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ANTIFUNGAL ACTIVITY AND BIOMEDICAL IMPORTANCE OF MARINE GASTROPOD CONUS ACHATINUS GMELIN, 1791

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ABSTRACT

The present study for *in-vitro* evaluation of antifungal activity on crude and column fractions obtained from the whole body tissue extract of marine gastropod *Conus achatinus* against six fungal pathogens and to identify the most potent compounds. Molluscs are known to be a rich source of chemically diverse secondary metabolites and most of the active metabolites are derived from shelled gastropods. In the study the crude extract showed only negligible activity whereas column fractionated extract showed significant antifungal activities at three different concentrations. In this, F2 (Chloroform) and F3 (Benzene: Methanol) showed most potent activity than other fractions and developed zones of inhibition varied from 2 mm (*Rhizopus* sp) to 8 mm (*Trichoderma* sp). F3 developed 8mm zone of inhibition against *Candida albicans* and 2mm against *Rhizopus* sp. at 100mg/ml concentrations. These promising results reveal, the presence of alkaloid, amino, nitrogen, aldehyde, alcoholic, sulphur, nitrogen and steroid compounds by GC-MS analysis of whole body tissue of *Conus achatinus* which might be responsible for the antifungal activity.

Keywords: Antifungal, marine natural products, *Conus achatinus*, GC-MS analysis

INTRODUCTION

The number of natural products isolated from marine organisms increases rapidly, and now exceeds with hundreds of new pharmacological compounds with novel compounds being discovered every year (Putra and Muniarsih, 2016). Natural products have always been a major role to treat various kind of emerging diseases in accordance with the changed life style diseases, global climate change and its impact in the environment, in turn, the entry and survival of new pathogenic

microorganism in all kinds of ecosystems. Marine natural products have been shown to display antibacterial, antifungal, anticancer, antiviral, antiparasitic, anti-inflammatory activities (Kelman *et* al., 2001, Washida *et al.*, 2006; Kato, 2015 and Sun *et al.*,2017), and several pharmacological activities of benefit to humankind.

Most marine organisms produce metabolites in response to ecological stress such as competition for space and food, maintenance of unfouled surface, deterrence for predation to reproduce and live successfully. They exhibit unique physiological and structural characteristics by producing secondary metabolites which are enabling them to survive in hostile environment and are endowed with pharmacodynamic properties (Benkendorff, 2010). Innumerable micro organisms inhabit the benthic marine environment and thus these animals particularly the sessile forms are more vulnerable to microbial infections. To combat the microbial pressure, these animals have evolved chemical defense mechanisms by producing metabolites. In case of living marine surfaces, the colonization process can additionally be affected by organic metabolites produced by the host organism. The role of secondary metabolites as a chemical defense against epibiosis has been discussed by (Pawlik, 1993, Piel, 2009, Benkendorff, 2010 and Datta *et al*, 2015). Like other animals, antimicrobial activity has also been observed in the extracts of various species of gastropods by (Sugesh, 2010, Dolashka *et al.*, 2016 and Nightingale *et al.*, 2018).

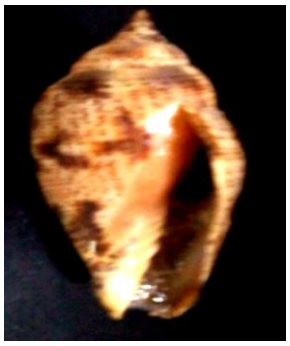
Finding out of new drugs is the need of the hour and hence an attempt is made in the present study for *in-vitro* evaluations of antifungal activity on column fractions obtained from the whole body tissue extract of a marine gastropod *C. achtinus* to identify and characterize the most potent compounds.

MATERIALS AND METHODS

a) Systematic Position

***Conus achatinus* Gmelin, 1791**

Phylum : Mollusca
Class : Gastropoda
Order : Neogastropoda
Super family : Conoidae
Family : Conidae
Genus : *Conus*
Species : *achatinus*



Conus achatinus were collected from Gulf of Mannar in Thoothukudi coastal region during low tides from the sea in its natural habitat. The freshly collected samples were cleaned and washed with sea water to remove all impurities. The shells were broken and the tissues were cut into small pieces with aseptic

scissors and then dried in hot air oven at 56ºC for 48 hours. Air dried materials were immersed in 100% AR grade methanol for 10 days in room temperature. The obtained extract was filtered using Whatman No.1 filter paper. The filtrate was taken in the petriplates and evaporated to dryness and the dried substance was used for further studies.

b) Fractionation

The crude methanol extract was fractionated by silica gel column chromatography with four different solvent systems. Elutions with Hexane: Chloroform (F1), Chloroform (F2), Benzene : Methanol (F3) and Distilled water (F4) in the order of their polarity afforded four fractions viz., F1, F2, F3 and F4. A known amount of extract was taken and their organic solvents were removed by vacuum evaporation. Solids were dissolved in deionized water in respect of the concentration series of 1mg/ml, 10mg/ml and 100mg/ml.

c) Sample Preparation

Specimens were collected from the Gulf of Mannar at Thoothukudi Coastal region during low tides from the sea in their natural habitat that is intertidal zone and from reefs by divers. The collected samples were brought to the laboratory, cleaned and washed with fresh sea water to remove all the impurities. The shells were broken using a hammer and the animals were carefully removed from the shells as coiled nature of muscle arrangement. After removing from the shell, the animals were cut into pieces and dried in hot air oven at 40˚C for 24hours. Dried tissues were soaked in 100% A.R. grade methanol for 10 days at room temperature. The extract from the solvent was filtered using Whatman No. 1 filter paper. The filterate was poured in petriplates and evaporated to dryness. For doing experimental works, different concentrations of extracts were prepared and stored at 0˚C for further use.

d) Antifungal Assay

In vitro antifungal activity was determined using the techniques of Kelman *et al*., (2001). The fungal pathogens such as *Aspergillus niger, Fusarium moniliform, Trichoderma* sp., *Penicillium citrinum, Rhizopus sp.* and *Candida albicans* were obtained from Tamil Nadu Agriculture University, Coimbatore. Pathogenic fungal strains were incubated in potato dextrose agar medium and incubated for 48 hours. *In vitro* antifungal activity of *Conus achatinus* extract were determined against Czapex Dox agar using inoculum of 48 hours old culture of all fungal pathogens. 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 and column fractionated 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 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 activity were fractionated using normal phase silica gel 160-120 mesh (Glaxo-Bombay) column chromatography with low polar to high polar solvent Hexane:Chloroform (F1), Chloroform (F2), Benzene : Methanol (F3) and Distilled water (F4). The fractions thus obtained were evaporated, concentrated and assayed for antifungal activity. The extracts showing broad spectrum activity was examined at different concentrations viz., 1mg/ml, 10mg/ml and 100mg/ml**.** One of the most potent fraction F3 (Benzene: Methanol) was characterized to know the functional groups through GC-MS study. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC 20i auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) at Indian Institute of Crop Processing Technology, Tanjore. Interpretation on mass spectrum GC-MS was conducted using the data base of National Institute of Standards and Technology (NIST) from Dr. Duke's phytochemical and Ethnobotanical databases.

RESULTS AND DISCUSSION

In the present investigation, crude and column fractionated extract of *C. achatinus* was tested against six fungal pathogens and are represented in Figures (1&2) and Plate (1). In this study, all the four fractions inhibited the fungal species namely *Aspergillus niger, Fusiarium moniliform, Trichoderma* sp. *Penicillium citrinum, Rhizopus* sp. and *Candida albicans*. Rocio Garcia-Rubio et al, (2020) reported that the fungi were more resistant to the tested compound, this could lead to the nature of fungal cell wall made up of chitin, which is relatively resistant, including microbial decomposition.

The crude extract showed negligible activity whereas the column fractionated extracts developed significant activity against *Trichoderms Sp, Penicillium citrinum*, *Fusiarium moniliform* and *Candida albicans* species. At 100mg/ml of Chloroform extract, maximum activity (8mm) was noticed against *Trichoderma* sp., and minimum 2 mm was developed against *Rhizopus* sp. F3 fraction developed maximum inhibition against *Candida albicans* and minimum 2mm against *Rhizopus* sp. *Candida albicans* remains the most common infection-causing fungus, about 45% of clinical fungal infections are caused by *C. albicans* Gupta *et al.,* (2004). Harekrishna Jana *et al.*, (2017) reported moderate antifungal activity from the extract of various bivalve molluscs. This study corroborates the present study.

In the present study, *C. achatinus* inhibited the fungal species namely *Aspergillus*

niger, Fusiarium moniliform, Tricoderma sp. *Penicillium citrinum, Rhizopus* sp., and *Candida albicans* at 100mg/ml concentrations. Santhi *et al.*, (2016) reported that the extract of *Purpura persica* inhibited *F. moniliform, Trichoderma sp., P. citrinum* and *A. niger* at 100ml/mg concentrations level. The fungal activity of *C. achatinus* was found to be high which may be due to species specific characteristics. Moreover these activities can depend upon the nature of solvent and compound extracted, different types of strains and different assays and methods used (Sugesh *et al.*, 2019).

In conclusion, F_2 (Chloroform) and F_3 (Benzene: Methanol) extracts showed significant antifungal activity against most of the tested pathogens. This could be attributed that the test extract might contain secondary metabolites like Aziridine, 1-methyl- (Alkaloid), Cyclohexanol, 2-amino-, trans (Amino), Azocine, octahydro- (Nitrogen), Butanol, O-methyloxime (Aldehyde), L- Homocitrulline (Nitrogen), Pentanol, oxime (Aldehyde), E-2-Tetradecen-1-ol (Alcoholic), Z-10-Pentadecen-1-ol (Alcoholic), Trimethylamine, compd. with borane (11) (Amino), 1,1-Cyclopropane dicarbonitrile, 2,2-dimethyl- (Nitrogen), Cyclopentanol, 2-(aminomethyl)-, cis- (Amino), Tricyclo [4.2.1..1 (2,5) decan-3-ol (Alcoholic), Thimorpholine (Sulphur), Semioxamazide (Amino) and Cholan-24-oic acid, 3-oxo-, methyl ester, (5a)- (Steroid) are the compounds which inhibit the fungal growth and they have been recognized as potential sources of antifungal substances.

Fig. 1. Antifungal activity of F2 fraction of *Conus achatinus*

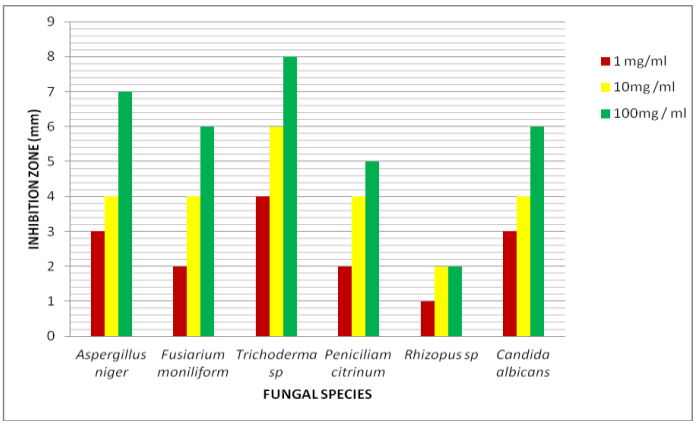


Fig.2. Antifungal activity of F3 fraction of *Conus achatinus*

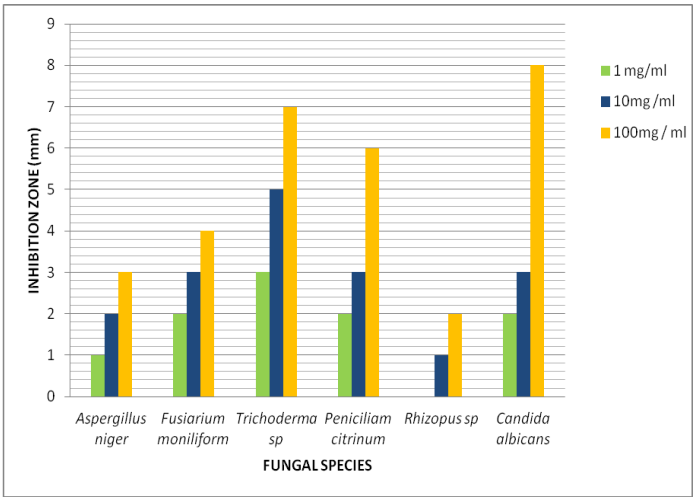
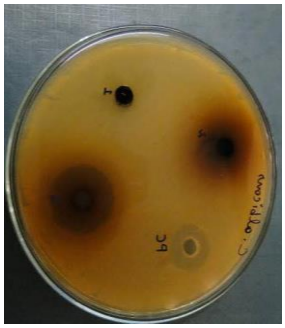
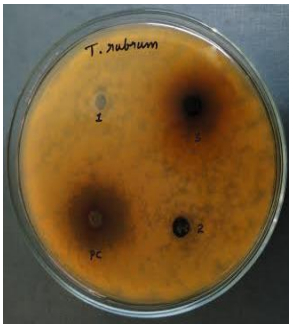


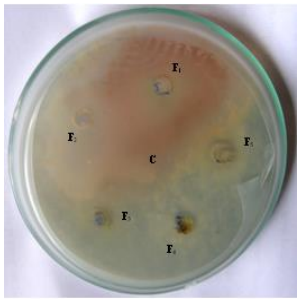
Plate 1. Antifungal activity from the extract of marine gastropod *Conus achatinus*



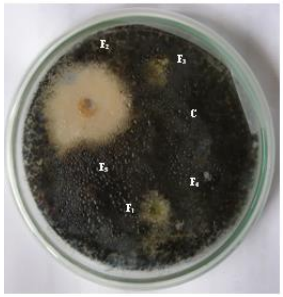
Candida albicans



Trichoderma sp.



Fusiarium moniliform



Aspergillus niger

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