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In vitro antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents

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***Trapa natans* L. fruit rind was extracted in different solvents with increasing polarity; 1,4-dioxan, chloroform, acetone, dimethylformamide, ethanol and water. The extractive yield ranged from 0.62 – 12.62%. The antibacterial activity of all the extracts was determined by agar disc diffusion method. Maximum antibacterial activity was observed against Gram negative bacteria. The resistant Gram negative strains were *C. fruendii*, *E. aerogenes*, *E. coli*, *P. vulgaris*, *P. aeruginosa* and *S. typhimurium*. Amongst Gram positive bacteria, *M. flavus* was the most susceptible bacteria and *B. subtilis* was most resistant. The moulds showed better antifungal activity than yeast. *A. niger* was the most resistant fungal strain. The best antimicrobial activity was with 1,4-dioxan extract and the least activity was with petroleum ether extract. The inhibitory effects of the extracts were comparable with the standard antimicrobics used. This work has highlighted the antimicrobial effects of fruit rind of *Trapa natans* L. on some of the medically important pathogens.**

Key words: *Trapa natans* L, antimicrobial, pathogenic microorganisms, polar solvents, non-polar solvents.

INTRODUCTION

The quest for plants with medicinal properties continues to receive attention as scientists survey plants for a complete range of biological activities, which range from antibiotics to antitumor. Natural products from some plants, fungi, bacteria and other organisms continue to be used in pharmaceutical preparations either as pure compounds or as extracts. There is a great variety of compounds that can be extracted and characterized from plants. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity (Pamplona-Roger, 1999, Manna and Abalaka, 2000). Furthermore, the active components of herbal remedies have the advantage of being combined with many other Substances that appear to be inactive.

In light of the recent emergence of bacteria which are resistant to multiple antimicrobial drugs posing a challenge for the treatment of infections (Service, 1995), the need to discover new antimicrobial substances for use in combating such microorganisms become pertinent. There is an urgency to find new drugs that can be less toxic to humans and also can be used for the treatment of many diseases. Thus there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000). Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seeds, fruit rind, etc (Gordon and David, 2001); i.e. any part of the plant may contain active components. *Trapa natans* L. belongs to the family Trapaceae; shingoda is its local Indian name. The fruits of *Trapa* are sweet, astringent, cooling, diuretic and tonic. They are used in dyspepsia, haemorrhages, diarrhea, dysentery, etc (Anjaria et al., 2002). However, there are hardly any reports on the antimicrobial activity of fruit rind. Therefore, it was thought of interest to study the efficacy of *T. natans* fruit rind in combating the multi drug resistant microbial strains. The fruit rind of *T. natans* was evaluated for antimicrobial activity against some medically important microbial strains.

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MATERIALS AND METHODS

Plant material

T. natans L. fruit was collected from local market of Rajkot, India. The taxonomic identity of this plant was confirmed by Dr. N. K. Thakrar Department of Biosciences, Saurashtra University, Rajkot, India. The fresh fruits were collected locally, rind was separated and then air dried. The dried plant material was homogenized to fine powder and stored in airtight bottles.

Extraction of plant material

The air-dried, powdered plant material (10 g of each) was extracted with 100 ml each of petroleum ether, 1,4-dioxan, chloroform, acetone, dimethylformamide, ethanol and water, kept on a rotary shaker for 24 h. Thereafter it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and evaporated to dryness to give the dried crude extract. The extractive yield (%) of all the extracts is shown in Table 1.

Microbial strains

The test microbial strains investigated are listed in Table 1. All the bacterial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth and maintained on nutrient agar slants at 4°C while fungal strains were grown in Sabouraud broth and maintained on MGYP slants (yeast) and potato dextrose agar slants (mould) at 4°C.

Antimicrobial assay

A modified agar diffusion method (Bauer et al., 1966) was used to determine the antimicrobial activity. Molten Mueller Hinton agar No. 2 (HiMedia) was inoculated with microbial cell suspension (100 µl) and poured into sterile Petri dishes. Sterile filter paper discs of 7 mm diameter were impregnated with 20 µl extract solution equivalent to 500 µg of the each dried extract in 100% DMSO (dimethylsulphoxide) and air dried. Thereafter the discs were placed on the surface of the seeded agar plates. Piperacillin (100 µg/disc), gentamicin (10 µg/disc) and amphotericin B (100 units/disc) were used as positive controls. Paper discs loaded with 20 µl of DMSO served as negative control. The plates were incubated at 37ºC for 24 h for all the bacterial strains while that of fungal strains were incubated at 28ºC for 48 h. The experiment was done three times to minimize error. After incubation period the antibacterial activity was evaluated by measuring the inhibition zones. An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antibacterial activity.

RESULTS AND DISCUSSION

The antimicrobial activities of the plant extracted in different solvents varied greatly because there are many factors that influence the active principle present in the plant. Here the polarity of the extracting solvents was different and it greatly influenced the antimicrobial property (Table 1). This is in agreement with our earlier reports (Parekh et al., 2006). The extractive yield of different solvents is given in Table 1. Maximum yield obtained was with dimethylformamide (12.62%) while minimum was with petroleum ether (0.62%) (Table 1). Traditional healers use primarily water as solvent but in our studies we found that the plant extracts, extracted in different organic solvents showed profoundly distinct antibacterial activity than aqueous extract. These observations can be rationalized in terms of the polarity of the compound being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the media used in the assay.

The activity of the plant against both Gram positive and Gram negative bacteria and some fungal strains may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Generally Gram negative bacteria are more resistant than Gram positive bacteria (Rabe and van Staden 1997; Kelmanson et al., 2000; Parekh et al., 2005) but the result of this present work is different. Here the maximum antibacterial activity was seen against Gram negative bacteria (Table 1). *P. aeruginosa, P. vulgaris* and *P. pseudoalcaligenes* were completely resistant while best antibacterial activity was shown against *P. putida* followed by *P. testosterone* and *P. morganii* respectively while *P. mirabilis* was inhibited by 4 of the solvents only. Amongst Klebsiella strains, *K. pneumoniae* showed good antibacterial activity with all the solvents while *K. aerogenes* showed slightly less antibacterial activity. The resistant strains were *C. fruendii*, *E. aerogenes*, *E. coli*, *P. vulgaris*, *P. aeruginosa* and *S. typhimurium*. Amongst Gram positive bacteria, *M. flavus* was the most susceptible bacteria and *B. subtilis* was the most resistant. All others showed intermediate effects. The moulds showed better antifungal activity than yeast. Maximum activity was shown by *A. candidus* followed by *M. hiemalis*. Except *T. beigelii*, none of the yeast showed any antifungal activity. *A. niger* was the most resistant fungal strain.

Amongst the 7 solvents used water and petroleum ether extracts showed minimum activity, most of the microbial strains being resistant. The antimicrobial activity increased as the polarity of the solvents increased. Maximum antibacterial and antifungal activity was with 1, 4-dioxan extract.

This work has highlighted the antimicrobial effects of fruit rind of *T. natans* on some of the medically important pathogens. Some antibiotics have become almost obsolete because of the problem of drug resistance (Ekpendu et al., 1994) and the consequence of drug resistance implies that new drugs must be sought for and to treat diseases for which known drugs are no longer useful. The results of the antibacterial and antifungal activity of the different extracts of *T. natans* were compared with the standard antimicrobics (Table 1). The extracts showed significant activity against more than 50% of the investigated microbial strains, which is in fact a promising result, which was also comparable with standard antimicrobics. It is interesting to note that the extracts are not pure compounds and in spite of it, good results were obtained which only suggests the potency of these extracts. Hence

Table 1. Antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents.

Microbial Strains	Inhibition Zone (mm)[a]							Antimicrobics[d]		
	Trapa natans L. Extracts (500 µg/disc)[b] [Extract yield in %[c]]									
	TPe	TDi	TCi	TAc	TDf	TEt	TAq	Pc	G	Ap
	[0.62]	[1.87]	[2.45]	[3.17]	[12.62]	[2.49]	[12.57]	(100 µg /disc)	(10 µg/disc)	(100 units/disc)
Gram-positive bacteria										
Bacillus cereus ATCC 11778	-	13.5	12	12	11.5	11	9	18	14	-
Bacillus megaterium ATCC 9885	-	10	-	9.5	-	-	-	12	32	-
Bacillus subtilis ATCC 6633	-	-	-	-	-	-	-	20	13	-
Corynebacterium rubrum ATCC 14898	-	12	-	11	-	-	-	24	21	-
Micrococcus flavus ATCC 10240	12	20	17	16	15	16	14	35	37	-
Staphylococcus aureus ATCC 25923	-	11	9	10	-	-	-	28	17	-
Staphylococcus aureus ATCC 29737	-	13	12	13	14	12	11	27	15	-
Staphylococcus epidermidis ATCC 12228	-	12	-	11	13	10	-	11	22	-
Staphylococcus subflava NCIM 2178	-	11	-	9	-	-	-	22	15	-
Gram-negative bacteria										
Alcaligenes fecalis ATCC 8750	-	13	-	11.5	11	10	-	10	18	-
Citrobacter fruendii ATCC 10787	-	-	-	-	-	-	-	12	12	-
Enterobacter aerogenes ATCC 13048	-	-	-	-	-	-	-	12	10	-
Escherichia coli ATCC 25922	-	-	-	-	-	-	-	13	25	-
Klebsiella aerogenes NCTC 418	-	13	11	11.5	10.5	9	-	17	17	-
Klebsiella pneumoniae NCIM 2719	13	21	13	13	11	10.5	10	25	22	-
Proteus mirabilis NCIM 2241	-	12	-	11	9	9	-	25	23	-
Proteus morganii NCIM 2040	13	18	12	11	11	12	9	19	28	-
Proteus vulgaris NCTC 8313	-	-	-	-	-	-	-	20	25	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	-	-	-	-	23	20	-
Pseudomonas putida ATCC 12842	10	19	11.5	17	16	15	10	45	35	-
Pseudomonas pseudoalcaligenes ATCC 17440								25	27	-
Pseudomonas testosteroni NCIM 5098	9	22	19	22	2	17	-	-	15	-
Salmonella typhimurium ATCC 23564	-	-	-	-	-	-	-	19	25	-
Yeast										
Candida albicans ATCC 2091	-	-	-	-	-	-	-	-	-	13
Candida albicans ATCC 18804	-	-	-	-	-	-	-	-	-	17
Candida glabrata NCIM 3448	-	-	-	-	-	-	-	-	-	18
Candida tropicalis ATCC 4563	-	-	-	-	-	-	-	-	-	12
Cryptococcus luteolus ATCC 32044	-	-	-	-	-	-	-	-	-	15
Cryptococcus neoformans ATCC 34664	-	-	-	-	-	-	-			16

Table 1. Contd.

Trichosporon beigelii NCIM 3404	9	10	9	9	-	-	-	-	-	15
Mould										
Aspergillus candidus NCIM 883	-	15	12	18	10	-	-	-	-	20
Aspergillus flavus NCIM 538	-	12	10	-	-	-	-	-	-	18
Aspergillus niger ATCC 6275	-	-	-	-	-	-	-	-	-	20
Mucor hiemalis wehmer NCIM 873	-	10	-	10	9	10	-	-	-	17

-: No activity. Negative controls did not show any activity.
[a]Inhibition zones are mean of three replicates including the diameter of the paper disc (7 mm).
[b]TPe-petroleum ether, TDi-1,4 dioxan, TCl-chloroform, TAc-acetone, TDf-dimethylformamide, TEt-ethanol, TAq-aqueous.
[c]Percentage extract yield (w/w) was estimated as dry extract weight/dry material weight × 100.
[d]Pc-Piperacillin, G-Gentamicin, Ap- Amphotericin-B.

T. natans L. fruit rind could be used as a guide in our continuing search for new natural products with potential medicinal properties.

The potential for developing antimicrobial from higher plants is rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic compounds. The results obtained are encouraging as the organic solvent extracts have shown considerable antibacterial activity.

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