British Microbiology Research Journal
13(6): 1-5, 2016, Article no.BMRJ.24031
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Phytochemical Analysis and Antibacterial Efficacy of *Mentha piperita* (L) Ethanolic Leaf Extract against Clinical Isolates of Uropathogens

Sowjanya Pulipati[1*], O. Sai Koushik[1] and P. Srinivasa Babu[1]

[1]Vignan Pharmacy College, Vadlamudi- 522213, Guntur, Andhra Pradesh, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed the literature searches. Authors OSK and PSB managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/24031
Editor(s):
(1) Giuseppe Blaiotta, Department of Food Science, Via Università, Italy.
Reviewers:
(1) R. Jasmine, Bishop Heber College, Tamil Nadu, India.
(2) Amit Raj Sharma, Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal.
(3) Dorota Wojnicz, Wroclaw Medical University, Poland.
Complete Peer review History: http://sciencedomain.org/review-history/13811

Original Research Article

Received 31st December 2015
Accepted 1st March 2016
Published 22nd March 2016

ABSTRACT

Aim: The present study was designed to evaluate the antibacterial activity of *Mentha piperita* (L) leaf extract against clinical isolates of urinary tract infections.
Introduction: *M. piperita* L. (Peppermint) is a strongly scented herb belonging to family Lamiaceae. The plant is stimulant, aromatic and used for headache, vomiting and allaying nausea. In India the leaves are used to relieve sore throat. The most common form of bacterial infections is urinary tract infections (UTIs). They affect people of all age groups throughout their lifespan.
Methodology: The *M. piperita* ethanolic extract (MPEE) was prepared by cold maceration. The presence of phytoconstituents was determined using standard protocols. Clinical isolates of UTI pathogens such as *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated from urine samples and identified by biochemical tests. The antibacterial property was determined by agar well diffusion method.
Results and Discussion: The preliminary phytochemical screening revealed the presence of amino

**Corresponding author: E-mail: sowjypulipati@gmail.com;*

acids, carbohydrates, alkaloids, proteins, glycosides, steroids, tannins and flavonoids. MPEE exhibited pronounced antibacterial activity against tested microorganisms. The extract exhibited antibacterial activity at 1000 µg concentration against *S. aureus* (21.50±1.22 mm), *E. coli* (19.33±0.81 mm), *P. aeruginosa* (15.33±1.69 mm) from high to low respectively. The minimum inhibitory concentration was ranged from 62.5 to 125 mg/ml.

Conclusion: The results of antibacterial studies confirm that MPEE was found to contain bioactive constituents that exhibited notable antibacterial activity. However, further isolation and characterization of phytoconstituents will be needed to evaluate the antimicrobial activities against a wider range of microbial pathogens.

Keywords: Mentha piperita L.; UTI pathogens; antibacterial activity.

1. INTRODUCTION

M. piperita L belongs to the family Lamiaceae. *M. piperita* is a non-native herbaceous plant and perennial plant that is found as cultivated and wild [1-3]. It has been reported that *Mentha piperita* is used internally as an herbal tea and externally tincture, oil or extracts, and applied as liniment. Botanists consider it as an antispasmodic, anticatarrhal, astringent, antiseptic, antipyretic, rubefacient, stimulant, emmenagogue and anti-aging properties [4-6]. The *M. piperita* possess essential oils such as menthol, menthone and their derivatives as active constituents. Less well recognized is peppermint's potential role in the management of numerous medical problems, including colonoscopy [7-9]. Peppermint oil or peppermint tea is often used to treat gas and indigestion; it may also increase the flow of bile from the gall bladder. Peppermint oil relaxing action also extended to topical use, it acts as counterirritant and analgesic with the ability to reduce pain and improve blood flow to the affected area when applied topically. Menthol and Peppermint oil have moderate antibacterial effects against both gram-positive and gram negative bacteria. Peppermint is also found to have fungicidal and antiviral activity [10-11]. Menthol is virucidal against influenza, herpes and other viruses. However, no serious efforts have been made to test the antibacterial properties of *M. piperita* against clinical isolates of uropathogens. Therefore the present study is designed to evaluate the antibacterial efficacy of *M. piperita* (L) leaf extract against clinical isolates of uropathogens.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant *M. piperita* L was collected from local market in Guntur, Andhra Pradesh, India. The plant specimen was identified and authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. The collected leaves were shade dried and pulverized to coarse powder.

2.2 Extraction

The *M. piperita* ethanol extract (MPEE) was prepared by cold maceration. The 100 g of leaf powder was macerated with 1000 ml of ethanol (Research lab Fine Chemical Industries) at 37℃ shaking occasionally for 48 h. The extract was filtered through 5 layers of muslin cloth and concentrated at low temperature. The extract was preserved in a desiccator for further study.

2.3 Test Bacteria

Two Gram positive bacteria: *Staphylococcus aureus, Enterococcus faecalis* and three Gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* used were isolated from subjects suffering with UTIs.

2.4 Phytochemical Screening

The qualitative screening of phytoconstituents for the MPEE was carried out by standard procedures [12,13]. Alkaloids (Mayer's test), glycosides (Legal's test), saponins (froth formation test), carbohydrates (Molisch's test), proteins (Xanthoproteic test), amino acids (Ninhydrin test), flavonoids (Lead acetate test), steroids and terpenoides (Salkowski test), tannins (Ferric chloride test) were analyzed.

2.5 Isolation and Identification of UTI Bacteria

The microorganisms present in urine samples of UTI infected patients were differentiated using the gram staining procedure into gram positive and gram negative organisms. The organisms

were transferred to cystine lactose electrolyte deficient (CLED) agar medium (Himedia, Mumbai) for further differentiation. The biochemical tests catalase activity, indole production test, citrate utilization test, urease test, methyl red and voges proskauer's test were performed to confirm the organisms.

2.6 Antibacterial Activity

The antibacterial efficacy of MPEE was determined by agar well diffusion method [14]. The Muller Hinton agar (Himedia, Mumbai) plates were inoculated with the clinical isolates of UTI pathogens. The inoculated plates were punched with sterile borer to get 6 mm diameter wells. A stock solution of 2.5, 5, 7.5 and 10 mg/ml were prepared in dimethyl sulfoxide (DMSO) (Thermo Fischer Scientific Pvt. Ltd, Mumbai). The wells were filled with aliquots of 100 µl of each concentration. The plates were incubated at 37℃ for 24 h in inverted position and the zones of inhibition were recorded. The activity was compared with positive control (Amikacin, 100 µl of 1 mg/ml in DMSO) and negative control (50 µl of DMSO). The activity assays were conducted in triplicate.

2.7 Minimal Inhibitory Concentration (MIC)

The broth dilution technique was used where the plant extract was prepared to the highest concentration of 500 mg/ml (stock concentration) in DMSO and serially diluted (two-fold) to a working concentration ranging from 0.97 mg/ml to 300 mg/ml using peptone broth. And the tubes were inoculated with 0.1 ml suspension of the test organisms. Control was used with peptone broth, plant extract and without test organism. After 24 h of incubation at 37℃, the tubes were observed for turbidity. The least concentration where no turbidity was observed determined as MIC value.

3. RESULTS AND DISCUSSION

Urinary tract infections are the most common form of bacterial infections that affects human beings throughout their life span. The conditions such as pregnancy, diabetes, immunosuppression and other urological disorders trigger the occurrence of urinary tract infections. To provide appropriate therapy for UTIs, the knowledge on antibiotic susceptibility of the organisms is essential. The clinical isolates of UTIs were confirmed by biochemical tests and are reported in Table 1. The therapeutic value of plants depends on the presence of phytoconstituents. The preliminary phytochemical screening showed the presence of amino acids, proteins, cardiac glycosides, steroids, terpenoides, alkaloids, flavonoids and tannins (Table 2). The results obtained in the study showed that different bacterial species exhibit different sensitivities towards the extract. The extract inhibited all the bacteria with variable extent. The antibacterial activity against selected bacteria was in the following order *S. aureus* > *E. coli*> *E. faecalis* > *K. pneumoniae* > *P. aeruginosa* (Table 3). In the present study, the MPEE effectively inhibited all bacteria tested. The zone inhibition values of the extract against tested bacteria ranged from 8.33±0.81 to 21.50±1.22 mm in increasing order of concentration. Amikacin showed inhibition zones that ranged from 15.6±0.57 to 22.3±0.57 mm. The ethanolic extract exhibited maximum activity against *S. aureus* (21.50±1.22 mm) followed by *E. coli* (19.33±0.81 mm) and *K. pneumoniae* (17.66±1.24 mm) and then against *P. aeruginosa* (15.33±1.69 mm). The results in the present study indicate that the antibacterial activity depends according to type of bacteria used for the study. The MICs of MPEE were 62.5 mg/ml against *S. aureus* and *E. coli,* whereas 125 mg/ml against *E. faecalis, P. aeruginosa, K. pneumonia* (Table 4).

The results obtained in the study indicate that the MPEE is very active against tested bacteria. The essential oils of Mentha are used as antibiotics in the treatment of various infectious diseases. The peppermint oil was reported for its strong effect against *Salmonella* [15]. The roots of *M. piperita* were studied for antimicrobial, antioxidant and anti-inflammatory activities. The activities were tested for hexane, petroleum ether and methanol extracts [16]. Hexane and petroleum ether produced less significant antimicrobial activity compared to methanol. It was reported that *S. mutans* (25.3 mm) exhibited the highest inhibitory effect and *S. aureus* (15.0 mm) exhibited the lowest inhibitory effect. The phytochemical analysis of MPEE showed the presence of alkaloids, glycosides, flavonoids and tannins. Thus, it is concluded that the antibacterial activity is attributed due to the presence of various phytoconstituents.

Table 1. Biochemical tests of recovered clinical isolates of uropathogens

S. no	Catalase	Indole	MR	VP	Citrate	Urease	Organism confirmed
1	+	+	+	-	-	-	*E. coli*
2	+	+	-	-	+	+	*Pseudomonas*
3	+	-	-	+	+	+	*Klebsiella*
4	-	-	-	+	-	-	*Enterococcus*
5	+	-	+	+	+	+	*Staphylococcus*

Note: "+" indicates positive, "-" indicates negative

Table 2. Preliminary phytochemical screening of MPEE

S. no	Name of the test	MPEE
1	Carbohydrates	-
2	Phenols	+
3	Amino acids	+
4	Steroids	+
5	Terpenoids	+
6	Glycosides	+
7	Flavonoids	+
8	Alkaloids	+
9	Tannins	+

Note: "+" indicates positive and "–" indicates negative

Table 3. Antibacterial activity of MPEE against selected UTI pathogens

S. no	Name of the organism	Zone of inhibition in mm					
		250 µg	500 µg	750 µg	1000 µg	Amikacin (100 µg)	DMSO
1	*S. aureus*	12.33±0.81	14.66±1.24	17.66±1.24	21.50±1.22	19.3±0.57	-
2	*E. faecalis*	9.66±1.69	13.33±0.81	14.50±1.22	16.66±1.69	15.6±0. 57	-
3	*E. coli*	11.50±1.22	13.33±0.81	16.66±1.24	19.33±0.81	22.3±0.57	-
4	*K. pneumoniae*	10.66±1.69	13.33±1.22	14.66±1.24	17.66±1.24	17.6±0.57	-
5	*P. aeruginosa*	8.33±0.81	11.66±1.24	13.50±1.22	15.33±1.69	18.6±0.5 7	-

Note: Values are mean ± SD of triplicates

Table 4. Minimum inhibitory concentration (MIC) of MPEE

Name of the organism	Extract concentration mg/ml								
	0.97	1.95	3.90	7.81	15.6	31.2	62.5	125	250
S. aureus	+	+	+	+	+	+	β	-	-
E. faecalis	+	+	+	+	+	+	+	β	-
E. coli	+	+	+	+	+	+	β	-	-
K. pneumoniae	+	+	+	+	+	+	+	β	-
P. aeruginosa	+	+	+	+	+	+	+	β	-

Note: + = Turbidity observed, - = No turbidity observed, β= MIC value

4. CONCLUSION

The results from the current study indicate that *M. piperita* L leaves contained various potential bioactive compounds that exhibited antibacterial activity against uropathogens. The observed antibacterial activity confirms the effectiveness of this herb against microbes. Further isolation and characterization of bioactive compounds from this plant may helpful in formulating a potential natural antibacterial agent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sujana P, Sridhar TM, Josthna P, Naidu CV. Antibacterial activity and phytochemical analysis of *Mentha piperita* L. (Peppermint)—an important

multipurpose medicinal plant. AJPS. 2013;(4):77-83.
2. Alade PI, Irobi ON. Antimicrobial activity of crude leaf extracts of *Acalypha wilkensiana.* J Ethanopharmacol. 1993; 39:171-4.
3. Deans S, Kitchie G. Antibacterial properties of plant essential oils. Int J Food Microbiol. 1987;5:165-180.
4. Essawi T, Srour M. Screening of some palestian medicinal plants for antibacterial activity. J Ethanopharmacol. 2000;70: 343-9.
5. Mimica Dukic N, Bozin B, Sokovic M, Mihailovic B, Matavulj M. Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Medica. 2003;69:413-9.
6. Sowjanya P, Srinivasa Babu P, Lakshmi Narasu M. Phytochemical analysis and antibacterial efficacy of *Amaranthus tricolor* (L) methanolic leaf extract against clinical isolates of urinary tract pathogens. Afr J Microbiol Res. 2015;9(20):1381-5.
7. Koushik OS, Himaja V, Babu PS, Karthikeyan R. *In vitro* anti - bacterial activity of flowers of *Nymphaea alba*. Indian Journal of Drugs. 2015;3(2):47-8.
8. Valsaraj R, Pushpangadan P, Smith UW, Adsersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol. 1997;58: 75-83.
9. Samy RP, Ignacimuthu S. Antibacterial activity of some medicinal plants from Eastern Ghats, South India, Solai Bull. J Ethnopharmacol. 2000;72(1):39-41.
10. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar well diffusion method. Acta Biological et Medica Experimentalis. 1990;15(1):113-5.
11. Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991;30(12): 3875-83.
12. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Nirali Prakashan, Pune. 2005;39:607-11.
13. Evans WC. Trease and evans pharmacognosy. WB. Saunders Company Ltd., London, 2002;15:191-393.
14. Bauer AW, Kirby WMW, Sheries JC. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 1966;45:493-6.
15. Sartoratto A, Machado ALM, Delarmelina C. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol. 2004;34(4): 1517-20.
16. Abhishek M, Reena P, Deepika M, Prasad GBKS, Dua VK. Pharmacological investigation of methanol extract of *Mentha piperita* L. roots on the basis of antimicrobial, antioxidant and anti-inflammatory properties. Pelagia Research Library. 2011;2(1):208-16.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13811