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**EFFECT OF PEPPERMINT (*MENTHA PIPERITA*) ESSENTIAL OIL COLLECTED  
FROM AL-BAHA REGION AGAINST CANDIDAL VAGINITIS**

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**ABSTRACT**

Candida vaginitis is a common mucosal infectious disease in women caused by *Candida albicans* infection. The high resistance to antifungal treatment makes alternative therapies increasingly necessary. Therefore, this study aimed to investigate the antifungal activity of *Mentha piperita* essential oil for candidal vaginitis. *Mentha piperita* essential oil (E.O.) was extracted with *n-hexane* and a Soxhlet extractor and tested for activities. Thus the agar diffusion method, MIC and MFC were collected *in vitro*. A dose of *Mentha piperita* (500 µg/mouse) E.O. was given intravaginally to vaginal candidiasis mice every two days until day +21. Histological diagnosis using PAS staining was examined *in vivo* after mice were sacrificed and vaginas excised.

The *Mentha piperita* E.O. showed a high ability to inhibit candidal growth with a 30.7±2.1 mm inhibition zone, with MIC & MFC 0.125 mg/ml and 0.250 mg/ml, respectively. The efficacy of *Mentha piperita* E.O. was significant with a semi-normal appearance in histological examination of the treated group compared to non treated group. This study demonstrated the use of *Mentha piperita* E.O. as an effective and suitable alternative antifungal against candidal vaginitis infection in candidal vaginitis mice model.

**Keywords:** *Mentha piperita*, Essential Oil, *Candida albicans* and Candidal Vaginitis

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## INTRODUCTION

Al-Baha is a region located in the southwestern part of Saudi Arabia. It is characterized by varying terrain that affects climate and vegetation. The geographic characteristics of the Al-baha region are described by distinct forests, high *Juniperus procera* woodland and diverse vegetation [1].

*Mentha piperita*, (family Lamiaceae) is a species seen in different areas around the world, including the Al-Baha region. These mint plants contain large amount of several minerals like iron and magnesium, which play an important role in human nutrition [2]. *Mentha* spp essential oils have important medicinal properties that have been described in many medical studies [3].

The most traditional medical usage of the peppermint (mint) plant is for mint tea, which is made from mint leaves, and is effective for digestive system disorders, and also flu and musculoskeletal pain. Menthol is an effective material that consists of cyclic terpene alcohol, which is a form of prescribed mint used as a medication [4].

Phytochemicals analysis has been done in past research [5], and flavonoids exhibit anti-microbial, inflammatory, anti-oxidant, and vascular activities along with other medicinal properties [6]. The flavonoids, steroids and triterpenes from

crude extracts have significant activity with bacterial strains such as *Escherichia coli*, *Streptococcus faecalis* and *S. aureus* [7]. Tannin is toxic to the pathogens [5] and has a potential antiviral, and antibacterial activity [8].

Alkaloids have many uses, especially as anesthetic [9]. Terpenoids have demonstrated anti-inflammatory, and antimicrobial activities [10]. Plant extracts have also been utilized as an alternative medicine to control many fungal diseases [11]. Various roots, barks, stems, leaves and seeds extracts have been studied as antimicrobial agents [12] and offer a variety of chemical constituents.

Various studies show the efficacy of various plant extracts as antifungal agents [13]. Candidal vaginitis a common gynecological infection and approximately 75% of women have had at least one candidal vaginitis occurrence [14]. Clinical studies have noted numerous infections among pregnant women with candidal vaginitis [15].

Multiple medications, especially antimicrobial agents, are facing resistance if overused for infectious disease, and can have associated adverse effects. Therefore, a new and safe antimicrobial, which treats the

infectious diseases, from new natural sources (such as plants) is necessary. Plants extract, especially essential oil, is reliable and an attractive alternative when, compared with expensive synthetic medications and their possible adverse effects [16].

The resistance and virulence of pathogens, including *Candida albicans*, has led to a search for alternative therapies. As such, this study aimed to test the effectiveness of *Mentha piperita* essential oils collected from the Al-Baha region in Saudi Arabia on *Candida albicans* infection in a candidal vaginitis mice model.

#### MATERIAL AND METHODS

This study was conducted in the Biology Department, Faculty of Science, Al-Baha University from October 2017 to August 2018.

***Candida albicans* samples:** The *Candida albicans* sample was obtained from a microbiology laboratory, Al-Borg Medical Laboratories. The yeast cells were grown 2-3 times in a broth medium (SDB), (pH:  $5.6 \pm 0.2$ ) and preserved on Sabouraud agar slopes at 4°C until use.

**Plant sample collection and essential oil extraction:** Plant samples of *Mentha piperita* were collected from the Al-Baha Region and identified at the Biology Department, Faculty of Science, Al-Baha University.

Approximately 60g of finely ground powder of *Mentha piperita* leaves was dried and placed in a thimble, then extracted using a Soxhlet extractor for 8 h using 250 ml of hexane as solvent. The solvent was then removed. The obtained plant oil was dissolved in 10% dimethyl sulfoxide (DMSO) W/W. The extracts were saved at 4°C [17].

**Agar-well diffusion method:** The Agar-well diffusion test, as previously described [18], was utilized for the present study. Agar plates were incubated for 48 hr. at 37°C. The zone of inhibition was measured in (mm).

**Determination of minimum inhibitory concentrations (MIC) and minimal fungicidal concentration (MFC):** The minimal inhibitory concentrations of the extracts against *C. albicans* were determined by the modified broth microdilution test according to the CLSI method in 96 microtiter plates [19]. Red phenol was used as a color indicator, which turned to red in a basic condition, neutral in a medium condition, and yellow in an acidic medium. Yeast growth was indicated by the color. The Minimal fungicidal concentrations (MFCs) were recorded in wells that had no visible growth in the microtiter plates before minimum inhibitory concentration (MIC) wells.

**Induction of vaginal candidiasis in mice**

**model:** Thirty female BALB/C mice (6 weeks old) weighing 20–25 gm were obtained from an experimental animal store. The experiment was carried out according to international guidelines for the use of the animals in the laboratory. All animals were randomly arranged into three groups: Group (1) Control positive non-infected were treated with a physiological solution, Group (2) Control negative infected non-treated, and Group (3) were infected and treated with *Mentha piperita* E.O.

All mice infected with *C. albicans* were given treatment as described by Pietrella *et al.*, [20] with some modifications, except for mice in the Control positive group. The intravaginal treatment with a physiological solution for the Control positive group was *Mentha piperita* E.O. with a value of (500 µg/mouse), and the treatment was taken every two days until day +21.

**Light microscope study (LM):** After treatment, mice were sacrificed by cervical dislocation. Vaginas were excised and immediately fixed in 10% (vol/vol) of buffered formalin and the histological changes in the vaginas were evaluated according to the level of tissue injury and recovery using H&E and PAS stains. The

histological changes were analyzed using a 40x objective lens of a light microscope.

**Statistical analysis:** The values of the inhibition zone are expressed as mean±SD.

**RESULTS AND DISCUSSION****Effect of *Mentha piperita* E.O on *C. albicans* *in vitro*:**

The essential oils activities against *Candida albicans* were obtained by developing and calculating inhibition zones. Mean diameters ±SD were used as a criterion for antifungal activity. *Mentha piperita* E.O. resulted in an inhibition zone of 30.7±2.1mm. Meanwhile, the MIC & MFC of *Mentha piperita* E.O against *Candida albicans* were 0.125 mg/ml and 0.250mg/ml, respectively (Figure 1) (Table 1).

**Effect of *Mentha piperita* E.O on *C. albicans* *in vivo*:**

Histological examination of a PAS stained section of the vagina in the Control negative group, which was infected and non-treated showed a considerable amount of a *C. albicans* yeast, hypha and pseudohypha which attacked the stratified squamous epithelium of the vagina lumen (Figure 2-A). Meanwhile, a PAS stained section of the vagina in the *Mentha piperita* group showed no biofilm formation of *C. albicans* yeast or hyphae with a normal distribution of stratified squamous epithelium in the vagina lumen and no

appearance in the treatment section (Figure 2-B).

This study successfully extracted *Mentha piperita* E.O. using a Soxhlet extractor with hexan as a solvent. This extraction of *Mentha piperita* E.O. showed a high effect *in vitro* and *in vivo*, and the inhibition zone was  $30.7 \pm 2.1$  mm. Meanwhile, MIC and MFC were 0.125 mg/ml and 0.250 mg/ml, respectively. Agarwal *et al.*, [21] found a 26.7 mm inhibition zone for *Mentha piperita* E.O. against *Candida albicans*, which was similar to this study results. This variation may reflect to the climate, season of plant collection, and site of plant growth, which can impact the presence and concentration of certain chemical constituents of E.O. [22].

The antifungal characteristic of *M. piperita* methan E.O. improved recovery from vaginitis infection in an experimental mice model. Histological results showed high protective effects against *C. albicans* infection, proving that the antifungal activity of *M. piperita* E.O. corresponded with previous reports, which suggested the redacting of microbial colonisation through the use of *M. piperita* extracts, which subsequently leads to the prevention of mucosal infections [23]. The preventive activity of *Mentha piperita* E.O. against pathogens acts as an effective anti-biofilm

that prevents microbial access to the epithelial mucosa [24]. *Candida albicans* is associated with biofilm formation, and this phenomenon helps in survival of the fungus, and drug resistance. The hydrophobicity characteristic of E.O. enables their incorporation into the cell membrane, as described by Agarwal *et al.* [25].

Many studies have investigated the antimicrobial and antioxidant activities of *M. piperita* E.O., and methanolic extracts. A report by Yadegarinia *et al.*, [26] confirmed the high vulnerability of *C. albicans* to *M. piperita* oil. This is due to the tannins and flavonoids extracted from *M. piperita* methane oil and is composed of monoterpenes, menthone, menthol and their derivatives which have antibacterial and antifungal activities [27] [28]. The most important pharmacological activities of *M. piperita* E.O. as an antifungal agent for *C. albicans* were alkaloids and terpenoids that provided anti-inflammatory and antibacterial activities [4], which have been showed to exhibit inhibitory effects against a wide range of pathogens including the *Aspergillus* species of aflatoxins [29] [30].

Looking forward, the promising results from this study support further research on Al-Baha region plants to discover additional antimicrobial agents that are effective against

various pathogens. Additionally, various chemical tests can be applied to determine the active substances in these medical plants

and to examine their activity against resistant pathogens.



Figure 1: The inhibition zone of *Mentha piperita* E.O against *C. albicans* in agar plate

Table 1: *In vitro* antifungal activity of *Mentha piperita* E.O. against *Candida albicans*

	<i>Mentha piperita</i> E.O		
	Zone of inhibition	MIC	MFC
<i>Candida albicans</i>	30.7±2.1mm	0.125 mg/ml	0.250mg/ml

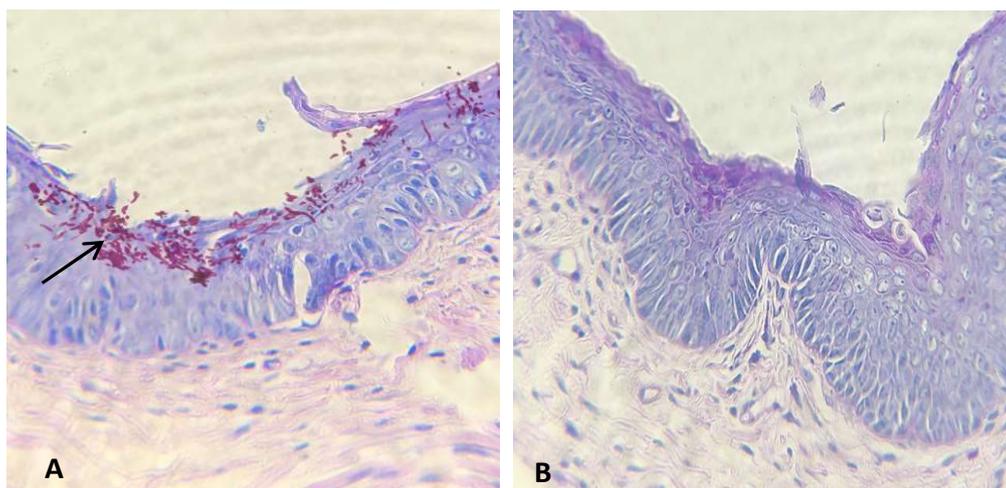


Figure 2: Sagittal section of the histological examination in vaginal tissue from BALB/c mice Day +21: A) Microscopic observation in control negative group showed invasion of *C. albicans* yeast and hypha that attacked the vaginal epithelium (arrow). B) Microscopic observation in vaginal tissue of *Mentha piperita* group showed no *C. albicans* yeast or hyphae in the treatment sections. A&B stained with PAS stain at a magnification of 400 X

## CONCLUSION

This study showed that *Mentha piperita* E.O. collected from the Al-Baha region had efficiency in protection and recovery from candida vaginitis infection. This essential oil is Promising as a *C. albicans* antifungal treatment that is economical and widely available. Further, studies are necessary to identify active substance from *Mentha piperita* E.O. and to determine other therapeutic activity. This investigation of *Mentha piperita* E.O. demonstrated the applicability of natural antimicrobial and antioxidants agents toward various maladies.

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