



Antimicrobial Activity of *Phoma* Species against Pathogenic Bacteria

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Abstract: This study aim to investigate the antibacterial activity of the endophytic fungus. It deals with an antimicrobial activity of fungi *Phoma* against human pathogenic bacteria. Species of *Phoma* i.e. *Phoma sorghina*, *Phoma exigua*, *Phoma herbarum* and *Phoma fimeti* produces pigments in culture. The pigments were extracted and the antimicrobial activity was evaluated against pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus vulgaris*. Evaluation of antimicrobial activity was studied using the agar diffusion method and liquid fermentation process. The activity test from the extract was carried out using a disk diffusion method with the endophytic extract containing the antibacterial activity. Four tests were performed and minimum and maximum inhibitory concentration was determined)

Index Terms: Antimicrobial activity, Endophytic fungi, Pathogenic bacteria, *Phoma* i.e., *Phoma exigua*, *Phoma fimeti*, *Phoma herbarum* and *Phoma sorghina*

I. INTRODUCTION

Rabenhorst (1878) was the first mycologist who initiated the work on *Phoma* in India by reporting *P. conscorae* and *P. desmonchi*. Shortly afterward (1880), Theumen described *P. keckii* (theum) Sacc. and Sydow and Butler (1916), gave a significant contribution and reported species of *Phoma* from different hosts. In the first half of nineteenth century, a little contribution was made on Indian *Phoma*.

The genus *Phoma* fungus is distributed world widely. It is distributed over a large area of soil and found in plants and plant materials. In plants, *Phoma* species are common pathogens i.e. disease causing agent. The fungi can cause a condition known as *Phoma* blight, which can cause withering and fading of the leaves of the plant and this blight will kill the plant as well as spread to other nearby plants and trees. They are also common plant pathogens causing disease in crucifers, celery, beets, tomatoes and peppers.

Phoma species are rarely cause infections in humans particularly to individuals with weak immune system.

Sometimes *Phoma* species may infect humans and causing cutaneous or subcutaneous infections.

II. MACROSCOPIC CHARACTERISTICS OF PHOMA

Colonies of *Phoma* grow rapidly on the flat surface of the medium. Their texture is powdery to velvety and often largely submerged in the medium. From dorsal side of petriplate, the color is initially white and later becomes olive-grey with an occasional tint of pink. From the reverse, it is dark-brown to black. Some species (particularly, *Phoma cruris-hominis* and *Phoma herbarum*) produce reddish-purple to yellowish-brown diffusible pigment, which is readily visible from the reverse.

III. MICROSCOPIC CHARACTERISTICS OF PHOMA

The genus *Phoma* is a fungi which do not reproduce sexually and therefore have no genetic exchange. *Phoma* produce their pycnidiospores in enclosed structures called pycnidia. Pycnidia generally have an apical ostiole (opening) through which the pycnidiospores escape. The pycnidia in *Phoma* are rounded and are brown to black in color. The pycnidiospores are unicellular, hyaline and ellipsoidal to cylindrical. Each conidium typically has two oil droplets inside which are called as guttules. Some *Phoma* produce brown chlamydospores that are arranged singly or in chains. These chlamydospores may be unicellular or multicellular and "alternarioid" in appearance.

Although over 2000 *Phoma* species have been reported throughout the world, many of these have been characterized by host substrate alone. The cultural and morphological studies of Indian species of *Phoma* were made and on the basis of these studies, the following broad morphological groups were formed. These groups include *P. medicaginis*, *P. medicaginis* var. *medicaginis*, *P. pomorum*, *P. herbarum*, *P. exigua* var. *exigua*, *P. tropica*, *P. glomerata*, *P. sorghina*, *P. multirostrata*, *P. capitulum*, *P. betae*, *P. jolyana*, *P. fimeti*, *P. chrysanthemicola*, *P. complanata*, *P. destructiva eupyrena*, and *P. arachidicola*.

Phoma causes various serious diseases to plants as well as to humans. Besides these harmful aspects, certain *Phoma* species also contain antibiotic potential and economically useful. There are important antibiotics (Table I.) produced by some species of *Phoma*.

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B. Endophytic Extracts

Phoma cultures preserved at 4°C on PDA slants were carefully inoculated on PD broth for 7 days at 23°C. It was grown in 250 ml flasks. Twelve flasks were taken for experiments and incubated at 23°C in dark condition.

Table I. Antibiotic production by species of *Phoma*

<i>Phoma</i> sp.	Active chemical/ product	Reference
<i>Phoma exigua</i> var. <i>exigua</i>	Antibiotic E (antibacterial and antifungal) Cytochalasin B	Boerema & Howeler (1967)
<i>Phoma pigmentivora</i>	LL-D253alpha	McIntyre et al. (1984)
<i>Phoma lingam</i> (Tode) Desm.	Phomenoic acid and phomenolactone (antifungal and antibacterial)	Topgi et al. (1987)
<i>P.exigua</i> var. <i>heteromorpha</i>	Cytochalasin F	Capasso et al. (1991a) (1991b)
<i>Phoma</i> sp. (Marine)	Phomactin A	Sugano et al. (1991)
<i>Phoma</i> sp.	Squalestatin 1, 2 (S1, S2)	Baxter et al. (1992)
<i>Phoma</i> sp.	Squalestatin (antiinfective agents)	Dawson et al. (1992)
<i>Phoma</i> sp.	Antitumour (Fusidienol A)	Singh et al. (1997)
<i>Phoma</i> sp.	Antibiotic activities	Sponga et al. (1999)
<i>Phoma</i> sp. (NRRL 25697)	Phomadecalins A-D and Phomapentenone A	Yongsheng et al. (2002)
<i>Phoma</i> sp.(Q60596)	YM-202204, antifungal	Nagai et al. (2002)
<i>Phoma</i> sp.	FOM-8108, inhibitors of neutral sphingomyelinase	Yamaguchi et al. (2002)
<i>Phoma</i> sp. TC 1674.	TMC-264	Sakurai et al. (2003)
<i>Phoma medicaginis</i>	Brefeldin A	Weber et al. (2004)
<i>Phoma</i> sp. QN04621	YM-215343, antifungal compound	Shibazaki et al. (2004)
<i>Phoma</i> sp. FKI-1840	Spylidone	Koyama et al. (2005)

IV. MATERIALS AND METHODS

A. Endophytic fungi: Isolation and Identification

In the present study, the four *Phoma* species were selected. These include *P. sorghina*, *P. exigua*, *P. herbarum* (MTCC 2319) and *P. fimeiti* (MTCC 2323). Out of these *P. exigua* was isolated from soil and leaf litter while *P. sorghina* from leaf of *Carica papaya*.

Isolations of *P. sorghina* and *P. exigua* were made by cutting the infected portions from the junctions of healthy and diseased region surface sterilizing with 70% ethanol and putting it into petriplate containing sterilized PDA (Potato Dextrose Agar) and malt agar. Isolations from soil were performed by serial dilution and Warcup (1950) method. Remaining two species i.e. *P. herbarum* and *P. fimeiti* were obtained from MTCC (Microbial Type Culture Collection Centre and Gene Bank,

After 7 days a well grown mycelia mat on PD broth in 250 ml flasks were observed. The extract was then harvested through normal filter assembly and washed with water 3 to 4 times using Buchner filtration equipment. Extract of *Phoma* were extracted from mycelium of *Phoma* with ethanol solvent using Soxhlet extractor and the temperature at 55°C. This extract was used for the purpose of antimicrobial activity of pathogenic bacteria.

V. ANTIMICROBIAL ACTIVITY

Four sterile nutrient agar plates were prepared. In each plate 10-15 ml of nutrient agar media was poured. One hundred µl (100 µl) sample of pathogenic bacteria i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus vulgaris* were poured and spread in agar plate 1,2,3 and 4 respectively. These plates were incubated at 37°C for 72 hours. Sterile discs (5 mm diameter of Whatman filter paper No.42) were soaked in extracts of *P. sorghina*, *P. exigua*, *P. herbarum*

and *P. fimeti* up to saturation. These saturated discs were placed on the center of the petridishes containing nutrient agar and incubated at 37°C for 72 hours. For each extract, triplicates were maintained. The zones were observed and result was recorded.

VI. RESULT AND DISCUSSION

Evaluation of extracts of four *Phoma* species, viz., *Phoma sorghina*, *Phoma exigua*, *Phoma herbarum* and *Phoma fimeti* was carried out against four pathogenic bacteria including *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgaris*. At 100% concentration, extracts of species of *Phoma* showed significant antibacterial activity against *P. aeruginosa*, *B. subtilis*, *S. aureus*, *P. vulgaris* (Table II.)

Table II. Antimicrobial activity of different species of *Phoma* by disc diffusion technique

Test Microorganisms	Inhibition Zones [mm] Endophytic Fungi				
	<i>Phoma sorghina</i> 100 %	<i>Phoma exigua</i> 100 %	<i>Phoma herbarum</i> 100 %	<i>Phoma fimeti</i> 100 %	Control 100 %
<i>Pseudomonas aeruginosa</i>	11 (± 0.86)	16 (± 0.47)	12 (± 1.28)	14 (± 1.34)	18 (± 1)
<i>Bacillus subtilis</i>	12 (± 1.28)	13 (± 1.25)	11 (± 0.86)	12 (± 1.28)	17 (± 1.52)
<i>Staphylococcus aureus</i>	8 (± 0.5)	14 (± 0.76)	15 (± 2.08)	10 (± 0.29)	16 (± 0.47)
<i>Proteus vulgaris</i>	14 (± 0.76)	10 (± 0.29)	8 (± 0.5)	11 (± 0.86)	15 (± 2.08)

NOTE: a Control – Ampicillin concentration (100%) used for bacteria
The data in parenthesis are Standard Deviation

Different species of *Phoma* exhibited different inhibitory effect on the test bacteria. *Phoma sorghina* showed the maximum inhibition of *Proteus vulgaris* and the minimum inhibitory effect was recorded against *Staphylococcus aureus*. *Phoma exigua* demonstrated a fairly high antibacterial effect against *Pseudomonas aeruginosa* and the minimum against *Proteus vulgaris*.

The extract of *Phoma herbarum* exhibited considerably high activity against *Staphylococcus aureus* and the minimum against *Proteus vulgaris*. The maximum efficacy of *Phoma fimeti* was found against *Pseudomonas aeruginosa* and the minimum against *Staphylococcus aureus*.

Finally it can be concluded that the extract of *Phoma* species showed remarkable antibacterial activity and hence can be used

as antibacterial agents after more studies in experimental animals.

CONCLUSION

This study concluded that *Phoma* species have antibacterial activity and it has the most potential bactericidal properties. New therapeutic agents can be developed by using *Phoma* species which have antibacterial supplement. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of *Phoma* extract as an antibacterial agent in topical or oral applications.

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