GC-MS ANALYSIS OF *Sonchus asper* ROOT EXTRACT FOR IDENTIFICATION OF FUNGICIDAL COMPOUNDS AGAINST *Rhizoctonia solani*

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ABSTRACT

Black scurf disease incited by Rhizoctonia solani causes a heavy burden on the potato crop due to occurrence of large number of persistent sclerotia on tubers, while the current conventional practices against diseases are limited and are associated with toxicity and resistance. Botanical fungicides have shown enormous antifungal potential against many sclerotial forming phytopathogens. In the present study, methanolic root extract of Sonchus asper was assessed for its antifungal activity against R. solani. The methanolic extract was prepared by soaking dried root biomass in methanol and analyzed by GC-MS. Antifungal potential of the extract was detected in test tubes filled with malt extract broth. The results revealed fungal biomass was significantly reduced (52–97%) with increase in concentrations of the extract (1.56–200 mg mL^{-1}). GC-MS analysis revealed that benzoic acid, 4-hydroxy-3-methoxy-, methyl ester; 13-cis-retinoic acid; pyridine, 2-pentyl-; 9-octadecenamide, (Z)- and L-proline were important compounds responsible for the antifungal activity. Hence, methanolic root extract might be a potential library for fungicidal compounds against R. solani.

Keywords: GC-MS, *Rhizoctonia solani*, *Sonchus asper*, weed.

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INTRODUCTION

Rhizoctonia solani is a necrotrophic, soil and seed-borne phytopathogen causes black scurf on potato affecting stem and stolons, and the disease is responsible for 30–50% yield losses (Carling *et al.*, 1989). The disease is prevalent in all the eight potato growing zones of Pakistan (Rauf *et al.*, 2015). The most noticeable sign of black scruf appears as sclerotia (superficial, hard and irregularly shaped dark brown to black masses) on daughter tubers, which causes deformed and cracked tubers. The pathogen also causes root damage, shoot stunting, upward leaf rolling and purple pigmentation in the uppermost leaves. In case of severe infection, the aerial tubers start appearing in the axils of branches and petioles. All of these symptoms may appear on infected potato plants either separately or in combination (Muzhinji *et al.*, 2018). Generally, certified seeds, tillage, crop rotation and fungicides are considered as the appropriate integrated strategies to combat this menace (Ajayi-Oyetunde and Bradley, 2018). The over-wintering nature as long-lived sclerotia or as mycelia and extensive host range of *R. solani* make it hard to manage through traditional cultural and chemical methods (Ajayi-Oyetunde and Bradley, 2018).

Currently, the search for natural products, particularly related to pest management is very active. Aromatic and medicinal plants have attracted interest in the field of plant disease control and have shown enormous antifungal potential against many sclerotial forming robust phytopathogens (Javaid *et al.*, 2018). Moreover, plenty of literature is available on antifungal activity of extract of many botanical fungicides against notorious phytopathogens including *R. solani*. Iranbakhsh *et al.* (2010) reported that extract of different plant parts (seed, flower, leaves and root and stem) of *Datura stramonium* had differential inhibitory effect on growth of *Certocystis ulmi*, *Fusarium semitectum* and *R. solani* due to occurrence of varied type and proportion of phytochemicals in each part. Seema *et al.* (2014) reported ethyl acetate extract of *Lawsonia inermis*, *Pelargonium graveolens* and *Piper betel* completely halted *R. solani* growth. Meela *et al.* (2019) used acetone extracts of different weeds for their antifungal potential against *R. solani, F. oxysporum, Pythium ultimum* and *Phytophthora nicotiana* and observed better activity against some pathogens as compared to available commercial fungicides. Persaud *et al.* (2019) evaluated eleven plant extracts, three biocontrol agents and five fungicides against *R. solan*i. The extracts (15%) of lemon grass (*Cymbopogon citratus*), thick leaf thyme (*Plectranthus amboinicus*), marigold (*Tagetes minuta*) and clove (*Syzygium aromaticum*) showed greater retardation in hyphal growth of *R. solani* and helped in managing sheath blight disease in rice.

Sonchus asper of family Asteraceae is annual or biennial herb, which is native to Europe, North Africa, and western Asia, distributed worldwide and abundantly available in Pakistan. It has a varied nutritional and pharmacological spectrum as anti-cancer, anxiolytic and anti-inflammatory due to occurrence of important compounds in its different parts (alkaloids, terpenoids, phenols, quinones, flavonoids and glycosides, saponins and tannins) (Li and Yang, 2018). These chemical constituents also exhibit antimicrobial potential through alternating cell membrane permeability and inhibiting enzyme activity (El-Mogy and Alsanius, 2012). Therefore, the present *in vitro* investigation was undertaken to determine fungicidal components in root extract of *S. asper* against *R. solani.*

MATERIALS AND METHODS

Isolation and culturing of the pathogen

The pathogen, *R. solani* was isolated from potato tubers infected with black scurf (diseased and deformed tubers having sclerotia on the surface). The infected potato tubers were dipped in 1% solution of sodium hypochlorite for 3 min followed by continuous rinsing with

autoclaved distilled water. Small pieces of infected tubers were placed on autoclaved 2% malt extract agar (MEA) medium in Petri plates. After one week of incubation at 25 °C, the emerging hyphae from samples were re-inoculated on fresh MEA plates to get pure culture of the fungus and for further use (Javaid *et al.,* 2019).

Preparation of methanolic root extract

S. asper was collected from Lahore, Pakistan. Roots were separated, washed, sun dried and finally in the electric oven at 40 ºC. Two hundred grams of root material was dipped in 1 L of methanol for 2 weeks in air tight jar. Solvent was filtered through Whatman No. 1 filter paper. Excess methanol was removed by using the rotary evaporator at 45 ºC. The filtrates were transferred to pre-weighed glass beakers and the solvent was completely dried at 45 ºC in an oven to get to 11.01 g root extract of *S. asper*.

Antifungal assays

Protocol described by Banaras *et al*. (2017) was employed for antifungal assays with 8 concentrations of methanolic root extract (1.562, 3.125, 6.25, 12.50, 25.00, 50.00, 100 and 200 mg mL^{-1}) in 10 mL test tubes. Extract (1.2 g) was dissolved in 1 mL of dimethyl sulphoxide (DMSO) and added ME broth to get 6 mL of 200 mg mL^{-1} concentration. For preparation of same volume of the control solution, 1 mL of DMSO was mixed with 5 mL of autoclaved ME broth. Later, 3 mL of medium (200 mg mL^{-1}) was mixed with 3 mL control solution to make 1st dilution (100 mg mL^{-1}); and the process was repeated 8 times for the serial dilution. The inoculum of the fungus was prepared in distilled water, 10 µL of which was added in each test tube containing 1 mL of the growth medium. Each treatment was replicated three times. The test tubes were incubated at 27 ºC for one week. Thereafter, the fungal biomass was taken on pre-weighed filter papers to record dry biomass of the fungus after drying in an oven at 70 ºC.

GC-MS analysis of methanolic root extract

For identification of constituents in methanolic root extract, the GC-MS analysis of was performed on a machine (Agilant Technologies Model GC-7890A), attached with mass spectrometer (MS 5975C) and fused with a capillary column (30 × 0.25 µm ID × 0.25 µm df). An electron ionization system operated in electron impact mode was used for GC-MS detection with 70 eV ionization energy and helium gas (99.999%) at rate of 1 mL min^{-1} was used as a carrier. The temperature of injector was kept at 260 °C, the temperature of ion-source was 200 °C, the temperature of oven was started from 50 °C, keeping it constant for 2 min leading to an increase of 10 °C min^{-1} and it went to 310 °C. The retention catalogues were used and mass spectral fragmentation patterns were compared with already reported compounds in the literature, which were stored in the NIST database.

RESULTS AND DISCUSSION

Various concentrations (1.56–200 mg mL^{-1}) of methanolic root extract of *S. asper* significantly ($P \leq 0.05$) inhibited biomass of *R. solani* by 52–97% (Fig. 1). Previous reports have also indicated marked antimycotic activity of methanolic fraction of *S. asper* against *Aspergillus niger*, *A. fumigatus*, *A. flavus, Fusarium solani* (Khan *et al*., 2010); *Candida albicans, Aspergillus flavus* (Upadhyay *et al*., 2013); *Botrytis cinerea* and *R. solani* (Ejaz *et al.*, 2017).

GC-MS chromatogram shows the presence of 50 compounds in methanolic root extract of *S. asper* (Table 1), while potential antifungal compounds are listed in Table 2. Of the 50 compounds, benzoic acid, 4-hydroxy-3-methoxy-, methyl ester (12.89%), 13-cis-retinoic acid (12.32%) and D-ribofuranose (11.49%) were identified as the most abundant compounds, while benzoic acid, 3-hydroxy (9.63%) was moderately abundant compound (Fig. 2; Table 1). Antifungal and antimicrobial activities of benzoic acid derivatives have been reported

extensively (Parra-Amin *et al*., 2019) suggesting that antifungal action in root extract is mainly caused by benzoic acid derivatives. The benzoic acid can restrict the pathogen growth by diffusing and dissociating inside cell membrane to lower the intracellular pH to an extent, which can easily jeopardize microbial cell activities (Krebs *et al*., 1983). Retinoic acid (isotretinoin) is the biologically active metabolite of vitamin A, which has shown fungistatic action against *Aspergillus fumigatus* and *Candida albicans* by modeling both innate and adaptive immune response in the host (Campione *et al*., 2016) (Table 2).

Seventeen peaks (1.01–4.45%) corresponded to less abundantly compounds and the remaining 28 identified compounds were found in negligible amounts (<1% each) (Fig. 2; Table 1).

However, among the less abundant compounds, pyridine, 2-pentyl has been reported as potential antifungal biomaterial against *Fusarium oxysporum* and *Phomopsis asparagi* (Wei *et al*., 2018), while these pyrimidine are known inhibitors of bacterial DNA replication (Nguyen *et al.,* 2015). Another less abundant compound was bis(2-ethylhexyl) phthalate, which is used as plasticizers or solvents in many industrial products and has been isolated from many microorganism culture filtrate and plants (El-Sayed, 2012), while exhibited antibacterial and antifungal activity against pathogenic bacterial strains and the fungus *Aspergillus flavus* (Habib and Karim, 2009).

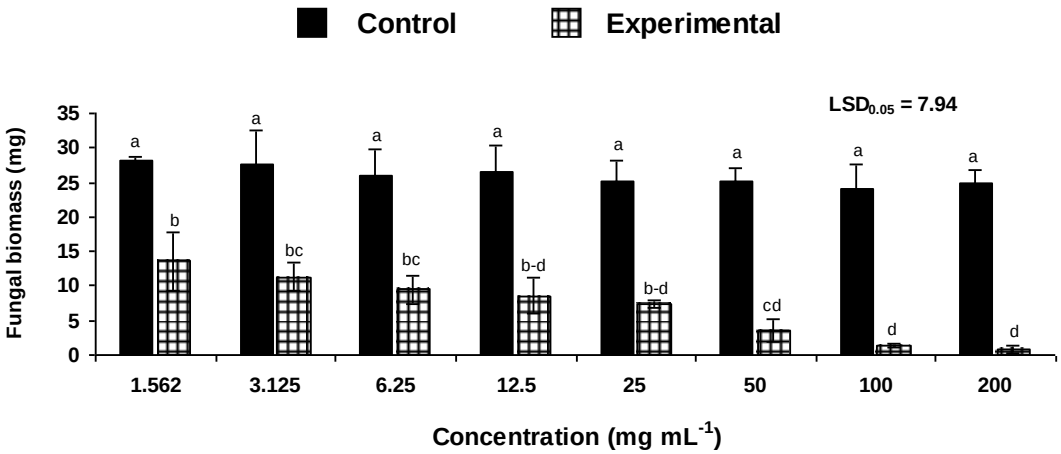

Fig. 1: Effect of different concentrations of methanolic root extract of *Sonchus asper* on growth of *Rhizoctonia solani*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

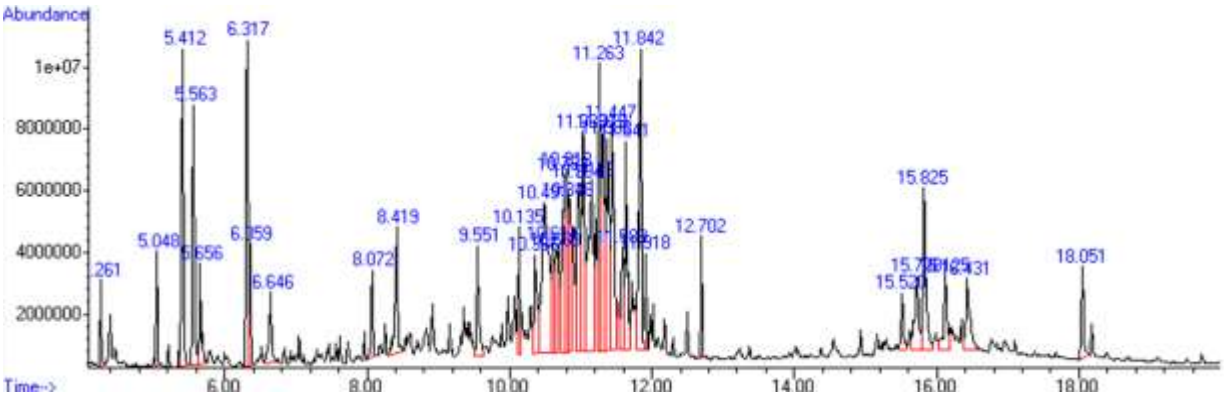

Fig. 2: GC-MS chromatogram of methanolic root extract of *Sonchus asper*.

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

Comp. No.	Names of compounds	Formula	Weight	Retention time (min)	Peak area (%)
1	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_9H_{10}O_4$	182.17	8.48	12.89
2	13-*cis*-Retinoic acid	$C_{20}H_{28}O_2$	300.25	15.27	12.32
3	D-Ribofuranose	$C_5H_{10}O_5$	150.16	8.42	11.49
4	Benzoic acid, 3-hydroxy	$C_7H_6O_3$	138.12	8.83	9.63
5	Pyridine, 2-pentyl-	$C_{10}H_{15}N$	149.23	5.65	4.45
6	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56	15.12	4.10
7	Phosphoric acid, monomethyl ester	CH_5O_4P	112.02	5.42	4.04
8	2-methylidene-6,10,14-trimethylpen2-methylidene-6,10,14-trimethylpentadecanoic acid silylated	$C_{22}H_{44}O_2Si$	368.31	11.73	3.56
9	Galactopyranose	$C_6H_{12}O_6$	180.26	17.98	3.27
10	5-O-Coumaroyl-D-quinic acid	$C_{16}H_{17}O_8^-$	337.30	17.32	2.87
11	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666.17	10.72	2.78
12	Decanedioic acid, dibutyl ester	$C_{18}H_{34}O_4$	314.46	12.96	2.20
13	Octahydro-1H-cyclopenta[b]pyridin-4-ol	$C_8H_{15}NO$	141.12	4.26	2.05
14	Pentan-3-ol	$C_5H_{11}O$	87.11	5.07	1.80
15	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.47	14.11	1.73
16	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.46	13.26	1.68
17	L-Proline	$C_5H_9NO_2$	115.12	8.52	1.43
18	Bohlmann k2631	$C_{15}H_{20}O_2$	232.15	11.92	1.18
19	10-Undecenoic acid	$C_{11}H_{20}O_2$	184.27	12.91	1.11
20	2,3-Dihydroxybutanoic acid	$C_4H_8O_4$	120.16	7.01	1.03
21	l-Isoleucine, N-trifluoroacetyl-	$C_8H_{12}F_3NO_3$	227.08	6.49	1.00
22	5-Amino-8-hydroxyquinoline	$C_9H_8N_2O$	160.17	16.36	0.94
23	4-Hydroxybutanoic acid	$C_4H_8O_3$	104.1	5.91	0.94
24	n-Tetracosanol-1	$C_{24}H_{50}O$	354.39	14.23	0.74
25	Stigmasterol	$C_{29}H_{48}O$	412.69	19.12	0.70

26	4-Hydroxy-2,2',4',6'-tetrachlorobiphenyl	$C_{12}H_6Cl_4O$	308	16.12	0.69
27	Dehydroabietic acid	$C_{20}H_{28}O_2$	300.4	14.38	0.69
29	Octanoic acid	$C_8H_{16}O_2$	144.21	6.16	0.58
30	Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	426.37	16.35	0.53
31	7,12-Dithia-14-azadispiro[4.0.5.3]tetradeca-9,13-diene, 9,10-dimethyl-13-phenyl-	$C_{19}H_{23}NS_2$	329.13	15.59	0.52
32	Benzoic acid, 3,4,5-trihydroxy	$C_7H_6O_5$	170.11	11.73	0.50
33	Pantothenic acid	$C_9H_{17}NO_5$	219.23	11.98	0.37
34	Benzeneacetic acid	$C_8H_8O_2$	136.15	6.52	0.37
35	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	$C_{14}H_{20}O_2$	220.03	8.08	0.36
36	L-Valine	$C_5H_{11}O_2$	117.14	4.53	0.34
37	Pentanedioic acid	$C_5H_8O_4$	132.11	7.45	0.33
38	Glycerol	$C_3H_8O_3$	90.09	5.55	0.31
39	Pimelic acid	$C_7H_{12}O_4$	160.17	9.09	0.31
40	Triethylene glycol	$C_6H_{14}O_4$	150.17	8.30	0.26
41	4-Coumaric acid	$C_9H_8O_3$	164.13	11.57	0.24
42	6-Phthalazinecarboxylic acid, 1,2,3,4-tetrahydro-2,3-dimethyl-1,4-dioxo-	$C_{11}H_{10}N_2O_4$	234.06	7.57	0.21
43	Stigmastanol	$C_{29}H_{52}O$	416.07	19.62	0.20
44	Acetylaminoethanol	$C_4H_9NO_2$	103.12	5.64	0.20
45	Diethylene glycol	$C_4H_{10}O_3$	106.12	6.00	0.20
46	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.26	11.85	0.19
47	Undecanedioic acid	$C_{11}H_{20}O_4$	216.27	11.85	0.19
48	Levoglucosenone	$C_6H_6O_3$	126.03	4.88	0.17
49	1-Monomyristin	$C_{17}H_{34}O_4$	302.45	14.29	0.17
50	5-Propyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrochloride	$C_{18}H_{19}N$	249.15	12.85	0.15

Table 2: Potential antifungal compounds in methanolic root extract of *Sonchus asper*.

Compound name	Target fungus	Reference
Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	*Cladosporium cladosporioides* *C. sphaerospermum*	Lago *et al.* (2004)
13-*cis*-Retinoic acid	*Aspergillus fumigatus* and *Candida albicans*	Campione *et al.* (2016)
Pyridine, 2-pentyl-	*Fusarium oxysporum* and *Phomopsis asparagi*	Wei *et al.* (2018)
Bis(2-ethylhexyl) phthalate	*Aspergillus flavus*	Habib and Karim (2009)
Pentan-3-ol	*Candida* species	Gallucci *et al.* (2014)
9-Octadecenamide, (Z)-	*Candida* species	dos Reis *et al.* (2019)
L-Proline	*Magnaporthe grisea*	Balhadère *et al.* (2019)

CONCLUSIONS

The results highlighted the promising antifungal potency of methanolic root extract of *S. asper* against *R. solani* due to occurrence of several phytocompounds, which foreseeing a potential in preparation of natural product-based fungicides for management of black scurf disease of potato. Further studies will be required to investigate their cost, applicability, safety, and phytotoxicity against crops as potential botanical fungicides.

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