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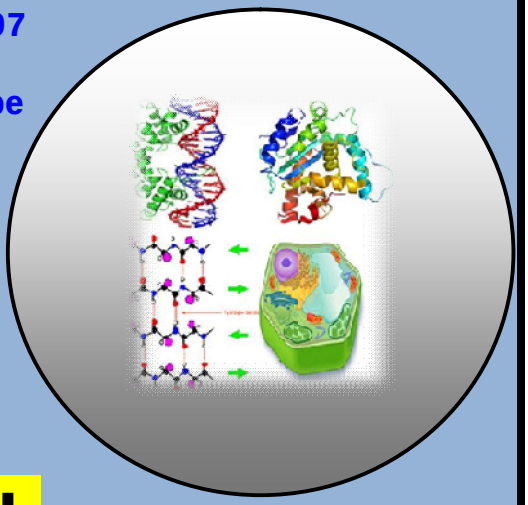

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RESEARCH PAPER
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Biosynthesis of Antibacterial and Antifungal Compounds from Fungal Origin

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ABSTRACT

The present study was undertaken to isolate and test the efficacy of culture extracts of 11 fungal species Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus tamarii, Trichoderma harzianum, Fusarium oxysporum, Fusarium chlamydosorum, Rhizopus stolonifer, Cladosporim herbarum, Mucor sp. and Penicillium chresogenum. Certain species including A. fumigatus, T. harizianum and P. chresogenum exhbited antimicrobial activites againist Gr+ ve , Gr-ve bacteria and unicellular yeast (Candida albicans) and filamentous fungus A. niger. The two isolates T. harzianum and P. chresogenum crude extract from rest fungal isolates given promising results on bacteria therefore its chosen for fourther study. The inhibition zones were found to decrease as the concentration of the crude extract of fungal isolate decreased. The predominant minimum inhibitory concentration of tested crude extracts of T. harzianum was 10mg/ml for Escherichia coli , Enterobacter aerogenes and Candida albicans . On the other hand MIC of Penicillium chresogenum was 25 mg/ml for Bacillus subtilis and Enterobacter aerogene.

Key Words: Biosynthesis, Antibacterial, Antifungal Compounds, Inhibitory effect and Fungi.

INTRODUCTION

It is well known that fungi remain one of the most important resources for the discovery of new bioactive compounds against bacteria, fungi , insects and nematoads as well as antitumor compounds (Pelaez, 2005). Bull *et al*. (1992) stated that the history of drug discovery from microorganisms, fungal secondary metabolites have provided a number of important drugs, such as the antibiotic penicillin, the immunosuppressant cyclosporine and the antihypercholesterolemic agents lovastatin and compactin**.** Soil microbial communities are the most complex, diverse and important assemblages of organisms in the biosphere; and they participate in various biological activities.

Accordingly, they are an important source for new antimicrobial agents and molecules with biotechnological importance (Hackl *et al.,* 2004). Fungi are recognized as prolific secondary metabolite producers. Fungi have provided several bioactive compounds and chemical models currently used as pharmaceuticals, and the soils are traditionally the main source of fungal genetic resources for bioprospection programs (Adrio and Demain, 2003). Several biocontrol agents have been identified from soils with added compost, including many fungi such as Trichoderma, Penicillium, Aspergillus and *Gliocladium virens* (Hoitink and Boehm, 1999). Certain fungal extracts were assessed for antibacterial activity against three standard pathogenic bacterial strains. Most of the tested extracts showed *in vitro* inhibition of *Bacillus subtilis, Staphylococcus aureus and Escherichia coli* growth **(Al-mahi Idris *et al*.**, 2013).

The genus *Trichoderma* produced a wide range of antibiotic substances and parasitizes other fungi. Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, *Trichoderma* species exert a property that is known as rhizosphere competence (Chet and Baker, 1981; Saravanan, and Jayaraaj, 2004). Langfied *et al.,* (2004) verified that cyclic hexadepsipeptide beauvericin isolated from *Fusarium redolens* which showed strong and broad antibacterial activity against the six test bacteria (i.e. *Bacillus subtilis*, *Staphylococcus haemolyticus*, *Pseudomonas lachrymans*, *Agrobacterium tumefaciens*, *Escherichia* coli and *Xanthomonas*. Filamentous fungi belonging to fifteen different genera were isolated by Mara *et al* (2009) were screened to discover novel antimicrobial and cytotoxic compounds. A total of 1422 extracts were prepared by solid phase extraction of the culture broths or by biomass solvent extraction. 47 (29%) filamentous fungi showed antimicrobial activity; most of them inhibited the growth of gram-positive *Staphyloccusaureus* (14%), gram negative *E. coli* (10%), *Candida albicans* (11%) and *Cryptococcus neoformans* (8%). Less activity was detected against representatives of enterobacteria and filamentous fungi. Many antibiotics have been discovered from microorganisms. The process of discovering antibiotics usually includes several steps: including; isolation of microorganisms mainly from soil or other source; pure culture of isolated microorganisms; identification of isolated microorganisms mainly by morphological characteristics and biochemical tests to eliminate duplicate strains; testing whether or not these culture broths or extracts of microorganisms show antimicrobial activity to select candidate microorganisms that produce antibiotics and re-culture of candidate microorganisms to observe the reproducibility of antimicrobial activity. This routine process (named the traditional method in this study) usually takes 5-7 weeks.

MATERIAL AND METHODS

Media used

Czapek-Dox Agar (CzDA): Containing of (Sucrose, 30.00 g;Sodium nitrate,2.00 g; Potassium dihydrogen orthophosphate, 1.00 g; Potassium chloride, 0.50 g ;Magnesium sulphate 0.50 g; Ferrous sulphate, 0.002g; Agar 20.00 g; Distilled water 1 L.

Malt Extract Agar (MEA): Containing of Malt extracts, 20 g; Peptone 1 g ; Glucose20 g; Agar,20 g; Distilled water.

Potato Dextrose Agar

Potato extract	4.0*
Glucose	20.0
Agar	15.0
pH 5.6 ± 0.2 @ 25°C	

Fungal isolation and their identification:

Collecting samples from different soil were processed using the soil plate method**).** About 1 g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (40-45 °C) agar medium Malt extract agar (MEA) and Czapek-Dox agar medium (CzDA) was added, which was then rotated gently to disperse the soil particles in the medium. Duplicate plates were prepared from each dilution. The plates were then incubated for 3-7 days, at 27˚C. The fungal colonies were picked up on tap water agar (TWA) medium for checking the purity of culture and picked up on the plate of the isolating medium and purified by streaking several times on the same isolating medium, potato-dextrose agar, Czabek agar. Light microscopy was used for examination and identification of the fungal isolates according to **Raper and Fennel (1965) Samson *et al.*, (1988).**

Production and Separation of Antimicrobial metabolites

Fungal isolates were inoculated in broth medium (Fig. 1). After 10 days of incubation period, the growth medium (potato dextrose broth medium of fungal isolates containing extracellular metabolites was extracted twice with Chloroform/methanol (2:1v/v) and defated using n-hexane in a separating funnel (Fig. 2), then concentrated and loded on disc or well to determine the antimicrobial activity.

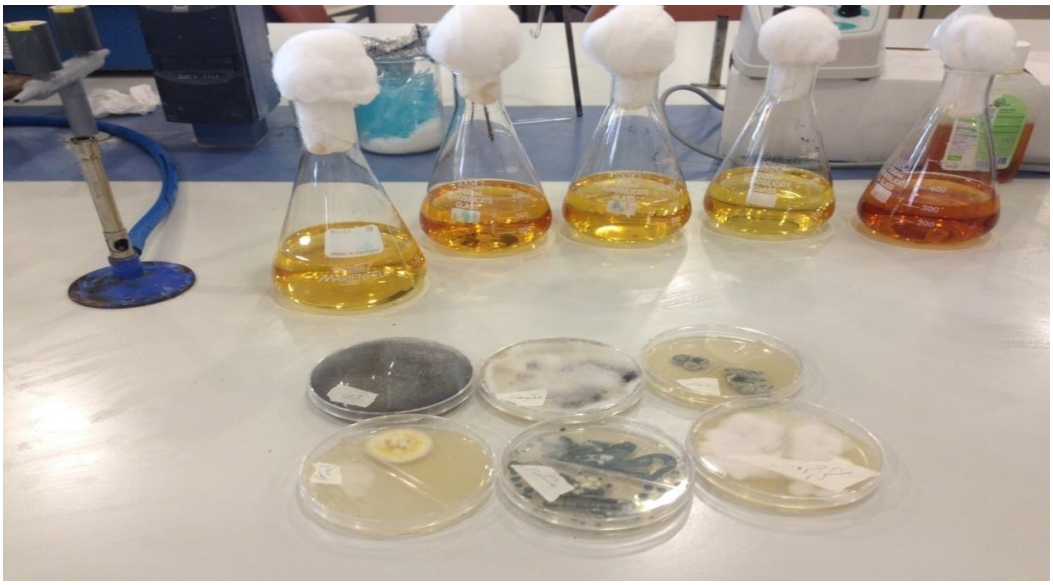

Fig. 1 Fungal isolates inoculated in broth medium for antimicrobial metabolites.

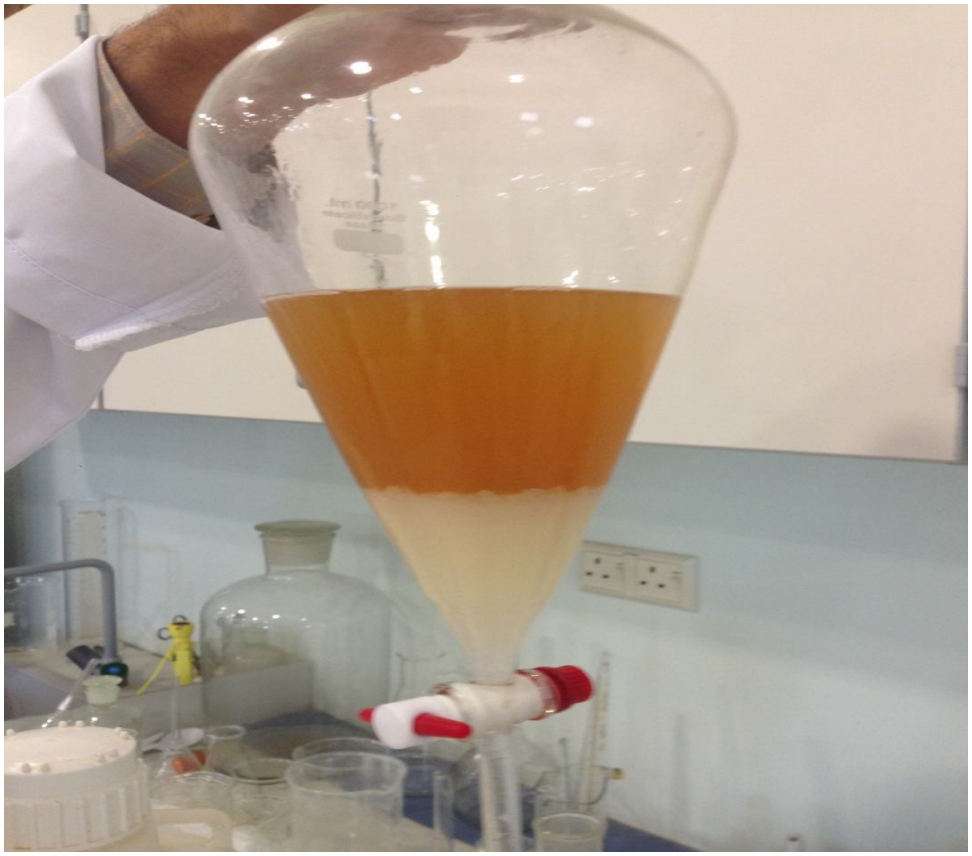

Fig. 2. Separation of antimicrobial compounds with using separation funnel.

Test organisms and antimicrobial screening

Representatives of Gram-negative bacteria; namely, *Enterobacter aerogenes*, *Escherichia coli*, and Gram-positive bacteria; namely, *Bacillus subtlis* and *Staphylococcus aureus* and unicellular fungi; namely, *Candida albicans*, and filamentous fungi; namely, *Aspergillus niger* were used as test organisms. The disc diffusion and well methods were used for the antimicrobial screening and determination of minimum inhibitory concentration (MIC) of the investigated isolates. The fungal culture was extracted with equal volume of chloroform: methanol (2:1, v/v), left to evaporate till dryness, and then dissolved in 3 ml distilled water. The agar disc diffusion method was employed for the determination of antimicrobial activities of extra-cellular extracts of the investigated fungal isolates. A standard blank paper discs (5 mm in diameter) were separately soaked in the solvent used in the extraction extracts and transferred onto the surface of growth media seeded with the test organism. After incubation period, following suitable conditions for the test organism, the diameter of the inhibition zone around the discs and well were measured in millimeters. Disc diffusion method for making antimicrobial activity of fungal supernatants obtained. Suspensions of the test pathogenic bacteria and test pathogenic fungi were inoculated and spread on the surface media.

Sterile discs were saturated with fungal supernatants that obtained from previous steps and placed on the prepared plates. All the nutrient agar plates were incubated at 35°C for one day and at 25-28ºC for test fungi at three days and the results of Inhibition zone were measured, **(Fagbemi *et al*., 2009).**

Minimum inhibitory concentration will determine as follow:

The minimum inhibitory concentration was determined from readings on the culture plates after incubation (MIC) and also determine of minimum bactericidal and/or fungicidal concentration (**Fagbemi *et al*., 2009**).

RESULTS AND DISCUSSION

In the current study 11 fungal isolates were isolated from differnt sources including farmer soil, air conditionar and agricultural soil with using two grwoth media (Table 1). The isolates were related to ascomycetes and deuteromycetes. Soil is considered one of the most suitable environments for microbial growth (Cavalcanti et al., 2006). Most of fungal isolates were dominant on potato dextrose agar (PDA) medium, where zygomycetes including Rhizopus and Mucor were isolated only on PDA.

Table 1. Fungal isolates from different sources on two growth media.

Fungal isolates	Czapek-Dox Agar			Potato Dextrose Agar		
	Farmer animal soil	Agricultural soil	Air conditioner	Farmer animal soil	Agricultural soil	Air conditioner
Aspergillus niger	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+
Aspergillus fumigates	+	-	-	+	+	+
Aspergillus tamarii	+	+	-	+	+	+
Trichoderma harzianum	-	+	+	+	-	+
Fusarium oxysporum	+	+	-	+	+	-
Fusarium chlamydosorum	+	+	-	+	+	-
Rhizopus stolonifer	-	-	-	+	+	+
Cladosporim herbarum	+	-	-	+	-	-
Mucor	-	-	-	+	+	+
Penicillium chresogenum	+	+	-	+	+	-

The remarkable increase in antibiotics resistant bacteria species (Motta et al., 2004) lead to search for new sources of antibiotics through the isolation and identification of new types of microorganisms such as bacteria, fungi and actinomycetes (Alexander, 1982; Srividya *et al*., 2014). Five fungal species were isolated from the mixture of soil and saliva which were *Chaetomium globosum*, *Fusarium oxysorum* , *Aspergillus biplane , Cochliobolus lanatus* and *Emericella nidulans* The fungal suspensions of the isolates showed efficient antagonistic activities against the two tested pathogenic bacteria and completely prevent their growth on solid media (Huda, 2010).

In the current study, antimicrobial activity of fungal isolates (Table 2) was screened for research and selection of new antimicrobial metabolites with using two methods including Disc and well methods (Fig.3). Antibiotic producing microbes are abundant in soil, water, sewage and compost that serve source for isolation. Gram positive bacteria were less seriously studied compared to gram negative bacteria (Loeffler et al. 1986). The obtained results showed that certain fungal isolates capable of produces antibiotic compounds against Gr+ve and Gr-ve bacteria; antifungal compounds against unicellular and filamentus fungi. *Penicillium chresogenum* was the most potant fungal producers of antibacterial compounds. On the other hand *Trichoderma harizianum* was the most potant fungal producers of antifungal compounds. Five of six *A. fumigatus* and four of six *A. flavus* isolates had bactericidal effect against all tested bacterial strains. *A. niger* isolates and Trichoderma spp. isolates showed bactericidal effect against gram positive bacterial strains (Stefan et al., 2012). Amna *et al*. (2009) reported that the species of *Penicillum* showed marked antibacterial activity against all test bacterial species in their study. Todar (2002) stated that currently microorganisms of genera Penicillium, Streptomyces, Cephalosporium, Micromonospora and Bacillus are known to produce more than 5000 different antibiotics.

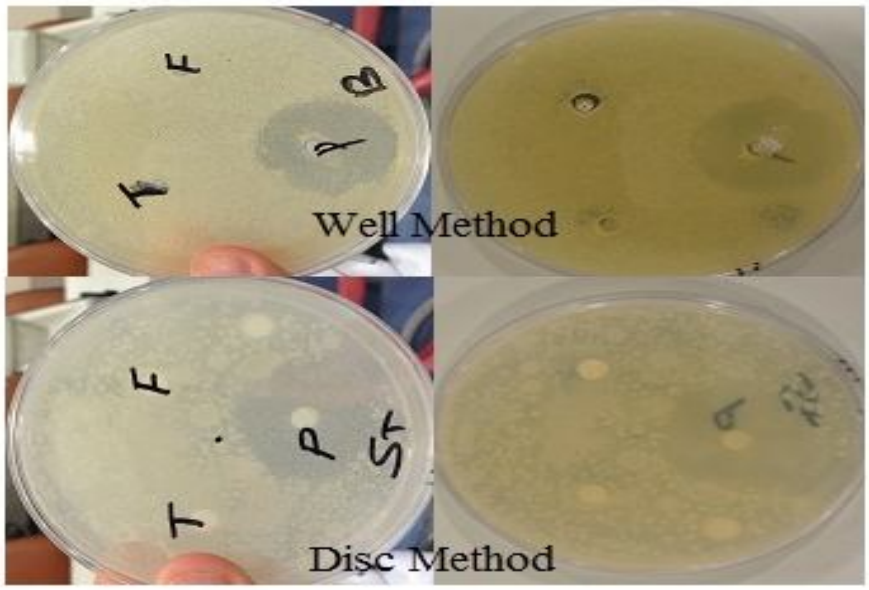

Fig. 3. Antimicrobial activity of fungal isolates with disc and well methods.

Table 2. Antimicrobial activity of fungal isolates.

Fungal isolates	Gram-negative bacteria		Gram-positive bacteria		Fungi	
	*E.c.	*E.a.	*B.s.	*S.a.	*C. a.*	*A. n.*
Aspergillus *niger*	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus flavus	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus *fumigatus*	0.9	0.8	0.9	1.3	0.0	0.0
Aspergillus *tamarii*	0.0	0.0	0.0	0.0	0.0	0.0
Trichoderma *harzianum*	1.2	1.3	0.0	0.0	1.5	2.2
Fusarium *oxysporum*	0.0	0.0	0.0	0.0	0.0	0.0
Fusarium *chlamydosorum*	0.0	0.0	0.0	0.0	0.0	0.0
Rhizopus *stolonifer*	0.0	0.0	0.0	0.0	0.0	0.0
Cladosporim *herbarum*	0.0	0.0	0.0	0.0	0.0	0.0
Mucor sp	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium chresogenum	3.2	2.9	2.2	2.5	0.0	0.0
Control	0.0	0.0	0.0	0.0	0.0	0.0

***E.a.=** *Enterobacter aerogenes*, ***E.c.**= *Escherichia coli* **,** ***B.s.** =*Bacillus subtilis*, ***S.a.=** *Staphylococcus aureus.* Control : Discs or wells loaded with separating solvent.

Table 3. Minimum Inhibitory concentration of fungal extract for Trichoderma harzianum and Penicillium chresogenum.

Fungal isolate	Test organism	**Diameter of inhibition zone(mm)** at different concentration (mg/ml)				
		100	50	25	10	5
Trichoderma harzianum	*Escherichia coli*	1.4	0.9	0.8	0.6	0.0
	Bacillus subtilis	1.2	0.0	0.0	0.0	0.0
	Staphylococcus aureus	0.0	0.0	0.0	0.0	0.0
	Enterobacter aerogenes	1.6	0.8	0.9	1.3	0.0
	Aspergillus niger	2.8	2.1	1.2	0.0	0.0
	Candida albicans	1.6	1.3	0.9	0.8	0.0
Penicillium chresogenum	*Escherichia coli*	3.4	3.2	2.5	1.2	0.0
	Bacillus subtilis	2.0	1.6	1.6	0.0	0.0
	Staphylococcus aureus	0.9	0.8	0.9	1.3	0.0
	Enterobacter aerogenes	2.9	1.8	0.8	0.0	0.0
	Aspergillus niger	1.2	1.3	0.0	0.0	1.5
	Candida albicans	0.0	0.0	0.0	0.0	0.0

The two isolates *Trichoderma harzianum* and *Penicillium chresogenum* crude extract from rest fungal isolates given promising results on bacteria therefore its chosen. According to data illustrated in the Table (3) the inhibition zones were found to decrease as the concentration of the crude extract of fungal isolate increased. According to data illustrated in the Table (3) the predominant minimum inhibitory concentration of tested crude extracts of T. *harzianum* was 10mg/ml for *Escherichia coli* , *Enterobacter aerogenes* and *Candida albicans.*

On the other hand MIC of *Penicillium chresogenum* was 25 mg/ml for *Bacillus subtilis* and *Enterobacter aerogene.* Also MIC explained to go with Suthep *et al,* (2004) when selected 360 distinct fungi for cultivation on malt Czapek broth and yeast extract sucrose broth, from which extracts were tested for biological activity against bacteria. Finally and according to Demain and Fang (2000) isolated fungi in the form of crude extracts and spore suspensions could be served as competitive weapons used against harmful bacteria, fungi, nematodes.

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