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Original article

Antibacterial activity of *Mentha piperita* extraction against uropathogenic bacteria

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ABSTRACT

Background: Urinary tract infections (UTIs) are widespread health issues affecting millions of people annually. Medicinal plants are gaining broad interest as an alternative source of productive and inexpensive bio-remedies to synthetic chemotherapeutic compounds, such as Peppermint. **Aim:** To evaluate the antibacterial activity of the crude leaf ethanolic peppermint extract against *Escherichia coli* and *Staphylococcus aureus.* Methods: The antibacterial activity of Peppermint alcoholic extract at different concentrations was evaluated against uropathogens (*Escherichia coli* and *Staphylococcus aureus*). **Results:** The peppermint extract showed closely sturdy activity towards tested bacterial strains, with inhibition zones showing differences depending on the extract concentration. The inhibition zone diameter in mm was recorded (18.20 ± 0.03 mm) for *E. coli* and (35.14 ± 0.04 mm) for *S. aureus* at a (400 mg/ml) concentration. The minimum inhibitory concentration values of the peppermint extract ranged (252 µg/mL and 480 µg/mL) regarding *E. coli* and *S. aureus*, respectively. **Conclusion:** The promising result obtained from the antimicrobial activity has the potential to control several bacterial pathogens.

Introduction

Urinary tract infections (UTIs) are problems involving public health that impact millions of people every year. Pathogenic microorganisms colonize the urinary tract, causing infection. [1]. The Gram family, including Positive and Negative bacteria, are the major players. [2]. The main bacterial source is *Escherichia coli*, which is the prevalent microorganism. [3]. Interest in the biological and antimicrobial properties of medicinal plant extracts has escalated in the past few years. [4].

The *Lamiaceae* family includes *Mentha piperita L. (M. piperita).* It is a perennial herbaceous non-native plant that can be found both in developed and natural settings [5]. It has been claimed that *M. piperita* is used internally as an herbal tea and externally as an ointment using tinctures, oils, or extracts. According to botanists, it has aftereffects against spasms, catarrhal, septic, temperature elevation, and aging [6]. The *M. piperita* possesses essential oils like menthone and menthol, which serve as active ingredients. The possible function of *M. piperita's* phytochemical components and antioxidant activity in the treatment of a variety of medical conditions, including its ability to treat urolithiasis and the justifications for its traditional use in kidney stone illness, were evaluated [7].

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Camele *et al*. (2021) reported moderate antibacterial effects of menthol and peppermint oil for Gram family bacteria, which have bactericidal and fungicidal effects [8]. An earlier local investigation by Ghazi *et al*. (2016) reported a promising antibacterial activity of peppermint extract against *E. coli* [9]. Therefore, the current investigation aims to evaluate the antibacterial activity of a crude leaf ethanolic peppermint extract against *Escherichia coli* and *Staphylococcus aureus*, which were determined in vitro using the disc diffusion method and minimum inhibitory concentration (MIC).

Methods

Uropathogens isolates

The Microbiology Laboratory Unit/Al-Kadhemiyian Teaching Hospital provided all tested bacterial isolates used in this study. A urologist referred the urine samples to the laboratory. The isolates were identified by colony morphology cultured on selective media. Further confirmation was obtained through Gram staining and biochemical characterization.

Bacterial suspension preparation

Three to five colonies of *E. coli* and *S. aureus* isolates were obtained after 18 hrs of incubation from Muller-Hinton agar (MHA) plates. A suspension of microorganisms was then prepared using five ml of Brain-Heart Infusion (BHI) agar, which was kept for 2 hrs at a temperature of 37ºC with shaking set at 200 rpm, by diluting the microbial suspension with a solution of 0.9% NaCl. The concentration was adjusted to reach 10^8 CFU/mL using the McFarland standard. Every time the suspension of microorganisms was utilized for the antibacterial test, 200 mL of it was taken.

Antimicrobial Sensitivity Test

It was determined using the Kirby-Bauer disk diffusion susceptibility test. A 200 mL of microbial suspension needed to be adjusted to reach CFU/mL of 10^8 using the McFarland standard. The above suspension was used to inoculate solid MH media. Ampicillin, Amoxicillin and Clavulanic Acid, Bacitracin, Cefixime, Ciprofloxacin, Chloramphenicol, Clindamycin, Gentamycin, and Keflex antibiotic discs were used and implanted in proper positions on the media. The incubation period was implemented overnight with a temperature of 37°C. A metric ruler and an inhibitory zone were generated and recorded to compare to the resistance benchmark for microbial drugs [10].

Plant preparation

Locally obtained peppermint leaves were washed with tap water, and the stems were cut off. The leaves were allowed to dry in the air for a few weeks before being ground into a fine powder. Following hot ethanol (70%) extraction of the powder, to achieve an extract residue devoid of alcohol, the entire extracts were filtered and then evaporated to dryness (air dried) at ambient temperature (25°C) for 4-5 days [9].

Peppermint Extract Production and Series Concentration

The Peppermint extract stock concentration was made at 800 mg/mL according to [11], and the concentration of 800 mg/mL in 500 µL was directly taken from the stock solution.

Peppermint Antibacterial Activity by Disk Diffusion Assay

This procedure was performed according to the previous method; the bacterial suspension (10^6 CFU/mL) from bacterial strains was prepared using saline from bacteria incubated for 18 hrs to compare with 0.5 McFarland turbidity. A bacterial suspension was taken in 200 µL and then inoculated on MHA. Flattened the suspension using a glass spreader, then allowed it to dry. Peppermint extract was prepared at several concentrations (100, 200, and 400 mg/mL). The extracted peppermint dilutions were taken with a volume of 20 µL. These concentrations were allowed to flow to a blank disk, then placed on MHA, and then poured into a plate with a 9 mm diameter that had already been cultivated with the tested bacterial solution. The incubation at 37 °C overnight was followed, and the mean inhibition zone was registered. Each test was performed three times. Gentamycin (30 µg) as a positive standard was used to mark the microorganism's sensitivity [12].

Minimum inhibitory concentration (MIC) assay

Micro-dilution broth standards suggested implementation of the peppermint fundamentals to get a concentration range from 8 µg/ml to 4096 µg/mL; the extract stock solution was serially diluted in a 96-well microtiter plate with MH broth. Until an inoculum size of roughly 5 x 10^5 CFU/mL in each well was attained, the inoculum standardized for each bacterial strain was used. Micro-titer plates were incubated for 18 hrs at 37°C. After the incubation period, the bacterial strain's observable growth was inhibited by the extract at the lowest

concentration, which was referred to as the MIC [13], [14].

Statistical analysis

ANOVA (one-way analysis of variance) was applied to compare the means of various extracts and concentrations. The LSD (least significant difference) test was used for the group means comparison. P values under five were deemed significant.

Results and Discussion

A complementary medication based on plant extracts is called phytotherapy [15]. It is a crucial non-antibiotic method that has been utilized for millennia to either prevent or treat various illnesses, including UTIs [16]. Consequently, the current study sought to examine the antibacterial activity of three significant uropathogenic isolates identified as the primary cause of UTIs.

Antimicrobial Sensitivity Test

The disk diffusion method was used to test bacterial isolates against several antimicrobial discs. The results revealed that the *E. coli* selected isolate was resistant to some empirical antimicrobial agents such as Amoxicillin, Chloramphenicol, Cefixime, and Ciprofloxacin; meanwhile, the *S. aureus* selected isolate was resistant to Keflex, Amoxicillin, and Bacitracin. While *E. coli* was sensitive to Gentamycin, meanwhile, *S. aureus* was sensitive to Clindamycin and Gentamycin, as illustrated in **Figure 1**.

Antibacterial Activity of Peppermint Extract

Leaves of the peppermint ethanol extract concentrations were (100, 200, and 400 mg/mL) had (4 mg/disk) concentration. Gentamicin (30 g) as a positive index was applied **(Table 1)**. The highest level of inhibition was found for the pathogenic isolates tested by the antibacterial activity of the positive control (Gentamycin 30 g). With an inhibition zone diameter of 35 mm, which is comparable to Gentamycin's zone of inhibition with a diameter of 36 mm, the peppermint extracts also demonstrated a considerable increase in antibacterial activity for *S. aureus*. There was moderate antibacterial activity towards *E. coli* (18 mm) compared to Gentamycin (20 mm), as shown in **Figure 2**.

The peppermint crude extraction (20 µL) exhibited a greater zone of inhibition against *S. aureus* nearly approached the positive control, Gentamycin. However, the peppermint extraction showed a lesser zone of inhibition than the positive control, Gentamycin, as for *E. coli*. Thus, it is more effective against the positive member tested strain (*S. aureus*) when compared to the gram-negative tested strain (*E. coli*) in this study. It can be explained that the outer membrane lipopolysaccharides of Gram-positive bacteria potentially play a role in boosting resistance to antibacterial substances [17].

The current study findings agree with a local study by Ghazi *et al*. (2016), which reported a (27.25±0.52 mm) inhibition zone for *E. coli* [9]. This study's findings also come with Indrayudha (2021) findings [11]. Meanwhile, Desam *et al*. (2019) revealed the peppermint antibacterial activity extract against *S. aureus* recorded (a 42.44 ± 0.10 mm) inhibition zone, while *E. coli* recorded (a 27.02 ± 0.13 mm) inhibition zone [18]. The result of this study disagrees with Hammadi *et al*. (2021), which reported that *in Vitro* activity of the mint extract against pathogenic bacteria (*E. coli, Klebsiella pneumonia, S. aureus*, and *Streptococcus pneumonia*), the inhibition zone for *E. coli* was 23 mm and for *S. aureus* 9 mm respectively [19]. Shawket *et al*. (2015) proposed that methanolic and aqueous leaf extracts of Peppermint showed a significant inhibitory effect on Acetylcholinesterase activity in Mice in a matter of decrease of AchE in the serum, liver, and brain, in comparison with mice fed ethanol liquid diet [20].

Antibacterial activity using MIC assay

The results of the MIC value of the antibacterial activity of Peppermint against tested bacteria are demonstrated in **Table 2**. The current study findings agree with a local study by Ghazi *et al*. (2016) regarding peppermint ethanol extract [9]. Another study by Pulipati *et al*. (2016) reported MIC results of 62.5 µg/mL for *S. aureus* and *E. coli* and 125 µg/mL for other tested bacteria (*E. faecalis, P. aeruginosa*, and *K. pneumonia*) [21]. The antibacterial activity of another plant essential oil, like *Rosmarinus officinalis* leaves, against intermediate-stage *S. aureus* treated with Vancomycin was assessed by Al-Hayali *et al*. (2023), and the results showed that the essential oil reduced resistance. The minimum inhibitory concentration was determined to be (MIC = 1.5 mg/ml) [22]. Determining the MIC assay is crucial in diagnostic labs because it helps confirm that microbes are resistant to an antimicrobial agent and tracks the activity of new antimicrobial agents [23].

The populace has used medicinal plants as alternative resources for healing purposes since

ancient times. According to estimates, ninety percent of subjects already have employed remedies in nature as their first choice means of easing plenty of the suffering brought on by illnesses [24]. The goal is to guarantee that the curing characteristics found in those products are naturally used for either prevention or curing diseases without harming well-being. In addition, the World Health Organization (WHO) welcomes research that focuses on the use of plants [25]. It is reasonable to conclude that the ethanolic extract of peppermint leaves represents a new class of antimicrobial medicines that may significantly affect urinary tract infections as a substitute treatment brought on by these two types of bacteria [26 – 28].

This work is limited by the absence of a phytochemical analysis utilizing gas chromatography-mass spectrometry to identify the active compounds in peppermint leaves. When doing this study, it is important to remember that peppermint essential oil has been shown to have a substantial impact in numerous studies. Innovative antimicrobial medicines without the issue of bacterial resistance and the active components require more investigation, and the profile of toxicity, if any, would be essential.

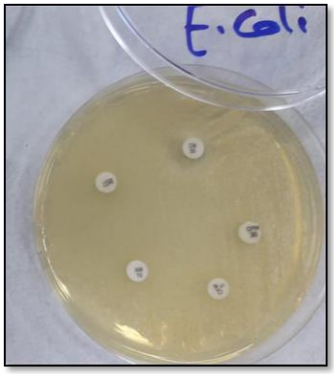
Table 1. Antibacterial activity of *M. piperita* against uropathogens using disc agar method.

Ethanolic extract	**Diameter of Inhibition Zones (mm)**		
	Concentration	***E. coli***	***S. aureus***
Peppermint (*Mentha piperita*)	100 mg/mL	13.48 ± 0.07^{c}	23.11 ± 0.08^{b}
	200 mg/mL	15.83 ± 0.04^{c}	25.98 ± 0.05^{b}
	400 mg/mL	$18 .20 \pm 0.03^{b}$	35.14 ± 0.04^{a}
Gentamycin	30 µg	20.18 ± 0.15^{a}	36.20 ± 0.08^{a}
Values for each tested microorganism in each column that are followed by a different letter are statistically different at p 0.05. Similar letters indicate differences that are not statistically significant. Data are presented as the three replicated means SD.			

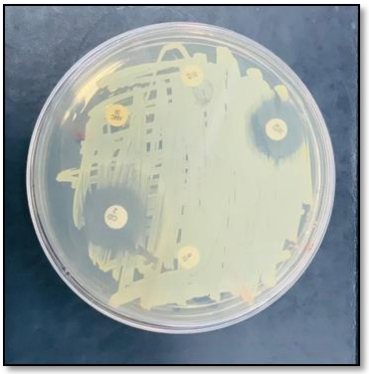
Table 2. Antibacterial activity of *M. piperita* against uropathogens using MIC assay.

Ethanolic extract	**MIC value (µg/mL)**	
Peppermint (*Mentha piperita*)	***E. coli***	***S. aureus***
	252	480

Figure 1. Antimicrobial Susceptibility Assay for antimicrobial disc against uropathogens.

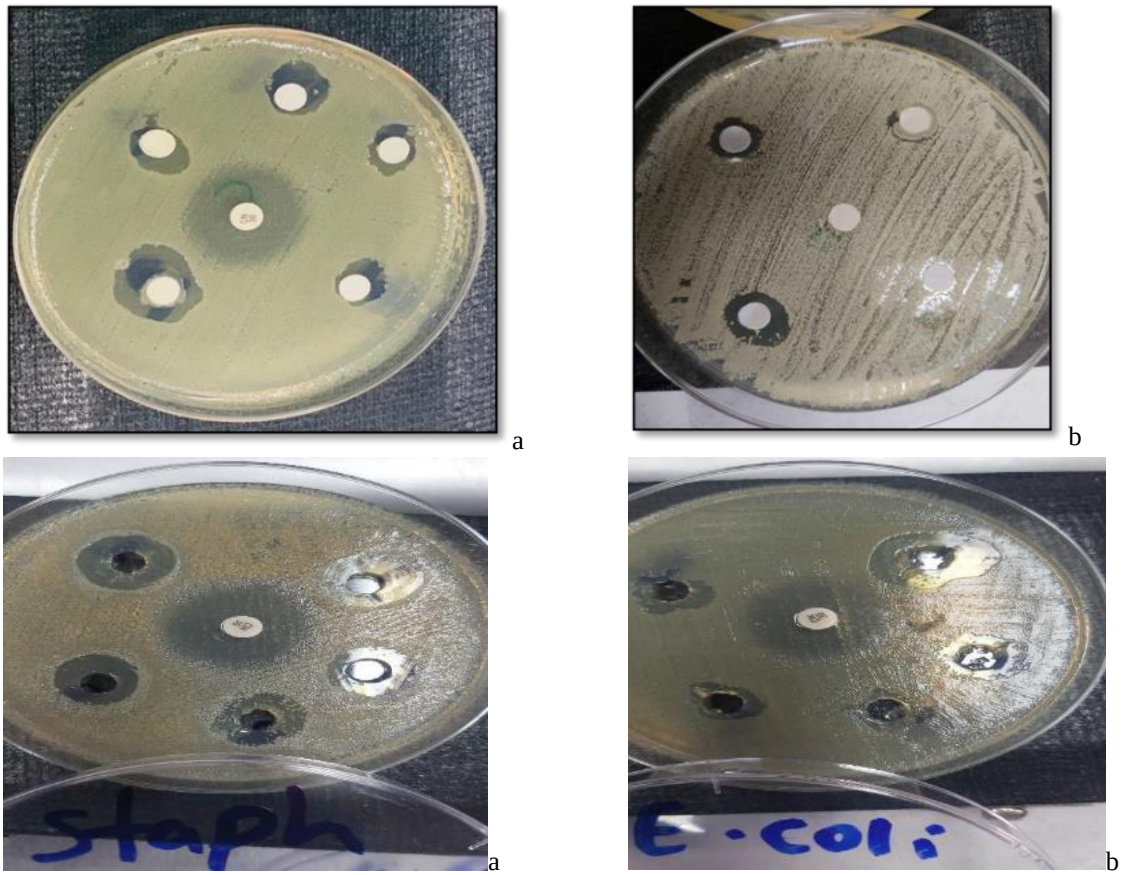


E. coli



S. aureus

Figure 2. Inhibitory zone of *Mentha piperita* crude extract using Disc diffusion method and well diffusion methods.



a: on Gram-positive bacterial strain (*Staphylococcus aureus*).
b: on Gram-negative bacterial strain (*Escherichia coli.)*

Conclusion

Both *E. coli* and *S. aureus* are susceptible to the peppermint leaf ethanol extract's antibacterial properties. Antimicrobial activity's encouraging outcome has the potential to be used as an alternative to traditional medicine. Further, peppermint alcohol-leaf extract may be employed in pharmaceuticals as a naturally occurring substitute for synthetic pathogen-killing chemicals. It is advised that other members of the *Enterobacteriaceae* family, such as *Pseudomonas aeruginosa* and *Klebsiella pneumonia*, as well as more multi-antimicrobial resistance pathogens, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), be tested for their susceptibility to peppermint alcohol extraction.

Competing interests

The authors reported no conflicts of interest.

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