

Antifungal activity of methanolic stem extract of *Sonchus asper* against *Rhizoctonia solani*

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Abstract

Rhizoctonia solani Kühn is a broad host ranged soil-borne pathogen that infects more than 200 plant species. This study was carried out to assess the antifungal potential of stem extract of a weed *Sonchus asper* (L.) Hill and to identify possible antifungal compounds using GC–MS method. In a laboratory bioassay, the methanolic stem extract at concentrations of 100 and 200 mg mL⁻¹ significantly inhibited fungal growth by 77% and 81%, respectively. The extract was subsequently subjected to GC–MS analysis, which identified 49 compounds. The predominant compound was benzoic acid, 4-hydroxy-3-methoxy-, methyl ester (15.65%), followed by D-ribofuranose, 5-deoxy-5-(methylsulfinyl) (13.68%), phenol, 4-ethenyl-2,6-dimethoxy- (8.98%), and 4-hydroxy-2,2',4',6'-tetrachlorobiphenyl (7.19%). Literature survey showed that compounds such as benzoic acid, 4-hydroxy-3-methoxy-, methyl ester; 10-undecenoic acid, pyridine, 2-pentyl-; 9-octadecenoic acid, (E)-; cetene; azelaic acid; and octanoic acid possess antifungal potential against a variety of humans and plants fungal pathogens. These findings indicate that *S. asper* stem extract exhibits antifungal activity against *R. solani* and may serve as a source of candidate antifungal compounds. More studies are required to validate the antifungal activity of individual compounds, establish their mechanisms of action, and evaluate their potential application in plant disease management.

Keywords: Biocontrol, Natural fungicides, Potato, *Rhizoctonia solani*, *Sonchus asper*.

Introduction

Fungal pathogens pose a notable threat to agricultural production globally, causing considerable economic losses and threatening food security (Ekwomadu and Mwanza, 2023). Among these pathogens, *Rhizoctonia solani* Kühn is an important soil- and seed-borne phytopathogen with a broad host range. It causes several economically important diseases, including black scurf and stem canker in potato and various root and stem diseases in other crops (Rafiq *et al.*, 2021; Chaudhary *et al.*, 2024). In Pakistan, the disease is widespread across all the eight potato-growing zones (Majeed and Muhammad, 2018). The management of *R. solani* relies heavily on synthetic fungicides; however, their repeated and excessive use may contribute to fungicide resistance, environmental pollution, and hostile effects on non-target organisms, including beneficial soil microorganisms (Yang *et al.*, 2011; Deresa and Diriba, 2023). Furthermore, the extensive host range and persistence of this pathogen in soil and crop residues complicate disease management programs that encourage the scientists to search for alternative environmentally sustainable strategies

(Rauf *et al.*, 2015). Integrated disease management methods, including cultural practices, resistant cultivars where available, biocontrol agents, and plant-derived antifungal compounds, offer promising opportunities to reduce reliance on synthetic fungicides (Rafiq *et al.*, 2024; Zohaib *et al.*, 2024). In particular, plant extracts contain diverse secondary metabolites that may inhibit phytopathogenic fungi and serve as potential sources of natural antifungal agents (Javaid and Iqbal, 2014; Khan and Javaid, 2023; Cenobio-Galindo *et al.*, 2024).

In recent years, natural resources have attracted much attention as potential sources of bioactive compounds with antimicrobial properties (Woo *et al.*, 2023). *Sonchus asper* (L.) Hill, an herbaceous species belonging to the family Asteraceae, has been investigated for its diverse activities, including reported anti-inflammatory, anxiolytic, and anticancer effects (Boukhary *et al.*, 2026). The presence of various phytochemicals, including alkaloids, terpenoids, phenolic compounds, and flavonoids, in different parts of *S. asper* suggests that this species may contain compounds with

potential antifungal activity (Mostafa and El-Sayed, 2021; Zulkefle *et al.*, 2025). Importantly, Rafiq *et al.* (2021) investigated the use of *S. asper* root extract against *R. solani* and characterized its chemical constituents using GC-MS, providing a basis for further investigation of this plant as a potential source of antifungal agents. GC-MS is a valuable analytical technique for profiling volatile and semi-volatile constituents of plant extracts and generating preliminary information about their chemical composition (El-Rafie *et al.*, 2023; Javaid *et al.*, 2023). However, the chemical composition and biological activity of plant extracts may vary among plant organs, making it important to investigate different tissues to identify potentially useful antifungal constituents. Therefore, this study aimed to investigate the *in vitro* antifungal activity of methanolic stem extract of *S. asper* against *R. solani* at different concentrations and to characterize its chemical constituents using GC-MS. By investigating the antifungal potential of the stem extract and identifying its major chemical constituents, this study seeks to provide preliminary evidence supporting the further evaluation of *S. asper* as a source of plant-derived antifungal agents for sustainable management of *R. solani*.

Materials and Methods

Isolation and culturing of the pathogen

Infected potato tubers exhibiting deformities and visible sclerotia were collected from the local market and brought to the laboratory. The tubers were surface-sterilized using 1% sodium hypochlorite solution to remove surface contaminants and were subsequently rinsed three times with autoclaved distilled water. The infected portions of the tubers were cut into small pieces, and each piece was placed in a Petri plate containing autoclaved malt extract agar. The plates were incubated at 25 °C for one week, after which the emerging hyphae were transferred to freshly prepared MEA plates for further use (Javaid *et al.*, 2019).

Preparation of methanolic stem extract

The weed *S. asper* was collected from different locations in Lahore and stems were separated to dry them in shade initially and then in an oven at 40 °C. The dried stem material was crushed into a fine powder (200 g) was dipped in pure methanol (1.0 L) for 15 days. Thereafter, the solvent was filtered and allowed to evaporate in rotary at 45 °C to reduce its volume. Then this concentrated extract was put in a pre-weighed beaker completely dried at 45 °C in an electric oven. After the drying process almost 11 g stem extract of *S. asper* was obtained.

Antifungal bioassays

Serial dilutions of methanolic stem extract (1.562, 3.125, 7.19, 12.50, 25.00, 50.00, 100 and 200 mg mL⁻¹) in 10-mL test tubes were prepared for antifungal bioassays by following the method described by Banaras *et al.* (2017). The 1.2 g of the extract that was obtained after drying in hot oven, was dissolved in DMSO (1 mL) followed by addition of autoclaved malt extract broth to have a total volume of 6 mL. To prepare the control solution, 5 mL of broth medium was added to 1 mL of DMSO to prepare a stock solution that was repeatedly double diluted 7 times. A volume of 10 µL of *R. solani* inoculum was added to each test tube having 1.0 mL of broth media. The treatments were replicated three times and the test tubes were placed in an incubator for 7 days at 27 °C. Fungal material after 7-day growth was collected on filter papers, dried at 70 °C and weighed.

GC-MS analysis

The GC-MS analysis was done for the identification of constituents in methanolic stem extract on a machine (Agilent Technologies Model GC-7890A) that was attached with mass spectrometer (MS 5975C). The dimensions of capillary column were 30 × 0.25 µm ID × 0.25 µm df. The machine was operated in an electron impact mode that uses ionization system for GC-MS detection with 70 eV ionization energy and 99.99% helium gas at rate of 1 mL min⁻¹ was used as a carrier. Injector temperature was maintained at 260 °C by keeping 200 °C temperature for the ion-source while the oven was started with 50 °C and it was kept constant for 2 min followed by 10 °C min⁻¹, eventually attaining 310 °C. The NIST database, mass spectral fragmentation patterns and retention catalogues were used for the identification of compounds.

Statistical analysis

Data were analyzed by one-way ANOVA. Means were separated using LSD test at P≤0.05 using Statistix 8.1 software.

Results and Discussion

Different concentrations of the methanolic stem extract of *S. asper* (1.56–200 mg mL⁻¹) inhibited the growth of *R. solani* by 0–81% (Fig. 1). The effect of two higher concentrations was significant. Previous studies have also reported the antimicrobial activity of methanolic fractions of *S. asper* against *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Fusarium solani* (Samanta *et al.*, 2020); *Candida albicans*, *A. flavus* (Singh *et al.*, 2018); *Botrytis cinerea* and *R. solani* (Ejaz *et al.*, 2021). GC-MS analysis of the stem extract of *S. asper* showed 49 compounds in it (Table 1), while potential antifungal compounds are listed in Table 2. Among these, benzoic acid, 4-hydroxy-3-methoxy-,

methyl ester (15.65%) and D-ribofuranose, 5-deoxy-5-(methylsulfinyl) (13.68%) were the most abundant compounds, followed by phenol, 4-ethenyl-2,6-dimethoxy- (8.98%), 4-hydroxy-2,2',4',6'-tetrachlorobiphenyl (7.19%), and pyridine, 2-pentyl- (6.03%) (Table 1, Fig. 2). Other identified compounds included benzeneacetic acid; L-isoleucine, and 5-O-coumaroyl-D-quinic acid, with their respective relative abundances reported in Table 1. Seventeen peaks, each accounting for 1.01–4.45%, represented compounds of relatively lower abundance, whereas the remaining 32 identified compounds were present at less than 1% each (Table 1, Fig. 2). Previous studies have reported the antifungal properties of derivatives of benzoic acid. Cuca *et al.* (2021) suggested that benzoic acid derivatives may contribute to the antifungal activity of plant extracts. Benzoic acid and related weak organic acids can enter microbial cells in their undissociated form and dissociate within the cytoplasm, disrupting intracellular pH homeostasis and cellular metabolism and thereby inhibiting microbial growth (Krebs *et al.*, 1983). Similarly, Chen *et al.* (2018) investigated the antimicrobial potential of pyridine derivatives and reported their ability to inhibit the growth of *Fusarium oxysporum* and *Phomopsis asparagi*.

Among the relatively abundant compounds identified in the present study, phenol, 4-ethenyl-2,6-dimethoxy- has been reported to have applications in food preservation and the perfume industry, as well as antimicrobial activity against various bacteria and fungi. Its antimicrobial effects may involve interactions with microbial cell envelopes and disruption of cellular integrity; however, the specific mechanism and contribution of this compound to the antifungal activity of *S. asper* stem extract require further investigation. This compound has also been reported as a constituent of preparations used in alcohol production (Bardant *et al.*, 2023). L-isoleucine, which was detected at a lower relative abundance, has diverse biological functions, and products associated with it have been investigated

for antioxidant activity (Bibi *et al.*, 2019). In another study, Talie *et al.* (2020) evaluated different concentrations of *n*-hexane extract of *Rhizopogon* species against *Penicillium chrysogenum*, *Aspergillus niger*, and *Alternaria alternata*, which are associated with postharvest diseases of apple. Their findings indicated significant antifungal activity of the tested extract concentrations against these fungal pathogens. Together, these findings provide context for the antifungal potential of plant-derived extracts and support further investigation of the chemical constituents associated with the activity of *S. asper* stem extract against *R. solani*.

Conclusion

The methanolic stem extract of *S. asper* exhibited significant antifungal activity against *R. solani*, inhibiting fungal growth up to 97% at the tested concentrations. GC-MS analysis identified several reported antifungal constituents, including benzoic acid, 4-hydroxy-3-methoxy-, methyl ester; 10-undecenoic acid; 9-octadecenoic acid, (E)-; pyridine, 2-pentyl-; cetene; azelaic acid; and octanoic acid, which may contribute to the observed antifungal activity. However, further studies are required to confirm the antifungal activity of individual compounds and elucidate their mechanisms of action against *R. solani*. These findings highlight the potential of *S. asper* stem extract as a source of candidate antifungal agents for the sustainable management of *R. solani*.

Contribution of authors

MR: Carried out experiments; **SA:** Data analysis; **AJ:** Conceptualization and data curation, final editing; **AS:** Writing original draft; All other contributed equally to this work.

Conflict of interests

Authors declare that there is no conflict of interest.

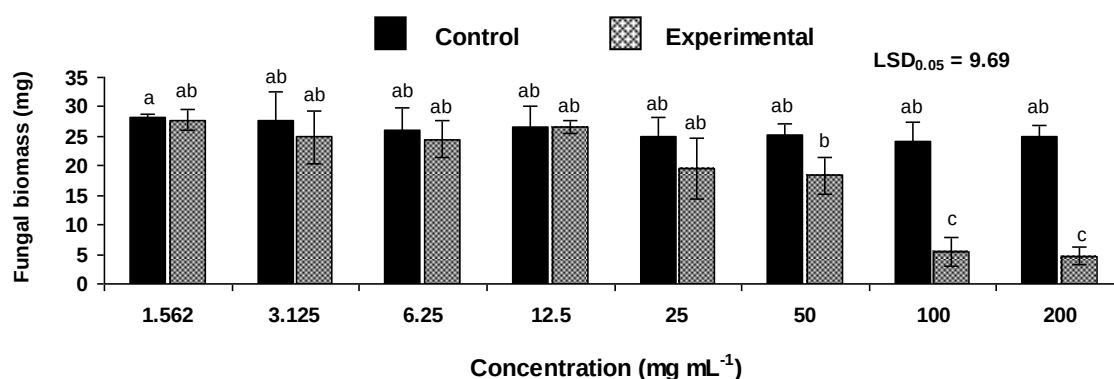


Fig. 1: Effect of different concentrations of methanolic stem extract of *Sonchus asper* on growth of *Rhizoctonia solani*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

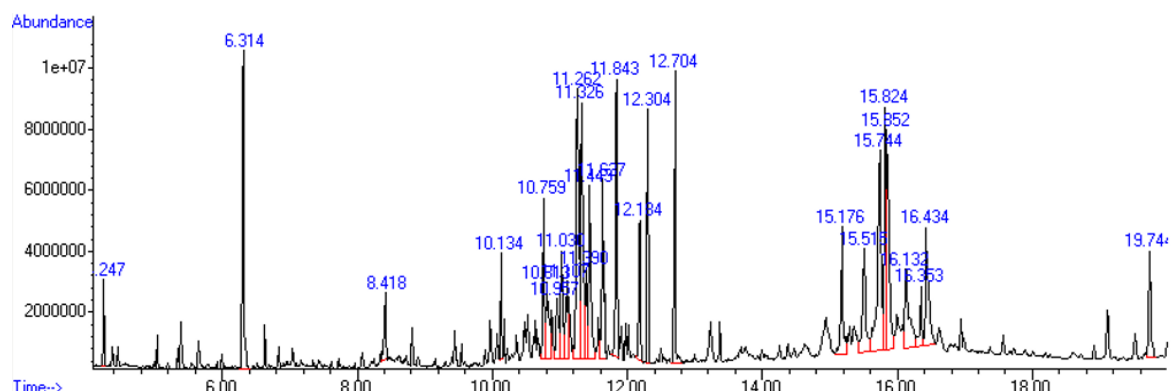
Table 1: Compounds identified from methanolic stem extract of *Sonchus asper* through GC-MS analysis.

No.	Names of compounds	Formula	Molecular Weight	Retention time (min)	Peak area (%)
1	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	C ₁₀ H ₁₂ O ₄	196.07	8.48	15.65
2	D-Ribofuranose, 5-deoxy-5-(methylsulfinyl)	C ₈ H ₈ O ₂	136.15	8.42	13.68
3	Phenol, 4-ethenyl-2,6-dimethoxy-	C ₁₀ H ₁₂ O ₃	180.08	8.85	8.98
4	4-Hydroxy-2,2',4',6'-tetrachlorobiphenyl	C ₁₂ H ₆ Cl ₄ O	308.00	16.12	7.19
5	Pyridine, 2-pentyl-	C ₁₃ H ₁₃ N	183.10	5.65	6.93
6	Benzeneacetic acid	C ₈ H ₈ O ₂	136.15	6.52	6.45
7	L-Isoleucine	C ₈ H ₁₂ F ₃ NO ₃	227.08	6.49	5.30
8	5-O-Coumaroyl-D-quinic acid	C ₁₆ H ₁₇ O ₈	337.30	17.32	3.18
9	10-Undecenoic acid	C ₁₁ H ₂₀ O ₂	184.27	12.91	3.17
10	Decanedioic acid, dibutyl ester	C ₁₈ H ₃₄ O ₄	314.25	12.96	2.91
11	Dehydroabietic acid	C ₂₀ H ₂₈ O ₂	300.40	14.38	1.887
12	9-Octadecenoic acid, (E)-	C ₁₈ H ₃₄ O ₂	282.46	13.26	1.77
13	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z,Z)-	C ₁₇ H ₁₉ NO ₃	285.14	16.20	1.40
14	Galactopyranose	C ₆ H ₁₂ O ₆	180.26	17.98	1.36
15	Cetene	C ₁₆ H ₃₂	224.25	8.98	1.35
16	Levoglucosenone	C ₆ H ₆ O ₃	126.03	4.88	1.28
17	4-Hydroxybutanoic acid	C ₄ H ₈ O ₃	104.10	5.91	1.28
18	Pimelic acid	C ₇ H ₁₂ O ₄	160.17	9.09	1.26
19	Octahydro-1H-cyclopenta[b]pyridin-4-ol	C ₈ H ₁₅ NO	141.12	4.26	1.20
20	Azelaic acid	C ₉ H ₁₆ O ₄	188.10	9.34	1.12
21	Cyclononasiloxane, octadecamethyl-	C ₁₈ H ₅₄ O ₉ Si ₉	666.17	10.72	1.01
22	Hexacosane	C ₂₆ H ₅₄	366.42	13.76	0.88
23	Myo-inositol	C ₆ H ₁₂ O ₆	180.16	11.84	0.86
24	9-Hexadecenoic acid, methyl ester, (Z)-	C ₁₇ H ₃₂ O ₂	268.24	11.28	0.80
25	Glycerol	C ₃ H ₈ O ₃	90.09	5.55	0.62
26	Benzene, [(3-butynyloxy)methyl]-	C ₁₁ H ₁₂ O	160.09	17.08	0.62
27	Benzoic acid, 3,4,5-trihydroxy	C ₇ H ₆ O ₅	170.11	11.73	0.59
28	Decanedioic acid, bis(2-ethylhexyl) ester	C ₂₆ H ₅₀ O ₄	426.37	16.35	0.57
29	3-(1-ethoxyethoxy)-1-phenyl-6-heptene	C ₁₉ H ₃₂ O ₃	308.24	6.00	0.55
30	Silane, dimethyl(4-(2-phenylprop-2-yl)phenoxy)tridecyloxy-	C ₃₀ H ₄₈ O ₂ Si	468.34	17.38	0.54
31	Bohlmann k2631	C ₁₅ H ₂₀ O ₂	232.15	11.92	0.51
32	Pentan-3-ol	C ₅ H ₁₁ O	87.11	5.07	0.44
33	Benzoic acid, 3-hydroxy	C ₇ H ₆ O ₃	138.12	8.83	0.44
34	9-Octadecenamide, (Z)-	C ₁₈ H ₃₅ NO	281.27	14.11	0.41
35	5-Amino-8-hydroxyquinoline	C ₉ H ₈ N ₂ O	160.17	16.36	0.37
36	5-Propyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrochloride	C ₁₈ H ₁₉ N	249.15	12.85	0.35
37	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.27	12.90	0.32
38	Pentanedioic acid	C ₅ H ₈ O ₄	132.11	7.45	0.32
39	(3R,4R)-2,5-dimethoxy-2,5-dimethyl-hexane-3,4-diol	C ₁₀ H ₂₂ O ₄	206.15	7.92	0.29
40	(3β)-Olean-18-en-3-ol	C ₃₀ H ₅₀ O	426.71	19.53	0.28
41	Stigmasterol	C ₂₉ H ₅₂ O	416.07	19.62	0.26
42	1-Pentanol, 5-chloro-, acetate	C ₇ H ₁₃ ClO ₂	164.06	4.35	0.21
43	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₀ O ₂	196.07	8.08	0.21
44	5-Oxatricyclo[8.2.0.0.4,6]dodecane, 4,12,12-trimethyl-9-methylene-, (1R,4R,6R,10S)-	C ₁₅ H ₂₄ O	220.18	9.13	0.17
45	Diethylene glycol	C ₄ H ₁₀ O ₃	106.12	6.00	0.17
46	Octanoic acid	C ₈ H ₁₆ O ₂	144.21	6.16	0.17
47	2-(4-Cyclopropyl-4-hydroxy-4-phenylbutyl)-	C ₁₆ H ₂₂ O ₃	262.16	10.82	0.15

	1,3-dioxolane				
48	Octacosane	C ₂₈ H ₅₈	394.45	16.28	0.15
49	Docosanoic acid, methyl ester	C ₂₃ H ₄₆ O ₂	354.35	14.95	0.13

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

Compound No.	Compound name	Target fungus	Reference
1	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	<i>Cladosporium cladosporioides</i> <i>C. sphaerospermum</i>	Lago <i>et al.</i> (2004)
5	Pyridine, 2-pentyl-	<i>Fusarium oxysporum</i> and <i>Phomopsis asparagi</i>	Wei <i>et al.</i> (2018)
9	10-Undecenoic acid	<i>Candida albicans</i>	McLain <i>et al.</i> (2000)
12	9-Octadecenoic acid, (E)-	<i>Candida albicans</i>	Karthikeyan <i>et al.</i> (2014)
15	Cetene	<i>P. chrysogenum</i> , <i>Aspergillus niger</i> and <i>Alternaria alternata</i>	Talie <i>et al.</i> (2020)
20	Azelaic acid	<i>Scopulariopsis brevicaulis</i> , <i>Candida albicans</i> , <i>Candida glabrata</i> and <i>Pityrosporum ovale</i>	Brasch and Christophers (1993)
46	Octanoic acid	Yeasts and molds	Lee <i>et al.</i> (2020)

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

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