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RESEARCH ARTICLE

SCREENING OF MARINE GASTROPOD CYPRAEA ARABICA FOR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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ABSTRACT

Among marine invertebrates, mollusc is one of the potential source of bioactive substances. The number of natural products isolated from marine organisms increases rapidly and now exceeds. The antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents. In the present study a total of 4 extracts from *Cypraea arabica* was assayed for its antimicrobial activity against ten bacterial and six fungal pathogens. Among all the four fractions fraction 3 (Benzene : Methanol) showed maximum level of inhibition zone 18mm against *Salmonella typhi*. The results of maximum inhibitory concentration revealed that among the concentrations, 100mg/ml concentration showed maximum activity and 1mg/ml concentration showed minimum activity. Crude methanol extract showed only negligible activity whereas column fractionated extracts showed significant antifungal activities. F_3 fraction of *Cypraea arabica* developed maximum zone of inhibition (14mm) against *Candida albicans*.

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INTRODUCTION

More than 70% of the earth's surface is covered by the ocean and it has become the biggest resources for the discovery of chemotherapeutic agents (Chau *et al*., 2005). Emergence of new diseases and increasing incidence of bacterial resistance has necessitated the mankind to look constantly of new alternative source of medicines. Marine organisms have been the source of medicines and pharmaceuticals since ancient times, and over the ages several claims have been made for the use of various kinds of marine animal extracts. Marine invertebrates offer a source of potential antimicrobial drugs (Bazes *et al*., 2009). Molluscs are widely used in world research institution for various studies, but only recently they have been recognized as potential sources for antibacterial and antifungal substances. A number of molluscs were ranked very high in the priority list of species exhibiting antimicrobial activity. Studies of antimicrobial compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insights into bioactive compounds in marine molluscs (Sri Kumaran *et al*., 2011). In this view, the present study has been carried out to determine the antibacterial and antifungal activities of the marine gastropod *Cypraea arabica* from Gulf of Mannar coastal region.

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MATERIAL AND METHODS

Antibacterial activity

The specimens of *Cypraea arabica* used in the present study were collected from Gulf of Mannar coastal region of Thoothukudi during low tides from the sea. In the present study whole body tissue extract of *C.arabica* was used for the antibacterial and antifungal assays. The freshly collected samples were cleaned and washed with fresh sea water to remove all impurities. The shells were removed and the tissues were then dried in hot air oven at 56^0C for 48 hours and used for further studies. Dried tissues were soaked in 100% A.R. grade methanol for 10 days at room temperature. After filtration with Whatman No. 1 paper, the methanol extract was reduced by vacuum evaporation. The extract residue was resuspended in 20ml of 100% A.R grade methanol. The methanol soluble extracts were dried and solubilized in deionized water and stored at 0^0C for further use.

Crude methanol extract was fractionated by silica gel column chromatography with four solvent systems viz Hexane: Chloroform (F_1), Chloroform (F_2), Benzene: Methanol (F_3), and Distilled water (F_4) in the order of their polarity afford four fraction viz., F_1, F_2, F_3 and F_4. A known amount of extracts were taken and their organic solvents were removed by vacuum evaporation. Solids were dissolved in deionized water and concentration series of 1mg/m1, 10mg/ml and 100mg/ml

were prepared and used. Antibacterial activity of the extract of *Cypraea arabica* was determined against ten bacterial strains viz., *Pseudomonas aerogenosa, Bacillus cereus, Shigella flexneri, Vibrio cholerae, Salmonella typhi, Escherichia coli, Proteus vulgaris*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*. These pathogens were obtained from the Microbiology Department of Sri Paramakalyani College, Alwarkurichi. Nutrient broth medium was prepared and sterilized and autoclaved at 151b pressure for about 30 minutes. Ten bacterial species were inoculated in the nutrient broth and incubated at 28 ± 2^0C for 24hr. The autoclaved nutrient agar medium was transferred aseptically into sterile petridishes. The 24hr old bacterial broth cultures were inoculated by using a sterile cotton swab.

In vitro antibacterial assay was carried out by slightly modified disc diffusion technique (Gunthorpe and Cameron, 1987). Whatman No.1 Paper discs with 6 mm diameter were impregnated with a known amount of extract of *Cypraea arabica.* The impregnated discs along with the control (incorporated with solvent along) were kept at the centre of agar plates, seeded with test bacterial cultures. After incubation at room temperature for 24hr, the inhibition zones were measured with the outer side of the disc to inner side of the inhibition zone. The extracts showing broad spectrum activity were examined for minimum inhibitory concentrations by testing at different concentrations viz., 1mg/ml, 10mg/ml and 100mg/ml. Each activity of extract was tested thrice for confirmation of activity. The same procedure was adopted for crude as well as all the factions.

Antifungal activity

In vitro antifungal activity was determined using the techniques of Kelman *et al.,*2001. The fungal pathogens *Aspergillus niger, Fusarium moniliform, Trichoderma sp, Penicillium citrinum, Rhizopus sp* and *Candida albicans* were obtained from TNAU, Coimbatore. Pathogenic fungal strains were incubated in potato dextrose agar medium and incubated at 48 hours. *In vitro* antifungal activity of *Cypraea arabica* extract was determined in Czapex Dox agar using inoculums of 48 hours old culture of *Aspergillus niger*, *Fusarium moniliform, Trichoderma sp*, *Penicillium citrinium, Rhizopus* sp and *Candida albicans*. Fungal strains were gently swabbed on the surface of the sterile petridishes containing 20 ml Czapex Dox solidified nutrient agar with the help of a sterile cotton swab. The 20 ml of the crude methanol extract was pipetted out on a 6 mm sterile paper disc.

The solvent was allowed to evaporate and the disc was placed on the surface of sterilized and fungal seeded agar plate. Control disc was also placed with solvents to access the effect of solvent on pathogens. Areas of inhibited fungal growth were observed after 48 hrs. Antifungal activity was measured as diameter of zone of inhibition excluding the paper disc diameter. After initial screening the extracts showing broad spectrum were fractionated using normal phase silica gel 160-120 mesh (Glaxo; Bombay) column chromatography by using the solvents viz., Hexane: Chloroform(F_1), Chloroform (F_2), Benzene: Methanol (F_3) and distilled water(F_4). The fraction thus obtained were once again evaporated, concentrated and assayed for antifungal activity. The extracts showing broad spectrum activity was examined for MIC by testing at different concentrations viz., 1mg/ml, 10mg/ml, 100mg/ml.

RESULTS

In the present study, a total of 4 extracts from *Cypraea arabica* was screened against 10 human pathogenic bacterial strains for antibacterial activities. F_1 (Hexane : Chloroform) fraction of the *Cypraea arabica* extract showed activity with the inhibition zones ranging from 1 mm to 8mm. Among the three concentrations, 100 mg/ml showed maximum activity 8mm against *Salmonella typhi* and at 1mg/ml concentration minimum activity was exhibited by *Staphylococcus aureus* (1 mm) (Fig.1). F_2 fraction (Chloroform) of *Cypraea arabica* developed maximum zone of inhibition 14mm against *V.cholerae* and minimum zone of inhibition 1mm against *P.aerogenosa* and *S.flexneri* (Fig.2).

Among all the four fractions fraction 3 (Benzene : Methanol) recorded highest level of inhibition zone 18mm against *S.typhi* and lowest level of inhibition zone 1mm against *K.pneumoniae* (Fig.3). Fraction 4 (Distilled water) of *C.arabica* showed activity with the inhibition zones ranging from 2mm to 15mm. At 100mg/ml concentration F_4 developed the inhibition zones of 15mm against *S.flexneri* and at 1mg/ml concentration the distilled water extract showed minimum activity (2mm) against *B.cereus* and *P.vulgaris* (Fig.4). The results of maximum inhibitory concentration revealed that among the concentrations, 100mg/ml concentration showed maximum activity and 1mg/ml concentration showed minimum activity. Antifungal activity of F_3 fraction of *Cypraea arabica* are presented in Fig (5 - 8). Crude methanol extract showed only negligible activity whereas column fractionated extracts showed significant antifungal activities. F_1 (Hexane : Chloroform) fraction of *C.arabica* showed maximum activity of 11 mm against *Candida albicans* and minimum inhibition zone (1mm) was developed against *F.moniliform* and *Rhizopus* sp (Fig.5).

F_2 (Chloroform) fraction of *C.arabica* developed inhibition zones varied from 1mm to 8mm. At 100mg/ml concentration maximum inhibition zone (8mm) was developed against *C.albicans* and at 1mg/ml concentration minimum activity (1mm) was recorded against *A.niger*, *F.moniliform*, *P.citrinum* and *Rhizopus sp* (Fig.6). Of the 4 fractions, F_3 (Benzene : Methanol) fraction of *C.arabica* developed maximum zone of inhibition (14mm) against *C.albicans* and minimum inhibition zone (2mm) against *F.moniliform* and *P.citrinum* (Fig.7).

At 100mg/ml F_4 (Distilled water) fraction of *C.arabica* developed maximum inhibition zone (7mm) against *C.albicans*. At 10mg/ml concentration F_4 extract of *C.arabica* developed 4mm zone of inhibition against *C.albicans* and minimum zone of inhibition 1mm against *F.moniliform*. At 1mg/ml concentration maximum zone of inhibition 2mm was observed in *T.rubrum* and *C.albicans* and minimum inhibition zones of 1mm against *A.niger*, *F.moniliform*, *P.citrinum* and *Rhizopus sp* (Fig.8).

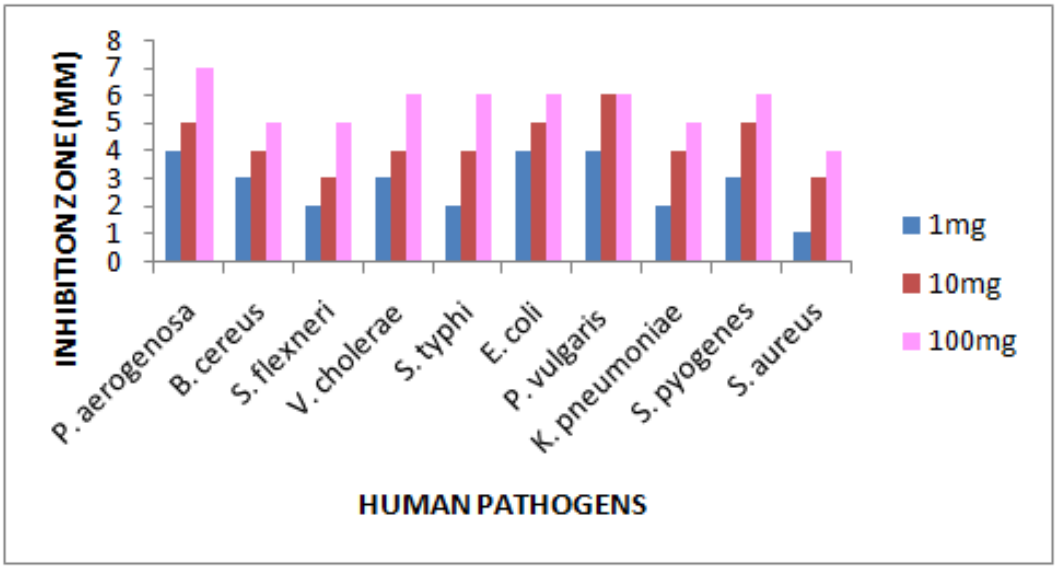

Fig. 1. Antibacterial activity of (F_1) Hexane: Chloroform extract of *Cypraea arabica*

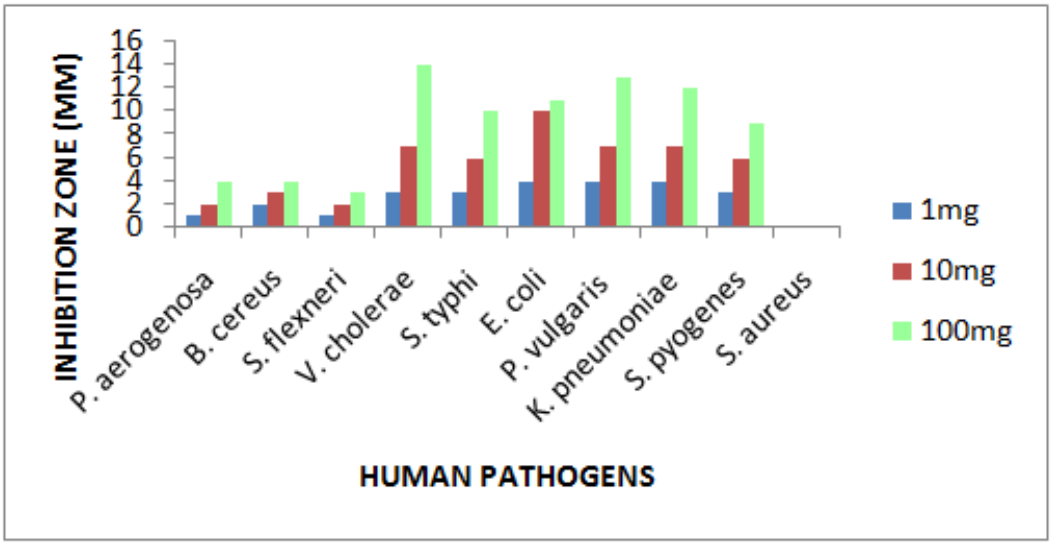

Fig. 2. Antibacterial activity of (F_2) chloroform extract of *Cypraea Arabica*

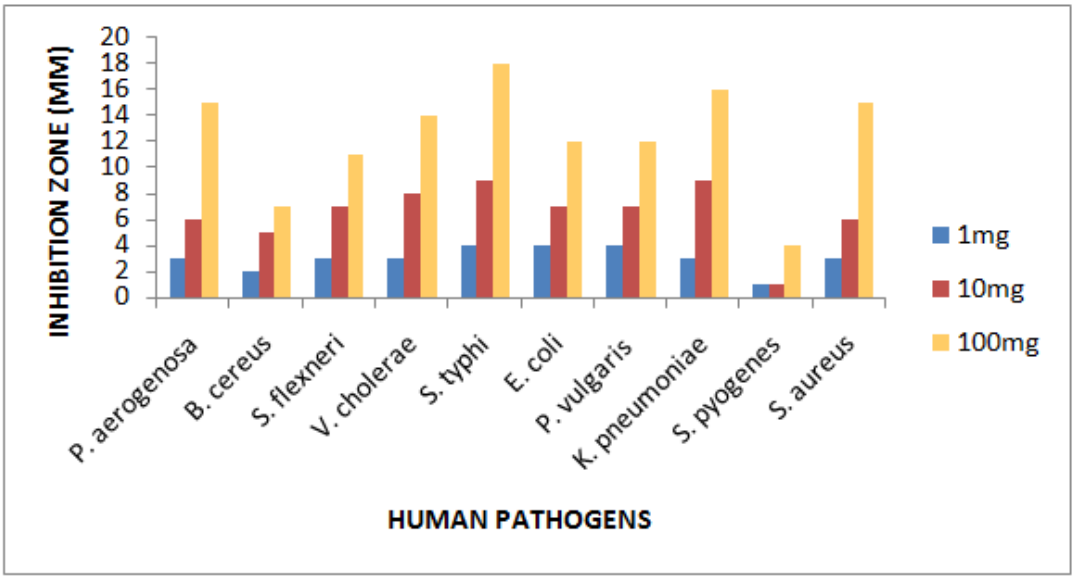

Fig. 3. Antibacterial activity of (F_3) Benzene: Methanol extract of *Cypraea arabica*

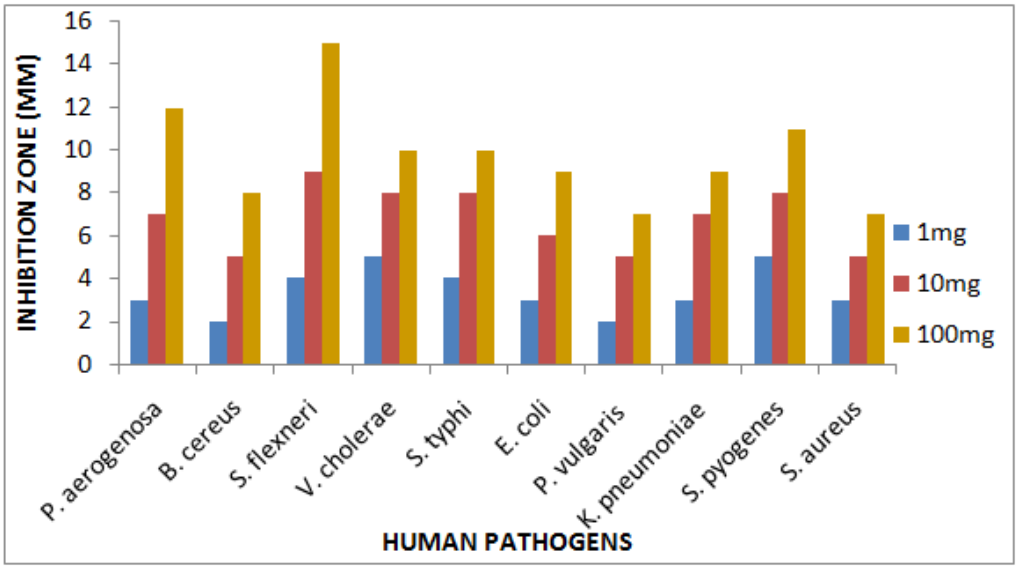

Fig 4. Antibacterial activity of (F_4) Distilled water extract of *Cypraea arabica*

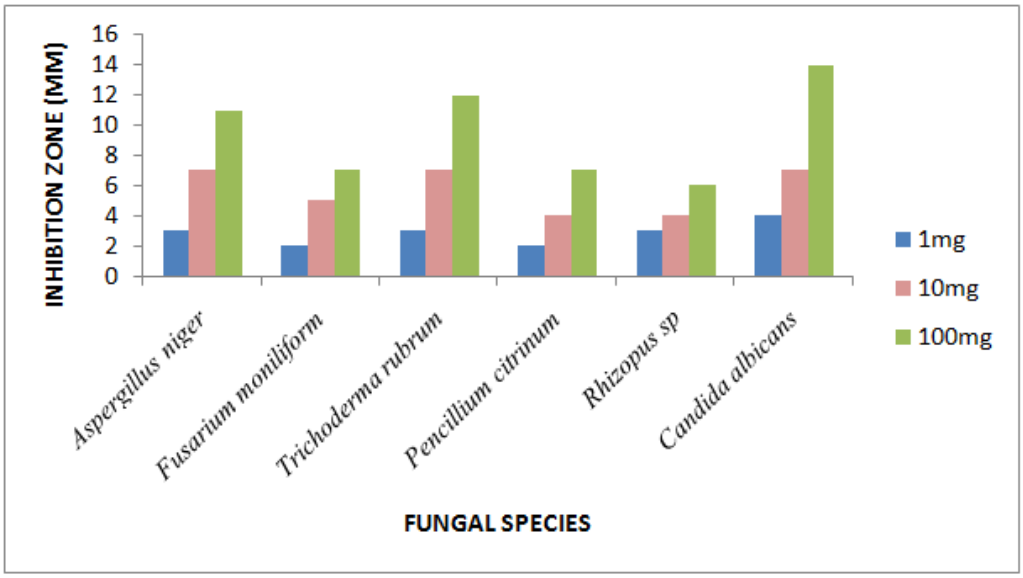

Fig. 5. Antifungal activity of (F_1) Hexane: Chloroform extract of *Cypraea arabica*

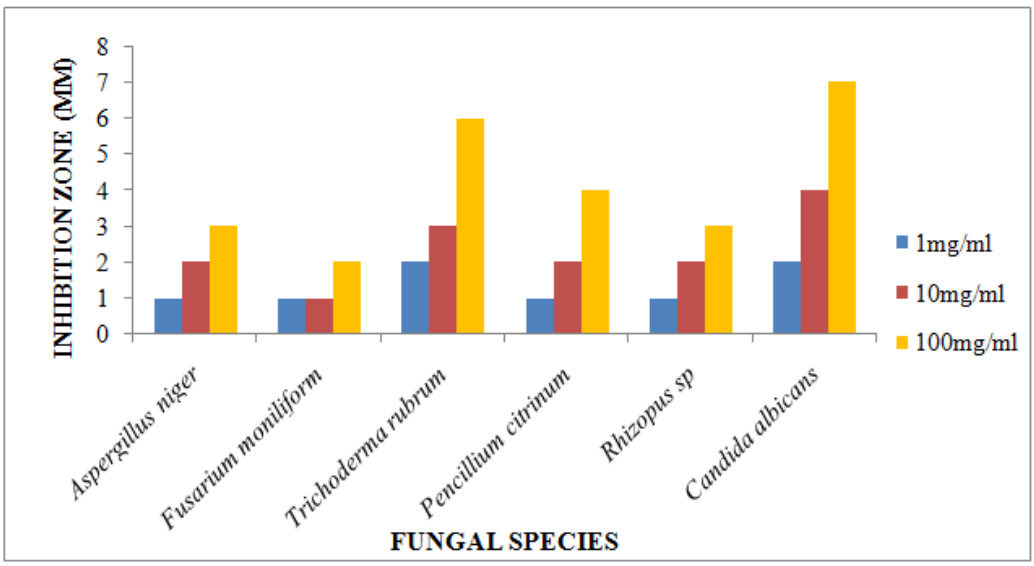

Fig. 6. Antifungal activity of (F_2) Chloroform extract of *Cypraea arabica*

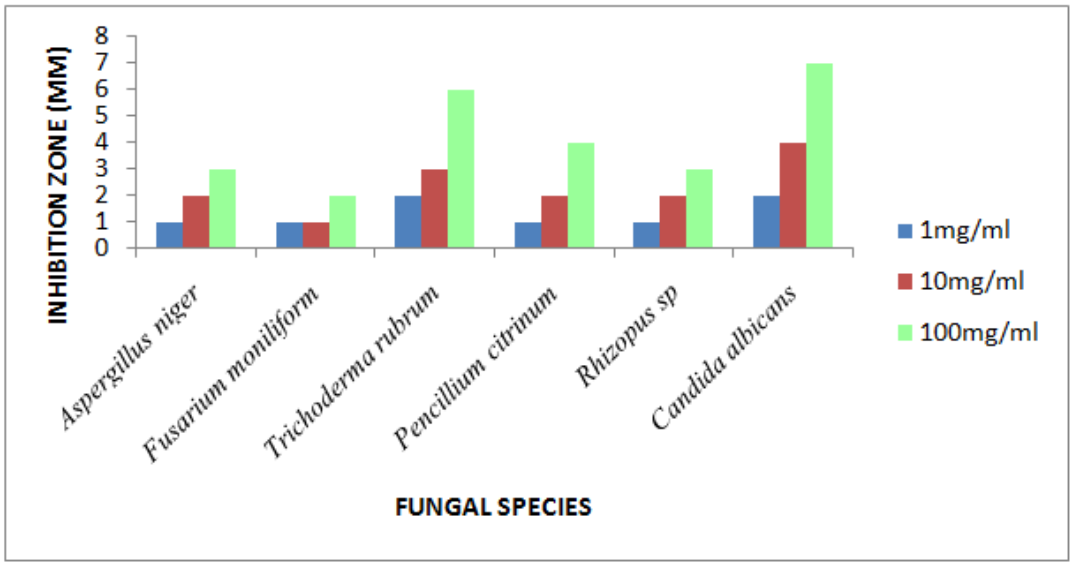

Fig. 7. Antifungal activity of (F_3) Benzene: Methanol extract of *Cypraea arabica*

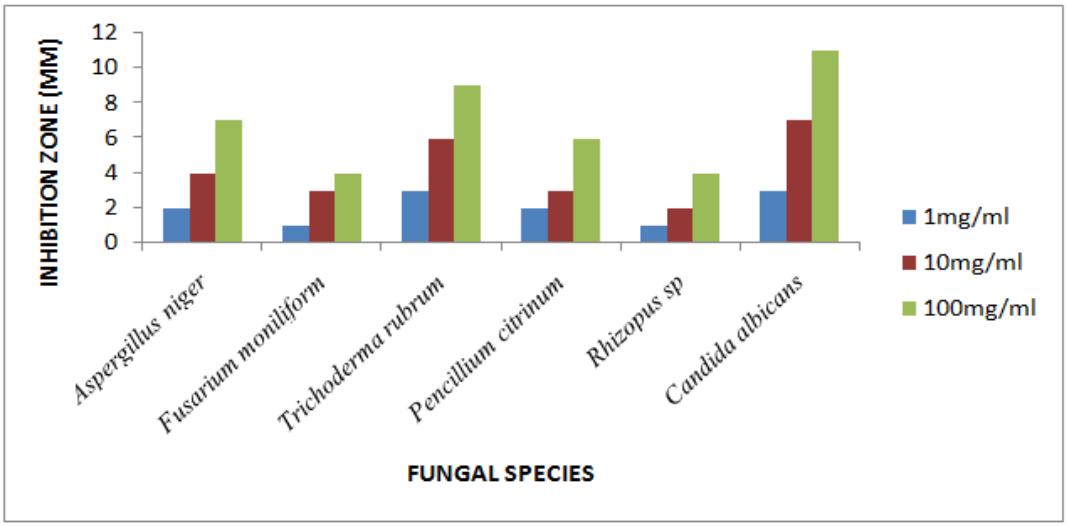

Fig. 8. Antifungal activity of (F_4) Distilled water extract of *Cypraea arabica*

DISCUSSION

Marine invertebrates, which develop in a different environment from terrestrial animals, are the source of a broad range of pharmacological substances. They either express constitutively or the expression is induced by exposure to pathogens (Sri kumaran *et al.,* 2011). By the virtue of such excellent property marine organisms are of interest in terms of pharmaceutical potentials particularly invertebrates (Keivan *et al.,* 2007). A lot of structurally and pharmacologically important substances have been isolated from marine gastropods with novel antimicrobial, antitumour and anti-inflammatory properties (Bhadury and Wright, 2004).

Numerous pathogenic microorganisms have developed their resistance to commonly available antibiotics; hence the need for developing new virulent drugs against these harmful pathogens becomes more important. Chemical drugs may lead to adverse effects and recent researchers have focussed on pharmacologically active compounds from natural sources. Extracts of marine molluscs exhibited antibacterial, antifungal and antiviral activities against fish and human pathogenic bacteria and the extract also may be applied in aquaculture (Defer *et al.,* 2009).

In the present investigation among the various strains tested, maximum zone of inhibition (18mm) was recorded in F_3 fraction against *S.typhi* and minimum zone of inhibition was recorded in F_4 fraction of *Cypraea arabica,* similar observation was made by Suresh *et al.* (2012) in *Sunetta scripta*. The hypobranchial gland extracts of *C.ramosus* was found to inhibit the growth of bacterial strains; among these the broad inhibition zone was formed against *Streptococcus faecalis* and *Staphylococcus aureus* (Kagoo *et al.,* 1992). The ethanol extracts of hypobranchial gland of *C.virgineus* showed 10 mm of inhibition zone against *S.typhi.* Distinct antibacterial activity of ethanol extract of *Hemifusus pugilinus* was observed against

almost all the pathogenic bacteria (Dhinakaran *et al.,* 2011). In the present study F_3 fraction of *Cypraea arabica* developed inhibition zones against all bacterial pathogens. Similar study was carried out by Jayaseeli *et al.,* 2001. They found antibacterial activity of four bivalves against few pathogens and the extracts showed significant activity against *Bacillus subtilis.* Antibacterial activity of some gastropods against *S.typhi* was reported by Rajaganapathy 1996, also supporting present study. Anand and Patterson Edward (2001) studied the antibacterial activities in ethanol extracts of gastropod *Babylonia spirata* and *Turbo brunneus* and observed highest activity against *E.coli, K.pneumoniae, P.vulgaris* and *S.typhi.* Very similar to the present study Annamalai *et al.,* 2007 noticed highest antibacterial activity with extracts of *Perna viridis* against *S.aureus* and *E.coli.* These results encourage the idea that marine molluscs are potent sources for antibacterial drug development.

In the present investigation a screening for antifungal activity of a crude and column fractionated extracts of *Cypraea arabica* were conducted. Prem Anand and Patterson Edward (2002) reported a moderate antifungal activity from the extract of various bivalve molluscs. Highest inhibitory activity was observed against *Aspergillus niger* with methanol extract of *Microcosmus curvus* (Karthikeyan *et al.,* 2009). Earlier, Bhosale *et al.* (1999) reported the strong antifungal activity of marine mollusc such as *Elysia grandifolia* against 3 fungal strains such as *A.fresenii, A.japonica* and *A.niger.* Lakshmi *et al.* (2010) studied on the antifungal activity of marine sponge *Heliclona exigna* against *Candida albicans, Cryptococcus neoformans* and *A.fumigatus.* Further she has purified the fungicidal compound as araguspongin C, which was responsible for the antifungal activity. Shanthi (2012) studied the antifungal activities of *P.persica* which inhibited *A.flavus, F.moniliform, A.terreus, Trichoderma sp, P.citrinum* and *P.oxallicum.* In the present investigation F_3 fraction of *Cypraea arabica* exhibited maximum antifungal activities. Due to its broad spectral activity *Cypraea arabica* is considered to be a promising antifungal source, which definitely expected to be a potential producer of new antibiotics.

Conclusion

The marine environment is a huge source for discovering many novel drugs. Apart from the food that is derived from the marine environment, wide varieties of drugs are being isolated and characterized with great promise for the treatment of human diseases. Antimicrobial compounds from natural sources would be the alternative to overcome the resistance problems. It is promising that the tested gastropod exhibited antibacterial and antifungal activities.

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