue to be the most reliable source for the discovery of useful compounds and screening of plants (Ben *et al.* 1992, Broekaert *et al.* 1997, Dubery *et al.* 1999, Hanawa *et al.* 1992, Kruger and Manion 1994, Mohamed and Sehgal 1997, Pernas *et al.* 2000). It has opened another source of compounds useful inhibitory activities against different microbes. Hence there is a constant need to establish and develop antimicrobial drugs from natural origin that are much safe, reliable and less expensive. There are hundreds of medicinal plants which have a long history of curative properties against various diseases. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the higher plant.

Over the last 20 years, a large number of 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). The screening of the plants for their biological activity is done on the basis of their chemotaxonomic investigation or ethno-botanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging issue. The increase in resistance to microorganisms due to the indiscriminate use of antimicrobial drugs forced scientists to search for new antimicrobial substances from various sources including medicinal plants (Karaman *et al.* 2003). A significant portion of the agricultural products in the world has become unfit for human consumption due to mycotoxins contamination of grains (Chandra and Sarbhoy 1997). The main toxic effects are carcinogenicity, genotoxicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immune-suppression (Desjardins *et al.* 2000, Lacey 1998). A large portion of world population is living below poverty line in the developing and under-developing countries. People are also suffering from health problems associated with consuming mycotoxin contaminated grains and cereals (Majumder *et al.* 1997). Besides this the infectious diseases also account for high proportion of health problems in the developing countries (Sashi *et al.*, 2003).

As plant extract of many higher plant have been reported to exhibit antibacterial activities under laboratory trials (Bouamama *et al.* 2006, Ergene *et al.* 2006). Thus it need to search for a compound to protect against various infectious diseases without any toxicity problem that are eco-friendly and inexpensive. *Trapa bispinosa* Roxb., is such an aquatic annual fruit plant of Trapaceae family comprising about 30 species that are distributed in tropical, sub tropical and temperate zone (Daniel *et al.* 1983, Kumar *et al.* 1985, Kusum and Chandra 1980, Mazumdar 1985, Srivastva and Tandon 1951) with high pharmaceutical value.

Keeping all these in mind in this study, an attempt was made to investigate in details of the water chestnut fruits using locally available two varieties (green and red). Although it has pharmaceutical value but there are some limited work done on water chestnut. Therefore the present study was undertaken with the view to investigate the antibacterial activities of the two varieties (red and green) of water chest nuts.

## Materials and Methods

### Plant materials:

Mature fresh fruits of two varieties of *Trapa* sp. for extraction were used as plant material. Two varieties (Red and Green) of *Trapa bispinosa* Roxb were collected from the Fruit Research Substation, Shyampur, Rajshahi, Bangladesh.

### Test organisms

Eight pathogenic bacteria including five strains of gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea* and *Bacillus cereus*) and three strains of gram-negative (*Escherichia coli*, *Salmonella typhi* and *Shigella sonnei*) were used for the bioassay study. The pure strain was identified and obtained from Genetic Engineering and Biotechnology Laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh.

### Preparation of fresh culture:

The nutrient agar medium was prepared and dispersed in a number of clean test tubes to prepare slants (5ml in each test tube). The test tubes were plugged with cotton and sterilized for 30 mins. After sterilization, the test tubes were kept in an inclined position (45° C) 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 37.5°C for 24 hrs to ensure the growth of test organisms. These fresh cultures were used for the sensitivity test.

### Preparation of the test plates:

Nutrient agar media were transferred to sterile Petri dishes in aseptic condition. The media were poured into Petri dishes in such a way to keep a uniform depth of approximately 4mm. The Petri dishes were rotated several times, initially clockwise and then anticlockwise. Test organism (200 µl) cultured in nutrient broth media was spread on the surface of solid nutrient agar media and kept preserved for applying samples and standard discs.

### Preparation of test sample:

For preparing the test sample, three different concentrations (5 mg, 10 mg and 20 mg) of methanol extract (ME) of each water chestnuts sample (Red and Green) were dissolved in 0.5 ml water in separate glass vial. The concentrations were 10 µg/µl, 20 µg/µl and 40 µg/µl, respectively for each extract.

### Preparation of discs:

Three types of discs were prepared for antibacterial screening. Sterilized (BBL, USA) filter paper discs (5 mm in diameter) were prepared with the help of punch machine and were taken in blank Petri dishes. Sample solution of desired concentration (10µl/ disc) was applied on the discs with the help of a micropipette in an aseptic condition. These were used to compare the antibacterial activity of test material. In our investigation, Kanamycin (30 µg/disc) was used as standard disc. These were prepared by using identical filter paper (5 mm diameter) and same volume of residual solvent in the same condition. These were used as negative control to ensure that the residual solvent and the filter paper themselves was not active.

### Placement of the discs and incubation:

The dried crude extract discs and standard discs were placed gently as 20 mm apart from each other and 15 mm far from the edge of the plate to prevent overlapping the zones of inhibition on the solidified agar plates seeded with the test organisms. The plates were kept in a refrigerator at 4°C for 24 hrs in order to provide sufficient time to diffuse the antibiotics into the medium. Then the plates were incubated at 37.5° C for 24 hrs in an incubator.

### Measurement of the zones of inhibition:

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

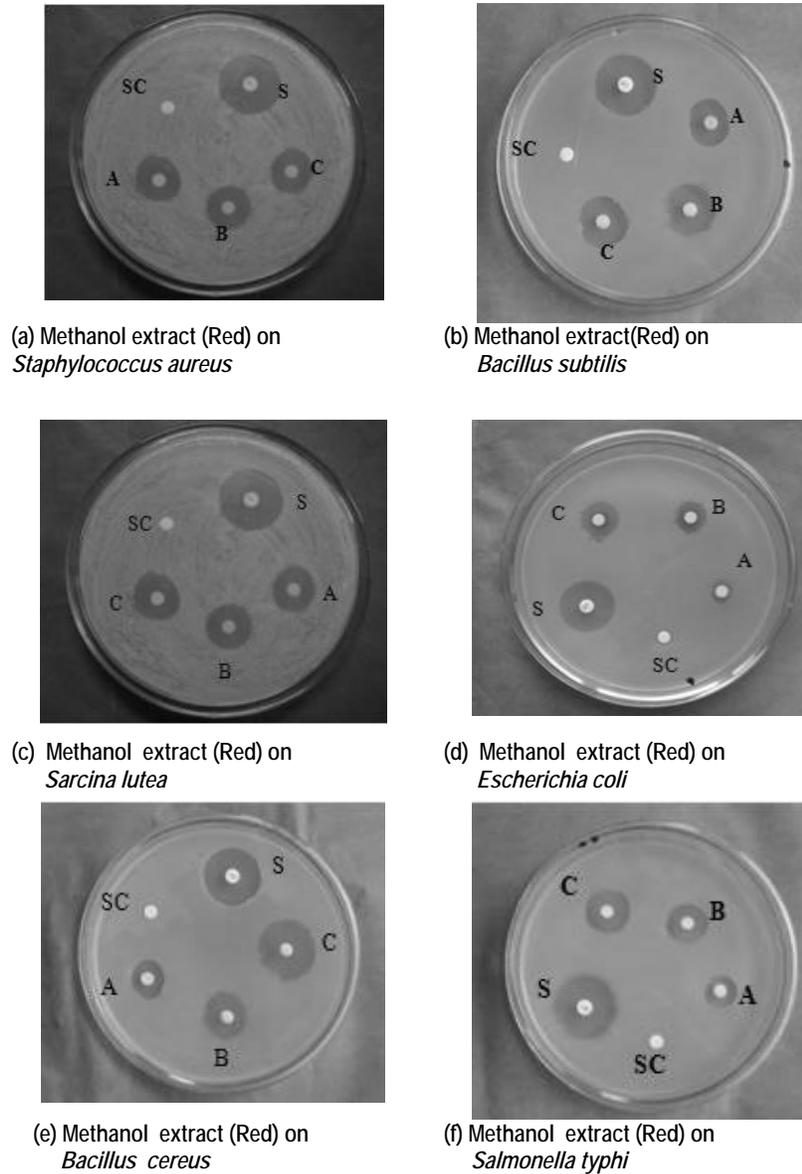
### Results

The antimicrobial activity of the methanol extract having different concentrations (200µg/disc, 400µg/disc and 600 µg/disc), was tested against eight pathogenic bacteria. Kanamycin disc (30µg/disc) was used for comparing the antibacterial activity. Figure 1 (a-f) and Table 1 represent the inhibition zone, found by the activity of isolated methanol extract (ME) of red variety of water chestnut having different concentration (200 µg/disc, 400 µg/disc and 600 µg/disc) tested against eight bacteria and standard antibiotic Kanamycin (30 µg/disc). At 200 µg/disc dosage methanol extract of red variety of water chestnut showed 12, 23, 18, 18, 15 and 13 mm zone of inhibition against *S. aureus*, *B. subtilis*, *S. lutea*, *B. cereus*, *E. coli* and *S. typhi*, respectively. However no antimicrobial activity was observed against *Bacillus megaterium* and *Shigella sonnei* at the same concentration.

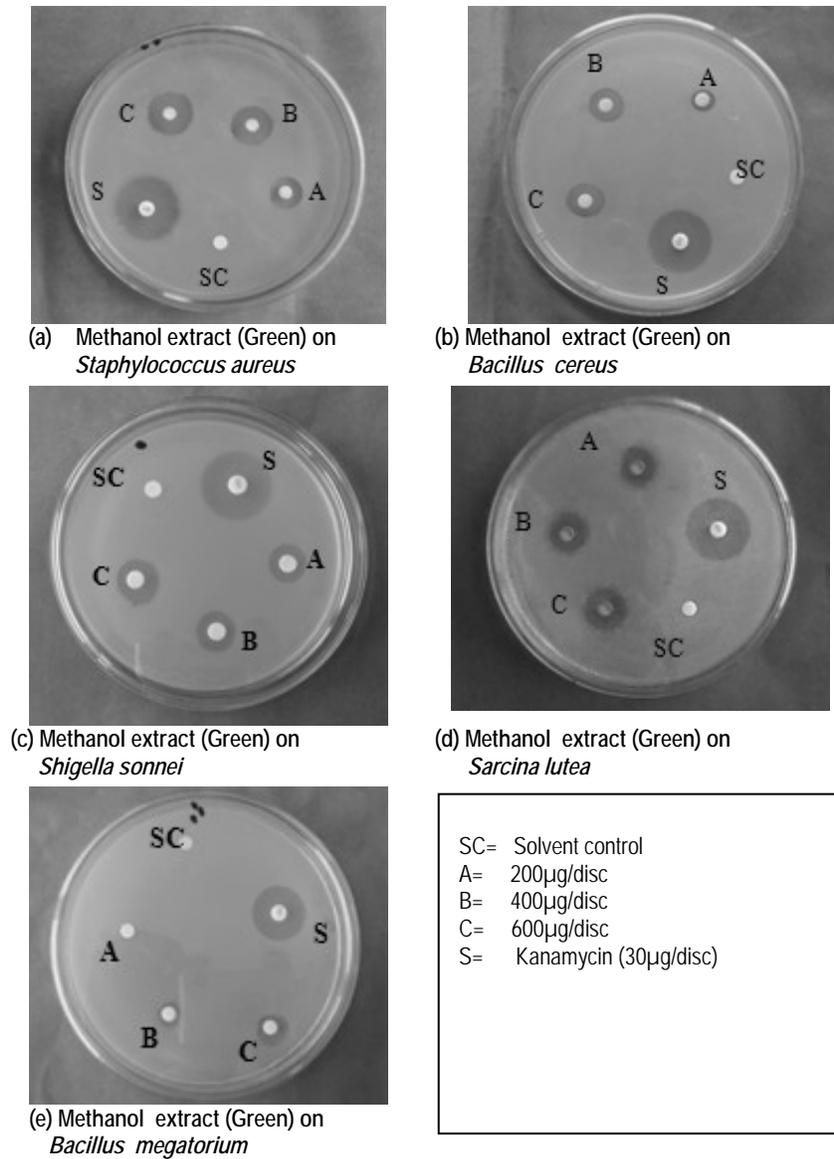
**Table 1.** Antibacterial activity of methanol extracts of red and green variety of water chestnut.

Test bacteria	Red variety (µg/disc)			Green variety (µg/disc)			(µg/disc) Kanamycin
	200	400	600	200	400	600	
	Zone of inhibition (Diameter in mm.)						
<i>Staphylococcus aureus</i>	12	12	15	9	10	12	33
<i>Bacillus subtilis</i>	23	13	31	R	R	R	27
<i>Bacillus megaterium</i>	R	R	R	0	9	10	28
<i>Sarcina lutea</i>	18	16	23	10	10	11	26
<i>Escherichia coli</i>	18	16	23	R	R	R	22
<i>Bacillus Cereus</i>	15	19	22	0	10	10	27
<i>Shigella sonnei</i>	R	R	R	12	11	10	28
<i>Salmonella typhi</i>	13	15	18	R	R	R	26

R = Resistant



**Fig.1.** Antimicrobial activity of methanol extract of water chestnuts (Red) on six pathogenic bacteria as compared to standard antibiotic Kanamycin by using disc diffusion method. (a-f) indicates the antibacterial activities of methanol extract of water chestnut (Red) against six bacteria (a) *Staphylococcus aureus* (b) *Bacillus subtilis* (c) *Sarcina lutea* (d) *Escherichia coli* (e) *Bacillus cereus* and (f) *Salmonella typhi* were used at concentration 200µg/disc, 400µg/disc and 600µg/disc respectively. Standard antibiotic disc Kanamycin(30µg/disc) was used for comparison. SC- indicates solvent control A- indicates 200µg/disc B- indicates 400µg / disc C- indicates 600µg /disc and S- indicates Kanamycin (30µg /disc), respectively.



**Fig. 2.** Antimicrobial activity methanol extract of water chestnuts (Green) on five bacteria as compared to standard antibiotic Kanamycin by using disc diffusion method. (a-e) indicates the antibacterial activities of methanol extract of water chestnut (Green) against five bacteria (a) *S. aureus* (b) *B. cereus* (c) *S. sonnei* (d) *S. lutea* and (e) *B. megaterium* were used at concentration 200µg/disc, 400µg/disc and 600µg/disc respectively. Standard antibiotic disc Kanamycin (30µg/disc) was used for comparison. SC- indicates solvent control A- indicates 200 µg/disc B-indicates 400 µg /disc C- indicates 600 µg/disc and S- indicates Kanamycin (30 µg/disc), respectively.

As shown in Fig. 1 and Table 1 in case of methanol extract of red variety of chestnut at 400µg/disc concentration, the zone of inhibition against the same bacteria were 12, 13, 16, 16, 19, and 15mm respectively. In case of highest dose of 600 µg/disc, the zones of inhibition were found to be 15, 31, 23, 23, 22, and 18 mm, respectively. As shown in Table 1 also two bacteria *Bacillus megaterium* and *Shigella sonnei* were resistant to 200 µg/disc, 400 µg/disc and 600 µg/disc dosages methanol extract of red variety (Not shown in Fig. 1). But the standard antibiotic disc Kanamycin (30 µg/disc) showed the zone of inhibition against eight bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *Escherichia coli*, *Bacillus cereus*, *Shigella sonnei* and *salmonella typhi*. The zones of inhibitions were 33, 27, 28, 26, 22, 27, 28 and 26mm respectively (Table 1).

On the other hand the antimicrobial activity of the methanol extract of green variety of water chestnut against *Staphylococcus aureus*, *Bacillus megaterium*, *Sarcina lutea*, *Bacillus cereus* and *Shigella sonnei*, were 9, 0, 10, 0 and 12,mm at 200 µg/disc dosage respectively (Table 1 and Fig. 2). At 400 µg/disc dosage green variety of methanol extract produced 10, 9, 10, 10 and 11mm of inhibition zone against the same bacteria respectively (table 1 and Fig. 2). Highest dosage of (600 µg/disc) of methanol extract of green variety was also able to produce zone of inhibition resulting 12, 10, 11, 10 and 10 mm respectively (Table1 and Fig. 2). The resistance was observed in case of the methanol extract of green variety of water chest nut against three bacteria like *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* respectively ( not shown in Fig.2 but data shown in Table 1).

## Discussion

The aqueous extract of both the red and green variety of water chest nut found to be effective against all the pathogenic bacteria by disc diffusion assay. It exhibited reasonable antibacterial activity against all the tested bacteria. The methanol extract of water chestnut (green varieties) showed notable antibacterial efficiency (9-12 mm) against most of the tested organisms except, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*. Highest antibacterial activity was observed against *Shigella sonnei* (12 mm) bacteria when applied 200 µg/disc dosages (Table 1 & Fig. 2c).

Table 1 and Fig. 2a represent the poor efficiency against the *Staphylococcus aureus* (9mm) which is lower than Kanamycin (33 mm). From Table 1 and Figure 1 it is also revealed that fruit extract of water chestnut (red variety) showed the most effectiveness (12- 31mm) against all pathogenic bacteria except *Bacillus megaterium* and *Shigella sonnei*. Highest inhibitory activity was found against *Bacillus subtilis* (31 mm) (Table 1 and Fig.1b) having concentration of 600µg/disc, whereas lowest activity was observed against *Staphylococcus aureus* (12 mm) ( Fig.1a and Table 1) *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* were resistant to the green variety but sensitive to red variety of water chestnut fruit (table 1). Beside this *Bacillus megaterium* was found to be resistant to red variety though it showed a little bit sensitivity against green variety of water chestnut fruit extract. This result indicates that the fruit extract from green & red varieties of water chestnut also act as inhibiting agent against some bacteria. Nascimento *et.al* 2000 also reported that *Caryophyllus aromaticus* and *Syzygyum joabolanum* extracts showed promising inhibitory growth of *Pseudomonas aeruginosa*.

In the present study the fruit extract of red water chestnut was more effective than green water chestnut against all the pathogenic bacteria tested by the disc diffusion assay. It is also reported that the leaves extract of *Pistacia lentiscus* and *Pistacia arlantica* have been proved to be very effective against eight bacteria, five moulds & yeast by disc diffusion method (Benhammou *et al.* 2008).

The methanol extract of red variety of water chestnut fruit was found to be the most potential antibacterial extract that showed inhibitory activity against both gram (+ ve) and gram (- ve) bacteria. This study reveals that water chestnut can be used potentially as a broad spectrum antibacterial agent. Considering the cost,

availability and extractability percentage of the aqueous extract, it can also be used as a cheap alternative to substitute antibiotics. The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents (Kone *et al.*, 2004).

It can be concluded that water chestnut (*Trapa spp.*) has a wide range of antibacterial activity and supports the traditional use of these plants as medicines. This study also demonstrated that herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms. This work has highlighted the antimicrobial effects of fruit extract of *T. bispinosa* on some of the medically important pathogens and opens a new window and suggests the potentialities of these extracts as antibacterial agent. Hence, *T. bispinosa* fruit could be used as a guide in our continuing search for new natural products with potential medicinal properties as it will lead to the development of a phyto-medicine to act against microbes.

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