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Efficacy of Some Medicinal Plants Extracts for Potential Antifungal Activity

Javad Abkhoo,[1,*] and Somayeh Jahani[2]

[1]Institute of Plant Biotechnology, University of Zabol, Zabol, IR Iran
[2]Infectious Disease and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, IR Iran

[*]*Corresponding author*: Javad Abkhoo, Institute of Plant Biotechnology, University of Zabol, Zabol, P. O. Box 98616, IR Iran, E-mail: javad.abkhoo@yahoo.com

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Abstract

Background: *Peganum harmala*, *Glycyrrhiza glabra*, *Mentha spicata* and *Rosmarinus officinalis* are often used in traditional medicine.
Objectives: The current study aimed to screen the antifungal activity of ethanolic extracts of *P. harmala*, *G. glabra*, *M. spicata* and *R. officinalis* leaves collected from Sistan region, Iran against *Fusarium oxysporum*.
Methods: Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of herbal extracts were determined.
Results: Of the total herbal extracts, four showed fungistatic activities and two showed fungicidal activities. MIC values, ranged 6.25 - 50 ppm and MFC values, ranged 12.5 - 100 ppm. The ethanolic extract of *G. glabra* had the lowest MIC (6.25 ppm); while the ethanolic extract of *R. officinalis* had the highest MIC (50 ppm). Minimum bactericidal concentration (MBC) of the alcoholic extract of *G. glabra* showed the highest efficiency against *F. oxysporum* at the low value of 12.5 ppm.
Conclusions: The results of the study showed the antifungal activity of medicinal plants against *F. oxysporum*. Especially, the bioactive compounds of *G. glabra* were effective to inhibit the growth of *F. oxysporum*.

Keywords: Antimicrobial Effect, Ethanol Extract, Medicinal Plants, *Fusarium oxysporum*

1. Background

The *Fusarium oxysporum* that is one of the most important *Fusarium* species does not show sexual reproduction. Crown and root rot caused by these fungi are reported (1). The species were isolated from several European countries, Asia, Africa and North America (2). In Iran, for the first time in 1964, the causal agent was isolated from melon in Mashhad (3). Although the fungi is an option, due to practical difficulties including non-target effects, high cost and environmental and health threatening risks, it is not at least always preferred (4). The growing popularity of organic production requires the development and adoption of other methods to control fungi diseases.

Iran has a long history of traditional medicine with recent academic facilities. At present, there is little evidence on the antimicrobial properties of herbal medicines under review against *F. oxysporum*.

2. Objectives

The current study aimed to investigate the potential antifungal activity of aqueous extracts of herbal medicines collected from Sistan region, Iran, against *F. oxysporum* to verify possible inhibitory effects.

3. Methods

3.1. Plant Material

Different herbal tissues of various species used in traditional medicine (Table 1) were collected from Sistan region, Iran, in 2016.

Table 1. List of Herbal Medicines Used in the Research

Species	Family	Organ Used
Mentha spicata	Lamiaceae	leaf
Glycyrrhiza glabra	Fabaceae	leaf
Rosmarinus officinalis	Lamiaceae	leaf
Peganum harmala	Zygophyllaceae	leaf

3.2. Preparation of Plant Extracts

After collecting the plants, they were dried at 25°C. To prepare the extracts, 10 g dry powder of the plants was placed in a liter flask containing 100 mL of 96% ethanol.

Extracts of *Peganum harmala*, *Glycyrrhiza glabra*, *Mentha spicata* and *Rosmarinus officinalis* were prepared using a rotary device (5).

3.3. Fungal Strain

Spores of F. oxysporum were collected from corn meal agar cultures after seven days (6). Sporangial suspension concentration was prepared using a cell counting chamber adjusted to 1×10^6 spores mL^{-1} (7).

3.4. Minimal Inhibitory Concentration and Minimum Fungicidal Concentration

A microplate method was utilized to determine MIC values of the herbal extracts (8). The extracts were diluted ranging from 1/1 to 1/64 of crude extract. In each well, 100 microliters of each dilution of the extract with 10 microliter of the spore suspension (1×10^6 mL^{-1} spores) were mixed. Microplates were incubated for 72 hours at 28°C. The MIC was measured by microplate reader at 595 nm and was determined by comparing the growth in the control wells. The MIC was defined as the lowest concentration of the extract that inhibits the growth of more than 90% at 72 hours as compared to that of the control.

4. Results

The effect of different concentrations of herbal extracts on *F. oxysporum* is summarized in Table 2. Thirteen ethanol extract dilutions (ranging from 1/1 to 1/32) inhibited the growth of *F. oxysporum* more than 90%. The ethanol extract of *G. glabra* performed in 1/32 dilution showed growth inhibition at low concentrations.

Table 2. Antifungal Activity of Various Dilutions of Ethanolic Herbal Extracts[a]

Plant Species	Ethanolic Herbal Extract						
	1/1	1/2	1/4	1/8	1/16	1/32	1/64
Peganum harmala	+	+	-	-	-	-	-
Glycyrrhiza glabra	+	+	+	+	+	+	-
Menthaspicata	+	+	-	-	-	-	-
Rosmarinus officinalis	+	+	+	-	-	-	-

[a] +, growth inhibition $\geq$ 90%; -, growth inhibition < 90%.

MIC and MFC were performed for ethanol extract of each of the two species. Table 3 shows MIC and MFC of active herbal extracts. Of the total extracts, four showed inhibitory effects on fungi and two showed no activity.

MIC values ranged 6.25 - 50 ppm and MFC values ranged 12.5 - 100 ppm. The ethanol extract of *G. glabra* showed the lowest MIC (6.25 ppm), while the ethanol extract of *R. officinalis* had the highest MIC (50 ppm). MBC of the alcoholic extract of *G. glabra* had the highest inhibitory effects on *F. oxysporum* at 12.5 ppm (Table 3).

Table 3. Minimal Inhibitory Concentration and Minimum Fungicidal Concentration of Ethanolic Herbal Extracts Against *Fusarium oxysporum*[a]

Plant Species	MIC	MFC
Peganum harmala	100	Nf
Mentha spicata	100	Nf
Glycyrrhiza glabra	6/25	12/5
Rosmarinus officinalis	50	100

[a] -, no inhibitory activity; Nf, no fungicidal activity.

5. Discussion

The antifungal effects of *G. glabra* extract, and its active constituent glabridin, on antibiotic-resistant *Candida albicans* species were studied in different studies (9). The current research showed antifungal effects of *G. glabra* extracts on *C. albicans* species. The inhibitory effects of glycyrrhetinic acid derived from *G. glabra* on *Candida* spp. were evaluated in different studies and the results showed the efficiency of the combination (10). The researches indicated that *G. glabra* can inhibit the growth of *Aspergillus parasiticus* and prevent the production of aflatoxin (11). Another research reported that the methanol extract of *G. glabra* had high fungicidal effects on *Chaetomium funicola* and *Arthrinium sacchari* (12).

In addition, *Glycyrrhiza* species include glycyrrhizin, which prevents protein synthesis in bacteria (13). A study indicated the antipseudomonal activity of *G. glabra* and one of its compounds (14). The inhibitory effect of *G. glabra* on *Helicobacter pylori* was studied in-vitro and indicated that it can have effects similar to those of metronidazole (15). Another research also indicated the inhibitory effects of *G. glabra* extract on *H. pylori* (16). Some *Glycyrrhiza* species, e g, *G. glabra*, inhibit the growth of some Gram-negative bacteria such as *Shigella* spp. and *Salmonella* spp. (17). Glycyrrhizin also showed anti-viral and anti-tuberculosis properties (18, 19).

5.1. Conclusion

The results of the current research showed antifungal activities of herbal medicines against *F. oxysporum*. In particular, *G. glabra* extract presented bioactive compounds effective to inhibit the growth of *F. oxysporum*.

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Footnotes

Authors' Contribution: All authors had equal role in designing, performing the experiments, statistical analyses and manuscript writing.

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