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Antifungal Activity of Fruit Extracts of Different Water Chestnut Varieties

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Abstract

The antifungal activity of three varieties (red, green and wild) of water chestnut fruit extracts was studied against a number of fungal species. A strong antifungal activity of ethanol and petroleum extract was found against the treated fungi resulting remarkable inhibition zone in comparison to both Dithane-M_{45} fungicide and control. It has also been evident that wild variety of water chestnut was comparatively more efficient in respect to antifungal activity compared to the red and green variety of the same plant.

Keywords: antifungal activity, fruit extract, inhibition zone, solvents

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources and many of them are based on their use in traditional medicine. 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 plant 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.

Fungi are significant destroyers of crop, food stuffs and grains. A significant portion of the agricultural products in the world has become unfit for human consumption due to mycotoxins contamination of grains, especially those produced by species of *Aspergillus* (Chandra and Sarbhoy, 1997; Devi *et al*., 2001; Janardhana *et al*., 1999). The main toxic effects are carcinogenicity, genotoxicity, terratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immune-suppression (Desjardins *et al*., 2000; Lacey, 1988). A large portion of world population is living below poverty line in the developing and underdeveloped countries. People are also suffering from health problems associated with consuming mycotoxin contaminated grains and cereals (Majumder *et al*., 1997).

Though effective control of different fungi can be achieved by the use of synthetic chemical fungicides, but these are not environmentally safe. Thus, there is a need to search for alternative compound to protect the damage of crop, store grains or cereals and various diseases or infections without toxicity problems that are eco-friendly and expensive. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Bouamama *et al*., 2006; Ergene *et al*., 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006; Okigbo and Ogbonnaya, 2006; Satish *et al*., 1999; Shariff *et al*., 2006). Plant metabolites and plant-based pesticides appear to be one of the better alternatives as they are known to have minimal environmental danger to consumers in contrast to the synthetic pesticides (Verma and Dubey, 1999).

The present study was undertaken in order to investigate the antimicrobial properties of different varieties of water chestnut fruits found on rainy season in Bangladesh. Antifungal activities of different extracts were tested against seven fungi by using disc diffusion technique. Because it is basically a quantitative or semi-quantitative test which indicates the sensitivity or resistance of fungus to the test material.

Materials and methods

Plant materials

Mature fresh fruits of three varieties of *Trapa* spp for extraction were used as plant material. One wild variety (*Trapa quadrispinosa* Roxb.) and two cultivars (Green and Red) of *Trapa bispinosa* Roxb were collected from the

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experimental field of Botanical Garden at Rajshahi University, Bangladesh. These plants were previously collected from Niamatpur and Sapahar Upazila, Naogaon, Bangladesh.

Fungal strain

Total seven pathogenic fungal strains, namely *Penicilium* sp, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium species, Aspergillus fumigatius* and *Candida albicans,* were used for the present sensitivity test.

Test materials

Three solvents (aqua's, ethanol and petroleum ether) were used for the antifungal activity study. The concentrations of test sample in different extract were 100 µg/l, 200 µg/l and 250 µg/l, respectively. The standard antifungal reagent was Dithane- M_{45} fungicides at a fixed concentration (100 µg/l) used as positive control.

Preparation of culture media

Potato dextrose agar (PDA) media was used to perform the antifungal activity test as well for the subculture of the test organism. The composition of the culture media was 200 g peeled and sliced potato, 40 g dextrose, 20 g agar and 1000 ml distilled water.

Preparation of the test plates

About 10 ml distilled water was poured in several clean test tubes and plugged with cotton. Then 6 ml of the medium was poured carefully in the medium sized petri-dishes. The petri-dishes were rotated several times, firstly clockwise followed by anticlockwise to assure homogenous thickness of the medium and allowed to cool and solidify at 30°C. The test tubes containing distilled water were inoculated with fresh culture of the test fungi and were shaken gently to form a uniform suspension of the organism because of their high prevalence sporulation process. Then a piece of cotton was immerged in the test tubes with the help of individual glass rod. Then the medium was gently rubbed and the cotton was discarded. Finally the plates were stored in a refrigerator (4°C) for whole night.

Preparation of discs

Two types of antifungal discs were prepared for antifungal screening. One was the sample disc and another type discs was the standard disc prepared by antifungal reagent Dithane- M_{45} fungicide. Sample discs were sterilized (BBL, Cocksrvile, USA) and filter paper discs (5mm diameter) were taken in a blank petri-dishes. Sample solution of desired amount was applied on the discs with the help of micropipette in aseptic condition. The discs were left for a few minutes in an aseptic condition for complete removal of solvent and standard discs was prepared with the concentration of (Dithane-M_{45} fungicides) 100 µg/disc.

Placement of the discs and incubation

Both the dried crude extract discs and standard disc were placed gently on the solidified agar plates sealed with the test pathogenic fungus ensuring contact with the medium by the sterile forceps. The plates were then kept in a refrigerator at 4°C for 24 hours in order to provide sufficient time to diffuse the antibiotics into the medium. After that the plates were incubated at 37.5°C for 24 hours in an incubator. After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of inhibitory zones (millimeter) with a transparent scale.

Results and discussion

Different concentrations of aqua's, ethanol and petroleum extract of water chestnut fruit extract were tested against fungal growth. In was observed that all three varieties of water chestnut exhibited some sort of antifungal activity resulting inhibition zone ranging from 1.1±0.03 mm to 9.9±0.71 mm in diameter. Highest inhibition zone (9.9±0.71 mm) was found against *Aspergillus flavus* when treated with 250 µg/disc of ethanol extract of wild variety (Fig. 1). It was revealed that both ethanol and petroleum extracts seemed to be more efficient to inhibit the growth of all *Aspergillus* species compared to aqua's extract (Tab. 1). It was reported that ethanolic extract of *Anogeissus leiocarpus* and *Terminalia avicennioides* is more efficient than the methanolic, chloroform, or aqueous extracts against all the test fungi (Mann *et al*., 2008). In comparison, wild variety showed highest antifungal activity against all the fungi used in this study, which reveals the presence of toxic substances in wild water chestnut compared to red and green. It was interesting to be noted that petroleum extract

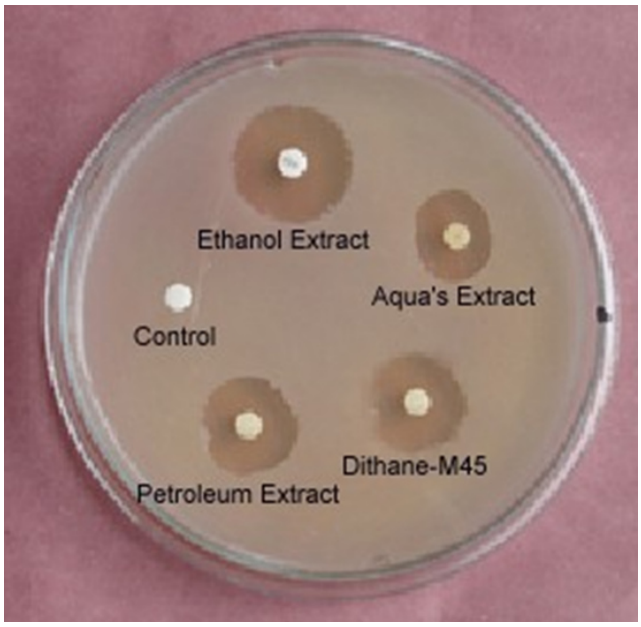

Fig. 1. Antifungal activity of water chestnut (wild variety)

found to be more efficient than other two solvents against *Penicilium* sp., *Fusarium* sp. and *Candida albicans* (Tab. 2). Lowest growth (4.2±0.07 mm) was recorded against *Candida albicans* while highest (7.1±0.58 mm) was obtained against *Penicilium* sp. (Tab. 2). Hypothetically increase in the antifungal activity of any extract supposed to be found by increase concentration, which was not observed in this study. Application of plant extract to inhibit fungal or bacterial growth is a common practice. In this study, fruits of water chestnut especially the wild variety found to be efficient against some fungal genotypes and it also indicates the potential inhibitory effect on other fungi or molds (Tab. 1 and Tab. 2). Use of different parts of plant has also been noticed in many literatures. Leaves extract of *Pistacia lentiscus* and *Pistacia atlantica* have been proved to be very effective against eight bacteria, five moulds and yeast by disc diffusion method (Benhammou *et al*., 2008). Plant extracts also act as an inhibiting agent against some bacteria. It was reported that *Caryophyllus aromaticus* and

Tab. 2. Evaluation of *in vitro* antifungal activity against other fungi

PM	Ext. µg/disc	Disc/Zone of inhibition (mm)								
		Penicilium sp			*Fusarium* species			*Candida albicans*		
		100	200	250	100	200	250	100	200	250
V_1	AQ	1.1±0.03	1.7±0.01	1.9±0.01	1.3±0.12	2.2±.02	2.0±0.22	2.4±0.16	2.9±0.09	2.6±0.04
	ET	3.0±0.22	2.0±0.22	3.0±0.22	1.5±0.34	2.0±0.32	2.0±0.21	3.0±0.30	3.0±0.06	3.0±0.01
	PE	4.0±0.01	3.0±0.34	3.1±0.30	3.1±0.54	4.0±0.41	3.0±0.07	3.0±0.45	4.0±0.09	4.0±0.42
V_2	AQ	1.3±0.11	1.3±0.11	1.2±0.28	1.7±0.70	2.2±0.11	2.9±0.11	2.3±0.31	2.3±0.34	2.1±0.55
	ET	4.3±0.19	4.3±0.51	5.2±0.35	4.1±0.24	4.7±0.44	3.8±0.23	4.5±0.43	3.3±0.41	3.1±0.21
	PE	5.1±0.08	3.9±0.22	4.8±0.22	4.4±0.54	3.8±0.11	4.1±0.29	4.3±0.22	4.1±0.44	3.8±0.07
V_3	AQ	2.3±0.28	2.3±0.44	2.8±0.38	3.1±0.23	3.5±0.10	3.6±0.11	2.5±0.21	2.9±0.21	2.9±0.22
	ET	6.1±0.21	5.9±0.24	5.9±0.11	5.3±0.66	5.2±0.25	5.5±0.05	4.2±0.07	4.9±0.07	4.8±0.36
	PE	7.1±0.58	6.8±0.34	6.5±0.25	5.2±.08	5.1±0.44	4.9±0.08	6.1±0.35	6.3±0.02	6.4±0.11
NC	AQ	+	+	+	+	+	+	+	+	+
	ET	+	+	+	+	+	+	+	+	+
	PE	+	+	+	+	+	+	+	+	+

PM= Plant Materials, AQ= Aqua's extract, ET= Ethanol Extract, PE= Petroleum Extract, PC= Positive control (Disc containing Antifungal reagent), NC= Negative control (Disc containing only solvent), (+)= Growth, (-)= no sensitivity, V_1= Green, variety, V_2= Red variety, V_3= Wild variety, Inhibition zone excluding disc (5 mm) space

Syzygyum joabolanum extracts found to be promising for inhibiting the growth of *Pseudomonas aeruginosa* (Nascimento *et al*., 2000).

Conclusions

It could be concluded that fruit extract of water chestnut has great prospective as antimicrobial compounds against microorganisms. This may open a new window in the treatment of infectious diseases caused by resistant microbes.

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