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Phytochemical Screening and Antibacterial Activity of Libyan *Mentha Piperita* L. and *Mentha Spicata* L. Leaves

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ABSTRACT

Medicinal plants are widely used worldwide and play an important role in their bioactive compounds. The Lamiaceae family contains many genera of medicinal plants that are used in traditional medicine. Among them are *Mentha piperita L*. (peppermint) and *Mentha spicata L*. (spearmint). This study investigates the phytochemical composition and antibacterial activity of different solvent extracts of Libyan *M. piperita* and *M. spicata* leaves. Petroleum ether, ethyl acetate, and methanol were used to extract bioactive components from selected Mentha species. Phytochemical screening was performed by standard methods. Antibacterial efficacy was evaluated using the agar well diffusion method against four bacterial strains, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using a macrodilution method. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolic compounds, and essential oils in both Mentha species. The antibacterial assay illustrated varying inhibition levels among bacterial strains, with the petroleum ether extract of *M. piperita* exhibiting the strongest effect against *E. coli*, while *M. spicata* showed moderate inhibition against *E. coli* and *K. pneumoniae*. The methanol extract of *M. piperita* shows a moderate antibacterial effect on *E. coli*, while a low effect for the same extract of *M. spicata*. Regarding *S. aureus* bacteria, *M. spicata* methanol extract and *M. piperita* ethyl acetate extract had moderate effects. MIC and MBC values indicated that *M. spicata* petroleum ether extract had the strongest effect against *K. pneumoniae* (MIC = 50 mg/ml), while *M. piperita* ethyl acetate extract had a significant impact on *S. aureus (*MIC = 50 mg/ml). The study concludes the medicinal significance of Libyan *M. piperita* and *M. spicata* in bacterial infection treatment. These findings support their potential as natural antibacterial agents

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INTRODUCTION

Medicinal plants play an important role in healthcare systems, serving as valuable sources of compounds for drug development. It has been estimated that approximately 40,000 plants have been used in traditional medicine practices around the world. Additionally, many medicinal plants contain a wide variety of secondary metabolites such as alkaloids, flavonoids, and tannins, which can be used for therapeutic properties and drug synthesis (1). Herbal medicines are shown to be the main therapy in the traditional medicine system. Two-thirds of the world's population relies on herbal medicine as their primary form of healthcare. This is because of their reduced likelihood of side effects and greater cultural acceptance (2). In Libya, there are 2,103 plant species spread across 856 genera and 155 families. Among these are 450 medicinal plants, with 208 extensively utilized as traditional medicine. Nearly 30% of Libya's population depends on traditional medicinal practices (3).

The Lamiaceae (Labiatae) family is the most common family, which contains around 7000 species and 245 genera, and covers a wide range of aromatic and medicinal plant species with strong pharmaceutical applications in folk medicine, such as cosmetics and perfume, due to aromatic volatile oils that are involved in the plant's aerial parts, particularly the leaves. The Mentha genus can be classified into 42 species. *Mentha piperita* L., and *Mentha spicata* L., are edible aromatic herbaceous plants in Libya, belonging to this family (4).

Taxonomical classification of the Mentha genus

Kingdom: Plantae;
Subkingdom: Tracheobionta, Vascular plants;
Superdivision: Spermatophyta, Seed plants;

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Division: Magnoliophyta, Flowering plants;
Class: Magnoliopsida, Dicotyledons;
Subclass: Asteridae;
Order: Lamiales;
Family: Lamiaceae, Labiatae; Mint family
Genus: Mentha L. – Mint (5).

Mentha piperita L. (peppermint) is a perennial species, cultivated globally; the height of this plant is nearly 30–90 cm. The leaves have an oval shape and their size is about 1.5-3.5 inches long and 2.5 inches wide. The stems are square and smooth, and they are dark green with reddish veins. This plant species is utilized as a traditional folk remedy and has notable medicinal values. It is employed in complementary and alternative herbal therapies for the treatment of many disorders, as well as in the development of fragrances and cosmetics (6,7). *M. piperita* leaves contain mainly phenolic acids, flavonoids, and some other components such as menthol, menthone, limonene, cardiac glycosides, caffeic acid, acetaldehyde, tannins, and tocopherols. The essential oil of *Mentha piperita* contains limonene, citronellol, menthyl acetate, menthol, menthone, and isomenthone (8).

Mentha spicata L. (spearmint) represents a cultivated perennial herb, it has been regarded as one of the most significant crops yielding essential oils. Spearmint is an aromatic botanical that can be used in a fresh state or as dried leaves or powder, functioning as a flavouring herb or as an herbal infusion (9). Spearmint essential oils are mainly composed of carvone (22%–73%) and limonene (8%–31%), with small amounts of 1,8-cineole (4%–7%), menthone (1%–5%), menthol, eucalyptol, and other minor compounds (9).

This study aimed to carry out phytochemical screening and evaluate the antibacterial activity of different solvent extracts of Libyan peppermint (*Mentha piperita* L) and Spearmint (*Mentha spicata* L) leaves.

MATERIALS AND METHODS

Preparation of leaf extracts (by maceration method)

Healthy plants were collected, the leaves were separated from the plant, gently rinsed with tap water, and subsequently with distilled water to remove dirt before being dried at room temperature. They were then dried in a shaded place for 15 days. Using a grinding machine, the leaves of both mints were transformed into a fine powder (6). The powdered leaves (70g) were soaked in petroleum ether, ethyl acetate, and methanol (350 ml for each), and placed in a shaker for 3 days. The filtered extracts were concentrated in a rotary evaporator at a temperature of 50 – 55 C^0 to obtain semisolid material. Then they were kept for several days to get dry, after that the extracts were stored in small, tightly closed containers in a dark place for phytochemical screening and antibacterial evaluation (10).

The phytochemical screening of the Mentha extracts was conducted using standard procedures.

Plant extracts preparation for antibacterial assay: Extracts were prepared by weighing 200mg of each extract and dissolving them in 1 ml of DMSO (dimethyl sulfoxide), Mixed using a Vortex.

Bacteria species used

Four types of bacteria stored at -80°C at the Libyan Biotechnology Research Centre laboratory were used .The bacteria used were isolated from patient samples in hospitals (clinical samples); the species of bacteria used were *E. coli, K. pneumoniae, P.aeruginosa* (gram-negative*),* and *S.aureus* (gram-positive).

Bacterial Preparation

The bacteria were reactivated by culturing them on the following media: Nutrient agar and MacConkey agar. A suspension of growing bacteria was made in 5 mL of the Nutrient broth. The turbidity was adjusted to match the 0.5 McFarland scale.

Agar Well Diffusion Assay

Two types of antibiotics (Ampicillin 10µg, and cefotaxime 30 µg) were used; amikacin 30 µg was used with *K. pneumoniae* only instead of Ampicillin (because it has intrinsic resistance to Ampicillin) to compare the effect of the extract. Some wells were filled with solvents and DMSO as a control.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was measured by the macrodilution method in tubes. The two Mentha extracts that were found active in primary screening were similarly diluted to obtain the following concentrations, 200,100, 50,25,12.5, 6.25, 3.125, 1.56, 0.8, 0.4 mg/ml (11).

RESULTS

Methanolic extracts have the highest percentage yield when compared to other extracts, where *M. piperita (*7.41%) and *M. spicata (*4.75%).

Preliminary phytochemical screening

Preliminary phytochemical screening shows the presence of many different secondary metabolites in different *M. piperita* and *M. spicata* extracts. The results are shown in Table 1.

Antibacterial activity results

Mentha extracts were studied for their antimicrobial activities. The results were classified into levels represented by plus and minus signs, using the agar well diffusion method, and the zones of inhibition were measured by using the well diffusion method as demonstrated in Table 2. The petroleum ether extract of *M. spicata* leaves has moderate (++) antibacterial activity against *K. pneumonia* and *E. coli*, while the same extract of *M. piperita* reveals strong (+++) antibacterial activity against *E.coli* and weak antibacterial activity against *S. aureus*. On the other hand, the methanol extract possesses moderate antibacterial activity against *E. coli* for *M. Piperita* and *M. spicata,* showing the same effect against *S. aureus.* Ethyl acetate *of M. Piperita*

exhibited *a* moderate effect against *S. aureus*. In the case of activity against *P. aeruginosa*, ethyl acetate and methanol of both *M. Spicata* and *M. Piperita* had weak (+) antibacterial activity; also, there was no effect of different solvents alone on bacterial growth. MIC of the most biologically active plant extracts was measured as shown in Table 3. The MIC for *M. spicata* petroleum ether extracts was found to be 200mg/ml, and MBC more than 200mg for *E. coli*, while the MIC for *K. pneumoniae* was equal to 50 mg/ml, and MBC = 100mg/ml. whereas, the MIC of *M. piperita* petroleum ether extracts was 100 mg/ml when applied to *E. coli*. On the other hand, the MIC of *M. piperita* ethyl acetate extract was found to be 50mg/ml when applied to *S. aureus***.**

Table 1. Phytochemical screening of various solvent extracts of M. piperita and M. spicata

Test	Petroleum ether		Ethyl acetate		Methanol	
	M. spicata	***M. piperita***	***M. spicata***	***M. piperita***	***M. spicata***	***M. piperita***
Dragendorff's test	+	+	+	+	+	+
Mayer`s test	+	+	+	+	+	+
Wagener`s test	+	+	+	+	+	+
Alkaline reagent test	+	+	+	+	+	+
Benedict's test	+	+	+	+	+	+
Fehling`s test	+	+	+	+	+	_
Ferric chloride test	+	+	+	+	+	+
Gelatin test	+	+	+	_	_	+
Salkowski`s test	_	_	_	_	_	_
Spot test/Stain test	+	+	+	+	+	+
NaOH test	+	+	+	_	_	_
Frothing test	_	+	_	_	_	+
Volatile oil	+	+	+	+	+	+

Table 2. Antibacterial activity of *M. spicata* and *M. piperita* extracts

Types of bacteria	***M.spicata* extracts**			***M.piperita* extracts**			**Antibiotic discs**		
	Petroleum ether	**Ethyl acetate**	**Methanol**	**Petroleum ether**	**Ethyl acetate**	**Methanol**	AMP	CTX	AK
K.Pneumonia	++ve	-ve	-ve	-ve	-ve	+ve	IR	+ve	+ve
E. coli	++ve	+ve	+ve	+++ve	+ve	++ve	R	+++ve	ND
P.aeruginosa	-ve	+ve	+ve	-ve	+ve	+ve	R	R	ND
S.aureus	-ve	-ve	++ve	+ve	++ve	+ve	+ve	+ve	ND

Table 3. MIC and MBC of the most potent extracts of Mentha species (mg/ml)

Type of extract	*E. coli*	*K. pneumoniae*	*S. aureus*	*P.aeruginosa*
M.spicata petroleum ether extract	MIC = 200mg/ml MBC >200mg/ml	MIC=50mg/ml MBC=100mg/ml	ND	ND
(*M.piperita*) petroleum ether	MIC =100mg/ml MBC >200mg/ml	ND	ND	ND
(*M.piperita*) Ethyl acetate extract	ND	ND	MIC=50mg/ml MBC=100mg/ml	ND

DISCUSSION

Medicinal plants and their components have been used in various traditional medicine systems for centuries to treat many ailments, and several therapeutic agents have been successfully isolated or extracted from natural sources (6).

Mentha piperita L. and *Mentha spicata* L. are two common medicinal plants in Libya that have important uses in folk and traditional medicine. *Mentha piperita* L. is a plant that grows in all climates and exists worldwide. The *M. piperita* is an aromatic herb. It is known to have antifungal, antioxidant, and other therapeutic activities (12).

Mentha spicata L. is an aromatic plant that is commonly used in traditional medicines as an antimicrobial agent, for gastrointestinal and respiratory problems, and as a preservative in food, mainly on account of the terpenoid and phenolic content (9).

Phytochemical screening revealed the presence of a variety of bioactive secondary compounds, such as alkaloids, flavonoids, phenolic compounds, and essential oils. These components are known for their antimicrobial properties and may be responsible for the antibacterial effects observed in this study. The selection of solvents plays a critical part in extracting and identifying bioactive compounds from plant materials. The highest

percentage yield was obtained using methanol extraction, which suggests its effectiveness in isolating secondary metabolites. Similar phytochemical analysis results were reported on various extracts of the selected plant by other authors (6).

The antibacterial activity of different extracts of *M. piperita and M. spicata* was determined against bacterial strains by using the agar well diffusion assay. The minimum inhibitory concentration (MIC) was measured by the macrodilution method in tubes. our results show that both *M. piperita* and *M. spicata* exhibited inhibitory effects against gram-positive and gram-negative bacteria. *M. piperita* petroleum ether extract revealed strong antibacterial activity against *E. coli*, while *M. spicata* showed moderate inhibition against *E. coli* and *K.pneumoniae*.

Compared with the selected antibiotic, the methanol extract of *M. piperita* shows a moderate antibacterial effect on *E. coli,* while a low effect for the same extract of *M. spicata.* Regarding *S. aureus* bacteria, *M. spicata* methanol extract and *M. piperita* ethyl acetate extract had moderate effects.

A comparison with other literature revealed some differences, while in previous studies done in 2023, *M. piperita* methanolic extracts displayed moderate antibacterial effects against *S. aureus* but no effects on *E.coli* (12). The microbial activity of *M. piperita* fractioned extracts was tested with the agar gel method in Oman in 2022. The different polarity extracts at different concentrations showed no activity against the tested *S. aureus*, *E.coli*, and *P. aeruginosa* bacterial strains (6). Another study in 2007 revealed that petroleum ether and ethyl acetate leaf extracts of *M. piperita* were more effective on *P. aerogenosa* than *S. aureus* (13). Concerning *Mentha spicata,* the study that was done in 2022 concluded that methanol extracts possessed good activity against *S. aureus* and *P. aeroginosa* (14).

Differences in antibacterial activity compared to previous studies may be attributed to many factors such as plant genotype, environmental conditions, extraction methods, and variations in the resistance genes of different bacterial strains.

Additionally, the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) further validated the antibacterial potency of these plant extracts. *M. spicata* petroleum ether extract displayed the strongest effect against *K.pneumonia*, with a MIC of 50 mg/ml, while *M. piperita* ethyl acetate extract showed the strongest effect against *S. aureus* with a MIC of 50 mg/ml. The results suggest that Mentha species could act as potential sources for developing antibacterial agents.

CONCLUSION

The Lamiaceae family contains many genera of medicinal plants that are used in traditional medicine for food preparation and the treatment of human diseases due to their several pharmacological properties. *Mentha spicata* and *M. piperita* are some of the medicinal plants that belong to this family, characterized by their ability to synthesize secondary metabolites, such as essential oils, alkaloids, phenolic compounds, and flavonoids .Many people use the aerial parts of these herbal plants for tea preparation. This study highlights the significant antibacterial activity of Libyan *M. piperita* and *M. spicata*, supporting their therapeutic potential. The phytochemical screening revealed the presence of bioactive components that may be responsible for their antimicrobial activity. Antibacterial activity results confirmed the efficacy of both plant extracts against many bacterial strains, including *E. coli* and *S. aureus*, *P. aeruginosa,* and *K. pneumoniae,* with different potencies. Overall, this study strengthens the traditional use of Libyan peppermint and spearmint in treating bacterial infections and suggests further research to use these Mentha species for future drug development.

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