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

Research Article

# EVALUATION OF ANTIMICROBIAL POTENTIAL OF RHIZOSPHERIC SOIL FUNGI ISOLATED FROM *TINOSPORA CORDIFOLIA*, *MENTHA ARVENSIS* AND *OCIMUM TENUIFLORUM* MEDICINAL PLANTS

Geetanjali, Pranay jain* and Tarun Kumar
Department of Biotechnology, University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra, India
*Corresponding Author Email: drpranayjain@gmail.com

Article Received on: 16/07/16 Revised on: 25/08/16 Approved for publication: 22/09/16

**DOI: 10.7897/2230-8407.079111**

**ABSTRACT**

Apart from producing phytohormones, plant associated microbes like rhizospheric microbes are also found to be the producers of pharmaceutically active compounds. The rhizosphere is a narrow region around plant roots, a hot spot for diverse and active population of soil microbes where various ecological and biological complex interactions occur. Medicinal plants harbour a distinctive microbiome in their rhizosphere due to their unique and structurally divergent bioactive secondary metabolites that are most likely responsible for the high specificity of the associated microbes. In the present investigation, fungi were isolated from rhizosphere of three medicinal plants *Tinospora cordifolia*, *Mentha arvensis* and *Ocimum tenuiflorum* of Kurukshetra and some nearby districts and screened for antimicrobial activity by agar well diffusion method. Out of 10 fungal isolates, TF1 isolate was found to be potent antimicrobial metabolite as showed highest activity against all bacteria and yeast pathogens. The Minimum inhibitory concentration calculated by agar dilution method showed that Minimum Inhibitory Concentration of yellow oily metabolite extracted by ethyl acetate by solvent extraction method was 0.039mg/ml against *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pyogenes* and *Candida albicans*. According to microscopic examination TF1 fungal isolate was found to belong to *Aspergillus* species. The potent isolate could be exploited for optimization of various parameters like pH, temperature, carbon and nitrogen sources etc. to increase the yield of metabolite.

**KEYWORDS:** Rhizosphere, medicinal plant, antimicrobial activity, minimum inhibitory concentration.

## INTRODUCTION

Plants have application in various industries including pharmaceutical, herbal, agricultural, food as well as cosmetic industries[1]. The plants which have medicinal properties as antimicrobial, antipyretic, anti-cancerous etc., are termed as medicinal plants[2]. Medicinal plants are thought to be safe to human beings and the environment compared to the synthetic medicines for the treatment of many diseases[3]. In past research was focused on bioactive phytochemicals but there is also a significant number of antimicrobial compounds produced by associated microbes as endophytic and rhizospheric microbes.

The rhizopshere is soil plant interface, a hot spot for diverse population of soil microbes in vicinity of roots of plant where various ecological and biological complex interactions occur. In this region microbes are driven via plant root exudates, the chemical components belong to the major groups of carbohydrates, proteins, lipids, phenolic compounds, organic acids and other cellular components[4]. The rhizosphere can host up to $10^{11}$ microbial cells per gram root with more than 30,000 different prokaryotic species. Besides plant species, the composition and diversity of rhizospheric microbes is influenced by soil type, moisture, condition of plant and developmental stage, climate and season, pesticide treatments and other biotic and abiotic factors[5]. Organisms found in the rhizosphere include bacteria, fungi, actinomycetes, nematodes, protozoa, algae, viruses, archaea etc.[6]. Medicinal plants harbour a distinctive microbiome in their rhizosphere due to their unique and structurally divergent bioactive secondary metabolites that are most likely responsible for the high specificity of the associated microbes.

Microorganisms are natural producers for potential drugs, agrochemicals and biocatalysts. Microbial secondary metabolites include antibiotics, pigments, toxins, enzyme inhibitors, immunomodulating agents, receptor antagonists and agonists, pesticides and antitumor agents. They have a major effect on society health and economy. Secondary metabolites are produced in idiophase of microbe growth cycle and have no physiological role in growth phase[7].

Infectious diseases caused by opportunistic microbes are an important cause of morbidity and mortality among the general population, particularly in developing countries. Antimicrobial agents are essentially important to reduce the global burden of diseases. Because of massive and improper use of antibiotics, resistant pathogens also develop and spread[8]. Therefore, pharmaceutical companies have been focused to develop new antimicrobial drugs. Consequently, common strategies adopted for production of new antimicrobial drugs include changing the molecular structure of the existing medicines in order to make them more efficient or recover the activity lost due to resistance mechanisms[9] but natural products offer a vast source of chemical diversity and economic and efficient solution to emergence of drug resistant microbes. In lieu of the above justification, the objective of present study was to search for antimicrobials from fungi isolated from rhizopsheric soil of *Tinospora cordifolia*, *Mentha arvensis* and *Ocimum tenuiflorum* medicinal plants.

## MATERIAL AND METHODS

### Collection of Soil Sample

Samples were collected using clean and sterile polythene bags from 5 to 7cm depth from surface of soil of three medicinal plants viz. *Tinospora cordifolia* (Geloy), *Mentha arvensis* (Pudina) and *Ocimum tenuiflorum* (Tulsi) of Kurukshetra and some nearby districts. Samples were directly transferred to laboratory, air dried and used for isolation of fungi within 24 hours of collection.

### Isolation of Fungi from Rhizosphere

Isolation of fungi from soil sample was performed by serial dilution method[10]. One gram of each soil sample was added in 9ml of sterilized distilled water and shaken well and dilution named as $10^{-1}$. Then further serial dilutions are made upto $10^{-4}$ and 0.1ml of each dilution was spread on Potato Dextrose Agar (PDA) supplemented with Streptomycin (0.2g/l) to prevent bacterial growth. Plates were incubated for 5 days at 30ºC. After incubation, the morphological appearances were observed, isolates were selected, sub cultured and maintained for further studies.

### Preparation of Culture Filtrate of Fungi

Fungal isolates were grown in conical flasks (250 ml) containing 100 ml Potato Dextrose Broth (PDB), autoclaved at 121°C for 20 minutes. After cooling the medium, each flask was inoculated with fungal disc, cut from the margin of vigorously growing culture of fungi. These flasks were incubated for 11 days at 30°C. After 11 days, fungi were filtered through Whatman No.1 filter paper. The culture filtrates were separated from mycelium.

### Procurement of Microbial Cultures

Selected test pathogens were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh which included Gram negative bacteria, *Pseudomonas aeruginosa* (MTCC-424) and *Escherichia coli* (MTCC-40); Gram-positive bacteria, *Streptococcus mutans* (MTCC-497), *Staphylococcus aureus* (MTCC-87), *Bacillus subtilis* (MTCC-441) and *Streptococcus pyogenes* (MTCC-1924) and two yeasts namely *Candida albicans* (MTCC-227) and *Candida tropicalis* (MTCC-3421). All slants were kept at 4ºC in the refrigerator for future studies. The inoculum of different test pathogens were adjusted according to 0.5 McFarland standard. The McFarland tubes were stored at 4-5$^0$C and prepared afresh after every 3 to 4 months[11].

### Screening of Antimicrobial Activity

Culture filtrates obtained were screened for their antimicrobial activity by Agar Well Diffusion method[12]. Nutrient agar (NA) was prepared, sterilized and poured into petriplates. Test microbes were spread on plates on which wells were made. To each well, culture filtrate, Ciprofloxacin and Fluconazole as positive control were added. Uninoculated broth was added as negative control. Plates were incubated at 37ºC for 24 hours. After incubation, diameter of zone of inhibition was measured surrounding the well containing filtrate. All experiments were performed in triplicates.

### Extraction of Bioactive Metabolite from Culture Filtrate

The antimicrobial compound was extracted by solvent extraction using ethyl acetate. Equal volume (1:1) of culture filtrate and ethyl acetate were taken in a separating funnel and agitated for about 30 minutes. Then the mixture was allowed to stand till complete separation to upper and lower phase was formed. The phase containing solvent was separated and aqueous phase was again extracted with ethyl acetate. The solvent layers were evaporated to dryness at 40$^o$C in rotary evaporator to get concentrated crude extract[13]. Then crude metabolite was dissolved in Dimethyl sulphoxide (DMSO).

### Minimum Inhibitory Concentration (MIC)

MIC represents the lowest concentration of metabolite that inhibits the growth of microorganism. MIC value was determined by Agar Dilution method. This method involves the incorporation of varying desired concentrations of the antimicrobial agent into wells on agar medium using serial two-fold dilutions, after the inoculation of a defined microbial inoculum onto the agar plate surface[12]. The lowest concentration which completely inhibits the growth of microorganism under suitable incubation conditions is recorded as minimum inhibitory concentration. DMSO was taken as negative control.

### Microscopic Characterization of Fungi

The fungal isolates were identified using lactophenol cottonblue staining. The fungal genera were identified on the basis of colony morphology, sporulation and pigmentation[10].

## RESULTS

### Sample Collection and Isolation of Fungi

A total of 10 morphologically different fungi were isolated from three soil samples collected from rhizosphere of *Tinospora cordifolia*, *Mentha arvensis* and *Ocimum tenuiflorum* medicinal plants of Kurukshetra and some nearby districts. All the isolates were sub cultured on PDA and preserved on PDA slants for further use.

### Screening of Antimicrobial Activity

Antimicrobial activity of 10 fungal isolates (GF1, GF2, GF3, GF4, PF1, PF2, PF3, PF4, TF1, TF2) which included genera of *Aspergillus*, *Penicillum*, *Alternaria* sp. were screened against 8 test microbes out of which six were bacteria (2 Gram negative; *P.aeruginosa*, *E.coli* and 4 Gram positive; *S.mutans*, *S. pyogenes*, *B.subtilis* and *S.aureus*) and two yeasts; *C.tropicalis, C.albicans*. The isolate designated as TF1 was found to be most effective against all the test bacteria and yeast as zone of growth inhibition was 22mm against *B.subtilis* and *E.coli*, 21mm against *S.aureus* and *S.pyogenes*, 20mm against *C.tropicalis*, 18mm against *P.aeruginosa* and *C.albicans* and 16mm against *S.mutans* and seems to be wide spectrum in its mode of action as inhibited the growth of all test pathogens. The isolates GF1, PF1, PF3 and TF2 were active against 4 bacteria and 2 yeast. The isolates GF2 and PF4 were suppressing growth of only 3 and 2 bacteria respectively. The isolate GF3 was active against 2 yeast and 3 bacteria. The isolate GF4 was active against 5 bacteria and 2 yeast. The isolate PF2 did not show activity against any test microbe. The results of antimicrobial activity are shown in Table 1.

### Identification of Promising Fungal Isolate

Identification of the fungal isolate TF1 was done on the basis of colony characteristics (colony growth, colour and production of exudate) and sporulating structures (conidiogenous cells, vesicle, conidial head and conidia) by following various

manuals[14, 15, 16, 17]. On Potato dextrose agar (PDA), the colony color was Black, powdery and buff to yellow reverse.

Microscopically, the conidiophores were arising from foot cell, conidial head was biseriate, conidia were smooth walled and globose. The fungal isolate was thus identified as *Aspergillus* sp. on the basis of colony morphology, color of the colony and the sporulating structures.

**Crude Metabolite Preparation**

Based on screening results the fungal isolate TF1 was selected for mass cultivation. The culture broth of selected isolate (1000ml) was extracted with ethyl acetate and metabolite was evaporated to dryness. The crude metabolite of isolate was approx. 400mg yellow oily residue.

**Determination of MIC of Crude Metabolite**

The potency of crude metabolite was quantitatively assessed by the MIC values. Metabolite of selected fungal isolate TF1 inhibited the growth of both bacteria and yeast strains with MIC value of 0.039mg/ml against *P.aeruginosa*, *E.coli*, *S.pyogenes* and *C.albicans*. The MIC value of crude metabolite against *S.aureus, C.tropicalis* and *S.mutans* was 1.25mg/ml. The MIC of 2.5mg/ml was observed against bacterium *B. subtilis* (Table 2).

**Table 1: Antimicrobial activity of fungal isolates obtained from rhizospheric soil sample of medicinal plants**

| Code of Sample | Diameter of Growth Inhibition Zone (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | | Gram negative bacteria | | Yeast | |
| | *B.subtilis* | *S.aureus* | *S.mutans* | *S.pyogenes* | *E.coli* | *P.aeruginosa* | *C.albicans* | *C.tropicalis* |
| GF1 | 19±0.37 | 14±0.57 | - | - | 17±0.37 | 16±0.81 | 14±0.37 | 15±0.37 |
| GF2 | 14±0.57 | 11±0.57 | - | - | 12±0.81 | - | - | - |
| GF3 | 15±0.37 | 12±0.37 | - | - | 15±0.81 | - | 12±0.81 | 13±0.57 |
| GF4 | 17±0.37 | 14±0.37 | 16±0.81 | 17±0.37 | - | 15±0.57 | 16±0.57 | 16±0.57 |
| TF1 | 22±0.81 | 21±0.81 | 16±0.57 | 21±0.57 | 22±0.37 | 18±0.37 | 18±0.57 | 20±0.37 |
| TF2 | 20±0.37 | 16±0.57 | - | - | 17±0.81 | 13±0.81 | 14±0.37 | 15±0.37 |
| PF1 | 22±0.57 | 16±0.57 | - | - | 19±0.37 | 14±0.37 | 17±0.37 | 18±0.57 |
| PF2 | - | - | - | - | - | - | - | - |
| PF3 | 20±0.81 | 16±0.57 | - | - | 16±0.37 | 16±0.37 | 14±0.57 | 17±0.57 |
| PF4 | - | 17±0.37 | - | 12±0.37 | - | - | - | - |
| Ciprofloxacin | 35±0.57 | - | 38±0.81 | 39±0.57 | 40±0.37 | 50±0.37 | * | * |
| Fluconazole | * | * | * | * | * | * | - | - |
| Uninoculated broth | - | - | - | - | - | - | - | - |

Results are expressed as mean ± standard deviation; - means No activity; * means Not determined

**Table 2: Minimum inhibitory concentration of isolate TF1 against eight test microbes**

| Code of Sample | Diameter of Growth Inhibition Zone (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| TF1 | Gram positive bacteria | | | | Gram negative bacteria | | Yeast | |
| Conc. | *B.subtilis* | *S.aureus* | *S.mutans* | *S.pyogenes* | *E.coli* | *P.aeruginosa* | *C.albicans* | *C.tropicalis* |
| 5mg/ml | 20±0.37 | 21±0.57 | 20±0.81 | 25±0.57 | 22±0.37 | 22±0.81 | 28±0.37 | 20±0.37 |
| 2.5mg/ml | 15±0.57 | 18±0.57 | 18±0.61 | 22±0.37 | 20±0.81 | 20±0.57 | 24±0.57 | 17±0.57 |
| 1.25mg/ml | 12±0.37 | 14±0.37 | 13±0.81 | 19±0.81 | 17±0.81 | 19±0.37 | 22±0.81 | 15±0.57 |
| 0.625mg/ml | - | - | - | 17±0.37 | 15±0.37 | 17±0.57 | 19±0.57 | - |
| 0.312mg/ml | - | - | - | 16±0.57 | 14±0.37 | 16±0.37 | 18±0.57 | - |
| 0.156mg/ml | - | - | - | 16±0.37 | 14±0.81 | 14±0.81 | 17±0.37 | - |
| 0.078mg/ml | - | - | - | 15±0.57 | 13±0.37 | 13±0.37 | 14±0.37 | - |
| 0.039mg/ml | - | - | - | 14±0.37 | 12±0.57 | 13±0.81 | 13±0.57 | - |
| DMSO | - | - | - | - | - | - | - | - |

Results are expressed as mean ± standard deviation; - means No activity

**DISCUSSION**

Soil microorganisms constitute the world's largest reservoir of bioactive substances. The rhizosphere is region of soil influenced by root exudates and characterized by greater microbiological activity than the soil away from plant roots[18]. In the present work, rhizosphere of three medicinal plants *Tinospora cordifolia* (Geloy), *Mentha arvensis* (Pudina) and *Ocimum tenuiflorum* (Tulsi) of Kurukshetra and some nearby districts were selected for isolation of fungi. Among soil microorganisms, actinomycetes and fungi have been the main producers of bioactive compounds. Since the 1940s, fungi have been used for the production of antibiotics such as penicillin from *Penicillium chrysogenum,* cephalosporin from *Cephalosporium acremonium* and griseofulvin isolated from *Penicillium griseofulvum*, used to treat infections[10]. Many other drugs like strobilurin A and B, antifungal antibiotics are initially isolated by fungus *Strobilurus tenacellus,* fusidic acid from mitosporic fungus *Fusidium coccineum* and fumagillin from *Aspergillus fumigatus* etc.[19].

The isolation of antibiotics from microorganisms is relatively easy as compared to chemical synthesis of antimicrobial agents. Antibiotic production from natural producers improved the discovery of novel antibiotics that could act as better chemotherapeutic agents. Various reports indicated the production of antimicrobial compound from fungi isolated from rhizospheric soil. In an investigation, the rhizosphere soil *Aspergillus terreus* from kuttralam hills station found as a potential source of highest antibacterial, phenol compound in biomedical applications. Strain was screened for antimicrobial activity against five test microbes (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus* sp.) and showed highest antibacterial activity against *Staphylococcus* (29mm)[20]. In the present study, out of 10 fungal isolates screened for antimicrobial activity against eight

test pathogens (*B.subtilis*, *S.aureus*, *P.aeruginosa*, *E.coli*, *S.mutans*, *S.pyogenes*, *C.albicans* and *C.tropicalis*), one isolate TF1 showed highest antimicrobial activity against *B.subtilis* and *E.coli* (22mm) and found to have a broad spectrum activity as showed activity against all bacterial and yeast strains.

In a report NF00659 A1, A2, A3, Bl and B2 novel metabolite produced by *Aspergillus* sp. NF00659 showed antimicrobial activity upto a concentration of 1mg/ml against Gram-positive and Gram-negative bacteria and fungi[11]. The results in present study showed MIC of metabolite produced from TF1 isolate against *P.aeruginosa*, *E.coli*, *S.pyogenes* and *C.albicans* is 0.039mg/ml. Based on antimicrobial potential, results revealed that bioactive metabolite produced from TF1 isolate (*Aspergillus* sp.) could be used as potent drug against infectious disease causing microorganisms. Further work is needed to optimize the various parameters like temp., pH, media, inoculum size, vessel size etc. to increase the yield of antimicrobial metabolite. Purification and characterization of antimicrobial metabolite will be performed.

**ACKNOWLEDGEMENT**

The authors are grateful to Hon'ble Vice-Chancellor, Kurukshetra University, Kurukshetra and the Director, University Institute of Engineering and Technology, Kurukshetra University, Kurukshetra for providing basic infrastructure to carry out research.

**REFERENCES**

1. Thapa T, Kumar De U, Chakraborty B. Association and Root Colonization of Some Medicinal Plants with Arbuscular Mycorrhizal Fungi. Journal of Medicinal Plant Studies 2015; 3(2):25-35.
2. Pandey A, Malviya T. Production of Antibiotics Isolated from Soil Bacteria from Rhizospheric and Non-rhizospheric Region of Medicinal Plants. Indian Journal of Applied Research 2014; 4(8):25-32.
3. Solaiman ZM, Anawar HM. Rhizosphere Microbes Interactions in Medicinal Plants. In: Egamberdieva D, Shrivastava S, Varma A, editors. Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants. 1st ed. Switzerland: Springer 2015.p.19-41.
4. Andreote FD, Gumiere T, Durrer A. Exploring Interactions of Plant Microbiomes. Scientia Agricola 2014; 71:528-539.
5. Koberl M, Schmidt R, Ramadan EM, Bauer R, Berg G. The Microbiome of Medicinal Plants: Diversity and Importance for Plant Growth, Quality, and Health. Frontiers in Microbiology 2013; 4:1-9.
6. Mendes R, Garbeva P, Raaijmakers JM. The Rhizosphere Microbiome: Significance of Plant Beneficial, Plant Pathogenic, and Human Pathogenic Microorganisms. FEMS Microbiology Reviews 2013; 37:634-663.
7. Demain AL. Induction of Microbial Secondary Metabolism. International Microbiology 1998; 1:259-264.
8. Azza S. Zakaria. Mechanism of antibacterial action of honey on pathogenic wound bacterial strains: A proteomic analysis. Int. Res. J. Pharm. 2015; 6(11):778-788http://dx.doi.org/10.7897/2230-8407.0611151
9. Silva NCC, Fernandes JA. Biological Properties of Medicinal Plants: a Review of their Antimicrobial Activity. The Journal of Venomous Animals and Toxins including Tropical Diseases 2010; 16:402-413.
10. Helen PAM, Shiny M, Ruskin S, Sree SJ, Nizzy AM. Screening of Antibiotic Producing Fungi from Soil. Journal of Environmental Science, Computer Science and Engineering & Technology 2012; 1:141-151.
11. Jain P, Pundir RK. Effect of Fermentation Medium, pH and Temperature Variations on Antibacterial Soil Fungal Metabolite Production. Journal of Agricultural Technology 2011; 7(2):247-269.
12. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro Evaluating Antimicrobial Activity: A Review. Journal of Pharmaceutical Analysis 2016; 6:71-79.
13. Kekuda PTR, Rakesh KN, Junaid S, Dileep N. Antibacterial and Antioxidant Activities of *Streptomyces s*pecies SRDP-H03 Isolated from Soil of Hosudi, Karnataka, India. Journal of drug delivery & therapeutics 2013; 3(4):47-53.
14. Domsch KH, Gams W, Anderson TH. Compendium of Soil Fungi. 1st ed. London: Academic Press; 1980.
15. Sutton BC. The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Kew, England: Commonwealth Mycological Institute; 1980.
16. Alexopoulos CJ, Mims CW, Blackwell MM. Introductory Mycology. 4th ed. New York: John Wiley; 1996.
17. Kirk PM, Cannon PF, David JC, Stalpers JA. Ainsworth and Bisby'Dictionary of Fungi. 9th ed. U.K.: CAB International Publishing; 2001.
18. Ramesh G, Hari BNV, Dhevendaran K. Microbial Association with Selected Medicinal Plants in Rhizosphere and their Biodiversity. Advances in Natural and Applied Sciences 2012; 6(6):947-958.
19. Anke T, Oberwinkler F, Steglich W, Schramm G. The Strobilurins—New Antifungal Antibiotics from the Basidiomycete *Strobiluris tenacellus.* The Journal of Antibiotics 1977; 30:806-810.
20. Rajalakshmi S and Mahesh N. Production and Characterization of Bioactive Metabolites Isolated from *Aspergillus terreus* in Rhizosphere Soil of Medicinal Plants. International Journal of Current Microbiology and Applied Sciences 2014; 3(6):784-798.

**Cite this article as:**

Geetanjali, Pranay jain and Tarun Kumar. Evaluation of antimicrobial potential of rhizospheric soil fungi isolated from *Tinospora cordifolia*, *Mentha arvensis* and *Ocimum tenuiflorum* medicinal plants. Int. Res. J. Pharm. 2016;7(9):37-40 http://dx.doi.org/10.7897/2230-8407.079111

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