ANTIFUNGAL ACTIVITY OF *Senna occidentalis* ROOT EXTRACT AGAINST *Macrophomina phaseolina* AND ITS GC-MS ANALYSIS

*Arshad Javaid[1], Halima Qudsia[1], Iqra Haider Khan[1], Aneela Anwar[2] and Malik F. H. Ferdosi[3]
DOI: https://doi.org/10.28941/pjwsr.v28i1.1033

Abstract

Hundreds of plant species, including many economically important crop plants, are attacked by a highly destructive soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid. In the present study, methanolic root extract of septicweed [*Senna occidentalis* (L.) Link] was evaluated against this fungus. Different concentrations of the extract (0.5 to 3.0%) considerably decreased fungal biomass by 33–43%. Nine compounds were identified when the extract was analyzed by GC-MS. The major compound was 11-octadecanoic acid, methyl ester (26.49%) followed by (5β)pregnane-3,20β-diol, 14α d-mannose (13.85%);, 18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate (13.61%); ethyl iso-allocholate (11.37%); pentadecanoic acid, 14-methyl-, methyl ester (11.01%); and 9,12-octadecadienoic acid, methyl ester, (E,E)- (9.76%), which might be the cause of antifungal activity.

Keywords: Antifungal activity, *Macrophomina phaseolina,* Root extract, *Senna occidentalis,* Weed.

Citation: Javaid, A., H. Qudsia, I.H. Khan, A. Anwar and M.F.H. Ferdosi. 2022. Antifungal Activity of *Senna occidentalis* Root Extract against *Macrophomina phaseolina* and its GC-MS Analysis. Pak. J. Weed Sci. Res., 28(1): 73-80

[1]Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan.
[2]Department of Basic Sciences, University of Engineering and Technology, KSK Campus, Lahore, Pakistan.
[3]Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan.
*Corresponding author's email: arshad.iags@pu.edu.pk; arshadjpk@yahoo.com

Introduction

Macrophomina phaseolina is a seed- and soil-borne plant pathogen (Ghosh *et al.,* 2018). It has a wide geographic distribution, especially reported from tropical and subtropical regions with arid to semi-arid climates in Asia, Africa, South America, North America, and Europe (Jana *et al.,* 2003; Machado *et al.,* 2019). Being necrotrophic in nature, it causes seedling blight, root and stem rot, leaf blight, collar rot, and charcoal rot in over 500 plant species involving several economically important crops *viz.* cotton, sorghum, maize, soybean, chickpea, alfalfa, and sunflower (Amin and Javaid, 2007; Khan *et al.,* 2017; Lodha and Mawar, 2020). Over the decades, many fungicides have been recommended for effective control of plant pathogens but ever-increasing cost, fungicide usage restrictions, and environmental concerns clearly indicated the need to explore for substitute managing plans (Parmar *et al.,* 2017; Marquez *et al.,* 2021). In addition, presently there is not any registered fungicide for control of *M. phaseolina.* Scientists have recommended the likelihood of using natural products as a convincing option for the control of fungal pathogens (Javaid *et al.,* 2020; Khan *et al.,* 2020; Javed *et al.,* 2021). It provides an environmentally safe and potentially stable alternate to synthetic chemicals. Several plant species such as *Sonchus oleraceous, Chenopodium quinoa* and *Ageratum conyzoides* have been reported to possess antifungal agents that considerably deter the growth of *M. phaseolina* (Banaras *et al.,* 2020, 2021; Khan and Javaid, 2020).

Senna occidentalis, native to South America, also grows in tropical areas of Australia, Europe, Africa and Asia (Tokarnia *et al.,* 2000; Gotardo *et al.,* 2017). In Pakistan, this plant is generally found at waste areas and along roadsides (Fig. 1). Its different parts are used across the globe in various traditional medicines as an expectorant, laxative, anthelmintic analgesic and diuretic (Yadav *et al.,* 2010; Teles *et al.,* 2015). However, studies regarding antifungal activity of *S. occidentalis* are very rare. In general, the few studies carried out with respect to its antifungal activity were restricted to aerial parts of *S. occidentalis* (Yadave *et al.,* 2010; Javaid *et al.,* 2017). Therefore, this study was carried out to examine antifungal activity of *S. occidentalis* root extract against *M. phaseolina.*

Fig. 1: A mature plant of *Senna occidentalis* growing in Lahore, Pakistan.

Materials and Methods

Mature plants of *S. occidentalis* were collected from Lahore, Pakistan. Roots were detached from aerial portions and thoroughly washed with water. These were first dried under shade and then fully dried at 40 °C in an oven. These roots were cut into very small parts and crushed. For one week at room temperature, crushed roots (200 g) were drenched in 1.0 L methanol. Thereafter, a cheese cloth was used to filter the soaked plant material, followed by filtration by filter paper. Methanol in the extract was vaporized in a rotary evaporator and 5.64 g root extract was acquired. For *in vitro* bioassays, 4.725 g root extract was added in 4 mL of dimethyl sulfoxide (DMSO) followed by the addition of distilled water to prepare 10.5 mL of the stock solution. The control solution was made by adding 4 mL of DMSO in 6.5 mL autoclaved distilled water. To 42 mL of autoclaved malt extract broth (MEB), 3 mL of mixtures of stock and control solutions in different ratios were added to prepare 0.5, 1, 1.5, 2, 2.5, and 3% concentrations. The 45 mL of each treatment were separated into 3 uniform shares in 100 mL flasks to serve as replicates. For the control treatment, 3 mL of control solution was added to 42 mL of MEB. Flasks were inoculated with 5 mm discs of one-week old culture of *M. phaseolina* and incubated at 28 ^{o}C for seven days. Pre-weighed filter papers were used for the filtration of fungal mat in order to get the fungal harvest. The obtained fungal harvest was dried at 60 ^{o}C in order to obtain dry weight. Data were examined by ANOVA and afterwards LSD test was employed at $P \leq 0.05$ by using Statistix 8.1.

Methanolic root extract was analyzed by GC-MS procedure to obtain a profile of chemical constituents. The analysis was executed on Agilent 7890A GC system with 5975C mass spectrometer. The capillary column was 30 m × 250 µm × 0.25 µm. Helium was used as a carrier gas at 1 mL min^{-1} flow rate. An injection volume of 1 µL was used in splitless mode. Injector and ion-source were set at 250 °C and 280 °C, respectively. Temperature of the oven was first increased from 100 to 200 °C at 5 °C min^{-1} and later on up to 240 °C at a rate of 10 °C min^{-1} and continued at 240 °C for 10 min. Total GC running time was 23 min.

Results and Discussion

Antifungal effect of various concentrations of root extract is illustrated in Fig. 2. All the concentrations were highly effective against *M. phaseolina*. Maximum fungal biomass (0.218 g) was documented in the control that was decreased to 0.124–0.146 g in different concentrations of the extract. A 33–43% reduction in *M. phaseolina* biomass was recorded due to different concentrations of the root extract. In most of the previous studies, scientists investigated antimicrobial activities of above-ground parts of *S. occidentatlis*. Javaid *et al.* (2017) showed the similar result of leaf extract of *S. occidentatlis* against *M. phaseolina.* Its leaves and fruits are used as antifungal agents in Brazilian folk medicines, mainly to cure wounds and mycoses, for example, ringworm caused by *Tinea corporis* and the skin eruption caused by *Ptiriase versicolor* as stated by Fenner *et al.* (2006). Extracts of different parts of this plant have been discovered as very efficient against bacteria and fungi namely *Candida albicans*, *Aspergillus flavus*, *A. niger* and *Fusarium oxysporum* (Ali *et al.,* 1999; Abo *et al.,* 2000).

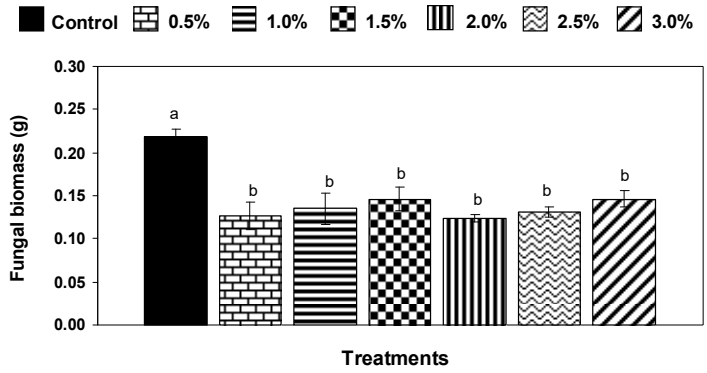


Fig. 2: Effect of different concentrations of methanolic root extract of *Senna occidentalis* on biomass of *Macrophomina phaseolina*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

GC-MS chromatogram of the root extract showing 9 compounds as shown in Fig. 3. These peaks were found at retention times ranging from 11.605 to 23.813 minutes. The names of various compounds that were identified in this extract along with percent peak areas are shown in Table 1. Principal compound in the extract was 11-octadecanoic acid, methyl ester with a peak area 26.49%. It belongs to fatty acid methyl esters group. The members of this group are quite renowned for their antifungal activities against a range of fungal species, including *Macrophomina phaseolina, Paracoccidioides* spp., *Candida albicans* and *C. krusei* (Aliyu *et al.,* 2017; Banaras *et al.,* 2017; Pinto *et al.,* 2017). Other frequently occurring compounds were (5β)pregnane-3,20β-diol, D-mannose (13.85%), 14α, 18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate (13.61%), ethyl iso-allocholate (11.37%), pentadecanoic acid, 14-methyl-, methyl ester (11.01%) and 9,12-octadecadienoic acid, methyl ester, (E,E)-(9.76%). D-manose is known to possess inhibitory effects against *Candida* and *Pichia* species as reported by Regente *et al.* (2014). Three compounds were classified as less frequent ones including 16-hydroxyhexadecanoic acid (4.98%), 2H-indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl (4.86%) and 2,7-diphenyl-1,6-dioxopyridazino[4,5:2′,3′]pyrrolo[4′,5′-d]pyridazine (4.02%). Structures of the compounds present in root extract are illustrated in Fig. 4.

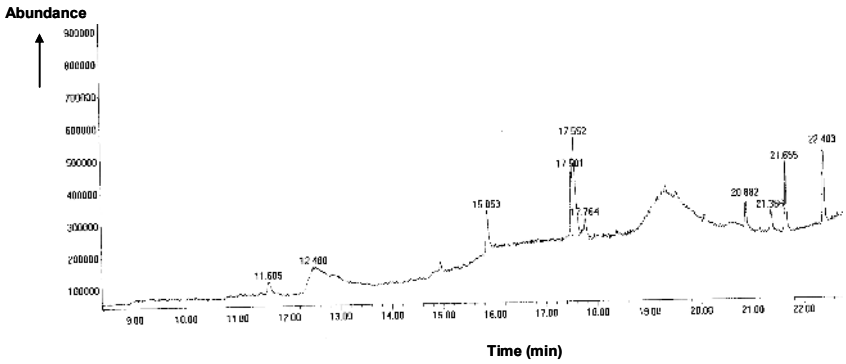
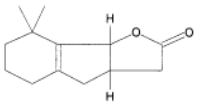
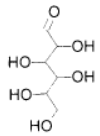


Fig. 3: GC-MS chromatogram of methanolic root extract of *Senna occidentalis.*

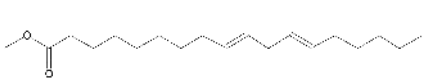


1- 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl



2- d-Mannose

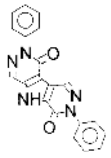
3- Pentadecanoic acid, 14-methyl-, methyl ester



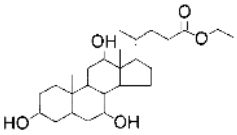
4- 9,12-Octadecadienoic acid, methyl ester, (E,E)-

5- 11-Octadecanoic acid, methyl ester

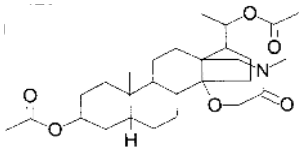
6- 16-Hydroxyhexadecanoic acid



7- 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine



8- Ethyl iso-allocholate



9- (5.beta.)Pregnane-3,20.beta.-diol, 14.alpha., 18.alpha.-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate

Fig. 4: Structures of compounds identified from methanolic root extract of *S. occidentalis* through GC-MS analysis.

Table 1: Compounds identified from methanolic root extract of *S. occidentalis* through GC-MS analysis.

Comp. No.	Names of compounds	Molecular formula	Molecular Weight	Retention time (min)	Peak area (%)
1	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}O_2$	206	11.605	4.86
2	D-Mannose	$C_6H_{12}O_6$	180	12.480	13.85
3	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	15.853	11.01
4	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	17.501	9.76
5	11-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296	17.552	26.49
6	16-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272	17.764	4.98
7	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2′,3′]pyrrolo[4′,5′-d]pyridazine	$C_{20}H_{13}N_5O_2$	355	21.366	4.02
8	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	21.655	11.37
9	(5β)Pregnane-3,20β-diol, 14α, 18α-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}NO_6$	489	22.403	13.61

Conclusion

This study concludes that root extract of *S. occidentalis* has the potential to control growth of *M. phaseolina.* The antifungal activity could be attributed to the occurrence of D-manose and various fatty acid methyl esters in the root extract.

References

Abo, K.A., A.A. Adeyemi and I.A. Jegede. 2000. Spectrophotometric estimation of anthraquinone content and antimicrobial potential of extracts of some *Cassia* species used in herbal medicine. Ibadan Sci. Forum., 3: 57-63.

Ali, M.S., I. Azhar, Z. Amtul, V.U. Ahmad and K. Usmanghani. 1999. Antimicrobial screening of some Caesalpiniaceae. Fitoterapia, 70: 299-304.

Aliyu, A.B., M.A. Ibrahim, H. Ibrahim, M.B. Dambatta and A.O. Oyewale. 2017. GC-MS analysis of *Pavetta corymbosa* lipophilic extract and its antimicrobial activity. Ife J. Sci., 19: 363-368.

Amin, M. and A. Javaid. 2007. Exploitation of allelopathic potential of *Chenopodium* species to control charcoal rot pathogen of sunflower. Pak. J. Agric. Res., 20: 130-136.

Banaras, S, A. Javaid, A. Shoaib and E. Ahmed. 2017. Antifungal activity of *Cirsium arvense* extracts against a phytopathogenic fungus *Macrophomina phaseolina.* Planta Daninha, 35: e017162738.

Banaras, S., A. Javaid and I.H. Khan. 2020. Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina*. Int. J. Agric. Biol., 24: 1376-1382.

Banaras, S., A. Javaid and I.H. Khan. 2021. Bioassays guided fractionation of *Ageratum conyzoides* extract for the identification of natural antifungal compounds against *Macrophomina phaseolina*. Int. J. Agric. Biol., 25: 761-767.

Fenner, R., A.H. Betti, L.A. Mentz and S.M.K. Rates. 2006. Plantas utilizadas na medicina popular brasileira com potencial atividade antifúngica. Rev. Bras. Ciên. Farmacêut., 42: 369-394.

Ghosh, T., M.K. Biswas, C. Guin and P. Roy. 2018. A review on characterization, therapeutic approaches and pathogenesis of *Macrophomina phaseolina*. Plant Cell Biotechnol. Mol. Biol., 19: 72-84.

Gotardo, A.T., M. Haraguchi, P.C.F. Raspantini, M.L.Z. Dagli and S.L. Górniak. 2017. Toxicity of *Senna occidentalis* seeds in laying hens and its effects on egg production. Avian Pathol., 46: 332-337.

Jana, T., T.R. Sharma, R.D. Prasad and D.K. Arora. 2003. Molecular characterization of *Macrophomina phaseolina* and *Fusarium* species by a single primer RAPD technique. Microbiol. Res., 158: 249-257.

Javaid, A., H. Qudsia and A. Shoaib. 2017. Bioassays guided fractionation of *Senna occidentalis* for identification of natural antifungal constituents against *Macrophomina phaseolina.* Planta Daninha, 35: e017163483.

Javaid, A., R. Munir, I.H. Khan and A. Shoaib. 2020. Control of the chickpea blight, *Ascochyta rabiei,* with the weed plant, *Withania somnifera.* Egypt. J. Biol. Pest Control, 30: 114.

Javed, S., Z. Mahmood, K.M. Khan, S.D. Sarker, A. Javaid, I.H. Khan and A. Shoaib. 2021. Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. Sci. Rep., 11: 1-11.

Khan, A.N., F. Shair, K. Malik, Z. Hayat, M.A. Khan, F.Y. Hafeez and M.N. Hassan. 2017. Molecular identification and genetic characterization of *Macrophomina phaseolina* strains causing pathogenicity on sunflower and chickpea. Front. Microbiol., 8: 1309.

Khan, I.H. and A. Javaid. 2020. Comparative antifungal potential of stem extracts of four quinoa varieties against *Macrophomina phaseolina*. Int. J. Agric. Biol., 24: 441-446.

Khan, I.H., A. Javaid, A.H. Al-Taie and D. Ahmed. 2020. Use of neem leaves as soil amendment for the control of

collar rot disease of chickpea. Egypt. J. Biol. Pest Control, 30: 98.

Lodha, S. and R. Mawar. 2020. Population dynamics of *Macrophomina phaseolina* in relation to disease management: A review. J. Phytopathol., 168: 1-17.

Machado, A.R., D.B. Pinho, D.J. Soares, A.A.M. Gomes and O.L. Pereira. 2019. Bayesian analyses of five gene regions reveal a new phylogenetic species of *Macrophomina* associated with charcoal rot on oilseed crops in Brazil. Eur. J. Plant Pathol., 153: 89-100.

Marquez, N., M.L. Giachero, S. Declerck and D.A. Ducasse. 2021. *Macrophomina phaseolina*: General characteristics of pathogenicity and methods of control. Front. Plant Sci., 12: 634397.

Parmar, H.V., H.J. Kapadiya and C.M. Bhaliya. 2017. Efficacy of different fungicides against *Macrophomina phaseolina* (Tassi) Goid causing castor root rot. Int. J. Commun. Syst., 5: 1807-1809.

Pinto, M.E.A., S.G. Araujo, M.I. Marcela and L.A.R.S. Lima. 2017. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. An. Acad. Bras. Cienc., 89: 1671-1681.

Regente, M., G.B. Taveira, M. Pinedo, M.M. Elizalde, A.J. Ticchi, M.S.S. Diz, A.O. Carvalho, L. de la Canal and V.M. Gomes. 2014. A sunflower lectin with antifungal properties and putative medical mycology applications. Curr. Microbiol., 69: 88-95.

Telesa, A.V.F.F., R.A. Fockb and S.L.Górniak. 2015. Effects of long-term administration of *Senna occidentalis* seeds on the hematopoietic tissue of rats. Toxicon, 108: 73-79.

Tokarnia, C.H., J. Dobereiner and P.V. Peixoto. 2000. Plantas tóxicas do Brasil (second ed.), Helianthus, Rio de Janeiro.

Yadav, J.P., V. Arya, S. Yadav, M. Panghal, S. Kumar and S. Dhankhar. 2010. *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia, 81: 223-230.