IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2023 Vol.:28, Issue:3

Novel Bioactive Metabolites of Macro Fungus *Phomopsis* against Multidrug-Resistant Bacterial Pathogens and Human Cancer Cell Lines

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INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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ISSN 2349-7203
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Submitted: 18 September 2023
Accepted: 23 September 2023
Published: 30 October 2023

Keywords: Phomopsis sps., Drug-resistant bacteria, Human cancer cell lines.

ABSTRACT

Microorganisms are a reservoir of novel secondary metabolites. More number of metabolites produced fungal endophytes compare to other endophytic microorganism class. In the present study, the fruiting body extracts of *Phomopsis* sp were obtained using methanol, diethyl ether and ethanol. Among them, the diethyl ether extract highly inhibit the bacterial and fungal pathogens growth. Based on the effective antimicrobial activity, the crude acetate extract was partially purified by column chromatography. All the fractions were tested for antibacterial activity against drug-resistant *Staphylococcus aureus* strains and *Pseudomonas aeruginosa* strains. The extract showed a maximum inhibition zone of 26.5 mm against drug resistant *S. aureus* and 30.3 mm against drug resistant *P. aeruginosa*. Minimal inhibitory concentration of extract was found to be 50µg/ml against *S. aureus* and *P. aeruginosa*. Further, the extract had significant spectral data of the extract exhibited C=O, C-C=C asymmetric, N-H and C-O stretches in frequencies. Thus, the results indicated that the metabolites of *Phomopsis* sp are the potential active principles source for the construction of new antibacterial and anticancer drugs.

INTRODUCTION

Endophytes, which occupy a unique biotope with global estimation up to one million species, are a great choice to avoid replication in the study of natural products to assist in solving not only plant diseases but also human and animal health problems. Endophytes are chemical synthesizers inside plants, in other words, they play a role as a selection system for microbes to produce pharmacologically active substances with low toxicity maximum number of natural drugs are produced by endophytes (Kaulet al., 2012). There are many pieces of evidence that bioactive compounds produced by endophytes could be alternative approaches for the discovery of novel drugs. Stierle *et al.* (1993) reported endophytic fungus as a sustainable alternative source of taxol. Natural products are adapted to a specific function in nature. Thus, the search for novel secondary metabolites should concentrate on organisms that inhabit novel biotypes. Endophytic fungi inhabit a biotype that is not well studied (Nithya and Muthumary, 2011). Endophytic microorganisms are a significant reservoir of novel bioactive secondary metabolites including antimicrobial, antiinsect, anticancer, antidiabetic, and immunosuppressant compounds with their great potential applications in agriculture, medicine, and food industry (Kharwar *et al.*, 2011). These bioactive compounds could be mainly classified as alkaloids, terpenoids, steroids, quinones, isocoumarins, lignans, phenylpropanoids, phenols, and lactones. World health problems caused by drug-resistant bacteria and fungi are increasing. Approximately 4,000 secondary metabolites of fungal origin have been described to possess biological activities. The number of secondary metabolites produced by fungal endophytes is larger than that of any other endophytic microorganisms (Zhang ***et al***., 2011). Most of the natural products from endophytic fungi are antimicrobial, anticancer agents biological control agents, immunosuppressive agents, and other bioactive compounds by their different functional roles.

MATERIAL AND METHODS

Collection, identification and processing of fruiting bodies

The endophytic fungi *phomopsis* sps isolated from the soil of tropical evergreen forest of Courtallum hills, Western Ghats, Tamilnadu, India. The fungi were grown in PDA medium. The fungus was identified as *Phomopsis* sps based on the morphological characteristics and confirmed by 18S ribosomal RNA gene sequence (Altschul *et al*. 1990). The 18S rRNA gene sequencing of the fungus was done at Synergy Scientific Services, Chennai, India. Sequence

comparisons were made using BLAST searches through the NCBI. The isolates were used to preparation of extracts with organic solvents.

Preparation of extracts

For extraction, 200 ml of organic solvents such as methanol, diethyl ether and ethanol were taken into a conical flask containing 20 ml of fungal extracts. This was allowed to stand for 24 h with intermittent agitation (150 rpm) and filtered through Whatman No. 1 filter paper. The residue was then extracted with two additional treatments with 200 mL of solvent as described above. The extracts were pooled and concentrated at 30^{o}C and the solvent was reduced pressure at 35°C using a rotary vacuum evaporator. The fungal extract was dissolved in 10% dimethyl sulfoxide(DMSO) and stored at 4°C for antimicrobial activity.

Partial purification by column chromatography

The crude fungal metabolites were mixed with silica gel to prepare the metabolite-silica gel slurry and air-dried. A glass column was packed with silica gel up to 25 cm height and washed with 200 ml of methanol. Then, the metabolite-silica gel slurry was loaded onto the column and eluted initially with methanol followed by different ratios of diethyl ether mixture by increasing polarity [9:1 to 1:9 (v⁄v)] followed by the ethanol and methanol [9:1 to 1:9 (v⁄v)]. About 100 ml of each solvent system was used for elution and fractions of 5 ml each were collected. The presence of compounds was analyzed by thin-layer chromatography (TLC) using pre-coated silica gel plate with methanol and diethyl ether at as solvent system. Fractions showing similar spots on TLC were pooled and concentrated. The partially purified metabolites were further fractionated using 230-400 mesh silica-gel column chromatography. Fractions showing a similar single spot on TLC were pooled together and concentrated. Totally six fractions were collected and each fraction was tested for preliminary *in-vitro* antimicrobial activity to select the active fractions. Further, the active fraction was tested for antibacterial activity against multidrug-resistant bacterial strains and for anticancer activity against human cancer cell lines.

Test microorganisms

Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and *Candida albicans* were purchased from IMTECH, Chandigarh, India. The clinical strains of

S. aureus and *P. aeruginosa* were obtained from Alex Clinical Laboratory (Sivakasi, Tamilnadu, India). All the isolates were maintained on nutrient agar slants at 4°C.

Antibiotic susceptibility test

The drug-resistant bacterial strains were confirmed for their identification based on gram's stain, catalase test, mannitol fermentation test and coagulase test. Antibiotic susceptibility was detected by disk diffusion technique using methicillin (5 µg/disk), penicillin (10 units/disk) and vancomycin (30 µg/disk) for clinical strains of *S. aureus.* Ciprofloxacin (5 µg/disk), cefotaxime (30 µg/disk), ofloxacin (5 µg/disk) and amikacin (30 µg/disk) were used for clinical strains of *P. aeruginosa*(Chandrasekaran*etal*.2009).Inoculated plates were inverted and incubated at 37°C for 18 h. After the incubation period, the diameter of the zone of inhibition was measured and results were interpreted according to the standards of the Clinical and Laboratory Standards Institute (NCCLS 2008).

Antimicrobial activity

For the preliminary screening, the disk diffusion method was used to determine the antimicrobial activity of the ethyl acetate extract of fruiting bodies of *Phomopsis* sps. (Acarand Goldstein 1996). Sterile disks (6 mm) were impregnated with 10 µl of extract at a concentration of 1 mg/ml. The media used for the antimicrobial susceptibility testing of bacteria and fungi were muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. Tetracyclinedisk10 mgfor gram-positive bacteria, chloramphenicol disk (10 mg)for gram-negative bacteria, and nystatin disk (10 units) for fungi were used as controls. The disks impregnated with 10% DMSO were also used to prove the absence of antimicrobial activity of DMSO. The plates were incubated at 37°C for 18 h and 48 h for bacteria and fungi, respectively. The diameter of the inhibition zone around each disk was measured at the end of the incubation period. Experiments were performed in triplicate and the antimicrobial activity was expressed as the average of inhibition zone diameters (in mm) produced by the extract.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of ethyl acetate extracts of culture filtrate and the fruiting bodies of the fungus was determined based on a broth microdilution method in a 96-well microplate (Al-bayati2008). Briefly, *S. aureus* strains (1–10) and *P. aeruginosa* strains (1–8) were cultured overnight at

37°C on Mueller hinton broth and adjusted to a final density of 10^8 CFU/ml by 0.5 McFarland standards. In the 96-well plate, each well had 90 µl of MH broth supplemented with 2% NaCl, 10 µL of bacterial inoculums, and 10 µl of different concentrations of fruiting body. The plate was incubated at 37°C for 18 h. After incubation, the bacterial growth was visually inspected, and the lowest concentration of fungal extract at which no observable bacterial growth or turbidity was taken as the MIC value. Each experiment was carried out in triplicate.

Determination of minimum bactericidal concentration (MBC)

The minimum inhibitory concentration of fraction E was determined based on the broth dilution method (Pattnaik et al. 1997). The fraction E was diluted with 10% DMSO to give concentration of 50-500 µg/ml. 20 µl (fresh culture) of the test organism was inoculated into 1 ml of culture medium containing various concentration of the fraction E. The tubes were incubated at 37°C for 18-24 h and the lowest concentration of inhibiting bacterial growth was considered as MIC.

Test for bactericidal activity

In order to evaluate the bactericidal activity of the fraction E, one loop from the MIC tube was subcultured on to the Muller hinton agar plates which were then incubated at 37°C overnight to check whether the fraction E merely had bactericidal activity.

Morphological observation

A-549 cells (35000 cells/wel) were grown in 24-well plates and treated with fraction Eat the concentration of 40 µg/ml and 60 µg/ml. Morphological changes of cells in both of the treated group and control group (ethyl acetate treated-cells) were analyzed at 8 h and 24 h under the OLYMPUS 1×71 Inverted Fluorescence microscope at 10x magnification.

RESULTS AND DISCUSSION

***Morphological description and identification of the Phomopsis* sps**

The fruiting bodies were 3-9 cm in length, 0.5-1.5 cm in broad, growing either singly or in groups which were emerging from partially decomposing wood with soil, black at the base. The fruiting bodies were arisen during spring season and found in decaying wood with soil.

Similarly, many species of *Phomopsis* were actively growing in decaying wood of angiosperms and are known to be saprobic (Rogers 1979).On the other hand, the morphological characters of this fungal isolate were identical with *X. angulosa* (AB274814) growing in soil (Rogers et al. 1987). Morphological characteristics allowed the identification of the macro fungal isolate as *Phomopsis* sps. which was reinforced by the sequence of their rRNA gene that gives a high sequence similarity to those accessible at the BLASTN of species of *Phomopsis*. Gene sequences were deposited into GenBank with Accession No. KC405623.

Antimicrobial activity of the extract of fruiting bodies

In the present investigation, hexane, ethyl acetate and methanol extracts of fruiting bodies of *Phomopsis* sp. were screened for antimicrobial activity against bacterial and fungal pathogens. The results indicated that the ethyl acetate extract showed an effective antimicrobial activity against the growth of bacterial and fungal pathogens compared to hexane and methanol extracts. The diethyl ether extract produced a maximum inhibition zone of 31.7 mm against *S. aureus* and minimum inhibition zone of 21.3 mm against *C. albicans* (Table 1). This result is similar to the findings of Ramesh *et al*. (2012) who reported that the ethyl acetate extract of *Phomopsis* inhibited the growth of *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*. Similarly, essential oil isolated from *Ganoderma japonicum* exhibited potent antibacterial activity against 18 pathogenic microorganisms including bacterial and fungal species (Liu *et al*. 2009).

Further, the diethyl ether extract was partially purified by column chromatography and the fractions were analyzed for antibacterial activity against multidrug resistant bacterial strains. Among the drug resistant bacteria, methicillin resistant *S. aureus* (MRSA) gained much attention in the last decades. MRSA is resistant to a large group of antibiotics called the β-lactams, including aminoglycosides, the third and fourth generation cephalosporins, and monolactams. Hence, the emergence of the MRSA strain possesses a substantial threat to public health. The clinical strains of *Staphylococcus aureus* (1-10) were found to be positive for various biochemical tests likes coagulase test, mannitol utilization test, DNase test and catalase activity and the strains of *P. aeruginosa* (1-8) were identified based on colony morphology, growth on CFC agar, a positive oxidase test, and growth at 42^{o}C. The antibiotic resistant profiles of *S. aureus* and *P. aeruginosa* strains were reported earlier (Ramesh *etal*.2012). The active fraction E produced maximum inhibition zone of 27.9 mm and 27.3

mm against *S. aureus* strains 3 and 5 respectively (Table 2). Similarly, Healy *et al*. (2004) reported that the xanthones from the microfungus *Phomopsis* had an effective antibacterial activity against *Escherichia coli*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. A maximum inhibition zone of 29.4 mm and minimum inhibition zone of 19.7 mm were observed against *P. aeruginosa* strain 4 and 2, respectively (Table 3). Recently, Hacioglu et al.(2011**)** reported that the ethanol extracts of *X. polymorpha* exhibited maximum inhibition zone of 13.8 mm and 12.6 mm against *S. aureus* and *P. aeruginosa*, respectively.

The MIC of the fraction E was found to be 75 µg/ml against *S. aureus* strain 1 & 3 and *P. aeruginosa* strain 3 (Fig. 1) and MBC value ranged between 0.2-0.6µgmL^{-1} against all clinical strains of *S. aureus* and *P. aeruginosa* (Fig. 2). Similarly, Zhang et al. (2009) reported that the MIC values of pleosporane isolated from the *pleosporalean* ascomycete ranged from 1-64 µg/ml against *S. aureus.* Recently, Schuffler et al. (2011) reported that the chryoxanthane isolated from the mycelia of ascomycete fungi of IBWF11-95A had MIC value of 2.5-20µg/ml against different types of bacterial pathogens.

Morphological analysis

The above cytotoxicity results revealed that the partially purified fraction E of *Phomopsis* sp. had an effective anticancer activity against human lung cancer cells (A549). Hence, the fraction E was used to study the morphological changes of cancer cells during treatment. The morphological changes of the A549 human lung carcinoma cells were observed by treating the cells with 40 µg and 60 µg of fraction E at 8h and 24 h incubation. The growth of the A549 cells was markedly inhibited by fraction E at 40 µg after 8 h treatment. At 60 µg, most of the cancer cells growth was arrested during cell division and the cell nuclei became condensed and segmented after 24 h treatment which was an indication of apoptosis (Fig. 4).

Preliminary nature of the active components

The physical appearance of the partially purified compound was a semi-solid yellow color, soluble in diethyl ether, methanol, dimethyl sulfoxide & water, and insoluble in ethanol. The partially purified compound isolated from *Phomopsis* sp. exhibited λ_{max} at 246 and 396 nm (Fig. 5). The absorption peak at 246 nm due to n - σ* transition indicates the presence of saturated compounds containing atoms with lone pairs (non-bonding electrons) such as alcohols, amines, halides, ethers etc. The high absorption peak at 396 nm indicates the

presence of chromophoric group due to the n - π* transition. Further the IR spectrum analysis revealed that the peak at 3438 cm^{-1} indicates the presence of hydrogen bonding (OH or NH). The band around 2935 cm^{-1} is due to H-C-H asymmetric and symmetric stretches. The broad peak at 1699 cm^{-1} is due to C=O stretching and the peaks at 1454, 1390-1228 & 1053indicateC-C=C asymmetric, N-H and C-O stretches (Fig. 6).

The present study revealed that the fruiting body extract of *Phomopsis* sp. had an effective broad spectrum antibacterial and cytotoxic activity that could be used as pharmacological substances having diverse therapeutic applications.

REFERENCES

1. Abate D, Abraham WR, Meyer H. Cytochalasins and phytotoxins from the fungus *Phomopsis obovata*. *Phytochem*1997; 44: 1443-1448.
2. Acar JF, Goldstein FW. 1996. Antibiotics in laboratory medicine.In: Lorian V, editor. Disk susceptibility test, 4th ed. Baltimore(MD): Williams and Wilkins, PA; p. 1-51.
3. Al-bayati FA. Synergistic antibacterial activity between *Thymusvulgaris* and *Pimpinellaanisum* essential oils and methanol extracts. *JEthnopharmacol* 2008; 116: 403-406.
4. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool.*JMolBiol* 1990; 215: 403-410.
5. Benedict RG, Brady LR. Antimicrobial activity of mushroom metabolites.*J Pharm Sci*1972; 61: 1820-1822.
6. Boonphong S, Kittakoop P, Isaka M, Pittayakhajonwut D, Tanticharoen M, Hebtaranonth Y. Multiplolides A and B, new antifungal 10-membered lactones from *Phomopsis multiplex*. *JNatProd* 2001; 64:965-967.
7. Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anticancer agents.*Chem Rev* 2009; 109: 3012-43.
8. Cragg GM, Newman DJ. Nature: a vital source of leads for anticancer drug development. *Phytochem Rev* 2009; 8: 313-31.
9. Deyrup ST, Gloer JB, O'Donnell K, Wicklow DT. Kolokosides A-D: Triterpenoid glycosides from a Hawaiian isolate of *Phomopsis* sp. *J Nat Prod* 2007; 70: 378-382.
10. Essers L, Radebold K. Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test.*JClinMicrobiol* 1980; 12: 641-643.
11. Hacioglu N, Akata I, Dulger B.Antimicrobial potential of *Phomopsispolymorpha* (Pers.) Grev.*Afr J Microbiol Res* 2011;5: 728-730.
12. Healy PC, Hocking A, Tran-Dinh N, Pitt JI, Shivas RG, Mitchell JK, Kotiw M, Davis RA. Xanthonesfrom amicrofungus of the genus *Phomopsis*.*Phytochem*2004; 65: 2373-2378.
13. Kaul, S., Ahmed, M., Zargar, K., Sharma, P., Dhar, M.K.2012. Prospecting endophytic fungal assemblage of *Digitalislanatahrh*. (Foxglove) as a novel source of digoxin: A cardiac glycoside. Biotech. 3(4):335-40.
14. Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D.2011. Anticancer compounds derived from fungal endophytes: Their importance and future challenges. Nat Prod Rep.28 (7):1208-28.
15. Liu D, Hu Z, Liu Z, Yang B, Tu W et al. Chemical composition andantimicrobial activity of essential oil isolated from the cultured myceliaof *Ganodermajaponicum. J Nanj Med Univ*2009; 23:168-172.
16. Lord CJ, Ashworth A. Biology-driven cancer drug development: back to the future. *BMC Biol* 2010; 8: 38.
17. NCCLS (2008): Performance Standards for Antimicrobial Susceptibility Testing; Ninth Informational Supplement. NCCLS document M100-S9. National Committee for Clinical Laboratory Standards, Wayne, PA, pp 120-126.
18. Neu HC. The crisis in antibiotic resistance.*Science* 1992; 257:1050-5.
19. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Nat Prod* 2007; 70:461-477.

20. Nithya K, Muthumary J. 2011.Bioactive metabolite produced by *Phomopsis* sp., an endophytic fungus in AllamandacatharticaLinn. Recent Res. Sci. Technol.3: 44-8.
21. Odds FC. Synergy, antagonism and what the checkerboard puts between them. *JAntimicrobChemother* 2003; 52: 1.
22. ParkinDM, Bray F, Ferlay J, Pisani P.Global cancer statistics 2002.*CA Cancer J Clin*2005 55: 74-108.
23. Pongcharoen W, Rukachaisirikul V, Isaka M, Sriklung K. Cytotoxic metabolites from the wood-decayed fungus. *Chem Pharm Bull* 2007; 55: 1647-1648.
24. Pourshadi M, Klaas J. Evaluation of latex agglutination and microtube coagulase tests for detection of *Staphylococcus aureus*. *DiagnMicrInfecDis* 1984; 2: 287-291.
25. Quang DN, Hashimoto T, Asakawa Y. Inedible mushrooms: a good source of biologically active substances. *Chem Record* 2006; 6: 79-99.
26. Ramesh V, Arivudainambi USE, Thalavaipandian A Karunakaran C, Rajendran A. Antibacterial Activity of Wild *Phomopsis* sp. Strain R005 (Ascomycetes) against Multidrug-Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Int J Med Mushr2012; 14: 47-53.
27. Ramesh V, Karunakaran C,Rajendran A. Evaluation of synergistic andantibacterial activity of *Phomopsiscurta* against drug-resistant *Staphylococcusaureus* and *Pseudomonasaeruginosa*, *Mycology* 2012; 3: 252-257.
28. Rogers JD, Callen BE, Samuels GJ. The Phomopsisceae of the rain forests of north Sulawesi (Indonesia).*Mycotaxon* ;1987; 29:113–172.
29. Rogers JD. The Phomopsisceae: Systematic, biological and evolutionary aspects. *Mycologia* 1979; 71: 1-41.
30. Rukachaisirikul V, Khamthong N, Sukpondma Y, Pakawatchai C, Phongpaichit S, Sakayaroj J, Kirtikara K. An 11 cytochalasin derivative from the marine-derived fungus *Phomopsis* sp. PSU-F100.*Chem Pharm Bull* 2009; 57: 1409-1411.
31. Scheffler A, Anke T. Antimicrobial Compounds from Tree Endophytes. In: Endophytes of Forest Trees, Pirttilä, A. M. & Frank, A. C. (eds.), Springer Netherlands, 2011: 265-294.
32. Shiono Y, Motoki S, Koseki T, Murayama T, Tojima M, Kimura KI. Isopimaranediterpene glycosides, apoptosis inducers, obtained from fruiting bodies of the ascomycete *Phomopsispolymorpha*. *Phytochem* 2009; 70:935-9.
33. Stierle, A, Strobel GA, Stierle DB. Taxol and taxane production by Taxomycesandreanae, an endophytic fungus of Pacific yew. Sci 1993;260:214-6
34. White RL, Burgess DS, MandruruM, Bosso JA. Comparison of three different *invitro* methods of detecting synergy: Time-kill, checkerboard and E-test. *AntimicrobAgents Chemother* 1996; 40:1914-1918.
35. World Health Organization Implementing antimicrobial drug resistance surveillance and contentment for HIV, TB, and malaria. An outline for National Programmes, Geneva: *WHO*; 2003.
36. Xia M, Wang D, Wang M, Tashiro S, Onodera S, Minami M, Ikejima T. Dracorhodinpercholarate induces apoptosis via activation of caspases and generation of reactive oxygen species. *J PharmacolSci* 2004; 95: 273-283.
37. Zhang C, Ondeyka JG, Zink DL, Basilio A, Vicente F 2009). Isolation, structure and antibacterial activity of pleosporone from a *pleosporalean* ascomycete discovered by using antisense strategy.*Bioorg Med Chem*2009; 17: 2162-2166.
38. Zhang G, Sun S, Zhu T, Lin Z, Gu J, Li D.2011. Antiviral isoindolone derivatives from an endophytic fungus *Emericella* sp. associated with *Aegicerascorniculatum*. Phytochem.72(11-12):1436-42.

Table 1

Antimicrobial activities of fruiting body extract of *Phomopsis* sp.against bacterial and fungal pathogens.

Microorganisms	Zone of inhibition (mm)		
	Ethanol extract	Diethyl ether extract	Methanol extract
Bacillus subtilis MTCC 619	12.0±0.12	31.2±0.15	15.0±0.14
Staphylococcus aureus MTCC 3160	11.8±0.15	31.7±0.12	14.5±0.19
Escherichia coli MTCC 4296	11.5±0.13	28.2±0.08	12.5±0.18
Pseudomonas aeruginosa MTCC 2488	13.5±0.21	29.3±0.05	12.3±0.15
Candida albicans MTCC 3018	12.8±0.19	21.3±0.12	11.7±0.11

Table 2

Antibacterial activity of partially purified fraction E of *Phomopsis* sp. against *Staphylococcus aureus.*

S. aureus strains	Zone of inhibition (mm)			
	Penicillin (10 units/ml)	Methicillin (10 µg/ml)	Vancomycin (30 µg/ml)	Fraction E
Strain 1	12.8 ± 0.15 (R)	10.8 ± 0.14 (R)	16.5 ± 0.08 (S)	22.9±0.27
Strain 2	12.8 ± 0.14 (R)	11.2 ± 0.31 (R)	15.4 ± 0.28 (S)	25.8±0.19
Strain 3	14.2 ± 0.28 (R)	12.1 ± 0.16 (R)	16.0 ± 0.22 (S)	27.9±0.21
Strain 4	11.5 ± 0.35 (R)	10.2 ± 0.31 (R)	17.2 ± 0.14 (S)	24.1±0.17
Strain 5	10.8 ± 0.14 (R)	8.9 ± 0.22 (R)	18.5 ± 0.28 (S)	27.3±0.35
Strain 6	10.3 ± 0.15 (R)	9.5 ± 0.35 (R)	12.4 ± 0.35 (R)	23.1±0.41
Strain 7	13.8 ± 0.16 (R)	15.5 ± 0.07 (S)	16.9 ± 0.14 (S)	25.0±0.09
Strain 8	10.5 ± 0.23 (R)	8.8 ± 0.14 (R)	13.5 ± 0.21 (R)	25.6±0.36
Strain 9	14.0 ± 0.21 (R)	12.2 ± 0.10 (R)	14.8 ± 0.22 (R)	22.4±0.15
Strain 10	14.9 ± 0.1 (R)	8.9 ± 0.18 (R)	17.4 ± 0.35 (S)	24.3±0.08

R- Resistant, **S** - Sensitive

Table 3

Antibacterial activity of partially purified fraction Eof *Phomopsis* sp. against *Pseudomonas aeruginosa* strains.

***P. aeruginosa* strains**	**Zone of inhibition (mm)**				
	CF (5 µg/ml)	**CE (30 µg/ml)**	**OF (5 µg/ml)**	**AK (30 µg/ml)**	**Fraction E**
Strain 1	14.5±0.4 (R)	12.4±0.4 (R)	12.5±0.35 (R)	15.5±0.17 (R)	27.7±0.32
Strain 2	21.6±0.1 (S)	23.5±0.14 (S)	20.3±0.28 (S)	20.5±0.14 (S)	19.7±0.09
Strain 3	15.4±0.28 (R)	14.9±0.14 (R)	13.5±0.4 (R)	12.9±0.28 (R)	27.9±0.12
Strain 4	23.7±0.23 (S)	16.8±0.1 (R)	18.6±0.31 (S)	14.5±0.31 (R)	29.4±0.41
Strain 5	24.3±0.04 (S)	24.8±0.15 (S)	19.5±0.14 (S)	19.4±0.22 (S)	23.7±0.32
Strain 6	22.3±0.15 (S)	25.4±0.07 (S)	18.4±0.28 (S)	18.8±0.07 (S)	20.8±0.41
Strain 7	16.5±0.22 (R)	23.7±0.18 (S)	13.9±0.4 (R)	10.5±0.28 (R)	21.6±0.06
Strain 8	18.7±0.14 (R)	16.5±0.35 (R)	16.5±0.14 (S)	14.4±0.23 (R)	25.8±0.09

R- Resistant, **S**- Sensitive, **CF**-Cefotaxime, **CE**- Ciprofloxacin, **OF**-Ofloxacin, and **AK**-Amikacin

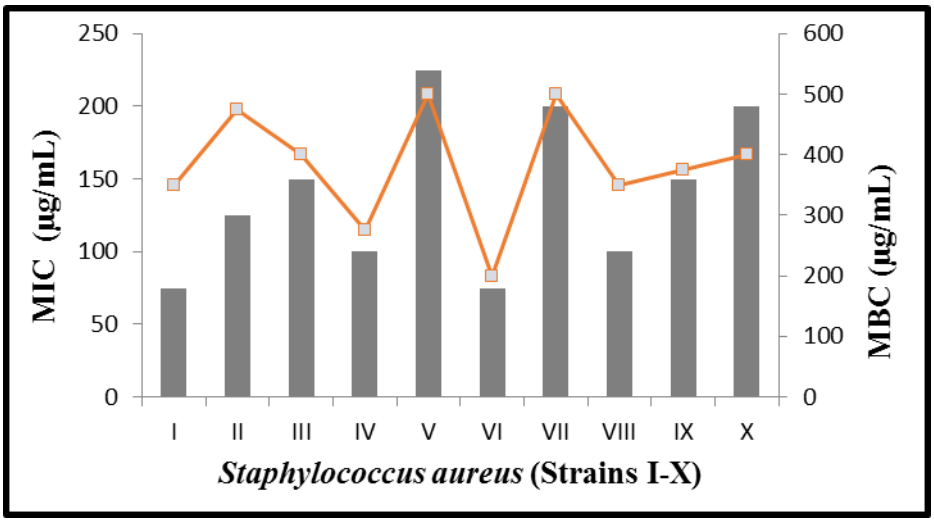

Fig.1. MIC/MBC values of fraction E of Phomopsis sp. against drug-resistantStaphylococcus aureus strains

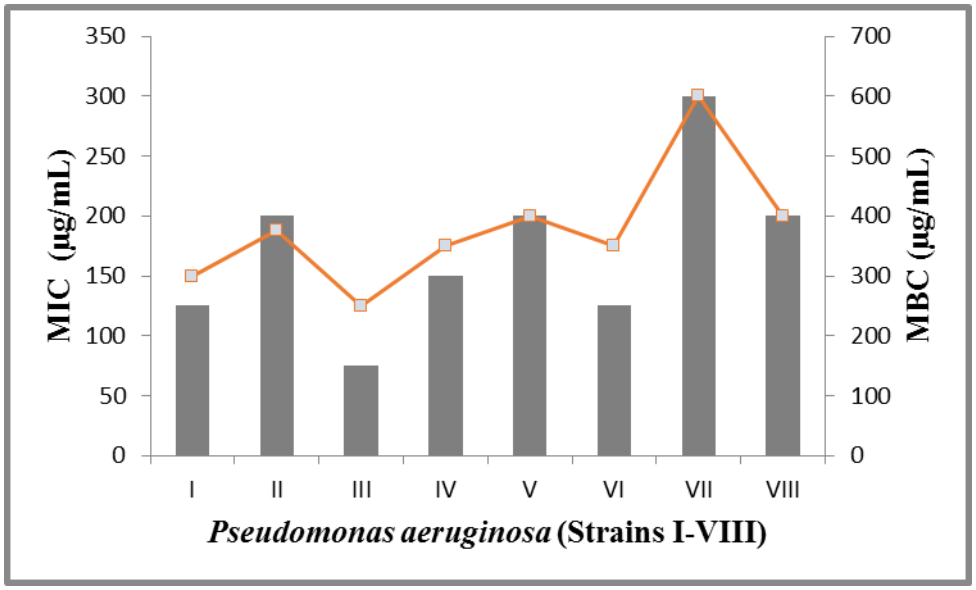

Fig.2. MIC/MBC values of fraction E of Phomopsis sp.againstdrug-resistant Pseudomonas aeruginosa strains.

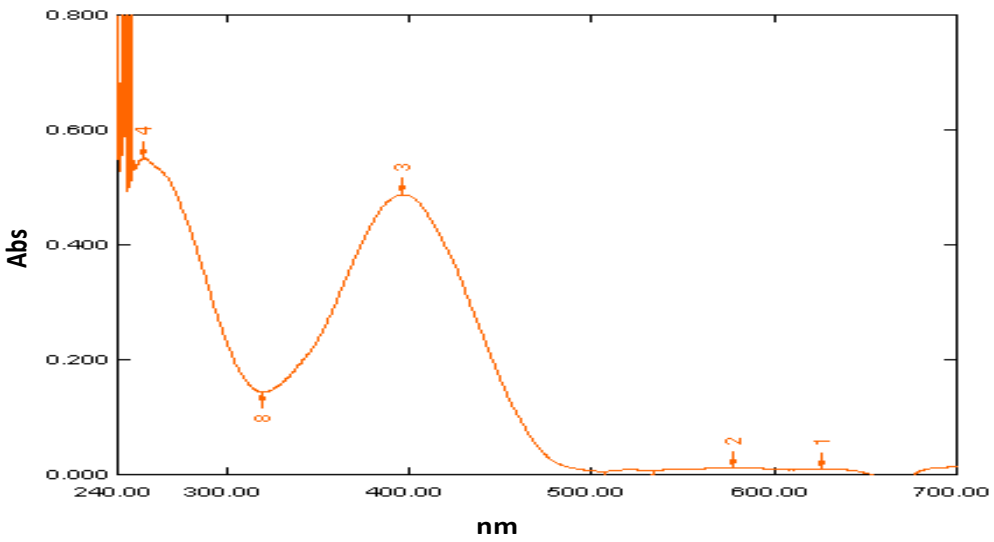

Fig. 3.UV spectrum of fraction E.

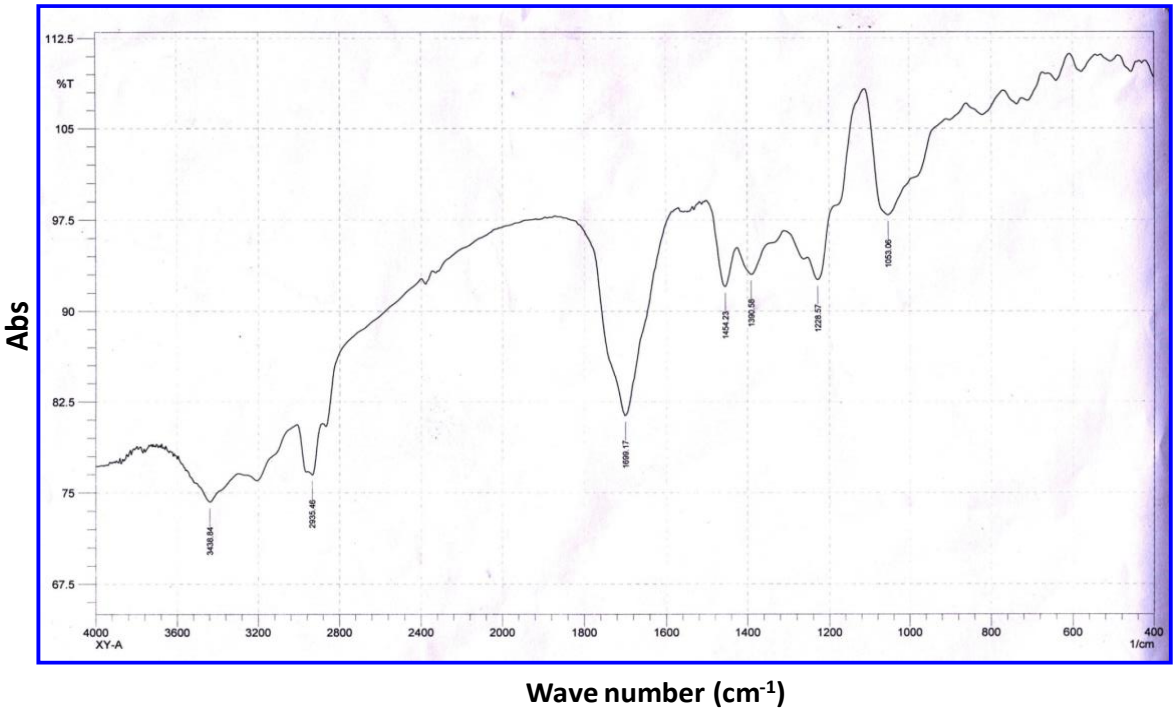

Fig. 4.IR spectrum of fraction E.