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In vitro antimicrobial study of *Aspergillus flavus* mycelial extract against different bacterial and fungal pathogenic strains

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Abstract

The aim of our research was to determine the antimicrobial activity of *Aspergillus Flavus* against some selected bacterial and fungal pathogen. The research work was done in the laboratory of Agricultural Chemistry, Department of Agricultural Chemistry, Agriculture University Peshawar, Khyber Pakhtunkhwa, Pakistan during the month of May 2013. The agar well diffusion method was used. The Acetonitrile and n-hexane extracts were used. The result of both the extracts of *Aspergillus flavus* was found to be effective against all the tested Bacterial i.e. *P. aeruginosa, E. coli, S. aureus, S. aureus* (Methicillin resistant), *S. aureus* (Vancomycin resistant), and fungal pathogen *A. niger, A. oryzae, C. albican, P. distalatum, F. oxysporum*. The result in Acetonitrile solvent was quite good from the result in n-hexane fraction, which confirmed that the secondary metabolites are more soluble in acetonitrile. Minimum inhibitory concentration (MIC), of the extracts against these bacterial and fungal strains were in the range of 0.20 mg/ml. Different phytochemical analysis result indicate the presence of secondary metabolites like mycotoxin, Aflatoxin, Kojic acids which may be responsible for antimicrobial properties. From our result is the concluded that extract of *Aspergillus flavus* have potential against fungal and bacterial strains.

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Introduction

Fungi are the important component of ecosystem. They play main role in the process of decomposition and mineralization. Kumar *et al.,* (2010) stated that thermophilic fungi are present worldwide due to self-heating masses, present in the natural habitat soil and where decomposition of plant, plants parts, animal, birds, and municipal refuse takes place. At 20-60 ^{o}C temperature the thermophilic fungi can grow.

Bridge and Spooner (2001) reported that fungal organisms are much diverse group consists of microscopic single celled yeast to large macro sized fungus like mushrooms and toad stools. Chatia and Ahmad (2012) opined that for ecosystem fungi are very important and they are the big decomposer of dead organic matter of forests, fruits etc. Due to saprobes nature they play a key role in mineralization and decomposition. Some of fungi form mycorrhiyzal association (symbiotic association of plant roots and fungi).

Several fungi produce bio-active compounds, secondary metabolite and chemical models having pharmaceutical importance (Zang *et al.,* 2009). Silva *et al.,* (2000) examined that some fungal species form beneficial associations with roots of higher plants known as mycorrhyizal association which is based on the plant part providing carbohydrates and other important organic compounds to the fungi while in return fungi helps the plant in uptake of nutrients of outreach from its roots system.

Many fungi produce biological active compounds, secondary metabolite and mycotoxin having pharmaceutical importance (suay *et al.,* (2000); Zang *et al.,* (2009). A fungal species *Penicillium notatum* gifted the first antibiotics named penicillin. Against microbes many fungal species have an antimicrobial active constituent which are used e.g. *Penicillium* was the fungus from which the first antibiotic drug was prepared, named as 'Penicillin' (Haven, 1994).

Aspergillus is a large genus belongs to family Trichocomaceae (Ascomycota) which includes 9 genera and 180 species (Pitt *et al.,* 2000).

Acosmopolitan fungus *Aspergillus flavus* has pathogenic and saprophytic nature (Machida and Gomi, 2010; Camejo *et al.,* 2012). Due to pathogenic nature and presence in soil it causes many diseases in various important agricultural crops. Cereal grain and legumes are common hosts of this pathogen. *A. flavus* specifically infect the ear rot in corn and yellow mold in peanuts **(**Saori and Keller, 2011).

The aim of the study was that to explore the antimicrobial activity of the mycelial extracts of *Aspergillus flavus* in acetonitrile and n-hexane solvent against different fungal and bacterial human pathogens strain. The fungi are very important and further study should be made to explore new antibiotic and antifungal drugs from fungal species.

Material and methods

Collection of Soil Sample

For collecting composite soil sample, a square was made on the ground & the soil was collected from each end of the pointed area through a sickle with a depth of 6 inches. The Sample was collected in sterile zipper polythene bags, mixed thoroughly & transferred to the Laboratory of Natural Products and Medicinal chemistry, Department of Agricultural Chemistry, Agriculture University, Peshawar, for further investigation. The samples were stored at 4 ^{o}C. For collection of soil samples, standard soil collection method was used (Rohilla *et al.,* 2011). The soil sample was 1st dried and then converted to powder through pestle and mortar. The fine soil was then filtered through 2 mm sieve and the sieved soil (2.5 g) was dissolved in distilled water (97.5) mL, to make the volume 100mL. The pH of distilled water was determined through pH meter. The mixture of soil & distilled water was shacked in a shaker for 20 minutes at 120 rpm (revolution per minute) (Rohilla *et al.,* 2012).

Isolation of Fungal Spore

Dilution plate technique was adopted for the isolation of fungal strains from the soil samples (Waksman,

1922). The Media used for the isolation were Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) Plates were incubated for 7 days at 28˚C (26). All this process was carried out in the laminar flow unit (LFU) under aseptic condition.

Extraction of Crude extract

The mature mycelia was collected in the flask, and then crushed with the help homogenizer. The crushed mycelia were kept on the stirring for 24 hour to dissolve all the secondary metabolites present. The active components were extracted using two solvent, Acetonitrile and n-hexane.

Media Sterilization and Pouring

The media was sterilized in the autoclave at 121 C^{o} for 15 mints. The sterilized media (PDA) was poured in glass Petri plates in Laminar Flow, which was pre-sterilized by use washing with 70% methylated spirit after that the UV light was run for 15 mints to kill or denature all the contaminants present if any. The petri-plates containing media were kept for 30 mints in the Laminar flow unit to solidify.

Tested Microorganisms

The in-vitro antimicrobial activity of Aspergillus flavus crude was checked against some selected bacterial. i.e. *P. aeruginosa, E. coli, S. aureus, S.aurues* (Methacillin resistant), *S.aureus* (Vancomycin resistant), and fungal pathogen *A.niger, A.oryzae, C.albican, P. distalatum, F. oxysporum*. For antifungal activity 100µl and for antibacterial 50 µl of suspensions were aseptically introduced and spread using pre-sterilized cotton swabs on surface of MHA plates.

Formation of Wells

Agar well diffusion method as stated by Adeniyi *et al*., (1996). With the help of sterilize cork borer 8mm diameter well was punched aseptically in the agar plates.

Antimicrobial Activity

The antimicrobial activity was carried out using agar well method. The crude extract of Aspergillus flavus was tested against both fungal and bacterial pathogen. For bacterial 50 µl and for antifungal 100 µl were poured into the well. All the plates were kept in the incubator by adjusting temperature 30 C^{o} for fungal species (7days) and at 37 C^{o} for bacterial species (48 hour). The zone of inhibition was measured with the help of Vernier caliper.

Data Analysis

All the experiments were carried out in triplicates. The statistical analysis was conducted by using Graph pad prism software. The data was arranged as mean and S.D (Standard deviation).

Results and discussion

The aims of our research were to determine the hidden potential of *Aspergillus flavus* extracts against different fungal and bacterial pathogen.

Table 1. Shows the characteristics of soil samples collected from Batkhela Malakand.

Soil sample	% Moisture	pH	Color	Temperature
Batkhela Malakand	65	6.9	Yellow	30

Table 2. Activity of *Aspergillus flavus* against bacterial strains.

Fungal specie	Solvent	Concentration used (ul)	Zone of inhibition(mm) Mean ± S.D				
			E.coli	*S. aurues* (Methacillin resistant)	*S. aurues* (Vancomycin resistant)	*P. aeruginosa*	*S. aurues*
Aspergillus flavus	Acetonitrile	50ul	12.1±0.4	19.4±0.5	23.4±1.2	16.1±0.7	21.8±0.4
	n-hexane	50ul	9.2±0.5	7.0±0.6	8.0±0.6	5.7±0.7	4.4±0.5

*Values with different letters in the same column are significantly different (P<0.05)

Soil Sample

The fungal specie *Aspergillus flavus* was isolated from the soil sample collected from the Batkhela, Malakand Agency, Khyber Pakhtunkhwa, Pakistan. The physiochemical properties of the soil sample were shown in the Table 1.

Table 3. Activity of *Aspergillus flavus* against fungal strains.

Fungal specie	Solvent	Concentrat in used (ul)	Zone of inhibition(mm) Mean ± S.D				
			A. niger	*C. albican*	*A. oryzae*	*P. distalatum*	*F. oxysporum*
Aspergillus flavus	Acetonitrile	100ul	26.1±0.2	23.3±0.7	20.8±0.1	18.0±0.5	15.5±1.2
	n-hexane	100ul	9.9±0.2	5.5±0.5	7.2±0.5	6.5±0.7	10.5±0.5

*Values with different letters in the same column are significantly different (P<0.05).

Antimicrobial Activity

The result of antimicrobial study revealed that *Aspergillus flavus* extract has good activity in acetonitrile and low activity in n-hexane against all the tested pathogens. It was confirmed from our result that the extract of *Aspergillus flavus* have secondary metabolites which may be responsible for antimicrobial activity. . MIC values of all the extracts tested against Bacterial isolates were summarized in Table 2, while table 3 showed the antifungal activity. The inhibitory activity varied significantly against all the clinical isolates with MIC value ranged between 0.20mg/ml.

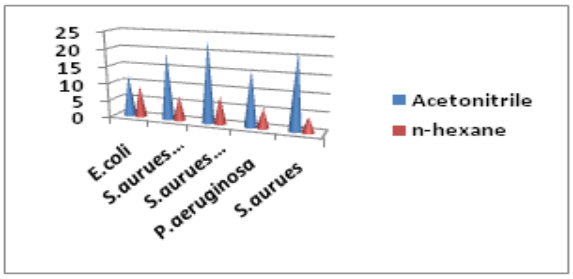

Fig 1. Extracts Showing Zone of Inhibition against Known Bacterial strains.

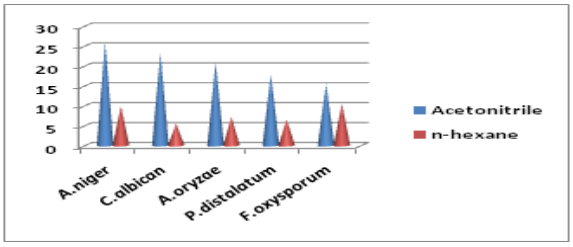

Fig. 2. Extracts Showing Zone of Inhibition against Known Fungal strains.

The extracts in Acetonitrile showed good activity while extracts in n-hexane gives satisfactory result. Some researcher suggested that observed antimicrobial activity of fungal extracts due the presence of secondary metabolite. (Nweze *et al.*, 2004). Idris *et al.*, (2013) investigated the antibacterial activity of *Aspergillus flavus* against *E. coli, Bacillus subtilis, Staphylococcus aureus*. The zones of inhibition ranged from 14-37 mm. Tawfik *et al.*, (2012) reported the antibacterial activity of fungal extracts of some fungi, using disk diffusion method. All the fungal extracts showed an inhibitory action against bacterial species, *E.coli* and *S. aureus*. The zone of inhibition by fungal extracts ranged between 22-28 mm diameters. Aspergillus flavus have good antifungal activity against *C. albicans, A. niger,* Makut and Owolewa (2011). Takahashi, *et al., (*2010) reported that total 200 fungal strain were isolated from the soil sample of Serra do Cipo National Park in Brazil. The fungal strain were checked for antibacterial activity against *S.aurues, E.coli, S.typhi, Streptococcus pyogenes, and Listeria monocytogenes, which show activity against the tested species. From my research work it was revealed that Aspergillus flavus have potential against both fungal and bacterial species.*

Conclusion

From the results of my research work it was concluded that the extracts of *Aspergillus flavus* have compounds which have ability to inhibit the growth of fungal and bacterial strain. Further study should be

made to study the compound or secondary metabolites of *Aspergillus flavus,* which can be utilize as medicines like antibiotics and fungicides.

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References

Adeniyi BA, Odelola HA, Oso BA. 1996. Antimicrobial potentials of *Diospyros mespiliformis* (Ebenaceae). African Journal of Medicine and Medical Sciences **25,** 221–224.

Bridge P, Spooner B. 2001. Soil fungi diversity and detection. *Plant and Soil* **232,** 147–154.

Chutia M, Ahmed GU. *2012.* Diversity, Antimicrobial activities and associated microbiota of soil pencilia from virigin forest floor Journal of Microbiology, Biotechnology **1(5),** 1279-1294 Food Sciences.

Haven, Kendall F. (1994). *Marvels of Science: 50 Fascinating 5-Minute Reads*. Littleton, CO: Libraries Unlimited. 182 p.

Kumar R, Rajavaram, Bathini S, Girisham S, Madhusudhanreddy S. 2010. Incidence of Thermophilic fungi from different substrates in Andhra Pradesh .International journal of pharma and biosciences..**1**/Issue-3.

Machida M, Gomi K. (2010). *Aspergillus: molecular biology and genomics*. Horizon Scientific Press. 157 p.

Makut DM, Owolewa AO. 2011. Antibiotic-producing fungi present in the soil environment of keffi metropolis, Nasarawa state, Nigeria. Trakia Journal of Sciences **9(2),** 33-39 p, 2011.

Nweze Ei, okafor JI, Njoku O. (2004).Antimicrobial activities of methanolic extract of *tremaguineensis* (schummand thorn) and *morindalucida Benth* used in nigerian herbal medicine practice. Journal of Biological Reseach Biotechnology **2(1),** 39-46.

Pitt JI, Samson RA, Frisvad JC. 2000. List of accepted species and their synonyms in the family Trichocomaceae. In: Integration of modern taxonomic methods for Penicillium and Aspergillus classification, Samson RA, Pitt JI (eds), Harwood Academic, Amsterdam. 9–49 p.

Rohilla S, Salar R. 2011, Isolation and Characterization of Various Fungal Strains from Agricultural Soil Contaminated with Pesticides. Research Journal Recent. Science **1,** 297-303.

Saori A, Nancy PK. 2011. *Aspergillus flavus*. *The Annual Review of Phytopathology* **49,** 107–133.

Silva MG, Furtado NAJC, Pupo MT, Fonseca MJV, Said S, Filho AAS, Bastos JK. 2004. Antibacterial activity from Penicillium corylophilum Dierckx. In Microbiological Research **159,** 2004, 317—322 p.

Suay I, Arenal F, Asensio FJ, Basilio A, Cabello MA, Diez MT, Garcia JB, Delval AG, Gorrochategui J, Hernandez P, Pelaez F, Vicente MF. 2000. Screening of basidiomycetes for antimicrobial activities. In Anton van Leeuwenhoek, **78,** 2000, 129–139 p.

Takahashi JA, Castro MC, Souza GG, Lucas EM, Bracarense AA, Abreu LM, Oliveira TS. (2008). Isolation and screening of fungal species isolated from Brazilian cerrado soil for antibacterial activity against Escherichia coli, Staphylococcus aureus, Salmonella typhimurium Streptococcus

pyogenes and Listeria monocytogenes. Journal of Medical Mycology **18(4),** 198-204.

Tawfik M. Muhsin, Halla M, Mohammad. 2012. .Preliminary screening of bioactive metabolites from three fungal species of *Drechslera* isolated from soil in Basrah, Iraq .Journal of Basrah Researches (Sciences) **38,** Number 2 .A.

Waksman SA. 1922. A method of counting the number of fungi in the soil. International Journal of Bacteriology **7,** 1922, 339-341 p.

Zhang Y, Mu J, Feng Y, Kang Y, Zhang J, Gu PJ, Wang Y, Ma LF, Zhu YH. 2009. Broad-spectrum antimicrobial epiphytic and endophytic fungi from marine organisms: isolation, bioassay and taxonomy. In Marine Drugs **7,** 2009, 97-112 p.