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Original Research Article

Testing to Determine the Antifungal Activity of Selected Medicinal Plants Extracts and Itraconazole Against Dermatophytes Isolated in Human Infections

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Abstract: Dermatophytosis is a category of superficial fungi infection caused by the keratinophilic fungi, like Trichophyton rubrum, which attacks the conventional antifungal drugs less often. This article compared the antifungal activity of aqueous and alcoholic extracts of Senna alata, Ficus carica and Ricinus communis against Trichophyton rubrum isolates obtained on human infections in Babylon Province in Iraq compared with itraconazole. The strongest antifungal activity was availed by Senna alata, aqueous extract and the alcoholic extract of Senna alata, in comparison with itraconazole. Statistical work was done with (One-way ANOVA) that revealed that there were significant differences between extracts ($p < 0.05$). These results suggest the possibility of Senna alata extract as a natural antifungal agent considering that it is safe, accessible, and can be used as an addition to managing dermatophytosis.

Keywords: Antifungal activity, Dermatophytosis, MIC, Hair perforation, Keratin.

1. INTRODUCTION

Causative of dermatophytosis which is one of the most common types of superficial fungal infection in humans (Deng *et al.,* 2023) despite not being life-threatening the infections of this type frequently lead to chronic irritation, mutilation, and economic burden, as they recur quite often and are not eliminated by treatment (Mahajan *et al.,* 2017). The traditional antifungal agents like the azoles, allylamines and griseofulvin continue to play the major role in the treatment process. Nevertheless, resistance especially on Trichophyton rubrum has become an international issue (Gupta *et al.,* 2025). Excessive exposure to such agents may lead to reduction in drug efficacy, hepatotoxic effects, and post-treatment relapse (Yang *et al.,* 2021). Thus, the use of alternative antifungal measures which are safe, cheap, and effective is gaining interest. Among the bioactive compounds that have antifungal activity, Senna alata, Ficus carica and Ricinus communis have been long known to possess therapeutic potential and bioactive compounds, which are widely used in the developing world as traditional medicine (Sezer *et al.,* 2024). In 12icinus communis (castor), there are ricinoleic acid and flavonoids that interfere with the integrity and function of fungal membranes and cause antifungal effects (Ramothloa *et al.,* 2025). Itraconazole is a broad-spectrum triazole antifungal that inhibits fungal cytochrome P450 -dependent enzyme Lanosterol 14 a -demethylase, preventing the synthesis of ergosterol and impairing the integrity of the membranes (Saleh *et al.,* 2024). This research study assesses and compares antifungal properties of Senna alata, Ficus carica, and Ricinus communis extracts aqueous and alcoholic extracts and Itraconazole therapy, to T.rubrum clinical isolates of patients taken in Babylon Province, Iraq. The research will also seek to find natural agents that have potential to be used in the management of dermatophytosis and limit the use of synthetic drugs.

2. MATERIALS AND METHODS

2.1. The sample was collected and identified using the following procedure.

During the period March to July 2025, A total of 160 clinical samples (e.g , skin scraping and hair stumps) were collected using aseptic precautions on patients with suspected cases of cutaneous fungal infections in three districts in

Babylon Province, Iraq: Al-Musayyib (60 samples), Al-Qasim (50 samples) and Al-Hashimiya (50 samples) (Figure 1). Al-Musayyib had the most samples but had the lowest positive rate of Trichophyton rubrum (23.3%) followed by Al-Qasim (28.0%) then Al-Hashimiya had the highest rate of infection (34.0%) with the same sample size as Al-Qasim. The general positivity rate in all regions was 28.1 that is similar to the 20 to 25 percent prevalence rates of global dermatophytes (Deng *et al.,* 2023). The samples were placed in Sabouraud Dextrose Agar (SDA) containing chloramphenicol and incubated at (27- 30 o C) (10-14) days (Figure 2) (SA *et al.,* 2021). The colony morphology and microscopic examination with lactophenol cotton blue stain were used to identify T. rubrum based on their distinguishing features: radiating colony form and microconidia (Figure 3) (Bitew, 2018, Jaishi *et al.,* 2022). hair perforation assay and keratin degradation assay are described below.

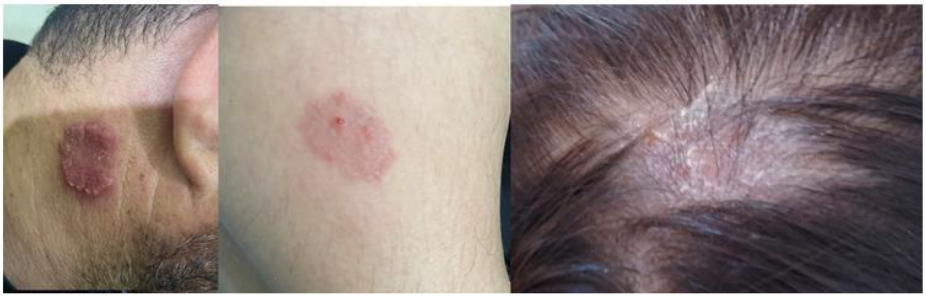

Figure 1: Clinical images showing the affected areas on the scalp, neck and hand /forearm which samples were collected for Dermatophyte isolation.

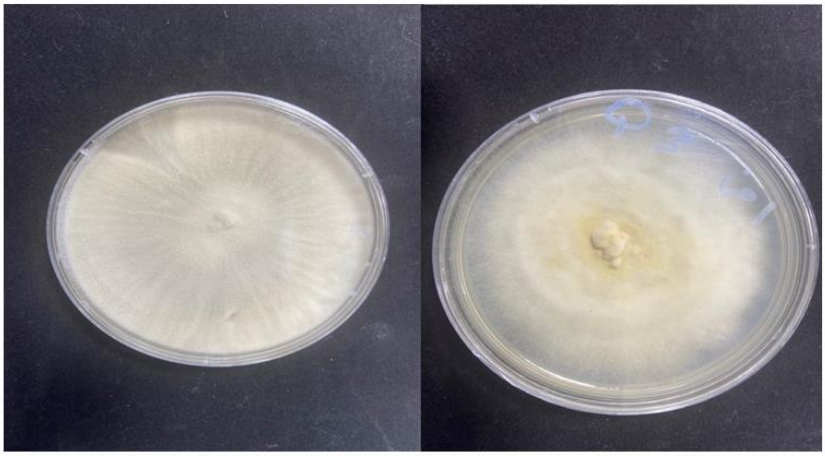

Figure 2: Growth of Trichophyton rubrum isolates on Sabouraud Dextrose agar (SDA) plates, illustrating colony morphology.

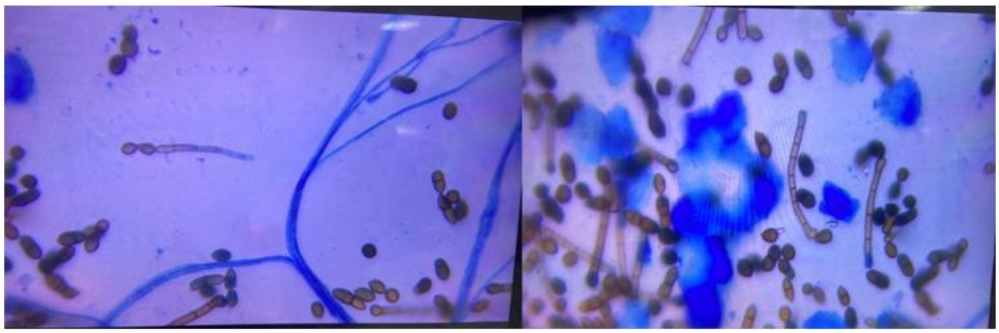

Figure 3: Light microscopy images of *T. rubrum* stained with lactophenol cotton blue showing characteristic filamentous structures.

2.2 Plant Extract Preparation

The fresh leaves of Senna alata, Ficus carica and Ricinus communis were taken, washed and dried in shade at the room temperature and ground to fine powder with the help of sterile grinder. Aqueous extracts: were obtained according to (Monica Rodriguez Garccia and LPE Peraza Echeverria, 2019). 50 g of powdered plant material was put in contact with 500 ml of distilled water at a room temperature in 24 hours and stirred intermittently. It was filtered using muslin cloth and Whatman No.1 paper followed by concentration using a water bath at 45 0 C. The extract was kept at 4 o C until it was used. The preparation of alcoholic extracts was performed (Savaririrajan *et al.,* 2021). 50g of powdered material was wet with 500 ml 95% ethanol (with frequent shaking). The extract was filtered and evaporated in the rotary evaporator at 45 o C and kept in amber bottles at 4 o C.

2.3 Itraconazole Treatment

One positive control was Itraconazole (Sigma-Aldrich, purity 98 and above). A stock solution was made in dimethyl sulfoxide (DMSO) and two-fold dilutions were made to get final concentration of (0.031 to 4 µg/mL) in compliance with CLSI M38 guidelines (Clinical and Laboratory Standards Institute (Clinica and Laboratory Standards Institute (CLSI), 2008). The methodology was based on antifungal assays (MIC determination). The effectiveness of the plant extracts against fungi was tested on the basis of the broth dilution method per the Clinical and Laboratory Standards Institute (CLSI,2008) guidelines with slight modification to allow fungi to test plant extracts.

2.4. Individual extracts

The extracts were made in Sabouraud Dextrose Broth (SDB). Each tube was inoculated by using standardized fungal inoculate (1 x 104 CFU/mL) and incubated at 28 o C between 48-72 hours. Fungal growth was measured qualitatively and Minimum Inhibitory Concentration (MIC) was defined as the minimum concentration of extract that showed no growth (Dannaoui & Espinel-Ingroff, 2019). This method and the meaning of distributions of MIC are justified by comparative data in recent cross section studies that apply updated CLSI standards in the determination of dermatophyte susceptibility to extracts (Das *et al.,* 2025). Each assay was done thrice to provide accuracy and reproducibility.

2.4.1 Agar diffusion assay

The six T. rubrum isolates were placed as young cultures on Dextrose Agar Sabouraud (SDA). cork borer was used to develop wells of 0.5 cm diameter to measure the inhibitory action and diffusion characteristics of the plant extracts and itraconazole. The plates. were incubated at 28 o C up to 48-72 hours and the inhibition zones were recorded to determine the diffusion capacity and the antifungal activity of the respective treatments. The was distilled water (negative control). (Table 1).

Table 1. Agar diffusion response of *T. rubrum* isolates to tested treatment

Isolate	Senna alcoholic	Senna aqueous	Ficus alcoholic	Ricinus alcoholic	Itraconazole	Distilled water
TR 1	+	+	+	±	++	-
TR 2	+	±	±	-	++	-
TR 3	+	+	±	-	++	-
TR 4	+	±	±	-	++	-
TR 5	+	+	±	-	++	-
TR 6	+	+	+	±	++	-

Note: (++), strong inhibition; (+) moderate inhibition; (±) weak inhibition; (-) no inhibition

2.4.2 Keratin Degradation Test

Keratin medium (with powder keratin (1%)) was made in line with the recently published standardized literature. Isolates of fungi underwent inoculation and incubation after 14 days at 28 o C. Keratin hydrolysis and keratinolytic enzymes activity in dermatophytes was assessed by measuring clear zones around colonies (Mercer & Stewart, 2019)

2.4.3 Hair Perforation Test

Sterile human hair strands were plunged in mineral salt solution to which each of the T. rubrum isolates had been inoculated. The tubes were incubated at 28 o C and 14 days. Hair strands were also analyzed using a microscope after incubation to determine the structure of the perforation as signs of fungal invasion.

2.5 Control Treatments

All the assays were performed in triplication to provide reproducibility.

2.6 Statistical Analysis

Tukey post hoc test was conducted against one-way ANOVA to compare the antifungal activity across treatments. They showed significant differences (p 0.05) and analysis was done using inhibition zone diameters, because there was an equal MIC.3.Results

3.1 Prevalence and Source Distribution

Out of 160 clinical samples, T. rubrum was most frequently detected in Al-Hashimiya (34.0%), followed by Al-Qasim (28.0%) and Al-Musayyib (23.3%). (Figure 4). Most positive cases were obtained from skin lesions (62%), followed by hair (25%) and nail samples (13%).

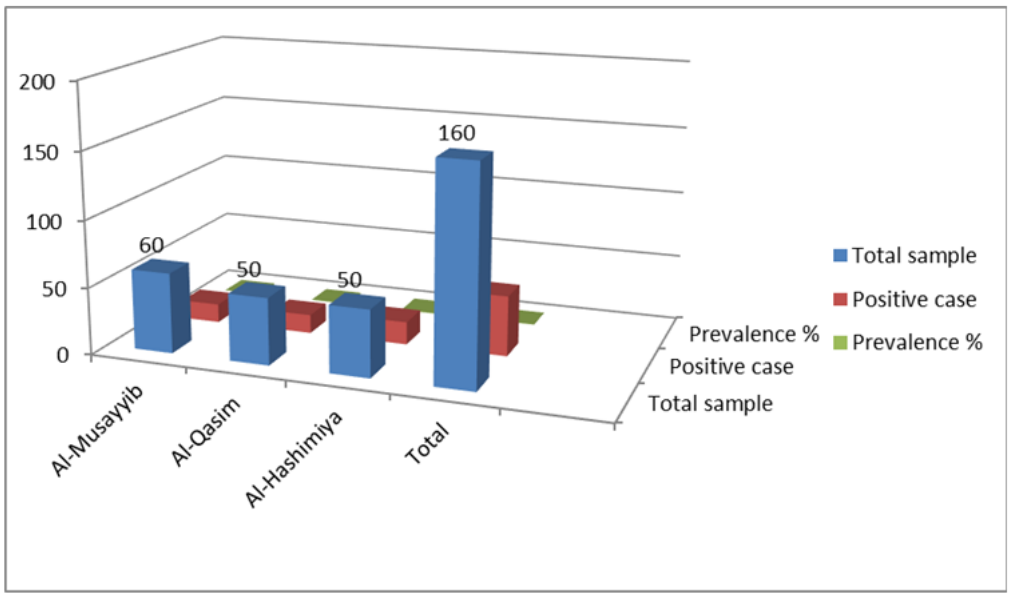

Figure 4: Comparative Prevalence of T. rubrum Infections in Al-Musayyib, Al-Qasim, and Al-Hashimiyah. This figure illustrates the percentage of confirmed dermatophytic infections in each district, emphasizing the higher burden observed in Al-Hashimiyah

Table 2. MIC and inhibition zone of alcoholic Senna extract against *T. rubrum* isolates.

Isolate	MIC (µg/ml)	Inhibition Zone (mm)
TR1	750	18
TR2	750	19
TR3	750	19
TR4	750	18
TR5	750	19
TR6	750	20
Mean ±SD	750 ± 0.00	18.83±0.75

Table 3. MIC and inhibition zone of aqueous Senna extract against *T. rubrum* isolates.

Isolate	MIC (µg/ml)	Inhibition Zone (mm)
TR1	1500	14
TR2	1500	13
TR3	1500	15
TR4	1500	14
TR5	1500	13
TR6	1500	14
Mean ±SD	1500 ±0.0	13.83±0.75

Table 4. MIC and inhibition zone of alcoholic Ficus extract against *T. rubrum* isolates.

Isolate	MIC (µg/ml)	Inhibition Zone (mm)
TR1	2000	12
TR2	2000	11
TR3	2000	13
TR4	2000	12
TR5	2000	11
TR6	2000	12
Mean ±SD	2000 ±0.0	11.83±0.75

Table 5. MIC and inhibition zone of alcoholic Ricinus extract against *T. rubrum* isolates.

Isolate	MIC (µg/ml)	Inhibition Zone (mm)
TR1	3000	9
TR2	3000	8
TR3	3000	9
TR4	3000	8
TR5	3000	7
TR6	3000	8
Mean ±SD	3000±0.0	8.17±0.75

Table 6. MIC and inhibition zone of itraconazole against *T. rubrum* isolates.

Isolate	MIC (µg/ml)	Inhibition Zone (mm)
TR1	0.25	26
TR2	0.25	25
TR3	0.25	27
TR4	0.25	26
TR5	0.25	25
TR6	0.25	26
Mean ±SD	0.25±0.0	25.83 ±0.75

Table 7. Comparative summary of MIC and inhibition zone for all treatment.

treatment	MIC (µg/ml)	Inhibition zone (mm)
Senna (Alcoholic)	750± 0.00	18.83± 0.75
Senna (Aqueous)	1500 ± 0.00	13.83± 0.75
Ficus (Alcoholic)	2000± 0.00	11.83± 0.75
Ricinus (Alcoholic)	3000± 0.00	8.17±0.75
Itraconazole	0.25 ± 0.00	25.83±0.75
Distilled Water	No inhibitory inhibition	0

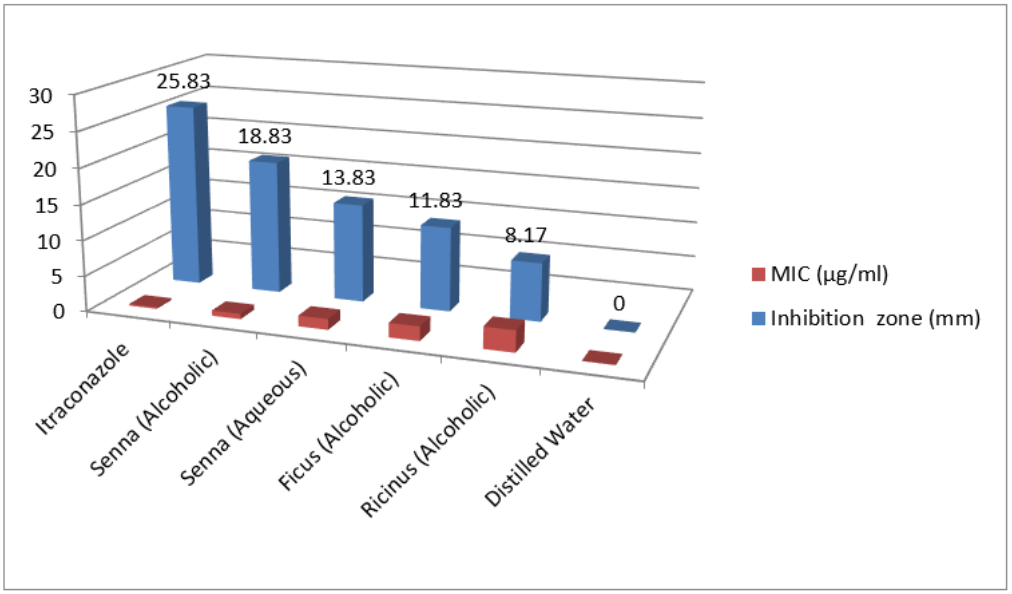

Figure 5. Comparative antifungal effect of all treatments on SDA plates.

The results of the 3.2 MIC and Inhibition Zone are presented below. The summaries of antifungal activity are presented in Tables 2-7 and in Figure 5. Senna alata alcoholic extract recorded the best activity among plant extracts (MIC = 750 µg/ml; inhibition zones = 18.83±0.75mm) (Table 2). and its aqueous extract (MIC = 1500 µg/ml) (Tables 3). Ficus carica and Ricinus communis alcoholic extracts had weak activity (MIC = 2000 µg/ml) (Tables 4,5). Itraconazole showed the best antifungal activity (MIC = 0.25 µg/ml; inhibition zones = 25.830.75mm) (Table 6). Distilled water did not exhibit any inhibitory activity. (Table 7).

4. DISCUSSION

Antifungal resistance of the tested plant extracts against T.rubrum showed a clear variation of inhibitory strength, as well as diffusion capacity. With the lowest -MIC values (750µg/ml) and fairly wide inhibition zones (18.83±0.7 mm), the safest bet was among the plant extracts the alcoholic extract of senna which showed the best antifungal effect (Ashfaq and Yosufaf,2022). The increase in activity could be explained by better extraction of phenolic and anthraquinone compounds. Compared to it; Senna alata aqueous extract was intermediate in its antifungal activity with more MIC values and smaller zone of inhibition but Ficus and Ricinus extracts had lower activity (Shafique *et al.,* 2021]. Reflected value MIC values =1.0 mg/ml and inhibition values =15 mm. These results indicate inconsistency in the efficacy of the antifungal in different species of plants and in different methods of extractions. Positive control, itraconazole. generated the largest zones of inhibition (25.831mm 2) attesting to its better fungistatic activity and diffusion power in the agar media (Krauße *et al.,* 2025). The combination of this and other results shows that though itraconazole still is that most useful antifungal agent, Senna alata can have potential in terms of natural antifungal agent.

5. CONCLUSION

Alcoholic extract of Senna alata has good antifungal action against T. rubrum, but itratecazole is still the best in terms of strength and diffusion.

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