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Original Article

MENTHA PIPERITA LINN: PHYTOCHEMICAL, ANTIBACTERIAL AND DIPTERIAN ADULTICIDAL APPROACH

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ABSTRACT

Objective: There is an immense need to develop an alternative antimicrobial source for the treatment of several infectious diseases and restrict its vectors. So, evaluation of phytochemicals from *Mentha piperita,* its antibacterial and adulticidal potential was undertaken in the present study.

Methods: The organic solvent of ethanol and water were used for the preparation of extract for the study. These extracts were analyzed for the qualitative and quantitative phytochemicals. Antimicrobial activity was determined by using agar well diffusion method, and Bioassay was carried out by "Sugar Bait" Method to check adulticidal potential**.**

Results: The *Mentha piperita* were found a rich source of phytochemical compounds like diterpenes, steroids, tannin, flavonoids, cardial glycosides, alkaloids, phenols, coumarin, and saponin. Both the extracts possesses active antibacterial compounds which shown antibacterial activity. The aqueous (5% of 50 µl) and ethanolic (5% of 100 µl) extract (EA) were found as effective concentration. *Salmonella typhimurium* showed resistance against both the extracts. The aqueous extract (AE) has found with effective adulticidal potential. Hence it can be used to control and repel most popular disease spreading Dipterian vector, *Musca domestica*.

Conclusion: These results confirm the presence of antibacterial, adulticidal compounds in *Mentha piperita* which gives an opportunity to explore the possible usage of *Mentha piperita* in the development of antibiotics and insecticides.

Keywords: *Mentha piperita* L., Phytochemical analysis, Antibacterial, Adulticidal potential

INTRODUCTION

The plant kingdom is a treasure house of potential drugs, and there has been an increasing awareness about their importance as medicinal plants. They are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. Different plants have been used as a source of inspiration in the development of novel drugs.

Phytochemicals are the chemical compounds that occur naturally in plants are responsible for color and organoleptic properties, such as the deep purple of blueberries and smell of garlic. Peppermint (*Mentha piperita*) is a hybrid mint, a cross between watermint and spearmint. The plant, indigenous to Europe and the Middle East, is now widespread in cultivation in many regions of the world. It is found wild occasionally with its parent species. Peppermint has a long tradition of use in folk medicine and aromatherapy. Peppermint is commonly thought to soothe or treat symptoms such as nausea, vomiting, abdominal pain, indigestion, irritable bowel, and bloating, although most of these effects have not been adequately demonstrated in human research.

Mentha piperita L. is a medicinally important plant which comes under the family Labiatae [1]. It is a non-native herbaceous plant, perennial, which can reach up to 40 inches in height and has a four-sided stem while leaves are stalked opposite and toothed. The flowers are irregular in shape with pinkish or purplish color [2].

Antibiotics are the main basis used in the therapy of various microbial infections. On the basis of evidence of the rapid global spread of drug-resistant in microbes, the need to find new antimicrobial agents have great importance [3]. Wide varieties of antibiotics are commonly used in the treatment of serious infections caused by some aerobic Gram-negative bacteria [4]. The increased use of antibiotics has resulted in the development of antibiotics resistant bacteria [5]. From recent years, misuse of antibiotics impacting in multi-drug resistance among bacteria which accelerated the search for drugs effective against such bacteria. In 1996, it has been reported that sales of botanical medicines peaked by 37% than that of 1995 [6]. In this relation, various parts of plants, herbs and spices have been used for many years to prevent infections. These parts are easily available and can be used in a domestic setting with self-medication. Most of their properties are due to the oils produced by their secondary metabolites [7].

So, as there is a need to develop an alternative antimicrobial source for the treatment of several infectious diseases from other sources like plants; however a large number of literature are available on the medicinal properties of essential oils present in *Mentha*; but, no much study has been directed towards its other useful properties which are locally available. Therefore, the present study was undertaken to evaluate antibacterial potential and qualitative phytochemical analysis with adulticidal effect from leaves of *Mentha piperita.*

MATERIALS AND METHODS

Collection of plant material

The fresh and healthy leaves of *Mentha piperita* L. was collected from the various localities of Chandgad Tahsil, Maharashtra, India; during October 2014. Leaves were washed with distilled water so as to remove dirt and soil particles. The leaves were crushed and dried in a shaded area at room temperature for a period of a week. Then the dried leaves were grinded with an ordinary grinder and then sieved through server.

Identification

Mentha piperita L**.** was authenticated by Mr. R. S. Sawant, Associate Professor, Department of Botany, Dr. Ghali College, Gadhinglaj, Kolhapur district, Maharashtra, India and Mr. Vinayak Sardesai, Assistant Professor, Department of Botany, R. B. M. College, Chandgad, Kolhapur district, Maharashtra, India and the voucher specimen (*RSP 001*) was deposited in Shivaji University, Kolhapur.

Preparation of test extract for antibacterial activity

The aqueous and ethanolic extract of leaves of *Mentha piperita* were prepared by addition of 0.5 gm of powder and 1 gm of powder into 10 ml of respective solvents for getting 5% and 10% concentration respectively. These extracts were kept at room temperature for overnight. Sample further used after centrifugation.

Preparation of extracts for phytochemical analysis

The collected leaves of *Mentha piperita* Linn were washed and then dried under shade. The coarse powder was soaked in 500 ml of distilled water and extracted in the cold for 3 d with occasional shaking. The solvent from the total extract was filtered & concentrated on a water bath for 8 h. The remaining was used for the analysis of phytochemicals test; same procedure was followed for ethanolic extraction using ethanol.

Identification tests for phytochemical analysis

The aqueous and ethanolic extracts of the plant were analyzed for the qualitative and quantitative phytochemicals analysis as shown in table 1 using standard methods [8-11].

Test. organisms

The standard test microorganisms were used in the present study as: *Staphylococcus aureus* NCIM 2654, *Salmonella typhimurium* NCIM 2501, *Bacillus cereus* NCIM 2703 and *Proteus vulgaris* NCIM 2813 and were obtained from National Collection of Industrial Microorganisms, Pune, (M. S.) India.

Antibacterial activity

Antimicrobial activity of the aqueous and ethanolic extract of *Mentha piperita* against various microorganisms (table 2) was determined by using agar well diffusion method by using Nutrient agar medium and respective solvent used as a control.

Preparation of bacterial suspension

A loop full suspension of the test organisms was aseptically streaked onto nutrient agar slants and incubated at 37 °C for 24 h. The obtained bacterial growth was harvested from the respective slant and suspension was prepared using sterile 1 ml normal saline (0.85 gm NaCl in 100 ml of distilled water). Then suspensions were stored in the refrigerator at 4 °C until used [12].

Adulticidal potential

Test insect

Musca domestica, house flies, was reared in the insect rearing cage in the laboratory at room temperature and 55-60% relative humidity. A standard rearing method [13] was adapted to provide adult flies of 0-24 h old for running bioassay tests. The adults of *Musca domestica* were collected from various poultry farms of Ajara Tahsil and kept under the starvation condition for a day.

Extraction

The method of Freedman *et al.* [14] was adapted with minor modification. The 20 gm of dried leaf powder was kept in 80 ml of distilled water for overnight to obtain 25 X 10^4 ppm. The extract obtained was double filtered and used as stock solution. The various concentrations (10%, 20%, 40% and 50% of 25 X 10^4 ppm) were prepared from the stock solution by diluting with distilled water and used as working extracts and kept in a deep freezer (-18 °C) until used.

Bioassay by "Sugar Bait" method

The Sugar Bait method of Mansour *et al.* [15] was adapted with minor modification. Quantities of 2 gm sugar with 10 ml of respective working solution was mixed to prepare bait and placed in petri dishes. Control sugar bait was prepared by dissolving 2 gm of sugar in 10 ml of distilled water. Each baited petri dish was placed in a rearing cage containing 50 adults of *Musca domestica* and maintained at room temperature for 24 h to estimate potency of the various concentrations. Usually, three replications were carried out for each tested concentration alongside with control tests.

RESULTS AND DISCUSSION

Phytochemicals analysis

According to Harborne and Willians [16], flavonoids exhibit anti-inflammatory, antimicrobial, antioxidant, vascular activities along with other medicinal properties. There are several reports on the antimicrobial activity of flavonoids [17-19]. The crude extracts with flavonoids, steroids, and triterpenes have significant activity against various strains of *Staphylococcus aureus, Escherichia coli* and *Streptococcus faecalis* [20]. Tannin may be toxic to the organisms like bacteria, yeast and filamentous fungi [21] having a potential antiviral [22] and antibacterial activity [23, 24]. Alkaloids are used as anesthetic agents [25]. Terpenoids exhibit various important pharmacological activities, i.e., anti-inflammatory, anticancer, antimalarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities [26].

The results (table 1) of chemical test with aqueous extract of *Mentha piperita* contain phytochemicals like diterpenes, steroids, tannin, flavonoids, Carbohydrates, alkaloids, phenols, coumarin, and saponin while the ethanolic extract also contains the same chemicals except tannin, cardiac glycosides and saponin; whereas the aqueous extract found rich one.

Table 1: Phytochemicals present in aqueous and ethanolic extract of *Mentha piperita*

S. No.	Phytochemical		Results	
			Aqueous extract	Ethanolic extract
1	Anthocyanin		-	-
2	Diterpenes: Copper acetate test		+	++
3	Steroids		+	++
4	Tannin:	Lead acetate test	++	-
		$FeCl_3$	+	-
5	Cardial Glycosides: Keller-Killani test		+++	-
6	Flavonoid:	Alkaline Reagent Test	+++	-
		NH_4OH	++	+
		Mg turning test	-	-
		Zn dust test	-	-
7	Phlobatannins		-	-
8	Phytosterol: Salkowski's test		+	+
9	Alkaloids	Wagner's reagent	+++	+
		Hager's reagent	++	+
10	Phenols: $FeCl_3$ test		+++	++
11	Leucoanthocyanin		-	-
12	Coumarin		++	+
13	Saponin: Foam test		+++	-

Key: (+) Positive test, (-) Negative test, n=2

Antibacterial activity

The aqueous extract of *Mentha piperita* showed an inhibitory effect against *Proteus vulgaris* and *Staphylococcus aureus* and its 5% of 50 µl solutions is most effective while *Bacillus cereus* and *Salmonella typhimurium* don't shown a zone of inhibition (table 2). The ethanolic extract is more effective and shown a significant zone of inhibition against *Proteus vulgaris* and *Bacillus cereus* and its 5% of 100 µl solutions is most effective concentration. *Salmonella typhimurium* shown resistance against both the extract (table 2). These results confirm the presence of antibacterial compounds in *Mentha piperita* and it may be used to fulfill the need of alternative antibiotics.

Table 2: Antibacterial activity of active components present in the leaf extract of *Mentha piperita*

Organism used	Zone of inhibition (in mm)							
	Aqueous extract (AE)				Ethanolic extract (EE)			
	5%		10%		5%		10%	
	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl
Staphylococcus aureus NCIM 2654	15.0±1.0	16.0±1.0	12.0±2.0	15.0±1.0	-	-	-	-
Salmonella typhimurium NCIM 2501	-	-	-	-	-	-	-	-
Proteus vulgaris NCIM 2813	13.0±1.0	11.0±2.0	13.0±1.0	13.0±1.0	14.0±2.0	16.0±1.0	20.0±1.0	21.0±1.0
Bacillus cereus NCIM 2703	-	-	-	-	16.0±1.0	19.0±1.0	18.0±2.0	17.0±1.0

Note: Each value is the mean of three readings±SD. n=3

Adulticidal potential

The adulticidal potential of *Mentha piperita* on *Musca domestica* was evaluated. The survival rate and Mortality rate are represented in table 3 and table 4 respectively. The present study revealed that 40% of working extract was effective as an adulticide. This proportion exhibited 100% Mortality. It might be due to various phytochemicals present in the leaf of *Mentha piperita.* Lower proportions have shown a comparatively low rate of Mortality ultimately due to the lower quantity of secondary metabolites. On the other hand, the rate of Mortality was expected higher in 50% but, it was found quietly similar to that of 10% of the extract.

This might be due to repellent properties of secondary metabolites. During the study, although Mortality rate at 10% was noticed low, the activity of adult flies got diminished. It indicates that higher quantity of secondary metabolites acts as repellent due to which files didn't show any interest in sugar bait along with the extract.

Table 3: Survival rate of adult *Musca domestica* at various concentrations of *Mentha piperita* with respect to time

Proportion of working solution	No. of adults introduced	No. of adults survived after 6 h	No. of adults survived after 12 h	No. of adults survived after 18 h	No. of adults survived after 24 h
Control	50	50±0.0	50±0.0	49±1.0	48±1.0
10 %	50	47±1.0	42±1.0	37±1.0	25±1.0
20 %	50	42±1.0	31±2.0	19±1.0	10±0.0
40 %	50	39±1.0	16±1.0	03±1.0	01±1.0
50 %	50	48±1.0	39±1.0	28±2.0	20±1.0

Note: Each value is the mean of three readings±SD. n=3

Table 4: Mortality rate of adult *Musca domestica* at various concentrations of *Mentha piperita* after 24 h

Proportion of working solution	No. of adults survived after 24 h	Percent mortality
Control	48±1.0	04±2.0%
10 %	25±1.0	50±2.0%
20 %	10±0.0	80±0.0%
40 %	01±1.0	98±2.0%
50 %	20±1.0	60±2.0%

Note: Each value is the mean of three readings±SD. n=3

CONCLUSION

The *Mentha piperita* were found a rich source of secondary metabolites. The aqueous and ethanolic extract of *Mentha piperita* possesses antibacterial activity. These results confirm the presence of antibacterial compounds in *Mentha piperita.* The aqueous extract was effective against *Staphylococcus aureus* might be used as a source for finding the antibiotics effective against MRSA. Aqueous extract of *Mentha piperita* has effective adulticidal potential, hence it can be used to control and repel most popular disease spreading Dipterian vector, *Musca domestica*.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. Kirethekar Basu I. Indian Medicinal Plants; 1985. p. 714-6.
2. Clark RK, Menory RC. Environmental effects or peppermint (*Mentha piperita*). Aust J Plant Physiol 1980;7:685-92.
3. Basheer A Al-Sum, Abdullah A Al-Arfaj. Antimicrobial activity of the aqueous extract of mint plant. Sci J Clin Med 2013;2:110-3.
4. Tumah H. Fourth-generation cephalosporins: *in vitro* activity against nosocomial Gram-negative bacilli compared with beta-lactam antibiotics and ciprofloxacin. Chemotherapy 2005;51:80-5.
5. Derrida M. What is bitter melon (*Momordica charantia*)?; 2003. Available from: http://www.brama.com/ business/ messages/ 40688.html. [Last accessed on 10 Nov 2015].
6. Thongson C, Davidson PM, Mahakarnchanakul W, Weiss J. Antimicrobial activity of ultrasound-assisted solvent-extracted spices. Lett Appl Microbiol 2004;39:401-6.
7. Adam KA, Sivropoulou SK, T Lanaras, M Arsenakis. Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. J Agric Food Chem 1998;46:1739-45.

8. Godghate A, Sawant R, Sutar A. Phytochemical analysis of ethanolic extract of roots of *Carrisa Carandus Linn*. Rasayan J Chem 2012;5:456-9.
9. Patil RS, Godghate AG, Sawant RS. Phytochemicals and antimicrobial activity of leaves of *Homonoia riparia L*. Int J Pharm Biol Sci 2014;5:352-6.
10. Patil RS, Bhise KK. Evaluation of phytochemicals and *in vitro* antimicrobial activity of aqueous and ethanolic extract from seeds of *Ricinus communis* Linn. Eur J Biotechnol Biosci 2015;3:19-23.
11. Patil RS, Harale PM, Shivangekar KV, Kumbhar PP, Desai RR. Phytochemical potential and *in vitro* antimicrobial activity of *Piper betle* Linn. leaf extracts. J Chem Pharm Res 2015;7:1095-101.
12. Patil RS, Desai AB, Wagh SA. Comparative study of antimicrobial compounds extracted from leaves of *Nicotiana Tabacum* and cigarette. World J Pharm Pharm Sci 2015;4:1511-8.
13. Sawicki RM. Some general considerations of housefly rearing techniques. Bull WHO 1964;31:535-7.
14. Freedman B, Nowak LJ, Kwalek WF, Berry EC, Gutnrie WD. A bioassay for plant derived pest control agents using the European corn borer. J Econ Entomol 1979;72:541–5.
15. Mansour SA, Reda FA, Bakr LS, Hamouda RIM. The adulticidal activity of some botanical extracts, commercial insecticides, and their binary mixtures against the housefly, *Musca domestica* L. Egyptian Academic J Biol Sci 2012;5:151-67.
16. Harborne JB, Willians CA. Advances in flavonoid research since 1992. Phytochem Oxford 2000;55:481-504.
17. Baez DA, Vallejo LGZ, Jimenez-Estrada M. Phytochemical studies on *Senna skinneri* and *Senna wishizeni*. Nat Prod Lett Berks 1999;13:223-8.
18. Ogundipe OO, Moody JO, Houghton PJ, Odelola HA. Bioactive chemical constituents from *Alchormea laxiflora (benth) pax* and *hoffman*. J Ethnopharmacol Lausanne 2001;74:275-80.
19. Xu HX, Lee SF. The activity of plant flavonoids against antibiotic-resistant bacteria. Phytother Res London 2001;15:39-43.
20. Chattopadhyay D, Maiti K, Kundu AP, Chakraborty MS, Bhadra R, Maudal SC, *et al*. Antimicrobial activity of *Alstonia macrophylla*: A folklore of bay islands. J Ethnopharmacol Lausanne 2001;77:49-55.
21. Harborne JB. Photochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall London; 1973. p. 279.
22. Lin LU, Shu-wen L, Shi-bo J, Shu-guang W. Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacol Sin 2004;25:213-8.
23. Akiyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrobial Chemother 2001;48:487-91.
24. Funatogawa K. Antibacterial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*. Microbiol Immunol 2004;48:251-61.
25. Hérouart D, Sangwan RS, Fliniaux MA, Sangwan-Norreel BS. Variations in the leaf alkaloid content of androgenic diploid plants of *Datura innoxia*. Planta Med 1988;54:14-7.
26. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochemistry 1997;44:1185-236.