INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

IN VITRO ANTI-BACTERIAL ACTIVITY OF THE SOIL FUNGAL METABOLITES

D. Thamilvanan, A. Ram kumar, R. Ramesh, B.S. Balakumar and S. Kumaresan *
PG and Research Department of Botany, Ramakrishna Mission Vivekananda College, Mylapore, Chennai-600004, India
*Corresponding Author Email: kumareshrkm@yahoo.co.in

Article Received on: 23/10/18 Approved for publication: 20/11/18

DOI: 10.7897/2230-8407.0911279

ABSTRACT

Soil microbial communities are the most diverse group on earth possessing a plethora of activities useful to mankind. Fungal species in particular, produce secondary metabolites that are potential bioactive compounds. In the present study, fungal species isolated from the soils of Kolli Hills, Tamil Nadu were subjected to a primary screening for their antibacterial activity. Ten fungal strains were selected and their extracts were tested against pathogenic bacteria by disc diffusion method. The most efficient species identified as *Chaetomium aureum* showed remarkable inhibitory effect against the pathogenic bacteria tested and hence growth conditions such as culture medium, pH, temperature, incubation period and the minimal inhibitory concentration were standardized for the maximum production of the secondary metabolites.

Keywords: Soil fungus, Secondary metabolites, *Chaetomium aureum*, Antibacterial, Optimization.

INTRODUCTION

The emergence of resistance in pathogenic microorganisms to commercial antibiotics is a common and alarming problem around the world. Among the infectious diseases, bacterial infections are one of the most harmful threats to human beings. Hence, researchers have started isolating new antimicrobial medicines from different sources. The progress in development of antibacterial agents as new drugs was necessitated by the development of multidrug resistant bacteria[1]. Microorganisms are the important sources of bioactive compounds with enormous potential to be developed as new molecules for drug discovery[2]. Microorganisms grow in unique and extreme habitats that provide them the capability to produce unique and unusual metabolites. These metabolites may act as chemical defense to extreme environments or as an adaptation of fungi competing for substrates[3]. The antimicrobial properties of secondary metabolites from various groups of fungi are widely reported[4]. They suggest that the competitive environment of microbial community as an important requirement for the production of bioactive molecules. There are many different sources where antibiotics can be discovered, among which medicinal herbs and soil[5]. Soil is a naturally occurring loose mixture of mineral and organic particles, considered as one of the most suitable environments for microbial growth[6]. It still remains the most important target for many researchers in their efforts to discover novel antibiotics. Soil fungi are also the major source of other industrially important compounds like enzyme inhibitors, anti-helminthic, antitumor agents, insecticides, vitamins, immunosuppressant and immune-modulators[7]. Fifty-four actinomycetes were isolated from soil and analyzed for antimicrobial activity against fifteen test organisms including three phytopathogens. Among these nine isolates, three efficient strains were found to have higher degree of antimicrobial potential including activity against plant pathogenic fungus[8].

The ability of fungal strains to produce antimicrobial substances is influenced by different conditions such as culture media, pH, temperature, carbon content and incubation period[9]. The aim of the present work is to obtain crude extracts of soil fungus to investigate their antibacterial activity against the pathogenic bacterial strains and to standardize the physical and chemical parameters under which the fungus produces maximum quantities of the compounds of interest.

MATERIALS AND METHODS

Collection and isolation of soil fungi

Soil samples were collected from the Kolli hills (Lat 11^{o} 55^{1} 05^{1} to 11^{0} $21^{1}10^{0}$ N, Long $17^{1}05^{1}$ to 78^{0} 27^{1} E), Tamil Nadu, India. Fungal strains were isolated by soil dilution plate method. The soil samples were sieved through a 2mm sieve in order to avoid large soil particles and decomposing plant materials. The samples were then dispensed into sterile polythene bags and were brought to the laboratory. Fungi were isolated by mixing 1g of soil samples containing decomposed plant material in 10ml of sterile distilled water and spun at 100rpm for 15min at room temperature. After 6-fold serial dilution, 0.1ml of the suspension was spread onto sterile Potato Dextrose Agar (PDA) medium containing chloramphenicol ($150mg^{-l}$) for fungal isolation.

Cultivation and extraction of fungal metabolites

Extraction of secondary metabolites was carried out by using the method of Radji *et al.*[10]. All the fungal isolates were inoculated into 250 ml Erlenmeyer flasks containing 100ml potato dextrose broth and incubated at room temperature for 21 days under stationary conditions. The broth culture was filtered to separate the mycelia and the filtrate. To the filtrate equal volume of ethyl acetate was added, mixed well for 10 minutes and kept for 5 minutes until the two clear immiscible layers were formed. The upper layer of ethyl acetate containing the extracted compounds was separated using a separating funnel. The culture filtrate extracts were pooled and evaporated to dryness in hot air oven. The extract residue was dissolved in Dimethyl sulfoxide (DMSO) and stored at 4°C to be used as stock solution for antimicrobial assay.

Test micro-organisms

For antibacterial assay three gram-negative bacteria (*Alcaligenes faecalis* (Acc No: MTCC3104)*, Acinetobacter baumannii* (Acc No: MTCC1425) and *Pseudomonas aeruginosa* (Acc No: MTCC1262) obtained from the King Institute of Preventive Medicine and Research Chennai, were used as test pathogens. The bacterial cultures were maintained on nutrient agar (NA) slants (Peptone 5g, yeast extract 2g, NaCl 5g and agar 18g, distilled water 1000ml, pH 7.0) and incubated at 37°C.

Antibacterial assay

Antibacterial activity assay was performed by disc diffusion method[11]. Muller Hinton Agar (Hi-Media) served as the basal medium to carry out the assay. To adjust the turbidity of bacteria 0.5 McFarland Standard was used prior to carrying out the microbial assay. The discs were placed on to the bacteria- seeded plate along with 5% DMSO which served as negative control to detect the solvent effects. Commercial antibiotic discs (Streptomycin 10mcg) served as the positive control. The plates were then incubated at 37°C for 24 hours. Each test was carried out in triplets.

Molecular characterization of the selected fungal isolate

DNA extraction

Total genomic DNA of the fungus was extracted using InstaGene TM Matrix (Catalog # 732-6030) genomic DNA isolation kit, following the instructions of the supplier. The freshly cultured cells were pelleted by centrifuging for 2 minutes at 10,000 rpm. The pellet was resuspended in 1ml of sterile double distilled water and centrifuged at 10,000 rpm for 1 minute. The supernatant was discarded and 200µl of InstaGene matrix was added to the pellet. This mixture was evenly mixed by using magnetic stirrer and incubated at 56°C for 15–30 minutes. Later the mixture was vortexed at high speed for 10 seconds and placed in 100°C heat block for 8 minutes. Again the mixture was vortexed at high speed for 10 seconds and spun at 10,000 – 12,000 rpm for 2-3 minutes. The supernatant was used for DNA amplification using polymerase chain reaction (PCR).

PCR amplification

PCR was performed using the diluted genomic DNA of the selected isolate. 18S rRNA amplification and sequencing were done using the forward primer, ITS-1: TCCGTAGGTGAACCTGCGG and reverse primer ITS2 (5'-TCCTCCGCTTATTGATATGC-3')[12]. The PCR product was purified using Montage PCR Clean up kit (Millipore) and then sequenced using Big Dye Terminator and ABI 3730xl sequencer (Applied Biosystems). The phylogenetic tree was constructed through alignment of the sequences of the NCBI GenBank data using molecular and evolutionary genetics analysis (MEGA) software (version 4.0).

Optimization

Selection of culture medium for bioactive compound

In order to select the best suitable medium for production of secondary metabolites, four different culture media namely Potato Dextrose Broth (PDB), Sabouraud Dextrose Broth (SDB), Czapek Doxbroth (CDB) and Glucose Yeast Broth (GYP) were used. The potential of bioactive compounds extracted from selected fungal isolate was assessed by measuring the activity of crude extract against bacterial pathogens.

Effect of pH

Initial pH range of medium was adjusted starting from 3, 5, 7, 9 and 11, and incubated for 21 days in room temperature under stationary condition. The bioactivity of metabolite production was estimated by measuring the zone of inhibition against target bacterial pathogen.

Effect of temperature

The selected culture was grown under different temperatures to observe the effect on bioactive metabolite production. The culture was inoculated in 250ml flask containing 100ml of PDB and incubated for 21days at three different temperatures 25°C, 30°C and 35°C.

Effect of incubation period

The selected fungal strain was incubated and sampled at seven-day intervals to observe the activity of the crude extract. Erlenmeyer flask of 250 ml volume containing 100 ml broth (PDB) was inoculated with equal diameter (5mm) of fungal 'mat cake'. All flasks were incubated at 30°C for 7, 14, 21 and 28 days respectively. Activity of crude extract against target bacterial pathogens was assessed by performing disc diffusion assay.

Determination of minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of fungal crude extract:

MIC and MBC were determined by macrobroth two-fold serial dilution method[13]. All tests were performed with Muller-Hinton broth. Bacteria were cultured overnight at 37°C. The fungal crude extract was dissolved in 10 per cent DMSO to obtain 2mg/ml. For determination of MIC and MBC, 8 sterile screw capped test tubes were used. A volume of 1 ml of nutrient broth was dispensed into each test tube, 1-6 and 2mL into test tube 7 served as control and incubated at 37 °C for 24 hrs. After incubation, the lowest concentrations, which did not show any growth of tested organism was considered to Minimum Inhibitory Concentration (MIC). From the test tubes with no growth (no turbidity), 0.1 ml was taken and spread over the surface of Mueller Hinton agar plates. After incubation at 37 °C for 24 hours, the colonies were observed, and the MBC was determined.

Statistical analysis

The results are expressed as mean± SD obtained from triplicates. Values were statistically significant ($P \leq 0.05$). The mean values and standard deviation were calculated using the Excel program of Microsoft Office 2010 package.

RESULTS AND DISCUSSION

From the soil samples collected from Kolli hills, a total no of 31 fungal isolates were isolated by spread plate method. These fungal isolates were grouped into 10 genera, based on their physical appearances and growth morphologies. The 10 fungal strains belong to 3 sp. of *Penicillium*, 2 sp. of *Aspergillus*, 2 sp. of *Trichoderma*, 1 sp. of *Fusarium* and 1 sp. of *Chaetomium*. The present investigation recorded a greater number of *Penicillium* sp. and *Aspergillus* sp. followed by *Chaetomium* sp. *Aspergillus* and *Penicillium* that were dominant in forest soils[14]. A similar observation was reported by Saravanakumar *et al*., in wet ever green forest soils of Tamil Nadu[15]. 12 sp. of *Penicillium*, 09 sp. of *Aspergillus,* 7 sp. of *Chaetomium,* 6 sp. of *Trichoderma*, 4 sp. of *Fusarium*, 4 sp. of *Absidia*, 4 sp. of *Cladosporium,* and 3 sp. of *Phoma* sp. were isolated from the soil samples of Mattavara forest, Chikamagalur, Karnataka[16]. Twenty-three sp. of *Aspergillus* and sixteen sp. of *Penicillium* were reported while studying the flora of *Penicillium* and *Aspergillus* in different

habitats[17]. The observations of the present study is similar to that of the results obtained by Reddy *et al.,*[18].

Screening for antibacterial activity

The increasing prevalence of drug-resistant bacteria necessitates the need to search for novel sources of antibiotics. The development of novel drugs against drug resistant pathogen is the need of the hour. Metabolites of fungal species are diverse and exhibit various biological activities[19]. A number of fungi are already known to produce antibiotics[20]. The results obtained from the present study lay emphasis on antibacterial activity of soil fungi. Though, the biodiversity of the fungi remains unclear, the expected number of species can be up to 5 million[21]. It is to conclude that the fungi will remain one of the significant bio-resources for antibiotic discovery. The crude ethyl acetate extracts of the ten fungal species were selected to analyze for the presence metabolites exhibiting inhibitory activity against selected pathogenic microbes (Table 1 & Plate 1). Results showed that all the fungal isolates used in this study showed antibacterial activity against one or more of the tested organisms. Among them *Chaetomium* sp. (Isolate 1) showed prominent activity against all the three pathogenic bacteria. The crude ethyl acetate extract of *Chaetomium* sp. showed the highest activity against *A. faecalis* (22.83±0.55), followed by *A. baumannii* (15±0.66) and *P. aeruginosa* (14.5±0.33). Positive control (streptomycin) shows inhibition zone ranging between 13 and 14mm and no zone of inhibition was observed in the negative control (DMSO). Moderate activity was exhibited by *Aspergillus* and *Penicillium* sp. with inhibition zone ranging between 12.5±0.33 and 14.66±0.22. The ethyl acetate extract showed antioxidant and antimicrobial activities. Several types of bioactive compounds have been reported from this unique fungal species. *Chaetomium* species are able to produce antimicrobial, cytotoxic, anticancer, antimalarial, and antioxidant compounds with chemical diversity[22]. *Chaetomium* species have been used to produce umpteen number of pharmacologically active metabolites, including chaetoglobosins, cytoglobins, anthraquinones, steroids, azaphilones, alkaloids, and depsidones[23]. *C. globosum* strain has been to possess a variety of biological activities including cytotoxicity, antibacterial activity, phytotoxicity, elicitor activity[24]. The antimicrobial metabolites produced by *Chaetomium* species displayed considerable antibacterial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (zone of inhibition, 9 - 15 mm)[25]. Based on the results, the most potential *Chaetomium* species (Isolate, 1) was selected for further studies.

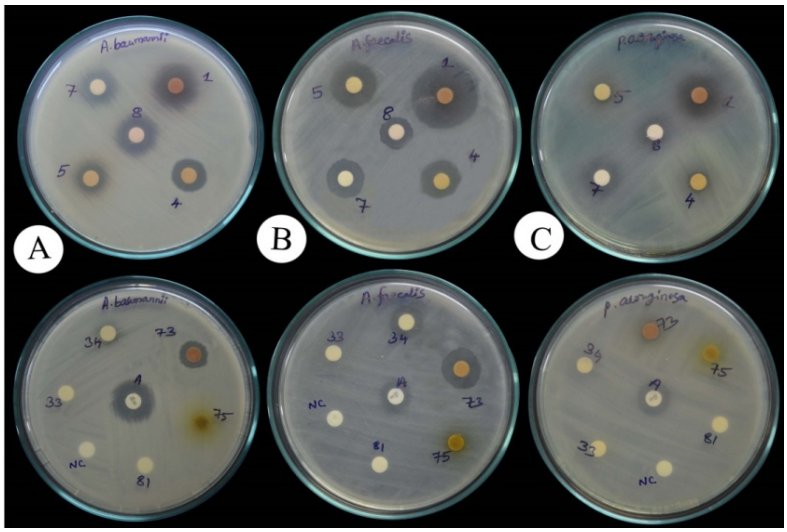

Plate 1: Plate assay for antibacterial activity of fungal extract
A- *Acinetobacter baumannii*; B- *Alcaligenes faecalis*; C- *Pseudomonas aeruginosa*;

Table 1: Bioactivity of fungal crude extracts against pathogenic bacteria (zone of inhibition)

Fungal Isolate No	*Acinetobacter baumannii*	*Alcaligenes faecalis*	*Pseudomonas aeruginosa*
1	**15.00±0.66**	**22.83±0.55**	**14.50±0.33**
4	14.66±0.22	14.83±0.55	-
5	10.00±0.00	14.10±0.22	-
7	10.00±0.00	18.00±0.66	-
8	13.00±0.66	13.00±0.00	-
33	-	-	-
34	-	12.43±0.37	-
73	12.50±0.33	15.33±0.66	-
75	-	-	-
81	-	-	-
Streptomycin	14.00±0.00	13.00±0.00	13.00±0.00
DMSO	-	-	-

Molecular characterization of fungal isolate

The selected fungus *Chaetomium* sp. (Isolate No:1) was characterized on the basis of 18S rRNA gene sequencing. Fig.1 illustrates the phylogenetic tree showing the evolutionary relationship of the isolate *Chaetomium* sp. with other closely related taxa. The isolate showed 98% similarity with *Chaetomium aureum* (KU597364.1) of the NCBI Gene bank data. The sequence has been deposited in Gene bank with Acc. No. MH198043.

GACGCGTACCTTTTAACGTTGCTTCGGCGGGCGGCCCGCTCCCCTGGAAAGCC
CCTGTGGCCGCCCGGGGCTGCGAGCCCCCCGGCCCCCCTCGCGGGGGCGCCC
GCCGGAGGATACCCAACTCTTGATTATTTTAGGCCTCTCTGAGTCTTCTGTAC
TGAATAAGTCTAACTTTCAACAGCGGATCTCTTGGTTCAGGCATCGATGAAGA
ACGCAGC

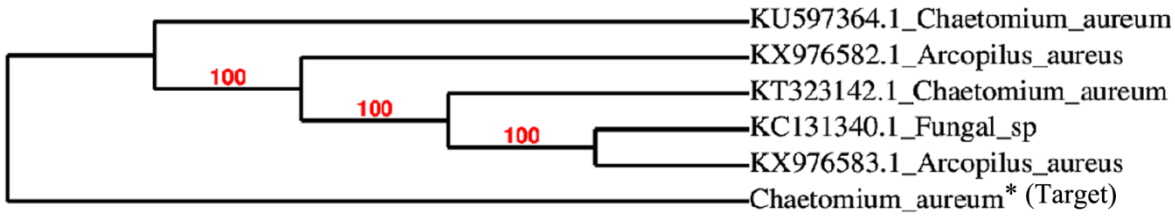

Fig.1 The phylogenetic tree of *Chaetomium aureum*

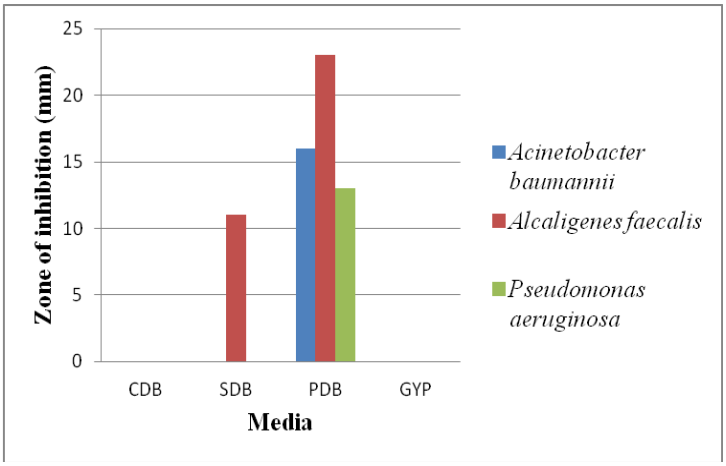

Fig 2: Effect of different media on metabolite production in *Chaetomium aureum*
Potato Dextrose Broth (PDB); Sabouraud Dextrose Broth (SDB); Czapek Dox broth (CDB); Glucose Yeast Broth (GYP)

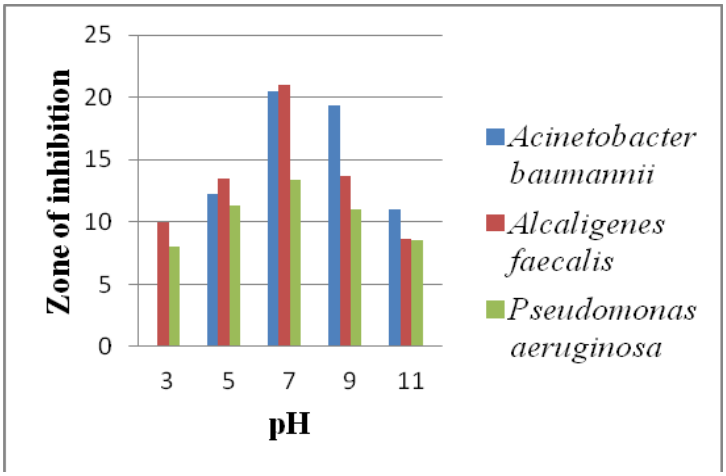

Fig 3: Effect of different pH on metabolite production in *Chaetomium aureum*

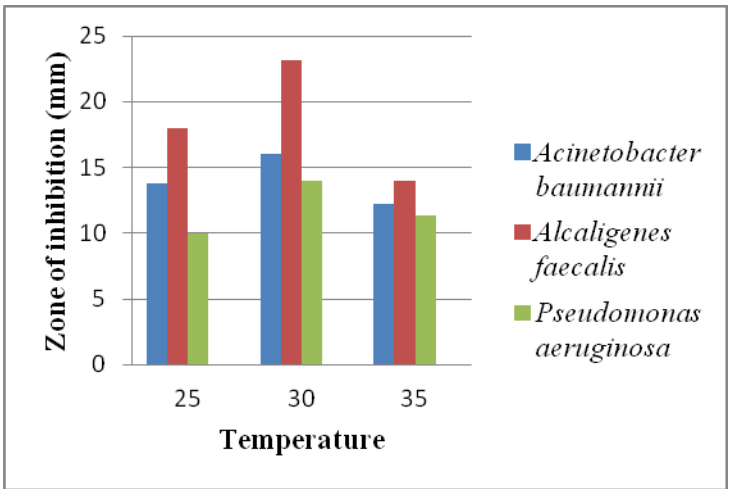

Fig 4: Effect of different Temperature on metabolite production in *Chaetomium aureum*

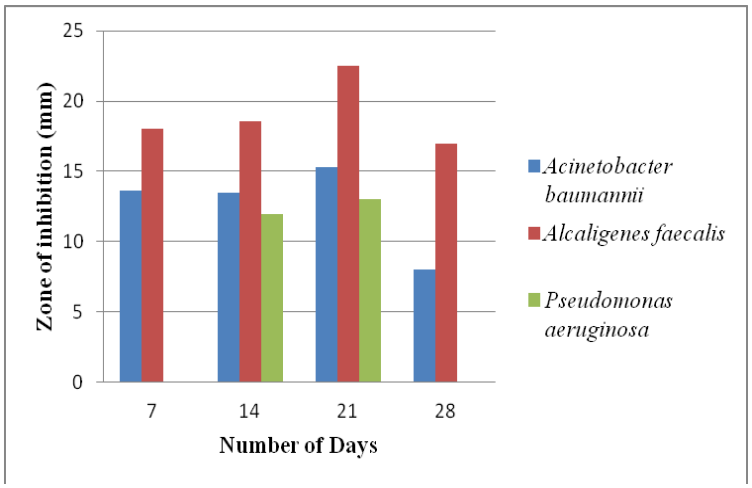

Fig 5: Effect of different Time periods of incubation on metabolite production in *Chaetomium aureum*

Effect of physical and chemical parameters on *Chaetomium aureum*

Selection of suitable culture media

Physiochemical requirements of fungi are known to vary significantly not only within genera but also between the strains[26]. In the present study, *Chaetomium aureum* was grown on different culture media such as PDB, CDB, SDB and GYP. The significant quantity of bioactive metabolite production was observed in Potato dextrose broth (PDB) with maximum zones of inhibition 23.16±0.22, 16±0 and 13±0 against *A. faecalis*, *A. baumannii*, and *P. aeruginosa* respectively. The crude extract obtained from SDB showed activity against the sole bacterium *A. faecalis* with an inhibition zone of 11.3±0.66. The least activity was recorded in SDB medium with an inhibition zone of 8±0 (Fig. 2). PDB is the best medium for the growth that enhanced the production of secondary metabolites from *Aspergillus terreus* considerably [27].

Effect of pH

The pH of a culture medium is usually not constant throughout fermentation and the changes that occur are highly dependent on composition of the medium. The optimum pH for growth rate may be entirely different from optimum pH of product formation[28]. In order to determine the optimum pH for maximum antimicrobial metabolite production, the selected isolate, *Chaetomium aureum* showed variations in antimicrobial activity when subjected to different pH values. Maximum activity was found at a pH value of 7, with a zone of inhibition of 21±0.66 against pathogenic bacteria (Fig, 3). Digrak and Eluk reports on the maximum production of biomass by *Fusarium equiseti* was at pH 8, whereas accumulation of toxic metabolites was high at the pH 5[29]. In the present study it was found that *Chaetomium aureum* grew well in pH ranging between 5 and 7. Rubini *et al*., also have reported the growth and production of antibacterial agent was at pH 7[30]. The pH of culture medium is one of the determining factors for the biosynthesis of secondary metabolites. The pH is related to permeability characteristics of the cell wall and membrane and thus has got an effect either on ion uptake or loss of ions to the nutrient medium[31].

Effect of temperature

Incubation temperature is known to influence directly the overall growth and development of any organism. It affects the physiology and subsequently the synthesis of various metabolites[32]. The effect of temperature is significant on the production of antimicrobial metabolites of the isolate *Chaetomium aureum* that remained active at all of the rendered temperature values. However, the most significant production was observed at 30°C, where the zone of inhibition against pathogens was noted to be 23.16±0.22 at 25°C (Fig, 4). Gunasekaran and Poorniammal have reported highest secondary metabolite production at a temperature of 30ºC in their study[33].

Effect of incubation period

The duration of incubation period varies significantly for different fungi for growth and metabolite production[34]. The effect of incubation period on the growth and production of antimicrobial metabolites was studied by incubating the *Chaetomium aureum* isolate for 7, 14, 21 and 28 days. Maximum antimicrobial activity was observed from the fungal cultures incubated for 21 days with inhibition range between 13±0.66 and 22.5±0.33 (Fig 5). Bioactive metabolite production increased significantly from 7 to 21 days. However, it declined after 21 days of incubation. Variations in antimicrobial metabolite production with different incubation periods have also been reported by earlier workers.[35]

MIC and MBC

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of antibiotics or pigment that did not show any growth of tested pathogens at a minimum concentration[36]. To determine the degree of antibacterial activity, the crude extract of *Chaetomium aureum* was subjected to minimum inhibitory concentration (MIC) assay by two-fold serial dilution method[37]. The present result revealed that the crude extract of the fungus, *Chaetomium aureum* exhibited good antibacterial activity against all the three test pathogens used in the study (Table 2). The MIC values were found ranging from 50µg/ml to 100 µg/ml of crude extract against test bacteria. The Minimum Bactericidal Concentration was recorded as 100 µg /ml of the crude extract. In fact, strongest activity was found against *Pseudomonas aeruginosa* (MIC of 50µg/ml and MBC of 100 µg/ml), followed by *Acinetobacter baumannii* (MIC of 100 µg/ml and MBC of 200 µg/ml). The obtained result is similar to the findings of Florey *et al*., [38]. Higher concentrations of antimicrobial substance yielded greater degrees of growth inhibition. Santos et al., have reported that the minimum inhibitory concentration of *Nigrospora sphaerica* against *Pseudomonas aeruginosa* with MIC as 1.56mg/ml and MBC 12.5mg/ml[39].

Table 2: MIC and MBC values of fungal crude extracts

Pathogens	Crude extract (µg /ml)		Standard (µg/ml)	
	MIC	MBC	MIC	MBC
Acinetobacter baumannii	100	200	25	100
Alcaligenes faecalis	50	100	25	50
Pseudomonas aeruginosa	50	100	25	100

CONCLUSION

This study indicates that fungi isolated from soil environment produce many pharmaceutically important bioactive compounds with antibacterial potential under optimum conditions. *Chaetomium aureum* showed better antimicrobial activity against test pathogens. Soil fungi revealed their potential to yield bioactive metabolite which may play an important role of providing biomolecules for drug designing in pharmaceuticals industry. Hence, further studies have to be taken to carry out purification, characterization and identification of bioactive metabolites of *Chaetomium aureum* for drug discovery and exploitation.

ACKNOWLEDGEMENT

Authors gratefully acknowledged the support and facilities extended by the secretary, Ramakrishna Mission Vivekananda College, Mylapore, for conducting the present study.

REFERENCES

1. Wise R, Hart T and Cars O. Antimicrobial resistance is a major threat to public health, British Medical Journal. 1998;317:(7159), 609–610.
2. Porras-Alfaro A and Bayman P. Hidden fungi, emergent properties: endophytes and microbiomes. Annual Review of Phytopathology. 2011;49:291–315.
3. John M, Krohn K, Florke U, Aust, HJ, Draeger S and Schulz B. Biologically active secondary metabolites from fungi. 12. (1) Oidiolactones A-F, labdane diterpene derivatives isolated from *Oidiodendron truncate*. Journal of Natural Products. 1999;62: 1218-1221.
4. Keller NP, Turner G and Bennett JW. Fungal secondary metabolism from biochemistry to genomics. Nature Reviews Microbiology 2005;3:937-947.
5. Dua A, Garg G and Mahajan R. European Journal of Experimental Biology. 2013;3(4): 203-208.
6. Nejad EMA, Abtahi A and Zareian G. Evaluation of physical and chemical properties of soils of Doroudzan dam region of Marvdasht province with respect to drainage conditions and elapsed time. European Journal of Experimental Biology. 2013;3(5):213-217.
7. Makut MD and Owolewa OA. Antibiotic-producing fungi present in the soil environment of Keffi metropolis, Nasarawa state, Nigeria. Trakia Journal of Sciences. 2011;9:33-39.
8. Sengupta Sohan, Pramanik Arnab, Ghosh Abhrajyoti, and Bhattacharyya Maitree. Antimicrobial activities of actinomycetes isolated unexplored regions of Sundarbans mangrove ecosystem. BMC Microbiology. 2015;15:170.
9. Dutta AC. Botany for Degree Students 18th (Ed). Oxford University Press, New York, 2005, 448-449.
10. Radji M, Sumiati A, Rachmayani R and Elya B. Isolation of fungal endophytes from *Garcinia mangostana* and their antibacterial activity. African Journal of Biotechnology 2011;10(1):103-07.
11. Bauer AW, Kirby WM MM, Sherris JC and Turch M. Antibiotic susceptibility testing by a standardized single disc method. American Journal Clinical Pathology. 1996;45(4):493- 496.
12. Paul NC, Kim WK, Woo SK, Park MS and Yu SH. Fungal endophytes in roots of *Aralia* species and their antifungal activity. Plant Pathology Journal . 2007;23(4):287–94. https://doi: 10.5423/ppj.2007.23.4.287.
13. Andrews M. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 2001;48: 5-16.
14. Moubasher AH and El-Dohlob SM. Seasonal fluctuation of Egyptian soil fungi. Transactions of the British Mycological Society, 1970;54:45-51.
15. Saravanakumar K, Nithya K and Kaviyarasan V. Seasonal distribution and abundance of soil fungi from forest soils of wet evergreen forest of Tamil Nadu, India. Journal of Modern Biotechnology. 2012;1(1):8-14.
16. Chandini and Rajeshwari. Isolation and identification of soil fungi in Mattavara forest, Chikamagalur, Karnataka. Journal of Pharmacognosy and Phytochemistry 2017;6(5):721-726.
17. Asan A. Trakya Bolgesimisirtarlalari micro fungus florasi. Turkish Journal of Botany, 1997; 21:89-101.
18. Reddy VN, Prakash P, Manoharachary C. Fungistasis in forest soil using the dominant soil fungi. Biome, 1987;2(1):126-128.
19. Pelaez F. Biological activities of fungal metabolites revised (Ed.), Chapter 3 Handbook of industrial mycology, Marcel Dekker, New York 2005; 49-92

20. Jensen PR, Mincer TJ and Fenical W. The true potential of the marine microorganism. Current Drug Discovery, 2003:17-19.
21. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics, 2015;40 (4) : 277-283.
22. Li H, Xiao J, Gao YQ, Tang JJ, Zhang AL and Gao JM. Chaetoglobosins from *Chaetomium globosum*, an endophytic fungus in *Ginkgo biloba*, and their phytotoxic and cytotoxic activities. Journal of Agricultural and Food Chemistry. 2014;62(17):3734–41.
23. Zheng QC, Kong MZ, Zhao Q, Chen GD, Tian HY, Li XX, Guo LD, Li J, Zleng YZ, Gao H. Chaetoglobosin Y, a new cytochalasan from *Chaetomium globosum*. Fitoterapia. 2014;93:126–31.
24. Zhang Q, Li HQ, Zong SC, Gao JM and Zhang AL. Chemical and bioactive diversities of the genus *Chaetomium* secondary metabolites. Mini-Reviews in Medicinal Chemistry, 2012;12(2):127–148.
25. Nighat Fatima, Usman Mukhtar Ihsan-Ul-Haq, Muneer Ahmed Qazi, Muniba Jadoon, and Safia Ahmed. Biological Evaluation of Endophytic Fungus *Chaetomium* sp. NF15 of *Justicia adhatoda* L.: A Potential Candidate for Drug Discovery. Jundishapur Journal of Microbiology, 2016;9(6): 29978.
26. Stanbury PF, Whitaker A and Hall SJ. Media for industrial fermentations. In: Principles of fermentation technology. Elsevier Science Ltd. Pergamon Oxford, New York, Tokyo, 1995, 93-122.
27. Suja M, Subramanian V and Nagamony S. Optimization and antimicrobial metabolite production from endophytic fungi *Aspergillus terreus* KC 582297. European Journal of Experimental Biology, 2012;3(4):138-4.
28. Lilly VG and Barnett HL. Physiology of fungi. 1st (Ed) Mc Graw Hill Book Co. Inc., 1951, 464.
29. Digrak M Eluk SZ, Determination of some fungal metabolite as influenced by temperature, time, pH and sugars by bioassay method. Turkish Journal of Biology, 2001;25: 197-203.
30. Rubini MR, Rute TSR, Pomella AW, Cristina SM, Arajo LW, Santos DRD and Azevedo JL. Diversity of endophytic fungal community of cocao (Thebroma cacao L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom disease. International Journal of Biological Sciences, 2005;1:24-33.
31. Hansen OH. Ecology, physiology, and biochemistry of blue green algae. Annual Review of Microbiology, 1968;22: 47-57.
32. Pandey AK, Singh AK, Quereshi S and Pandey C. Herbicidal activities of secondary metabolites of *Aspergillus* spp. against *Lantana camara*. Journal of Basic and Applied Mycology, 2005; 4: 65-67.
33. Gunasekaran S and Poorniammal R. Optimization of fermentation conditions for red pigment production from *Penicillium* sp. under submerged cultivation. African Journal of Biotechnology, 2008;7(12):1894-1898.
34. Alberts, JF, Gelderblom WCA, Thiel PG, Marasas WFO, Vanschalkwyk DJ and Behrend Y. Effects of Temperature and Incubation Period on Production of Fumonisin B1 by *Fusarium moniliforme*. Applied and Environmental Microbioogy, 1990;56(6):1729-1733
35. Merlin JN, Nimal IVS, Christhuda S, Praveen KP and Agastian P. Optimization of growth and bioactive metabolite production: *Fusarium solani*, Asian Journal of Pharmaceutical and Clinical Research, 2013;6(3):98-103.
36. Abere TA, Onyekweli AO and Ukoh GU. In vitro antimicrobial activity of the extract of *Mitracarpus scaber* leaves formulated as syrup. Tropical Journal of Pharmaceutical Research, 2007;6(1): 679-682.
37. Florey HW, Chain E and Florey ME. The Antibiotic, Oxford University Press, New York, 1989;1: 576-628.
38. Rasooli I, Rezaei MB and Allameh A. Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. Food Control, 2006;17: 359-364.
39. Santos IP, Silva LCN, Silva MV, Araújo JM, Cavalcanti MS and Lima VLM. Antibacterial activity of endophytic fungi from leaves of *Indigofera suffruticosa* Miller (Fabaceae). Frontiers in Microbiology, 2015;6:350. https://doi: 10.3389/fmicb.2015.00350

Cite this article as:

D. Thamilvanan *et al*. In vitro anti-bacterial activity of the soil fungal metabolites. Int. Res. J. Pharm. 2018;9(11):175-181 http://dx.doi.org/10.7897/2230-8407.0911279

Source of support: Nil, Conflict of interest: None Declared