

Original Research Article

Production and Characterization of Bioactive Metabolites Isolated from *Aspergillus terreus* in Rhizosphere Soil of Medicinal Plants

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ABSTRACT

In this investigation, a rhizosphere soil sample as capable of encouraging isolation of actinomycetes was found to be of potential candidate for antibacterial activities. The search shows that the rhizosphere region of hills soil is a rich source of clinically important microorganisms, especially from fungi, requires a large number of isolates in order to discover a novel compound of pharmaceutical interest. Thus, it can be concluded from the results of the present investigation that the rhizosphere soil *Aspergillus terreus* from kuttralam hills station are a potential source of highest antibacterial, phenol compound in biomedical applications. Since fungal isolates F-4 characterized macroscopic and microscopic morphology could merely provide a presumptive genus or species identification, prominent fungi F-4 should be further identified up to species level by molecular 18S rRNA assay and phylogenetic tree construction. These results indicate that the F-4 strain was showed highest antibacterial activity against *Staphylococcus*. Furtherly, Bioactive fraction F-4 was identified by FT-IR and HPLC analysis. Based on the GC-MS analysis, ten compounds were identified, out of ten compounds, one compound (TETRACONTANE) used in anti-microbial effect.

Keywords

Aspergillus terreus,
Actinomycetes,
Phylogenetic,
GC-MS
analysis

Introduction

Antibiotics are (Greek anti-against, and Bios-life) microbial products or their derivatives that can kill susceptible microorganisms or inhibit their growth. Antibiotics are special kind of chemotherapeutic agent usually obtained from living organisms. Chemical agents, chemotherapeutic that are used to treat disease. A drug that disrupt a microbial function. The pharmaceutical industry has become increasingly interested in screening fungi for novel antibiotics and other secondary metabolites. Historical discovery

novel compounds from fungi usually resulted from accidental finds or screening of ubiquitous fungi.

Infectious diseases are leading health problems with high morbidity and mortality in the developing countries. The development of resistance to multiple drugs is a major problem in the treatment of these infectious diseases caused by microorganisms. This multidrug resistance is presently an urgent focus of research and new bio active

compounds are necessary to combat these multidrug resistant pathogens. The fungi have been widely studied for their bioactive metabolites and have proven to be a rich and promising source of novel anti cancer, antibacterial anti plasmodial, Anti inflammatory and Anti-viral agents.

Considering the number of novel bioactive compounds that have been isolated from endophytic fungi and the fact that the number of the compound is increasing rapidly. (Rajasekar and Balaji, et al 2012). Secondary metabolites is a prerequisite for the development of novel pharmaceuticals, and this is an especially urgent task in the case of antibiotics due to the rapid spreading of bacterial resistance and the emergence of multiresistant pathogenic strains, which severe clinical problems in the treatment of infectious disease. The thematic series of on the biosynthesis and function of secondary metabolites deals with the discovery of new biologically active compounds from all kinds of source, including plants, bacteria and fungi and also with their biogenesis. Biosynthetic aspects are closely related to functional investigation, because the deep understanding of metabolic pathway of natural products. New secondary metabolites available from microorganisms may be used to optimize their availability by fermentation for further research and also for production in the pharmaceutical industry.

Investigations of the mode of action of β -lactam antibiotics have focused on the interaction of these drugs with sensitive enzymes and penicillin-binding proteins in vitro and on the correlation of these data with physiological responses to the drugs. The classical response is inhibition of growth and cell death followed by lyses; however, the bacteriostatic response to penicillins, which is seen in certain bacterial species such as *Streptococcus mutants* and in mutants of species such as *Streptococcus pneumoniae*, is

also described.

The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases known as penicillin-binding proteins (PBPs). PBPs bind to the D-Ala-D-Ala at the end of mucopeptides (peptidoglycan precursors) to crosslink the peptidoglycan. Beta-lactam antibiotics mimic the D-Ala-D-Ala site, thereby irreversibly inhibiting PBP crosslinking of peptidoglycan. The present study is focused on the production and characterization of bioactive metabolites isolated from *Aspergillus terreus* under submerged fermentation.

Materials and Methods

Collection of soil sample

The rhizosphere medicinal plant soil samples were collected from various locations of in and around Kuttalam, Tirunelveli (Dt). Ten soil samples were collected for the isolation of fungal strains. The soil samples were collected up to a depth of 1-5 cm from the soil surface under aseptic condition and transferred in to sterile labeled plastic bags to the laboratory.

Screening of antibiotic strains using crowded plate technique

Fungal stock solution was prepared by diluting 1gm of soil in 9 ml of sterile distilled water and shaken by using vortex and mixer. From the stock solution, 1 ml was used to prepare the final volume of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} by serial dilution method. Finally, 1ml of suspension from 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} were transferred to respective labeled plates and potato dextrose agar medium was transferred aseptically.

The plates were observed periodically for the growth of fungi. After incubation, the results were noted based on the presence and absence of colonies. The pure colonies were selected,

isolated and maintained in potato dextrose agar slants (Usha nandhini and Masilamani selvam, 2013). The isolated soil fungi were screened for their antibacterial activity against human bacterial pathogens.

Antibacterial activities of screened isolates were performed by agar well diffusion method. (Kirby Bayar method) Six pathogenic bacteria used as the antibacterial activity. Such as *Staphylococcus aerues* ATCC8739, *Bacillus subtilis* ATCC6633, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC8739, *Protease vulgaris* ATCC 13315, *Klebsiella pneumoniae* ATCC10031.

The fungal strain was isolated based on its antibacterial activity. The selected strains were further purified and maintained in agar slants at 4°C. The selected fungi are designated as F4.

Isolation and identification Phenotype characterization

The two identified fungal isolates were characterized by macroscopic and microscopic characterization. These two isolates were identified at genes level, based on substrate mycelia color, shape, and spore surface aerial, appearance of colony were identified.

Genetic Analysis

The strain was identified using 18S rRNA analysis and construct phylogenetic tree analysis. Genomic DNA was extracted from culture grow on PDA using the method (Altschul *et al* (1990). Add one µl of template DNA in 20µl of PCR reaction solution use ITS1/ITS4 primers for fungi initial Denaturation at 94°C for 5 min and performed 35 amplification cycles at 94°C for 45 sec, 55°C for 60 sec and 72°C for 60 sec. final extension at 72°C for 5 min. DNA fragments

are amplified include a positive control on (*E. coli* Genomic DNA) and a negative control in the PCR. Removed unincorporated PCR primers and dNTPs from PCR product by using Montage PCR clean up Kit (Millipore) the PCR products was sequenced using the ITS1/ITS4. Sequencing were performed using a ABI PRISM Big Dye TM terminator cycle sequencing with ampli Taq DNA polymerase (Fs enzyme)(applied biosystem) Single-pas sequencing was performed on each template using below 18S rRNA universal primers. the fluorescent labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples was resuspended in distilled water and subjected to Electrophoresis in an ABI 3730xl sequencer (applied biosystem).

Production of antibiotics by submerged fermentation

The fermentation broth was prepared (Potato dextrose broth). The fermentation was carried out by the submerged state culture. The strain F-4 was grown on PDA plates. The spore suspension was inoculated in 250 ml Erlenmeyer flask containing 50 ml of the fermentation medium and incubated on a rotary shaker at 180 rpm and 28°C for 120 hrs. The incubated period fermentation broth was able to grow on the production of antibiotic.

Recovery of secondary metabolites:

The production of crude antibiotic cultures was centrifuged at 5000rpm for 15 mins. After centrifugation the supernatant was collected and the fermentation product was extracted by solvent extraction method. (Chacko *et al.*, 2012). Ethyl acetate was added to the filtrate in the ratio of 40:40(v/v) and shaken vigorously for 1 hr for complete extraction. The aqueous phase having antibacterial property and it was dried for evaporated in water bath at 40°C. The residue obtained was weighed.

Purification of antibiotics by TLC

The extracted fractions were purified using TLC method. The fractions were dissolved in small amount of ethyl acetate and with the help of capillary tube used to apply on the spots on silica gel plates. The spots are placed about 1 cm from the edge bottom of one side and the spots were allowed to air dry. The silica gel plates were placed on the mobile phase solvent system presence of chloroform and methanol (24:1). The mobile phase was travelled on the half of the plate and the plates were removed from the mobile phase. The solvent were allowed to air dry at 37°C. the TLC plates were exposed to iodine vapor .after the distance travelled of each spots were observed and R_f values are measured.

Determination of antibacterial activity

The antibacterial activity was determined by agar well diffusion method (Suja Mathan et al., 1995). The purified isolate obtained by the evaporation of the ethyl acetate extract was dissolved in 1 ml of ethyl acetate. The test pathogens (*E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis*) were swabbed in Muller Hinton agar plates. The wells were bored in 0.9cm in diameter. 100µl of fungal extracts was loaded on the bored wells. The plates were incubated at 37°C for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured.

Absorbance using UV spectral photometer

UV-visible spectra for best active fraction for recorded on a PerkinElmer lambda 25 UV-VIS p=spectrophotometer equipped with 1.0 cm quartz cells description of (Nupur et al., 2012). The width of excitation slits were set to 1.0 nm. The spectra collected with subsequent scanning spectrum from 250 to 1100 nm at 1.0 nm increments.

FTIR-Analysis

FTIR analysis was performed in order to investigate the presence of compounds in extracted metabolites. FTIR analysis was done on Shimadzu 8400S spectrophotometer (Shimadzu Corporation, Japan) in the mid IR region of 400e 4000 cm⁻¹ with 16 scan speeds. The samples were prepared using spectroscopic pure KBr (5:95), pellets were fixed in the sample holder and analyzed.

HPLC (High Performance Liquid Chromatography)

The antibiotic fraction was monitored by analytical HPLC system employed consisted of a JASCO high-performance liquid chromatography coupled with a UV-visible multi wavelength detector (MD-910 JASCO). The analytical data were evaluated using a JASCO data processing system (DP-L910/V). The separation was achieved on a Supelco C₁₈ Silica, ODS (250× 4.6mm× 5µm) column at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: Water (25:35:40) (v/v/v). The monitoring wavelength was 280 nm. The identification of each compound was based on a combination of retention time and spectral matching.

GC-MS Analysis

The purified active compound were examined for the chemical composition using GC-MS Engine model, GC clarus 500,(Perkin Elmer, USA) and computer mass spectral library (NIST,2011 version.). Capillary column elite-5MS (polyethylene teripalate).The active best fraction was diluted with hexane and injected volume of 1:10 Electron ionization (EI) mass spectra were measured at 70 Ev over the mass range 45-450 amu. The chromatographic condition helium (carrier gas) Flow rate 1ml/min was used as an injector temperature 250°C at 5°C/9min and MS total time was 36 min. the constituents

were identified after compared with available data in the GC-MS library in the literatures.

Results and Discussion

Isolation of fungi from rhizosphere soil

The fungi were isolated from rhizosphere soil by serial dilution method. Based on morphological characteristics of fungi was isolated in selective media of potato dextrose agar media. This result has been observed in seven days. Conidial heads in shades of buff to brown colonies were observed in agar medium this may be due to secondary metabolite production. Characteristics of isolated fungi with a clear zone of inhibition around them were observed in pour plate technique. This may also be due to that the enriched medium provides certain nutrients for triggering of genes for the conversion as well as expression of other metabolic products, which in turn, leads to different areal lead substrate mycelial coloration.

Selection of fungal strain

From the crowded plate technique the strains F2, and F4 were positive results. The antibacterial assay for the isolated strains is tested against clinical pathogens of (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *E.coli*, *Staphylococcus aureus*) by agar well diffusion method. Finally, F4 sample showed the maximum antagonistic activity against *Staphylococcus aureus* (29 mm) (Plate 1)

Isolation and identification of selected fungal strains

Phenotypic characterization

The two isolate of spore chain

morphology, spore surface, aerial, substrate mycelia color and growth condition of antagonistic were determined. Morphological, Microscopical and microscopical characteristics of fungal strains were determined using specific media light and compound microscope [Table1, Plate 2]. The biochemical characterization was performed using proper way [Table 2].The strains are designated morphological different. The F-4 strain was showed maximum antagonistic activity.

Genetic analysis

The DNA of f4 strain was isolated and the 18S rRNA gene was amplified using universal eubacterial gene primers and it was phenotypically identified as *Aspergillus terreus* (F4) (Figure 1) have 1533 base pairs of 18S rRNA. Gene was determined and compared with previous sequence in the gene bank database by using the BLAST program.

Phylogenetic tree construction

Molecular phylogenetic tree was constructed F-4 strain by using neighbor joining method and the results are shown in (Fig 2.)

Production of secondary metabolites

The 250ml of fermentation broth (PDA broth) which contain 100µl of the standardized fungal suspensions were used to inoculate the flasks and incubated at 37°C on a shaker at 90 rpm for 7 days. After fermentation, the secondary metabolites were produced by isolated microorganisms.

Recovery of secondary metabolites

The fermentation was carried out for the putative antibiotic-producing cultures were centrifuged at 10,000 rpm for 20 minutes and the supernatants were collected. The crude extract of the fermentation product were recovered from the culture filtrate by solvent extract using ethyl acetate in accordance with the description of (Chacko *et al.*, 2012). Ethyl acetate was added to the antibiotic producing filtrates in the ratio 1:1 (v/v) and shaken vigorously for complete extraction.

The ethyl acetate phase that contains the crude antibiotic metabolites was separated from the aqueous phase and concentrated in vacuum at 40°C using a rotary evaporator. The residue obtained was weighed. The results are shown in [Table 3]

Purification of secondary metabolites by TLC

The extracted fractions were purified using TLC method. The fractions were dissolved in small amount of ethyl acetate and with the help of capillary tube used to apply on the spots on silica gel plates. The spots are placed about 1 cm from the edge bottom of one side and the spots were allowed to air dry. The silica gel plates were placed on the mobile phase solvent system presence of chloroform and methanol (24:1). The mobile phase was travelled on the half of the plate and the plates were removed from the mobile phase.

The solvent were allowed to air dry at 37°C. the TLC plates were exposed to iodine vapor. The fractions was isolated and purified from crude ethyl acetate

extract in chloroform and methanol mixture (24:1) by using TLC method. The R_f Value of selected compound (F4) is 0.94 and followed by the compounds recorded in [Table-4, Plate-3].

Antibacterial activity

Totally five clinical pathogens selected for antibacterial activity namely, (*klebsiella pneumoniae*, *pseudomonas aeruginosa*, *bacillus subtilis*, *E.coli*, *staphylococcus aureus*. Among the purified (F-4) compounds was tested and maximum zone formation against *Staphylococcus aureus*. The results are shown in [Table-5, Plate-4].

Absorbance using UV Spectra Photometer

The maximum absorbance μ_{max} of F-4 Strain was performed by varying the wavelength using scanning mode. The wavelength of 250 to 900 was measured. The results showed the μ_{max} in the region of 250.18 nm. The results are shown in figure (3)

FT-IR analysis

FT-IR spectra exhibits a sharp band at 1573 due to C=C-h stretching at 1401cm due to C=O stretching at 1243 due to C-O stretching at 2992cm due to C-CH stretching. On the basis of the above said spectral data, the compound was assumed to be a long chain unsaturated phenol (Figure 4).

HPLC Analysis

In current study we used shimadzu for the analysis of unknown compound against the reference antibiotic mentioned in USP 34. For identification of active compound

in in extract of F-4 isolate, coloumn 11 was used with flow rate 0.7 ml/min and injection volume 200 µl. peaks observed at retention time of 7.650 mines and run time was 15 mines. The active fungal isolate F-4 showed four peaks in HPLC graph, with retention times of 3.1min, 3.3 mines, 3.5mins, and 3.6 mines [Figure 5]. The results indicate the F-4 strain may produce antibiotics

GC-MS Analysis

GC-MS Analysis of *Aspergillus terreus* extract of F-4 showed totally ten peaks. The compounds were Cyclooctasiloxane, Hexadecamethyl (8.02%), Heptasiloxane, hexadecamethyl- (2.05%), 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (4.78%), 9,12-Octadecadienoic acid (Z,Z)- (31.23%), 9,12-Octadecadienoic acid \$\$ Linolsaeure (23.88%), 10,12-Hexadecadien-1-ol (5.82%), Docosane (cas) n-Docosane (7.49%), Hexacosane (CAS) n-Hexacosane (6.15%), TETRACONTANE (5.59%) and Hexacosane (CAS) n-Hexacosane (4.51%). The compounds present in peaks are given in the figure-6 and table-6. F-4 fraction from *Aspergillus terreus* were analyzed by gas chromatography with flame ionization detection and identified or characterized by gas chromatography-mass spectrometry. Among the F-4 compounds were esters, ketones, lipid compounds. Out of ten compounds, one compound (TETRACONTANE) used in anti-microbial effect.

Antibiotics are the most important bioactive compounds for the treatment of infectious disease. But now, because of the emergencies of multi-drug resistant pathogens, there are basic challenges for effective treatment for infectious disease.

Thus, due to the burden for high frequency for multidrug resistant pathogens in the world, there has been increasing interest for searching effective antibiotics from soil actinomycetes in diversified ecological niches (*Rajasekar et al.*, 2012). Previous studies showed that selection of different potential areas such as rhizosphere soil samples were important activity for isolation of different types of potent antibiotic producing endophytic fungi (*Tayung et al.*, 2007). Two isolates were collected from terrestrial rhizosphere soil samples with the aid of potato dextrose agar (*Panday et al.*, 2004).

Two isolates (F-4 & F-2) were found to showed antibacterial activity, F-4 was showed maximum activity, were showed high antibacterial activity. The isolates of F-4 were selected for further studies based on their high activity against the tested microbes. (*Duraipandian et al.*, 2010) reported that six fungi strains were isolated from the soil samples of the Himalaya and were screened for their antimicrobial activity (*Anupama et al.*, 2007).

A total of 10 fungal strains were isolated from the soil samples collected from the protected forest soil from two states in Northeast India. These were then characterized by conventional methods and assessed for their antagonistic activity against test microorganisms. The characterization was done by the phenotypic characterization and species affiliation by physiological and biochemical characteristics described by (*Rex et al.*, 2008).

Compounds were observed in F-4 strain though it exhibit antibacterial activity, it may because of lipid content of isolate. A broad-spectrum antibiotic from *Aspergillus terreus* was also purified by TLC

(Gebreselema *et al.*, 2013). The production of antifungal substances by these isolates was investigated using several criteria: antibacterial activity, ergosterol inhibition and UV-Vis spectra of active extracts (Tabaraie *et al.* 2012). Further, the bioactive fraction F-4 strain was characterized by using UV-Vis spectra showed the μ_{max} in the region of 250nm, FT-IR and GC-MS spectral analysis. Considering UV and FTIR Spectroscopy and according to the result of absorbance peak ranges 215 to 270nm. As well as the characteristics of adsorption peaks which signifies a highly polygene nature of the extract it is besides similarities in the general UV spectra and maximum absorbance peaks. Similarly the result presented in this investigation could explain the ability of the *Aspergillus terreus* to produce antibiotics. Furthermore the pointed spectral data are consistent with (saadoun *et al.*, 1999c) (Sharma *et al.*, 2005) reported that the maximum absorbance peaks of UV spectral data ranged between 215 to 270 nm of streptomyces isolates from the soil sample of southeastern Serbia which identical to data presented by this study (Sharma *et al.*, 2005) accordingly there is a demanding need for new and more effective antimicrobial for use in more economical through industries. Similarly the result on antibiotic production potential of isolate it might be cited that *aspergillus terreus* potential in antibiotic production could meet this demand.

The variation in the HPLC profiles in extracts obtained at different temperature could possibly be attributed in the presence of different metabolites, which in turn influence antimicrobial activity. Since manipulation of the culture conditions can improve yield and bioactivity by (Sigi *et al.*, 2013)

Similarly the variation of four peaks could possibly be attributed in the presence of different metabolites which in turn influence antimicrobial activity.

Use of multiple extraction methods, along with a sensitive measuring method (e.g., GC-MS) and possibly isolation of Ten compounds allowed us to evaluate the entire potential of secondary metabolite production in that specific environment.

Similarly F-4 fraction from *Aspergillus terreus* were analyzed by gas chromatography with flame ionization detection and identified or characterized by gas chromatography-mass spectrometry. Among the F-4 compounds were esters, ketones, lipid compounds. Out of ten compounds, may be one compound (TETRACONTANE) used in anti-microbial effect.

In this study, a rhizosphere soil sample as capable of encouraging isolation of actinomycetes was found to be of potential candidate for antibacterial activities. The search shows that the rhizosphere region of hills soil is a rich source of clinically important microorganisms, especially from fungi, requires a large number of isolates in order to discover a novel compound of pharmaceutical interest. Thus, it can be concluded from the results of the present investigation that the rhizosphere soil *Aspergillus terreus* from kuttralam hills station are a potential source of highest antibacterial, phenol compound in biomedical applications. Since fungal isolates F-4 characterized macroscopic and microscopic morphology could merely provide a presumptive genus or species identification, prominent fungi F-4 should be further identified up to species level by molecular 18S rRNA

assay and phylogenetic tree construction. These results indicate that the F-4 strain was showed highest antibacterial activity against *Staphylococcus*. Furtherly, Bioactive fraction F-4 was identified by FT-IR and HP-LC analysis. Based on the GC-MS analysis, ten compounds were identified, out of ten compounds, one

compound (TETRACONTANE) used in anti-microbial effect.

Further studies are required for the production and characterization of bioactive compounds are to be carried out with F-4 strain. The results can be applied in the pharmaceutical industries.

Table.1 Morphological characteization of selected fungi

S No	Characteristics	F-4
1.	Aerial mycelia color	Un colored
2.	Substrate mycelia color	brown
3.	Source	Rhizosphere soil
4.	Habitat	Hills station

Table.2 Morphological characterization of selected *Aspergillus terreus*

S.No	Characteristics	Best effective selected antibiotic <i>Aspergillus sp</i> F-4
1.	Shape	Convex
2.	Spore surface	Smooth
3.	Aerial mycelia color	uncolored
4.	Substrate myceial color	brown
5.	Source	Rhizosphere soil
6.	Habitat	Hills station

Table.3 Recovery of secondary metabolites

S. No	Isolated strains	Yield of secondary metabolites solvent recovery
1.	F-4	58.5 g

Table.4 Screening of compounds by using Thin Layer Chromatography (TLC)

Strain	Purified compounds	R _f values
1	F-4	0.94

Table.5 Antibacterial activity of purified F-4 fraction of compounds

S.No	Selected clinical pathogens	Zone of inhibition (in mm)
1.	<i>Escherichia coli</i>	15
2.	<i>Klebsiella pneumoniae</i>	24
3.	<i>Pseudomonas aeruginosa</i>	25
4.	<i>Bacillus subtilis</i>	20
5.	<i>Staphylococcus sp.</i>	29

Table.6 GC-MS studies of purified bioactive compound

No	Name of the compound	Peak Area %
1.	Cyclooctasiloxane, hexadecamethyl	8.02
2.	Heptasiloxane, hexadecamethyl-	2.54
3.	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	4.78
4.	9,12-Octadecadienoic acid (Z,Z)-	31.23
5.	9,12-octadecadienoic acid \$\$ linolsaeure	23.88
6.	10,12-hexadecadien-1-ol	582
7.	Docosane (CAS) n-Docosane	749
8.	Hexacosane (CAS) n-Hexacosane	6.15
9.	Tetracontane	5.59

Plate.1 Antimicrobial activity of fungal isolates

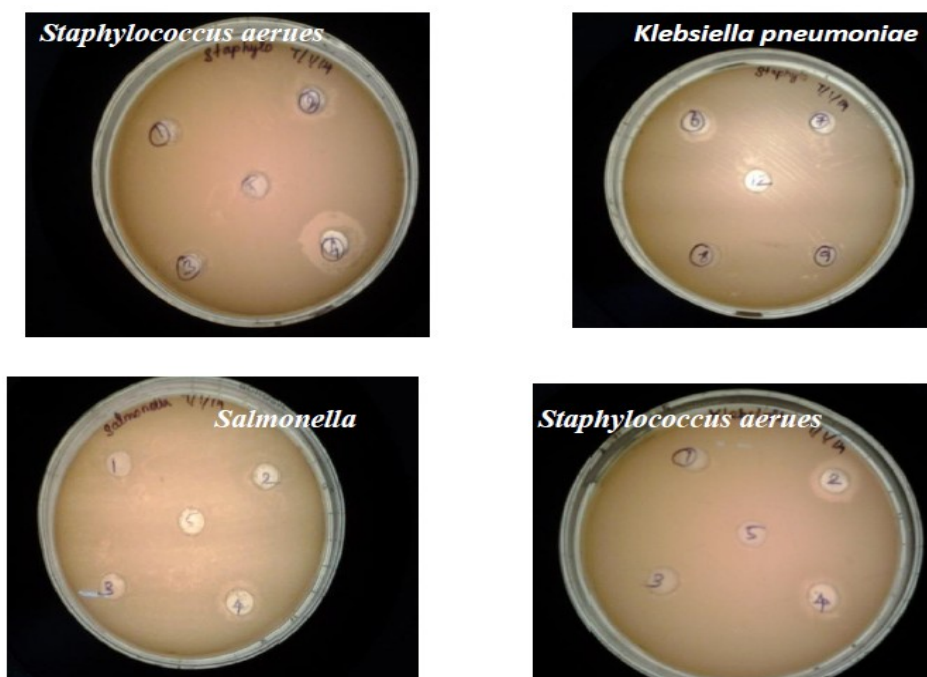


Plate.2 Morphological characterization of fungi in potato dextrose agar medium

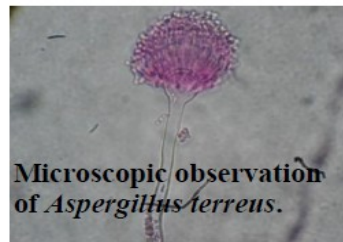


Plate.3 Thin layer chromatography (F-4)

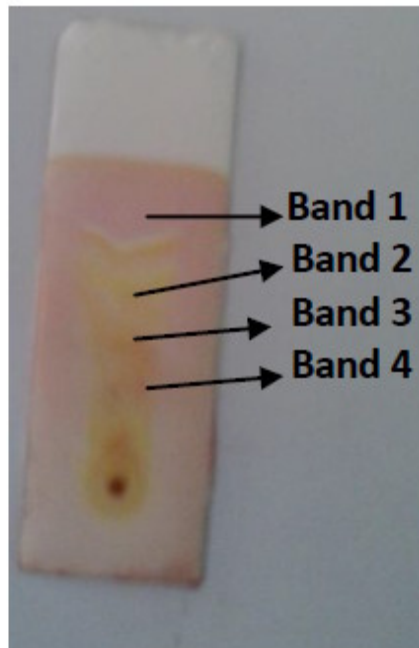


Plate.4 Antibacterial activity of extracted two antibiotics [F-4, F-2]

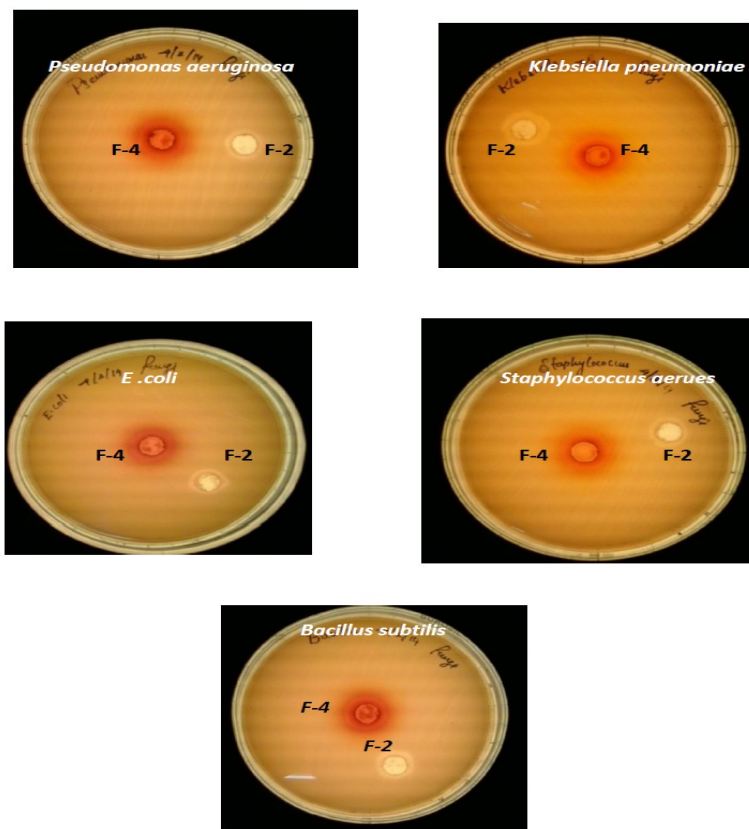


Figure.1 Genitic Analysis -18S rRNA sequencing analysis of selected F-4 strain

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CCTTCCGTAGGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCTTTATGGCCC
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GCCGCCGGGGGGCGACTCGCCCCGGGCCCGTGCCCGCCGGAGACCCCAACATGAA
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ACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAA
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GGGGAAGTGAAAGAATTCTTACACCCCTCGCGGCGGGGGTGGGTCCCACCCCCCCC
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AAGCAA
    
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Figure.2 Phylogenetic tree analysis- Phylogenetic Tree analysis of F-4 strain

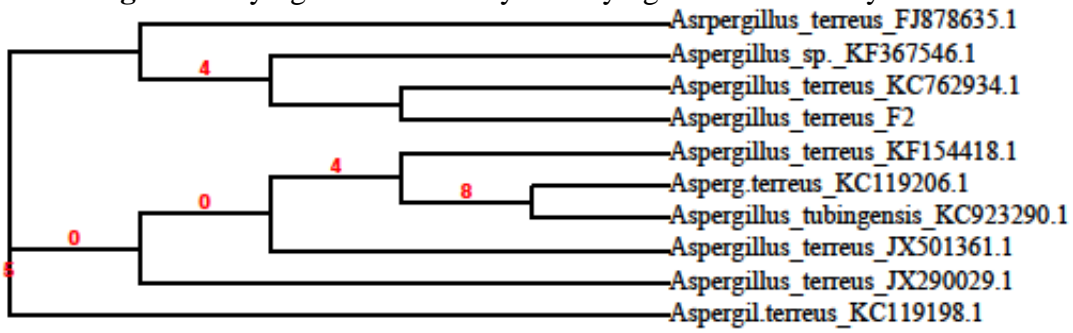


Figure.3 UV-Visible spectrum

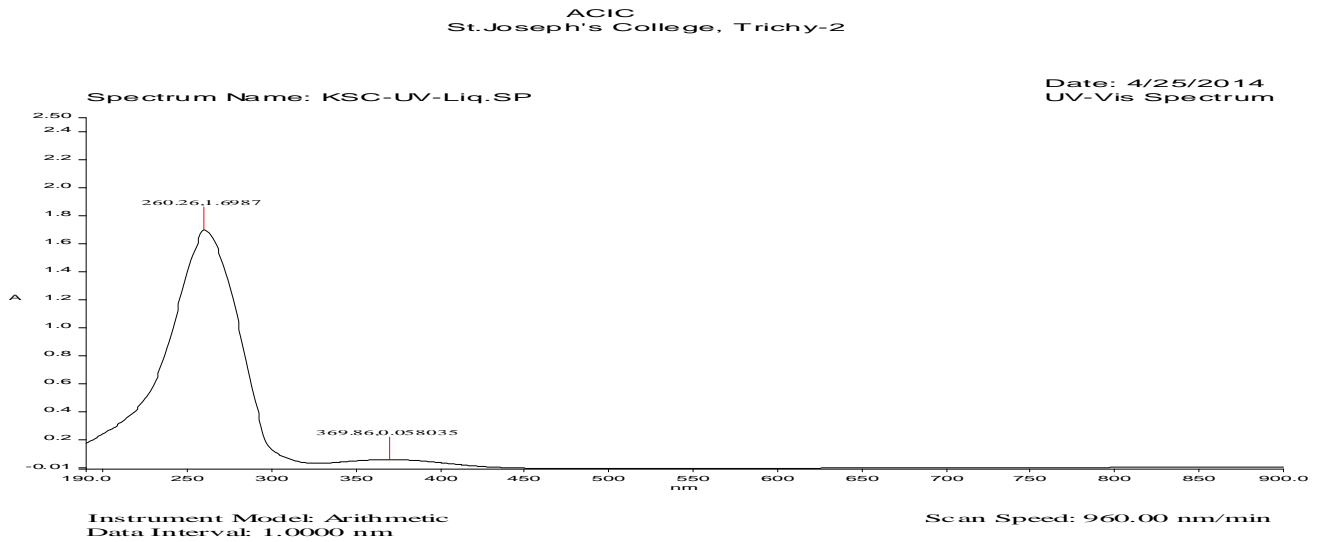


Figure.4 FT-IR-Analysis

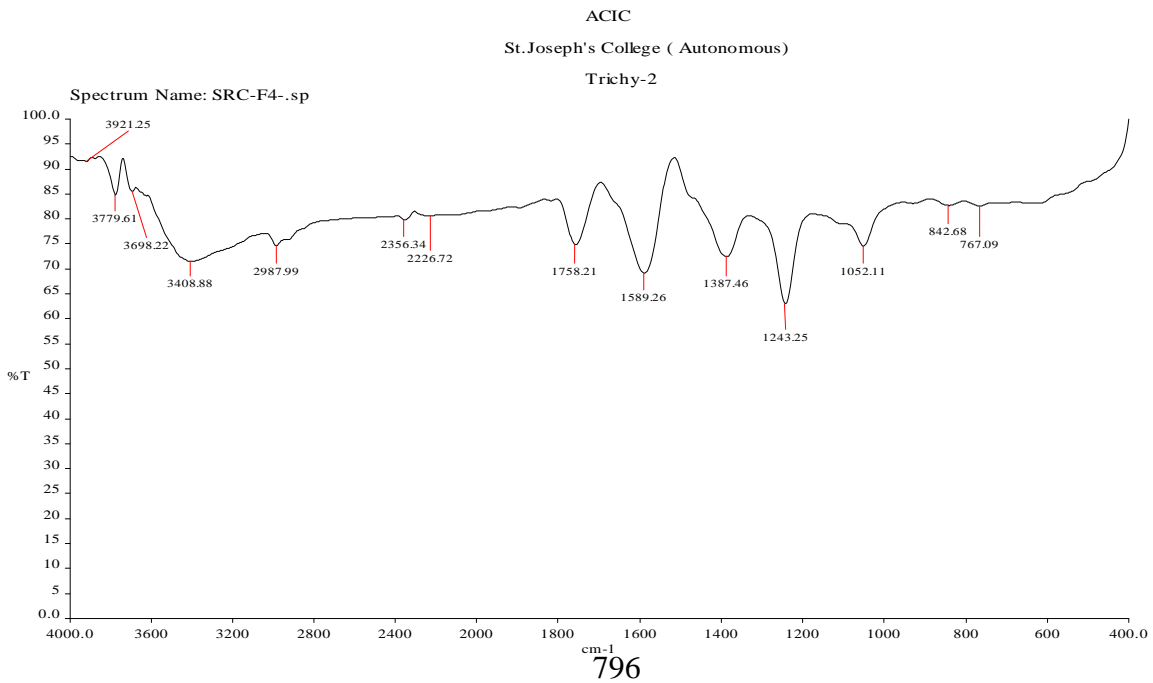


Figure.5 HPLC Analysis

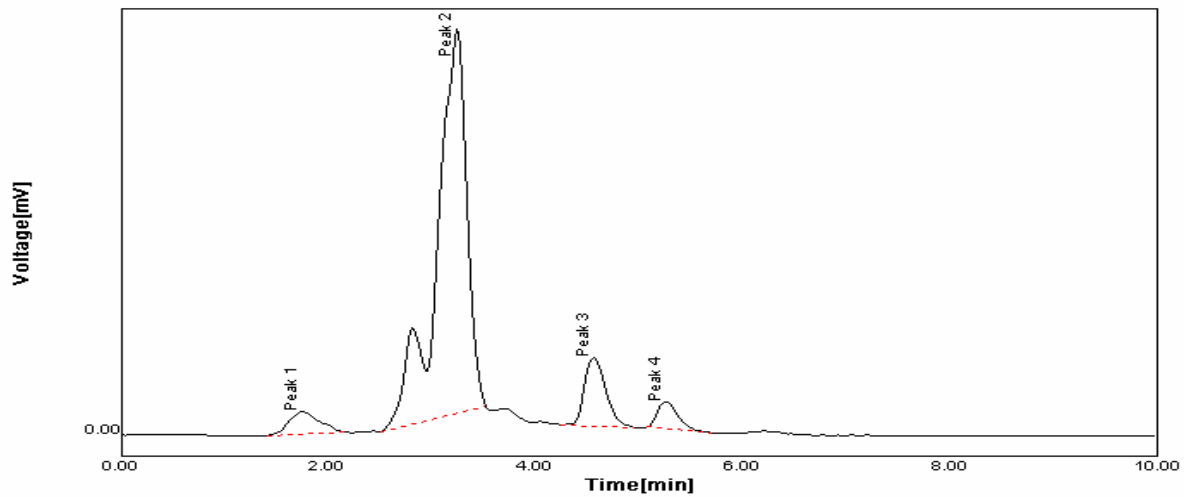
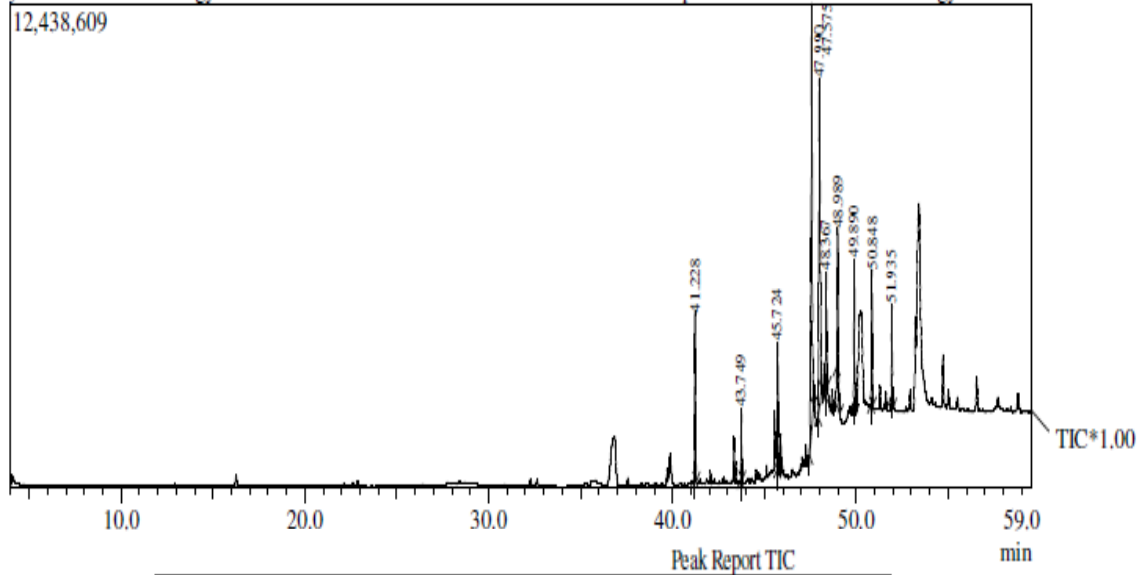


Figure 6 GC-MS Analysis

gram M.Sc Microbiology SASTRA Univ D:\GCMS E Drive\GCMS Data\2014\Apri\Data file\M.Sc Microbiology SASTRA Univ 11.04



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	41.228	12609215	8.02	CYCLOOCTASILOXANE, HEXADECAMB
2	43.749	3996672	2.54	Heptasiloxane, hexadecamethyl-
3	45.724	7514755	4.78	1,2-Benzenedicarboxylic acid, butyl 2-methyl
4	47.575	49089345	31.23	9,12-Octadecadienoic acid (Z,Z)-
5	47.990	37536361	23.88	9,12-OCTADECADIENOIC ACID \$\$ LINO
6	48.367	9155989	5.82	10,12-HEXADECADIEN-1-OL
7	48.989	11766969	7.49	Docosane (CAS) n-Docosane
8	49.890	9666942	6.15	Hexacosane (CAS) n-Hexacosane
9	50.848	8781099	5.59	TETRACONTANE
10	51.935	7084685	4.51	Hexacosane (CAS) n-Hexacosane
		157202032	100.00	

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