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Full Length Article

Bioassays Guided Fractionation of *Ageratum conyzoides* Extract for the Identification of Natural Antifungal Compounds against *Macrophomina phaseolina*

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Abstract

Macrophomina phaseolina (Tassi) Goid. is a soil-borne fungal pathogen causing diseases in more than 500 plant species. The present study aimed to identify possible antifungal constituents in different parts of billygoat-weed (*Ageratum conyzoides* L.) through bioassays guided fractionation for the control of *M. phaseolina.* Extracts of different parts of the weed were made in methanol and antifungal bioassays were conducted using 1, 2, 3, 4 and 5% concentrations of the extract. Stem extract caused the highest inhibition in fungal biomass (20–83%) followed by leaf extract (16–67%). Methanolic stem extract was partitioned using four organic solvents namely *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Bioassays carried out with different concentrations (3.125, 6.25, 12.5, 25, 50, 100 and 200 mg mL^{-1}) of the sub-fractions of stem extract revealed the highest antifungal potential of chloroform sub-fraction with 56–93% reduction in the fungal biomass followed by *n*-butanol, ethyl acetate and *n*-hexane sub-fractions causing 24–76%, 7–75% and 5–70% reduction in fungal biomass over control, respectively. Chloroform sub-fraction with the highest antifungal potential was analyzed by GC-MS. Out of 10 compounds identified in this sub-fraction, 2H-1-benzopyran, 7-dimethoxy-2,2-dimethyl- (27.58%) was the most abundant followed by hexadecanoic acid, methyl ester (18.85%); 11-octadecenoic acid, methyl ester (15.28%) and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (10.88%), which could be responsible for antifungal activity.

Keywords: *Ageratum conyzoides*; Antifungal activity; Asteraceae; Billygoat weed; *Macrophomina phaseolina*

Introduction

Macrophomina phaseolina, a soil-borne fungus, is well known for causing a number of diseases mostly charcoal rot in more than 500 plant species including sunflower, chickpea (Lakhran and Ahir 2020), maize (Emayavarman *et al*. 2019), soybean (Yasmin *et al*. 2020), and other economically important crop plants (Degani *et al*. 2020). The pathogen is highly destructive under dry and hot conditions (Pickel *et al*. 2020). The name charcoal rot is because of production of large number of minute black microsclerotia by the fungus which give the plant tissues a black appearance (Sarr *et al*. 2014). Management strategies of charcoal rot pathogen include biological and cultural methods as well as application of fungicides to seeds, however, these methods have provided only limited disease control (Pandey and Basandrai 2020). Moreover, synthetic agro-chemicals create environmental problems and also pose risks to human health causing mutagenic and carcinogenic effects (Singh *et al*. 2009; Westlund *et al*. 2018). There is need of an alternate environmentally friendly strategy for management of *M. phaseolina* and other fungal pathogens.

Recent studies have shown that natural compounds from plants and other organisms or their derivatives can be used as fungicides (Li *et al*. 2018; Akbar *et al.* 2020). Various studies revealed that crude plant extracts and pure compounds are effective in controlling *M. phaseolina.* Methanolic fruit and leaf extracts of *Datura metel* were found highly effective against *M. phaseolina* (Javaid and Saddique 2012). Moreover, soil amendment with dry materials of *D. metel* also significantly reduced charcoal rot disease in mungbean in pot trial (Javaid and Saddique 2011). Likewise, methanolic and *n*-hexane extracts of *Chenopodium album, C. quinoa, C. murale* and *C. ambrosioides* showed profound potential in suppressing *in vitro* growth of *M. phaseolina* (Javaid and Amin 2009; Khan and Javaid 2020). Flavonoids isolated from *Azadirachta indica* and *Mangifera indica* had significant effects in arresting mycelial growth of this pathogen (Kanwal *et al*. 2010, 2011). Recently, Javaid *et al*. (2017a) investigated through bioassays guided fractionation that leaf extract of *Senna occidentalis* possess highly antifungal constituents for the management of *M. phaseolina.* Similar

effects of extracts of *Sisymbrium irio*, *Azadirachta indica* and *Sonchus oleraceous* have also been reported against this fungal pathogen (Javaid *et al*. 2017b; Munir *et al*. 2018; Banaras *et al*. 2020).

Previous studies have shown that extracts of asteraceous weeds such as *Cirsium arvense*, *Sonchus oleraceous* and *Eclipta alba* were highly antifungal effective in inhibiting growth of *M. phaseolina* (Banaras *et al*. 2015, 2017, 2020). However, studies regarding the antifungal effects of asteraceous weeds *Ageratum conyzoids* against *M. phaseolina* are lacking. This annual tropical weed is common in West Africa as well as in parts of South America and Asia where it has been used against a number of diseases (Marks and Nwachuku 1986). In West Africa, it is used for wound healing, skin diseases, curing malaria, gastrointestinal pain, measles, headache and eye diseases (Okunade 2002; Ukwe *et al*. 2010). Keeping in view antifungal activity of asteraceous weeds and unavailability of a registered fungicide against *M. phaseolina*, the present study was undertaken to investigate for the antifungal activity of extracts of different parts of *A. conyzoides* against *M. phaseolina* and identification of possible antifungal compounds through GC-MS analysis.

Materials and Methods

Bioassays with methanolic extracts

A. conyzoides plants were collected from Lahore, Pakistan and its different parts *viz.* leaf, stem, root and inflorescence were separated. Hundred grams of each part were soaked in 1000 mL of 80% methanol for 2 weeks. Thereafter, materials were passed through muslin cloth followed by filtration through Whatman No. 1 filter papers. After evaporation of the solvent on a rotary evaporator (Model ROTVAP, UTECH Products INC. Albani NY, U.S.A.), the final traces of the solvent were evaporated in an electric oven at 45°C to get 16, 13, 12 and 10 g of leaf, stem, root and inflorescence extracts, respectively (Akhtar and Javaid 2018).

For antifungal bioassays, 9 g of each extract were dissolved in 5 mL dimethyl sulfoxide (DMSO) and sterilized distilled water was added to prepare 15 mL of stock solution. Likewise, 15 mL of a control solution were made by mixing the same amount of DMSO in distilled water. Measured quantities of stock and control solutions were added to 55 mL pre-autoclaved malt extract broth (MEB) to get 60 mL of growth medium of each concentration that were divided into 4 equal portions to serve as replicates. There were six concentrations *viz.*, 0, 1, 2, 3, 4 and 5% (w/v). Experiment was carried out in 100-mL conical flasks with 15 mL growth medium in each flask following Javaid *et al*. (2018). The flasks were inoculated with 5 mm plugs of *M. phaseolina* (isolated from charcoal rot infected mash bean plants) followed by incubation at 27°C for one week. Thereafter, fungal biomass was weighed after filtering and drying at 70°C.

Bioassays with sub-fractions of methanolic stem extract

Methanolic stem extract was selected for further experimentation on the basis of its best antifungal activity in laboratory bioassays. Methanolic extract was obtained by soaking 3 kg of crushed stem of the weed for 2 weeks, filtration and evaporation on a rotary evaporator. To this extract, 200 mL of distilled water was added. It was partitioned with *n*-hexane in a separating funnel. After repeating the process several times for complete separation of *n*-hexane soluble components, the remaining aqueous phase was serially partitioned with 500 mL of each of chloroform, ethyl acetate and *n*-butanol. All the solvents were evaporated on a rotary evaporator and the obtained sub-fractions were used in antifungal bioassays against the target fungal pathogen (Javaid *et al*. 2017b).

Antifungal bioassays were carried out in 10-mL volume test tubes. A stock solution of 200 mg mL^{-1} was prepared by dissolving 1.2 g of each sub-fraction in 1 mL DMSO followed by addition of autoclaved malt extract broth to raise the volume up to 6 mL. Three milliliters of this growth media were used in antifungal bioassays (1 mL in each test tube) while rest of the volume was serially double diluted to prepare 100, 50, 25, 12.5, 6.25 and 3.125 mg mL^{-1} concentrations. For preparation corresponding control treatments, 1 mL of DMSO was added to 5 mL autoclaved MEB followed by serial double dilutions as in case of experimental treatments following the procedure given by Javaid *et al*. (2017b). To each test tube, 20 μL suspension of *M. phaseolina* was added and tubes were incubated at 27ºC for one week. Biomass of *M. phaseolina* was filtered and weighed after drying at 70ºC.

GC-MS analysis

GC-MS analysis of chloroform sub-fraction of methanolic stem extract was performed on Agilant Technologies Model GC-7890A attached with mass spectrometer MS 5975C.

Statistical analysis

All the data regarding fungal biomass in different laboratory bioassays were analyzed by ANOVA. Mean separation was carried out by applying LSD test at *P* 0.05 using Statistix 8.1.

Results

Bioassays with methanolic extracts

ANOVA indicated significant differences in plant parts (P), extract concentration (C) as well as their interaction for biomass production of *M. phaseolina* (Table 1). Stem extract exhibited the highest antifungal activity followed by leaf extract causing 20–83% and 16–67% reduction in biomass of *M. phaseolina*, respectively. Root and inflorescence extracts showed lower antifungal effects than

Table 1: Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem, root and inflorescence extracts of *A. conyzoides* on biomass of *M. phaseolina*

Sources of variation	df	SS	MS	F values
Plant parts (P)	3	44838	14946	2137*
Concentration (C)	5	73857	14772	2112*
P × C	15	19646	1310	187*
Error	72	503	7	
Total	95	138845		

*, Significant at $P \leq 0.001$

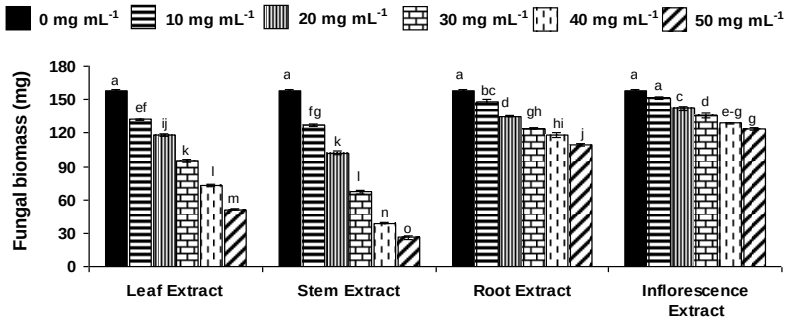

Fig. 1: Effect of methanolic extracts of different parts of *A. conyzoides* on biomass of *M. phaseolina*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's HSD test

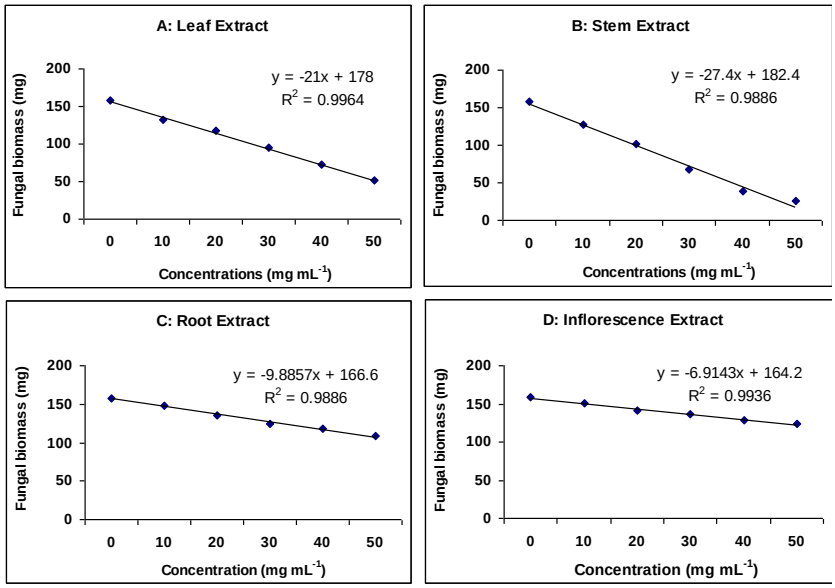

Fig. 2: Regression analysis for the effect of different concentrations of methanolic leaf, stem, root and inflorescence extracts of *A. conyzoides* on biomass of *M. phaseolina*

the extracts of other two parts of the weeds resulting in 6–31 and 4–21% suppression in fungal biomass over control, respectively (Fig. 1). In general, fungal biomass was gradually reduced by increasing the extracts concentrations. A linear association was recorded between fungal biomass and extract concentration with R^2 = 0.9964, 0.9886, 0.9886 and 0.9936 for leaf, stem, root and inflorescence extracts, respectively (Fig. 2).

Bioassays with sub-fractions of methanolic stem extract

Generally, higher concentrations of all the sub-fractions significantly reduced growth of *M. phaseolina* in terms of its biomass production. Chloroform fraction showed the highest antifungal potential and all of its concentrations significantly reduced fungal biomass by 56–93%. The inhibitory activity was concentration dependent (Fig. 3B,

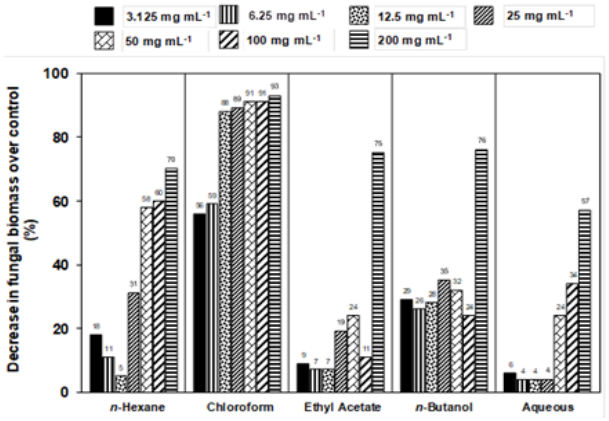

Fig. 4: Percentage decrease in biomass of *M. phaseolina* due to different sub-fractions of methanolic stem extract of *A. conyzoides* over control

4). In *n*-butanol sub-fraction, although all the concentrations significantly reduced fungal biomass, however, the effect was less obvious as compared to chloroform sub-fraction and there was only 24–76% decrease in fungal biomass (Fig. 3D, 4). In case of *n*-hexane and ethyl acetate sub-fractions, the effect of 25–200 mg mL^{-1} concentrations was significant (P = 0.05). Different concentrations of these sub-fractions reduced fungal biomass by 5–70% and 7–75%, respectively (Fig. 3A and C, 4). The aqueous sub-fraction exhibited the least activity against *M. phaseolina* where only 50% and higher concentrations showed significant effect and different concentrations reduced fungal biomass just by 4–57% over control (Fig. 3E, and Fig. 4).

GC-MS analysis

GC-MS chromatogram, presented in (Figs. 5) revealed the presence of 10 compounds in chloroform sub-fraction. Names and other details regarding retention time, molecular weights and chemical formulae of the identified compounds are presented in Table 2. Structures of the compounds are shown in Fig. 6. The most abundant compound was 2H-1-benzopyran, 7-methoxy-2, 2-dimethyl- (2) followed by hexadecanoic acid, methyl ester (5); 11-octadecenoic acid, methyl ester (7); 9, 12-octadecanoic acid (Z,Z)-, methyl ester (6) and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (10) with 27.58, 18.85, 15.28, 13.67 and 10.88% peak areas, respectively. Other compounds namely octadecanoic acid, methyl ester (8); morphinan, 7,8-didehydro-4,5-epoxy-3,6-dimethoxy-17-methyl-, (5.alpha, 6.alpha)- (9); 1-hexadecanol, 2-methyl- (3); 2-pentadecanone, 6, 10, 15-trimethyl- (4) and 2H-1-benzopyran, 7-methoxy-2, 2-dimethyl- (1) were present in lower concentrations with peak areas ranging from 2.42 to 3.46%.

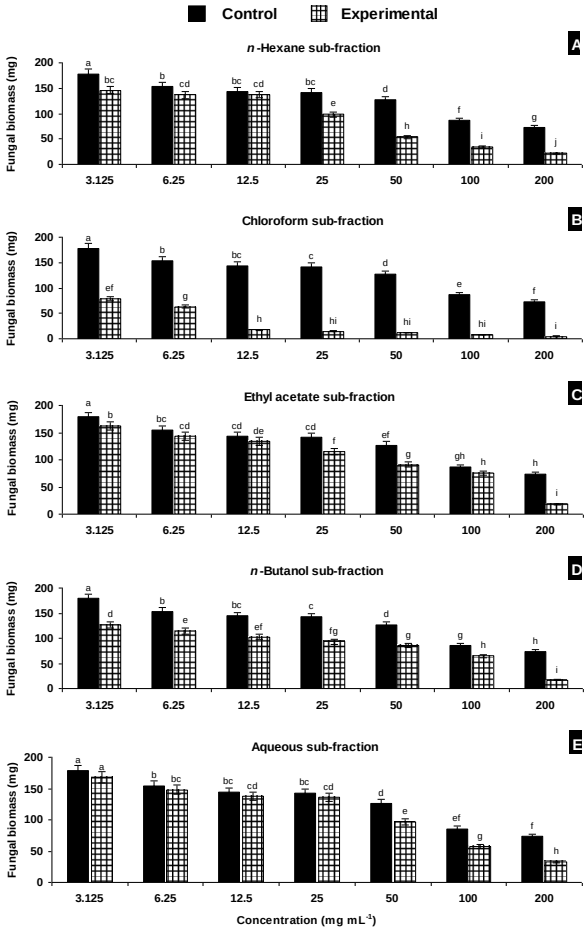

Fig. 3: Effect of different concentrations of sub-fractions of methanolic stem extract of *A. conyzoides* on growth of *M. phaseolina*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's HSD test

Discussion

In the present study, methanolic stem, leaf, root and inflorescence extracts of *A. conyzoides* significantly reduced the *M. phaseolina* fungal biomass. Earlier studies have shown similar antifungal activities of extracts and essential oils of *A. conyzoides* against different fungal species such as *Puccinia arachidis* (Yusnawan and Inayati 2018), *Fusarium oxysporum* (Lian *et al*. 2019), *Penicillium notatum*, *Rhizopus stolon* and *Aspergillus niger* (Omole *et al*. 2019). Ethanolic extract of this weed markedly reduced the growth of *F. lateritium*, *F. solani*, *Cochliobolus lunatus* (Ilondu 2013) and *Phytophthora megakarya* (Ndacnou *et al.* 2020). A coumarin compound isolated from acetone fraction of leaves of *A. conyzoides* showed remarkable activity against *Aspergilus niger* (Widodo *et al.* 2012). Likewise, essential oils of *A.*

Table 2: Compounds identified from chloroform sub-fraction of methanolic stem extract of *A. conyzoides* through GC-MS analysis

Comp. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak Area (%)
1	2H-1-Benzopyran, 7-methoxy-2, 2-dimethyl-	$C_{12}H_{14}O_2$	190	13.695	2.42
2	2H-1-Benzopyran, 6,7-methoxy-2, 2-dimethyl-	$C_{13}H_{16}O_3$	220	16.099	27.58
3	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	17.340	2.56
4	2-Pentadecanone, 6, 10, 15-trimethyl-	$C_{18}H_{36}O$	268	17.926	2.53
5	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	18.758	18.85
6	9, 12-Octadecanoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	20.406	13.67
7	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	20.466	15.28
8	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	20.653	3.46
9	Morphinan, 7,8-didehydro-4,5-epoxy-3,6-dimethoxy-17-methyl-, (5.alpha, 6.alpha)-	$C_{19}H_{23}NO_3$	313	23.839	2.77
10	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	24.246	10.88

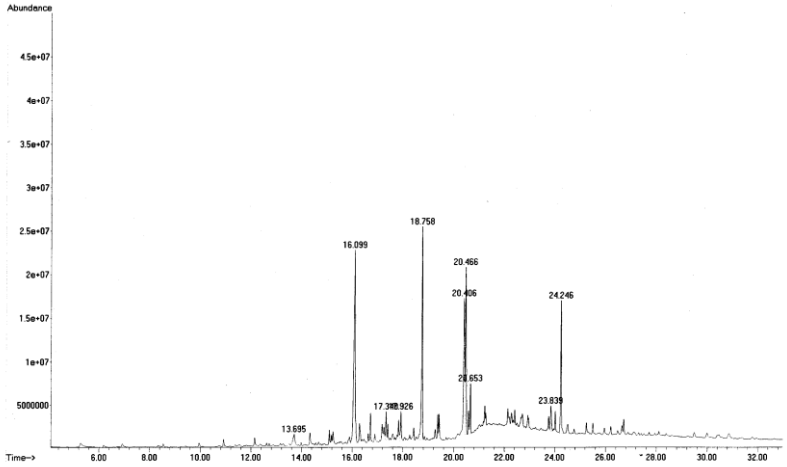

Fig. 5: GC-MS chromatogram of chloroform subfraction of methanolic stem extract of *A. conyzoides*

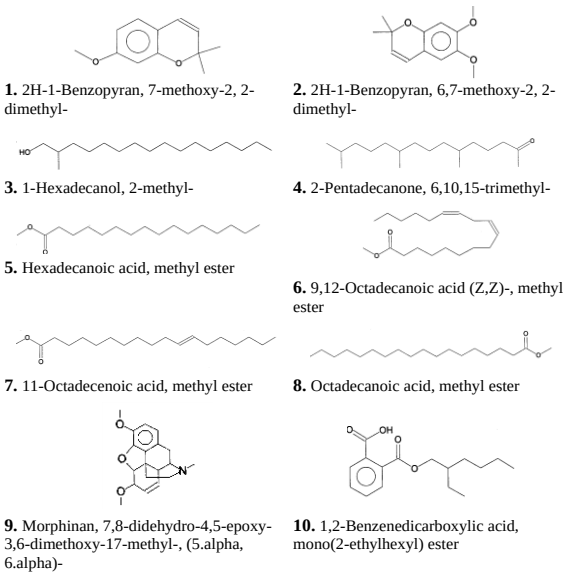

Fig. 6: Structures of compounds identified in chloroform sub-fraction of methanolic stem extract of *A. conyzoides* through GC-MS analysis

conyzoides are known to exhibit antifungal activity against *A. parasiticus* and *A. flavus* (Nogueira *et al*. 2010; Patil *et al*. 2010). Compounds belonging to flavonoids glycosides, tannins, resins, saponins and alkaloids are reported to be present in different parts of *A. conyzoides* (Aja *et al*. 2016), most of which are known for their antifungal activity (Kanwal *et al*. 2010).

Generally, sub-fractions prepared from methanolic stem extracts of *A. conyzoides* retarded the pathogen growth variably at different concentrations. The variations in antifungal activities of different sub-fractions of methanolic extracts of other plant species namely *Chenopodium album*, *C. quinoa*, *C. murale*, *Coronopus didymus*, *Senna occidentalis* and *Sisymbrium irio* have also been reported in other similar studies (Rauf and Javaid 2013; Javaid and Iqbal 2014; Javaid *et al*. 2017a, b; Naqvi *et al*. 2019; Khan and Javaid 2020). This variation may be attributed to different polarity natures of the organic solvents used for separation of compounds in methanolic stem extract of *A. conyzoides*. These solvents comprised of non-polar *n*-hexane on one side and highly polar *n*-butanol on the other hand. Compounds present in stem extract were dissolved in various solvents on the bases of their polarity natures during partitioning process and thus different sub-fractions showed different antifungal activities. Similar to that of the present study, in previous studies higher antifungal activities of

chloroform sub-fractions of other plant species have also been reported against *M. phaseolina* (Javaid *et al*. 2017a, b; Khan and Javaid 2020). Recently, Banaras *et al.* (2020) have reported similar antifungal activity of chloroform sub-fraction of an asteraceous weed *S. oleraceous* against *M. phaseolina.*

Chloroform fraction was proved to be very effective in suppressing the growth of *M. phaseolina* in comparison to the other sub-fractions. Therefore, it was selected for GC-MS analysis that revealed the presence of ten phytoconstituents, some of which also known for their antifungal activities against other fungal species. For intense, compound 2 commonly known as precocene (Kouame *et al.* 2018), has been identified as one of the major components in essential oil of *A. conyzoides* ranging from 30–93% and inhibited the growth of *A. flavus* (Castro *et al.* 2004; Esper *et al.* 2015). Iqbal *et al*. (2004) isolated precocene from *A. conyzoides* and reported that 80–100 ppm concentration of this compound can completely control growth of *Sclerotium rolfsii* and *Rhizoctonia solani.* Similarly, compound 5, 6, 7 and 8 belong to fatty acid methyl esters group. Members of this group are generally known for their antifungal activity against a number of fungal species (Agoramoorthy *et al*. 2007; Lima *et al*. 2011; Ali *et al.* 2017). In the present study, compound 10 was also found as an important compound present in reasonable concentration in the chloroform sub-fraction. This plasticizer compound has been identified in a number of plants (*Polygonum chinense* and *Chenopodium album*), bacteria (*Streptomyces* spp.) and fungi (*Alternaria* spp.), and exhibited cytotoxic, anti-inflammatory and anti-oxidant properties (Ezhilan and Neelamegam 2012; Govindappa *et al*. 2014; Krishnan *et al*. 2014; Ali *et al*. 2017). Recently, Zhang *et al*. (2018) have identified this compound in *Trichoderma longibrachiatum* showing antifungal activity against a number of phytopathogenic fungi.

Conclusion

The present study concludes that methanolic stem extract of *A. conyzoides* and its chloroform sub-fraction are highly antifungal against *M. phaseolina.* The antifungal activity of the chloroform sub-fraction is possibly because of 2H-1-benzopyran, 7-methoxy-2, 2-dimethyl- as well as 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester and various fatty acid methyl esters.

Author Contributions

SB conducted the study, AJ supervised the work and wrote a part of this paper. IHK contributed in paper writing.

Conflict of Interest

The authors declare no conflict of interest among them of any sort

Data Availability Declaration

We hereby declare that the data relevant to this paper is available and will be provided on request

References

Agoramoorthy G, M Chandrasekaran, V Venkatesalu, MJ Hsu (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Braz J Microbiol* 38:739–742

Aja PM, OC Enechi, VEO Ozougwu, EA Onya-Mmaghiri, KA Agu, A Ali, OL Nweke (2016). Phytochemical composition, gas chromatography-mass spectrometric (GC-MS) analysis and antibacterial activity of ethanol leaf-extract of *Ageratum conyzoides*. *Afr J Basic Appl Sci* 8:34–40

Akbar M, IN Sherazi, T Khalil, MS Iqbal, S Akhtar, SN Khan (2020). Identification of antifungal compounds from slender amaranth. *Planta Danin* 38; Article e020207096

Akhtar R, A Javaid (2018). Biological management of basal rot of onion by *Trichoderma harzianum* and *Withania somnifera*. *Planta Danin* 36; Article e018170507

Ali A, A Javaid, A Shoaib (2017). GC-MS analysis and antifungal activity of methanolic root extract of *Chenopodium album* against *Sclerotium rolfsii*. *Planta Danin* 35; Article e017164713

Banaras S, A Javaid, IH Khan (2020). Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina*. *Intl J Agric Biol* 24:1376–1382

Banaras S, A Javaid, A Shoaib, E Ahmed (2017). Antifungal activity of *Cirsium arvense* extracts against a phytopathogenic fungus *Macrophomina phaseolina*. *Planta Danin* 35; Article e017162738

Banaras S, A Javaid, SM Iqbal (2015). Use of methanolic extracts of an asteraceous weed *Eclipta alba* for control of *Macrophomina phaseolina*. *Pak J Weed Sci Res* 21:101–110

Castro HG, LOE Oliveira, LCB Almeida, FA Ferreira, DJH Silva, PR Mosquim, EA Nascimento (2004). Content and composition of the essential oil of five accesses of mentrasto. *Quím Nova* 27:55–57

Degani O, S Dor, D Abraham, R Cohen (2020). Interactions between *Magnaporthiopsis maydis* and *Macrophomina phaseolina*, the causes of wilt diseases in maize and cotton. *Microorganisms* 8:249-270

Emayavarman P, P Renukadevi, R Ravikesavan, S Nakkeeran (2019). Antifungal efficacy and growth promotion by *Trichoderma virens* TRI 37 and *Bacillus amyloloiquefaciens* (VB7) against *Macrophomina phaseolina*-the maize charcoal rot pathogen. *Intl J Curr Microbiol Appl Sci* 8:2682–2693

Esper RH, E Gonçalez, RC Felicio, JD Felicio (2015). Fungicidal activity and constituents of *Ageratum conyzoides* essential oil from three regions in São Paulo state, Brazil. *Arq Inst Biol* 82:1–4

Ezhilan BP, R Neelamegam (2012). GC-MS analysis of phytocomponents in the ethanol extract of *Polygonum chinense* L. *Pharm Res* 4:11–14

Govindappa M, P Somu, V Vinay, C Ryavalad (2014). Chemical composition of methanol extract of endophytic fungi, *Alternaria* spp. of *Tebebuia argentea* and their antimicrobial and antioxidant activity. *Intl J Biol Pharm Res* 5:861–869

Ilondu EM (2013). Phytochemical composition and efficacy of ethanolic leaf extracts of some *Vernonia* species against two phytopathogenic fungi. *J Biopest* 6:165–172

Iqbal MCM, ULB Jayasinghe, HMTB Herath, KB Wijesekara, Y Fujimoto (2004). A fungistatic chromene from *Ageratum conyzoides*. *Phytoparasitica* 32:119-126

Javaid A, A Saddique (2012). Control of charcoal rot fungus *Macrophomina phaseolina* by extracts of *Datura metel*. *Nat Prod Res* 26:1715–1720

Javaid A, A Saddique (2011). Management of Macrophomina root rot of mungbean using dry leaves manure of *Datura metel* as soil amendment. *Span J Agric Res* 9:901–905

Javaid A, D Iqbal (2014). Management of collar rot of bell pepper (*Capsicum annuum* L.) by extracts and dry biomass of *Coronopus didymus* shoot. *Biol Agric Hortic* 30:164–172

Javaid A, M Amin (2009). Antifungal activity of methanol and *n*-hexane extracts of three *Chenopodium* species against *Macrophomina phaseolina*. *Nat Prod Res* 23:1120–1127

Javaid A, GR Shahzad, N Akhtar, G Ahmed (2018). *Alternaria* leaf spot disease of broccoli in Pakistan and management of the pathogen by leaf extract of *Syzygium cumini*. *Pak J Bot* 50:1607–1614

Javaid A, H Qudsia, A Shoaib (2017a). Bioassays guided fractionation of *Senna occidentalis* for identification of natural antifungal constituents against *Macrophomina phaseolina*. *Planta Danin* 35; Article e017163483

Javaid A, L Afzal, A Shoaib (2017b). Antifungal potential of a brassicaceous weed *Sisymbrium irio* against *Macrophomina phaseolina*. *Planta Danin* 35; Article e017164280

Kanwal Q, I Hussain, HL Siddiqui, A Javaid (2011). Antimicrobial activity screening of isolated flavonoids from *Azadirachta indica* leaves. *J Serb Chem Soc* 76:375–384

Kanwal Q, I Hussain, HL Siddiqui, A Javaid (2010). Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves. *Nat Prod Res* 24:1907–1914

Khan IH, A Javaid (2020). Comparative antifungal potential of stem extracts of four quinoa varieties against *Macrophomina phaseolina*. *Intl J Agric Biol* 24:441–446

Kouame BKFP, D Toure, L Kabla, G Bedi, I Tea, R Robins, JC Chalchat, F Tonzibo (2018). Chemical constituents and antibacterial activity of essential oils from flowers and stems of *Ageratum conyzoides* from Ivory Coast. *Rec Nat Prod* 12:160–168

Krishnan K, A Mani, S Jesmine (2014). Cytotoxic activity of bioactive compound 1, 2- benzene dicarboxylic acid, mono 2- ethylhexyl ester extracted from a marine derived *Streptomyces* spp. VITSJK8. *Intl J Mol Cell Med* 3:246–254

Lakhran L, RR Ahir (2020). *In vivo* evaluation of different fungicides, plant extracts, biocontrol agents and organics amendments for management of dry root rot of chickpea caused by *Macrophomina phaseolina*. *Legum Res* 43:140–145

Li D, TTM Loung, WJ Dan, JM Jao (2018). Natural products as sources of new fungicides (IV): Synthesis and biological evaluation of isobutyrophenone analogs as potential inhibitors of class-II fructose-1,6-bisphosphate aldolase. *Bioorg Med Chem* 26:386–393

Lian F, R Xue, X Lin, Y Yang, H Zhang, R Zeng, Y Song (2019). Inhibitory effects of aqueous leachates and volatiles from *Allium tuberosum* and *Ageratum conyzoides* on *Fusarium oxysporum* f. spp. *cubense*. *J South Chin Agric Univ* 40:40–46

Lima LARDS, S Johann, PS Cisalpino, LPS Pimenta, MAD Boaventura (2011). *In vitro* antifungal activity of fatty acid methyl esters of the seeds of *Annona cornifolia* (Annonaceae) against pathogenic fungus *Paracoccidioides brasiliensis*. *Rev Soc Bras Med Trop* 44:777–780

Marks MK, AC Nwachuku (1986). Seed-bank characteristics in a group of tropical weeds. *Weed Res* 26:151–158

Munir M, A Shoaib, A Javaid, Z Arshad, M Rafiq (2018). Anti-mycotic potential of *Trichoderma* spp. and leaf biomass of *Azadaricta indica* against the charcoal rot pathogen *Macrophomina phaseolina* (Tassi) Goid in cowpea. *Egypt J Biol Pest Cont* 28:26-32

Naqvi SF, A Javaid, MZ Qureshi (2019). Evaluation of antifungal potential of leaf extract of *Chenopodium murale* against *Fusarium oxysporum* f. sp. *lycopersici*. *Planta Danin* 37; Article e019199050

Ndacnou MK, A Pantaleon, JBS Tchinda, ELN Mangapche, F Keumedjio, DB Boyoguemo (2020). Phytochemical study and anti-oomycete activity of *Ageratum conyzoides* Linnaeus. *Indust Crop Prod* 153; Article 112589

Nogueira JHC, E Gonçalez, SR Galleti, R Faanali, MOM Marques, JD Felício (2010). *Ageratum conyzoides* essential oil as aflatoxin suppressor of *Aspergillus flavus*. *Intl J Food Microbiol* 137:55–60

Okunade AL (2002). *Ageratum conyzoides* L. (Asteraceae). *Fitoterapia* 73:1–16

Omole OA, JO Oladipo, BO Orimolade, OO Ajetomobi, KS Olorunmaiye, OO Dosumu (2019). Anti-oxidant and anti-microbial activities of the root and leaf extracts of *Ageratum conyzoides* L. *Agric Consp Sci* 84:295–304

Pandey AK, AK Basandrai (2020). Will *Macrophomina phaseolina* spread in legumes due to climate change? A critical review of current knowledge. *J Plant Dis Prot* 96:1–10

Patil RP, MS Nimbalkar, UU Jadhav, VV Dawkar, SP Govindwar (2010). Antiaflatoxigenic and antioxidant activity of an essential oil from *Ageratum conyzoides* L. *J Sci Food Agric* 90:608–614

Pickel B, N Dai, M Maymon, M Elazar, Z Tanami, O Frenkel, S Freeman (2020). Development of a reliable screening technique for determining tolerance to *Macrophomina phaseolina* in strawberry. *Eur J Plant Pathol* 157:707–718

Rauf S, A Javaid (2013). Antifungal activity of different extracts of *Chenopodium album* against *Fusarium oxysporum* f. sp. *cepae* the cause of onion basal rot. *Intl J Agric Biol* 15:367–371

Sarr M, M Ndiaye, J Groenewald, P Crous (2014). Genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot. *Phytopathol Mediterr* 53:250–268

Singh B, DK Sharma, AA Gupta (2009). Study towards release dynamics of thiram fungicide from starch–alginate beads to control environmental and health hazards. *J Hazard Mater* 161:208–216

Ukwe CV, OI Ekwunife, EA Epueke, CM Ubaka (2010). Antimalarial activity of *Ageratum conyzoides* in combination with chloroquine and artesunate. *Asian Pac J Trop Med* 3:943–947

Westlund P, D Nasuhoglu, S Isazadeh, V Yargeau (2018). Investigation of acute and chronic toxicity trends of pesticides using high-throughput bioluminescence assay based on the test organism *Vibrio fischeri*. *Arch Environ Contamin Toxicol* 74:557–567

Widodo GP, EY Sukandar, IK Adnyana (2012). Mechanism of action of coumarin against *C. albicans* by SEM/TEM analysis. *J Math Fundament Sci* 44:145–151

Yasmin H, R Naz, A Nosheen, MN Hassan, N Ilyas, M Sajjad, Z Geng (2020). Identification of new biocontrol agent against charcoal rot disease caused by *Macrophomina phaseolina* in soybean (*Glycine max* L.). *Sustainability* 12:6856-6879

Yusnawan E, A Inayati (2018). Antifungal activity of crude extracts of *Ageratum conyzoides*, *Cyperus rotundus*, and *Amaranthus spinosus* against rust disease. *J Agric Sci* 40:403–414

Zhang S, B Xu, J Zhang, Y Gan (2018). Identification of the antifungal activity of *Trichoderma longibrachiatum* T6 and assessment of bioactive substances in controlling phytopathgens. *Pest Biochem Physiol* 147:59–66