

Prosopis farcta: Potent Antifungal Activity Against *Trichophyton mentagrophytes* Strains; A Research Based on an Ethnobotanical Study

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ABSTRACT

Background and Aim: Dermatophytosis is a superficial fungal disease. *Prosopis farcta* has attracted attention for ethnobotany and medical purposes. The present study aimed to investigate the antifungal properties of *Prosopis farcta* extracts against *Trichophyton mentagrophytes* (PTCC 5054) and five archived terbinafine resistant clinical isolates of *T. mentagrophytes*, based on an ethnobotanical report in Yazd province (Iran).

Materials and Methods: *In vitro* drug susceptibility for methanol extract and amphotericin B was carried out according to the CLSI-M38-A2. A topical solution (1%) was formulated by root extract of *P. farcta*. The nine male Sprague rats were infected by *T. mentagrophytes* and assessed for *in vivo* anti-dermatophytic activity.

Results: The MIC value of amphotericin B was $\leq 0.5 \mu\text{g/mL}$ against all strains. The methanol extract showed the lowest MIC and MFC values on fungal activity (both with 0.00625 mg/mL). The complete cure of 21-day period with terbinafine is reduced to 10 days with methanol 80% root extract of *P. farcta* solution.

Conclusion: Compared with amphotericin B, *P. farcta* could be considered a potential antifungal agent in terbinafine-resistant clinical isolates of dermatophytes.

Keywords: Dermatophytes, Ethnobotanical Report, *Prosopis farcta*, Terbinafine Resistant, *Trichophyton mentagrophytes*

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1 Introduction

Fungal infections are among the most prevalent infections affecting approximately a quarter of the world's population, which is observing an increasing trend since the number of immunocompromised patients is rising due to the increased number of patients receiving immunosuppressive drugs in such conditions as cancers and HIV infection (1, 2). Dermatophytosis, also known as tinea or ringworm, is

a well-known superficial fungal disease, mostly caused by *Trichophyton* species such as *Trichophyton mentagrophytes* and *Trichophyton rubrum* (3). *Trichophyton mentagrophytes* are divided into two distinct strains, namely anthropophilic and zoophilic (3).

Various synthetic components with different structural properties are available adopted to treat

dermatophytosis. In this regard, today, terbinafine (TRB) and azoles are used as the main treatments of dermatophytosis (4). However, the emergence of TRB and azole-resistant *Trichophyton* strains showed that these strains should be included in searching for new inhibitory agents to treat drug-resistant organisms (5-7).

Plants are considered the main source of secondary metabolites showing degrees of antifungal activity

such as flavonoids, phenols, saponins, and terpenoids (8).

Prosopis farcta (Banks & Sol.) J.F. Macbr. from the Leguminosae family, commonly known as mesquite, is a native plant of Asia, Eastern Europe, and Northern Africa (mostly distributed from India to Iran). Similar to other species of genus *Prosopis*, this plant is mostly less than 1 m (usually 40 cm) tall and well adapted to drought (Figure 1) (9, 10).



Figure 1. *Prosopis farcta* grown in Yazd province, Iran.

P. farcta has been mainly used as a traditional medical means in the south of Iran, and nowadays, it has attracted attention for ethnobotany and medical purposes, such as antimicrobial activity, anti-tumor, and antioxidant properties (11, 12). To the best of our knowledge, few studies have been performed investigating the biological activities of *P. farcta* root. The previous studies merely determined the antibacterial, anti-diabetic, and nitrogen fixative activity of root parts (12, 13) and aerial parts (9, 14) of *P. farcta*.

In a report obtained from the researchers, Afghans living in Dehnow region of Yazd province used the root of this plant as an anti-parasitic and topical antifungal. It is called "Jenjengok" in the Yazdi dialect. The purpose of this study is to assess the antifungal activity of *P. farcta* extract on *T. mentagrophytes* strain *in vitro* and *in vivo* for the first time.

2. Materials and Methods

2.1 Plant Materials and Extraction Procedure

The roots of *P. farcta* were collected from Dehnow area (Yazd Province, Iran) in July 2018. The plants were identified by Dr. V. Mozaffarian at Iran Agriculture and Natural Resources Research and Education Center, Tehran, Iran. The root parts were cleaned, dried, and grounded to a fine powder. Subsequently, 100 g of

each powdered part was immersed in 300 mL methanol: water (4:1 or 80%) (Chem-Lab, Belgium) and held in a shaking incubator (GFL, Germany) at room temperature for 24 h (maceration method). This process was performed three times more, followed by the filtration (Whatman, UK) and evaporation of solvents using a rotary evaporator (Heidolph, Germany). The concentrated extracts were dried in a dry-oven (Dena, Iran) at 40°C to remove the remained solvents, and the yield of extracts was calculated. For further tests, the resulting extracts were kept in sterile containers at 4°C. To prepare the topical solution of 1%, the above-mentioned amount was solved in water. Then it was subjected to the ultra-sound waves for ten minutes. It is worth mentioning that the solution is made under GLP conditions in a reliable laboratory.

2.2. Fungi Isolates and *in vitro* Anti-dermatophytic Assay of *P. farcta*

The five archived clinical isolates of terbinafine resistant *T. mentagrophytes* strains were used in this study. *T. mentagrophytes* reference strain (PTCC 5054) was obtained from the Iranian Research Organization for Science and Technology.

The strains were cultured on potato dextrose agar (PDA; Merck, Germany). Inoculum suspensions were prepared by covering fresh cultures of *T. menta-*

grophytes with saline solution and tween 80, according to the Clinical and Laboratory Standards Institute guidelines (CLSI, M38-A2) (15). The suspensions were added into a tube containing 2 mL of saline solution, and densities were adjusted at 530 nm. The final density of inoculum was 1×10^3 to 3×10^3 CFU/mL. The inocula were diluted 1:50 in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, USA).

The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were evaluated with methanol plant extract and amphotericin B (AMB; as a positive control).

The first, 100 μ L of RPMI 1640 medium was added to a 96-well microplate, and 100 μ L extract suspension (64 mg of concentrated extracts in 1 mL distilled water) was added to the first well. The contents were mixed, and 100 μ L was transferred to a second well. This serial dilution was repeated through to the ninth well. An aliquot (100 μ L) was discarded from the ninth well.

The serial dilution of plant extract was prepared with concentrations ranging from 0.00625-32 μ g/mL. Then 100 μ L of inoculum was added to wells 1-10, and the contents were mixed. The microplate was incubated at 30°C for 72 h.

50 μ L was inoculated onto PDA from the solution without growth in the MIC test regarding MFC. Moreover, the lowest concentration without growth was recorded as the MFC value. The tests were performed in duplicate.

2.3. In vivo Anti-dermatophytic Activity of *P. farcta*

A suspension of *T. mentagrophytes* (PTCC 5054) was prepared by washing the surface of the fresh culture tube (on PDA media after 7-10 days at 25°C) with sterile distilled water and tween 80 adjusted to 1×10^6 conidia/mL (16).

Male Swiss albino mice (*Mus musculus*) of approximately 5-7 weeks old and 30-40 g weight were used for the present investigation. Mice were immunosuppressed by subcutaneous injection of 500 mg of estradiol valerate 4 days before the infection (17). The hair of the back of each mouse (2 cm²) was shaved, and the skin was slightly scraped by a single-use scalpel. Afterward, 50 μ L of the suspension of *T. mentagrophytes* was inoculated to the surface within the shaved zone and was gently rubbed with the flat part of a sterile blade (17).

The animals were assigned to three groups, including a test group (n=3), a positive control group (n=3) receiving treatment with TRB (the reference antifungal drug), and a negative control group taking only distilled water without infection (n=3). After 21 days of primary infection, when the dermatophytosis appeared, methanol extract of *P. farcta* root was used as a solution (1%) in the test group. The animals were fed with autoclaved water and food in clean cages. The skin lesions were scored on a scale ranging from 0-3 (no visible lesions to significant crusting and erythema) based on the results of a previous study (18). The effect of the *P. farcta* solution against *T. mentagrophytes* strains was examined by shaving the hair and scraping the skin of infected mice (Figure 2).



Figure 2. A: Preparing the animal for the test. B: Dressing the contaminated area with a solution containing the extract of *P. farcta*.

2.6. Ethical Statement

The ethics approval was obtained from the animal ethics committee of Tehran University of Medical Science, Tehran, Iran (IR.TUMS.VCR.REC.1398.196).

2.7. Statistical Analysis

The values of MIC and MFC were calculated in SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA) and the differences between the groups were determined

using ANOVA. A P-value of ≤ 0.05 was considered significant.

3. Results

3.1. Yield of Extraction

The extraction yield of *P. farcta* was obtained as 11.8%.

3.2. In vitro Anti-dermatophytic Activity

The hydro-alcoholic extract revealed a growth inhibition effect against tested isolates ($P=0.042$). The MIC and MFC of the methanol extract are tabulated in Table 1.

Table 1. Minimum inhibitory concentration and minimum fungicidal concentration of the plant extracts

Test isolates	Methanol extract		amphotericin B	
	MIC* ($\mu\text{g/mL}$)	MFC* ($\mu\text{g/mL}$)	MIC ($\mu\text{g/mL}$)	MFC ($\mu\text{g/mL}$)
<i>T. mentagrophytes</i> (PTCC 5054)	0.125	0.25	0.031	0.062
1	0.125	0.25	0.25	0.25
2	0.031	0.062	0.062	0.125
3	0.031	0.062	0.125	0.25
4	0.062	0.125	0.125	0.25
5	0.031	0.031	0.125	0.25

*MIC: Minimum Inhibitory Concentration, MFC: Minimum Fungicidal Concentration, Clinical isolates of *Trichophyton mentagrophytes*: 1, 2, 3, 4, 5

Based on the results, The MIC value of AMB was $\leq 0.5 \mu\text{g/mL}$ against all strains. The highest MIC and MFC values were determined by the methanol extract (both with 0.0312 mg/mL).

3.3. In vivo Anti-dermatophytic Activity

It was revealed that *P. farcta* solution (1%) was a perfect cure compared with terbinafine ($P<0.05$). The complete cure using the terbinafine occurred on day 21, while using *P. farcta* solution (1%) reduced this period to 10 days. The hair culture exhibited 100% recovery within 6-15 days; however, the positive control group was grown in the hair culture.

4. Discussion

One of the major concerns in modern medicine is the organisms developing resistance to synthetic drugs (7, 19). This issue made conventional medicine a point of interest to search for any evidence allowing it to be applicable for drug-resistant organisms (20). Over time, the application of herbal medicine in treating superficial fungal lesions has received attention among different parts worldwide (21, 22). Secondary metabolites produced by herbal medical species are the main mediators to achieve the desired effect of herbal medicine on the subject (23). The antimicrobial activity of *P. farcta*, is related to active metabolites in all parts of *P. farcta* like; flavonoids,

saponins, phenols, alkaloids, tannins, resins, and glycosides.

It was reported that the root of *P. farcta* contained high concentrations of flavonoids, saponins, and phenols and moderate concentrations of other active compounds noted previously (12).

For anti-parasite activity, the fresh root extract was used as a herbal remedy in Iran (24).

The source of variable degree of resistance or sensitivity of fungi against plant extract may be due to the combinations and nature of compounds present in the plant extract (25). On the other hand, the inherent tolerance of the fungi species can play a role in this pattern. The main phytochemicals in *P. farcta* are alkaloids, tannins, and glycosides. These phytochemicals have antimicrobial activity (12).

Recent reports have indicated that terbinafine-resistant *T. mentagrophytes* harbors a mutation in the squalene epoxidase (*SQLE*) gene, which aroused concern for the spread rate of this resistance throughout the world (26). In 2020, this mutation-mediated resistance was reported in Iran and India and made this organism a target for herbal medicine research (27, 28).

According to the review of previous studies, anti-organism topics were mainly investigated on aerial parts of *P. farcta* (9, 29). In those with the root parts examined, only the antibacterial properties under-

went evaluation (30). To the best of our knowledge, the present study was the first to investigate both *in vitro* and *in vivo* antifungal activity of the root part of *P. farcta*. According to Saad *et al.*, ethyl-acetate extract of aerial parts of *P. farcta* demonstrated significant antifungal activity against *Candida albicans* with a 7.3 mm inhibition zone in the filter paper disc method (29). Based on another study, a MIC value of 32 µg/mL against *C. albicans* was reported for *P. farcta* ethanol fruit extract. In the mentioned research, the MIC value was estimated at 256 µg/mL for *Aspergillus niger* (9). The only study investigating the *in vitro* antifungal activity against dermatophytes was performed by Maoz and Neeman. Accordingly, a MIC of 10 % was reported for aqueous extract of upper parts of *P. farcta* against *Microsporum canis* and *Trichophyton rubrum* (31).

A comparative review of previous studies concerning the antifungal activity of herbs against *T. mentagrophytes* revealed the potentiality of *P. farcta* against this fungus species. According to Balakumar *et al.*, the *Ocimum sanctum* showed antifungal activity against *T. mentagrophytes* with a MIC of 125±25 µg/mL (32). Essential oils of *Thymus serpyllum* (MIC=0.1 %, area percentage), *Origanum vulgare* (MIC=0.5 %), and *Rosmarinus officinalis* (MIC=5 %) were demonstrated to have *in vivo* anti-dermatophytic activity on *T. mentagrophytes* (33). In a study, the antifungal activity of ethyl-acetate extracts of 36 herbs grown in Japan was evaluated against *T. mentagrophytes* based on an agar diffusion test. The results of the mentioned research showed the best inhibitory diameter for *Monarda fistulosa* seeds (48 mm), *Lavandula x intermedia* flowers (36 mm), and *Salvia officinalis* flowers and leaves (35 mm) (34). *Inula helenium* and *Curcuma longa* were demonstrated to have antifungal activity against *T. mentagrophytes*

with an inhibition zone of 23 mm and 14 mm, respectively (35).

5. Conclusion

Herein, the hydro-alcoholic root extract of *P. farcta* showed excellent anti-dermatophytic properties compared with AMB in terbinafine resistant clinical isolates. The results of this study confirmed the ethnobotanical uses of this plant in fungal skin infections. This finding can be considered a promising antifungal agent. Furthermore, to reveal the effective compounds in the methanol extract, it is essential to screen the anti-dermatophytic assay.

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Author's Contribution

E.S.S and E.L designed the project. E.S.S prepared the extracts and solution. E.L performed an antifungal susceptibility assay. M.F performed the animal assay. K.R and A.Kh wrote the draft of the manuscript. E.S.S, M.F, and E.L revised the manuscript. All of the authors approved the final version of the manuscript.

Conflict of Interest

The authors declared no conflict of interest.

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