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RESEARCH ARTICLE

EVALUATION OF BIOLOGICALLY ACTIVE MOLECULES ISOLATED FROM OBLIGATE MARINE FUNGI

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ABSTRACT

The marine environment is a tremendous source of natural products. Marine microorganisms have become an important source of pharmacologically active metabolites Fungi are well known for their vast diversity of secondary metabolites that include many life-saving drugs and highly toxic mycotoxins. In general, fungal cultures producing such metabolites are immune to their toxic effects. However, some are known to produce self-toxic compounds that can pose production optimization challenges if the metabolites are needed in large amounts for chemical modification. Objective: The main objective of the present study was the isolation of new and preferably biologically active secondary metabolites from marine microorganisms, especially marine-derived fungi. Method: Marine fungi had isolated from marine soil by serial dilution method from Rose Bengal medium. Single colony was isolated by microscopic and macroscopic observation. Secondary metabolites are produced by marine fungi. Biological evaluation was performed by microbial studies. TLC is performed to identify the number of sub compounds in the crude extract. Further species level identification and structure elucidation of the compound are to be done. Results: The isolated marine fungi *Aspergillus sp*, showed maximum activity against the *Candida rugosa* with a zone diameter of 16mm at a concentration of 200µg and for bacterial strains it showed maximum activity against the E.coli with a diameter of 24mm at a concentration of 200µg. From the thin layer chromatography it has nearly 2-3 compounds to be purified. Conclusion: The selected organism which produces the compounds contains the biological activities which include anti-bacterial and anti fungal activities.

Keywords: *Aspergillus sps* fungi, crude extract, biological activities.

INTRODUCTION

Marine microorganisms have become an important source of pharmacologically active metabolites. More specifically, fungi from the marine environment have shown great potential as suggested by the diversity of secondary metabolites. The intent of this work is to summarize the new marine-derived fungal metabolites. Fungi in the marine environment and taxonomic considerations of marine-derived fungi are intended to be introductory. In addition, most fungi will be referred to as “marine-derived” since the marine ecosystem is comprised of fungi that are obligate fungi and facultative species. A more detailed discussion about marine fungi and obligate marine species has been presented by Kohlmeyer and Kohlmeyer, 1979. Briefly, obligate marine fungi grow and sporulate in a marine environment while facultative marine fungi are from terrestrial or freshwater habitats, but may also grow in the marine environment [1]. Over 75% of the identified fungi belong to the monophyletic ascomycota which is the most important and diverse phylum [2].

Bioactive compounds from marine fungi have extensive use in the treatment of many diseases and these compounds act as the templates for synthetic modification. Several molecules isolated from various fungi are currently involved in the advanced stage of clinical trials. From the fungi Aspergillus sp, the biologically active molecules show strong antibiotic, analgesic and anti-inflammatory properties[3]. Some of the *Aspergillus sps* produced the secondary metabolites are Aspergillus *niger, Aspergillus oryzae*, are important production organisms used in industries. Many secondary metabolites produced by selected strains of these fungi are capable of eliciting toxicity in animals. The Secondary metabolites produced by *A. niger* are Naphtho-r-pyrones, Nigerazine B, Nigragillin, Ochratoxin A. and *A. oryzae* are Aspergillomarasmine, Cyclopiazonic Kojic acid , Maltoryzine , Naphtho-r-pyrones[4]**.** The present study describes the antimicrobial ability for the crude extracts against the Gram positive and Gram negative bacterial and fungal organisms.

MATERIALS AND METHODS

The marine soil sample is collected from surya lanka Beach, Guntur. District, Andhra Pradesh, India.

Test organisms

Bacteria used: *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Pseudomonas aurogenosa, Klebsiella pneumonia.*

Fungi used: *Candida albicans, Candida rugosa, Saccharomyces cerevisiae,*

Media Used

Potato Dextrose Broth, Potato Dextrose Agar, Nutrient Broth, Rose Bengal chloramphenicol, Agar:

METHODOLOGY

Soil Sample Collection:

In the collected samples, first marine water sample is collected into sterile water bottle The samples were stored carefully and capped tightly till they reached the laboratory. The samples were processed using serial dilution method in the laboratory.

Isolation of Fungi from Soil using serial dilution method:

Weigh about 2 g of soil sample in a 500ml conical flask containing 200ml sterile distilled water. Keep the conical flask on obituary shaker for 30 min at 200rpm. Prepare about 250ml Rose Bengal medium and sterilize it at 121 °c, 15lbs pressure. After cooling down pour it into Petri plates leave it for some time for solidification. The soil solution (100µl) from the conical flask was spread over the Petri plates with Rose Bengal medium. Incubate the plates at 25°C -27°C for 24-72 hrs. After incubation, check for the individual colonies and subculture unique and isolated colonies onto the potato dextrose agar slants.

Preparation of Rose Bengal Media

Weigh 32gm of Rose Bengal medium into a conical flask of 1000 ml of distilled water.

Microscopic observation

After incubation of slide culture, slides are stained with Lactophenol blue for microscopic examination. First the slides are observed

under light microscope at 10x resolution and then at 45x and 100x (oil immersion) for morphology of fungi.

Extraction of Secondary Metabolites

Preparation of Potato Dextrose Broth Media: Weigh 24 gm of potato dextrose broth Hi-media and it was dissolved in 1000 ml of distilled water. Sterilization: This prepared media was sterilized in an autoclave at 121°C, 15 lbs pressure for 15-20min. Production of the isolated fungi: Inoculate the pure culture of *Aspergillus sps* in the sterilized media and the culture flasks were incubated at 27°C for 15 days. Extraction of Secondary Metabolites: After incubation ethyl acetate was added to the culture flasks and kept for 5 hr. Minimal shaking is required for dissolving the metabolites into ethyl acetate solvent.

Separation of Metabolites

The metabolites which are now dissolved in ethyl acetate solvent are separated by using separating funnels. In the separating funnel add the media with ethyl acetate. To that add some amount of ethyl acetate, shake well and allow it to settle for few minutes. Later two layers were observed in the separating funnel. The bottom layer is Broth Layer which is discarded and the upper layers of ethyl acetate with metabolites are collected which is called the organic layer. The washes were repeated for three times to extract the complete metabolites. This separated extract was Rota vapored for the collection of crude extract.

Antibacterial Activity

The *in vitro* antibacterial activity of the collected crude extract was studied against the bacterial strains, viz., Gram-positive organism's viz. *Bacillus subtilis, Staphylococcus aureus, Streptococcus. epidermidis* and Gram-negative organisms viz *Escherichia coli*, *Pseudomonas aurogenosa*, *klebsiella pneumoniae* by agar cup diffusion method[5]. The ready-made Nutrient agar (NA) medium (Himedia, 39 g) was suspended in distilled water (1000 ml) and heated to boiling until it is dissolved completely. The medium and Petri dishes were autoclaved at pressure of 15 lb/inc2 for 20 min. Agar cup bioassay was employed for testing antibacterial activity**.** The medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates is solidified, 0.5 ml of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving in DMSO and different concentrations were made. After inoculation, cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, different concentrations of test solutions were added. Controls were maintained with DMSO. The plates were kept at 37^0 C for 48 h. Inhibition zones were measured and the diameter was calculated in millimetres.

Antifungal activity

The in vitro antifungal activity of the collected crude extract was studied against the fungal strains, viz., *Candida albicans*, *Candida rugosa,* Saccharomyces *cerevisiae*, and by agar cup diffusion method and the strains were obtained from the Institute of Microbial Technology, Chandigarh. Controls were maintained with DMSO. The treated and the controls were kept at 27^0 C for 48 h. Inhibition zones were measured and the diameter was calculated in millimetre

RESULTS

Macroscopic Observation

Colonies on potato dextrose at 250C are olive to lime green. Rapid growth was observed .Texture is wooly to cotton and also appears as granular. Sclerotia were present and appears as dark brown**.**

Macroscopic Observation

Conidial heads are radiate and loosely columnar with age. Conidiophores are coarsely roughened, uncolored. Conidia are smooth and measures 3-6 mm in diameter.

Antimicrobial activity

Table 1: Antibacterial activity.

Organisms	50 µg	100 µg	150 µg	200µg
Control (DMSO)	--	--	--	--
S. aureus	16	17	18	18
S. mutant	20	22	24	24
L. casei	17	18	20	22
Lactobacillus acidophilus	13	13	14	14
Enterococcus faecalis	15	16	16	16
Bacillus megatisium	19	20	20	22
E. Coli	20	22	22	24

Aspergillus crude extract is characterized by antibacterial activity. Crude extract is dissolved in DMSO. Crude extract is prepared in 50µg, 100µg, 150µg and 200µg and tested against S*. aureus, S. mutant, L. casei, Lactobacillus acidophilus, Enterococcus faecalis, Bacillus megatisium* and E. *coli*. At a concentration of 150µg good activity was found against *S.mutants* (zone of diameter is 24 mm) and at 200 µg zone of diameter is 24 mm was observed against *S.mutants* and *E.coli.*

Table 2: Antifungal activity.

Concentration	*C.rugosa*	*C. albicans*	*S.cerveceae*	*R. oryzae*
control	--	--	--	--
50 µg	12	14	10	12
100 µg	12	20	10	14
150 µg	14	20	11	15
200 µg	16	16	14	16

Measurement of Zones for Anti Fungal Activity

The above tables show the activity of crude extract against different fungi. The best activity was observed against *Candida rugosa* at a concentration of 200µg (zone diameter is 16), which shows that the crude extract showed good activity against *Candida rugosa.* The best activity against *Candida albicans* was observed at a concentration of 150µg (zone diameter is 20mm). For *Rhizopus oryzae* the best activity was observed at a concentration of 200µg (zone diameter is 16mm). For *Aspergillus niger* and *Saccharomyces cerevisiae* the best activity was observed at a concentration of 200µg (zone diameter is 11 and14mm)

Separation of Compounds by Thin Layer Chromatography (TLC)

TLC is performed with hexane and ethyl acetate solvent in different proportions. After performing in all proportions, among them crude extract showed 2-3 compounds in 6(Hexane): 4(Ethyl acetate). Hence the crude extract is known to have 2-3 sub compounds.

Figure 1 : Purification of crude by TLC plate.

DISCUSSION & CONCLUSION

The aim of our present study is characterization of novel secondary metabolites with antimicrobial activity against microbial pathogens. The marine soil sample was taken as source for the isolation of secondary metabolites with good biological activity. The Fungus *Aspergillus spp.* was isolated from soil source. So far, literature show only 12 species were identified. We carried out extraction of secondary metabolites from *Aspergillus spp. Aspergillus spp* was cultivated in 1-2 liters of sterilized potato dextrose broth and incubated for 10 days. After the incubation period, the broth was soaked in ethyl acetate for overnight and was extracted with ethyl acetate. The organic layer was separated and concentrated in vaccum using Rota vapour. The crude extract *of Aspergillus spp*. was studied for antibacterial and antifungal activities using agar well diffusion method was done. When compared these activities with the *Aspergillus sps* namely *Aspergillus awamori*. The crude extract was active against both fungi and bacteria. For fungal strains it showed maximum activity against the *Candida rugosa* with a zone diameter of 16mm at a concentration of 200µg and for bacterial strains it showed maximum activity against the E.coli with a diameter of 24mm at a concentration of 200µg. From the thin layer chromatography it has nearly 2-3 compounds to be purified. Further the crude extract can be purified by using the column chromatography. Our comparative study of *Aspergillus sps* with *Aspergillus awamori* revealed that it has totally suppressed the growth of *Staphylococcus aureus* and *Bacillus subtilis* with MIC values. This indicates that the *Aspergillus sps*. has a great potential to act against pathogenic bacterial and fungal strains. On the other side in the literature it showed that the *Aspergillus* sps namely *Aspergillus conicus* was tested against the human bacterial pathogens there it showed a moderate activity against those pathogens**.** In this present work this *Aspergillus sps* was tested against the bacterial and fungal strains where it showed good antibacterial and antifungal activities.

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