



# Antimicrobial Properties of some Fungi active against Pathogenic Fungi of Banana Fruits

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## Abstract

The present study was aimed to evaluate antimicrobial activity of fungal strains against four bacterial & four fungal pathogens isolated from ripen Banana fruits. The antimicrobial activity of fungal strains against fruit deteriorating pathogens was evaluated by co-inoculation method, followed by confirmatory test by pour plate method. The solvent extraction and evaluation for antimicrobial properties were studied by agar well diffusion method. In the co-inoculation and pour plate method there was a formation of significant zone of inhibition against pathogens. Methanolic and ethanolic extracts of selected fungal strain showed effective antibacterial effect and the methanolic extract of selected fungal strain also demonstrated strong antifungal effect against pathogens. These findings may stimulate the search for new antibacterial and antifungal agents.

**Keywords:** Antibacterial, Antifungal, Bacteria, Plant pathogen, Test organisms.

## Introduction

Fruits play an important role in daily human life by supplying vitamins and essential minerals are the necessary growth factors, which help to maintain a healthy body<sup>1</sup>. These are highly nutritious and economically important but now a day they experience a different marketing problem. Attack of pathogens is one of the limiting factor which influence the economic and nutritional value. Pathogenic bacteria and fungi are generally absent in fresh fruit and processed fruit products because of their high acid content but present in ripen fruits or fruit spoilage. Recent outbreaks of *E. coli* and *Salmonella spp.* in apple and orange juices have challenged the belief that high acid foods cannot harbor viable pathogenic bacteria<sup>2</sup>. During growth, harvesting and processing, microbial infections such as pathogenic bacteria, fungi, protozoa and viruses are introduced through contaminated water and results small amounts of spoilage or illness. Unsanitary field conditions are another factor for spoilage and early ripening due to source of pathogens. During the growth of fruit, fungal spores attached but remain in dormant condition and after ripening begins, the attached spores start to germinate. Fruit pathogenic fungi are basically present in apple, corn, grapes, guava, mango, orange, papaya and pomegranate pawpaw, orange, tomato, pineapple, and watermelon<sup>3,4</sup>. The genus *Colletotrichum* and its teleomorph *Glomerella* are considered to be the major banana fruit pathogens worldwide. They cause significant economic damage to banana fruits in tropical, subtropical, and temperate regions<sup>5</sup>. Other than *Collectotrichum*, *Aspergillus*, *Rhizopus*, *Alternaria*, *Penicillium*, *Fusarium*, *Lasiodiplodia*, *Curvularia*, *Syncephalastrum*, *Trichoderma* are the fruit deteriorating fungi

isolated from spoiled fruit<sup>6,7</sup>. In case of bacteria, several studies have been reported the occurrence of this in spoiled fruits including *Pseudomonas*, *Erwinia*, *Xanthomonas*, *Enterobacter*, *Flavobacterium*, *Chromobacter*, *Lactobacillus*, *Bacillus*, and *Clostridium*. Bacteria like *Escherichia coli*, *Salmonella typhi*, *Shigella Flexneri*, *Staphylococcus aureus*, *Salmonella paratyphi A*, *Salmonella paratyphi B* and *Vibrio cholera* are present in banana which leads to its spoilage<sup>8</sup>.

As the infectious diseases are a global problem due to the spread of pathogens so we a need to search for new antimicrobial agents. In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Several antimicrobial assay like disk diffusion, well diffusion, broth agar diffusion are well known and commonly used in microbial laboratory now adays<sup>9</sup>. The present work was to isolate the fungi and bacteria from ripen fruit like banana and grapes & systemically investigate the antimicrobial potential of the micro-organisms.

## Materials and Methods

**Isolation and Characterization of fungal isolates:** Rippen Fruit sample like banana was collected from the street market and washed with running tap water to remove the debris and air-dried. Fruit samples were cut into small fragments using sterile scalpels before surface sterilization. Sample fragments were successively surface sterilized by immersion in 70% ethanol for 3mins, 2.5% sodium hypochlorite solution for 5

mins and sterile distilled water for 3-4times for 1min each. The surface-sterilized fragments were placed on Sabouraud dextrose agar medium and incubated for 4-5days until the micro-organisms had grown properly<sup>10</sup>. Purification of pathogens was done by streak plate method, so that the microbial cells were well spaced from each other. The process like streak plate and central inoculation were repeated for 3-4times until we got purified pathogens. Gram staining was conducted to study the cellular morphology of isolated bacteria. The smear of bacteria was first heat fixed, then crystal violet added followed by Iodine solution, then washed with Gram's decolorizer and then lastly covered with safranin. All were added for 30 sec. The slides were washed with distilled water and then shown under microscope.

**Screening for antimicrobial activity:** Total 29 test organisms were tested for their antimicrobial activity against four pathogenic fungi and four pathogenic bacteria isolated from ripen fruits like banana by inoculating them through co-inoculation method on Sabouraud dextrose agar media and kept incubation at 30°C for 5-7 days<sup>11</sup>. In the first phase of confirmatory test, pathogens were mixed with Sabouraud dextrose media and then plated. After media solidification inoculation of test organisms was done. Similarly in second phase test, test organisms were added to Sabouraud Dextrose agar media and plated, after media solidification, those pathogens were centrally inoculated and then plates were incubated for 10 days. This test is repeated to find the best test organism for antimicrobial activity.

**Extraction and Evaluation for antimicrobial activity:** Two pieces of growing mycelial disc of selected fungi/test organism were inoculated into Sabouraud dextrose broth & incubated for 10 days. After the incubation period, filtration was occurred to separate the culture filtrate and mycelial mat through Whatmann no. 1 filter paper<sup>12</sup>. The culture filtrate was concentrated by Soxhlet apparatus and ethyl acetate was added to the concentrated filtrate for 72 hours. The upper layer was separated and the lower layer was evaporated by Soxhlet. Evaporated samples were dissolved in Ethanol and DMSO (Dimethyl sulfoxide) respectively. Both the samples were passed through two different columns. Then partially purified samples were obtained through Column chromatography. Collected fractions were pooled and again passed through two separate columns by using solvents according to the polarity<sup>13,14</sup>. Both antibacterial and antifungal activity was performed by agar cup diffusion method against 3numbers of bacterial pathogens and 4numbers of fungal pathogens. Nutrient agar plates were prepared by inoculating bacterial pathogens through pour plate technique. After solidification, agar cups were prepared by scooping out the media with cork borer (10mm in diameter). The cups were then filled with 500µL of different extracts and incubated at 37°C for 48 hours for bacteria pathogens. Similarly, Sabouraud dextrose agar plates were prepared by adding 500µL of different extracts in the molten agar medium and poured into plates by pour plate technique.

Then fungal pathogens were inoculated by cutting 10mm disc by cork borer centrally in the medium and incubated at 30°C for 72 hours. The zone of inhibition was observed and photographed<sup>15</sup>.

## Results and Discussion

**Isolation and Characterization of fungal isolates:** A total of 4 bacterial pathogens named as B1, B2, B3 & B4 and 4 fungal pathogens named as *Colletotrichum sp.*, *Penicillium sp.*, *Aspergillus sp.*, *Fusarium sp.* was purified from ripen fruit sample. According to classical bacteriology, most species of bacterial isolate can be differentiating based on simple Gram staining technique. Out of 4 bacteria, B2 was identified as gram negative, rest were gram positive.

**Screening for selection of organisms for antimicrobial activity:** In the screening part, Out of 29 fungi, 10 nos. of test organisms showed positive result against 4 bacterial pathogens and 11 nos. of test organisms showed positive result against 4 fungal pathogens respectively. All 29 fungi were different and morphologically identified in genus level and these are mostly *Aspergillus sp.*, *Penicillium sp.*, *Fusarium sp.*, *Trichoderma sp.* described in Table-1, Co-inoculation plate assay for screening of fungi for antibacterial activity described in Table-2 and Co-inoculation plate assay for screening of fungi for antifungal activity described in Table-3.

**Confirmatory test for antimicrobial activity:** For bacterial pathogens, 2 bacterial pathogens named as B3 and B4 showed antimicrobial activity against 4 different test organisms (T3, T4, T9 and T13). For Fungal pathogens, *Penicillium sp.* and *Aspergillus sp.* showed positive result against two test organisms named as T28 and T1, described in Table-4 and Table-5.

**Antimicrobial Activity of selected fungi extracts:** According to the polarity, ethanol, methanol, acetic acid and water extracts of the test organism *Aspergillus sp.* were collected and used for antibacterial tests. Highest zone of inhibition was shown in ethanolic extracts (passed through DMSO column) of the test organism as compared to positive control and highest zone was shown in ethanolic and methanolic extracts (passed through Ethanol column) as compared to positive control against bacterial pathogen B2 in comparison to other extracts. In case of B3, methanolic extract (passed through DMSO column) and ethanolic extracts (passed through Ethanol column) showed highest zone of inhibition and exhibiting antibacterial activity. Ethanolic and methanolic extracts (passed through DMSO column) showed positive result against B4 as compared to other extracts which shown in Table-6.

According to the polarity, ethanol, methanol, acetic acid and water extracts of the test organism *Trichoderma sp.* were collected and used for antifungal tests. In case of *Colletotrichum sp.*, highest mycelia growth of inhibition exhibited in methanolic extracts (passed through Ethanol column) as compared to positive

control and antifungal activity was shown. In case of *Penicillium sp.*, methanolic extracts (passed through DMSO column) and (passed through Ethanol column) showed better antifungal activity by inhibiting the growth of pathogenic fungi as compared to positive control. Again methanolic extracts (passed through

DMSO column) and ethanolic extracts (passed through Ethanol column) showed positive result in case of *Aspergillus sp.* as compared to other extracts. Negative results were found in case of *Fusarium sp.* which shown in Table-7.

**Table-1:** Name of 29 fungal strains.

Sl no	Genus name
1	<i>Aspergillus sp.</i>
2	<i>Penicillium sp.</i>
3	<i>Fusarium sp.</i>
4	<i>Trichoderma sp.</i>
5	<i>Aspergillus sp.</i>
6	<i>Trichoderma sp.</i>
7	<i>Penicillium sp.</i>
8	<i>Aspergillus sp.</i>
9	<i>Trichoderma sp.</i>
10	<i>Aspergillus sp.</i>
11	<i>Aspergillus sp.</i>
12	<i>Aspergillus sp.</i>
13	<i>Aspergillus sp.</i>
14	<i>Aspergillus sp.</i>
15	<i>Trichoderma sp.</i>
16	<i>Fusarium sp.</i>
17	<i>Fusarium sp.</i>
18	<i>Aspergillus sp.</i>
19	<i>Penicillium sp.</i>
20	<i>Trichoderma sp.</i>
21	<i>Fusarium sp.</i>
22	<i>Aspergillus sp.</i>
23	<i>Aspergillus sp.</i>
24	<i>Penicilium sp.</i>
25	<i>Trichoderma sp.</i>
26	<i>Fusarium sp.</i>
27	<i>Aspergillus sp.</i>
28	<i>Trichoderma sp.</i>
29	<i>Fusarium sp.</i>

**Table-2:** Co-inoculation plate assay for screening of fungi for antibacterial activity.

Test Organisms	Pathogens			
	B1 (Gram +ve)	B2 (Gram -ve)	B3 (Gram +ve)	B4 (Gram +ve)
<i>Aspergillus sp.</i>	-	-	-	-
<i>Penicillium sp.</i>	-	-	+	+
<i>Fusarium sp.</i>	-	-	+	+
<i>Trichoderma sp.</i>	+	-	+	+
<i>Aspergillus sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	+	+
<i>Penicillium sp.</i>	-	-	+	+
<i>Aspergillus sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	+	+
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	+	+	-	-
<i>Aspergillus sp.</i>	-	-	+	+
<i>Trichoderma sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Penicillium sp.</i>	-	-	+	+
<i>Trichoderma sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	+	+
<i>Penicilium sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	-

+ : results indicates the formation of zone against pathogens, - : results indicates no formation of zone against pathogens.

**Table-3:** Co-inoculation plate assay for screening of fungi for antifungal activity.

Test Organisms	Pathogens			
	<i>Colletotrichum sp</i>	<i>Penicillium sp.</i>	<i>Aspergillus sp.</i>	<i>Fusarium sp.</i>
<i>Aspergillus sp.</i>	-	-	-	+
<i>Penicillium sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	+	-	-
<i>Trichoderma sp.</i>	+	-	-	+
<i>Aspergillus sp.</i>	-	-	-	+
<i>Trichoderma sp.</i>	-	-	-	-
<i>Penicillium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	-	+
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	+	-
<i>Trichoderma sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	+
<i>Fusarium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Penicillium sp.</i>	-	-	+	-
<i>Trichoderma sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	+	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Penicillium sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	+	-	-	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	+	-	-	+
<i>Fusarium sp.</i>	-	-	-	-

+: results indicates the formation of zone against pathogens, -: results indicates no formation of zone against pathogens.

**Table-4:** Pour plate quantitative assay for antibacterial properties of selected fungi.

Test Organisms	B1 (Gram +ve)	B2 (Gram -ve)	B3 (Gram +ve)	B4 (Gram +ve)
<i>Penicilium sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	+	+
<i>Trichoderma sp.</i>	-	-	-	+
<i>Trichoderma sp.</i>	-	-	-	+
<i>Aspergillus sp.</i>	-	-	-	+
<i>Aspergillus sp.</i>	-	-	-	-

+: results indicates the formation of zone against pathogens, -: results indicates no formation of zone against pathogens.

**Table-5:** Pour plate quantitative assay for antifungal properties of selected fungi.

Test Organisms	<i>Colletotrichum sp.</i>	<i>Penicillium sp.</i>	<i>Aspergillus sp.</i>	<i>Fusarium sp.</i>
<i>Aspergillus sp.</i>	-	-	+	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Fusarium sp.</i>	-	-	-	-
<i>Penicilium sp.</i>	-	-	-	-
<i>Aspergillus sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	-	-	-
<i>Trichoderma sp.</i>	-	+	+	-

+: results indicates the formation of zone against pathogens. -: results indicates no formation of zone against pathogens.

**Table-6:** Antibacterial Activity of solvent extracts of selected fungal strain.

<i>Aspergillus sp.</i>	B2 (Gram -ve)				B3 (Gram +ve)				B4 (Gram +ve)			
	W	A	M	E	W	A	M	E	W	A	M	E
DMSO	-	-	-	+	-	-	+	-	-	-	+	+
Ethanol	-	-	+	+	-	-	-	+	-	-	-	-

W-Water, A-Acetic acid, M-Methanol and E-Ethanol.

**Table-7:** Antifungal Activity of solvent extracts of selected fungal strain

Trichoderma sp.	Colletotrichum sp.				Penicillium sp.				Aspergillus sp.				Fusarium sp.			
	W	A	M	E	W	A	M	E	W	A	M	E	W	A	M	E
DMSO	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
Ethanol	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-

W-Water, A-Acetic acid, M-Methanol and E-Ethanol.

## Conclusion

Collectively, based on our findings, we conclude that methanolic and ethanolic extracts of selected fungi showed effective antibacterial effect and the methanolic extract of selected fungi also demonstrated strong antifungal effect against *Colletotrichum sp.*, *Penicillium sp.*, *Aspergillus sp.* These findings may stimulate the novelty for new antibacterial and antifungal agents and also could be promising and an unaffected asset of therapeutic compounds.

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