Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

CHEMICAL PROFILE AND ANTIFUNGAL ACTIVITY OF LEAF EXTRACT OF *TABERNAEMONTANA DIVARICATA* AGAINST *MACROPHOMINA PHASEOLINA*

Arshad Javaid, Iqra Haider Khan
Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan.

ARTICLE INFO

Article history
Received: 18th August, 2022
Revised: 24th September, 2022
Accepted: 16th October, 2022

Keywords
Antifungal
Leaf extract
Macrophomina phaseolina
Tabernaemontana divaricata

ABSTRACT

Macrophomina phaseolina is a highly problematic fungus that causes diseases in hundreds of plant species. In order to search for an alternative to chemical fungicides for the control of this devastating pathogen, leaf extract of *Tabernaemontana divaricata* was assessed for the control of *M. phaseolina.* Five concentrations of leaf extract in methanol *viz.* 1, 2, 3, 4 and 5% (w/v), were checked against *M. phaseolina.* All these concentrations significantly suppressed the fungal growth resulting in 34–74% decreased biomass of *M. phaseolina* over control. GC-MS analysis of this extract showed the presence of 54 compounds. Squalene was the predominant compound with 24.11% peak area followed by vitamin E (8.96%). Other important compounds were hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (6.21%), thiophene, tetrahydro-2-methyl- (5.68%), cyclopentanol (5.61%), neophytadiene (6.25%), 1-tert-butoxypropan-2-yl 2-methylbutanoate (6.03%), ibogamine-18-carboxylic acid, 12-methoxy-, methyl ester (2.95%), phytol (2.26%), and *n*-hexadecanoic acid (2.20%). Some of these major compounds might be responsible for antifungal property of methanolic leaf extract of *T. divaricata* against *M. phaseolina.*

Corresponding Author: Arshad Javaid
Email: arshad.iags@pu.edu.pk; arshadjpk@yahoo.com

INTRODUCTION

Macrophomina phaseolina, a pathogen distributed worldwide, causes charcoal rot, seedlings blight, root rot and other diseases in more than 500 plant species (Banaras et al., 2021; Hyder et al., 2018). It is also an opportunistic human pathogen (Iqbal and Mukhtar, 2014; Javed et al., 2021; Uroos et al., 2022). The adverse effects of this pathogen are more severe at high temperature (30–35°C) and low moisture (less than 60%) as reported by Kaur et al. (2012). No registered fungicide against *M. phaseolina* is available in the market. However, some fungicides such as benomyl, carbendazim, dazome, difenoconazole and azoxystrobin have been tried *in vitro* and *in vivo* against *M. phaseolina* (Iqbal and Mukhtar, 2020a; Marquez et al., 2021). In order to reduce environmental pollution, scientists are in search of alternatives to fungicides such as the use of biological agents (Iqbal and Mukhtar, 2020b; Khan and Javaid, 2021, 2022a) and botanicals (Iqbal et al., 2014; Khan and Javaid 2022b) to control *M. phaseolina.* There are reports of control of *M. phaseolina* by extracts of *Chenopodium quinoa* (Khan and Javaid, 2020), *Sonchus oleraceous, Ageratum conyzoides* (Banaras et al., 2020, 2021), *Alternanthera philoxeroides (*Amin et al., 2022) and *Coronopus didymus* (Javaid et al., 2020).

Tabernaemontana divaricata is an evergreen shrub of family Apocynaceae. It is widely cultivated in gardens and along the roads in urban areas of Punjab, Pakistan as

an ornamental plant. It is also grown in gardens in Tropical Asia, India, Polynesia and Australia as an ornamental plant. Many studies have shown medicinal importance of this plant. It is known to possess many biological activities including antimicrobial (Singh et al., 2011), antidiabetic and anti-inflamatory (Satyanarayan et al., 2004; Rahman et al., 2011) activities. Most of the previous studies have been carried out regarding antibacterial activities of this plant while studies regarding its antifungal activity are rare. Especially, studies about antifungal activity of this plant against *M. phaseolina* are lacking. Therefore, the present study was undertaken to explore activity of methanolic leaf extract of *T. divaricata* against *M. phaseolina* and identification of the possible antifungal compounds through GC-MS analysis of the extract.

MATERIALS AND METHODS

Extraction of leaves in methanol

Leaves were collected from *T. divaricata* plants growing in Lahore, Pakistan (Figure 1). After washing, the leaves were cut into pieces and dried in an electric oven at 4ºC. After thorough crushing, 100 g of it was soaked in 500 mL of pure methanol. After two weeks, the material was filtered and the filtrates were evaporated on a rotary evaporator at 45 ℃. Finally, a gummy mass was obtained, called as methanolic extract, was used in the antifungal bioassay (Ferdosi et al., 2022).

Figure 1. *Tabernaemontana divericata* plant, branches and flowers.

Antifungal study

Nine grams of methanolic extract of *T. divaricata were* mixed in 5 mL dimethyl sulfoxide (DMSO) followed by addition of appropriate quantity of distilled water to have 15 mL of a stock solution. Same amount of the control solution was prepared by mixing 5 mL DMSO and 10 mL distilled water. Control and stock solutions were mixed in different ratios to have a 5 mL mixture that was added to 55 mL sterilized malt extract broth (MEB). This volume (60 mL) of the growth medium was divided into 4 parts to serve as replicates for each concentration viz. 0, 1, 2, 3, 4 and 5% (w/v). The flasks were inoculated with the pathogen and incubated at 27 ℃ for 7 days. The fungal material was separated by filtration and weighed after drying at 70ºC (Banaras et al., 2021).

GC-MS analysis

The GC machine 7890B and MS machine 5977A, both of Agilent USA, were used for chemical profiling of leaf extract of *T. divaricata*. The column dimensions were 30 m × 0.25 µm × 0.25 µm; 1 µL volume was used; and helium was used as a carrier gas. Oven was 80 ℃ at the start that was raised to 300ºC by increase at a rate of 10ºC min^{-1}. The processing time was 30 min. MS conditions were set as: source temperature 230 ℃ and scan range 50–500 m/z. The spectra were compared with NIST library of 2017 version following Ferdosi et al. (2021).

Statistical analysis

All the data regarding antifungal activity of the leaf extract of *T. divaricata* was analyzed by one-way ANOVA

and the treatment means were delineated through LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Antifungal activity of methanolic leaf extract

Leaf extract of *T. divaricata* was found highly inhibitory to the growth of *M. phaseolina.* All the concentrations used in the experiment significantly ($P \leq 0.05$) retarded the growth target pathogen. The lowest concentration (1%) reduced fungal biomass by 34% while the highest one (5%) resulted in 74% decline in fungal biomass (Figure 2 A and B). There was a polynomial relationship between the extract concentration and biomass of *M. phaseolina* (Figure 2 C). Earlier, Singh et al. (2011) reported antifungal activity of ethanolic extract of *T. divaricata* against *Penicillium chrysogenum*. They identified coronaridine as the major antifungal compound in the ethanolic extract. Likewise, dichloromethane fraction of ethanolic extracts exhibited a pronounced antifungal activity against *Candida albicans*, *C. neoformans*, *C. glabrata*, *Aspergillus fumigatus* and *A. flavus* (Boligon et al., 2015).

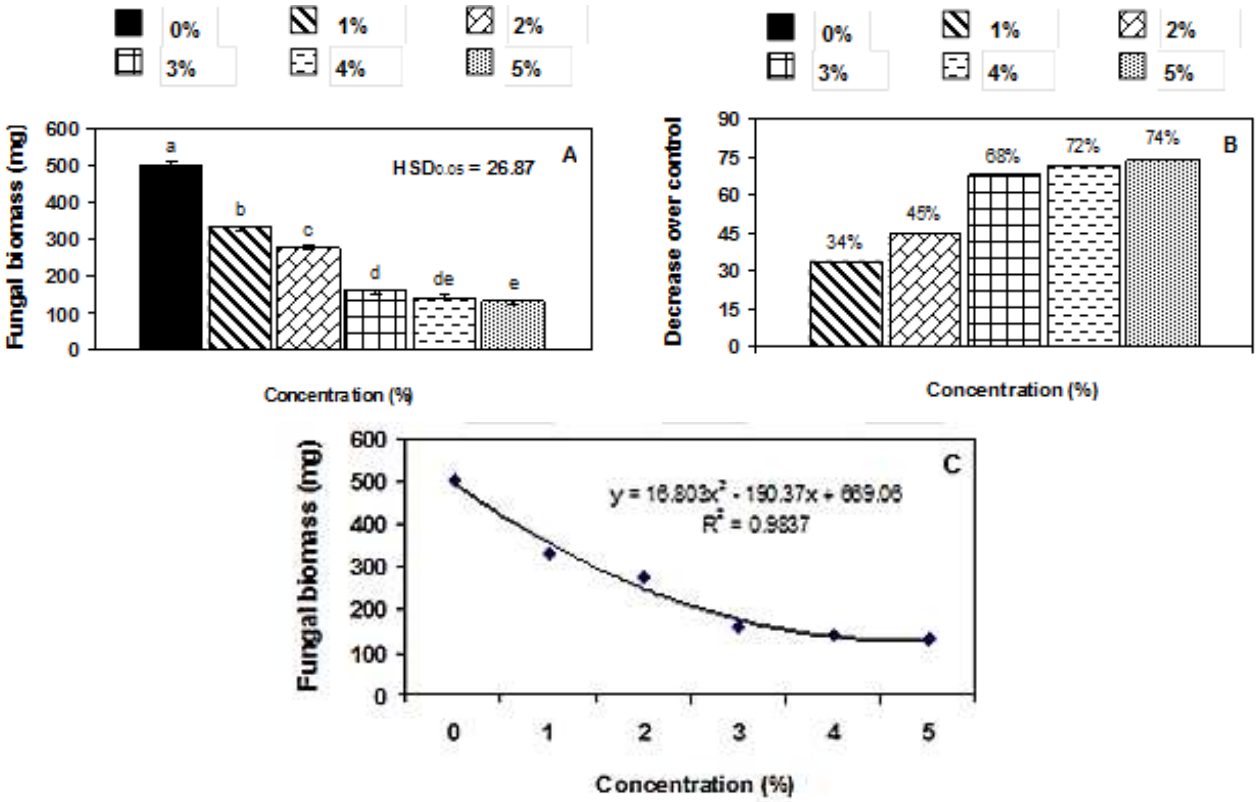

Figure 2. Antifungal activity of methanolic leaf extract of *Tabernaemontana divericata* against *Macrophomina phaseolina.* Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's HSD Test.

GC-MS analysis

A total of 54 compounds were identified in the extract as shown in GC-MS chromatogram presented in Table 1. Squalene was the predominant compound with 24.11% peak area followed by vitamin E (8.96%). Earlier, Javaid et al. (2021) have reported this compound in roots of *Cannabis sativa* as a moderately occurring compound. It is a triterpene that mostly occurs in adequate amount in oils of amaranth, rice bran olive and palm, and has antioxidant and antitumor properties (Huang et al., 2009). In addition, it also possesses antimicrobial properties. Squalene isolated from liver of a shark *Carcharhinus sorrah* inhibited the growth of both Gram-positive and Gram-negative bacteria (Dordab et al., 2021). Other frequently occurring compound was hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (6.21%). It was previously reported from *Chenopodium quinoa* leaves

(Khan and Javaid, 2022c), and known to have pesticidal and nematicide activities (Lalitha et al., 2015). Thiophene, tetrahydro-2-methyl- (5.68%), was also found among the frequently occurring compounds. Thiophene ring is a part of many natural products, and its derivatives possess antifungal properties (Mikami et al., 1990; Mabkhot et al., 2016). Other frequently occurring compounds were cyclopentanol (5.61%), neophytadiene (6.25%), and 1-tert-butoxypropan-2-yl 2-methylbutanoate (6.03%).

Table 1: Compounds identified in methanolic leaf extract of *Tabernaemontana divaricata* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak Area (%)
1	Erythro-3-bromo-2-pentanol	$C_8H_{18}O$	130.22	6.288	0.14
2	2,3-Pyrazinedicarboxylic acid	$C_6H_4N_2O_4$	168.11	6.609	0.44
3	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{21}O_2Si_3$	221.50	6.796	0.34
4	Piperazine, 1,4-dimethyl-	$C_6H_{14}N_2$	114.18	7.278	0.29
5	1-Ethyl-2-pyrrolidinone	$C_6H_{11}NO$	113.15	7.433	0.33
6	1-Ethyl-1H-pyrazole-3,4-diamine	$C_5H_{10}N_4$	126.11	7.572	0.39
7	Benzoic acid, hydrazide	$C_7H_8N_2O$	136.15	7.797	0.06
8	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370.76	8.230	0.18
9	N-Methylglycine	$C_3H_7NO_2$	89.09	9.385	0.24
10	Benzaldehyde, 2-methyl-	C_8H_8O	120.14	9.647	0.34
11	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444.92	10.658	0.15
12	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	10.952	1.01
13	Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl-	$C_8H_{18}OSi$	158.31	12.439	0.34
14	2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl]guanine	$C_{10}H_{15}N_5O_5$	285.26	12.862	0.84
15	Butanoic acid, 2-oxo-	$C_4H_6O_3$	102.08	12.947	0.85
16	1-tert-Butoxypropan-2-yl 2-methylbutanoate	$C_{12}H_{22}O_3$	214.30	13.300	6.03
17	1-Nonanamine	$C_9H_{21}N$	143.26	13.338	1.29
18	Cyclopentanol	$C_5H_{10}O$	86.13	13.423	5.61
19	Phenol, 4-ethenyl-2,6-dimethoxy-	$C_{10}H_{14}O_3$	182.21	14.156	0.85
20	N-propyl-butyramide	$C_7H_{15}NO$	129.20	14.964	0.16
21	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162.14	15.258	0.94
22	Docosanoic acid 1-methyl-butyl ester	$C_{27}H_{54}O_2$	410.7	15.435	0.98
23	Thiophene, tetrahydro-2-methyl-	$C_5H_{10}S$	102.20	15.980	5.68
24	Neophytadiene	$C_{20}H_{38}$	278.51	17.200	6.25
25	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280.53	17.253	0.24
26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	17.446	0.98
27	1,4-Eicosadiene	$C_{20}H_{38}$	278.51	17.644	1.95
28	*cis*-10-Heptadecenoic acid, methyl ester	$C_{18}H_{34}O_2$	282.46	18.050	0.17
29	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.45	17.104	1.74

30	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	18.483	2.20
31	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{50}O_7Si_8$	579.2	18.676	0.25
32	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	$C_{19}H_{34}O_2$	294.25	19.740	0.34
33	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.45	19.799	2.22
34	Phytol	$C_{20}H_{40}O$	296.53	19.890	2.26
35	Methyl stearate	$C_{19}H_{38}O_2$	298.5	20.034	0.92
36	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264.44	20.173	0.78
37	Acetamide, N,N-diethyl-	$C_6H_{13}NO$	115.17	21.460	0.17
38	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	$C_{23}H_{32}O_2$	340.49	22.527	0.37
39	1-Propanamine, N-ethyl-	$C_5H_{13}N$	87.16	22.912	0.33
40	Tryptamine	$C_{10}H_{12}N_2$	160.22	23.003	1.34
41	2,4-Disilapentane, 2,4-dimethyl-	$C_5H_{14}Si_2$	130.33	23.153	0.19
42	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.50	23.430	6.21
43	Mercaptoacetic acid, 2TMS derivative	$C_{13}H_{22}O_2SSi_2$	298.54	23.907	0.20
44	Indole, 3-methyl-	C_9H_9N	131.17	24.559	0.82
45	Cyclooctene, 3-ethenyl-	$C_{10}H_{16}$	1336.23	24.757	0.95
46	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	358.55	24.912	3.71
47	Valeramide, N-hexyl-	$C_{11}H_{23}NO$	185.30	24.961	0.38
48	Squalene	$C_{30}H_{50}$	410.7	25.608	24.11
49	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl	$C_{15}H_{26}O$	222.36	26.490	0.24
50	trans-Sesquisabinene hydrate	$C_{15}H_{26}O$	222.36	26.603	0.18
51	Sarcosine, N-valeryl-, pentyl ester	$C_{13}H_{25}NO_3$	243.34	26.699	0.05
52	Ibogamine-18-carboxylic acid, 12-methoxy-, methyl ester	$C_{22}H_{28}N_2O_3$	368.5	27.105	2.95
53	γ-Tocopherol	$C_{28}H_{48}O_2$	416.68	27.833	0.84
54	Vitamin E	$C_{29}H_{50}O_2$	430.70	28.805	8.96

Moderately abundant compounds included octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.71%), ibogamine-18-carboxylic acid, 12-methoxy-, methyl ester (2.95%), phytol (2.26%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (2.22%), *n*-hexadecanoic acid (2.20%), 1,4-eicosadiene (1.95%), pentadecanoic acid, 14-methyl-, methyl ester (1.74%), tryptamine (1.34%), and 2-methoxy-4-vinylphenol (1.01%). Phytol is a terpene that possesses antifungal activity against *Aspergillus niger* and *Candida albicans* (Ghaneian et al., 2015). Palmitic acid or *n*-hexadecanoic acid is reported to inhibit the virulence factors of the fungus *Candida tropicalis* (Prasath et al., 2020).

The remaining compounds were less abundant with peak area lesser than 1%. These included 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.98%), docosanoic acid 1-methyl-butyl ester (0.98%), cyclooctene, 3-ethenyl- (0.95%), 3-deoxy-d-mannoic lactone (0.94%), methyl stearate (0.92%), phenol, 4-ethenyl-2,6-dimethoxy- (0.85%), butanoic acid, 2-oxo- (0.85%), γ-tocopherol (0.84%), 2R,3S-9-[1,3,4-trihydroxy-2-butoxymethyl]guanine (0.84%), indole, 3-methyl- (0.82%), 9,12,15-octadecatrien-1-ol, (Z,Z,Z)- (0.78%), 2,3-pyrazinedicarboxylic acid (0.44%), valeramide, N-

hexyl- (0.38%), 1-ethyl-1H-pyrazole-3,4-diamine (0.39%), phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl (0.37%), 1,1,1,3,5,5,5-heptamethyltrisiloxane (0.34%), benzaldehyde, 2-methyl- (0.34%), 9,15-octadecadienoic acid, methyl ester, (Z,Z)- (0.34%), 1-ethyl-2-pyrrolidinone (0.33%), 1-propanamine, N-ethyl- (0.33%), piperazine, 1,4-dimethyl- (0.29%), octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (0.25%), N-methylglycine (0.24%), mercaptoacetic acid, 2TMS derivative (0.20%), 2,4-disilapentane, 2,4-dimethyl- (0.19%), cyclopentasiloxane, decamethyl- (0.18%), *trans*-sesquisabinene hydrate (0.18%), *cis*-10-heptadecenoic acid, methyl ester (0.17%), Acetamide, N,N-diethyl- (0.17%), N-propyl-butyramide (0.16%), cyclohexasiloxane, dodecamethyl- (0.15%), erythro-3-bromo-2-pentanol (0.14%), benzoic acid, hydrazide (0.06%), and sarcosine, N-valeryl-, pentyl ester (0.05%).

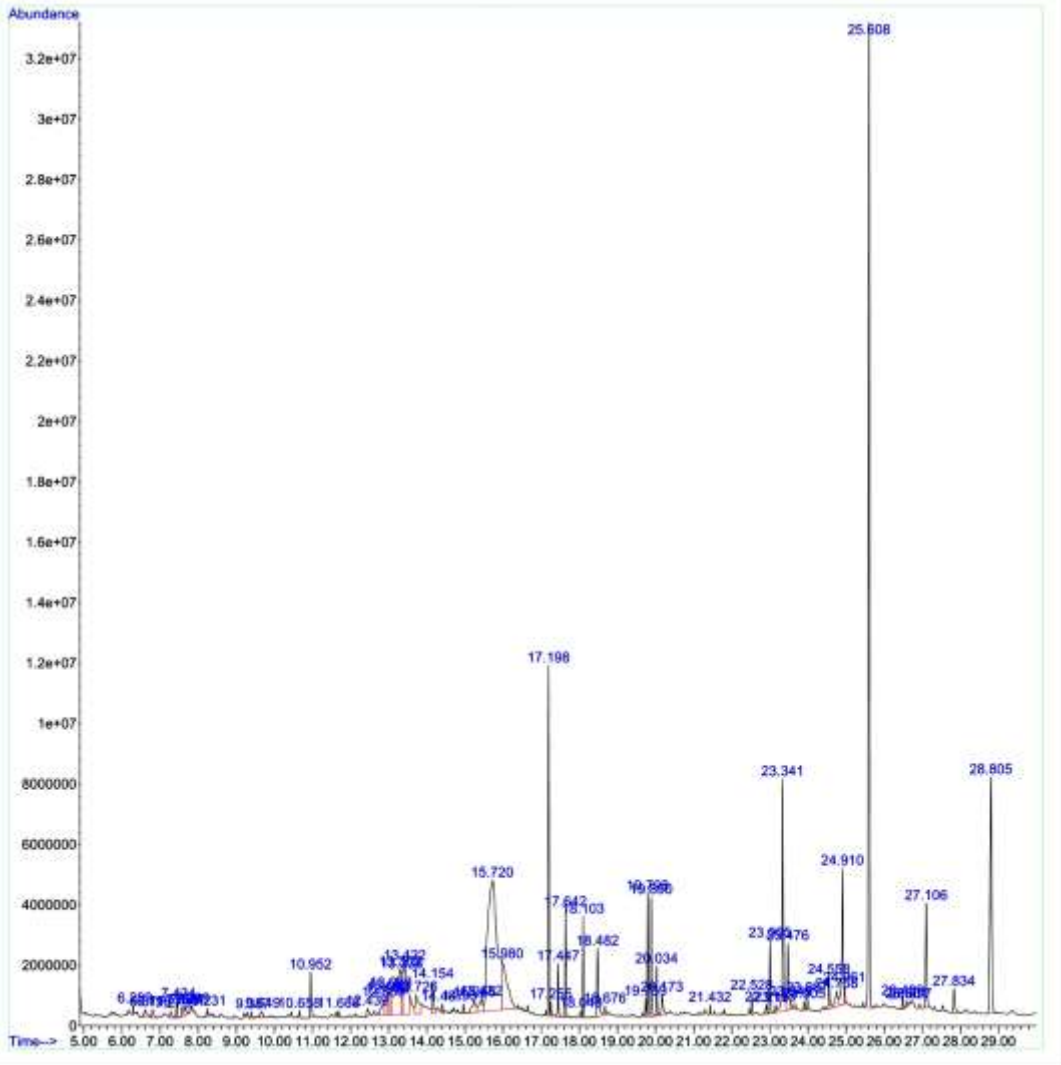

Figure 3. GC-MS chromatogram of methanolic leaf extract of *Tabernaemontana divericata.*

CONCLUSION

This study concludes that leaf extract of *T. divaricata* is highly effective against *M. phaseolina.* Its 5% concentration can reduce biomass of the fungus by 74%. Squalene with 24.11% peak area was found the most abundant compound in the leaf extract followed by neophytadiene (6.25%), and 1-tert-butoxypropan-2-yl 2-methylbutanoate (6.03%).

AUTHORS' CONTRIBUTION

AJ prepared graphs, carried out statistical analysis and wrote the manuscript, IHK did experimental work and

collected the data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

University of the Punjab provided funds (Rs. 360,000) during the fiscal year 2021-22 to carry the project entitled "Exploiting natural compounds of *Tabernaemontana divaricata* to control *Macrophomina phaseolina* and aspergilli".

REFERENCES

Amin, A., Akbar, M., Khalil, T., Akram, W., Ahmad, A., 2022. Antifungal activity of *Alternanthera philoxeroides* organic solvent extracts against plant pathogenic fungi. Pakistan Journal of Botany 54(1), 337-344.

Banaras, S., Javaid, A., Khan, I.H., 2020. Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina.* International Journal of Agriculture and Biology 24(5), 1376-1382.

Banaras, S., Javaid, A., Khan, I.H., 2021. Bioassays guided fractionation of *Ageratum conyzoides* extract for the identification of natural antifungal compounds against *Macrophomina phaseolina.* International Journal of Agriculture and Biology 25(4), 761-767.

Boligon, A.A., Piana, M., Kubiça, T.F., Mario, D.N., Dalmolin, T.V., Bonez, P.C., Weiblen, R., Lovato, L.T., Alves, S.H., Campos, M.M., 2015. HPLC analysis and antimicrobial, antimycobacterial and antiviral activities of *Tabernaemontana catharinensis* A. DC. Journal of Applied Biomedicine 13, 7-18.

Dordab, T., Sourinejad, I., Nazemi M., 2021. Antibacterial effect of the squalene extracted from the liver of the Persian Gulf spot tail shark Carcharhinus sorrah (Müller & Henle, 1839). Journal of Fisheries Science and Technology 10(2), 251-258.

Ferdosi, M.F.H., Ahmed, H., Khan, I.H., Javaid, A., 2022. Fungicidal potential of flower extract of *Cassia fistula* against *Macrophomina phaseolina* and *Sclerotium rolfsii.* Journal of Animal and Plant Sciences 32(4), 1028-1034.

Ferdosi, M.F.H., Khan, I.H., Javaid, A., Nadeem, M., Munir, A. 2021. Biochemical profile of *Calotropis procera* flowers. Pakistan Journal of Weed Science Research 27(3), 341-349.

Ghaneian, M.T., Ehrampoush, M.H., Jebali, A., Hekmatim, O., Mahmoudi, M., 2015. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal 2, 13-16.

Huang, Z.R., Lin Y.K., Fang, J.Y. 2009. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules 14, 540-54.

Hyder, S., Sahi, S.T., Gondal, A.S., Hannan, A., Rehman, A., Ahmed, R., 2018. First report of tomato charcoal rot caused by *Macrophomina phaseolina* (Tassi) *Goid* from Pakistan. Plant Disease 102(7), 1459.

Iqbal, U., Mukhtar, T., 2014. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiata* L.) Wilczek from Pakistan. The Scientific World Journal 2014, Article ID 950175, 9 pages.

Iqbal, U., Mukhtar, T., 2020a. Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. Pakistan Journal of Zoology 52(2): 709-715.

Iqbal, U., Mukhtar, T., 2020b. Evaluation of biocontrol potential of seven indigenous *Trichoderma* species against charcoal rot causing fungus, *Macrophomina phaseolina*. Gesunde Pflanzen, 72(2), 195–202.

Iqbal, U., Mukhtar, T., Iqbal, S.M., 2014. *In vitro* and *in vivo* evaluation of antifungal activities of some antagonistic plants against charcoal rot causing fungus, *Macrophomina phaseolina*. Pakistan Journal of Agricultural Sciences 51 (3): 689-694.

Javaid, A., Khan, I.H., Ferdosi, M.F.H., 2021. Bioactive constituents of wild *Cannabis sativa* roots from Pakistan. Pakistan Journal of Weed Science Research 27(3), 359-368.

Javaid, A., Khan, I.H., Shoaib, A., 2018. Management of charcoal rot of mungbean by two *Trichoderma* species and dry biomass of *Coronopus didymus*. Planta Daninha 36, Article e018182795.

Javed, S., Mahmood, Z., Khan, K.M., Sarker, S.D., Javaid, A., Khan, I.H., Shoaib, A., 2021. Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. Scientific Reports 11, 8417.

Kaur, S., Dhillon, G.S., Brar, S.K., Vallad, G.E., Chand, R.,

Chauhan, V.B., 2012. Biology, economic importance and current diagnostic trends. Critical Review in Microbiology 38, 136-151.

Khan, I.H., Javaid, A., 2020. Antifungal activity and GC-MS analysis of *n*-butanol extract of quinoa (*Chenopodium quinoa* Willd.) leaves. Bangladesh Journal of Botany 49(4), 1045-1051.

Khan, I.H., Javaid, A., 2021. *In vitro* screening of *Aspergillus* spp. for their biocontrol potential against *Macrophomina phaseolina.* Journal of Plant Pathology 103(4), 1195-1205.

Khan, I.H., Javaid, A., 2022a. DNA cleavage of the fungal pathogen and production of antifungal compounds are the possible mechanisms of action of biocontrol agent *Penicillium italicum* against *Macrophomina phaseolina. Mycologia* 114(1), 24-34.

Khan, I.H., Javaid, A., 2022b. Histopathological changes in root and stem of mungbean exposed to *Macrophomina phaseolina* and dry biomass of *Chenopodium quinoa*. Microscopy Research and Technique 85(7), 2596-2606.

Khan, I.H., Javaid, A., 2022c. Hexane soluble bioactive components of leaf extract of quinoa*.* Journal of Animal and Plant Sciences 32(2), 309-314.

Lalitha, S., Parthipan, B., Mohan, V.R., 2015. Determination of bioactive components of Psychotria nilgiriensis Deb & Gang (Rubiaceae) by GC-MS analysis. International Journal of Pharmacognosy and Phytochemical Research 7, 802-809.

Mabkhot, Y.N., Alatibi, F., El-Sayed, N.N.E., Bawazeer, S, Hadda, T.B., 2016. Antimicrobial activity of some novel armed *Thiophene derivatives* and petra/osiris/molinspiration (POM) analyses. Molecules 21, 222.

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

Mikami, Y., YazawaUno, K.J., Matsumae, A., 1990. *In vitro* activity of amphotericin B, flucytosine, fluconazole and miconazole against clinically isolated *Candida albicans*, *Aspergillus funigatus* and *Trichosporon beigelii*. Chemotherapy 38, 1039-1047.

Prasath, K.G., Tharani, H., Kumar, M.S. Pandian, S.K., 2020. Palmitic Acid Inhibits the Virulence Factors of Candida tropicalis: Biofilms, Cell Surface Hydrophobicity, Ergosterol Biosynthesis, and Enzymatic Activity. Frontiers in Microbiology.

Rahman, M., Islam, M.S., Ali, M.S., Islam, R., Hossain, Z., 2011. Antidiabetic and cytotoxic activities of methanolic extract of *Tabernaemontana divaricata* (L.) flowers. International Journal of Drug Development & Research 3, 270-276.

Satyanarayan, D., Joshi, A.B., Chandrasekhar, K.S., Vijaynarayan, K., 2004. Anti- inflammatory activity of the flowers of *T. divaricata* (L.) R.Br. Indian Drugs 41, 405- 409.

Singh, B., Sharma, R.A., Vyas, G.K., 2011. Antimicrobial, antineoplastic and cytotoxic activities of indole alkaloids from *Tabernaemontana divaricata* (L.) R.Br. Current Pharmaceutical Analysis 7, 125-132.

Uroos, M., Javaid, A., Bashir, A., Tariq, J., Khan, I.H., Naz, S., Fatima, S., Sultan, M., 2022. Green synthesis of coumarin derivatives using bronsted acidic pyridinium based ionic liquid [MBSPy][HSO_4] to control an opportunistic human and a devastating plant pathogenic fungus *Macrophemina phaseolina*. RSC Advances 12, 23963-23972.