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RESEARCH PAPER

Environment friendly antibacterial activity of water chestnut fruits

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Abstract

Antibacterial activities of the fruit extract of two varieties (Green and red varieties) of water chestnut by the disc diffusion method from methanol extract were studied. The extract of red variety of water chestnut showed high antibacterial potential (31mm) against *Bacillus subtilis* with the concentration of 600μ. On the other hand, green variety showed highest antibacterial activities (12mm) against both *Staphylococcus aureus* and *Shigella sonnei* with the concentration of 600μg kanamycin used as standard. In this disc diffusion assay, the methanol extract of red variety was found to have a significant antibacterial efficiency than the extract of green variety of water chestnut. 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.

Introduction

Trapa bispinosa Roxb. is an annual aquatic fruit plant found in tropical, sub-tropical and temperate zone of the world. It's a starchy fruit and used as minor fruit in Bangladesh. The modern medicine has brought with it an array of drugs, none of which is non-toxic and quite safer for human consumption. Over 50% of all advanced clinical drugs are made of natural product (Stuffness and Douros, 1982). Natural products play an important role in drug development programs of the pharmaceutical industry (Baker *et al*., 1995; Cordell, 1995). 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. 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. Importance of the plant lies in their biologically active principles.

Higher plants have the capacity to produce a large number of organic phyto-chemicals with complex structural diversity that is known as secondary metabolites. Some of these secondary metabolites are produced for plant's self defense (Evans *et al*., 1986). Over the last 20 years, a large number of secondary metabolites from different plant species have been evaluated for their antimicrobial activity. 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. Microorganisms have developed resistance to many antibiotics and this has created vast clinical inconvenience in the treatment of infectious diseases (Davis, 1994). 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). Another driving factor for the renewed interest in past 20 years has been the rapid rate of plant species extinction. Infectious diseases account for high proportion of health problems in the developing countries (sashi *et al*., 2003). *Trapa bispinosa* Roxb. is an aquatic annual fruit plant of Trapaceae family comprising about 30 species that are distributed in tropical, subtropical and temperate zone (daniel *et al*., 1983; Kumar

et al., 1985; Kusum and Chandra, 1980; Mazumder, 1985; Srivastava and Tandon, 1951). Trapa has two varieties, one of them green in colour with green stem, swollen nand fruit, another type is red in colure with red stem, swollen and fruit. The nutritive value of the fruit is not less than wheat (Kusum and Chandra, 1980). The plant has a folkloric reputation as a cure for various diseases. The acrid juice is used for diarrhoea and dysentery (Vhotracharcho, 1987) and fruit 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 good reputation in Yunani and Ayurvedic medicine in Indian subcontinent, still the plant is being used by the rural people of the northern part of Bangladesh in the treatment of diarrhea and dysentery.
The present study was undertaken to characterize some secondary compounds from the two varieties (green and red) of water chestnut and investigate their antibacterial effect.

Materials and methods

Plant materials

Plant materials used as mature fresh fruit of *Trapa bispinosaI* Roxb., were collected from experimental field of Botanical garden at Rajshahi University campus, Rajshahi 6205, Bangladesh.

Microbial strain

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 Gene Engineering and Biotechnology Laboratory, Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh.

Microbial culture media

Bacterial cultures were pre-grown on nutrient broth at 37.5°C for 24h. Cultures were spread on nutrient agar nutrient agar media (Difco). For preparing of 100ml nutrient agar media, 0.5g peptone, 1g yeast extract, 0.5g sodium chloride and 2g agar were

dissolved in distilled water. This composition of the nutrient was maintained constantly throughout the work.

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 minutes. After sterilization, the test tubes were kept in an inclined position (45^{o}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^{o}C 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 transferred to the sterile petridishes in sterile area. The media were poured into petridishes in such a way to keep a uniform depth of approximately 4mm. The petridishes were rotated several times, initially clockwise and then anticlockwise. The 200µl of test organism 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 amounts (5mg, 10mg and 20mg) of methanol extract (ME) of each water chestnuts sample (Green and Red) were dissolved in 0.5ml water in separate glass vial. The concentrations were 10µg/µl, 20µg/µl and 40µg/µl, respectively for each extract.

Preparation of discs

Three types of discs were prepared for antibacterial screening. Sterilized (BBL) filter paper discs (5mm 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 (30µg/disc) was used as standard disc. These were prepared by using identical filter paper (5mm 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 20mm apart from each other and 15mm 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^{o}C for 24 hours in order to provide sufficient time to diffuse the antibiotics into the medium. Then the plates were incubated at 37.5^{o} C for 24 hours 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. The antimicrobial activity of the methanol extract having different concentration (200μg/disc, 400μg/disc and 600μg/disc), was tested against eight bacteria. Kanamycin disc (30μg/disc) was used for comparing the bioassay.

Results and discussion

Inhibition zone found by the activity of isolated methanol extract (ME) of green variety of water chestnut against *Staphylococcus aureus, Bacillus Cereus*, *Shigella sonnei, Sarcina lutea, Bacillus megatorium* were 9, 0, 10, 10 and 0mm at 200 μg/disc dosage respectively. Another dosage (400μg/disc) produced 10, 10, 11, 10 and 9mm of inhibition zone against the same bacteria respectively. Highest dosage (600 μg/disc) of methanol extract was also able to produce zone of inhibition resulting 12, 10, 12, 11 and 10mm respectively. The other red variety of water chestnut formed 23, 15, 13, 18 and 13mm zone of inhibition at 200μg/disc dosage, respectively against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Sarcina lutea, Escherichia coli and salmonella typhi.* In 400μg/disc, zone of inhibition against the same bacteria were 12, 13, 19, 18, 16, and 15mm respectively (Table 1). In highest 600 μg/disc dosage, the zone of inhibition was found to be 15, 31, 22, 15, 23, and 18mm, respectively.

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)			Kanamycin (μg/disc)
	200	400	600	200	400	600	30
	Zone of inhibition (Diameter in mm.)						
Staphylococcus aureus	12	12	15	9	10	12	33
Bacillus subtilis	23	13	31	R	R	R	27
Bacillus megaterium	R	R	R	R	9	10	28
Sarcina lutea	18	16	23	10	10	11	26
Escherichia colli	18	16	23	R	R	R	22
Bacillus Cereus	15	19	22	R	10	10	27
Shigella sonnei	R	R	R	12	11	10	28
Salmonella typhi	13	15	18	R	R	R	26

R = Resistance

The aqueous extract was 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- 12mm) against most of the tested organisms except, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*. Highest antibacterial activity was observed against *Shigella sonnei* (12mm) bacteria when applied 200 and 600 μg/disc dosages. Poor efficiency was found against the *Staphylococcus aureus* (9mm) which were lower than kanamycin (33mm, 12mm). It was revealed that fruit extract of water chestnut (red variety) showed the most effectiveness (12-31mm) against all pathogenic bacteria except *Bacillus megaterium*. Highest inhibitory activity was found against *Bacillus subtilis* (31mm) bacterium having concentration of 600 μg/disc, whereas lowest activity was observed against both *Staphylococcus aureus* and *Shigella sonnei* (12mm).

Bacillus subtilis, *Escherichia coli* and *Salmonella typhi* were resistant to the green variety but sensitive to red variety of water chestnut fruit. 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. 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.

Discussion

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. It reveals its potential use 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 this medicinal plant 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. These investigations open a new window and suggest 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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