Research Article

Antifungal Compounds of *Tribulus terrestris* Root for the Control of *Pyricularia oryzae*, the Cause of Rice Blast Disease

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Abstract | *Pyricularia oryzae* is a fungal pathogen that causes blast disease in rice. In the present study, methanolic root extract of a medicinal herb *Tribulus terrestris* was assessed for its antifungal activity against this fungal pathogen. Roots of *T. terretris* were collected from Lahore, Pakistan and shade dried. The dried and crushed roots were extracted in methanol for two weeks and the effect of 1, 2, 3, 4 and 5% concentrations of the extract was checked *in vitro* against *P. oryzae* using malt extract broth as a growth medium. All the applied concentrations of the extract exhibited significant antifungal activity and declined *P. oryzae* biomass by 45–56% over control. The extract was analyzed by GC-MS to identify the possible antifungal constituents. Sixteen compounds were detected in the root extract in GC-MS analysis. Among these, three compounds namely neotigogenin (29.30%), hexadecanoic acid, methyl ester (19.70%) and *cis*-13-octadecenoic acid, methyl ester (18.10%) were found the highly abundant ones. Likewise, three compounds namely methyl stearate (6.65%), stigmasterol (6.05%) and 9,12-octadecadienoic acid (Z,Z)-, methyl ester (7.11%) were categorized as moderately abundant ones. Different fatty acid methyl esters together with stigmasterol identified in the root extract have shown antifungal activity in various previous studies and might be responsible for antifungal activity against *P. oryzae* in the present study.

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Introduction

Rice serves as a staple food for nearly 50% of the global population. It is primarily consumed by 2.5 to 3.5 billion individuals across the globe particularly living in low-income countries (Asibi *et al.*, 2019). It is a significant cash crop and ranks as the second most essential staple food after wheat. It is highly susceptible to numerous diseases, with rice blast being the most destructive. It is caused by the fungus *Pyricularia oryzae* (Wang *et al.*, 2024). The pathogen has the ability to attack rice crop at different growth stages. It produces lesion of varying colors on different aboveground parts of rice plants such as leaves, culms and panicles (Asibi *et al*., 2019). This filamentous fungus belongs to Ascomycete. The disease is polycyclic in nature and spreads by means of conidia, which have the ability to spread in an area of 230 m from the source. Darkness together with high relative humidity and a wind velocity of 3.5 ms^{-1} favor the dispersal of conidia (Kingsolver *et al*., 1984). Yield losses due to rice blast generally range from 10–30% in different rice-growing countries (Skamnioti and Gurr, 2009). The losses may reach up to 50% when there are severe disease outbreaks (Ashkani et al., 2014).

Fungal plant diseases are an increasing concern with significant economic impacts (Khan and Javaid, 2022, 2023a). Nature provides a vast array of chemical diversity, making natural products a vital source for developing new antifungal agents (Ali *et al*., 2017). While the synthetic fungicides application is an effective method for controlling rice blast, excessive reliance on these chemicals can negatively impact beneficial microorganisms, and also harms the environment consumer's health risks (Awla *et al*., 2017). Plants produce a variety of medicinal components capable of inhibiting pathogen growth, and numerous studies have been conducted recently to evaluate the antifungal activity of plant extracts against plant pathogens (Ferdosi *et al*., 2021; Naqvi *et al*., 2023; Rafiq *et al*., 2024).

Tribulus terrestris, a natural herb from the Zygophyllaceae family, is used to treat different diseases, including hypertension. It is found in many tropical and temperate regions worldwide, such as the US, Mexico, the Mediterranean, and throughout Asia (Hussain et al., 2009). Its different parts contain medicinally important chemical constituents such as alkaloids, flavonoids, saponins and flavonol glycosides (Chhatre *et al*., 2014). *T. terrestris* is used to treat asthma, urinary problems and ophthalmia (Qureshi *et al*., 2010). In addition, it exhibits antitumor, vasodilatory, antihelminthic, antihypertensive and cytotoxic properties (Hashim *et al*., 2014). The roots of *Tribulus terrestris* contain several bioactive compounds such as saponins, flavonoids, alkaloids, and glycosides, most of which are also antifungal in nature. Some of these root compounds can inhibit essential enzymes for fungal growth and metabolism, thereby preventing their proliferation. Additionally, the plant antioxidant properties can induce oxidative stress in fungal cells, causing damage and reducing their viability (Negri *et al*., 2014). Results of an earlier study revealed that the stem extract of *T. terrestis* can control *P. oryzae* growth *in vitro* (Javaid *et al*., 2019). There is a need to conduct similar studies using extracts of other parts of this plant. This study was, therefore, carried out to evaluate the antifungal potential of root extract of *T. terrestis* and identification of possible antifungal constituents in it.

Materials and Methods

Preparation of root extract

Roots of mature *T. terrestis* plants were collected from Lahore, Pakistan. After washing, these roots were dried in shade and crushed. A weighed amount (200 g) of root material was soaked in 1.0 L methanol for two weeks followed by successive filtration through muslin cloth and filter papers. Methanol was evaporated on a rotary evaporator and 5.45 g methanolic extract was obtained (Javaid *et al*., 2023).

Antifungal bioassay

Antifungal bioassays with methanolic root extract of the weed was carried out following procedure of Khan and Javaid (2023b) with some modifications. For preparation of stock solutions, an amount of 4.725 g of root extract was dissolved in 4 mL of dimethyl sulfoxide (DMSO) and raised the volume to 10.5 mL by adding distilled water. Same amount of DMSO was mixed in 6.5 mL of distilled water to get 10.5 mL of control solution. For preparation of different concentrations of the extract in growth medium, appropriate volumes of the two solutions (stock and control) were added to 42 mL autoclaved malt extract broth to acquire a final volume of medium up to 45 mL. This amount was divided into three equal portions to have 3 replicates of each concentration. Actively growing discs (5 mm) of *P. oryzae* were used as inoculum. After inoculation, the flasks were incubated at 27 °C and the fungal biomass was harvested on pre-weighed filter papers after 10 days. Fungal biomass along with filter papers was dried in an electric oven at 60 °C and weighed. Fungal dry weight was calculated by subtracting the weight of filter paper from the total weight.

GC-MS analysis

Methanolic root extract of the test weed species was analyzed by GC-MS on an Agilent 7890A GC system with 5975C mass spectrometer. Details of various conditions set for GC-MS analysis are presented in

Javaid *et al.* (2022).

Statistical analysis

Antifungal bioassay was carried out using three replicates of each treatment. Standard errors of means of the four replicates were calculated using MS Excel software. The data were analyzed by one-way ANOVA followed by LSD test at P= 0.05 for calculation of significant differences among the treatment means using software Statistix 8.1.

Results and Discussion

Antifungal activity of methanolic root extract

Root extract of *T. terrestris* showed remarkable antifungal activity and reduced the biomass of *P. oryzae* by 45–56% over control. Antifungal activity of the methanolic extract was increased gradually as the concentration was increased from 1% to 5% (Figure 1). Earlier, Al-Bayati and Al-Mola (2008) reported that root of *T. terrestris* collected from Iraq were extracted in water, ethanol and chloroform and their antifungal activity was evaluated against *Candida albicans*. All the extracts showed insignificant antifungal activities. Information regarding antifungal activity of root extract of this weed is rare. Previous studies have shown that extract of aerial parts of this weed such as stem extract suppressed the growth of *P. oryzae* by 35–43% when same concentrations of the extract were used (Javaid *et al.*, 2019). Previous studies on *T. terrestris* were generally carried to assess its antibacterial activity. Root extract of *T. terrestris* showed antibacterial activity against *Escherichia coli* and various other bacterial species (Chhatre *et al.*, 2014). Likewise, Sasikala *et al.* (2014) reported that acetone root extract (80 µL) of *T. terrestris* caused wide zones of inhibition against the growth of *Escherichia coli* (33.6 mm) and *Bacillus cereus* (23.6 mm).

GC-MS analysis

Sixteen compounds were detected in GC-MS analysis in the root extract of *T. terrestris* as shown in Table 1 and Figure 2. The most abundant compound was neotigogenin (29.30%) followed by hexadecanoic acid, methyl ester (19.70%) and *cis*-13-octadecenoic acid, methyl ester (18.10%). Neotigogenin was previously identified in *T. terrestris* and *Solanum paniculatum* with antibacterial and anticancer activities, respectively (Valadares *et al.*, 2009; Batoei *et al.*, 2016). Hexadecanoic acid, methyl ester and *cis*-13-octadecenoic acid, methyl ester belong to fatty acid methyl esters group. Many studies have shown that fatty acid methyl esters showed antifungal activity against a variety of fungal species including *Aspergillus flavus*, *A. ochraceus*, *A. fumigatus* and *A. niger* (Shen *et al.*, 2012; Abdelillah *et al.*, 2013), *Candida albicans* and *C. parapsilosis* (Agoramoorthy *et al.*, 2007).

Table 1: *Compounds identified methanolic root extract of Tribulus terrestris through GC-MS analysis.*

Comp No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	3-Methoxyacetophenone	$C_9H_{10}O_2$	150.17	12.696	0.99
2	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.39	17.227	1.10
3	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.42	18.885	0.94
4	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	20.516	19.70
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	23.099	7.11
6	*cis*-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48	23.206	18.10
7	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.48	23.281	0.95
8	Methyl stearate	$C_{19}H_{38}O_2$	298.50	23.602	6.65
9	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.84	26.533	1.22
10	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	$C_{23}H_{32}O_2$	340.49	27.705	1.19
11	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.66	33.738	1.03
12	Squalene	$C_{30}H_{50}$	410.71	35.455	1.71
13	Cholesterol	$C_{27}H_{46}O$	386.65	42.328	2.21
14	Stigmasterol	$C_{29}H_{48}O$	412.69	45.714	6.05
15	Tigogenin	$C_{27}H_{44}O_3$	416.63	46.730	1.30
16	Neotigogenin	$C_{27}H_{44}O_3$	416.63	47.276	29.30

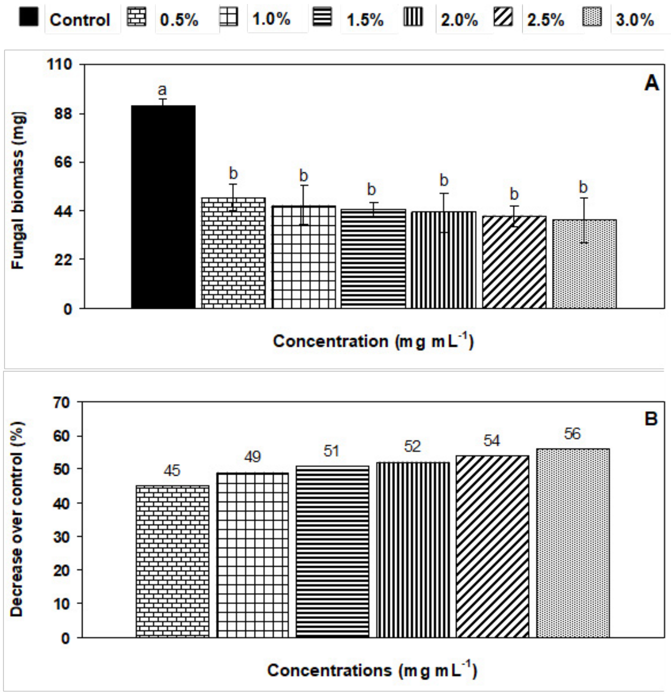

Figure 1: *Effect of different concentrations of methanolic root extract of Tribulus terrestris on biomass of Pyricularia oryzae. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD test.*

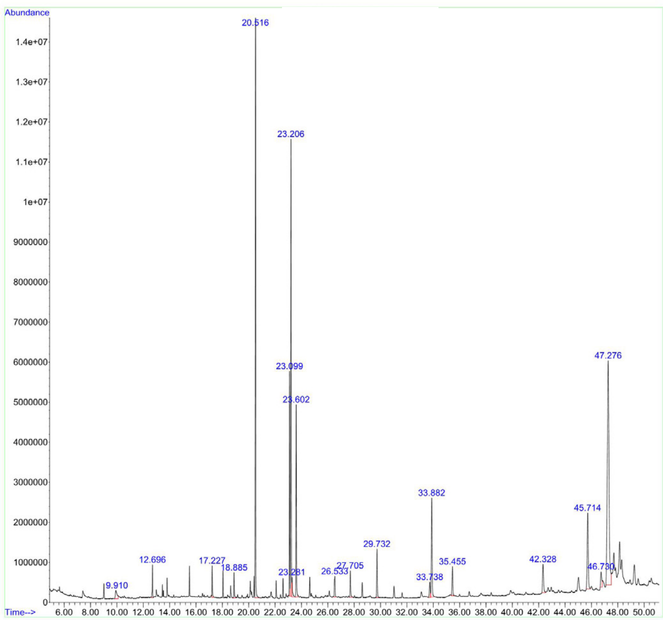

Figure 2: *GC-MS chromatogram of methanolic root extract of Tribulus terrestris.*

Moderately abundant compounds included methyl stearate (6.65%), stigmasterol (6.05%) and 9,12-octadecadienoic acid (Z, Z)-, methyl ester (7.11%) (Table 1). Methyl stearate possess antifungal activity in addition to various other biological activities (Adnan *et al*., 2019; Mazumder *et al*., 2020). Stigmasterol isolated from aerial parts of *Neocarya macrophylla* and *Spillanthes acmella* presented antifungal activity against different *Candida* spp. that was comparable with the fungicide fluconazole (Yinusa *et al*., 2014; Yusuf *et al*., 2018). 9,12-Octadecadienoic acid (Z, Z)-, methyl ester is a natural compound with a number of biological activities such as antimicrobial, wound healing, skin repair and anti-inflammatory (Salem *et al*., 2022).

Ten constituents were ranked as less abundant, which included cholesterol (2.21%), squalene (1.71%), tigogenin (1.30%), tetracosamethyl-cyclododecasiloxane (1.22%), phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (1.19%), methyl tetradecanoate (1.10%), tetracosanoic acid, methyl ester (1.03%), 3-methoxyacetophenone (0.99%), 9-octadecenoic acid, methyl ester, (E)- (0.95%), and pentadecanoic acid, methyl ester (0.94%) (Table 1). Most of these compounds are fatty acid methyl esters which are famous for inhibitory activity against fungi (Abdelillah *et al*., 2013).

Conclusions and Recommendations

This study concludes that methanolic root extract of *T. terrestris* is effective in controlling growth of rice blast pathogen *P. oryzae*. GC-MS analysis showed that it contains a number of antifungal compounds such as fatty acid methyl esters, stigmasterol and neotigogenin.

Novelty Statement

Tribulus terrestris is a weed plant whose medicinal and pharmacological importance is well-documented. A previous study showed that stem extract of this weed has the ability to control a highly damaging fungal pathogen of rice, *Pyricularia oryzae*. However, such studies regarding antifungal activity of root extract of this weed are lacking. Therefore, the present study was carried out to explore the potential of methanolic root extract of this weed in the management of rice blast pathogen, *P. oryzae*.

Author's Contribution

Arshad Javaid supervised the whole study, carried our statistical analysis, prepared graphs and did final editing of the mnauscript. Freeha Anjum conducted the antifungal bioassay. Aneela Anwar supervised the GC-MS study. Sadia Ahmad and Mahrukh Asif contributed in paper writing. Malik F.H. Ferdosi collected and processed the plant material, and also helped in GC-MS analysis.

Conflict of interest

The authors have declared no conflict of interest.

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