Antifungal activity and phytochemical analysis of *Zanthoxylum armatum* seeds against *Sclerotium rolfsii*

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Abstract

The present study was conducted to examine the antifungal efficacy of methanolic seeds extract of *Zanthoxylum armatum* DC against fungal pathogen *Sclerotium rolfsi* Sacc. Methanolic seed extract of 50, 25 and 12.5 mg mL^{-1} significantly decreased the fresh fungal biomass (61 to 70%). The methanolic extract was subjected to GC-MS analysis that revealed the presence of various phytoconstituents. The predominant compounds identified were *n-hexadecanoic acid*, constituting 44.07% of total fifteen compounds detected. Following closely was oleic acid with 17.84% peak area. This study concludes that methanolic seed extract of *Z. armatum* possess strong antifungal potential against *S. rolfsii.* These compounds may inhibit the growth and reproduction of fungal pathogens. These phytochemicals compounds can act as potential candidates for the development of organic fungicides.

Keywords: Methanolic fraction, Phytochemical constituents, *Sclerotium rolfsii*, *Zanthoxylum armatum.*

Introduction

Pesticides are substances or mixtures designed to help us control pests. They work by preventing, destroying, repelling, or reducing the impact of unwanted insects, plants, or other organisms that can harm our crops and health (Souto *et al*., 2021). Plants can be thought of as nature's perfect laboratories, producing a wide range of organic compounds. These organic compounds divided into primary metabolites and secondary metabolites. Plant derived compounds have been extensively explored for their potential as alternative to synthetic fungicides due to their ecofriendly nature and low toxicity (Rafiq *et al.*, 2024; Alsalih, 2025). Among these, *Zanthoxylum armatum* DC., a member of the Rutaceae family has pharmacological properties and antimicrobial activities (Shanker *et al*., 2023). *Sclerotium rolfsii* Sacc., a soil-borne fungal pathogen poses a significant threat to various crops including *Capsicum annuum* (Jabeen *et al.*, 2021) and *Cicer arietinum* (Ali *et al.*, 2020). It is a highly harmful, soil-borne, versatile pathogen that is easy to spot due to its white mycelial growth on infected plant tissues. It has an unusually broad range of hosts (Anil *et al*., 2025).

Z. armatum, evergreen, tiny, sub-deciduous, and spiny tree commonly known as winged prickly ash, is indigenous to Pakistan, India, Nepal and China. Traditionally various parts of this plant have been employed in folk medicines for analgesic, anti-inflammatory and antimicrobial properties. It is a common shrub of family Rutaceae, with approximately 250 species found worldwide. Its fruit is green in color and has characteristics dots of oil glands. It has tempting aromatic fruits and it is the key seasoning agents in the food industry (Peng *et al.*, 2025). Function of phytochemicals in plant. The formation of color, flavor and smell in plants are mainly due to the role that phytochemical play. They are released into the environment to protect the plant from harmful environmental factors, such as UV radiation, insect pathogens, atmospheric pollutants and biotic and abiotic stress or dry condition (Gurav *et al*., 2022). Recent studies have shed light on the antifungal potential of *Z. armatum* extracts against a range of phytopathogenic fungi, suggesting its efficacy as natural fungicidal agents (Yang *et al*., 2022; Shah *et al*., 2025). Phytochemical analysis of *Z. armatum* seeds analyzed the peaks of different bioactive compounds like alkaloids, flavonoids, terpenoids and phenolics (Phuyal *et al.*, 2020; Peng *et al*., 2025). These secondary metabolites showed antimicrobial activities through multiple mechanisms such as disruption of cell membrane, inhibiting activity of enzymes and interference with essential cellular processes (Ahmed *et al*., 2023; Shah *et al*., 2025). However, the specific compounds responsible for the antifungal activity against *S. rolfsii* remain to be elucidated. The present study aimed to investigate the antifungal activity of crude methanolic extract of *Z. armatum* seeds against *S. rolfsii* and to investigate the photochemical profiling of seed extracts to identify key bioactive constituents responsible for the antifungal activity.

Materials and Methods

Collection of seeds of *Z. armatum*

Z. armatum was selected for the collection of seeds. During summer, seeds of *Z. armatum* were collected from hilly areas of Abbottabad, Pakistan. Plant was identified by a taxonomist Dr. Qadeer Butt, School of Botany, Minhaj University Lahore.

Preparation of methanolic extracts of seeds

After collection, seeds were dried under shade and subjected to grinding to prepare fine powder. Seed powder (250 g) was soaked for 30 days in methanol. After one month, the methanolic mixture of seed powder of *Z. armatum* was filtered by Whatman filter paper 1. Solvent underwent for evaporation in dry air oven at 50 °C till formation of semi-solid biomass.

Stock solution preparation

Stock solutions were arranged in two sets. One set of experiment had methanolic seed extract of *Z. armatum*. Second set served as control group, without the methanolic seed extract. Stock solutions of control and experiment were used prepared in dimethyl sulfoxide (DMSO) followed by addition of malt extract broth (MEB).

In array to generate the tentative stock elucidation, methanolic seed extract (0.5 g) was dissolved in 0.5 mL DMSO. Final volume of 12 mL stock solution was made by adding 2% MEB to prepare a concentration of 50 mg mL^{-1}. Stock solution was double diluted by repeatedly adding MEB stock solution to prepare lower concentrations, 25, 12.5, 6.25, 3.125 and 1.562 mg mL^{-1} (Jabeen *et al.*, 2022).

Procurement of *S. rolfsii*

S. rolfsii pure culture was taken from Fungal Culture Bank of Pakistan, University of the Punjab Lahore, Pakistan. Microscopic and macroscopic observations were made to confirm the purity of the fungal culture. Mycelium was abundant, course and white in color. Fungus hyphae were about 9 µm in diameter. The primary branched hyphae were hyaline and thin walled. Main hyphal branches were cross walled and had clump connections. Dark brown sclerotia were abundant in the mature colony and were easily visible with nacked eye.

***In vitro* bioassay**

In vitro bioassay was conducted by using completely randomized design. Both sets *viz.* experimental and control stock solutions were poured in sterilized test tubes. Each concentration had six replicates. The solutions pouring was performed under sterilized environmental conditions of laminar flow cabinet. *S. rolfsii* fungal inoculum (50 µL) was loaded in each respective concentration. Each test tube was sealed properly with cotton plug to prevent any potential contamination. Test tubes were incubated at 25 ºC for 10 days. Thereafter, fungal mycelia were visualized in test tubes. The fungal biomass was collected through filtration and weighed.

Statistical analysis

Data were analyzed using one-way ANOVA (Statistix 8.1 software) to determine significant difference among the treatments. Means were separated using Least Significant Difference (LSD) test at $P \leq 0.05$.

GC-MS analysis

The gas chromatography (GC) 7890B replica and the mass spectroscopy (MS) 5977A model were utilized for identification of compounds from the sample. The column DB 5 was used. Carrier gas helium, and the injection volume 1 µL were used. Oven start temperature was 80 ºC the temperature was increased by 10 ºC each miniature until it reached to 300 ºC. The inlet warmth was 280 ºC. The foundation temperature was 230 ºC as well as quadruple hotness was 150 ºC. Substances were evaluated by comparing spectrum and parted ways in rising categorize of their maintenance periods and preservation keys. Peak areas were used stated the relative abundance.

Results and Discussion

Antifungal activity of seed extract *S. rolfsii*

Different concentrations of methanolic *Z. armatum* seeds were used in a series of control and experimental group. Fungal biomass of *S. rolfsii* was gradually inhibited by increasing concentrations of DMSO in control group as shown in Fig. 1. Fungal biomass was significantly reduced due to DMSO in control group from 0.0678 mg to 0.026 mg. However, 50, 25 and 12.5 mg mL^{-1} were insignificantly different from each other. The consequence of methanolic seed extort of *Z. armatum* on *S. rolfsii* fungal biomass was very evident. Fungal biomass was inhibited (0.0383 to 0.0108 mg) by using various concentrations of the fractions. Greater concentrations of DMSO and methanolic seed extract of 50, 25 and 12.5 mg mL^{-1} significantly decrease the fresh fungal biomass (61 to 70%) of *S. rolfsii* as shown in Fig. 1.

The present study finding demonstrates significant antifungal activity of the methanolic seed extract of *Z. armatum* against *S. rolfsii* suggesting its potential application as natural fungicide in agriculture. In the control experiment, where only DMSO was utilized, the fresh fungal biomass was consistently higher as compare to all experimental concentrations (Santra *et al.*, 2022). In some previous studies, it was observed that DMSO alone did not exhibit antifungal activity (Kumar *et al.*, 2025; Yahya *et al*., 2018). However, there are reports DMSO also reduced biomass of *S. rolfsii* and *Macrophomina phaseolina* (Banaras *et al.,* 2020). In the present study, in the experimental group where DMSO was combined with methanolic seed extract of *Z. armatum*, notable antifungal activity across all concentrations tested was recorded (Hamion *et al*., 2024). The antimicrobial activity of fruit extracts of *Z. armatum* were comparatively more effective than extracts of seed and bark due to the higher phenolic and flavonoid contents present in fruits than seeds and bark (Phuyal *et al*., 2020). Moreover, similar results of antibacterial and antioxidant activities of three different extracts (acetone, methanol, and *n*-hexane) of *Z. armatum* fruits and leaves are also reported (Irshad *et al*., 2021). Our findings are consistent with previous studies that have reported the antifungal potential of *Z armatum* extracts against various phytopathogenic fungi. Akbar *et al*. (2014) demonstrated the antimicrobial activity of *Z. armatum* against pathogenic fungi and bacterial strains. Phuyal *et al*. (2020) and Alam and Saqib (2017) have reported the broad-spectrum antimicrobial activity of *Z armatum* extracts.

GC-MS analysis

GC-MS examination of the methanolic extract of *Z. armatum* revealed 15 organic compounds. The predominant compounds were *n-hexadecanoic acid*, constituting 44.07% of total compounds detected. Following closely was oleic acid (17.84%). Phytochemical analysis revealed the presence of diverse bioactive compounds in methanolic seed extract of *Z. armatum* (Sharma *et al*., 2024). These compounds have been reported for their antimicrobial properties (Phuyal *et al.,* 2020). For instance, *n*-hexadecanoic acid has shown inhibitory effects against various fungi, including *Candida albicans* and *Aspergillus fumigatus* (Khan and Javaid, 2021), while oleic acid exhibited antifungal activity against plant pathogens such as *Fusarium oxysporum* (Liu *et al*., 2008).

Other organic compounds were found in moderate to low percentages as hexadecanoic acid methyl ester with 5.88% and doconexent with 4.87% peak areas. Similarly, 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z)- (3.64%), oxiraneoctanoic acid, 3-octyl-, *cis*- (3.35%), 10-octadecenoic acid methyl ester (3.10%). Chromatographic analysis revealed some more other compounds like *cis*-5,8,11,14,17-eicosapentaenoic acid (3.28%), oxiraneoctanoic acid, 3-octyl-, *cis*- (3.35%), *xanthoxylin* (2.52%) and terpinen-4-ol (3.07%). Compounds like linalyl acetate; terpinen-4-ol, and α-terpineol were relatively abundant during the fruit's maturation period (Peng *et al.*, 2025). All along with it, some other organic compounds were in low concentrations such as 2-cyclohexen-1-ol 1-methyl-4-(1-methylethyl)- *cis* (0.83%), caryophyllene oxide (0.56%) and 9 12 15-octadecatrienoic acid methyl ester (Z,Z,Z)- (0.93%) as shown in Fig. 2 and Table 1. Similar methanolic phyto-constituents namely alkaloids, flavonoids, glycosides, polyphenols, terpenoids, volatile oils, tannins, and saponins were reported in *Z. armatum* by other workders (Peng *et al*., 2025; Shah *et al*., 2025). The observed antifungal activity of *Z. armatum* seed extracts can be attributed to the presence of these bioactive compounds, along with other secondary metabolites such as alkaloids, flavonoids and terpenoids (Alam and Ashraf, 2019). These compounds may act individually or synergistically to inhibit the growth of *S. rolfsii* by disrupting cellular membrane (Manandhar *et al*., 2019), interfering with metabolic process or inhibiting enzyme activity. Isothiocyanates may inactivate the extracellular enzymes through the oxidative cleavage of disulphide bonds (Akhtar *et al.,* 2014).

Conclusion

The present study highlights the potential of methanolic *Z. armatum* seed extract as a foundation of natural antifungal agent against *S. rolfsii*. Phytochemical profiling provided an unprecedented look into the chemical arsenal of the seeds, identifying key bioactive compounds such as oleic acid and *n*-hexadecanoic acid. Additional investigation is necessary to elucidate the mechanism of individual compounds and to optimize extraction method for enhancing antifungal activity.

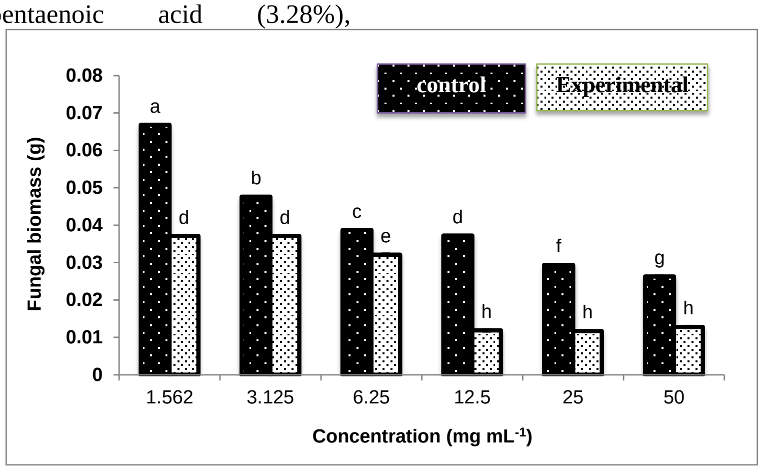

Fig. 1: Effect of methanolic seed extract of *Zanthoxylum armatum* on biomass of *Sclerotium rolfsii*. Different letters at the top of bars show significant difference ($P \leq 0.05$) as determined by LSD test.

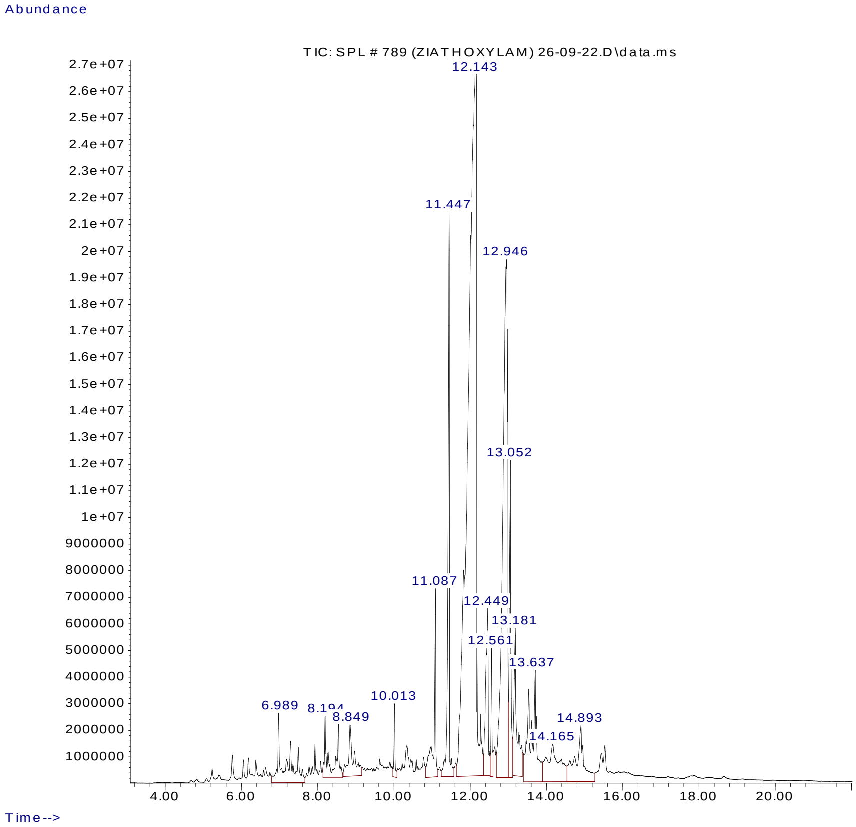

Fig. 2: GC-MS chromatogram of methanolic seed extract of *Zanthoxylum armatum.*

Table 1: Compounds acknowledged from methanolic seed extract of *Zanthoxylum armatum* throughout GC-MS analysis.

Area (%)	Compounds	Retention time (min)
0.56	Caryophyllene oxide	10.013
0.93	9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z)-	12.561
1.99	2-Cyclohexen-1-ol, 1-methyl- 4-(1-methylethyl)-*cis*-	8.194
2.52	Xanthoxylin	11.087
3.07	Terpinen-4-ol	6.989
3.10	10-Octadecenoic acid methyl ester	12.449
3.28	*cis*-5,8,11,14,17-Eicosapentaenoic acid	14.893
3.35	3-Octyl-, *cis*-oxiraneoctanoic acid	14.165
3.64	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	13.052
4.87	Doconexent	13.637
5.88	Hexadecanoic acid methyl ester	11.447
17.84	Oleic acid	12.946
44.07	*n*-Hexadecanoic acid	12.143

Contribution of authors

NJ contributed to the conception and design of the study, data collection, and drafting of the manuscript. NM contributed to the data analysis, interpretation of results, and critical revision of the manuscript. AF contributed to the literature review and data visualization.

Conflict of interests

It is declared that authors have no known conflict of interest related to publication of this manuscript.

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