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Original Article

Antimicrobial Efficacy of *Melissa officinalis* Extract Against Pathogenic Bacterial Isolates: Inhibition of Biofilm Formation and Adhesion

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ABSTRACT

Melissa officinalis, commonly known as lemon balm, is recognized for its broad-spectrum antimicrobial properties against various pathogenic bacteria, suggesting potential therapeutic benefits for treating infectious diseases. This study aimed to investigate the inhibitory effects of *M. officinalis* extract on human pathogenic bacteria and to assess its ability to prevent bacterial biofilm formation and adhesion. The antibacterial activity of the aqueous extract of *M. officinalis* was evaluated using disc-diffusion and agar-well diffusion methods. The antimicrobial efficacy of the extract was compared with that of standard antibiotics. Additionally, tests for adherence and biofilm formation were conducted. The *M. officinalis* extract demonstrated inhibitory zones ranging from 25 to 35 mm against all tested microorganisms. While some bacterial isolates were susceptible to imipenem, the majority exhibited resistance. Notably, certain isolated bacteria displayed strong adhesion and biofilm formation in response to the extract, whereas most Gram-negative bacteria showed moderate adherence and biofilm activity. The findings indicate that *M. officinalis* extracts are highly effective against a range of clinical isolates, including those associated with urinary tract infections. This suggests that these extracts may offer a more effective alternative to conventional antibiotics, particularly in combating bacterial adhesion and biofilm development.

Keywords: Antimicrobial Properties, *M. officinalis*, Biofilm Formation, Adherence Inhibition, Urinary Tract Infections

INTRODUCTION

The use of medicinal herbs and herbal remedies has a long-standing history, with practices dating back centuries. The advancement of modern therapeutics has further propelled the utilization of natural products for various illnesses and disorders worldwide [1]. Traditional medicine encompasses a range of methods, including the use of herbs to treat diseases [2]. This approach is gaining momentum globally, even in developed nations [3]. For example, traditional herbal medicines account for 30-50% of all pharmaceuticals used in China, and the global market for herbal medicine is estimated to exceed sixty billion dollars. Additionally, over eighty percent of the population in developing countries relies on plants as their primary source of medicine. Statistics indicate that a significant portion of the global population utilizes plants for medicinal purposes. However, in vitro analyses and clinical studies have only examined a limited number of these traditional medicinal plants [4].

Herbal remedies containing specific natural compounds may fulfill essential health needs and often represent the initial stage in disease treatment [5]. Notably, more than half of the medications used in clinical practice are derived from natural sources, including their derivatives and analogs; higher-plant-derived natural medicines constitute over one-fourth of this total. Due to the diverse range of natural compounds found in these herbs, their various roles in disease prevention and treatment, and their relative safety when used appropriately, both the public and medical professionals are increasingly interested in studying these plants for their potential therapeutic effects. Nevertheless, numerous questions remain unanswered regarding these plants [6].

To address this gap, extensive research has focused on identifying plants with curative properties that can also provide economic benefits to farmers. Factors such as cultivation conditions, ripening processes, and plant characteristics are crucial for obtaining optimal chemical and pharmacological compositions. From an economic perspective, cultivating *Melissa officinalis* is advantageous as it yields significantly more than other crops grown in fertilized soil [7].

M. officinalis L., commonly known as lemon balm or bee balm, is a perennial herb belonging to the Lamiaceae family. It is primarily native to Southern Europe but is also cultivated in regions such as Iran, Central Asia, North America, and New Zealand [8]. The plant thrives in sandy and scrubby areas but can also be found in damp wastelands from sea level up to hilly terrains. In Iran, it is known by local names such as Faranjmoshk, Varangboo, and Badranjbooye [7].

Historically, lemon balm has been attributed with various medicinal properties including treatment for asthma, flatulence, amenorrhea, bronchitis, arrhythmias, cardiac failure, wounds, and ulcers, as well as exhibiting antiviral and antioxidant activities. It also possesses antifungal, antiparasitic, and antispasmodic properties. Furthermore, it has been reported to influence thyroid function, menstruation regulation, memory enhancement, spasmolytic effects, hypotension reduction, anti-flatulent effects, fever reduction (febrifuge), and

antimicrobial functions. Lemon balm has been utilized in treating conditions such as migraines, anemia, vertigo, syncope (fainting), anorexia, insomnia, epilepsy, depression, psychosis, and hysteria [9].

The chemical composition of *M. officinalis* leaves includes flavonoids such as luteolin and rhamnocitrin; polyphenolic compounds like caffeic acid and rosmarinic acid; sesquiterpenes; tannins; triterpenes such as ursolic and oleanolic acids; along with monoterpenoid aldehydes [10]. In total, 33 components have been identified within its essential oil content. The primary constituents include geraniol (6.40%), citronellal (14.40%), nerol acetate (5.10%), caryophyllene (8.10%), geraniol acetate (10.20%), and caryophyllene oxide (11.00%), which collectively account for approximately 55% of the total oil content [7].

Free radicals are widely recognized as contributors to various diseases; thus antioxidants play a critical role in disease prevention. Reactive oxygen species (ROS) production can exceed cellular antioxidant capacity leading to oxidative stress—a condition linked to the progression of several diseases through mechanisms such as DNA mutation and lipid peroxidation. Research has highlighted the involvement of oxidative stress and ROS in conditions like diabetes and neurodegenerative diseases [11]. Studies have shown that essential oils from *M. officinalis* exhibit effective antioxidant activity due to their rich phenolic compound content [12]. The antioxidant capacity of these natural extracts is comparable to synthetic antioxidants like BHT and BHA.

This study aimed to investigate the antimicrobial efficacy of *M. officinalis* extract against a range of human pathogenic bacteria, with a particular focus on its ability to inhibit bacterial growth, prevent biofilm formation, and reduce bacterial adhesion. By comparing the effectiveness of the extract to standard antibiotics, the research seeks to provide insights into the potential use of *M. officinalis* as a natural alternative for treating bacterial infections, particularly those associated with urinary tract infections.

MATERIAL AND METHODS

Preparation of Extracts

Aqueous extracts of *Melissa officinalis* were prepared at a concentration of 25% according to established protocols [13]. The plant material was soaked in hot water for 24 hours and then filtered to obtain the liquid extract, which was subsequently evaporated to yield a concentrated powder. To prepare the working solution for antimicrobial assays, 2.5 g of the dried extract was dissolved in 10 mL of distilled water [14].

Bacterial Strains

A total of twenty-two bacterial isolates were investigated in this study. These isolates included fourteen Gram-negative and eight Gram-positive bacteria, some of which were sourced from urinary tract infections. The bacterial strains are listed in Table 1. After three consecutive culturing cycles on appropriate media, the isolates were preserved as nutrient agar slants at 4ºC. The identification of microbial organisms (MOs) was performed using a series of biochemical tests [15].

Table 1 List of Bacterial Isolates Used in the Study, Including Gram-Positive and Gram-Negative Pathogens Associated with Urinary Tract Infections

Gram-positive	Negative-bacteria
Staphylococcus saprophyticus	Aggregatibacter actinomycetemcomitans
Streptococcus pyogenes	Prevotella intermedia
Staphylococcus aureus	*Porphyromonas gingivalis,*
Streptococcus mutanus	*Pseudomonas flourscences*
Streptococcus pneumoniae	*Enterobacter aerugenes*
Streptococcus feacalis	*Escherichia coli*
Streptococcus aglagtia	*Proteus merabilis*
Staphylococcus epidermidis	*Proteus vulgaris*
	Acinetobacter
	Pseudomonas.aeroginosa
	Klebsiella pneumonia
	Serratia spp.
	Salmonella typhi
	Salmonella typhimurum

Antimicrobial Activity Assays

The antibacterial activity of *M. officinalis* extract was evaluated using the agar well diffusion method, as described by Forbes (2007) [15]. Triplicate tests were conducted for each bacterial isolate to ensure reproducibility. The results were measured by determining the diameter of inhibition zones around the wells containing the extract.

Adherence Test

Adherence to human epithelial cells is a critical virulence factor for pathogenic bacteria. This property was assessed using methods specified by previous studies [16,17], focusing specifically on Gram-negative bacteria.

Biofilm Formation Assay

Biofilm formation was evaluated using the Tissue Culture Plate (TCP) method, also known as the semi-quantitative microtiter plate test [18]. The results were quantified by measuring the optical density (OD) at 630 nm, with adherence categorized as follows: non-adherent (<0.120), medium (0.120–0.240), and elevated (>0.240) biofilm formation [19]. The results are summarized in Table 2.

Table 2 Bacterial Biofilm Formation and Adherence Assay Results

Mean of OD value at 630 nm	Adherence	formation Biofilm
0.120 >	non	non
0.240-0.120	Medium	Medium
>0.240	Elevated	Elevated

Statistical Analysis

The data obtained from the antimicrobial activity assays of Melissa officinalis extracts were analyzed using appropriate statistical methods to ensure the reliability and validity of the results. The following statistical analyses were performed:

Descriptive Statistics

Mean and standard deviation (SD) were calculated for the inhibition zones produced by Melissa officinalis extracts and imipenem across different bacterial isolates. This provided a summary of the central tendency and variability of the data.

Comparative Analysis

The antibacterial efficacy of Melissa officinalis was compared to that of imipenem using an independent samples t-test. This analysis determined whether there were statistically significant differences in the mean inhibition zones between the two treatments.

Biofilm Formation Assessment

The optical density (OD) values obtained from the biofilm formation assays were analyzed using one-way ANOVA to compare the means among different treatment groups (e.g., control, Melissa officinalis extract, imipenem). Post-hoc tests (e.g., Tukey's HSD) were conducted to identify specific differences between groups.

Correlation Analysis

A Pearson correlation coefficient was calculated to assess the relationship between the concentration of Melissa officinalis extract and the size of inhibition zones. This analysis provided insights into how changes in extract concentration affected antibacterial activity.

Significance Level

A p-value of <0.05 was considered statistically significant for all analyses, indicating that any observed differences or correlations were unlikely to have occurred by chance.

These statistical methods ensured a robust analysis of the data, supporting the conclusions drawn regarding the antimicrobial properties of Melissa officinalis extracts against various bacterial isolates. Future studies should continue to employ rigorous statistical approaches to further validate these findings and explore additional factors influencing antimicrobial efficacy.

RESULTS AND DISCUSSION

Efficacy of *M. officinalis* Against Bacterial Isolates

The antibacterial action of *M. officinalis* at a 25% concentration against various bacterial isolates was investigated using the agar well diffusion method. The results indicated that the extract produced inhibition zones ranging from 25 to 35 mm, demonstrating sensitivity across all identified Gram-positive and Gram-negative bacteria (Figures 1 and 2). This significant antibacterial activity highlights the potential of *M. officinalis* as a natural antimicrobial agent.

Imipenem, a commonly used antibiotic, was also tested using the disc diffusion method (Figures 3 and 4). While some isolated bacteria showed susceptibility to imipenem, the majority exhibited resistance. This finding underscores the growing concern of antibiotic resistance among clinical isolates, emphasizing the need for alternative treatments such as plant-derived extracts.

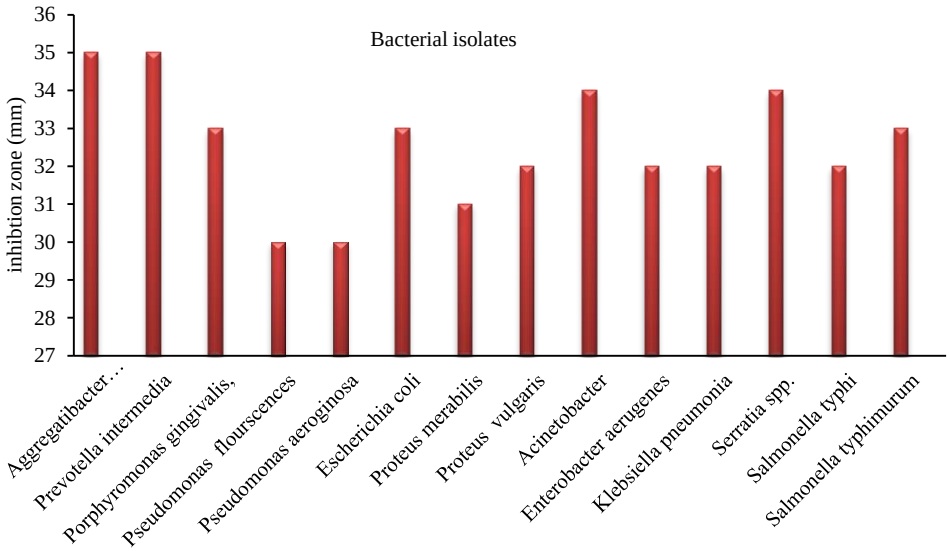

Fig. 1 Antimicrobial Activity of *M. officinalis* Against Gram-Negative Bacteria Using the Agar Well Diffusion Method

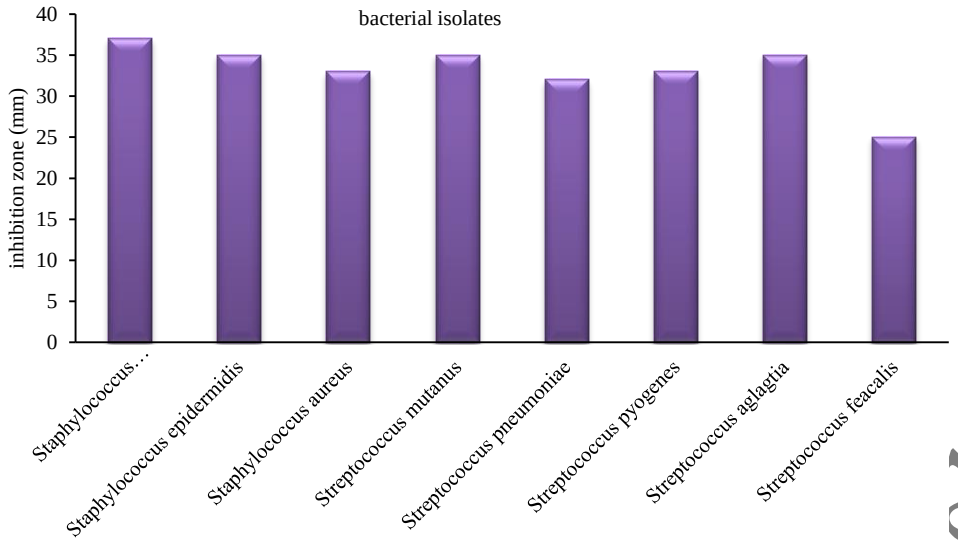

Fig. 2 Antimicrobial Activity of *M. officinalis* Against Gram-Positive Bacteria Using the Agar Well Diffusion Method

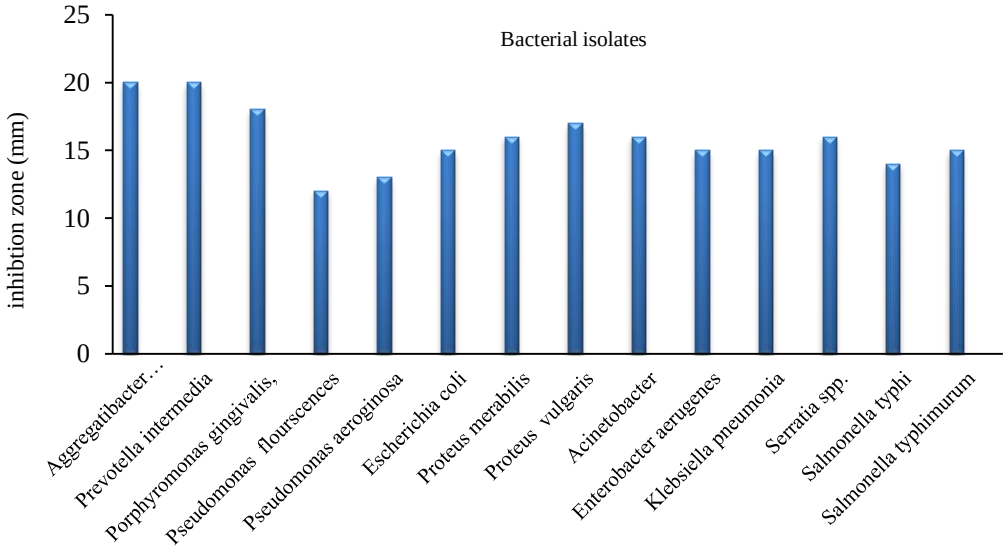

Fig. 3 Antibacterial Activity of Imipenem Against Gram-Negative Bacteria Using the Disc Diffusion Method

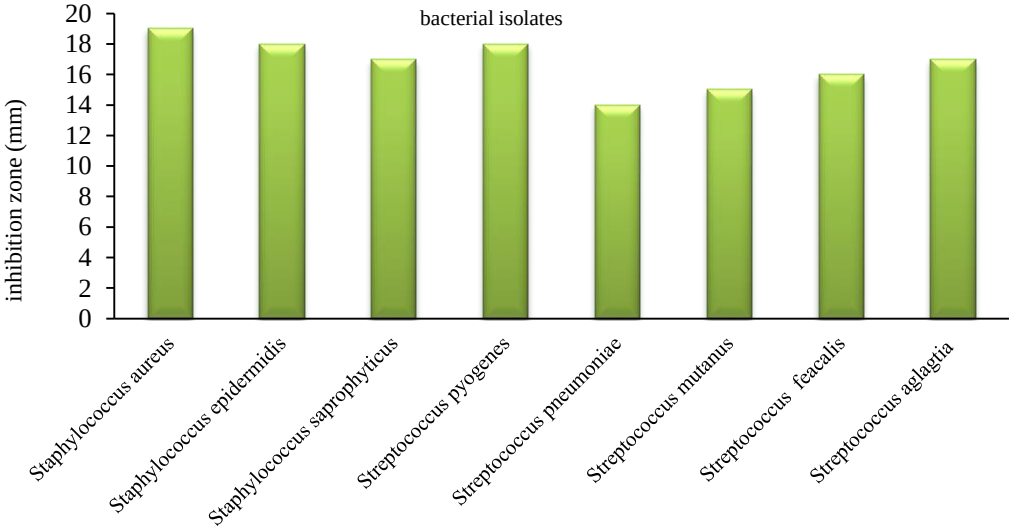

Fig. 4 Antibacterial Activity of Imipenem Against Gram-Positive Bacteria Using the Disc Diffusion Method

Anti-Biofilm and Adherence Effects of *M. officinalis* Extract

The anti-biofilm and anti-adherence properties of *M. officinalis* were evaluated, revealing that most isolated Gram-negative bacteria displayed moderate adherence and biofilm activity in response to the extracts. Conversely, certain isolated bacteria exhibited high adherence and biofilm formation (Figure 5). The ability to categorize biofilms into mild (OD630 <0.120), moderate (OD630 0.120–0.240), and strong (OD630 >0.240) formations allows for a clearer understanding of the extract's efficacy in inhibiting biofilm development.

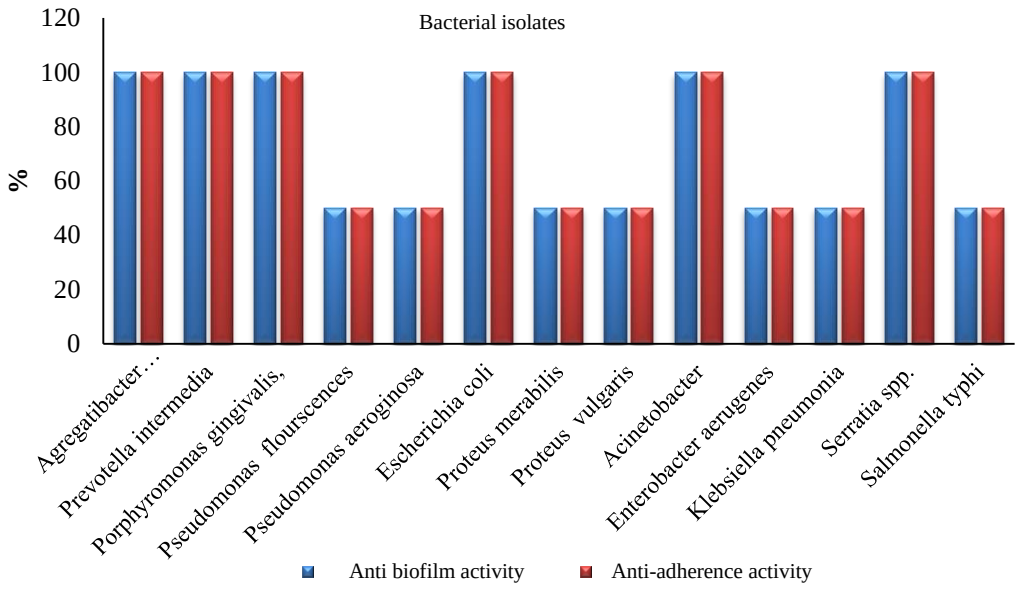

Fig. 5 Anti-Biofilm and Anti-Adherence Properties of *M. officinalis* Aqueous Extracts Against Gram-Negative

Antibacterial Properties of *M. officinalis* Extract

The findings align with previous research indicating that natural compounds possess significant antibacterial properties. Studies have shown that chlorogenic acid and other phytochemicals present in *M. officinalis* contribute to its antibacterial effects by disrupting bacterial cell membranes and inhibiting growth [20]. Furthermore, these compounds may work synergistically to enhance the overall antimicrobial activity of the extract.

The current study's results support the notion that *M. officinalis* extracts can effectively limit the growth of various clinical isolates. Herbal remedies have been utilized for centuries in traditional medicine, and their relevance continues to grow as more individuals seek natural alternatives for healthcare [21]. Over eighty percent of the global population relies on herbal medicines, underscoring their importance in primary healthcare systems despite the prevalence of synthetic pharmaceuticals [22].

Phytochemicals found in *M. officinalis* exhibit pharmacological benefits such as immunomodulation, antioxidant effects, and antibacterial properties [23, 24]. The extract's ability to inhibit biofilm formation is particularly noteworthy, as biofilms play a crucial role in chronic infections and antibiotic resistance. The lipophilic nature of these compounds allows them to interact with cellular receptors and disrupt bacterial function [25].

The formation of biofilms is a critical factor contributing to persistent infections, as they provide a protective environment for bacteria against external threats such as antibiotics [26]. This study reinforces the need for further research into antimicrobial-resistant phenotypes and biofilm-forming factors, which are essential for understanding health outcomes in infections [27].

In conclusion, *M. officinalis* extracts demonstrate considerable antimicrobial activity against a broad range of bacterial isolates, suggesting their potential as effective alternatives to commercially available antibiotics. Future studies should focus on elucidating the specific mechanisms through which these extracts exert their antimicrobial effects and exploring their applicability in clinical settings [28].

CONCLUSION

The findings of this study demonstrate that extracts of *M. officinalis* exhibit significant antimicrobial activity against a wide range of pathogenic bacteria, including both Gram-positive and Gram-negative strains. The aqueous extract, prepared at a 25% concentration, produced substantial inhibition zones, indicating its potential as an effective natural alternative to conventional antibiotics. Notably, the extract was particularly effective in preventing biofilm formation and bacterial adherence, which are critical factors in the persistence of infections and antibiotic resistance. Given the increasing prevalence of antibiotic-resistant bacteria, the use of plant-derived compounds such as those from *M. officinalis* offers a promising avenue for developing new therapeutic strategies. The phytochemicals present in the extract, including flavonoids and polyphenolic compounds, contribute to its antibacterial properties and may work synergistically to enhance its efficacy. These results support the traditional use of *M. officinalis* in herbal medicine and underscore the importance of further research into its mechanisms of action. Future studies should focus on isolating specific active compounds within the extract and exploring their potential applications in clinical settings. Overall, *M. officinalis* represents a valuable resource for addressing bacterial infections, particularly in light of growing concerns regarding antibiotic resistance.

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Authors' contributions

All authors contributed equally in writing and approving this manuscript.

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