AMERICAN JOURNAL OF BIOMEDICINE AND PHARMACY
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Article

Antifungal Activity of Urtica dioica Extracts Against Clinical Dermatophyte Isolates from Male Patients

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Citation: Mohammed A., Idan A. K., Jihad A. K., Al-Obadi Z. H. K., Rashed H. J. Antifungal Activity of Urtica dioica Extracts Against Clinical Dermatophyte Isolates from Male Patients. American Journal of Biomedicine and Pharmacy 2026, 3(7), 44-51.

Received: 10th Apr 2026
Revised: 11th May 2026
Accepted: 24th Jun 2026
Published: 13th Jul 2026

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Abstract: Fungi can cause infection with different species of fungi and may present in various clinical manifestations in humans. Dermatophytes cause one of the most prevalent superficial fungal infections in the world – known as dermatophytosis. A few synthetic and natural antifungal agents have been used in controlling this infection. The Urtica dioica is a medicinal plant that contains several bioactive compounds, such as saponins, tannins, phytosterols, flavonoids, chlorophylls, proteins, vitamins and amino acids. Few studies have been reported on its antifungal effect against dermatophytes, however. Thus, the present study was aimed to investigate the antifungal activity of U. dioica extracts against clinical isolates of dermatophytes. The present study was carried out in Al-Zahraa Teaching Hospital in Microbiology Unit, Wasit Governorate in Iraq. A total of 50 clinical specimens (skin scraping, nail, and hair) were taken from male patients attending the hospital during the time period of October 2024 to February 2025. Culture of dermatophytes on Sabouraud dextrose agar and direct microscopic examination with potassium hydroxide was employed for the isolation and identification of dermatophytes. The extracts, aqueous and ethanolic of U. dioica, were prepared at three concentrations 1g/mL, 0.5g/mL and 0.25g/mL and tested by poisoned food technique and disc diffusion method. 40 out of 50 (80.0%) of clinical specimens were positive for dermatophytes while 10 (20.0%) were positive for non-dermatophytes fungi in male patients. The dermatophyte isolates were T. rubrum (15; 37.5%), T. mentagrophytes (11; 27.5%), T. tonsurans (9; 22.5%), and M. gypseum (5; 12.5%). Non-dermatophyte fungi comprised of C. albicans and A. fumigatus. The ethanolic extract of U. dioica exhibited higher antifungal activity compared to the aqueous extract and the inhibitory effect was enhanced with increasing concentration of extract. Lowest Rg values were obtained at 1g/mL of ethanolic extract in poisoned food technique. The disc diffusion method also produced the highest inhibition zones at the concentration of 1g/mL of ethanolic extract. Ethanolic extract of U. dioica showed higher antifungal activity against dermatophytes than aqueous extract. The effect was found to be more pronounced with higher concentration and hence U. dioica might be useful as natural antifungal agent to control the growth of dermatophytes.

Keywords: Urtica dioica, dermatophytes, Sabouraud dextrose agar (SDA), aqueous extract, ethanolic extract, antifungal activity.

Introduction

Fungi are eukaryotic organisms which consist of yeasts, molds and mushrooms. Traditional classifications [1] classify them as a separate kingdom, one that is separate from the kingdom Plantae, the kingdom Animalia, the kingdom Protozoa, and the kingdom Chromista. Mycosis or fungal infection is a disease caused by fungi. Fungal infections are generally categorized as superficial, subcutaneous and systemic infections based on the body part infected. Superficial infections involve the superficial tissues of the skin, hands, feet, nails and beard area, while subcutaneous infections are those that involve deeper skin tissue. Systemic infections tend to be more severe as they can be accompanied with infection of internal organs [2][3].

Fungi are widely distributed in the environment; however, only some species are able to infect humans. Fungal infection can happen if the fungus gets into contact with a susceptible skin or tissues [4]. The fact that fungal infections are a major health problem and have been on the rise in recent years, especially in susceptible patients and immunocompromised persons [5][6], highlights their importance. Dermatophytosis is one of the most common superficial fungal infections of humans. It is caused by the keratinophilic fungi (dermatophytes), which can infect keratinized tissues like hair, skin and nails [7]. The most frequent species of dermatophytes found are T. rubrum, T. mentagrophytes and M. gypseum [8].

There are different antifungal drugs available for the treatment and control of dermatophytes infections. Variations in susceptibility, however, and the occurrence of recurring and/or resistant infections underscore the need for other or adjunctive antifungal drugs [9]. Today, herbal medicine is gaining more and more popularity due to the fact that several medicinal plants have biologically active compounds that could have antimicrobial or antifungal properties. Therefore, the plant sources can be considered as potential natural resources for discovering new antifungal agents. The medicinal plants are rich in a number of classes of bioactive organic compounds such as phytosterols, saponins, flavonoids, tannins, sterols, fatty acids, carotenoids, chlorophylls, proteins, amino acids and vitamins [10].

Urtica dioica, commonly known as stinging nettle, is a medicinal plant that has been used traditionally for different therapeutic purposes. There are a few pharmacological activities reported for U. dioica, but investigations into its antifungal activity against dermatophytes are limited. The present study was thus set to assess the antifungal activity of the two extracts of U. dioica against the clinical dermatophyte isolates recovered from patients who visited Al-Zahraa Teaching Hospital in Wasit, Iraq.

Materials and Methods

Sample Collection

Fifty clinical samples were obtained from male patients attending Al-Zahraa teaching hospital, Wasit Governorate, Iraq. The skin scrapings, nail samples and hair samples were taken from all the specimens included, and they were all from male patients. Skin scrapings were taken from the active perimeter of lesions after cleaning the area with 70% ethanol and using new and sterile blades to do so. Nail samples were taken after surface was cleaned with 70% ethanol and hair samples were placed in sterile Petri dishes.

Carefully all specimens were transferred into Petri dishes filled with Sabouraud dextrose agar (SDA) added with chloramphenicol (0.05 g/L) and cycloheximide (0.5 mL/L). Pathogenic fungi were isolated and cultured in the agar media. Identification was carried out based on the colony morphology and direct microscopic examination by potassium hydroxide (KOH) test.

Preparation of the Aqueous and Ethanolic Extracts of Urtica dioica.

The leaves and stems of U. dioica were dried and finely ground into powder using a grinder. The plant powder (200 g) was macerated separately in distilled water for the aqueous extract and in ethanol for the ethanolic extract for extract preparation. The mixtures were kept for 24 hours at room temperature and then filtered using filter paper.

A rotary evaporator was used to reduce the solvents at 40°C for 30 minutes. In case of necessity, the ethanolic extract was prepared in dimethyl sulfoxide (DMSO). Dry residues were frozen at 4°C until they were used.

The Poisoned Food Technique was used to investigate the effect of Urtica dioica Extracts on Dermatophytes.

The antifungal activity of extracts of U. dioica against dermatophytes was determined by poisoned food technique. 1 mL of distilled water (aqueous extract) or 70% ethanol (ethanolic extract) was used to make the various sample concentrations: 1 g/mL, 0.5 g/mL and 0.25 g/mL. 9 mL of SDA medium was added to 1 mL from each concentration and poured into sterile Petri dishes.

SDA medium without plant extract was used as the control. Mycelial discs of the target fungi were placed on the plates and the plates were incubated at room temperature for 14 days. Following the incubation, the diameter of fungal growth in mm was recorded.

The effect of Urtica dioica extract on the growth of dermatophytes was demonstrated by disc diffusion method.

The disc diffusion method was also used to evaluate the antifungal activity of aqueous and ethanolic extracts of U. dioica. Five mm diameter sterile paper discs were impregnated with extract solutions (1g/mL, 0.5g/mL and 0.25g/mL) respectively. The plates (SDA) were then inoculated with the target fungi and the discs placed on them.

The Agar plates were left at 25°C for 2 hours to let the extract diffuse into the Agar before incubated under appropriate conditions. Inhibition zone of the plates measured in mm and compared with control plates.

Results

Isolate and identify fungi.

The results obtained were 80.0% positive in dermatophyte and 20.0% in non-dermatophyte fungi out of 50 clinical specimens obtained from male patients. Only male patients were included in the study (n = 50). Dermatophyte isolates consisted of T. rubrum, T. mentagrophytes, T. tonsurans and M. gypseum. T. rubrum, T. mentagrophytes, T. tonsurans, and M. gypseum were the most common species isolated.

The culture, regardless of being negative or positive, was performed for the dermatophytes, T. rubrum (15 isolates, 37.5 %), T. mentagrophytes (11 isolates, 27.5 %), T. tonsurans (9 isolates, 22.5 %), and M. gypseum (5 isolates, 12.5 %). Non-dermatophyte fungi were the C. albicans and A. fumigatus.

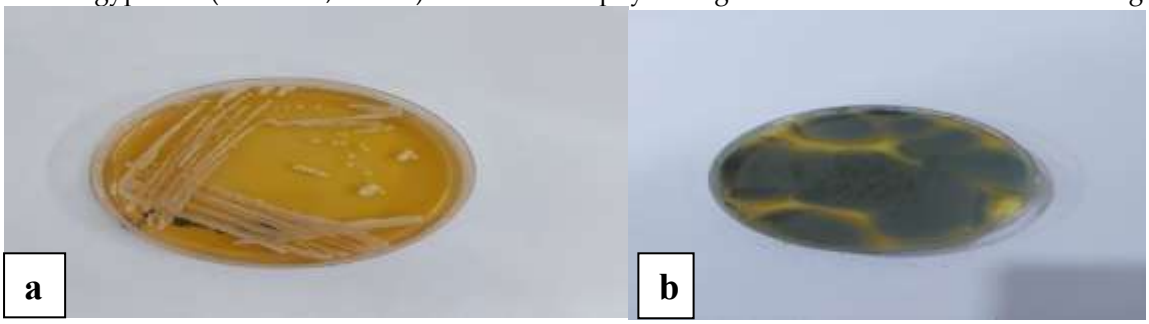

Figure 1. Isolated non-dermatophyte fungi: (a) Candida albicans and (b) Aspergillus fumigatus.

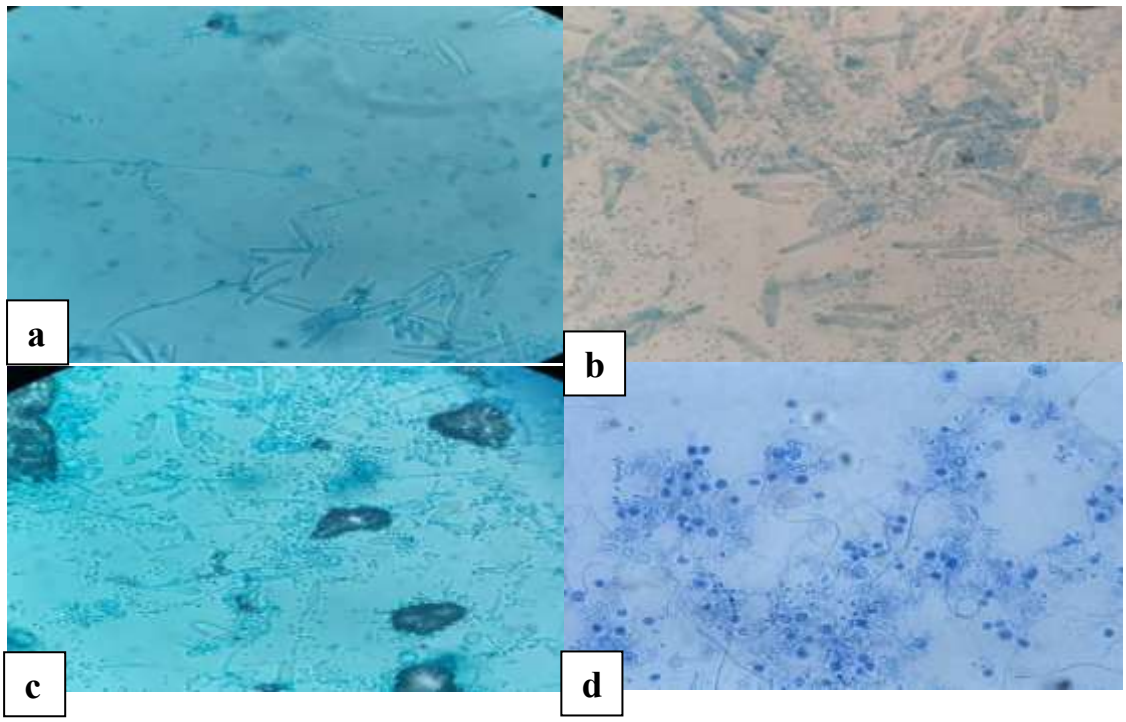

Figure 2. Isolated dermatophytes: (a) Trichophyton rubrum, (b) Trichophyton mentagrophytes, (c) Trichophyton tonsurans, (d) Microsporum gypseum.

Effective anti-dermatophyte activity of extracts from Urtica dioica plant using the Poisoned food technique

Table 1 shows that the extracts of U. dioica inhibited the growth of the dermatophytes when using the poisoned food technique. The ethanolic extract showed greater antifungal activity than the aqueous extract. The inhibitory effect was higher as the concentration of the extract was raised. The lowest radial growth values were obtained with the 1 g/mL of ethanolic extract, at which the values of 27 mm, 22 mm, 26 mm and 27 mm, were obtained for M. gypseum, T. rubrum, T. tonsurans and T. mentagrophytes, respectively.

In comparison, the maximum radial growth of the treated plates was obtained at 0.25 g/mL of the aqueous extract with 49 mm, 43 mm, 46 mm and 45 mm respectively against the 85 mm of the control plate. The results indicated that ethanolic extract yielded a significant difference ($p = 0.002$) while that of the aqueous extract was not significant ($p = 0.100$) between the groups. All extract treatments, however, had significant decreases in fungal growth when compared with the control.

Table 1. Effect of aqueous and ethanolic extracts of Urtica dioica on dermatophyte growth using the poisoned food technique.

Extract	Conc. (g/mL)	M. gypseum	T. rubrum	T. tonsurans	T. mentag.	Control	P within	P vs. control
Aqueous	0.25	49	43	46	45	85	0.100	0.005
Aqueous	0.5	45	35	37	36	85		
Aqueous	1.0	35	23	15	29	85		
Ethanolic	0.25	45	42	46	45	85	0.002	0.001
Ethanolic	0.5	36	30	27	40	85		
Ethanolic	1.0	27	22	26	27	85		

Note. Values represent radial fungal growth in millimeters. M. gypseum = Microsporum gypseum; T. rubrum = Trichophyton rubrum; T. tonsurans = Trichophyton tonsurans; T. mentag. = Trichophyton mentagrophytes. Significant at LSD = 0.05.

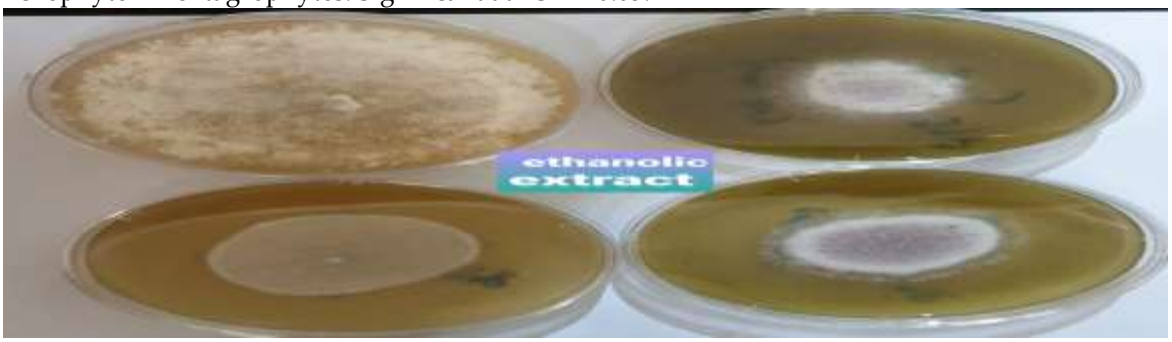

Figure 3. The effect of the ethanolic extract of Urtica dioica on dermatophyte growth.

3.3. The effect of Urtica dioica extracts on the growth of dermatophytes, through disc diffusion method.

The ability of U. dioica extracts to inhibit the growth of dermatophytes by disc diffusion method is listed below in Table 2. The ethanolic extract exhibited better antifungal activity than the aqueous extract. As the concentration increased the inhibition zones got bigger. The inhibition zones were 34 mm, 31 mm, 28 mm, 30 mm for M. gypseum, T. rubrum, T. tonsurans and T. mentagrophytes, respectively at 1 g/mL of ethanolic extract.

The lowest inhibition zone was obtained with aqueous extract at the concentration of 0.25 g/mL, which were 13 mm, 14 mm, 12 mm and 14 mm, respectively. The results indicated that there was significant difference for both aqueous and ethanolic extracts as compared to the control (p = 0.004).

Table 2. Inhibition of the growth of dermatophytes by extracts of Urtica dioica by disc diffusion.

Extract	Conc. (g/mL)	M. gypseum	T. rubrum	T. tonsurans	T. mentag.	Control	P within	P vs. control
Aqueous	0.25	13	14	12	14	0	0.014	0.004
Aqueous	0.5	25	17	15	24	0		
Aqueous	1.0	32	29	26	28	0		
Ethanolic	0.25	15	16	14	16	0	0.005	0.004
Ethanolic	0.5	27	19	17	26	0		
Ethanolic	1.0	34	31	28	30	0		

Note. Values represent inhibition zone diameters in millimeters. M. gypseum = Microsporum gypseum; T. rubrum = Trichophyton rubrum; T. tonsurans = Trichophyton tonsurans; T. mentag. = Trichophyton mentagrophytes. Significant at LSD = 0.05.

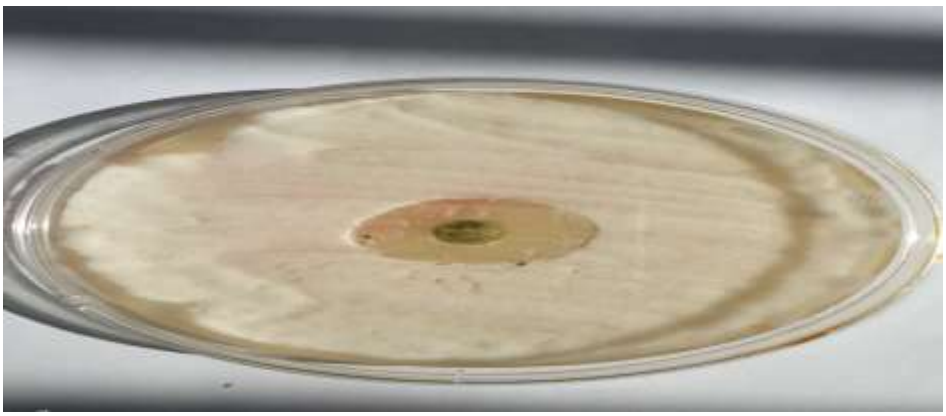

Figure 4. Effect of Urtica dioica extracts on Trichophyton rubrum growth.

Discussion

The present work gives information relating to dermatophyte fungi isolated from 50 clinical specimens from male patients in Wasit Governorate. It is noteworthy that the majority of fungi found in this study were dermatophytes, a finding which corroborates the other findings that dermatophytosis is one of the most prevalent superficial fungal infections in clinical practice [11][12]. As per the recent taxonomic concepts, the dermatophytes consist of about 50 species grouped under the genus of Microsporum, Trichophyton, Epidermophyton and Guarromyces. Conventional identification is often done by colony morphology and the microscopic characteristics, such as pigmentation and the appearance on the reverse side [13].

The epidemiology of dermatophytes is very significant for the planning of the preventive measures; also for dermatophytosis management. Moreover, accurate knowledge of the prevalence of the pathogenic dermatophyte species and the clinical picture they cause is necessary for good epidemiological surveillance [14]. More than 25% of the world population is affected by dermatophytosis and it is a worldwide public health problem. Recurrent and resistant dermatophytosis is on the rise, generating new therapeutic challenges in recent years [15].

The predominance of T. rubrum in the present study is similar to several studies which found that T. rubrum is one of the major causative agents in dermatophytosis particularly in the skin and nail infections. Shen et al. [16] reported high incidence of T. rubrum infection in Europe and this species is well known as one of the important cause of dermatophytosis in all over the world. Dermatophytosis is a common disorder seen in dermatology clinics and T. rubrum is still one of the most prevalent agents in human infections [17]. During recent times several studies have been done to assess the antimicrobial potential of medicinal plant products. Nettle has been reported to have antifungal activity on some fungi pathogenic to plants [18]. Other herbal materials are also traditionally employed in the treatment of dermatological disorder like eczema and acne [19]. The fungal inhibitory activity of ethanolic extract of U. dioica was higher than that of aqueous extract by poisoned food technique in the present study.

The results obtained are consistent with those of Hadizadeh et al. [20] that found in vitro antimycotic activity of ethanolic extract of nettle against some plant pathogenic fungi. U. dioica extracts (supercritical carbon dioxide extraction, SCE) also showed similar antimicrobial activity [21]. The present results, however, are not in line with the findings of Mikaeili et al. who did not find direct antifungal activity in extracts of stinging nettle, whether aqueous or hydroalcoholic. This difference might be due to the different extraction methods, plant part employed, concentration, fungal species and experimental conditions.

Herbs and plants have been found to be very useful and available in traditional medicine for treating various diseases which are natural, readily available and relatively inexpensive. U. dioica belongs to the family Urticaceae, and is a herbaceous, perennial plant with fine hairs on leaves and stems. These hairs are filled with substances, including: histamine, formic acid, acetylcholine and acetic acid, which can irritate the skin following contact (Fattahi, Golpour, and Akhavan Niaki 2016). Several phytochemicals including alkaloids, saponins, tannins, flavonoids, steroids, terpenes, polyphenols and cardiac glycosides have been found in the leaves of U. dioica (Roslon and Weglarz 2001). Stinging nettle

was found in higher quantities of polyphenolic acids in the male than in the female plant in the previous investigations, and polyphenolic acid content was found to increase at the full-blooming stage (Hryb et al. 1995). The antimicrobial activity of U. dioica might be attributed to these phytochemical constituents. In the present study, ethanolic extract was more active as compared to aqueous extract indicating that ethanol is more effective to isolate the antifungal compound from the plant material. All the specimens were taken from male patients in the present study, so the results in this study are applicable to male patients but should be interpreted with caution in female patients. The antifungal activity of U. dioica extracts is recommended to be further investigated with larger sample sizes, different age groups, additional dermatophytes and both sexes to confirm their possible clinical efficacy and usefulness.

Conclusion

In the current study, the extracts of U. dioica were found to have inhibitory activity against clinical dermatophyte isolates from males. The ethanolic extract had higher antifungal activity than the aqueous extract in both poisoned food technique and disc diffusion technique. The antifungal effect was raised with increase in the extract concentration. The results indicate that U. dioica could be a valuable natural source to develop antifungal compounds for further work in the treatment of dermatophyte infections.The extracts of U. dioica are therefore recommended for further studies on the identification of the active antifungal compound, establishing the minimum inhibitory concentrations and the evaluation of toxicity and safety, and investigating the potential use of these extracts in the treatment of dermatophytosis.

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