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Antifungal Activity of *Mentha Piperita* Ethanolic Extract against *Candida albicans* and Synergistic Potential with Diclofenac Sodium

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ABSTRACT:

This study investigated the antifungal effects of the ethanol extract of *Mentha piperita* (*M. piperita*) against standard and clinical *Candida albicans* isolates, compared its activity with that of fluconazole 100 mg (reference drug), and evaluated its potential synergistic effect when combined with diclofenac sodium (50 mg). The ethanolic extract of *M. piperita* was prepared using the maceration method with ethanol (75%). Antifungal activity was evaluated using the agar well diffusion method and was measured by observing the inhibition zones (mm). The results demonstrated that *M. piperita* ethanolic extract exhibited antifungal activity with inhibition zones (mm) ranging from (9.00±0.80 to 16.3±0.90 mm), and it was highly effective against 19% of *C. albicans* tested, while Fluconazole was effective against 9.5%. Furthermore, the combination of the extract and diclofenac sodium did not exhibit a synergistic effect; instead, a decrease in the activity of the extract was observed. These findings indicate that *M. piperita* ethanolic extract possesses various activities against the tested *C. albicans* isolates. Of all tested isolates, 71.4% demonstrated resistance to fluconazole. No synergism was observed between the extract and diclofenac sodium.

النشاط المضاد للفطريات للمستخلص الإيثانولي لنبات النعناع البري ضد المبيضات البيضاء والإمكانات التآزرية مع ديكلوفيناك الصوديوم

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الكلمات المفتاحية:
النعناع البري، النشاط مضاد للفطريات، المبيضات البيضاء، تأثير تآزري، ديكلوفيناك الصوديوم، فلوكونازول.

المستخلص:

تناولت هذه الدراسة دراسة التأثيرات المضادة للفطريات للمستخلص الإيثانولي لنبات النعناع البري ضد عزلات المبيضات البيضاء القياسية والسريرية، ومقارنة نشاطه بنشاط الفلوكونازول 100 ملغ (دواء مرجعي)، وتقييم تأثيره التآزري المحتمل عند دمجه مع ديكلوفيناك الصوديوم (50 ملغ). تم تحضير المستخلص الإيثانولي لنبات النعناع البري باستخدام طريقة النقع مع الإيثانول (75٪). تم تقييم النشاط المضاد للفطريات باستخدام طريقة انتشار بئر الأجار وتم قياسه من خلال ملاحظة مناطق التثبيط (مم). أظهرت النتائج أن المستخلص الإيثانولي لنبات النعناع البري أظهر نشاطًا مضادًا للفطريات مع مناطق تثبيط (مم) تتراوح من (9.00 ± 0.80 إلى 16.3 ± 0.90 مم)، وكان فعالًا للغاية ضد 19٪ من الكانديدا البيضاء المختبرة، في حين كان الفلوكونازول فعالًا ضد 9.5٪. علاوة على ذلك، لم يُظهِر الجمع بين المستخلص وديكلوفيناك الصوديوم تأثيرًا تآزريًا؛ بل لوحظ بدلاً من ذلك انخفاض في نشاط المستخلص. تشير هذه النتائج إلى أن المستخلص الإيثانولي لنبات النعناع يمتلك أنشطة مختلفة ضد عزلات المبيضات البيضاء المختبرة. من بين جميع العزلات المختبرة، أظهرت 71.4٪ مقاومة للفلوكونازول. لم يُلاحظ أي تآزر بين المستخلص وديكلوفيناك الصوديوم.

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INTRODUCTION

Candida spp. are commonly identified as one of the most prevalent fungal infections in dermatology. *Candida albicans* is specifically responsible for 80–90% of infections (Bolognia et al., 2018, Browning, 2018).

The azole antifungal is the most frequently used class of drugs for treating Candida infections. Azole antifungal agents, such as fluconazole, are often preferred for the treatment of numerous Candida infections because of their cost-effectiveness and availability for oral administration; however, owing to the inappropriate use of antifungal agents, fluconazole resistance has developed in several Candida species (Whaley et al., 2017). The toxicity, interactions, and resistance of the currently employed antifungal therapies are well-documented (Nami et al., 2019). The toxicity of antifungal drugs is extensively documented due to the similarities between fungal cells and human host cells, as they are both eukaryotes (Denning and Hope, 2010) ; (Scorzoni et al., 2017*)*; (Silva et al., 2019).
Similar to bacteria, fungi can develop resistance to multiple drugs, increasing the risk of infection. A recent study on fungal infections found that there are 150 million cases of severe fungal infections annually, with 1.7 million of those cases resulting in mortality (Kainz et al., 2020). The resistance rate continued to increase.

The resurgence of interest in natural remedies and traditional medicine has contributed to the support for herbal medicine, which purports to treat a wide range of ailments without adverse effects on patients (Loolaie et al., 2017). *Mentha piperita* L. is an aromatic plant belonging to the Lamiaceae family and is frequently used as a food condiment (Raja, 2012). The family comprises 250 genera and over 7000 species (Stankovic, 2020). Botanists classify it as astringent, antiseptic, antipyretic, antispasmodic, anticatarrhal, antimicrobial, stimulant, and anti-aging (Mukhtar, 2017). *M. piperita* extracts exhibit significant antibacterial and antifungal properties against numerous pathogens (Patil et al., 2023). This study aimed to investigate the antifungal effects of the ethanol extract of *M. piperita* against standard and clinical *C. albicans* isolates and to examine the potential synergistic interactions between the extract and diclofenac sodium.

MATERIALS AND METHODS

Materials

Chemicals and Drugs

The chemical used in this investigation was ethanol (Sigma Aldrich, Germany). Sabouraud Dextrose Agar (SDA) and Mueller-Hinton Agar (Hi Media Laboratories Pvt. Ltd., India) were employed. The pharmaceutical agents used were diclofenac sodium 50 mg (Votrex, Hikma, batch no. 6251065012062) and fluconazole 100 mg (Fluzole, Biofarma, batch no.869957151102).

Candida albicans Used

This study included 21 samples. One standard (*C. albicans* ATCC 7596) was obtained from the Laboratory of Microbiology, Faculty of Pharmacy, Omar Al-Mukhtar University, and 20 pre-identified isolates were collected between May and September 2024 from Al-Akeed Laboratory, Benghazi, Libya. The isolates were obtained from diverse sources.

Collection of plant materials and preparation

Fresh of *M. piperita* leaves (Figure 1) were collected from Derna City in Aljabal Alakhdar, Libya, during the spring of 2024. Taxonomists at the Herbarium Department of Botany, Faculty of Sciences, Omar Al-Mukhtar University, Al-Bayda, Libya, identified and authenticated the plant specimen, thereby ensuring the validity and reliability of the research. The plant leaves were washed, aerobically dried at ambient temperature, ground, and stored in sealed containers for extraction.

Figure (1): Fresh *Mentha Piperita* leaves.

Methods

Preparation of crude extract

The plant was extracted using an overnight maceration process, following Harbone (1984). A weight of 40 g was macerated in 400 ml of 75% ethanol for three days at room temperature. The supernatant was decanted after random shaking for 24 hours at room temperature. The extract was then dried and concentrated using a hood. The residue was weighed (g), and the yield is listed in Table (1) after being calculated in Table 1 as follows:

$$\text{Yield (\%)} = (\text{weigh of extract/weigh of the plant}) \times 100$$

The extract was stored at 4 °C until further use.

Antifungal activity

The antifungal activity of each extract, diclofenac sodium, and fluconazole was evaluated using the Agar Well Diffusion Method, as described by Hossain et al. (2022). In this assay, the agar plate surface was inoculated by spreading 100 µL of the fungal suspension over the entire agar surface. The suspension was prepared from a fresh culture in 0.9% normal saline and standardized to 0.7 McFarland solution. A sterile cork borer was used to create aseptic circular wells (6–8 mm) on the agar. A volume of 100 µL of each tested extract and drug was introduced into each well (Magaldi et al., 2004); (Valgas et al., 2007). Each sample was analyzed in triplicate. The Petri dishes were incubated at 37°C for 24–48 h. The inhibition zones were measured in millimetres (mm).

Statistical analysis

All experiments were conducted in triplicate, and the results are presented as Mean ± Standard Deviation (M±SD) and percentages (%). All assay results were analyzed using Microsoft Excel (2019).

RESULTS

Yield of crude extract

Table 1 shows the crude extract percentage yield. *M. piperita* crude ethanolic extract yielded 13.7%. A viscous consistency and a blackish-green hue characterized the extracted material.

Table (1): Description and yield percentage of ethanolic extract of *M. piperita:*

Parameters	Ethanolic extract
Weight of plant (g)	80
Yield (g)	11
Yield (%)	13.7
Color	Blackish-green
Consistency	Gummy

Percentage and Distribution of Isolates from Various Sources

Fifty percent of the total *C. albicans* isolates were obtained from urine samples, 40% from vaginal swabs, and 10% from sputum samples, as depicted in Figure (2).

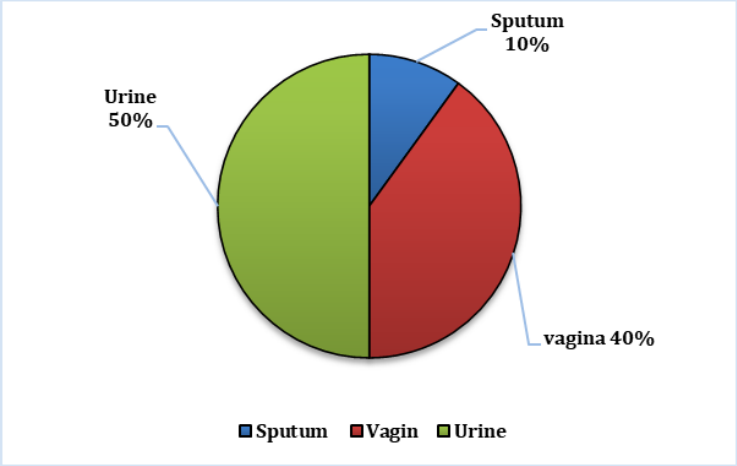

Figure (2): Percentage and Distribution of Isolates from Various Sources.

Antifungal activity of *M. piperita* extract against *C. albicans* isolates:

The results of this investigation indicated that the ethanolic extract at 100 mg/ml exhibited varying antifungal efficacy against *C. albicans* isolates. The observed inhibition zones (mm) ranged from (9.00±0.80 to 16.3±0.90 mm). Among the tested samples, the ethanolic extract was ineffective against 11(52.4%) of the tested samples, partially effective against 6(28.6%), and effective against 4(19%) of the tested samples, as illustrated in Figure (3).

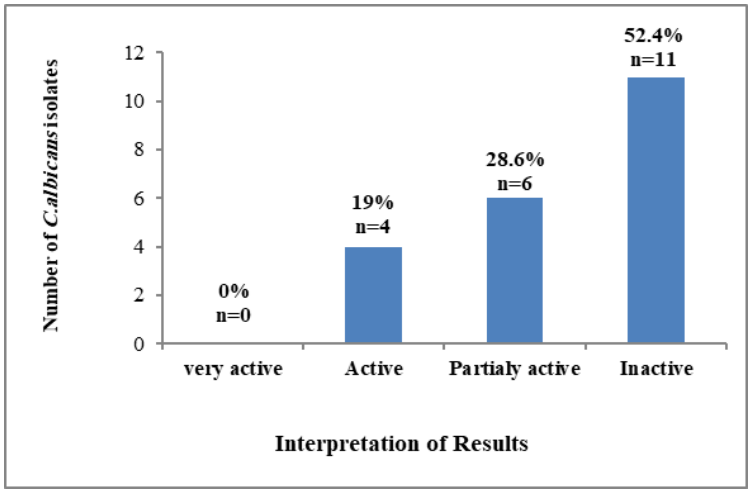

Figure (3): Antifungal activity of *M. piperita* extract against *C. albicans.*

Antifungal activity of combination of extract with Diclofenac sodium

This study investigated the synergistic effect of a combination of extract with diclofenac sodium against two isolates (more and less sensitive isolates).

In the lowest sensitive sample (code-11), the extract showed antifungal activity with an inhibition zone (mm) of 9.00±0.80, but exhibited no activity when combined with diclofenac. This result indicated a lack of synergistic or additive effects.

In the more sensitive sample (code-18), the extract exhibited antifungal activity with an inhibition zone (mm) of 16.3±0.90, and the ethanolic extract combined with diclofenac sodium showed a close effect to the extract alone (16.0±2.40 mm), as detailed in Table (2).

Table (2): Antifungal activity of extract combined with diclofenac sodium:

Samples No.	Extract	Diclofenac sodium	Extract with diclofenac sodium
Code-11	9.00±0.80	0.00±0.00	$0.00 \pm 0.00^{(A)}$
Code-18	16.3±0.90	0.00±0.00	$16.0 \pm 2.40^{(A)}$

Key: (A)= Antagonism.

DISCUSSION

Individuals experience infections caused by highly resistant fungal strains because although antifungal drugs are generally efficacious, certain fungal infections may resist specific medications, necessitating alternative treatment modalities (Kainz et al., 2020). Plants contain naturally occurring compounds with antimicrobial properties that can serve as sources of antimicrobial agents against infections (Mayekar et al., 2021). Plant-derived natural substances have been utilized in medicine for extended periods of time owing to their diverse therapeutic applications. Numerous researchers have considered natural resources to be essential for the development of antifungal medications.

The potential of *M. piperita* as a source of natural antifungal agents is significant because its extracts demonstrated inhibitory effects on the growth of various fungi in laboratory studies. However, the majority of published research has focused on investigating the antifungal or antimicrobial properties of *M. piperita* essential oils rather than exploring its other constituents (Ilboudo et al., 2016).

The primary objective of this study was to investigate the antifungal activity of *M. piperita* leaf ethanolic extract against *C. albicans* isolates. The results demonstrated that 52.4% of *C. albicans* isolates exhibited resistance to the ethanolic extract of *M. piperita* leaves. Previous studies have primarily focused on the antifungal properties of *M. piperita* essential oils, leaving other constituents less explored. Understanding the antifungal potential of the various constituents of *M. piperita* can contribute to the development of new natural antifungal agents. Our findings align with those of (Wenji et al., 2019), who also reported the weak antifungal activity of ethanolic extracts. In contrast, (Doddanna et al., 2013), reported a higher inhibition zone for ethanolic extracts. The study by (Höfling et al., 2010), supports our findings by reporting moderate activity of ethanolic extracts and no activity for dichloromethane extracts.

On the other hand, this study represents the first investigation into the combined effects of ethanolic *M. piperita* extract and diclofenac sodium on *C. albicans*. To the best of our knowledge, no previous research has explored this specific interaction, thereby contributing a novel perspective to the field of antifungal research.

Although this study contributes significantly to the screening and expansion of knowledge regarding the potential therapeutic applications of *M. piperita* leaf extract against standard and isolated *C. albicans* and its synergistic effects, it is constrained by the absence of phytochemical screening and cytotoxicity analysis of the active compounds. Therefore, further investigation of the phytochemicals and cytotoxicity of *M. piperita* is warranted.

CONCLUSION

In conclusion, the ethanolic extract of *M. piperita* exhibited significant antifungal activity against *C. albicans* isolates, demonstrating superior efficacy compared to fluconazole for a substantial proportion of isolates. Subsequent investigations should elucidate the mechanisms underlying the antifungal properties of *M. piperita* and examine potential modifications to enhance its efficacy and synergistic potential with other antifungal agents.

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Author contributions
A.M.K., S.A.E., and A.S.K.; conceptualized the work and wrote the manuscript, A.M.K., S.A.E., and A.S.K.; conducting experiments and wrote the manuscript, A.M.K., S.A.E., and A.S.K.; writing-original draft preparation, A.M.K., S.A.E., and A.S.K.; writing-review and editing, A.M.K., S.A.E., and A.S.K.; this work has been reviewed and approved by all authors.

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