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Antimicrobial activity of mycelial extracts of *Rhizopus stolonifer* against different fungal and bacterial pathogenic strains

Sohail[1*], Muhammad Afzal[1], Zafar Iqbal[3], Sheena[2], Shujaul Mulk Khan[1], Inayat Ur Rahman[1], Waqar Khan[1], Ali Asghar[1], Imran Ullah[4], Muhammad Numan[3]

[1]*Department of Botany, Hazara University, Mansehra, Khyber, Pakhtunkhwa, Pakistan*

[2]*Department of Zoology, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan*

[3]*Department of Agricultural Chemistry, Agriculture University, Peshawar, Khyber Pakhtunkhwa, Pakistan*

[4]*Department of Microbiology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan*

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Abstract

The aim of our research was to investigate the hidden antimicrobial potential of *Rhizopus stolonifer* against some selected fungal and bacterial pathogenic strains. 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 extracts in Acetonitrile and n-hexane were used. The result of all the extracts of *Rhizopus stolonifer* was found to be effective against all the tested fungal pathogenic strains i.e. *A. niger, A. oryzae, C. albican, P. digitatum, F. oxysporum* and Bacterial pathogens *P. aeruginosa, E. coli, S. aureus, S. aureus* (Methicillin resistant), *S. aureus* (Vancomycin resistant), The result of the extracts of *R. stolonifer* is quite effective in Acetonitrile solvent as compared to results in n-hexane. Minimum inhibitory concentration (MIC), of the extracts against these bacterial and fungal strains were in the range of 0.25 mg/ml. Different phytochemical analysis result indicate the presence of secondary metabolites like mycotoxin, Aflatoxin (B1, B2, G1and G2), which may be responsible for antimicrobial potential. From my result it is concluded that extracts of *Rhizopus stolonifer* have potential against all fungal and bacterial pathogenic strains.

* **Corresponding Author:** Sohail ✉ sohail.botanist@hotmail.com

Introduction

Fungi are the major components of bio-diversity, necessary for the survival of other living organism and have crucial role in ecological processes. Fungi are with versatile nature and adaptable in all habitats. The most important habitat for microorganism like fungi, bacteria, and actinomycetes is soil. Fungi mainly contribute in the soil biomass (Kumar *et al.,* (2010).

Chatia and Ahmed (2012) opined that fungi are very important for ecosystem and they are the main decomposer of dead organic matter of animals, fruits and forest etc. fungi are saprobes in nature so they play important role in mineralization and decomposition.

Nikapitiya (2012) reported that production of secondary metabolites is one of the characteristic features of microorganisms. More than 50,000 bioactive compounds have been isolated from the extracts of microorganisms with a diversified arrangement of chemical structures, which showed antimicrobial, antitumor and agrochemical activity. Devi *et al.,* (2012) stated that in microbial world, the top bioactive compounds producer are actinomycetes (45%), fungi (38%) and 17% bacteria.

It has been estimated that more than 22% of the total antibiotics available in today world market are produced by fungi. These fungal antibiotics comprise of many semisynthetic penicillins, the biosynthetic penicillin V, the semisynthetic cephalosporins and the natural penicillin G., some researchers confirmed that large numbers of fungal extract or extracellular products have antimicrobial activity, mainly from the filamentous fungi, e.g *Penicillium species* (Gharaei *et al.,* 2009). Many fungi produce bioactive compounds and secondary metabolites having pharmaceutical importance Suay *et al.,* 2000 : Zang *et al.,* 2009).

Rhizopus stolonifer (black bread mold) is a widely distributed thread-like mucoralean mold, belongs to family Mucoraceae. Most commonly found on bread surfaces. It takes food and nutrients from the bread and damages the surface where it lives. The growth of *Rhizopus stolonifer* is very fast at 15-30°C. The asexual spores are formed in pinhead like sporangia, which on maturity, burst and release spores (Schipper, 1984).

The aim of the research was to investigate the antimicrobial activity of *Rhizopus stolonifer* in acetonitrile and n-hexane solvent against bacterial and fungal strains. Fungi play very important in ecosystem. Further study is required for the discovery of new antifungal and antibacterial drugs to help Human being.

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 0C. 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 strain

For the isolation of fungal strain dilution plate method was used (Waksman, 1922). The medium used for the isolation was potato Dextrose Agar (PDA). Plate were than incubated for the period of 7 days at 28 °C. All this process was carried out in the cabinet Laminar flow unit (LFU) under aseptic condition.

Preparation of Fungal extracts

For the extraction, the mature mycelia were collected in flask and crushed with the help of homogenizer. The crushed mycelia were then subjected to stirring for the period of 24 hour. The extracts were then filter and dried in the open air. The active compounds were extracted using two solvent Acetonitrile and n-hexane.

Used fungal and bacterial strains

The extract of *R. stolonifer* was checked against different fungal and bacterial strains. The fungal strains were *C.albican, P.digitatum, A.niger, A.oryzae, F.oxysporum* and bacterial strains were *E.coli, S.aureus, S.aureus* (Methacillin resistant), *S.aureus* (Vancomycin resistant), *P.aeruginosa*. For antifungal 100µl and for antibacterial 50µl extract solutions were aseptically introduce and spread by using sterilized cotton swab on the surface of MHA plates.

Media Sterilization and Pouring

The media was sterilized in autoclave at 121 ºC for 15 mints. About 20 ml of Media (PDA) was added to each Petri-plate in laminar flow unit. The laminar flow unit was pre washed with 70% of methylated spirit after that, the UV light was run for 15 mints to kill or denature all contaminants present if any.

Antibacterial and antifungal screening by agar well method

Agar well method was adopted for antibacterial and antifungal activity as stated by Ahmed and Beg (2001). With the help of sterilized cork borer 8mm diameter well was punched aseptically in the agar plates. The antimicrobial activity was carried out in the liminar flow unit under aseptic condition. The extract of *Rhizopus stolonifer* was checked against both the selected fungal and bacterial pathogenic strains. For antifungal screening 100µl and for antibacterial 50µl extract were introduce into each well. All the petri-plates were incubated at 30 ºC for the period of 7 days for antifungal and at 38 ºC for 48 hour for antibacterial screening. The zone of inhibition was measure with the help of Vernier caliper.

Statistical Analysis

The statistical analysis of data was conducted by using SPSS software. All the experiments were performed in triplicates. The data was arranged as Mean and Standard Deviation (SD).

Result and discussion

The present study was to investigate the antimicrobial potential of the extracts of the *Rhizopus stolonifer*.

Soil Sample

The soil sample was collected from Batkhela, Malakand Agency, Khyber Pakhtunkhwa, Pakistan. The fungal spores were isolated in the laboratory and also the soil was tests were done for their physiochemical properties, which are shown in the Table 1.

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 *Rhizopus stolonifer* extracts 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*
Rhizopus stolonifer	Acetonitrile	50ul	16.0±1.0	25.0±1.0	19.0±1.0	14.3±0.5	21.0±1.0
	n-hexane	50ul	10.0±1.0	8.8±1.0	7.0±1.0	6.2±0.5	5.3±0.5

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

Table 3. Activity of *Rhizopus stolonifer* extracts against fungal strain.

Fungal specie	Solvent	Concentration used (ul)	Zone of inhibition(mm) Mean ± S.D				
			A. niger	*C. albican*	*A. oryzae*	*P. digitatum*	*F. oxysporum*
Rhizopus stolonifer	Acetonitrile	100ul	20.6±0.5	18.5±0.7	16.8±1.05	21.9±0.5	24.5±0.5
	n-hexane	100ul	5.2±0.7	9.5±0.3	7.3±0.5	11.8±0.5	3.7±0.8

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

Antimicrobial Activity

The result of antimicrobial study revealed that the extracts of *Rhizopus stolonifer* have ability to inhibit the growth of both the fungal and bacterial species. The extracts showed good activity in acetonitrile, while low activity was observed in n-hexane solvent against all the tested pathogens. It confirmed that *Rhizopus stolonifer* produce secondary metabolites and mycotoxin which may be responsible for antimicrobial activity. Minimum inhibition concentration (MIC) values of all the extracts tested against bacterial strains were summarized in table 2, while the antifungal were shown in table 3. Minimum inhibitory concentration (MIC), of the extracts against these bacterial and fungal strains was in the range of 0.25 mg/ml.

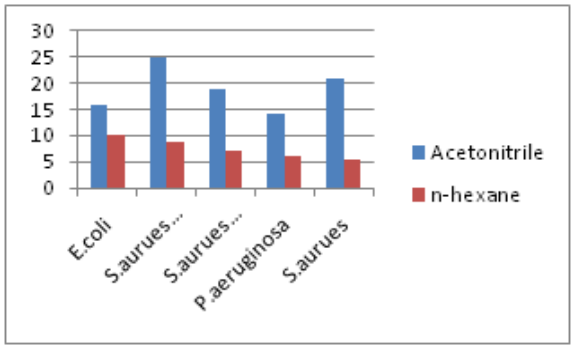

Fig. 1. Extracts Showing Zone of Inhibition against Known Bacterial strains.

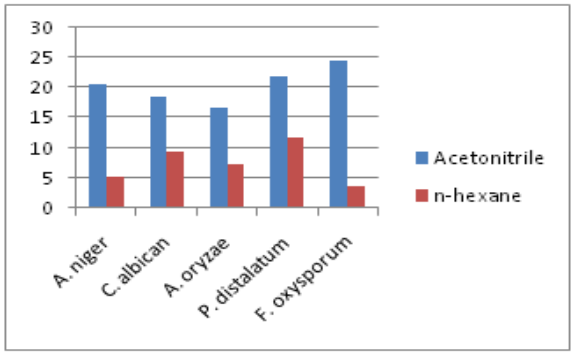

Fig. 2. Extracts Showing Zone of Inhibition against Known Fungal strains.

The extract of *Rhizopus stolonifer* showed good activity in acetonitrile solvent as compared to n-hexane solvent. Demain and Fang (2000) stated that large number of fungal have been found to have antimicrobial activity. Idris *et al.,* (2013) investigated the antimicrobial activity of soil isolated specie *Aspergillus flavus* against *E. coli, Bacillus subtilis, Staphylococcus aureus*. The zone of inhibition was ranged between 14.37 mm. Tawfik *et al.,* (2012) investigated the antibacterial activity of fungal extract of some fungi by disk diffusion method. The extract were found to be effective against both the bacterial species *E.coli* and *S.aureus* with the zone of inhibition ranged from 22-28 mm. Takahashi, *et al., (*2010) isolated total 200 fungal strains from soil samples, collected from Serra do Cipo National Park in Brazil. About 67% of the fungal extract showed antibacterial activity against tested species *S.aurues, E.coli, S.typhi, Streptococcus pyogenes,* and *Listeria monocytogenes*. Sohail *et al.,* (2014) investigated the antimicrobial Activity of fungal specie *Aspergillus flavus* against fungal strains *A. niger, A.oryzae, C.albican, P.digitatum, F.oxysporum* and bacterial strains *P. aeruginosa, E.coli, S.aureus, S.aureus* (Methicillin resistant), *S.aureus* (Vancomycin resistant). My results do similarity with sohail *et al.,* (2014). From the research work it was confirmed that *Rhizopus stolonifer* extracts have potential against both fungal and bacterial species.

Conclusion

From the result of my research work it was concluded that the extracts of *Rhizopus stolonifer* have compound which have ability to inhibit the growth of fungal and bacterial strains. It is recommended that further study should make available to discover new antibiotics and antifungal drugs, also in other aspects.

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