*Research Journal of Recent Sciences* _____E-ISSN 2277-2502
Vol. **5(10),** 36-38, October **(2016)** *Res. J. Recent Sci.*

***Short Communication***

# Evaluation of Antimicrobial Activities of Antibiotics from Marine-derived Fungi

**Siefeldeen Mohamed Hamed Mohamed Ahmed**
KMCH College of Pharmacy, Coimbatore-641046, Tamil Nadu, India
siefeldeenphar@gmail.com

**Available online at: www.isca.in, www.isca.me**
Received 30th April 2016, revised 18th September 2016, accepted 23rd September 2016

## Abstract

*Many microorganisms have been isolated and used as antibiotics against infections caused by other microorganisms because of their antimicrobial activities. Fungi are one of these microorganisms which have been isolated and evaluated for their antimicrobial activities. The objective of this study was to isolate fungi from marine sediments collected from the coastal regions of Ponnani and Chavakad of Kerala, India, and to evaluate their effects as antibiotics. Ten soil samples have been collected from different spots in the same area, serially diluted, maintained on starch casein agar slants by frequent sub-culturing and then spread on potato dextrose agar and nutrient agar media using streak plate method. Five isolates designated as AM1, AM2, AM3, AM4, and AM5 were evaluated for their antimicrobial activities. Out of the five isolates selected, only two strains AM2 and AM5 have shown good antibacterial activity against Staphelococcus aureus, Basilus subtilis, Salmonella typhi, and E.coli. The isolate AM5 exhibited more antibacterial activity than the isolate AM-2, and hence selected for further studies.*

**Keywords:** Fungi, Antibiotics, Zone of Inhibition, Antimicrobial Activity, Serial Dilution.

## Introduction

There are many sources for antibiotics, Fungi are one of them, it shows a variety of antimicrobial activities as most remarkable source for antibiotics. The Fungi Kingdom plays important roles in both term as ecological and economic .it is determined that about 1.5 million Fungi are present in the recent time throughout the world, but only 5% of these has been discovered[1].

Fungi are one of the major antibiotic-producing organisms, and one of the most diversified groups of organisms. the antibiotic have developed during the few past decades .In-fact in 1995 six of Fungi derived antibiotics reached the top 20 best selling medical drugs in the world[2].

Antibacterial is a class of antibiotics help in cure and prevent Micro-organism infection such as bacterial infection by destroying it or suppress its growth for other micro-organisms. For example fungi and protozoan which is very toxic for humankind as well as for animals.

Even thou when given as therapy quantity ,antibiotics play non-vital role against viruses as commonly as cold or infection , it may become harmful as cold or influenza and it may become harmful when it used inappropriate. The primary objective of this study was to evaluate the antimicrobial activities of fungi isolated from marine sediments collected from the coastal regions of Ponnani and Chavakad of Kerala, India.

## Materials and Methods

**Collection of Samples:** The samples were collected through core sampler from marine soil coastal regions of Ponnani and Chavakad in kerala, belongs to Indian nation. The character of the collected samples was brown to black in colored appearance and sandy texture.

**Media Used and Maintenance of Fungi:** Plenty of media were used in this study with different nutrient agar, potato dextrose agar, seed culture media were used for isolation, screening and optimization. The isolated has been maintained on starch casein agar slants through frequently sub-culturing[3].

**Monitoring of Activities Vs Bacterial Pathogens:** Medium as nutrient agar is prepared then allowed to pour into a sterile Petri plate, kept to solidify. The 24 hours old bacterial culture such as *Staphelococcus aureus, Basilus subtilis, Salmonella typhi, and E.coli* were swabbed to inoculate by plate method under aseptic condition. Then wells were made by the help of the sterile cork borer. The wells was measured in diameter as 0.9 cm to 1 cm, the difference concentrated of fungi filtrated culture were pour into, the different plate and incubated at room temperature, the activity of antibiotic was confirmed and measured by using clear zone of inhibition[1].

**Selection of Potential Fungi:** From the isolated fungi, sample number AM5 was selected to be screened for primary antibacterial and antifungal activity for analysis as well.

**Preparation of Seed Culture:** 10% of liter was taken from already prepared ,sterilized seed culture, heated then cooled and inoculated with 2 to 3 loops of fungi[4] . Then the flask kept in incubator shaker at maintained speed 200 rpm at 37ºC, for almost one week or day less, then the collected product used for further steps[1].

**Optimization of Metabolite Production:** The metabolite product optimized by taken 5 flasks of 250 ml with capacity of 100 ml of antibiotics isolated from produced medium, it was sterilized simultaneously. Then labelled by specific numbers from 1 to 5, date was mentioned too.

At the first day, flask inoculated with 1 ml to 1.5 ml of microorganism and allowed to stay for 24 hours in shaking incubator at room temperature. Then the similar procedure followed for the second, third, fourth and the fifth flask, then it was inoculated and kept in shaker incubator in sequence order, then after completion of the 10 days of continuously incubate with daily checking, the flask were harvested by filter 10 ml from each flask and 20 ml of ethyl acetate was added, then kept for half an hour in shaker at 100 rpm. It was mixed with highly spreader between the metabolite and solvent layer.

Then solvent layer collected in 5 different petri plates, evaporated by air dryer, then the metabolite collected the plate swirled with 100EL of metabolite with solvent, has been added to each individual plate and poured precisely into the medium wells present in the petri-plate, the medium contain nutrient agar, then it spread with this four microorganism *Staphelococcus aureus, Basilus subtilis, Salmonella typhi, and E.coli* one plate taken as control without metabolite. The plates were incubated till the end of this study duration 10 days and the vertex activity was found by zone of inhibition along with the exact day 8.

From the difference 10 days of optimization day number six ,has shown great activity .Then the sample were selected to produce the metabolites of antibiotics produced medium as it was form in various conical flask (150ml) Then it kept to autoclave .1% of seed culture of AM5 medium is inoculated in produced medium and incubated for 6 days under 28ºC , therefore the broth has become turbid, then it centrifuged at 10,000 for 20 minutes .the supernatant were isolated and double of the quantity of ethyl acetate was added and allowed to incubate at room temperature overnight, to continuously help in interchange the component of micro-organism solvent along with the compound layers. Then, the aqueous layer was collected. The aqueous layer was then transferred to Soxhlet apparatus.

However extraction proceeds took place. The condensate reactive phenomena occurred when solvent was separated and reservoir in well container. As result crude extracted was collected contain metabolites. The extract was separated using ethyl acetate to evaporate it, leaving behind the metabolites. The product was selected for addition process.

**Qualitative Analysis:** Assay for agar diffusion used to check and determine the antibacterial activity of the produced metabolite. The method succeed well in producing antibacterial agent. In the meanwhile, if the monitored extract contain an anonymous substance the result my lead to presence of false positive or negative output, sometimes factors may involved in causing problems. For example factor like, temperature, pH etc. Nutrient agar prepared was poured in 1 Petri dish for 24 hours growing culture *Staphelococcus aureus, Basilus subtilis, Salmonella typhi, and E.coli*, they were swabbed on the surface of the cultures. The culture were poured and walls made using cork borer, the different concentration of metabolite kept in the walls, incubated for 24hrs under 37ºC after that inhibition zone shown and was measured in diameter[1].

## Results and Discussion

**Screening of Fungi:** From the 5 fungi isolates (AM2 and AM5) exhibited antifungal activity for the production of fungi, this two isolates of fungi had pin point colonies with zone of inhibition and it is shown in Figure-1.

**Examination of Fungi Activity Host (Human) Bacterial Pathogen:** The isolated fungi (AM2 and AM5) produced antimicorganism activity against *Staphylococcus aureus, E.coli, Salmonella typhi, Bacillus substilis.* The antimicroganism activity was found to be vertex in the 5th isolated fungi. Then it was identified.

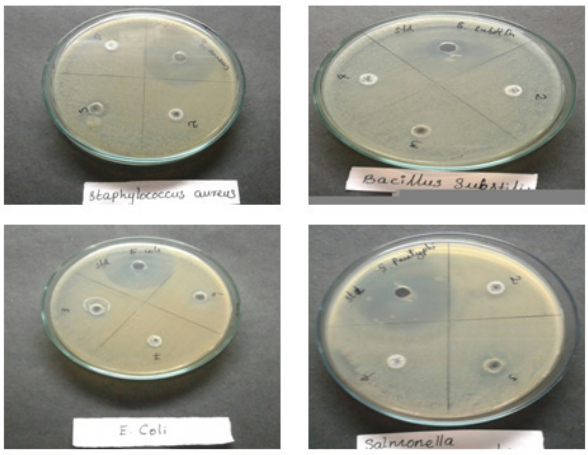


**Figure-1**
**Zone of inhibition by antibiotics isolated from fungi, during confirmation of secondary screening.**

**The Marine Fungi Identification:** The habitation of fungi was found to be coarse and lofty. The morphologic appearance monitored under illumined microscope which revealed series of spores that prove existence of fungi by this set of character.

**Optimization of Produced Antibiotic:** The day 6$^{th}$ of inhibition showed the climax productivity of the produced metabolite which was measured by the help of zone of inhibition. Out of the five isolates selected, only two strains

(AM2 and AM5) have shown good antibacterial activity against *Staphelococcus aureus, Basilus subtilis, Salmonella typhi, and E.coli* [Figure-1]. The three isolates (AM1, AM3, and AM4) have not shown any antibiotic activity. The isolate AM5 exhibited more antibacterial activity than the isolate AM2, and hence selected for further studies[1].

**Discussion:** For the treatment of infectious diseases, antibiotics have become one of the most important bioactive compounds. But nowadays, because of the multi-drug resistant pathogens outbreaks, it said to be challenges for treatment effective against diseases due to infection. There is need to broad spectrum antibiotics to demolish the resistance of some developed pathogens in the planet. Furthermore, there is noticeable interest in antibiotic research area from different sources of fungus in diversified ecological niches[5].

The antibiotics discovery, upsurges the mankind health state with its quality during the last two decades. The dramatically uplift phenomena of antibiotic resistance has the power to pass a completely change, in contrary to advance people health. Therefore, it will deliver an end to the newly born advanced medicine as we know it. In the recent time, there is core point plays a threaten to our life back to the olden days where people die due to infectious disease is widespread[6].

In the present study the fungi isolated have a capacity to produce secondary metabolites with antimicrobial activities. Nutrition plays a major role in the productivity of secondary metabolite due to the restriction of the growth depend upon the limitation and the supply of the nutrient to the metabolite. So it can regulate effectively. To reach the vertex point in the product output, it is advised to shape an ideal medium with all required nutritious to gain the maximum and effective result from the fermentation process.

## Conclusion

The production of metabolite was found to be higher in the day six and the zone of inhibition had clearly shown for this metabolite in (AM2 and AM5). Disc agar diffusion method was used regularly for this kind of assay. There is some possible incident my occur like some organism may result in variation in zone of inhibition , in contrary to the samples with a magnitude related to the amount of bioactive compounds present in the fermentation broth[3]. Among the isolated samples (AM2 and AM5), the 5th isolate exhibited maximum activity against bacterial pathogens and hence selected for further studies.

## References

**1.** Hawksworth D.L. (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological research*, 95(6), 641-655.

**2.** Ho W.H., To P.C. and Hyde K.D. (2003). Induction of antibiotic production of freshwater fungi using mix-culture fermentation. *Fungal Diversity*., 2003, 12, 45-51.

**3.** Bizuye A., Moges F. and Andualem B. (2013). Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. *Asian Pacific Journal of Tropical Disease*, 3(5), 375-381.

**4.** Kuamr Ritesh, Shrivastav A.K., Singha A.K., Kumar P. and Nirmala A. (2012). Antibiotic production from marine Strptomycyes sp. *Int J Pharm Bio Sci*, 3(4), 331-342.

**5.** Das P., Mukherjee S. and Sen R. (2008). Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. *Journal of applied microbiology*, 104(6), 1675-1684.

**6.** Prasad S. and Smith P. (2013). Meeting the threat of Antibiotic Resistance: building a new frontline defence. Office of the Chief Scientist.