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Phytochemical and antibacterial screening of different solvent extracts of *Mentha spicata*

V. R. Rakhi, T. S. Dhanaraj, V. Ramamurthy*

P.G & Research Department of Biochemistry, Maruthupandiyar College, Thanjavur, Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

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ABSTRACT

The aim of the present study was to investigate antibacterial activity of the leaf of *Mentha spicata* plant against common pathogenic bacteria. The agar diffusion method was used to examine the antibacterial activity of different solvent extracts of *Mentha spicata* tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. For qualitative phytochemical investigation, several solvent extracts of the complete *M. spicata* plant were investigated. The minimum inhibitory concentration (MIC) of *M. spicata* ethanol extract had significant antimicrobial effectiveness against *Escherichia coli* (489 µg/ml), and *Pseudomonas aeruginosa* (449 µg/ml). The ethanolic extract showed (437 µg/ml and 410 µg/ml) antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* respectively. The lowest antibacterial activity was found in acetone extract with MIC (216µg/ml) of *M. spicata*. Various parts of the plant have the presence of variety of phytoconstituents such as flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin.

***Corresponding author:** V. Ramamurthy ✉ v.ramamoorthy07@gmail.com

INTRODUCTION

Due to increasing consumers concerns regarding processed and ready-to-eat foods containing antibiotics, pesticides, hormones, and synthetic additives and also increasing demand to replace artificial antimicrobial agents with natural alternatives, the usage of natural and organic foods has been experiencing explosive market growth (Lv *et al.*, 2011; Moosavy and Shavisi, 2013; Sepahvand *et al.*, 2014). However, the untreated products and natural foods may be more susceptible to growth of food-borne pathogens than the conventional food version (Jay *et al.*, 2005). The most important food-borne pathogenic bacteria that have survived and grow in these products include *Staphylococcus aureus*, *Bacillus* spp., *Listeria monocytogenes*, *Salmonella* spp. *Escherichia coli*, *Yersinia* spp., and *Clostridium* spp. (Oroojalian *et al.*, 2010; Friedman *et al.*, 2002). These bacteria cause a great proportion of food-borne outbreaks in different foods such as dairy products, vegetables, and meat and fish products (Warriner and Namvar, 2009). In this context, plant essential oils are attracting interest as natural food preservatives in order to ensure the safety of food (Burt, 2004).

Mentha spicata L., commonly called spearmint, belongs to the Lamiaceae family, genus *Mentha*, which comprises about 25–30 species originating in Europe. It is one of Brazil's most cultivated varieties of spearmint and is well adapted to the subtropical climate.

The interest in cultivating Mentha is mainly related to the commercial importance of its essential oil, which is among the 10 most traded in the world. The oil is used in many industries, including pharmaceuticals, cosmetics, food, and chemicals. Spearmint is also known for its ability to improve memory. Besides being a stimulant, it has several biological uses, such as in insecticides, antimicrobials, antioxidants, antispasmodics, and anti-platelets (Scherer *et al.*, 2013).

M. spicata L. (spearmint) is an herbaceous rhizomatous perennial plant growing 30–100 cm tall, with variably hairless to hairy stems and foliage, and a wide-spreading fleshy underground rhizome. The leaves are 5–9 cm long and 1.5–3 cm broad, with a serrated margin. The stem is square-shaped, a trademark of the mint family of herbs. Spearmint produces flowers in slender spikes, each flower pink or white and 2.5–3 mm long and broad.

The leaves are popularly used as a tea flavoring agent and the whole plant is used as a carminative. The fresh and dried plants and their essential oils are widely used in the food, cosmetic, confectionary, chewing gum, toothpaste, and pharmaceutical industries (Bensabah *et al.*, 2013). This species is also often used in Indian and Italian cuisine and usually added fresh or dried to fish and shellfish plates before or after cooking. *M. spicata* possesses several biological activities and is used in folkloric medicine as a carminative, antispasmodic, diuretic, antibacterial, antifungal, and antioxidant agent, and for treatment of colds and flu, respiratory tract problems, gastralgia, hemorrhoids, and stomachache (Tawaha *et al.*, 2007; Kizil *et al.*, 2010; Tetika *et al.*, 2013).

Numerous medications have caused microbes to become resistant, which presents a substantial therapeutic issue in the management of infectious diseases. The overuse of commercially available antimicrobials, which are routinely used to treat illnesses, led to the development of the bacteria' tolerance (Lewis and Ausubel, 2006). In order to find new antimicrobial chemicals, researchers were motivated to investigate in other sources, particularly herbal resources.

MATERIALS AND METHODS

Collection and identification of plant material

For the study, the whole plant of *Mentha spicata* belongs to Lamiaceae family was collected from Kerala, South India. The whole plant were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Preparation of plant materials and extract

The leaves were carefully cleaned, shade dried and powdered. The powdered material was stored in a closed air-tight plastic container at low temperature. The powdered plant material (50 g) was extracted with 300 mL of each solvent ethanol by maceration (3×24 h) at room temperature. The collected solvents were concentrated by rotary vacuum evaporator at 45°C and then dried using a freeze dryer. All extracts and acyclovir (extracted from commercial tablet) were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was 0.1% v/v in cell culture environment.

Phytochemical analysis

The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described (Trease and Evans, 1983; Harborne, 1973). Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Test microorganisms

Escherichia coli, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* were used as test organisms in the current study. For the current experiment, the obtained cultures were repeatedly subcultured.

Antimicrobial activity by agar diffusion method

The antibacterial efficacy of several *Mentha spicata* solvent extracts was assessed using the agar diffusion method. For spreading agar media, a subcultured microbial suspension (100 µl) was prepared. Various concentrated varied extracts were used to measure antimicrobial activity (Magaldi *et al.*, 2004). The plates were filled with the sample and then left to allow for an hour to enable the extract to disperse. The plates were maintained in an incubator for 24 hours at 37°C, and the inhibitory zone was measured in millimeters (mm). Results are compared with those of conventional antibacterial drugs.

RESULTS AND DISCUSSION

Phytochemical studies of *Mentha spicata* showed that it contains a number of phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides and pectin. The main bioactive constituents found are bioflavonoid, amentoflavone with minute amount of cupressoflavone (Abinash *et al.*, 2012). All of the extracts from the *Mentha spicata* contained saponin, phenols, tannins, glycosides, terpenoids, flavonoids, alkaloids, and coumarins, according to a preliminary phytochemical examination. With the exception of the chloroform extract's lack of saponins, glycosides, and coumarins and the extract from ethyl acetate's absence of saponin. The results of the phytochemical analysis are displayed in Table 1. A higher degree of biological activity derives from the presence of a high concentration of phytochemicals in the plant.

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized (Sofowora, 1993). In the present study, it was clearly understood that the ethanolic extracted maximum amount of the different type of metabolites present in the *Mentha spicata*. Boominathan and Ramamurthy (Boominathan and Ramamurthy, 2009) reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti-cancer activity (Kunle and Egharevba, 2009). It also suggests that the plant might have diuretic properties (Jayvir *et al.*, 2002). The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns (Haslem, 1989). Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions (Oloyode, 2005). Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties (Evans, 2002). Volatile oils are used in the industries for various purposes, both as a pharmaceutical/ cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections.

Table 1. Qualitative phytochemical screening on extracts of *Mentha spicata*

Name of test	Test applied / Reagent used	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate
Alkaloids	A] Mayer's	+++	++	++	++	+++	++
	B] Wagner's	+++	++	++	++	+++	++
	C] Hagner's	+++	++	++	+++	+++	++
	D] Dragndorff's test	++	++	++	++	++	+
Flavonoids	HCl and magnesium turnings	+++	++	+	++	+	++
Carbohydrate	Molisch's test	+	+	+	+	+	+
Tannins & phenols	A] 10% Lead acetate	+++	+	++	++	++	++
	B] $Fecl_3$	+++	+	++	++	++	++
Test for steroids	A] Salkowski's test	++	++	++	++	++	++
	B] Libermann-Burchard's test	++	++	++	++	++	++
Gums & mucilages	Alcoholic precipitation	-	-	-	-	-	-
Fixed oil & fats	Spot test	+	-	+	+	-	-
Saponins	Foam test	+	+	+	+	+	+
Phytosterols	LB test	+	+	+	+	+	+
Volatile oils	Hydro distillation method	+	+	+	+	+	+
Protein & free amino acids.	A] Biuret test	++	++	++	++	++	++
	B] Ninhydrin test	+++	++	++	++	++	++
	C] Xanthoprotein test	+++	++	++	++	++	++

Table 2. Antimicrobial activity of the plant extracts *Mentha spicata*

Microorganism	Minimum inhibitory concentration (MIC)							
	Plant extract of *Mentha spicata* (µg/ml)							
	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate	Gentamicin (µg/ml)	Cephalosporins (µg/ml)
P. aeruginosa	489	386	287	263	227	312	ND	49
B. subtilis	437	385	294	242	209	274	ND	47
E. coli	446	392	323	257	230	302	ND	55
S. aureus	410	336	258	224	216	255	35	ND

The antibacterial properties of various solvent-based extracts of *Mentha spicata* are displayed in Table 2. The ethanol extract of *Mentha spicata* had the highest antimicrobial activity with MIC (489 µg/ml) against the *Escherichia coli*, (449 µg/ml) against the *Pseudomonas aeruginosa*, (437 µg/ml) against the *Bacillus subtilis* and (410 µg/ml) against the *Staphylococcus aureus*. The various extracts of *Mentha spicata* tested against *Escherichia coli* and showed considerable MIC results in water extract (392 µg/ml), chloroform extract (323 µg/ml), ethyl acetate extract (312 µg/ml), acetone extract (230 µg/ml), hexane extract (257 µg/ml). The results were compared with standard Cephalosporins as reference compounds with MIC (55 µg/ml). The different extracts of *Mentha spicata* were checked against the *Pseudomonas aeruginosa* and exhibited significant MIC values in water extract (386 µg/ml), chloroform extract (287 µg/ml), ethyl acetate extract (302 µg/ml), acetone extract (227 µg/ml), hexane extract (263 µg/ml). The obtained results were compared with Cephalosporins with MIC (49 µg/ml).

The individual extract of *Mentha spicata* was checked against *Bacillus subtilis* and found impressive MIC values in water extract (385 µg/ml), chloroform extract (294 μg/ml), ethyl acetate extract (274 μg/ml), acetone extract (209 μg/ml), hexane extract (242 μg/ml). The different solvent extract of *Mentha spicata* was evaluated against *Staphylococcus aureus* and found impressive MIC values in water extract (336 μg/ml), chloroform extract (258 μg/ml), ethyl acetate extract (255 μg/ml), acetone extract (216 μg/ml), hexane extract (224 μg/ml).

The gentamicin (35 μg/ml) was used as a standard compound. A considerable inhibitory zone may also be caused by the variety of phytochemicals present in the extract. The presence of different flavonoids, alkaloids, terpenoids, phenols, saponins, and coumarins has bactericidal properties (Kalidindi *et al.*, 2015). According to various scientific studies, high concentrations of phytochemicals and bioactive compounds are thought to have a stronger potential for treating a variety of pathogenic bacteria. Numerous plants and their various portions of them have historically been used to treat a variety of chronic illnesses, such as gastrointestinal problems, urinary tract infections, skin conditions, and various respiratory issues., etc. (Alzoreky and Nakahara, 2003). Several chronic illnesses caused by various bacteria may be prevented and managed with the use of plant-based remedies. Many societies still employ ethnomedicines to treat illnesses and overcome obstacles without creating negative side effects. The inclusion of several phytoconstituents, including alkaloids, flavonoids, coumarins, saponins, polyphenols, tannins, and terpenoids, is what gives herbal preparations their therapeutic effects (Bhalodia and Shukla, 2011). The presence of secondary metabolites prevents the growth of harmful microorganisms causing serious diseases (George *et al.*, 2016). The microorganisms are resistant to many antibiotics that is very harmful to humans. The researchers are finding an alternative to commercial antibiotics to prevent harmful infections against a variety of microorganisms using plant-based medicines (Khan *et al.*, 2013). The higher concentration of crude extracts sometimes may cause cytotoxicity in humans hence the dose-dependent values are determined using in vitro cell cytotoxicity assay (George *et al.*, 2016). As compared to commercial antibiotics, plant-based medicines have very small side effects if they are consumed in excess quantity (Talib and Mahasneh, 2010). In the world, 80 % of different pharmaceuticals are prepared from plant-based medicines and which are effective to cure any chronic disease.

CONCLUSION

Mentha spicata used for its wide therapeutic potential of antimicrobial agents. According to the studies, ethanol extract has the most potential, which may be because it includes the majority of the phytochemical compounds and bioactive compounds that have antibacterial activity. The complete plant extract of *Mentha spicata* has to be further studied in order to identify and purify chemicals that might be used as natural medicinal alternatives to synthetic commercial ones. The future aspects of the plant can be anti-microbial as it contains many of the phytochemicals and work has not been performed yet.

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