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Original article

Comparative Analysis of Imidazole and *Urtica dioica* Emulsions in the Management of Dermatophytic Infections

Salahaldin Alfurjany[1], Shamsi Shamsi*[2,3], Basma Alnaas[1], Selema Ibrahim[1] , Abdelkader Elzen[1]

[1]*Department of Microbiology, Faculty of Science, University of Sebha, Sebha, Libya*
[2]*Department of Botany, Faculty of Science , University of Sebha, Sebha, Libya*
[3]*Department of Medical Laboratory, Faculty of Medical Technology, University of Sebha Sebha, Libya*

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Corresponding Email. *Sha.saad@sebhau.edu.ly*

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ABSTRACT

This study explores the epidemiological distribution and antifungal efficacy of treatments against common dermatophytic infections, which predominantly include species from the genera Trichophyton, Microsporum, and Epidermophyton. These infections are significant due to their prevalence and the challenge posed by recurrence and resistance. Between January and September 2022, 35 fungal samples were collected from a dermatological clinic and analyzed using various growth media. Our research focused on evaluating the antifungal effectiveness of a synthetic imidazole emulsion (CSI), an antibiotic, and a natural emulsion from Urtica dioica (CSP). Results revealed that CSI and antibiotic treatments were significantly more effective across all tested species compared to CSP, with CSI often showing superior or comparable efficacy to antibiotics. The findings underscore the robust antifungal properties of imidazole derivatives and suggest a potential limitation in the therapeutic utility of U. dioica extracts. The study highlights the need for continued exploration of innovative treatments and the development of effective management strategies for fungal infections, considering the variance in species prevalence and treatment response.

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INTRODUCTION

Superficial fungal infections are among the most common diseases globally, mainly due to dermatophytes from the genera Epidermophyton, Microsporum, and Trichophyton [1]. These pathogenic fungi can thrive and spread in keratinized tissues like skin, hair, and, because of their capacity to produce various proteolytic enzymes that break down keratin [2]. These infected keratinized tissues offer the necessary temperature, pH, and nitrogen conditions to satisfy the nutritional requirements of the dermatophytes. Consequently, these infections typically remain restricted to the superficial layers of the skin and seldom penetrate into the deeper dermal tissues [3]. Although many antifungals have been reported to have primary activity against dermatophytes, these infections often relapse after discontinuing the drug because these organisms have demonstrated tolerance to treatment

Imidazole-based compounds are recognized for their potent antifungal properties [4], and their use in treating dermatophytic infections faces significant challenges. Primarily, the problem arises from the fungi's vigorous cellular structure, which relies heavily on ergosterol, a vital component that imidazoles target by inhibiting its synthesis [5]. Although this mechanism disrupts the fungal cell membrane and inhibits growth, the hydrophobic nature of imidazoles

often leads to poor solubility in aqueous formulations, complicating their delivery and effectiveness in medical applications [4].

Urtica dioica (stinging nettle) is a widespread medicinal plant throughout Europe, North America, North Africa, and Asia. It has a long history of use as a source of medicine, food, and fiber [6]. Many known phytochemical compounds from U. dioica include lectins, sterols, terpenes, volatile compounds, fatty acids, sugars, proteins, vitamins, minerals, and flavonoids such as kaempferol, isorhamnetin, quercetin, and many other critical medicinal compounds. It has been used as a diuretic, hemostatic, circulatory stimulant, nutritional, anticancer, anti-atherosclerosis, antihistamine, antidandruff, demulcent, and blood sugar lowering agent. For a long time, nettle has been used to treat arthritis and rheumatism. It also prevents inflammation of the urinary tract and digestive system, improves digestion and nutrient absorption, stimulates the immune system, and increases its resistance to microbial infections [7] .

Emulsions are a mixture of surfactants and emulsifiers that form a protective layer around certain oil particles, preventing their merging and separation [8]. This mixture is characterized by its significant effectiveness and antimicrobial action against many bacteria and fungi. Furthermore, it is more effective against aerobic and anaerobic microorganisms at only low concentrations [9]. Emulsions have been used in many pharmaceutical applications, such as the production of antimicrobial ointments and mouthwashes, as well as in several industrial fields, like manufacturing antimicrobial paint used in hospitals and health institutions [9] .

Despite the availability of various antifungal treatments, the recurrence of infections and the development of resistance among fungal species pose significant challenges. This study aims to evaluate the effectiveness of various antifungal treatments against specific Superficial fungal infections, and comparing the efficacy of synthesized emulsion treatments, including synthetic drugs imadizole , and natural plant extract of *Urtica dioica* -based treatments.

METHODS

Sample Collection

Samples were collected during the period from 22/1/2022 to 25/9/2022. Cotton swab samples were collected from patients with dermatological symptoms diagnosed by a physician, visiting the clinic (Al-Rahma Clinic), and samples were referred to the Ghosn Al-Salam Laboratory). A total of 4 samples were collected from the scalp, two from the hair, and the rest from the skin, amounting to approximately 45 fungal samples. Out of these samples, six contaminated samples were rejected, and four were negative, resulting in 35 fungal samples being used for research.

Preliminary Detection of Fungal Species

The detection of fungal species in the collected samples was done by isolation and cultivation on media such as Malt Extract agar, Demersal agar, Rose Bengal agar ,and Sabouraud Dextrose agar. The antifungal antibiotics Chloramphenicol and Nystatin were added to prevent the growth of bacteria and Saprophytic fungal species. The samples were then incubated at 28°C for six days.

Diagnosis of the Obtained Fungal Isolates

Morphological Examination

The mycelial growth morphology was examined, including the colony's color, shape, diameter, and height on the media (10).

Microscopic Examination

A glass slide was prepared from the fungal colonies with a drop of distilled water, covered with a glass coverslip, and examined under a light microscope to observe the shape, color, and size of conidia, spores, and hyphae of the fungus for species identification (11).

Preservation of Isolates

After confirming the identity of the obtained isolates, they were preserved in slanted tubes of SDA medium and stored in the refrigerator at 4°C until testing and use.

Preparation of Emulsions

For the formation of emulsions method mention (12, 13) by was followed with slight modification,the Imidazole compound and aqueous extract of *Urtica dioica* (stinging nettle) were chosen as the primary reaction materials for comparing pharmaceutical compounds and natural plant extracts at a final concentration of 500mg/ml . Tween-20 and distilled water were used as the surfactant and aqueous phase, respectively. Olive oil was used as the oil phase. Ethanol

and propylene at a concentration of 1% were used as surfactants. The mentioned compounds were mixed in a ratio of (1:2:1) sequentially on a magnetic stirrer for 24 Hrs at room temperature until the formation of the transparent emulsion, as shown in figure 1. The emulsion of the Imidazole compound was labeled (CSI), and the *Urtica dioica* extract emulsion was labeled (CSP).

Antifungal activity of the Prepared Emulsions

Method by was followed (14) , 10 selected isolate fungi were cultivated in an SDA medium for 5 to 7 days. Then, the fungal growth density was adjusted to OD620 = 1.2, equivalent to 10^6 CFU/mL, and suspensions were prepared for each fungal isolate. The test was carried out in SDA medium, where approximately 100 microliters of the tested emulsions were transferred and spread on the prepared SDA plates. The plates were left in the incubator at 37°C until the emulsions dissolved and dried on the surface of SDA. After that, 5 mm wells were made in each plate and filled with approximately 500 microliters of the tested fungal suspensions. The antibiotic fluconazole 150 mg was used as a positive control, and a plate containing only the fungus was used as a negative control for the experiment. The plates were then incubated at 28°C, and the radial growth of the fungi was monitored and recorded for the inhibition rate using the following equation:

Fungal Mycelium Growth Inhibition = (Diameter of control - Diameter of treatment / Diameter of control) x 100 .

Statistical Analysis

Descriptive statistics were conducted for the averages of the effects and percentages, and the significance of differences between the obtained results was tested using Minitab 19 software.

RESULTS

The distribution of fungal species isolated in our study as shown in figure 1, illustrating a disproportionate prevalence among the species. Trichophyton spp was 68% of the total, followed by Microsporum spp at 17%. Epidermophyton spp. were found to be 7% of the isolates, *Aspergillus spp.* Comprised 5%, and *Candida spp*. were the lowest, making up 3% of the isolates.

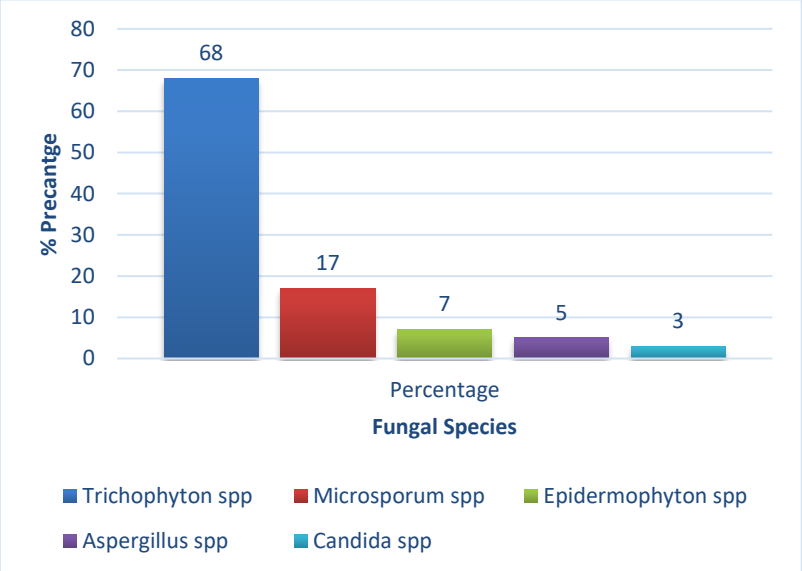

Figure 1. The distribution of Fungal Species Isolates

The variation in the frequency of the fungal species was statistically analyzed using a chi-square test, which yielded a chi-square statistic of approximately 149.8 and an extremely low p-value. These results indicate a statistically significant deviation from a uniform distribution of species isolation, signifying a disparity in the occurrence rates among the fungi studied.

Antifungal activity of different treatments against Trichophyton spp

Various treatments efficacy against Trichophyton spp. were quantified as shown in table 1. The negative control group exhibited a 100% growth rate, serving as a benchmark for comparison against the therapeutic interventions. The CSI

treatment showed substantial efficacy at a rate of 72% growth inhibition, followed by the antibiotic treatment at 65%. The CSP treatment displayed a notably lower efficacy at 22%.

Table 1. Activity of Different treatment against Tricophyton spp

Treatment	Effectiveness (%)
CSI	72
CSP	22
Antibiotic	65
Control	100
P-Vlaue	<0.003

A statistical analysis showed the significance of the differences in synthesized emulsion treatment effectiveness which is a highly significant p-value of approximately, The statistical evidence strongly suggests substantial differences in the effectiveness of the treatments tested. The CSI and Antibiotic treatments were both markedly more effective than the CSP treatment but less effective than the Control condition.

Antifungal activity of of different treatments against Microsporum spp

Table (2) demonstrates the effectiveness of different treatments against *Microsporum spp*. The control group again exhibits a 100% growth rate, suggesting perfect efficacy under control conditions. The CSI treatment shows a high effectiveness rate of 71%, whereas the antibiotic treatment displays 65% effectiveness. The CSP treatment demonstrates a considerably lower effectiveness at 20%. A statistical analysis using an Analysis of Variance (ANOVA) was performed to assess the significance of the differences observed in the effectiveness among treatments an which was significant p-value < 0.05 .

Table 2. Activity of Different treatment against Microsporum spp

Treatment	Effectiveness (%)
CSI	*71*
CSP	20
Antibiotic	*65*
Control	100
P-Vlaue	<0.001

These results indicate a contrast between the effectiveness of CSI treatment compared to CSP, with CSI being substantially more effective. Additionally, the Antibiotic treatment's effectiveness is also considerably higher than that of CSP. This variance in treatment response warrants further investigation into the mechanisms of action of these treatments and their suitability in treating Microsporum spp. infections.

Antifungal activity of different treatments against Epidermophyton spp.

The CSI and Antibiotic treatments both show 70% effectiveness, while the CSP treatment has a much lower effectiveness at 20%. The Control remains at 100% growth rate. The consistency in effectiveness between the CSI and Antibiotic treatments suggests similar efficacy against Epidermophyton spp., while the CSP treatment's lower effectiveness may warrant a review of its therapeutic utility.

Table 3 Activity of Different treatment against Epidermophyton spp

Treatment	Effectiveness (%)
CSI	70
CSP	20
Antibiotic	70
Control	100
P-Vlaue	<0.001

The ANOVA test conducted on the treatment effectiveness for Epidermophyton spp indicat that there are significant differences in treatment effectiveness.

The comparative effectiveness of CSI treatment, antibiotic treatment, and CSP treatment against three fungal species; *Trichophyton spp.*, *Microsporum spp.*, and *Epidermophyton spp*.was evaluated. The control group, indicating the uninhibited fungal growth, was set at 100% effectiveness, serving as a negative control to highlight the inhibitory impact of the treatments.

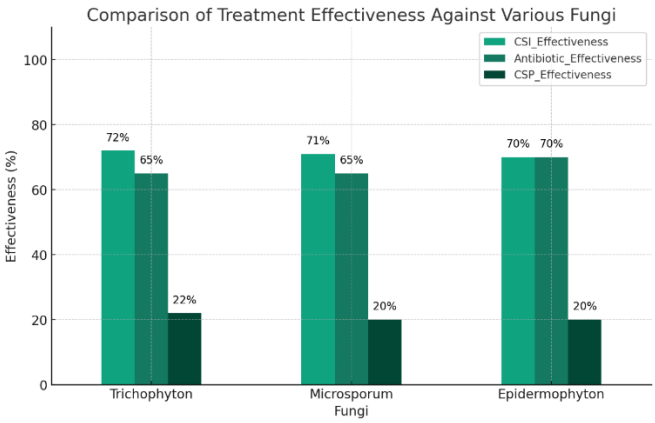

Figure 2 Comparsion of Different treatment against tested fungi

For Trichophyton spp., the CSI treatment demonstrated a superior effectiveness of 72%, compared to the antibiotic treatment at 65% and the CSP treatment at 22%. In the case of Microsporum spp., CSI treatment again showed a higher effectiveness (71%) relative to antibiotic treatment (65%), with CSP treatment lagging at 20%. When tested against Epidermophyton spp., CSI and antibiotic treatments both yielded an effectiveness rate of 70%, significantly surpassing the 20% effectiveness observed with CSP treatment. The results clearly illustrate that CSI treatment generally outperforms or matches the antibiotic treatment in inhibiting the growth of the tested fungal species, whereas CSP treatment consistently shows substantially lower effectiveness.

DISCUSSION

The distribution and treatment responsiveness of fungal species uncovered in our study present crucial insights into fungal infections' epidemiological and therapeutic landscape. Specifically, the dominant prevalence of Trichophyton spp. and the comparative effectiveness of treatments, including an imidazole emulsion (CSI) and Urtica dioica (nettle) plant extract emulsion (CSP), provide valuable implications for clinical management and future research avenues. Our findings illustrate a significant deviation from uniformity in the prevalence of fungal species, with Trichophyton spp. Represent the majority of isolates (68%). This observation is aligned with global reports [15,16], positioning dermatophytes, particularly Trichophyton spp., as prevalent agents in cutaneous mycoses. The statistical validation of these results emphasizes the reliability of the observed distribution pattern, pointing towards specific ecological or host-related factors influencing these disparities.

The representation of other species, such as Microsporum spp., Epidermophyton spp., Aspergillus spp., and Candida spp., though less prevalent, underscores the diverse fungal landscape confronting clinicians. This diversity necessitates a broad spectrum of diagnostic and therapeutic strategies to manage fungal infections effectively within varied clinical scenarios.

The evaluation of antifungal treatments revealed a significant variance in the effectiveness against Trichophyton spp., Microsporum spp., and Epidermophyton spp. The imidazole emulsion (CSI) consistently demonstrated superior efficacy across all tested species, underscoring the robust antifungal properties of imidazole derivatives. This is consistent with previous studies highlighting the efficacy of imidazole compounds in disrupting fungal cell membrane integrity [17-19], leading to cell death.

Conversely, the Urtica dioica plant extract emulsion (CSP) exhibited notably lower effectiveness, which, while unsatisfactory, opens new questions regarding its mechanism of action and potential role in combination therapies or as a preventive measure due to its natural origin and possibly lower side effect profile compared to synthetic compounds.

The analyses of our results confirm the significant differences in treatment efficacy, reinforcing the conclusion that CSI and antibiotic treatments are more effective than CSP treatment in controlling fungal growth.

The dominance of Trichophyton spp. among fungal isolates reinforces the need for targeted diagnostic and therapeutic strategies against this genus. The marked efficacy of the CSI treatment suggests that imidazole emulsions should be

considered a primary option in the pharmacological management of infections caused by the studied species. However, the reduced effectiveness of CSP highlights an essential area for further research. Investigating the phytochemical properties of Urtica dioica and its potential synergistic effects with other antifungal agents could uncover new, innovative approaches to managing fungal infections. Moreover, exploring the reasons behind the varying effectiveness of treatments against different fungal species could lead to a deeper understanding of fungal biology and resistance mechanisms.

This study's insights into the epidemiology and treatment responsiveness of fungal species lay the groundwork for future investigations. Longitudinal studies could elucidate trends in fungal prevalence and resistance patterns, while genomic studies might reveal insights into susceptibility and resistance mechanisms at the molecular level. Additionally, the exploration of novel natural compounds with antifungal properties could diversify the arsenal against fungal pathogens, potentially overcoming resistance issues and side effects associated with current treatments.

CONCLUSION

Our findings emphasize the significance of ongoing monitoring of fungal species occurrence and response to therapy. The demonstrated efficiency of imidazole emulsions against common fungal diseases supports its usage as a key component of antifungal treatment. Meanwhile, the research of alternative medicines, such as Urtica dioica extract, represents a promising route for increasing therapeutic choices, emphasizing the necessity for a diversified strategy in the ongoing war against fungal diseases.

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Conflicts of Interest

The authors declare no conflicts of interest

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تحليل مقارن لمستحلبات الإيميدازول والقراص في علاج الالتهابات الجلدية الفطرية

صلاح الدين الفرجاني[1]، شمسي سعد شمسي* [2،3]، بسمة النعاس[1]، سليمة إبراهيم[1] ، عبدالقادر الزين[1]

[1]قسم الأحياء الدقيقة، كلية العلوم، جامعة سبها، سبها، ليبيا
[2]قسم النبات، كلية العلوم، جامعة سبها، سبها، ليبيا
[3]قسم المختبرات الطبية، كلية التقنية الطبية، جامعة سبها سبها، ليبيا

المستخلص

تستكشف هذه الدراسة التوزيع الوبائي والفعالية المضادة للفطريات للعلاجات ضد العدوى الجلدية الفطرية الشائعة، والتي تشمل بشكل أساسي الأنواع من أجناس Trichophyton و Microsporumو Epidermophytonهذه العدوى مهمة بسبب انتشارها والتحدي الذي يفرضه تكرار الإصابة والمقاومة. بين يناير وسبتمبر 2022، تم جمع 35 عينة فطرية من عيادة جلدية وتحليلها باستخدام وسائط نمو مختلفة. ركز بحثنا على تقييم الفعالية المضادة للفطريات لمستحلب إيميدازول صناعي (CSI) ومضاد حيوي ومستحلب طبيعي من نبات القراص .(CSP) وكشفت النتائج أن علاجات مستحلب إيميدازول الصناعي والمضادات الحيوية كانت أكثر فعالية بشكل ملحوظ في جميع الأنواع التي تم اختبارها مقارنة بمستحلب القراص الطبيعي، حيث أظهر مستحلب إيميدازول الصناعي غالبًا فعالية متفوقة أو مماثلة للمضادات الحيوية. وتؤكد النتائج على الخصائص المضادة للفطريات القوية لمشتقات الإيميدازول وتشير إلى وجود قيود محتملة في الفائدة العلاجية لمستخلصات نبات القراص. وتسلط الدراسة الضوء على الحاجة إلى مواصلة استكشاف العلاجات المبتكرة وتطوير استراتيجيات إدارة فعالة للعدوى الفطرية، مع مراعاة التباين في انتشار الأنواع واستجابة العلاج.

الكلمات المفتاحية. العدوى الجلدية الفطرية، الفعالية المضادة للفطريات، مستحلب إيميدازول، نبات القراص.