"Screening of endophytic fungi *Xylaria* for antibacterial and antifungal activity "

A

DISSERTATION REPORT

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THE AWARD OF THE DEGREE OF

MASTERS OF SCIENCE

IN

MICROBIOLOGY

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CONTENTS

DECLARATION

I, hereby declare that the work which is being presented in this thesis entitled "Screening of endophytic fungi for antibacterial and antifungal activity", in partial fulfilment of the requirement for the award of the degree of Masters of science in Microbiology, Department of Biotechnology and Environmental Sciences (DBTES), Thapar University, Patiala, is an authentic record of my work during the period of six months from January 2013 to June 2013, under the guidance of Mrs. M. Vasundhara, Assistant Professor, Thapar University, Patiala. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any other degree or diploma.

Anjman

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This is to certify that the above statements made by the student are correct and true to the best of our knowledge and belief.

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CERTIFICATE

This is to certify that the thesis entitled "Screening of endophytic fungi for antibacterial and antifungal activity" submitted by Nilza Angmo in partial fulfilment of the requirement for the award of Degree of Master of Science in Microbiology to Thapar University, Patiala, is a record of student's own work carried out by her. The report has not been submitted for the award of any other degree or certificate in this or any other university or institute.

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DEDICATED TO MY PARENTS

To my parents, for all the support and motivation they have given me throughout my life.....

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ABSTRACT

The search for and exploitation of natural products and their properties has been the mainstay of biotechnology research. Natural product search and discovery from endophytes of medicinal plants represents a challenge to the scientist. All the available evidence, points to natural product discovery continuing strongly and accelerating as a consequence of new search strategies and innovative microbiology. Many new drugs with interesting structures and biological activities continue to be reported. Without such discoveries, there would be a significant therapeutic deficit in several important clinical areas. Endophytes are the plant-associated microorganisms that live within the living tissues of their host plants without causing any harm to them. Almost all groups of microorganisms have been found in endophytic association with plants may it be fungi, bacteria or actinomycetes. They stimulate the production of secondary metabolites with a diverse range of biological activities. They have been known to produce enormous variety of strange and wonderful secondary metabolites, some of which have profound biological activities that can be exploited for human health and welfare. Some of the endophytic microorganisms can produce the same secondary metabolites as that of the plant thus making them a promising source of novel compounds. During the present investigation, endophytic fungus *Xylaria* was isolated from different regions of Western Ghats of India from different host plants. The fungal metabolites were sub cultured, filtered and extracted using various methods and the extracts were tested for both antibacterial and antifungal activity. The highest activity was observed with XF-4 and XF-14. *E.coli* was susceptible to only 2 extracts were as *S.aureus* and *P.aeruginosa* were susceptible to almost all the extracts including intracellular. *B.megaterium* showed comparatively less susceptibility to all extracts except for XF-14 extracellular and

C.albicans was found to be susceptible to all extracts. So out of 4 strains of *Xylaria* XF - 14 extra showed highest inhibitory effect against all test organisms followed by XF-15. Further purification of the compound can be carried out and the final compound so obtained can be useful in treatment of various diseases.

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List of Abbreviations

S.aureus	*Staphylococcous aureus*
E.coli	*Escherichia coli*
P.aeruginosa	*Pseudomonas aeruginosa*
B.megaterium	*Bacillus megaterium*
C.albicans	*Candida albicans*
A.fumigatus	*Aspergillus fumigatus*
PDA	Potato dextrose agar
PDB	Potato dextrose broth
MHA	Muller hinton agar
MHB	Muller hinton broth
mg/ml	Milligram per millilitre
µg/ml	Microgram per millilitre
°C	degree(s) Celsius
g	gram
h	hour
mL	Millilitre
µg	Microgram
µl	Microlitre
Min	Minute
Mm	Millimetre
nm	Nanometer
%	Percentage
rpm	Revolutions per minute
Sec	Second
w/v	Weight by volume

Chapter 1

INTRODUCTION

1. INTRODUCTION

1.1 Fungi

Fungi are ubiquitous and a distinct class of microorganisms, most of which are free-living in nature where they function as decomposers in the energy cycle. Fungal life is found worldwide, in soil samples as well as deep sea vents and artic ice and often reveals symbiotic traits. Similar to plants, there is a long history of the utilization of fungi by mankind as remedies and in everyday life. Nearly 3000 years ago Mayans used fungi to treat intestinal ailments (Strobe *et al.*, 2004). It was only after the discovery of penicillin isolated from *penicillium notatum* by Sir Alexander Fleming in 1928 that resulted in a breakthrough in the treatment of bacterial infections, that fungi became an important source of drugs for the treatment of a variety of diseases. They are eukaryotes with a higher level of biological complexity than bacteria. They may be unicellular or may differentiate and become multicellular by the development of branching filaments.

Metabolism

Fungal metabolism is heterotrophic, requiring exogenous carbon for growth. Metabolic diversity is great, but most fungi grow with only an organic carbon source and ammonium or nitrate ions as a nitrogen source. In nature, nutrients for free-living fungi are derived from decaying organic matter. A major difference between fungi and plants is that fungi lack photosynthetic energy-producing mechanisms. Most are strict aerobes, although some can grow under anaerobic conditions. None are strict anaerobes.

Reproduction

Fungi may reproduce by either asexual or sexual processes. Reproductive elements produced asexually are termed conidia. Those produced sexually are termed spores (e.g., ascospores, zygospores, basidiospores). Asexual reproduction involves mitotic division of the haploid nucleus and is associated with production by budding spore-like conidia or separation of hyphal elements. In sexual reproduction, the haploid nuclei of donor and recipient cells fuse to form a diploid nucleus, which may then divide by classical meiosis. Some of the four resulting haploid nuclei may be genetic recombinants and may undergo further division by mitosis. Highly complex specialized structures may be involved.

Detailed study of this process in fungal species such as *Neurospora crassa* has been important in gaining an understanding of basic cellular genetic mechanisms.

Classification

Although conidia are more readily observed, the major classification of fungi primarily depends on the nature of sexual spores and siltation of hyphae as its differential characteristics. On this basis, fungi have been organized into four to six classes or phyla. A major problem of classifying the medically important fungi using these groups is that for most species, no sexual form has been demonstrated. This may be due to its loss during evolution or because the spores are so rarely produced that they have not been detected. One approach has been to give these fungi their own class (Deuteromycetes, or fungi imperfecti) and wait for the discovery of the sexual form to place it in one of the legitimate groups—the Ascomycetes, Basidiomycetes, or Zygomycetes. The application of molecular methods such as analysis of ribosomal RNA genes has allowed the placement of species pending discovery of the sexual forms. Discovery of the sexual form may not bring immediate clarity from the student's standpoint; for instance, when the sexual stage of *Trichophyton mentagrophytes* was demonstrated, it was found to be identical to that of an already named ascomycete (*Arthroderma benhamiae*). Most medically important species are now assigned to the Ascomycetes and a few to the Basidiomycetes or Zygomycetes.

1.2 Endophytes

Endophytes are those microorganisms that inhabit interior of plants especially leaves, stems, roots shows no apparent harm to host. Almost all classes of vascular plants and grasses examined till date are found to host endophytic organisms. Different groups of organisms such as fungi, bacteria, actinomycetes and mycoplasma are reported as endophytes of plants. The existence of endophytes has been known for over one hundred years. In literal translation, the word endophyte is derived from Greek, 'endo' >< 'endon' meaning within, and 'phyte'>< 'phyton' meaning plant. Endophytic fungi are to be found in virtually every plant on earth. These organisms reside in the living tissues of the host plant and do so in a variety of relationships ranging from symbiotic to slightly pathogenic. If the host plant is weakend, the endophytes can also become an aggressive saprophyte. The endophyte obtains nutrients and protection from the host and in return enhances the fitness of the host by producing certain functional metabolites. Because of what appears to

be their contribution to the host plant, the endophytes may produce a plethora of substances that may have potential use to modern medicine, agriculture and industry.

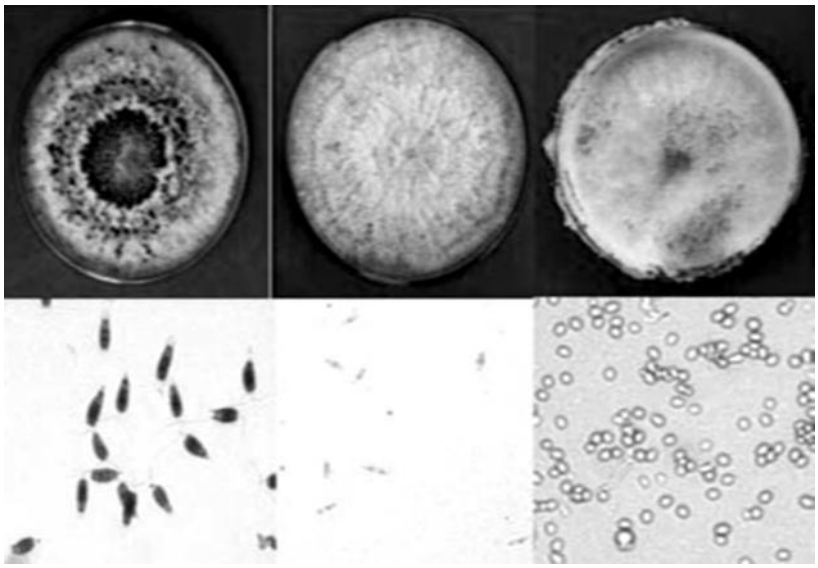


Fig 1. Pictures of various endophytic fungi and their spores

Novel anti tumor (taxol) antibiotics, antimycotics (quercine), immune suppressants, anticancer compounds are an only few good examples of what has been found after the isolation, culture, purification and characterization of some choice of endophytes in the recent past. The potential prospects of finding new drugs that may be effective candidates for treating newly developing diseases in human plants and animals are great.

The production of bioactive compounds by endophytes is directly related to the independent evolution of these microorganisms, which may have incorporated genetic information from higher plants, allowing them to better adapt to plant host and carry out some functions as protection from pathogens, insects and grazing animals. Endophytes are chemical synthesizers inside plants, in other words, they play a role as selection system for microbes to produce bioactive substances with low toxicity towards higher organisms (Strobel, 2003). Bioactive natural compounds produced by endophytes have been promising potential usefulness in safety and human health concerns, although there is still a significant demand of drug industry for synthetic products due to economic and time – consuming reasons (Strobel *et al*., 2004). Methods to obtain bioactive compounds include the extraction from a natural source, the microbial production via fermentation or microbial transformation. Extraction from natural sources presents some disadvantage as dependency on seasonal, climatic and political features and possible ecological problems

involved with extraction, thus calling for innovative approaches to obtain such compounds (Bicas *et al*., 2009).

Fungal endophytes mainly belong to ascomycetous family, basidomycetes, deuteromycetes and oomycetes are rarely found (Arnold *et al*., 2007). Although they don't show host specificity but certain fungal lineages appear frequently in plants representing families and denoting host preferences (Cannon and Simmons, 2002). Diversity in endophytes and the various bioactive compounds which they produce has spurged interest in research on the chemistry of endophytic fungi.

The *Xylariaceae* are a large and relatively well-known family which is representative of ascomycetous fungi. They are mainly found throughout topical and temperate regions and typically found on woody plants, woods, seeds, fruits, plant leaves and some are even associated with insect nests. Many species actively decay wood of living or dead angiosperms and are known to be saprophytic in most case.

TAXONOMICAL / SCIENTIFIC CLASSIFICATION

Kingdom	Fungi
Division	Ascomycota
Class	Sordariomycetes
Order	*Xylariales*
Family	*Xylariaceae*

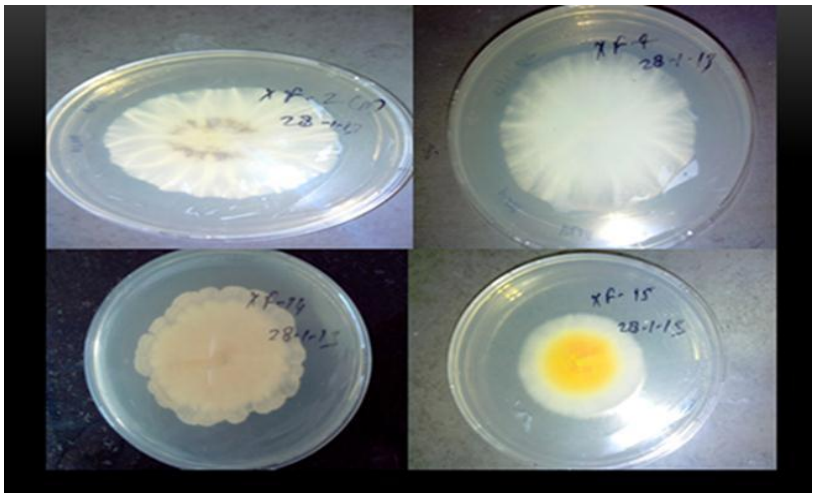


Fig 2. Pictures of 4 strains of *Xylaria* species after sub culturing on PDA

The members of Ascomycota division are known as sac fungi. They are the largest phylum of fungi with over 64,000 species (Kirk *et al.*, 2008). The distinct feature of this group is ascus, a microscopic sexual structure which consists of non-motile spores i.e., ascospores.

Xylaria is a large and the first described genus of the *Xylariaceae*. *Xylaria* species are saprophytic or sometimes weakly to strongly parasitic on woody plants and usually have erect elongated stromata. Although they are found mostly on wood, certain stroma are found on sawdust, leaf, dung or soil.

ASCOSPORES

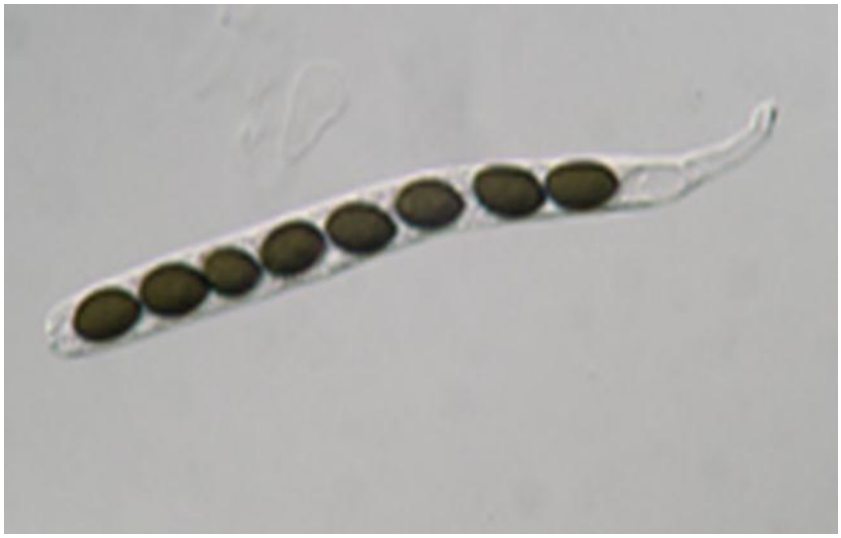


Fig 3. Ascospores

2. OBJECTIVE OF THIS PROJECT

Drug resistance in various bacteria and fungi, food borne diseases, consumer demand for natural foods, the abuse of toxic synthetic food substances, the threat posed by diseases such as cancer and other ever increasing infections has clearly proved the need for new antimicrobial drugs and novel modes of action. Recently there has been a considerable interest in endophytic fungi as they have the ability to produce various compounds with antimicrobial activities. Since these organisms have been least explored there is greater chance of encountering with many effective bioactive compounds which would be a boon to mankind.

The endophytes are poorly investigated group of microorganisms, but they represent an abundant and dependable source of novel bioactive compounds which has great potential for exploitation in variety of agriculture, medicine and industrial areas. The objective of this project was to screen various strains of endophytic fungi *Xylaria* for the production of various antimicrobial products and then test its antimicrobial susceptibility on various micro-organisms and then the extraction of the bioactive compounds and if possible within the given time, purification and characterization of the compound.

OBJECTIVES :

1.Screening of endophytic fungi for antimicrobial activity.

2.Characterization of the secondary metabolite having antifungal and antibacterial potential.

Chapter 3

REVIEW

OF LITERATURE

3. REVIEW OF LITERATURE

3.1 Endophytic fungi

Endophytic fungi are those organisms that reside within plant tissues without causing any immediate overt negative effects and they have been found in every plant species examined to date and have been recognized as the potential sources of various novel bioactive compounds (Bacon and White, 2000; Strobel and Daisy, 2003; Kumar and Sagar, 2007).

De bary (1866) first defined all organisms that colonize the internal plant tissue as endophyte (Gr. endon, within; phyton, plant) (De bary, 1866). Endophytic fungus was first identified by Freeman in 1904, and was isolated from *Loliumpersicum* (Persian darnel).

Of nearly 3,00,000 plant species that exist on the earth, each individual plant is the host to one or more endophytes (Strobel and Daisy, 2003). In this view, it is estimated that there may be as many as 1 million different endophyte species and only handful have been described, so there is more chance of finding new endophytes with novel compounds (Andrew and Hirano, 1991).

Fungal endophytes consist of two basic ecological groups : The Balansiaceous or "Grass endophytes" and the Non-Balansiaceous. Balansiaceous endophytes form a distinctive group of closely related fungi with ecological requirements and adaptation discrete from those of other endophytes (Petrini, 1996). They grow systemically, epicuticularly and intercellularly within all above ground plant organs of grasses, resulting in vertical transmission of the endophytes through the seeds. They belong to the clavicipitaceous genera *Epichloë* and *Balansia* and their anamorphs *Neotyphodium* and *Ephelis* (Schardl *et al*., 2004). The balansiaceous endophytes produce a diverse array of secondary metabolites.

Non- balansiaceous endophytes : These are diverse, both phylogenetically and with respect to life-history strategy. Most of them belong to the Ascomycota and colonize either inter or intracellular, localized or systemic. The majority of these isolates belonged to ubiquitous genera (e.g. *Acremonium, Alternaria, Cladosporium, Coniothyrium, Fusarium, Pleospora*) but some genera are common in both tropical and temperate climates (e.g. *Fusarium, Phomopsis, Phoma*) while members of the *Xylariaceace, Colletotrichum, Guignardia,*

Phyllosticta and *Pestalotiopsis* predominate as endophytes in the tropics (Schulz *et al*., 2005).

3.2 Endophytic fungi as a source of bioactive compounds

Endophytes are the chemical synthesizers inside plants (Owen and Hundley, 2004), in other words, they play a role as a selection system for microbes to produce bioactive substances with low toxicity towards higher organisms (Strobel, 2003).

Inmaculada *et al*., (2000) assayed 317 isolates of Basidiomycetes representing 204 species collected in Spain against a number of human pathogens and the amount of extracts from basidiomycetes shown antimicrobial activity (Anke *et al*., 1989) was similar or above that produced by orders of Ascomycete, example *Xylariales* and *Pezizales*. This suggested that there is no homogeneity in the ability to produce bioactive compounds among different classes of fungi.

Son Radu *et al.,* (2002) assayed 121 isolates of endophytic fungi isolated from medicinal plants in Malaysia. Sensitivity was found to vary among the microorganisms. Sixteen endophytic fungal isolates tested were also found to exhibit antitumor activity in the yeast cell-based assay.

Zhang *et al*., (2006) assayed about 140 novel products from endophytic fungi between the year 1987 and 2000 approx.

In a review by Cragg and Newman (2007), all approved agents were reported from 1981 to 2006, from which a significant number of natural drugs were produced by endophytes. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones and others (Tan and Zou, 2001). Such bioactive metabolites find wide-ranging application as agrochemicals, antibiotics, immune suppressants, antiparasitics, antioxidants and anticancer agents (Gunatilaka, 2006).

Xiang Lin *et al*., (2007) assayed about 174 endophytic fungi isolated from the pharmaceutical plant, *Camptotheca acuminate*. The results of the bioactivity test showed that 27.6% of the endophytic fungi displayed inhibition against more than one indicator microorganism. 4.0% and 2.3% of the endophytic fungi showed cytotoxicity and protease inhibition, respectively. The endophytic fungi with bioactivities were distributed in more

than 12 taxa including non-sporulating fungi, which are reliable sources for bioactive agents.

Jalgaonwala *et al*., (2010) assayed 142 endophytic fungal isolates from various parts of medicinal plants (Fisher, 1984) belonging to Jalgoan Maharashtra for evaluation of antimicrobial activity against various pathogenic and opportunistic microbes. 78 fungal isolates exhibited antimicrobial activity.

Yong-Mei Xing *et al*., (2011) assayed 53 endophytes, from roots and stems of *Dendrobium* species, 30 endophytic fungi in *D. Devonianum* were categorized into 11 taxa and 23 fungal endophytes in *D. thyrsiflorum* were grouped into 11 genera, respectively. 10 endophytic fungi in *D. Devonianum* and 11 in *D. thyrsiflorum* exhibited antimicrobial activity against at least one pathogenic bacterium or fungus among 6 pathogenic microbes (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Candida albicans, Cryptococcus neoformans,* and *Aspergillus fumigatus*).

L. Sathish *et al.,* (2012) assayed 30 endophytic fungi from leaves and twigs of *Eucalyptus globulus* and *Eucalyptus citriodora.* Among these almost all showed various antimicrobial activities and some produced various other enzymes as amylases, proteases etc.

Min-Yuan Ho *et al*., (2012) assayed a total of 156 isolates of endophytic fungi from 22 species of medicinal plants in Taiwan. Based on morphological characteristics and rDNA and ITS characteristics, 67 isolates, belonging to 21 genera in 15families, were from *Lauraceae* and 89 isolates, belonging to 27 genera in 16 families, were from *Rutaceae*. The most abundant genera were *Xylaria, Guignardia, Hypoxylon, Nigrospora, Phomopsis* and *Colletotrichum.* These presented fungal endophytes were preserved in Plant Fungal Parasite and Molecular Diagnosis of Fungicide Resistance Lab, Department of Plant Pathology, National Chung Hsing University.

3.3 Endophytic fungi *Xylaria* biological and chemical diversity

The *Xylariaceae* is a large and relatively well-known ascomycete family found in most countries (Whalley 1996), and it contains 35 genera (Eriksson & Hawksworth 1993). Species of *Xylaria* are difficult to identify and classify especially as the stromata of a given species often vary greatly in colour, size and sometimes in general shape (Whalley 1996).

Edward *et al.,* (1999) demonstrated chemical and biological diversity of *Xylariaceae*. It revealed that filamentous fungi have an excellent record of synthesizing a range of bioactive metabolites which have potential antimicrobial activity. The major metabolites produced could be grouped as dihydroisocoumarins and derivatives (Anderson *et al*., 1983), succinic acid and derivatives (Anderson *et al.,* 1985), butyrolactones (Edward *et al*., 1989), sesquiterpene alcohols, griseofulvin and derivatives (Whalley *et al*., 1995) etc.

Park *et al.,* (2004) isolated an endophytic fungi from *Abies holophyllla* producing griseofulvin and checked its antifungal activity against various plant pathogenic fungi in vivo. Two antifungal substances were purified from liquid cultures and their chemical identities were similar to those of griseofulvin and dechlorogriseofulvin through mass and NMR analysis. Comparing them showed that griseofulvin showed high in vivo and in vitro antifungal activity and effectively controlled the development of rice blast (*Magnaporthe grisea*), rice sheath blight (*corticium sasaki*), wheat leaf rust (*puccinia recondite*).

Xiaoli Liu *et al*., (2007) isolated an endophytic *Xylaria sp*., having broad antimicrobial activity, from *Ginkgo biloba* L. From the culture extracts of this fungus, a bioactive compound P3 was isolated by bioactivity-guided fractionation and identified as 7-amino-4-methylcoumarin by nuclear magnetic resonance and mass spectrometry spectral data. The compound showed strong antibacterial and antifungal activities in vitro against *Staphylococcus aureus* (MIC,16μg/ml], *Escherichia coli* (MIC,10μg/ml), *Salmonella typhia* (MIC,20μg/ml), *Salmonella typhimurium* (MIC,15μg/ml), *Salmonella enteritidis* (MIC,8.5μg/ml), *Aeromonas hydrophila* (MIC,4μg/ml), *Yersinia sp.* (MIC,12.5μg/ml), *Vibrio anguillarum* (MIC,25μg/ml), *Shigella sp.* (MIC,6.3μg/ml), *Vibrio parahaemolyticus* (MIC,12.5μg/ml), *Candida albicans* (MIC,15μg/ml), *Penicillium expansum* (MIC,40μg/ml), and *Aspergillus niger* (MIC,25μg/ml). This was the first report of 7-amino-4-methylcoumarin in fungus and of the antimicrobial activity of this metabolite (Zhu *et al*., 1993).

Sutjaritvorakul *et al*., (2011) Eleven fungal endophytes representing different morphotaxa were characterized from 68 cultures, which were isolated from 4 species of Dipterocapoustrees (*Dipterocarpu tuberculatus Roxb., Shoreaobtusa Wall., Shoreasia mensis Miq*. and *Dalbergiaoliveri Gamble*.) growing in the Dipterocapous forest at Viengsa district, Nan province. Species of *Phyllosticta spp.* (15 isolates), *Nodulisporium spp.* (13 isolates) and *Xylaria sp.* (10 isolates) were the most frequently found. All

endophytic fungal isolates were tested for potential production of bioactive metabolites. They were tested for antimicrobial activity against pathogenic microorganisms such as *S.aureus, B.subtilis, P.aerogenosa, E.coli* and *C.albicans* by the paper disk susceptibility test. They inhibited the growth of Gram positive bacteria more than Gram negative bacteria. *C.albicans* was inhibited only by *Nodulissporium sp.* and *Xylaria sp.*

Archana Nath *et al.,* (2012) assayed a total of four endophytic fungi belonging to Phylum Ascomycetes from different parts of the plant (*Emblica officinalis*) which were characterized morphologically and by using rDNA-internal transcribed spacer. The most frequently isolated endophyte was *Phomopsis sp*. and *Xylaria sp.*, and *Xylaria* showed higher levels of phenolics. Antimicrobial activity of fungal extracts was tested against four bacteria namely, *E.coli* MTCC 730, *Enteroccocus faecalis* MTCC 2729, *Salmonella enterica* ser. paratyphi MTCC 735 and *S.pyogenes* MTCC 1925, and the fungus *C.albicans* MTCC 183. In general, the fungal extracts inhibited the growth of test organisms except *E.coli.*

3.4 Techniques of screening and characterization of bioactive compounds

Obtaining secondary metabolites

Li *et al*., (2005) assayed a methodology in which the fungi were incubated in PD (Potato Dextrose) medium at 28º C for 15 days. The fermentation medium was centrifuged at 3,600 rpm for 10 minutes. The supernatant was transferred to a separatory funnel to which was added the same volume of crude ethyl acetate. The funnel was strongly agitated and then the separation of the phases occurred by polarity difference. The process was repeated twice. The obtained ethyl acetate extract was 98% concentrated in a R-3000 Büchi rotary evaporator at 40º C and the material obtained from the evaporation was suspended with 5 ml of absolute methanol and stored at 4º C.

Assessment of antimicrobial activity

Acar *et al.*, (1980) performed the antibacterial assay of the fungal extracts using a standard disc diffusion assay. Bacteria *Pseudoalteromonas spongiae* (Lau *et al*., 2005) and *Vibrio vulnificus* were used as a target because these two bacteria were found sensitive to the minor changes in bioactivity of fungal extracts in the primary screen. A 250-µg fungal extract was loaded onto a sterile filter paper disc (6 mm in diameter). The paper disc was air-dried and placed onto the nutrient agar plate that had already been inoculated with a

lawn of target bacteria. After incubation for 24 h at 30°C, the antibacterial activity was evaluated by measuring the width of the growth inhibition zones from the edge of each filter paper.

Rivers *et al*., (1988); Reis, (2006); Alves *et al*., (2008) assayed technique for determination of minimum inhibitory concentrations (MIC) and it is often considered as the best methodology for assessing antibiotics susceptibility or resistance of bacteria to antibiotics. According to Ostrosky *et al*., (2008), MIC has several advantages and one is that this method can be 30 times more sensitive than other methods used in the literatures.

Sambrook *et al.,* (2001) assayed a technique for the antimicrobial activity of metabolite extracts by cup plate diffusion technique. The test bacteria were grown on liquid LB (Luria Bertani) medium for 24 hours, adjusted at a concentration of 1×10^6 cells/ml. The bacteria (100 µl) were inoculated on the Petri dishes containing solid LB medium and spread with a Drigalsky spatula. Afterwards, four sterile Whatman No. 4 filter paper disks were placed (6 mm) equidistant and inoculated with 10 µl of the metabolite extract. The plates remained incubated at 37ºC for 24hours. The antimicrobial activity was detected by the formation of an inhibition halo. The diameter of the halo was measured in triplicate and compared with control.

Palombo *et al.,* (2001) described antimicrobial assays in PDA plates using the plate-hole diffusion with the exception that wells made in agar were 12 mm in diameter and 200µl of endophyte culture filtrate were added to each test well. PDB was used as a negative control, while ampicillin (25µg/ml) was used as a positive control in the antibacterial assays. NA plates were incubated at 37°C for 24 hours while PDA plates were incubated at 30°C for 24 hours. The filtrates were used directly in antimicrobial testing. Test bacteria, *S.aureus* (ATCC 25923) and *E.coli* (ATCC 25922) were grown in Nutrient Broth (Oxoid), while fungi, *C.albicans* (Food Science Australia Culture Collection 5580), were cultivated in PDB.

Suthep Wiyakrutta *et al*., (2004) extracted fungal metabolite in the mycelial mat by solvent extraction procedure with ethyl acetate as organic solvent. The mycelia mat was soaked in ethyl acetate for 2hrs and ground in a mortar pestle.

Valgas *et al.,* (2007) assayed a range of natural products of fungi, plant and lichen origin against 2 bacterial species *S. aureus* ATCC 25923 and *E.coli* ATCC 25922 by two varients

of the agar diffusion method (Smania *et al*., 1999), well and disc, bioautographic method (Hamburger *et al*., 1987) and by micro dilution assay (Vanden berghe, 1991) to determine antibacterial activity. It was concluded that the well -varient of diffusion method was more sensitive than the disc-varient, while the direct-varient of the bioautographic method exhibited a greater sensitivity if compared to indirect variant.

Vaz *et al*. (2009) assayed In vitro antimicrobial susceptibility tests with a panel of microorganisms: *E.coli* ATCC 25922, *S.aureus* ATCC 12600, *P.aeruginosa* ATCC 27853, *B.cereus* ATCC 11778, *S. typhimurium* ATCC 14028, *C.albicans* ATCC 18804, *C. krusei*. Inoculum of the target microorganisms were adjusted to a McFarland no. 1 standard. A McFarland 0.5 standard in optical density for bacteria corresponded to 1 to 2×10^8cfu/ml. The concentrations were confirmed via spectrophotometer readings at 626 nm. Bacterial samples were inoculated using a swab on MHA.

Maksum Radji *et al.*, (2011) evaluated the antimicrobial activity of the extracts by agar well diffusion method. The test cultures used were: *Candida albicans* ATCC 24433, *Escherichia Coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853. A volume of 50 ml of nutrient broth was prepared and dispensed into boiling tubes (10ml each) and they were sterilized. In each of sterilized nutrient broth tubes a loopful of test cultures were inoculated and kept in a shaker overnight at 37°C for growth. A volume of 500ml of nutrient agar was prepared and sterilized. 1 ml of test culture solution was added into the each 100ml of agar solution. The agar solutions with culture were poured in to the sterile Petri dishes and allowed to solidify. Wells was punched using a puncher on the plates. A volume of 50µl of the CCF and CME were loaded to each of the well. The test was carried out in triplicates. The negative control plate was loaded with DMSO on the well. Simultaneously a positive control was maintained with streptomycin. After 24 hrs incubation at 37°C, the plates were observed for zone of inhibition and measured.

Yu Li *et al*., (2011) isolated a product named cytochalasin H2 with cytochalasin H (Tao *et al.*, 2008) from the agar cultures of the strain *xylaria sp.* A23, which was isolated from *Annona squamosal* (Yang *et al.*, 2008, Chavan *et al.* 2010, Liaw *et al.* 2008). The antibacterial activities were tested against *Bacillus subtilis* (CMCC (B) 63501) using slip method. The compound had no effect on the test bacteria

Chapter 4

EXPERIMENTALWORK

4. EXPERIMENTAL WORK

4.1 Instrumentation

Apparatus and instruments used :

Petri plates, Separation funnel, Borer (7mm and 10mm), Spreader, Test tubes, Microtitre plate (Tarson), Auto pipettes (1ml, 20-200µl), Shaker at 25˚C/120rpm (Kuhner Shaker), Test tube stand, Cuvettes, Wash bottle, Eppendorfs, Eppendorf stand, Flasks, tubes, Mortar pestle, Filter paper.

Laminar air hood, Spectrophotometer (HITACHI, U-2900), Rota-evaporator (Yamato), Rota flask, Centrifuge (Sigma), Vortex (Genei), Incubator at 25˚C and 36˚C, Autoclave (Equitron), ELISA reader (Thermo Scientist MULTISKAN SPECTRUM).

Chemicals used : PDA (potato dextrose agar), PDB (potato dextrose broth), LB (luria broth), LA (luria agar), NB and NA (nutrient agar and nutrient broth), MHA (muller-hinton agar).

Antimicrobials : Streptomycin, Ampicillin, Penicillin and Fluconazole.

Other reagents : Barium chloride, Conc. H_2SO_4, Ethyl acetate (Merck), Methanol, Distilled water.

4.2 Source of endophytic fungi

Endophytic fungi were isolated from different regions of Western Ghats of India from different host plants. They were isolated from different parts of different plant species and then the plant samples were rinsed gently in running water to remove adhered dust and debris. Samples were cut into 2 mm segments and were surface sterilized with 70% ethyl alcohol for 1 min, soaked in 4% sodium hypochlorite solution for 3 min, and then rinsed with 70% ethyl alcohol for 1 min. They were finally rinsed with sterile distilled water and blot dried on sterile filter paper. The excess water was dried under laminar airflow chamber. These fungi were then cultured on PDA and after that sub cultured for this project. The fungal species used in this project were species of XF-2, XF-4, XF-14, XF-15.

CODE NO.	HOST	FAMILY
XF-2	*Memycylon*	*Melastomaceae*
XF-4	*Lasianthusvenulosus*	*Rubiaceae*
XF-14	*Randiadumetorum*	*Rubiaceae*
XF-15	*Neolitseascrobiculata*	*Lauraceae*

These fungal cultures from TIFAC-CORE, Thapar University were used in the project for investigating antimicrobial activity and the presence of novel bioactive compounds.

4.3 Subculturing of fungus "*Xylaria"*

From the master culture 1 piece of fungus was cut with the help of a sterile blade and then placed at the centre of PDA plate and then incubated for 14 days at 25˚C.

4.4 Composition of the media used for culturing of *Xylaria*

1. PDB (potato dextrose broth) (HiMedia laboratories Pvt. Ltd)

(Mac Faddin *et al*., 1985)

Suspend 24g of PDB in 1000ml distilled water. Boil to dissolve the medium completely. Sterlize by autoclaving at 15 lbs pressure and 121˚C temperature for 15 minutes.

Standard Formula

Ingredients	Gram/litre
Potato infusion	200
Dextrose	20

Final pH (at 25˚C) 5:1

4.5 Culturing of fungi

The fresh mycelia (grown on PDA) of representative endophytic fungi (XF-2, XF-4, XF-14, XF-15) were transferred to 250 ml Erlenmeyer flask containing 100 ml of the PDB. The culture flasks were incubated for 15 days at 25±1˚C and at 120 rpm. After the incubation period, the cultures were taken out and filtered through sterile mesh cloth to separate the mycelia from the culture broth (Prabavathy *et at*., 2011).

4.6 Extraction procedure

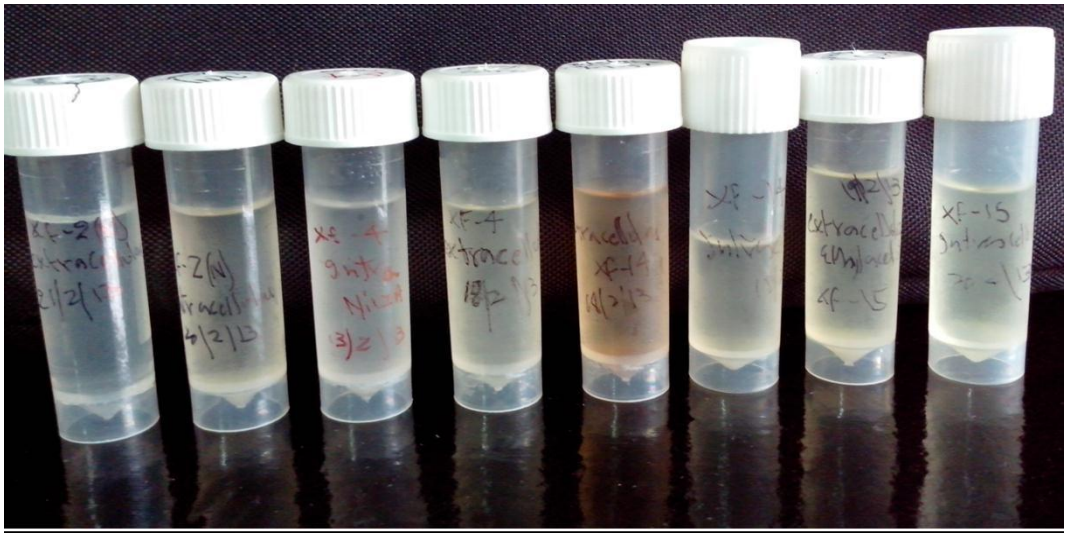
(Intracellular)

The mycelium of each fungal extract was separated from the fungal extract by passing through a funnel containing glass wool and then the mycelium was dried with the help of a blotting paper. After drying the dried mycelium were crushed in a mortar pestle by adding liquid nitrogen and then dissolved in methanol. After that the intracellular extract was centrifuged at 12,000 rpm at 4˚C for 10 mins. The pellet was discarded and the supernatant was taken in another ockrage tube. The supernatant obtained was evaporated using a rota evaporator at 40˚C for 20-40 minutes and the residual compound was dried and the weight of the residue was estimated. The dried compound was mixed with 5ml of methanol and then stored in a 5ml glass tubes. These extracts were further used to check for their antibacterial and antifungal activities.

(Extracellular)

The fungal metabolites were extracted by solvent extraction method using ethyl acetate as solvent. Equal volumes of the culture filterate/mycelial extract and ethyl acetate were taken in a separating funnel and were shaken vigorously for 5 mins and then allowed to settle for 5mins and then again followed the same procedure for 5 times and then the organic phase was collected. After that again added ethyl acetate and then followed the same procedure as above two more times where in the aqueous and organic phase got separated and the organic phase so obtained was collected. Ethyl acetate was then evaporated using a rota evaporator at 40˚C for 20-40 minutes and the residual compound was dried and the weight of the residue was estimated. The dried compound was mixed with 5ml of methanol and then stored in a 5ml glass tubes at 4˚C. These extracts were further investigated for their antibacterial and antifungal activities.

Fig.4 Rota evaporator for extraction



Various fungal metabolites obtained after extraction

4.7 Antibacterial and antifungal susceptibility testing

4.7.1 Media

1. MHA : Muller Hinton Agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing.

Composition : (w/v) (Atlas, 2004)

- 30% beef infusion
- 1.75% casein hydrolysate
- 0.15% starch
- 1.7% agar
- pH adjusted to neutral at 25˚C

4.7.2 Cultures used for antimicrobial susceptibility testing

1. Bacterial cultures

Gram positive bacteria : *Staphylococcus aureus, Bacillus megaterium*

Gram negative bacteria : *E.coli, Pseudomonas aeruginosa*

2. Fungal cultures

Candida albicans, Aspergillus fumigatus

4.7.3 Culturing of the test organisms

For *E.coli* LB (Lauria broth), NB (Nutrient broth) for *S.aureus, P.aeruginosa, B.megaterium* and PDB (Potato dextrose agar) for fungi *C.albicans* and *A.fumigatus* were used.

4.8 Preparation of Mcfarland standard

McFarland turbidity standard are used to standardize the approximate number of bacteria in a liquid suspension by visually comparing the turbidity of the same with the turbidity of test suspension. The McFarland standard is prepared by adding barium chloride to sulphuric acid to obtain a barium sulphate precipitate. By adjusting the volumes of these two reagents standard of varying degree of turbidity can be prepared to represent several different concentrations of bacteria. The standard most commonly used in clinical microbiology laboratory for routine antimicrobial susceptibility testing is 0.5 which represents 1.5×10^8 (generally range is 1.0×10^8 to 2.0×10^8 bacteria/ml).

A 0.5 McFarland standard is prepared by mixing 0.05ml of 1.175% barium chloride dihydrate ($BaCl_2.2H_2O$), with 9.95ml of 1% sulphuric acid (H_2SO_4).

Preparation of McFarland standard (NCCLS, 2003).

1. Added 85ml of 1 % H_2SO_4 to a 100ml volumetric flask.

2. Using auto pipette added 0.5ml of 1.175% $BaCl_2.2H_2O$ drop wise to H_2SO_4 while constantly swirling the flask.

3. Final volume was made upto 100ml with 1% H_2SO_4.

4. The flask was placed on magnetic stirrer for 3-5 minutes.

5. The solution was checked for any visible clumps. The O.D was recorded at 600 nm.

McFarland no.	1.0% $BaCl_2.2H_2O$ (ml)	1.0%H_2SO_4 (ml)	Approx.Cell density (cfu/ml)	Percentage transmittance	Absorbance at 600 nm
0.5	0.05	9.95	1×10^8	74.3	0.132

4.9 Screening of crude extract for antimicrobial activity

4.9.1 Agar Well Diffusion method

This method is the most commonly used method for testing antimicrobial activity of an extract. It mainly refers to the movement of molecules through a matrix that is formed by the gelling of the agar. In this the compounds are put in a well and allowed to diffuse away from the well which leads to the formation of a zone of inhibition. When performed under controlled conditions, the degree of the molecule's movement can be related to the concentration of the molecule. This forms the basis of the phenomenon of the Agar well diffusion assay which is mainly used in the determination of susceptibility or resistance of a bacterial or fungal strain to an antibacterial or antifungal strain including antibiotics. When the seaweed extract known as agar is allowed to harden, the resulting material is not impermeable. Rather, there are spaces present between the myriad of strands of agar that comprise the hardened polymer.

Various molecules of small size such as antibiotics are able to diffuse through the agar. On the MHA plates wells were made using borer and then antibiotics were applied to the wells. Thus the antibiotic will tend to move from this region of high concentration to the surrounding regions of lower antibiotic concentration. The larger the amount of material present the larger will be the zone of diffusion. Mainly the bacterial suspension set by comparing with 0.5 McFarland solution are spread on MHA plates on the surface and after sometime wells are made in the plates and then antibiotic is applied to a number of wells in the plates. We can take different concentrations of the antibiotic in the same plate or different plates. After a certain time period for the growth of the bacteria, the plates are examined and if the bacterial growth is right up to the antibiotic containing well, then the bacterial strain is deemed to be resistance to that particular antibiotic. If there is a clearing zone around the antibiotic well, then the bacteria has been killed or adversely affected by that particular antibiotic. The larger the zone the more potent is the antibiotic and the more susceptible is the bacteria. The zone of inhibition can be measured by using a scale and then compared with standards, in order to determine whether the bacterial strain is sensitive to the antibiotic. This method is reliable and allows the bacteria to be screened in a routine, economical and easy way detection of resistance and susceptibility of various cultures to various compounds or antibiotics. The various antimicrobial compounds present in the fungal extract are put in the well and allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The area of zone of inhibition can be then measured in millimetres and compared, (NCCLS 2009).

1. Media : MHA (Muller Hinton Agar)

The medium was prepared by dispensing 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia Pvt. Ltd.) in 1000ml of distilled water. The medium was dissolved and then autoclaved at 121˚C, 15 lbs pressure for 15 minutes. The autoclaved media was then poured onto 100mm petriplates (25-30ml/plate) and then allowed to solidify.

2. Materials required :

Petriplates, Autopipettes, Cork borer (4-10mm), Spreader, Vortex, Ethanol, McFarland containing test tubes, Saline etc

Cultures :*E.coli, S.aureus, P.aeruginosa, B .megaterium, C.albicans, A.fumigatus.*

3. Procedure :

Well grown colonies of test organisms from master plates were picked with the help of sterile loop and then transferred to flasks containing 25ml of broth i.e., *E.coli* in LB, *S.aureus, B.megaterium, P.aeruginosa* in NB and *C.albicans* in PDB. These flasks were then incubated at 37˚C in case of bacteria and 25˚C for fungus overnight. Adjusted the turbidity of the test cultures with 0.5 McFarland solution. After that swabbed the test cultures on MHA plates and then wells were bored with the help of a cork borer and 50µl of fungal extracts were added. After that the plates were kept for some time undisturbed for diffusion and then sealed with a cling film and then incubated at 37˚C in case of bacteria and 25˚C in case of fungi for 24hrs. After that the antibacterial and antifungal activities were assayed by measuring the zone of inhibition around the well (NCCLS, 1993). Three antibiotics were used as controls in case of bacteria viz Streptomycin, Ampicillin, Penicilin and in case of fungus, Fluconazole was used. For *Aspergillus,* after the boring of the plates and dispensing of the fungal extracts, a piece of *Aspergillus* was put on the top of the well. Also the control was same as for *C.albicans.*

4.9.2 Broth microdilution method (MIC)

The microdilution technique for determination of minimum inhibitory concentrations (MIC) is often considered as the best methodology for assessing susceptibility or resistance of bacteria to antibiotics (Rivers *et al*., 1988; Reis, 2006; Alves *et al.,* 2008). According to Ostrosky *et al.,* (2008), MIC has several advantages and one is that this method can be 30 times more sensitive than other methods used in the literature. Dilution susceptibility testing methods are used to determine the minimal concentration of an antimicrobial needed to inhibit or kill a microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold) and the lowest concentration that inhibits the visible growth of an organism is regarded as MIC. The concentration range used may vary with the drug, or the compound being used, the organism tested and the site of infection. The basic principles and method of micro dilution is essentially same as that of macrodilution method except that the antimicrobial dilutions are in 0.1ml volumes in the wells of micro dilution tray (96-well tray). Results obtained are then reported as the actual MIC i.e., the minimum

inhibition concentration of an antibiotic of any other compound that can inhibit the visible growth of an organism.

The miniaturization and the mechanization of the test by use of small, disposable, plastic trays has made broth micro dilution testing practical and popular. Standard trays contain 96-wells, each containing a volume of 0.1ml that allows approximately 12 antibiotics to be tested in a range of 8 two fold dilutions in a single tray (Jorgensen *et al.*, 2007 and clinical and laboratory standard 2009). Micro dilution panels are typically prepared using dispensing instruments that aliquot precise volumes of pre weighed and diluted antibiotics in broth into the individual wells of tray from large volumes vessels. Hundreds of identical trays can be prepared from a single master set of dilutions in a relatively brief period. Inoculation of panels with the standard 1×10^{8}cfu/ml is accomplished using a micropipette which transfers 0.01 to 0.05ml of standardized bacterial suspension into each well of the micro dilution tray. Following incubation, MICs are determined using a manual or automated viewing device for inspection of each of the panel wells for growth. The advantages of micro dilution method include generation of MICs, the reproducibility and convenience of having prepared panels, and the economy of reagents and the space that occurs due to the miniaturization of the test. The disadvantage of this method is inflexibility of drug selections available in standard commercial panels and also the process of filling up antibiotic and the broth is always not perfect which leads to errors in the result.

1. Media :

MHB (Muller Hinton Broth)

2. Materials required :

Suspension of test organism (Bacterial as well as fungal), Methanol (blank), Streptomycin, Ampicillin, Penicillin (Standard antibiotic), Fungal extracts, Microtitre plate (Tarson), Autopipette, ELISA plate reader, vortex etc.

3. Procedure :

A suspension of the test organism was prepared equal to 0.5 McFarland standard using isolated colonies. Also different concentrations of the fungal extracts were prepared from 1µg/ml to 10µg/ml from stock solution of 5ml. Similarly stock solution of all the

antibiotics were made i.e., 1mg/ml. To the wells of a 96-well ELISA tray 100 µl of MHB was added in all the wells selected for the test and then 100 µl of antibiotics and the fungal extracts respectively. After that added 10 µl of bacterial suspension to the wells except for the blank containing medium only. The absorbance of each well was determined using an automatic ELISA tray reader adjusted at 600 nm. As soon as the bacterial suspension was added, took the reading and considered this as 0 hr reading. The plate was incubated at 37˚C for 1hr, agitated and the absorbance was read again in the reader at the same wavelength. These absorbance values were subtracted from those obtained before incubation. Next absorbance was recorded after 1hr, 2hr, 3hr, 24hr and in case of

C.albicans absorbance was also calculated after 48hr. This procedure eliminated the interference of the tested substance. All tests were performed in triplicates and the MICs value of each test extract was expressed as the lowest concentration that inhibits the visible growth of bacteria. Graphs were plotted depicting the MIC values of the extract and the antibiotic standards and thus the comparisons could be made between the fungal extracts and the MICs of the standard antibiotics.

RESULTS

In the present study isolated endophytic fungi *Xylaria sp.* XF-2, XF-4, XF-14 and XF-15 from different host plants were screened for their antifungal and antibacterial activity using various methods such as agar well diffusion method and broth micro dilution method. The main objective of this study was to screen the fungal metabolites for bioactive compounds with antimicrobial activity from various strains of endophytic fungi *Xylaria* and then check their antimicrobial potential on various bacterial test cultures, *Staphylococcus aureus, Escherichia coli, Pseudomonas aerugenosa, Bacillus megaterium* and two fungal test cultures i.e., *Candida albicans* and *Aspergillus fumigatus.*

The various strains of the endophytic fungi were sub-cultured on PDA plates and then incubated at 25˚C for 15 days. Fig 5-8 shows various strains of endophytic fungi *Xylaria* with different cultural morphology on PDA plates.

Figure 5. Cultural morphology of XF-2

Figure 6. Cultural morphology of XF-4

Figure 7. Cultural morphology of XF-14

Figure 8. Cultural morphology of XF-15

Cultivation of the fungal strains was done in PDB, which were then incubated at 25˚C, 120 rpm for 15 days. Fig 9-10 shows the endophytic fungi growth on PDB broth after 15 days.

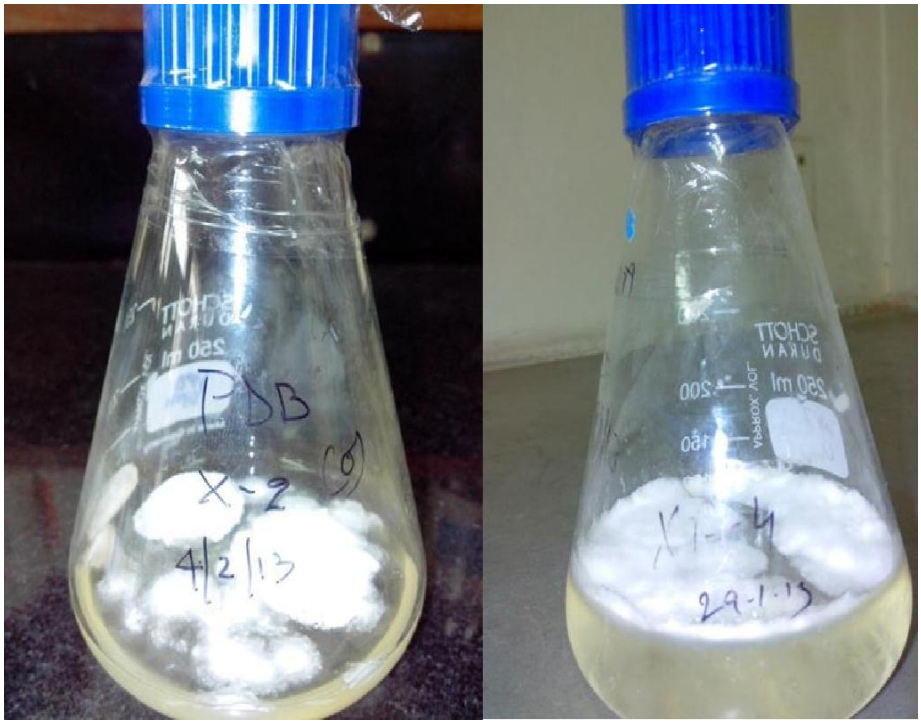


Figure 9.Cultivation of XF-2 and XF-4

Figure 10.Cultivation of XF-14 and XF-15

After filtration of the fungal broth, extraction was carried out in ethyl acetate. Both intracellular and extracellular extraction was done and then the solvent was evaporated with the help of a rota evaporator and the weight of the metabolites was calculated. The metabolites were then dissolved in 5ml of methanol and stored in 5ml glass tubes at 4˚C.

Yield of the extract obtained after evaporation :

1. XF-2 (intracellular) :

Initial weight of the rotary flask = 294.115gms

Final weight of the rotary flask = 294.481gms

Weight of the metabolite = 0.366gms

Concentration of the extract = 73.2mg/ml

2. XF-2 (extracellular):

Initial weight of the rotary flask = 294.112gms

Final weight of the rotary flask = 294.657gms

Weight of the metabolite = 0.545gms

Concentration of the extract = 109mg/ml

3. XF-4 (intracellular) :

Initial weight of the rotary flask =294.113gms

Final weight of the rotary flask= 294.475gms

Weight of the metabolite = 0.362gms

Concentration of the extract = 72.4 mg/ml

4. XF-4 (extracellular) :

Initial weight of the rotary flask =294.114gms

Final weight of the rotary flask = 294.428gms

Weight of the metabolite = 0.314gms

Concentration of the extract = 62.8 mg/ml

5. XF-14 (intracellular):

Initial weight of the rotary flask =294.114gms

Final weight of the rotary flask= 294.269gms

Weight of the metabolite = 0.115gms

Concentration of the extract = 31mg/ml

6. XF-14 (extracellular) :

Initial weight of the rotary flask =294.115gms

Final weight of the rotary flask= 294.430gms

Weight of the metabolite= 0.315gms

Concentration of the extract = 63mg/ml

7. XF-15 (intracellular) :

Initial weight of the rotary flask =294.115gms

Final weight of the rotary flask= 294.175gms

Weight of the metabolite= 0.06gms

Concentration of the extract = 12mg/ml

8. XF-15 (extracellular):

Initial weight of the rotary flask =294.115gms

Final weight of the rotary flask= 294.148gms

Weight of the metabolite= 0.033gms

Concentration of the extract = 6.6mg/ml

Fungal extracts	Yield of the metabolite (gms)	Concentration (mg/ml)
XF-2 I*	0.366	73.2
XF-2 E*	0.545	109
XF-4 I	0.362	72.4
XF-4 E	0.314	62.8
XF-14 I	0.155	31
XF-14 E	0.315	63
XF-15 I	0.06	12
XF-15 E	0.033	6.6

*(I) represents intracellular and (E) represents extracellular

Table 1. Concentration of various fungal metabolites in methanol

Results of antimicrobial activity of fungal extracts by agar well diffusion method:

Antimicrobial assay using Agar well diffusion method was performed in triplicates. Fungal extracts were tested on various test organisms and then incubated for an appropriate time and then zone of inhibition was measured in millimetres. Fig 11-15 shows zones of inhibition on MHA plates of various fungal extracts and control.

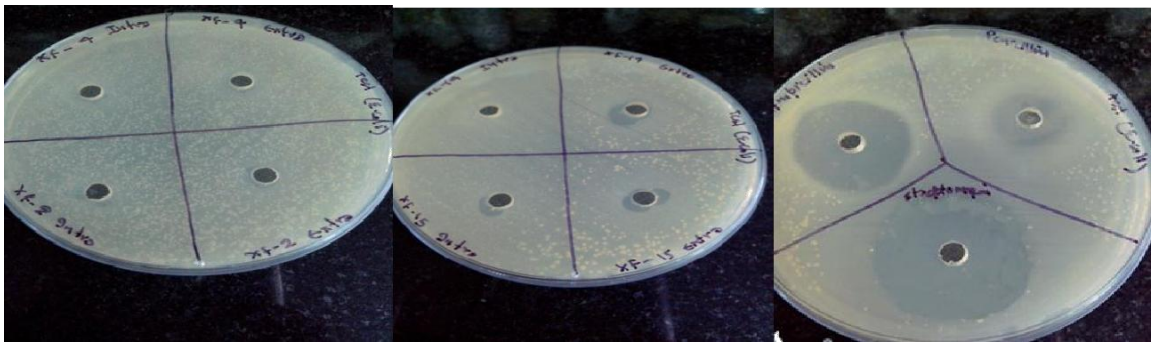


Figure 11. Zone of inhibition of fungal extracts and control against *E.coli*

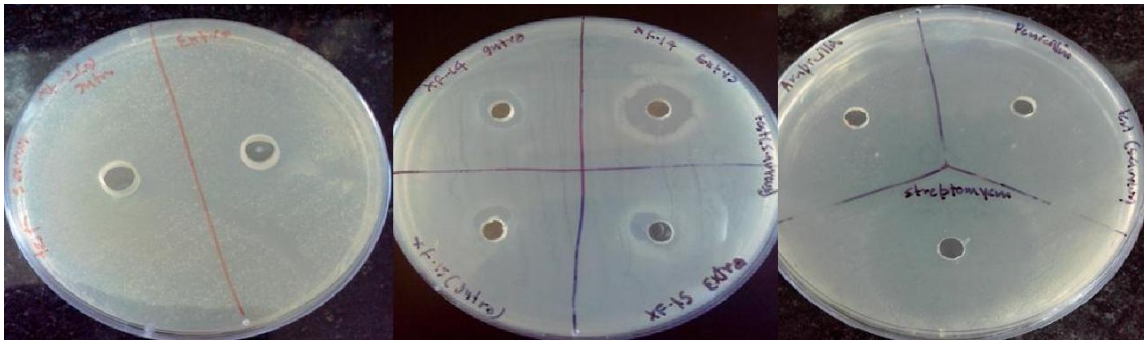


Figure 12. Zone of inhibition of Fungal extracts and controls against *S.aureus*

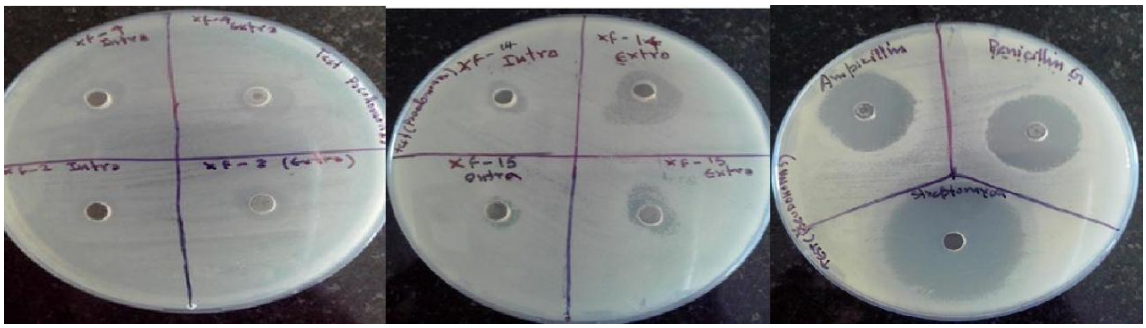


Figure 13. Zone of inhibition of fungal extracts and controls against *P.aeruginosa*

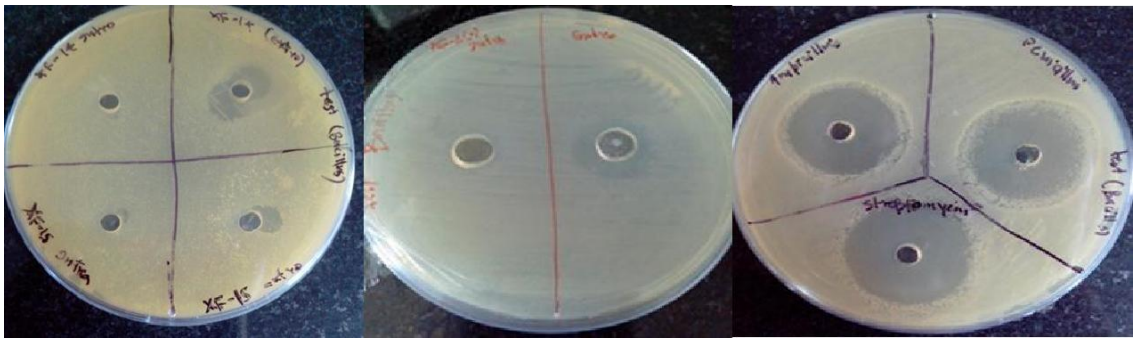


Figure 14. Zone of inhibition of Fungal extracts and controls against *B.megaterium*

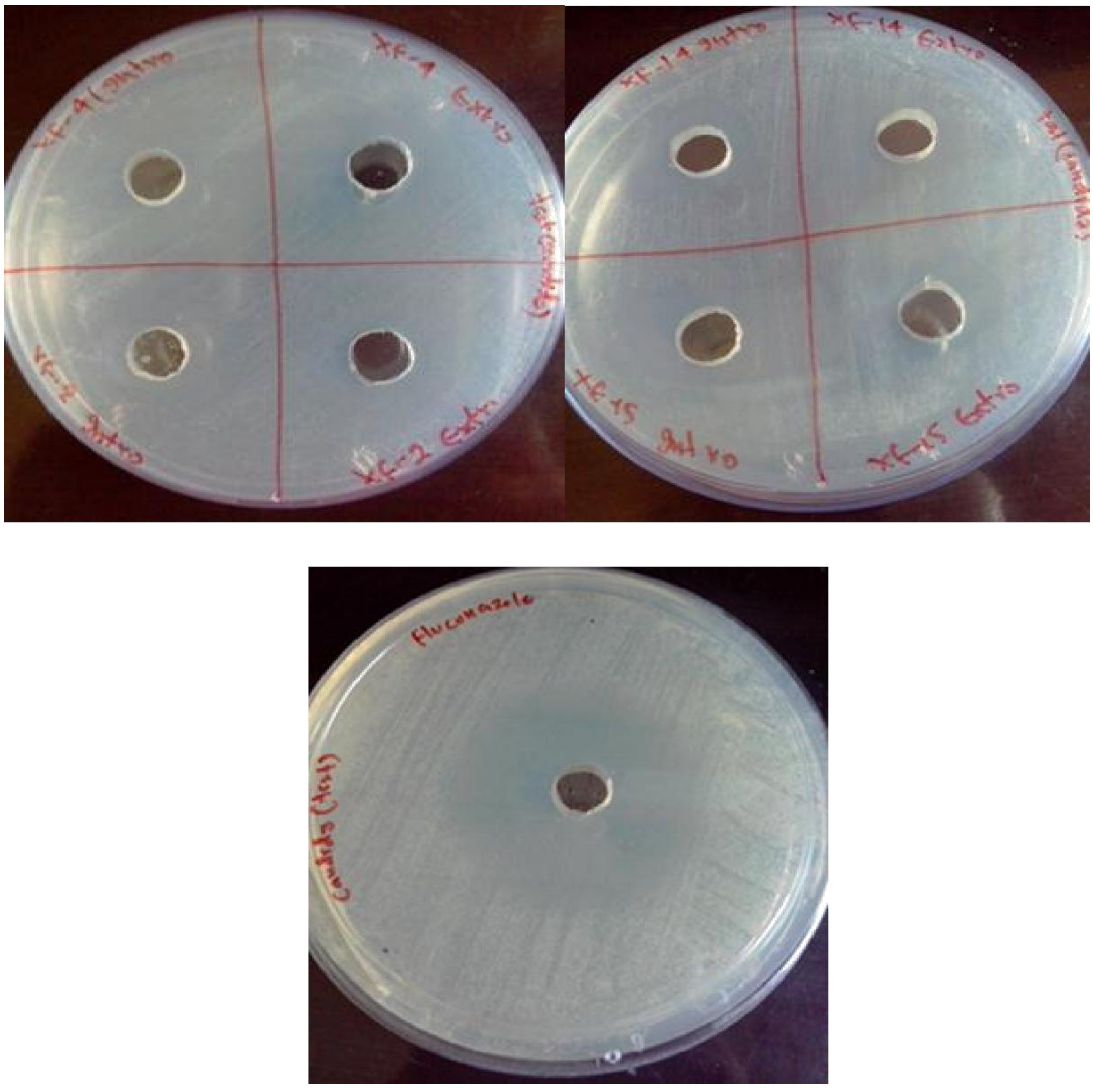


Figure 15. Zone of inhibition of Fungal extracts and control against *Candida albicans*

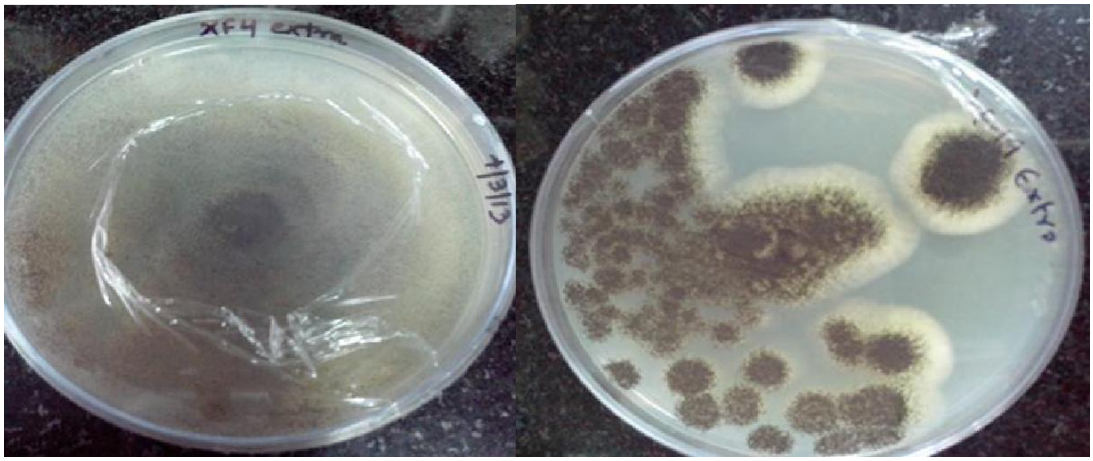


Figure 16. Fungal extract XF-4 and XF-14 (Extracellular) showing no effect on the growth of *A.fumigates*

Table 2. Comparison of zone of inhibition in mm of the control (Ampicillin, penicillin, streptomycin) and the fungal extracts against *E.coli.*

Control and fungal extracts	Zone of inhibition (mm)
Penicillin	15
Ampicillin	27
Streptomycin	29
XF-2 (I)	0
XF-2 (E)	0
XF-4 (I)	0
XF-4 (E)	0
XF-14 (I)	0
XF-14 (E)	12
XF-15 (I)	2
XF-15 (E)	10

Fig 17. Comparison of zone of inhibition in mm of control and fungal extracts against *E.coli.*

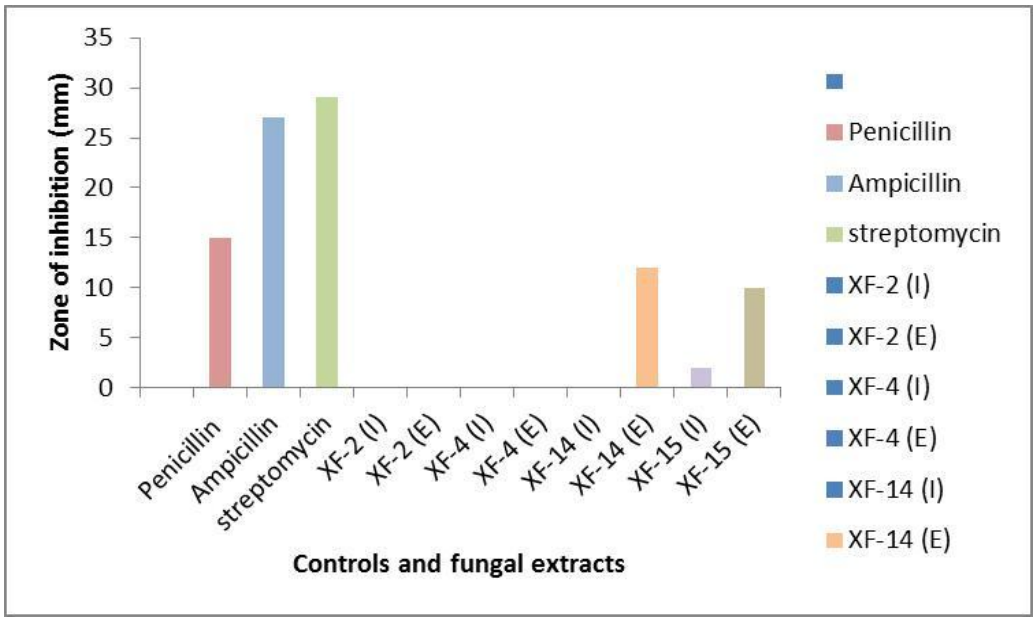


Table 3. Zone of inhibition in mm of control and fungal extracts against *S.aureus*

Controls and fungal extracts	Zone of inhibition (mm)
Penicillin	18
Ampicillin	25
Streptomycin	30
XF-2 (I)	0
XF-2 (E)	12
XF-4 (I)	0
XF-4 (E)	10
XF-14 (I)	10
XF-14 (E)	16
XF-15 (I)	2
XF-15 (E)	10

Fig 18. Comparison of zone of inhibition in mm of control and fungal extracts against *S.aureus.*

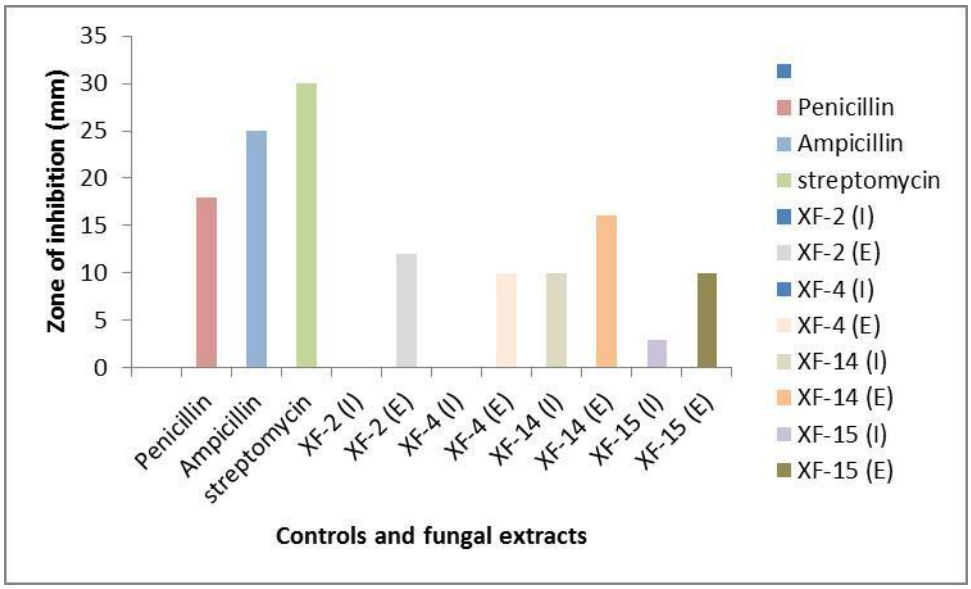


Table 4. Zone of inhibition in mm of control and Fungal extracts against *P.aerugenosa.*

Controls and fungal extracts	Zone of inhibition (mm)
Penicillin	24
Ampicillin	24
Streptomycin	31
XF-2 (I)	6
XF-2 (E)	11
XF-4 (I)	10
XF-4 (E)	11
XF-14 (I)	8
XF-14 (E)	17
XF-15 (I)	3
XF-15 (E)	13

Fig 19.Comparison of zone of inhibition in mm of control and fungal extracts against *P.aerugenosa.*

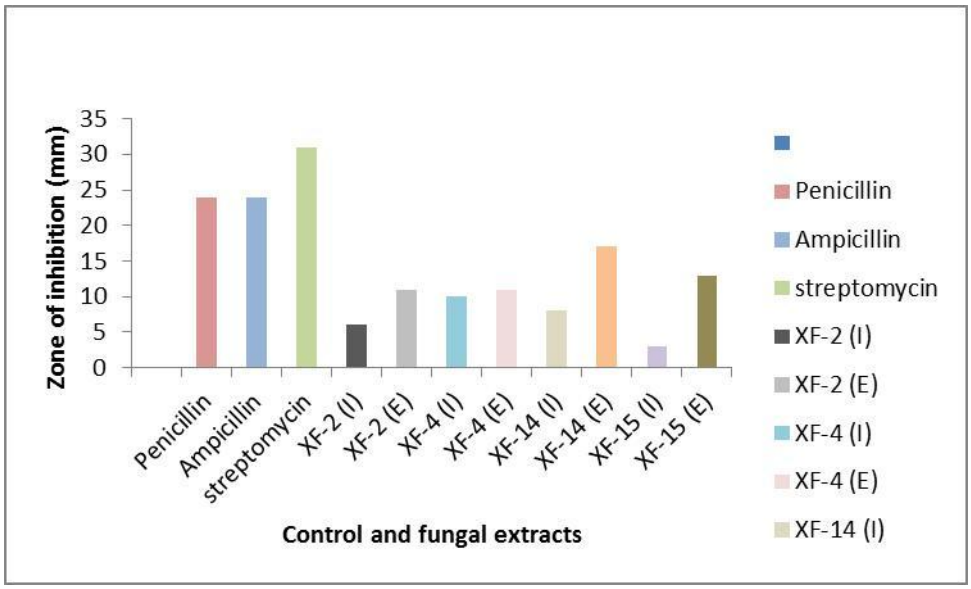


Table 5. Zone of inhibition in mm of of control and fungal extracts against *B.megaterium*

Controls and fungal extracts	Zone of inhibition (mm)
Penicillin	26
Ampicillin	24
Streptomycin	27
XF-2 (I)	3
XF-2 (E)	12
XF-4 (I)	3
XF-4 (E)	0
XF-14 (I)	10
XF-14 (E)	16
XF-15 (I)	10
XF-15 (E)	10

Fig 20. Comparison of zone of inhibition in mm of control and fungal extracts against *B.megaterium*

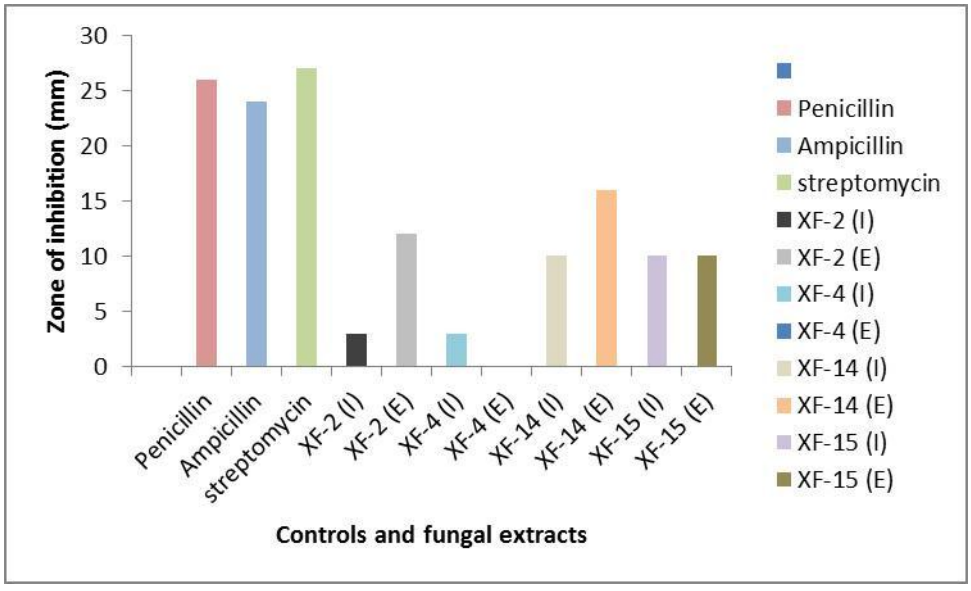


Table 6.Comparison of zones of inhibition in millimetres (mm) of fungal extracts and control Fluconazole against *C.albicans*

Control and Fungal extract	Zone of inhibition(mm)
Fluconazole	32
XF-2(I)	6
XF-2(E)	7
XF-4(I)	5
XF-4(E)	10
XF-14(I)	12
XF-14(E)	9
XF-15(I)	14
XF-15(E)	9

Fig 21.Comparison of zone of inhibition of various fungal extracts and Fluconazole against *C.albicans*

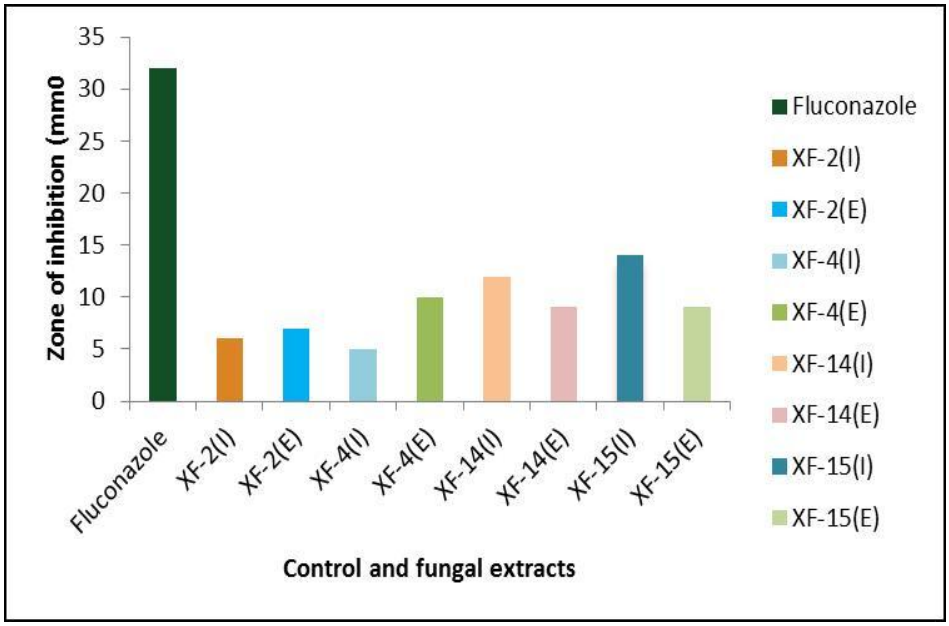


Table 7. Zone of inhibition in millimetre (mm) of intracellular fungal extracts and control against *Escherichia coli, Bacillus megaterium, Staphylococcus aureus and Pseudomonas aeruginosa.*

Controls and Fungal extracts	*E.coli*	*S.aureus*	*P.aerugenosa*	*B.megaterium*
Penicillin	15	18	24	26
Ampicillin	27	25	24	24
Streptomycin	32	30	31	27
XF-2 (I)	0	0	6	3
XF-4(I)	0	0	10	3
XF-14(I)	0	10	8	10
XF-15(I)	2	3	3	10

Figure 22. Comparison of zone of inhibition of intracellular fungal extracts and control against test bacterial cultures.

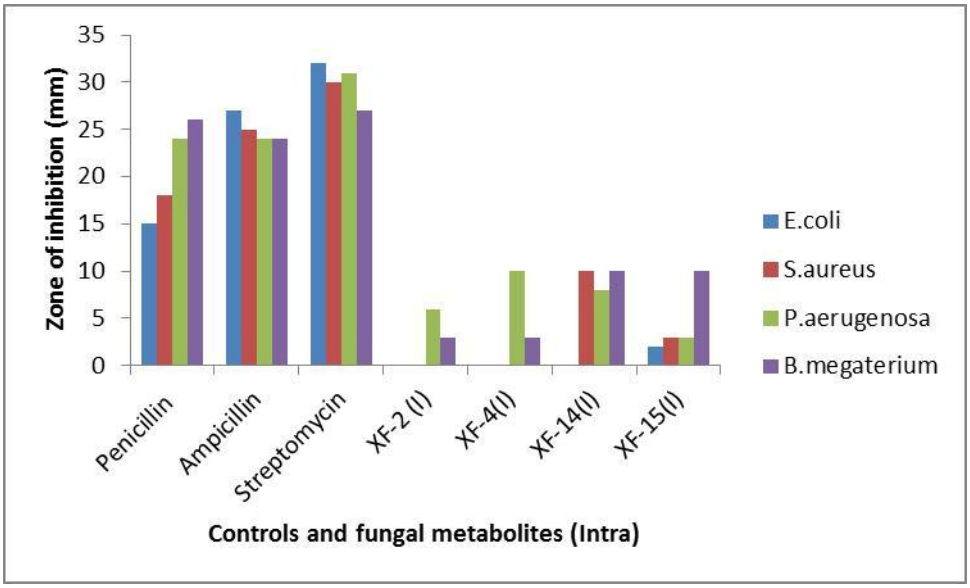


Table 8. Zone of inhibition in millimetre (mm) of extracellular fungal extracts and control against test bacterial cultures.

Controls and fungal extracts	*E.coli*	*S.aureus*	*P.aeruginosa*	*B.megaterium*
Penicillin	15	18	24	26
Ampicillin	27	25	24	24
Streptomycin	32	30	31	27
XF-2 (E)	0	12	11	12
XF-4 (E)	0	10	11	0
XF-14 (E)	12	16	17	16
XF-15 (E)	10	10	13	10

Figure 23. Comparison of zone of inhibition of extracellular fungal extracts and control against test bacterial culture.

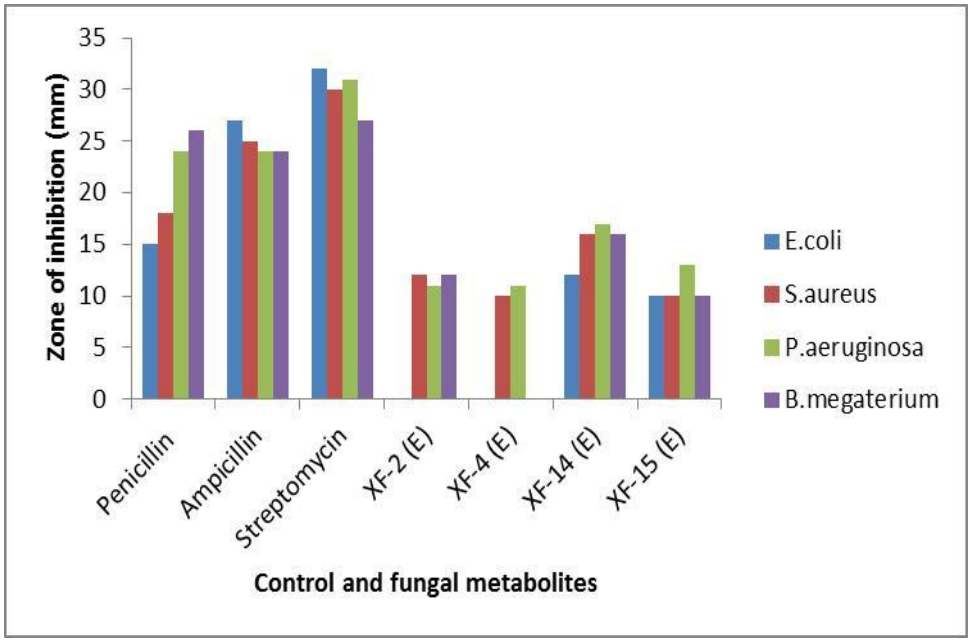
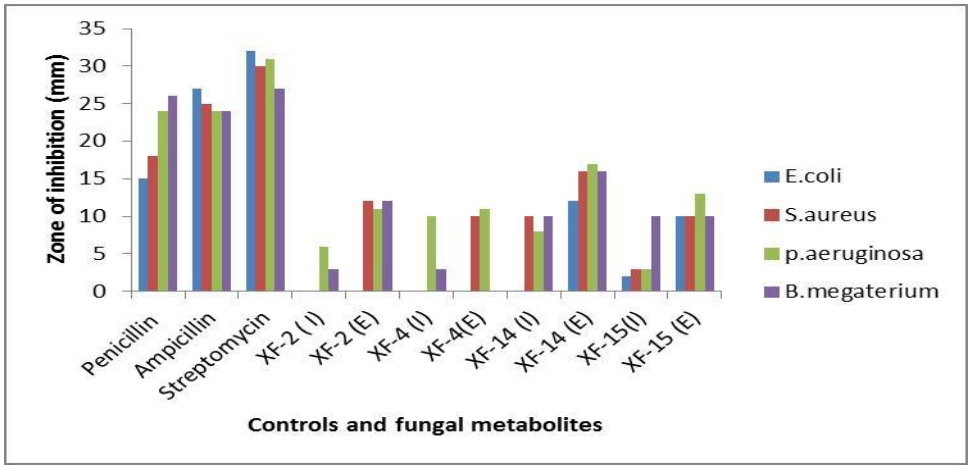


Table 9. Zone of inhibition in millimetre (mm) of all fungal extracts and controls against test bacterial culture.

Controls and Fungal extracts	Zones of inhibition (mm)			
	E.coli	*S.aureus*	*P.aerugenosa*	*B.megaterium*
Penicillin	15	18	24	26
Ampicillin	27	25	24	24
Streptomycin	32	30	31	27
XF-2 (I)	0	0	6	3
XF-2 (E)	0	12	11	12
XF-4 (I)	0	0	10	3
XF-4(E)	0	10	11	0
XF-14 (I)	0	10	8	10
XF-14 (E)	12	16	17	16
XF-15(I)	2	3	3	10
XF-15 (E)	10	10	13	10

Figure 24. Comparison of zone of inhibition of fungal extracts and control antibiotics against *S.aureus, E.coli, P.aeruginosa and B.megaterium,.*



*(I) represents Intracellular and (E) represents extracellular

*Bacterial cultures, E.coli = Escherichia coli, S.aureus = Staphylococcus aureus ,P.aeruginosa =Pseudomonas aeruginosa, B.megaterium = Bacillus megaterium

Results of Antimicrobial activity of fungal extracts by Broth microdilution method (Spectrophotometric method)

Broth micro dilution was performed and the O.D were noted at 0 hr,1 hr, 2 hr, 3 hr , 24 hr, and 48 hrs respectively, (Triplicates) and their average value was taken and the minimum inhibitory concentrations were noted.

Table 10. Effect of different concentration of control and Intracellular fungal extracts on the growth of *E.coli* after 24hrs.

Conc µg/ml	Amp	Strept	Pen	XF2 (I)	XF4 (I)	XF14 (I)	XF15 (I)
1	0.061	0.064	0.056	0.082	0.070	0.066	0.070
2	0.063	0.060	0.054	0.085	0.078	0.067	0.070
3	0.061	0.058	0.069	0.094	0.079	0.072	0.069
4	0.055	0.059	0.070	0.087	0.034	**0.064**	0.083
5	0.067	0.057	0.065	0.081	**0.026**	0.059	0.070
6	0.069	0.054	0.054	0.084	0.056	0.069	0.074
7	0.061	**0.051**	0.053	0.081	0.075	0.070	0.074
8	0.060	0.047	**0.057**	0.077	0.059	0.109	**0.069**
9	**0.054**	0.046	0.056	0.075	0.057	0.063	0.072
10	0.051	0.038	0.051	**0.070**	0.056	0.070	0.070

Fig 25. Comparison of MIC at 600nm of control and extracts against *E.coli.*

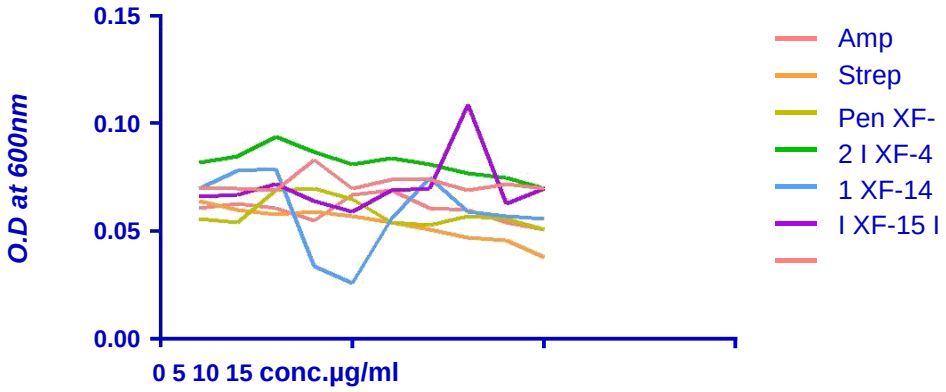


Table 11. Effect of different concentration of control and extracellular fungal extracts on the growth of *E.coli* after 24hrs.

Conc µg/ml	Amp	Strept	Pen	XF2 (E)	XF4 (E)	XF14 (E)	XF15 (E)
1	0.061	0.064	0.056	0.084	0.056	**0.79**	0.055
2	0.063	0.060	0.054	0.075	0.066	0.068	0.055
3	0.061	0.058	0.069	0.086	0.069	0.066	0.040
4	0.055	0.059	0.070	**0.070**	0.062	0.063	0.043
5	0.067	0.057	0.065	0.076	0.055	0.068	0.055
6	0.069	0.054	0.054	0.071	**0.045**	0.067	**0.045**
7	0.061	**0.051**	0.053	0.070	0.047	0.070	0.042
8	0.060	0.047	**0.057**	0.077	0.064	0.068	0.051
9	**0.054**	0.046	0.056	0.075	0.071	0.066	0.051
10	0.051	0.038	0.051	0.070	0.042	0.062	0.050

Fig 26. Comparison of MIC at 600nm of control and extracts against *E.coli.*

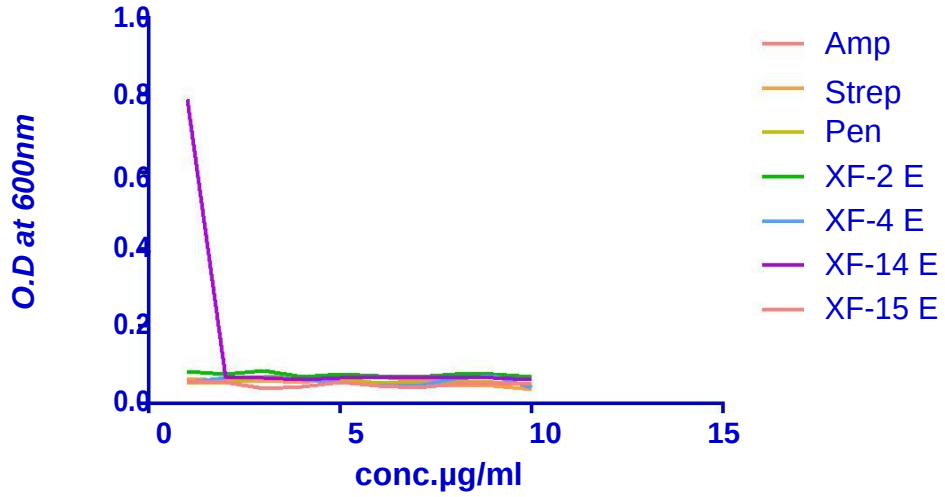


Table 12. Effect of different concentration of control and Intracellular fungal extracts on the growth of *S.aureus* after 24hrs.

Conc. (µg/ml)	Amp	Strept	Pen	XF-2 (I)	XF-4 (I)	XF-14(I)	XF-15(I)
1	0.036	0.026	0.031	0.042	0.046	0.088	0.082
2	0.031	0.027	**0.025**	0.031	0.047	0.079	0.089
3	0.034	0.029	0.029	0.029	0.042	0.078	0.089
4	**0.029**	**0.027**	0.035	**0.042**	0.040	0.075	0.088
5	0.030	0.026	0.030	0.031	0.043	0.097	0.110
6	0.034	0.029	0.031	0.039	0.037	0.101	0.109
7	0.039	0.029	0.030	0.038	0.036	0.097	**0.142**
8	0.033	0.025	0.029	0.041	**0.045**	0.116	0.069
9	0.033	0.025	0.024	0.045	0.053	0.121	0.149
10	0.032	0.027	0.026	0.042	0.037	**0.132**	0.104

Fig 27.Comparison of MIC at 600nm of control and extracts against *S.aureus.*

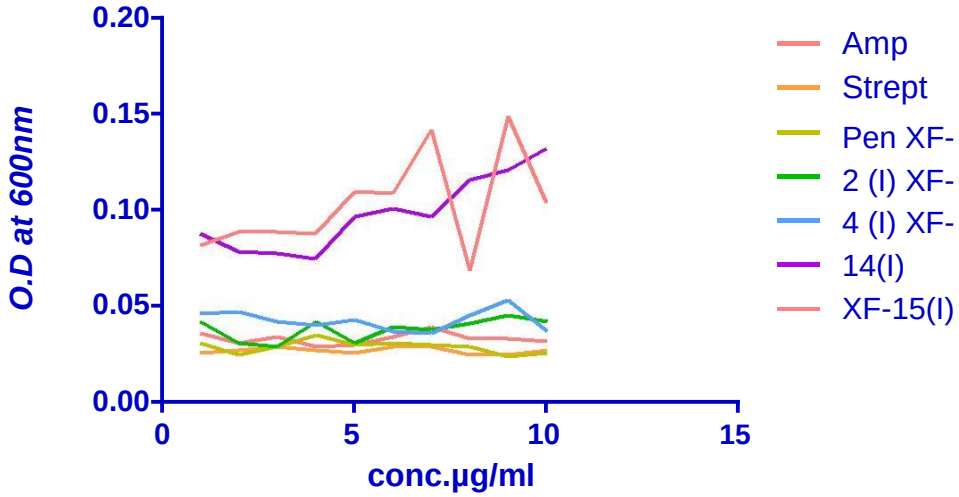


Table 13. Effect of different concentration of control and extracellular fungal extracts on the growth of *S.aureus* after 24hrs.

Conc. (µg/ml)	Amp	Strept	Pen	XF-2 (E)	XF-4 (E)	XF-14(E)	XF-15 (E)
1	0.036	0.026	0.031	0.79	0.055	0.086	0.074
2	0.031	0.027	**0.025**	0.071	0.045	0.095	0.088
3	0.034	0.029	0.029	0.066	0.040	0.120	0.072
4	**0.029**	**0.027**	0.035	0.063	0.045	**0.110**	**0.112**
5	0.030	0.026	0.030	0.059	0.042	0.124	0.100
6	0.034	0.029	0.031	0.067	**0.045**	0.104	0.093
7	0.039	0.029	0.030	0.051	0.042	0.081	0.077
8	0.033	0.025	0.029	0.051	0.039	0.086	0.183
9	0.033	0.025	0.024	0.043	0.041	0.099	0.144
10	0.032	0.027	0.026	**0.046**	0.037	0.089	0.081

Fig 28.Comparison of MIC at 600nm of control and extracts against *S.aureus*.

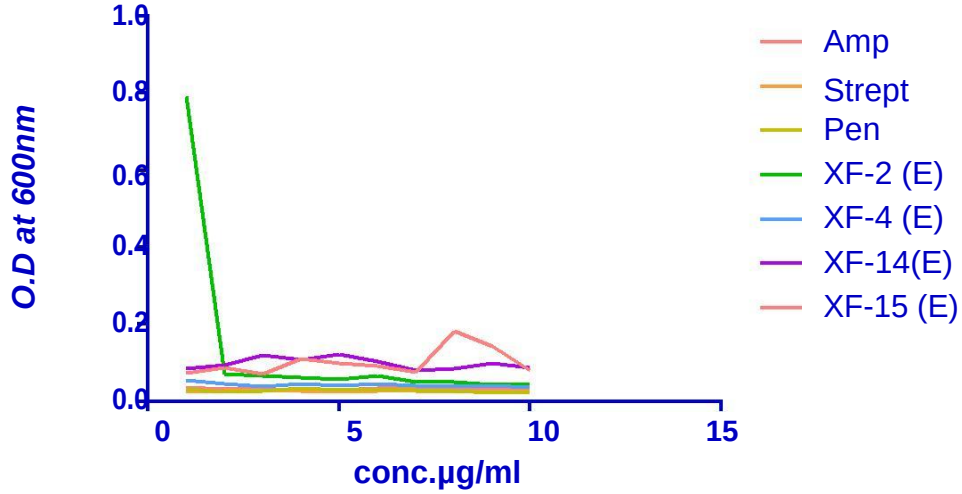


Table 14. Effect of different concentration of control and Intracellular fungal extracts on the growth of *P.aerugenosa* after 24hrs

Conc. (µg/ml)	Amp	Strepto	Pen	XF-2 (I)	XF-4 (I)	XF-14 (I)	XF-15 (I)
1	0.049	**0.048**	0.048	0.089	0.050	0.068	0.081
2	0.067	0.049	0.050	0.089	0.050	0.065	0.068
3	**0.056**	0.050	**0.052**	0.086	0.054	0.059	0.058
4	0.053	0.057	0.049	**0.71**	0.061	**0.102**	0.061
5	0.055	0.051	0.047	0.069	0.056	0.069	0.056
6	0.053	0.053	0.051	0.058	0.102	0.058	0.066
7	0.052	0.051	0.044	0.053	0.058	0.053	0.064
8	0.053	0.052	0.050	0.054	0.060	0.054	0.060
9	0.048	0.050	0.051	0.051	0.054	0.051	0.054
10	0.045	0.051	0.052	0.036	**0.052**	0.036	**0.052**

Fig 29 . Comparison of MIC at 600nm of control and against *P.aerugenosa.*

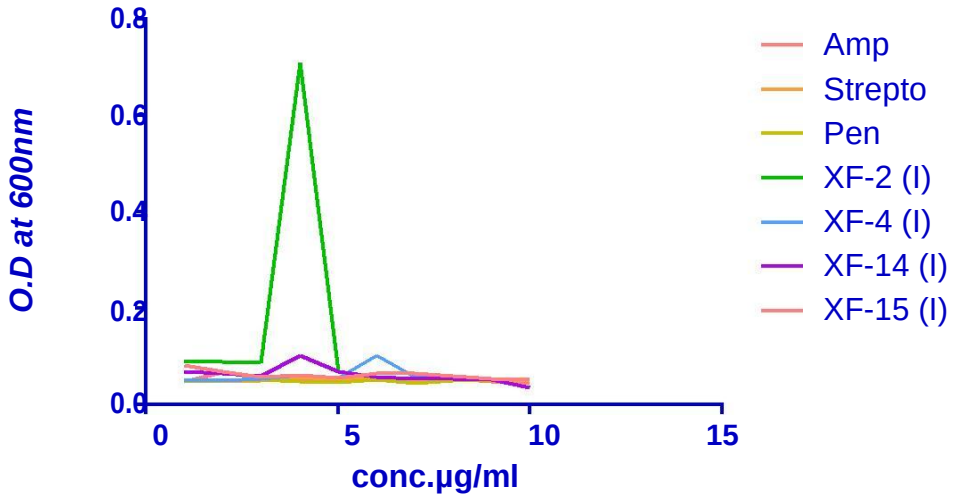


Table 15. Effect of different concentration of control and extracellular fungal extracts on the growth of *P.aerugenosa* after 24hrs.

Conc. (µg/ml)	Amp	Strepto	Pen	XF-2 (E)	XF-4 (E)	XF-14(E)	XF-15(E)
1	0.049	**0.048**	0.048	0.51	0.072	0.064	0.084
2	0.067	0.049	0.050	0.061	0.049	0.068	0.071
3	**0.056**	0.050	**0.052**	0.048	0.053	0.064	0.066
4	0.053	0.057	0.049	0.054	0.049	0.054	0.061
5	0.055	0.051	0.047	0.085	0.050	0.052	0.064
6	0.053	0.053	0.051	0.124	0.051	**0.063**	0.062
7	0.052	0.051	0.044	0.064	0.082	0.054	0.061
8	0.053	0.052	0.050	0.059	0.055	0.051	0.055
9	0.048	0.050	0.051	0.037	0.022	0.050	**0.053**
10	0.045	0.051	0.052	**0.014**	**0.011**	0.046	0.050

Fig 30. Comparison of MIC at 600nm of control and extracts against *P.aerugenosa*.

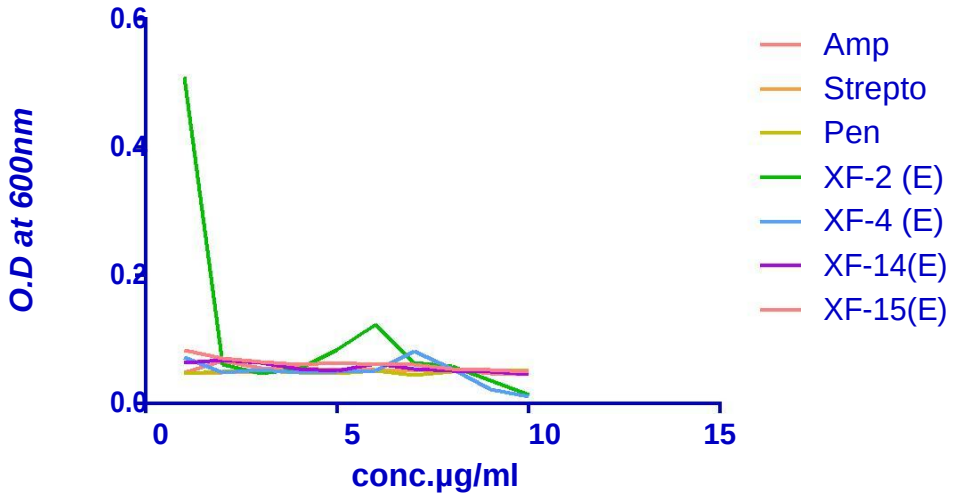


Table 16 Effect of different concentration of control and intracellular fungal extracts on the growth of *B.megaterium* after 24hrs

Conc. (µg/ml)	Amp	Strepto	Pen	XF-2 (I)	XF-4 (I)	XF-14 (I)	XF-15 (I)
1	0.164	0.178	0.182	0.059	0.082	0.372	0.283
2	0.183	0.099	0.164	0.085	0.085	0.321	0.238
3	0.139	0.098	**0.178**	0.086	0.080	0.199	0.220
4	0.183	0.093	0.177	0.072	0.081	**0.294**	0.216
5	**0.193**	0.098	0.172	**0.069**	0.072	0.267	0.261
6	0.183	0.088	0.165	0.068	0.064	0.250	**0.210**
7	0.160	0.084	0.169	0.062	0.058	0.254	0.196
8	0.151	**0.080**	0.150	0.059	0.054	0.250	0.192
9	0.139	0.093	0.144	0.051	0.053	0.262	0.174
10	0.135	0.078	0.103	0.049	**0.045**	0.236	0.168

Fig 31. Comparison of MIC at 600nm of and fungal extracts against *B.megaterium*

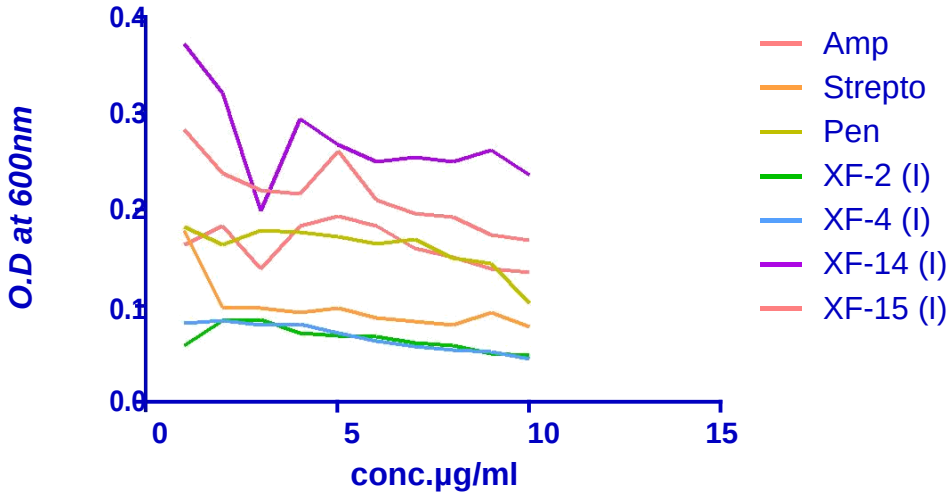


Table 17 Effect of different concentration of control and extracellular fungal extracts on the growth of *B.megaterium* after 24hrs.

Conc. (µg/ml)	Amp	Strepto	Pen	XF-2(E)	XF-4 (E)	XF-14(E)	XF-15(E)
1	0.164	0.178	0.182	0.072	0.074	0.381	0.225
2	0.183	0.099	0.164	0.083	0.079	0.339	**0.216**
3	0.139	0.098	**0.178**	0.114	0.085	0.323	0.220
4	0.183	0.093	0.177	**0.084**	0.076	0.300	0.203
5	**0.193**	0.098	0.172	0.063	**0.072**	0.285	0.206
6	0.183	0.088	0.165	0.066	0.063	0.239	0.178
7	0.160	0.084	0.169	0.153	0.053	0.231	0.177
8	0.151	**0.080**	0.150	0.131	0.052	0.271	0.172
9	0.139	0.093	0.144	0.121	0.050	**0.331**	0.114
10	0.135	0.078	0.103	0.048	0.051	0.294	0.129

Fig 32. Comparison of MIC at 600nm of control and extracts against *B.megaterium.*

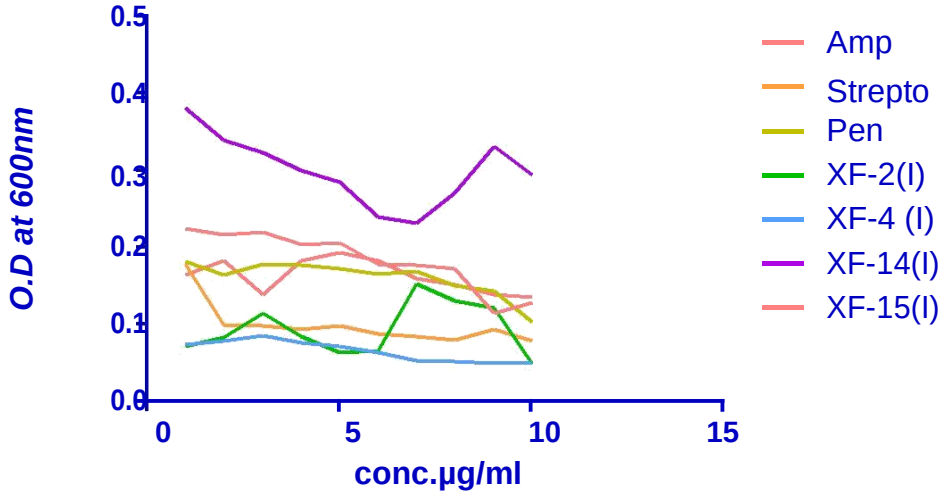


Table 18. Effect of different concentration of control and intracellular fungal extracts on the growth of *C.albicans* after 24hrs.

Conc. (µg/ml)	fluconazole	XF-2 (I)	XF-4 (I)	XF-14 (I)	XF-15(I)
1	0.554	0.540	0.516	0.640	0.450
2	0.417	0.429	0.358	0.531	0.358
3	**0.316**	0.308	0.251	0.543	0.411
4	0.328	0.292	0.353	0.312	0.326
5	0.345	**0.211**	0.291	0.294	0.360
6	0.293	0.334	0.296	0.260	0.282
7	0.287	0.261	**0.294**	0.242	0.264
8	0.370	0.299	0.251	**0.238**	0.246
9	0.391	0.372	0.241	0.229	**0.241**
10	0.321	0.333	0.187	0.215	0.216

Fig 33. Comparison of MIC at 600nm of Fluconazole and extracts against *C.albicans.*

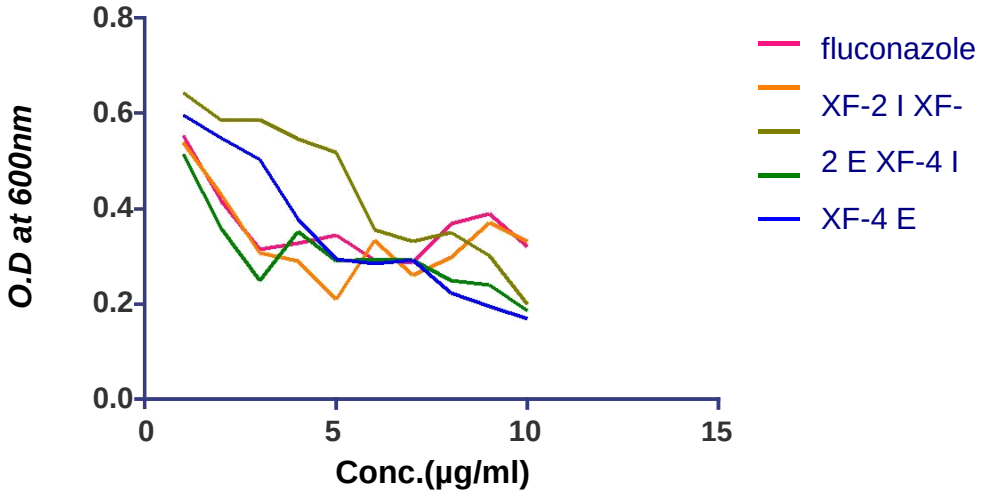


Table 19. Effect of different concentration of control and extracellular fungal extracts on the growth of *C.albicans* after 24hrs.

conc. (µg/ml)	Fluconazole	XF-2 (E)	XF-4 (E)	XF-14 (E)	XF-15 (E)
1	0.554	0.644	0.598	0.65	0.598
2	0.417	0.587	0.549	0.418	0.449
3	**0.316**	0.589	0.505	0.503	0.315
4	0.328	**0.548**	0.379	0.532	0.272
5	0.345	0.519	**0.296**	**0.355**	**0.296**
6	0.293	0.357	0.286	0.264	0.286
7	0.287	0.333	0.294	0.258	0.244
8	0.370	0.351	0.225	0.247	0.223
9	0.391	0.303	0.197	0.209	0.194
10	0.321	0.199	0.172	0.202	0.175

Fig 34. Comparison of MIC at 600nm of and extracts against *C.albicans.*

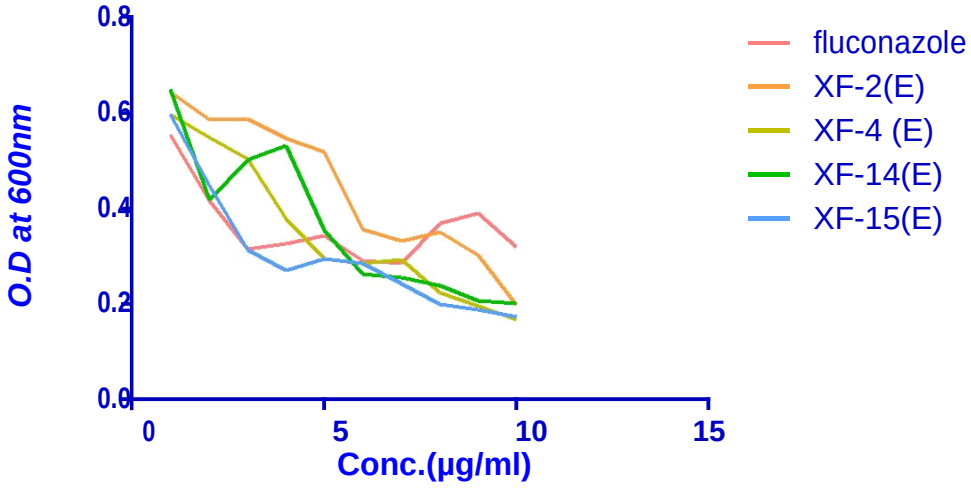


Table 20. Effect of different concentration of control and intracellular fungal extracts on the growth of *C.albicans* after 48hrs

Conc. (µg/ml)	fluconazole	XF-2 (I)	XF-4 (I)	XF-14 (I)	XF-15(I)
1	0.554	1.240	1.250	1.240	1.250
2	0.417	0.934	1.358	1.331	0.958
3	**0.316**	0.821	1.011	0.643	0.811
4	0.328	0.769	0.624	0.31	0.526
5	0.345	0.311	0.391	0.294	0.361
6	0.293	0.271	0.296	0.280	**0.272**
7	0.287	0.261	0.294	0.242	0.264
8	0.370	**0.230**	**0.251**	0.226	0.240
9	0.391	0.221	0.241	0.229	0.222
10	0.321	0.198	0.187	**0.215**	0.211

Fig 35. Comparison of MIC at 600nm of Fluconazole and extracts against *C.albicans.*

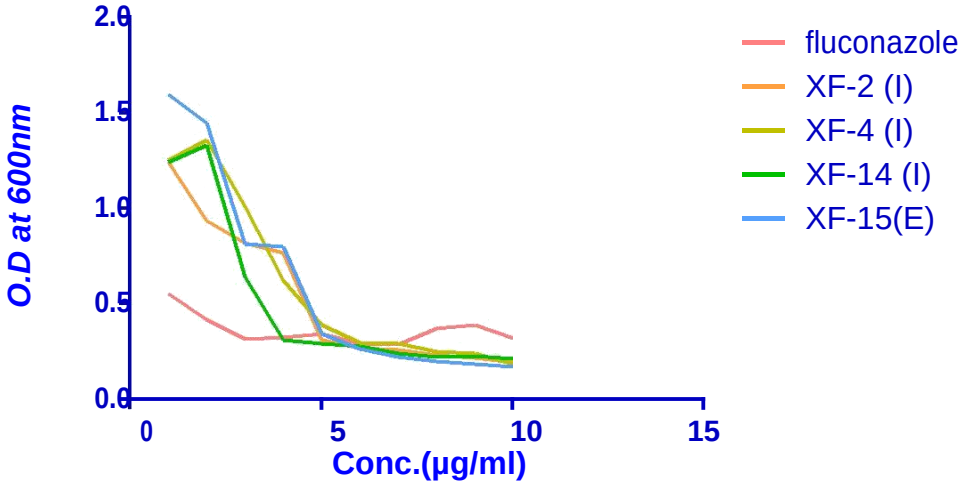


Table 21 Effect of different concentration of control and extracellular fungal extracts on the growth of *C.albicans* after 48hrs

Conc.(µg/ml)	Fluconazole	XF2(E)	XF-4 (E)	XF-14(E)	XF-15(E)
1	0.554	1.250	1.598	1.350	1.598
2	0.417	1.110	1.149	1.118	1.449
3	**0.316**	0.589	0.846	0.802	0.815
4	0.328	0.428	0.379	0.572	0.802
5	0.345	0.368	**0.296**	0.345	0.346
6	0.293	0.246	0.286	0.264	0.266
7	0.287	0.251	0.294	0.252	0.224
8	0.370	**0.249**	0.225	0.230	0.202
9	0.391	0.219	0.197	**0.209**	0.186
10	0.321	0.180	0.170	0.212	**0.175**

Fig 36. Comparison of MIC at 600nm of Fluconazole and extracts against *C.albicans.*

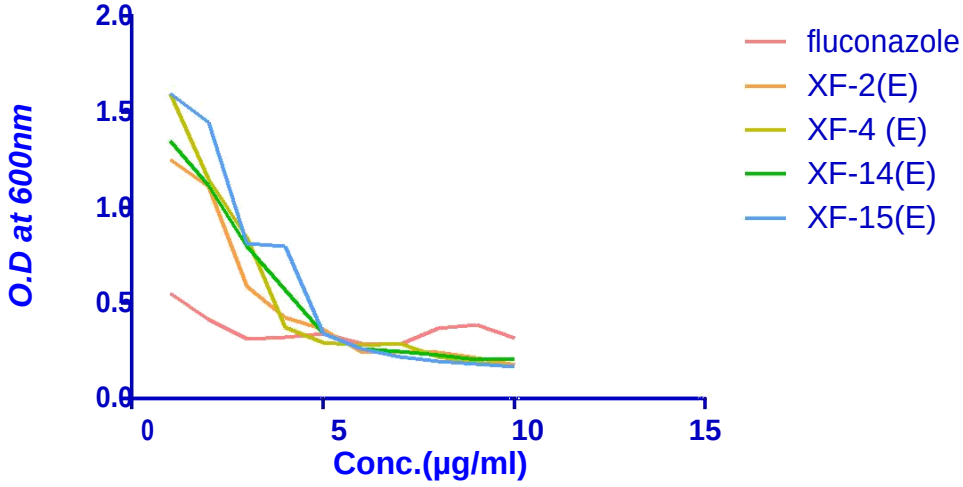
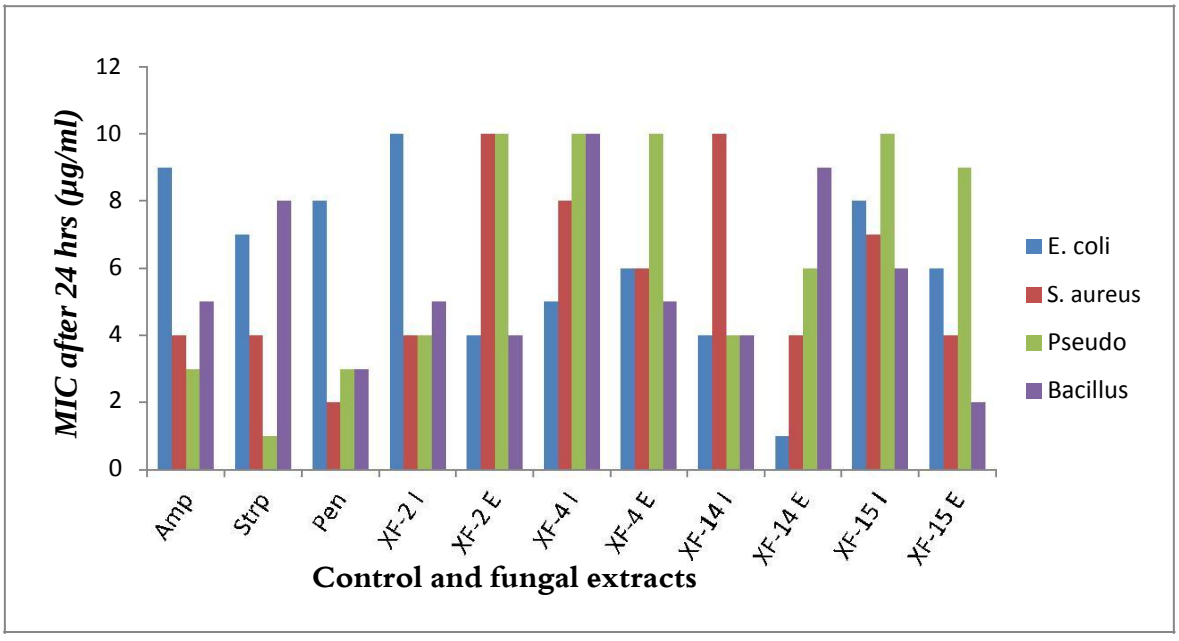


Table 22. MIC values of control and all the fungal extracts again 4 bacterial test cultures

Control and extracts	E.coli	S.aureus	Pseudo	Bacillus
Amp	9	4	3	5
Strp	7	4	1	8
Pen	8	2	3	3
XF-2 I	10	4	4	5
XF-2 E	4	10	10	4
XF-4 I	5	8	10	10
XF-4 E	6	6	10	5
XF-14 I	4	10	4	4
XF-14 E	1	4	6	9
XF-15 I	8	7	10	6
XF-15 E	6	4	9	2

Fig 37. Comparision of the MIC values of control and all the extracts against all test bacterial cultures



DISCUSSION

Many new and interesting bioactive metabolites such as antibiotics, antiviral, anticancer and antioxidant compounds, which are of pharmaceutical, industrial and agricultural importance have been reported andcharacterized from fungal endophytes. Strobel & Daisy (2003) suggested studying endophytic fungi since such plants may harbour unique and rare endophytes capable of producing important bioactive metabolites with multiple applications. The *Xylaria species* of endophytic fungi are an important group of fungi with many antimicrobial activities against various organisms.

Fungal broths were extracted and the concentrations were calculated. Table 1 shows the concentration of all the fungal metabolites. It was observed that the maximum concentration was of XF-2 extracellular followed by XF-2 intracellular, XF-4 intracellular, XF- 4 extra and XF-14 extracellular.

These extracts were then assayed for antimicrobial properties by agar well diffusion method and the zone of inhibition was measured. The diameter of the zone of inhibition in case of *E.coli* (Table 2) was observed to range from 2-12mm. The agar well diffusion studies indicate that XF-2 intra and extra, XF-4 intra and extra and XF-14 extra do not show any inhibition against *E.coli.* Some activity was seen with XF-14 and XF-15 extracellular extracts.

In case of *S.aureus* the range of zone of inhibition was from 2-16mm (Table 3). The zone of inhibition results indicate that XF-14 extra has the maximum inhibitory effect. Also XF-2 E, XF-4 E, XF-14 I and XF-15 E have an inhibitory effect. Zone oif inhibition was not seen with XF-2 and XF-4 intra.

P.aeruginosa showed zone of inhibition with all the compounds which represents that this test organism is susceptible to all the extracts. The range of zones was 3-17mm (Table 4) and again the highest zone was that of XF-14 extracellular. XF-2 intra and extra and XF-4, XF-15 intra and extra also showed activity.

In case of *B.megaterium* the range of zone was 3-16mm (Table 5). Again XF-14 extra showed the maximum inhibitory effect. XF-4 extra showed no activity. XF-2 intra and extra, XF-4 intra and XF-14 intra and XF-15 intra and extra showed some activity against *B.megaterium.*

Zone of inhibition of the extracts was in the range of 5-14mm against *C.albicans*. XF-15 intra showed the maximum inhibitory effect against *C.albicans*, (Table 6).

Figure 24. represents the comparison of zone of inhibition of all fungal extracts and control antibiotics against all the test bacterial cultures. It may be seen from the figure that XF-14 extra showed almost the same inhibitory effect against *S.aureus* and *E.coli* when compared with control penicillin.

Thus from the results it can be concluded Intracellular fungal extracts XF-2, XF-4, XF-14 and XF-15 showed activity against *P.aeruginosa* and *B. megaterium*. The extracts XF-2, XF-4, XF-14 intra were not effective against *E.coli*. XF-14 intra showed inhibition against *S.aureus*. The extracellular extract XF-14 showed max. inhibitory effect on all the extract except for *C.albicans*. XF-15 intra showed the highest activity with *C.albicans.*

Results of MIC are shown in table 10-21. Table 10 and Table 11 shows the effect of different concentration of control and extracts against *E.coli*. The MIC of Ampicillin was 9µg/ml, Streptomycin was 7µg/ml, Penicillin was 8µg/ml and the minimum inhibitory concentration obtained is 1µg/ml.

In case of *S.aureus* XF-2 intra, XF-14 extra, XF-15 extra showed activity comparable to ampicillin and streptomycin, (Table 22). MIC obtained against *P.aerugenosa* for Ampicillin was 3µ/ml, Streptomycin 1µg/ml, Penicillin 3µg/ml and the MIC of XF-14 I and XF-2 I were comparable to that of the control Ampicillin and Penicillin.

MIC obtained against *B.megaterium* (Table 16-17) of XF-15 extracellular is 2µg/ml and XF-4 extra and XF-2 intra are comparable to the control Ampicillin and XF-14 intra and XF-2 extra are comparable to Penicillin.

MICs obtained against *C.albicans* of Fluconazole (Table 22) was 3µg/ml, and the MIC of XF-4 extra was comparable to the control.

From the overall results it was found that the highest antibacterial and antifungal activities were obtained with XF-14 extracellular extract followed by XF-15 extracellular and these extracts can be further purified.

CONCLUSION

World health problems caused by drug-resistant bacteria and fungi are increasing. An intensive search for newer and effective antimicrobial agents is needed. Endophytic fungi have been recognized as useful sources of bioactive secondary metabolites. A recent comprehensive study has indicated that 51% of biologically active substances isolated from endophytic fungi were previously unknown (*Schulz et al.,* 2002). Many endophytic fungi have the ability to produce antimicrobial substances and are relatively unstudied and offer potential sources of novel natural products for exploitation in medicine, agriculture and the pharmaceutical industry. Also synthetic drugs have disadvantages as they are costly, have side effects and have harmful effects on the environment too. Although work on the utilization of this vast resource of poorly understood microorganisms has been initiated, it has already become obvious that an enormous potential for organism, product, and utilitarian discovery in this field holds exciting promise. Endophytic fungi are special group of organisms which spend their whole life or some times of their cycle in the plant tissue. They appear to have direct and induced effects on plant responses to biotic agents, but their interaction with abiotic agents remains largely unexplored. A number of bioactive compounds have been reported and many are yet to be discovered. So to add to the contribution towards finding novel compounds from different fungus, an attempt has been made in this study to screen endophytic fungi belonging to the *Xylariaceae* species for antimicrobial activity.

From the studies, it can be concluded that among the 4 strains of *Xylaria species*, the strain XF-14 extracellular showed the highest antibacterial and fungal activity with all the test organisms. XF-2 extracellular showed high antimicrobial activity with the test organisms *S.aureus* and *B.megaterium.* In case of XF-4 extracellular, highest activity was with *P.aeruginosa*. XF-14 showed highest activity with *P.aeruginosa* followed by *S.aureus* and *B.megaterium.* XF-15 showed highest activity with *P.aeruginosa.* It could be concluded from above that XF-14 extracellular contains a potent bioactive compound which could be beneficial for improving health of mankind.

SUMMARY

Natural products have rich source of therapeutic agents. Also there is a need to search new ecological niches for potential sources of natural bioactive agents for different pharmaceutical, agriculture, and industrial applications; these should be renewable, eco-friendly and easily obtainable (Liu *et al.*, 2001). Natural products discovery have played major role in the search for new drugs, and is the most potent source for the discovery of novel bioactive molecules. Natural products are chemical compounds derived from living organisms. This is an area of considerable interest to scientists due to the structural diversity, complexity and various bioactivities of isolated compounds. Crude natural products have been used directly as drugs which were low cost and important sources of traditional medicines. The aim of this project was determination of antibacterial, antifungal activity and characterization of the bioactive compound from *Xylaria* species. In the present study the endophytic fungi isolated from different hosts were screened for their antibacterial and antifungal activity using various methods as Agar well diffusion and Broth micro dilution. The test cultures used for determining antimicrobial activity were *S .aureus, E. coli, P. aeruginosa, B. megaterium* and *C. albicans.* From the results it was found that the extracellular metabolites of the 4 strains of *Xylaria*species were more potent for antimicrobial activity than the intracellular metabolites. Among the extracellular XF-14 showed the highest activity with almost all the test organisms, followed by XF-15 extracellular which was also effective against all the test cultures. In case of XF-15 intracellular, it showed highest activity against *C.albicans.* All the extracts were most effective against *P.aeruginosa* and least against *E.coli.*

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