Antibacterial activity of Mentha piperita L. (peppermint) from leaf extracts – a medicinal plant

Data · February 2015

CITATIONS
0

READS
1,833

2 authors, including:

Sureshkumar Periyasamy
Anna University of Technology, Tiruchirappalli
47 PUBLICATIONS **364** CITATIONS

SEE PROFILE

Agrovoc descriptors: mentha piperita; drug plants; leaves; plant extracts; antimicrobial properties; pathogens; bacteria

Agris category code: F60, Q04, S01

COBISS code 1.01

Antibacterial activity of *Mentha piperita* L. (peppermint) from leaf extracts – a medicinal plant

G. BUPESH[1], C. AMUTHA, S. NANDAGOPAL[2], A. GANESHKUMAR[3], P. SURESHKUMAR[1&]*, K. SARAVANA MURALI [4]

Received: December 23, 2006; accepted: June 20, 2007.
Prispelo: 23. decembra 2006; sprejeto: 20. junija 2007.

ABSTRACT

In the present study, we evaluated the antibacterial activity in the leaf extracts of *Mentha piperita* L. against pathogenic bacteria like *Bacillus subtilis, Pseudomonas aureus, Pseudomonas aerogenosa, Serratia marcesens* and *Streptococcus aureus*. The aqueous as well as organic extracts of the leaves were found to posses strong antibacterial activity against a range of pathogenic bacteria as revealed by *in vitro* agar well diffusion method. The ethyl acetate leaf extract of *Mentha piperita* showed pronounced inhibition than chloroform, petroleum ether and water, leaf extracts being more on *Bacillus subtilis, Pseudomonas aerogenosa* than *Streptococcus aureus, Pseudomonas aureus* and *Serratia marcesens.*

Keywords: *Mentha piperita* L., antibacterial activity, leaf extracts.

IZVLEČEK

ANTIBAKTERIJSKA AKTIVNOST LISTNIH EKSTRAKTOV POPROVE METE (*Mentha piperita* L.)

Ocenjena je bila antibakterijska aktivnost listnega ekstrakta poprove mete (*Mentha piperita* L.) na patogene bakterije *Bacillus subtilis, Pseudomonas aureus, Pseudomonas aerogenosa, Serratia marcesens* in *Streptococcus aureus*. Tako vodni ekstrakti, kot ekstrakti dobljeni z organskimi topili so imeli močan protibakterijski učinek proti vrsti patogenih bakterij v agarju po *in vitro* metodi. Listni ekstrakt poprove mete, dobljen z etilnim actetatom je imel bolj izrazit učinek kot ekstrakti dobljeni s kloroformom, etrom ali vodo, bolje so učinkovali na *Bacillus subtilis* in *Pseudomonas aerogenosa* kot na *Streptococcus aureus, Pseudomonas aureus* in *Serratia marcesens.*

Ključne besede: *Mentha piperita* L., antibakterijska aktivnost, listni ekstrakti

[1&]* Department of Animal Science and Animal Biotechnology, Bharathidasan institute of Technology, Bharathidasan University, Tiruchirappalli =96 620 024, India. Corresponding authors' e-mail: profsubbus@rediffmail.com. Tel.:+91-0431-2407222; Fax: + 91-2407333

[2] Dept. of Plant Science, Bharathidasan University, Tiruchirappalli – 620 024, India.

[3] Dept. of Micro Biology, Bharathidasan University, Tiruchirappalli – 620 024, India.

[5] Dept of Virology, King institute of Preventive Medicine Chennai – India.

1 INTRODUCTION

Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts (Hulin et al., 1998). *Mentha piperita* L. (peppermint) is a medicinally important plant that belongs to the family Labiate (Kirethekar and Basu, 1985). Peppermint is a non-native herbaceous plant, it is a perennial, which can reach 100 cm in height (40 inches) has four-sided stem. The leaves are stalked opposite and toothed. The flower are irregular in shape, they are pinkish or purplish (Clark and Menory, 1980). Peppermint leaves contains about 0.5-4 % volatile oil that is composed of 50-78 % free menthol, monoterpene, menthofurane and traces of jasmine (0.15 %) to improve the oils quality remarkably (Dew and Evans, 1984). Peppermint is largely cultivated in Indiana, Mexican and California for the production of peppermint oil. 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 Mimica et al. (2003) and Forster (1996). Peppermint oils relaxing action also extended to tropical use, when applied tropically it acts as counterirritant and analgesic with the ability to reduce pain and improve blood flow to the affected area. Peppermint oil and menthol have moderate antibacterial effects against both gram-positive and gram negative bacteria Diaz et al. (1988). Peppermint extracts are bacteriostatic against S*treptococcus pyrogens*, S*treptococcus aureus*, *Streptococcus pyrogens*, *Serratia marcescens*, *E.coli* and *Mycobacterium avium* (Gotshall, 1949). Peppermint is also found to have antiviral and fungicidal activity (Chaumont and Senet, 1978). Menthol is virucidal against influenza, herpes and other viruses. Aqueous extracts of peppermint leaves were antiviral against influenza A, newcastle disease virus in egg and cell culture system Hirobe (1994) and Alkofahi et al. (1990). Menthol and peppermint oil are fungicidal against *Candida albicans*, *Aspergillus albus* and *Dermatophytic* fungi. However, to the best of our knowledge, no serious efforts have been made to test the antibacterial properties of *Mentha piperita* so far. In the present study we established antimicrobial activity of *Mentha piperita* against pathogenic bacteria. The study confirms that both aqueous as well as organic solvent leaf extracts possess strong antibacterial properties against various pathogens viz., *Bacillus substillus*, *Pseudomonas aureus*, *Pseudomonas aerogenosa*, *Serratia marcesens* and *Streptococcus aureus.*

2 MATERIALS AND METHODS

2.1 Preparation of leaf extracts

Apparently healthy plants were collected, washed thoroughly in tap water and dried at dark room temperature for 15 days. The leaves was powdered and extracted following the published procedure with slight modification as standardized in our lab Alade and Irobi, (1993); Essawi and Srour (2000). The powdered material was soaked in petroleum ether, chloroform, ethyl acetate, ethanol, methanol and water by keeping it in a shaker for 3 days. The extracts were filtered through cheesecloth and the extracts were reduced to 10 % of its original volume. The filtrate organic solvents were concentrated in vacuum using a rotary evaporator, while aqueous extracts were dried using water bath.

2.2 Separation of the compounds

The menthol present in *Mentha piperita* leaf extracts were analysed for qualitative by using thin layer chromatography which is commercially available TLC, aluminum sheets with silica

gel $60F_{254}$ were used. The isolation separation of menthol was done by using the procedure of Stahl (1964).

2.3 Inoculums

The test microorganisms *Bacillus substillus*, *Pseudomonas aureus*, *Pseudomonas aerogenosa*, *Serratia marcesens* and *Streptococcus aureus* were obtained from culture repository of Best Biotech culture collection, Bangalore, India. The organisms were inoculated onto NB (Nutrient Broth), (0.5 % Peptone, 0.5 % Sodium Chloride, 0.15 % Yeast extract; pH 7.4) and incubated at 37 ºC for overnight. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer. The bacterial cells were diluted to approximately 10^5 CFU/ml before use (Owais et al., 2005).

2.4 Determination of antibacterial activity:

The antibacterial activity of the leaf extracts was determined using agar well diffusion method following published procedure with slight modification (Perez et al. 1990). Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) or standard antibiotic solution (positive control) viz., Chloromphenicol (100 µg/ml) were also run parallel in the same plate (Valsaraj et al., 1997). The plates were incubated at 37 ºC for 18 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard drugs viz., Choloramphenicol.

2.5 Statistical analysis

The resultant clear zones around the discs were measured in mm. The antibacterial activity of plant extracts were indicated by clear zones of growth inhibition. Five replicates were maintained for each treatment. The data were subjected to statistical analysis as per the method of Gomez and Gomez (Gomez and Gomez, 1976).

3 RESULTS

We used both polar as well as non-polar solvents for the extraction of active components from the leaves of *Mentha piperita* plant. The antibacterial activity of the *Mentha piperita* was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated (Table. 1). The results shown in (Table 1 and Figure 1) indicate that the aqueous and polar solvent extracts possessed strong antibacterial activity. In ethyl acetate, chloroform and water the highest antibacterial activity was retained in 50 µl and 100 µl concentration of leaf extracts. We found that both aqueous as well as ethyl acetate extracts of leaves were successful in killing the bacteria in a dose dependent manner. The MIC (Minimal inhibitory Concentration) for the aqueous extracts was found out to be 10 mg/ml for *Bacillus substillis* and *Pseudomonas aerogenosa*. While *Serratia marcesens* and *Streptococcus aureus* required about 0.25 mg/ml of the crude extract for effective killing. On the other hand *Pseudomonas aureus* was inhibited at the dose of 0.35 mg/ml of the crude extract. The zone of inhibition assay results demonstrated that the ~8 mg of crude leaf extract was able to produce same effect as that of 20 µg of Cholorompheniсol (data not shown). Beside the 50 µl concentration of leaf extracts, the 100 µl concentration of leaf extracts was found to possess maximum inhibition, although, overall potency was about half to that of the 50 µl concentration

of leaf extracts. (Table 1 and Figure 1,2). Upon chemical analysis, the extracts were found to possess glycosides and alkaloids. The extracts were found to be rich in menthol compound as revealed by specific assay. The presence of menthol was further confirmed by thin layer chromatogram, which showed presence of green fluorescent spots in the extracts of chloroform (Figure 3). The ethyl acetate extracts had very large zones of inhibition ranging from 9 mm and it also showed high degree of inhibition against *Bacillus subtilis, Pseudomonas aerogenosa, Streptococcus aureus* than *Pseudomonas aureus and Serratia marcesens.*. The chloroform leaf extracts showed moderate inhibition against *Bacillus subtilis, Pseudomonas aureus* and *Streptococcus aureus* than *Pseudomonas aerogenosa and Serratia marcesens* But the petroleum ether leaf extracts exhibited less inhibition against *Bacillus subtilis, Pseudomonas aureus* and *Serratia marcesens* than *Streptococcus aureus* and *Pseudomonas aerogenosa.*

4 DISCUSSION

We used both the aqueous and polar solvents for the extraction of active components from leaves of the plant. The result of the study reveals that an aqueous and polar solvent was actively against the strains of the bacteria that are common cause of infections. *Mentha piperita shows* significant activity as because the leaf contains many potent compounds such as menthol, menthone, menthyl acetate, menthofuran, and limnone (Fleming, 1998). These compounds have higher medicinal value especially in the treatment of dyspepsia, epigastric bloating, impaired digestion, eructions, and flatulence, tropically used to relieve nasal congestion in the common cold and itch relieving used as tropic protective agents Alkofahi et al. (1990). The biologically active compounds are screened by dissolving the crude powder on various solvents respective to the solubility of the compound specific solvents confirmed by the TLC (data not shown). The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent .The various crude extracts of *Mentha piperita* showed significant activity against all the bacteria tested. Similar to our result, the biological activity of *Mentha piperita* against the pathogenic bacteria were reported by (Deans and Baratta, 1998). Based on that, we used the three different solvent extracts of leaves of *Mentha piperita* showed activity against all bacteria at all dosages. The leaf extracts of *Mentha piperita* exhibited antibacterial activity only in ethyl acetate, petroleum ether, choloroform, menthanol and aqueous extracts against the bacteria tested in agar well diffusion method at 50 µl -100 µl concentration by the following method of Valsaraj et al. (1997). We observed maximum activity at 100 µl concentration against *Pseudomonas aerogenosa, Streptococcus aureus, Bacillus subtilis* than *Serratia marcesens* and *Pseudomonas aureus.*The present reports indicates that increased lipophillic compounds are extracted using the petroleum ether, chloroform and methanol increased the suspended higher compounds in the above solvents as stated by Tomas et al. (1988). We subjected the leaf extracts of different solvents to thin layer chromatography and column chromatography to identify the compounds dissolved in the solvents and quantifies the compounds using spectrophotometer techniques followed by the previous work (Essawi and Srour, 2000). The present work was similar to (Deans and Baratta, 1998) shows that the compounds from *Mentha piperita* possess potent antimicrobial activity and suggesting that the *Mentha piperita* leaf extracts should

contains the effective active constituents responsible for eliminating the bacterial pathogens. Finally, it can be concluded that the active chemical compounds present in *Mentha piperita* should certainly find place in treatment of various bacterial infections. The results from the present study are very encouraging and indicate this herb should be studied more extensively to explore its potential in the treatment of infectious diseases as well.

5 REFERENCES

Alade, P.I., Irobi, O.N. 1993: Antimicrobial activity of crude leaf extracts of *Acalypha wilkensiana*. J. Ethanopharmacol, 39: 171-174.

Alkofahi, A.S., Abdelaziz, A., Mahmoud, I, Abuirjie, M., El-Oqla, A., Hunaita, A. 1990: Cytotoxicity, mutagenicity and antimicrobial activity of forty Jordanian medicinal plants. International Journal of Crude Drug Research, 28: 139-144.

Chaumont, J.P., Senet, J.M. 1978: Antagonistic properties of higher plants against fungal parasites of man from food contaminants: screening of 200 fungi. Plant. Med. Phytother, 12: 186-196.

Clark, R.K., Menory, R.C. 1980: Environmental effects or peppermint (*Mentha piperita*). Aust J. Plant Physiology, 7: 685-692.

Deans, S., Kitchie, G. 1987: Antibacterial properties of plant essential oils. International Journal of Food Microbiology 5: 165-180.

Deans, S.G., Baratta, M.T. 1998: Antimicrobial & Antioxidant properties of some essential oils. Flau.Fragrance, 235-244.

Dew, M.J., Evans, J.R. 1984: Peppermint oil for the irritable bowel syndrome; a multi center trialm. Br. J. Clin Pract. 38: 394-395.

Diaz, R., Quevedo-Sarmiento, J., Ramos-Cormenzana, A., Cabo, P., Cabo, J. 1988: Phytochemical and antibacterial screening of some species of Spanish Lamiaceae. Fitoterapia, 59: 330-333.

Essawi, T., Srour, M. 2000: Screening of some palestian medicinal plants for antibacterial activity. J. Ethanopharmacol, 70: 343-349.

Fleming T, 1998: PDR for Herbal medicines: Medical Economic Company, JNC

Forster S. 1996: Peppermint: *Mentha piperita*, American Botanical Council – Botanical Series. 306: 3-8.

Gomez, Gomez, K.A. 1976: Statistical procedure for agricultural research with emphasis of Rice (Los Bans, Philippines International Rice Research Institute).

Gotshall, R.Y., Lucas, E.H., Lickfeldt, A. Roberts, J.M. 1949: The occurrence of antibacterial substances active against *Mycobacterium tuberculosis* in seed plants. Journal of Clinical Investigation, 28: 920-923.

Hirobe, C., Palevitch, D., Tayeka, K. Itokawa, H. 1994: Screening for antitumour activity of crude drugs (IV): Studies on cytotoxic activity of Israeli medicinal plants. Natural Medicine, 48: 168-170

Hulin, V., Mathot, A.G., Mafart, P., Dufosse L. 1998: Les proprietes anti-microbiennes des huiles essentielles et composes daromes. Sci. Aliments, 18: 563-582.

Kirethekar, Basu, I. 1985: Indian Medicinal Plants. 714-716 pp.

Mimica Dukic, N., Bozin, B., Sokovic, M., Mihailovic, B. Matavulj, M. 2003: Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Medica. 69: 413-419.

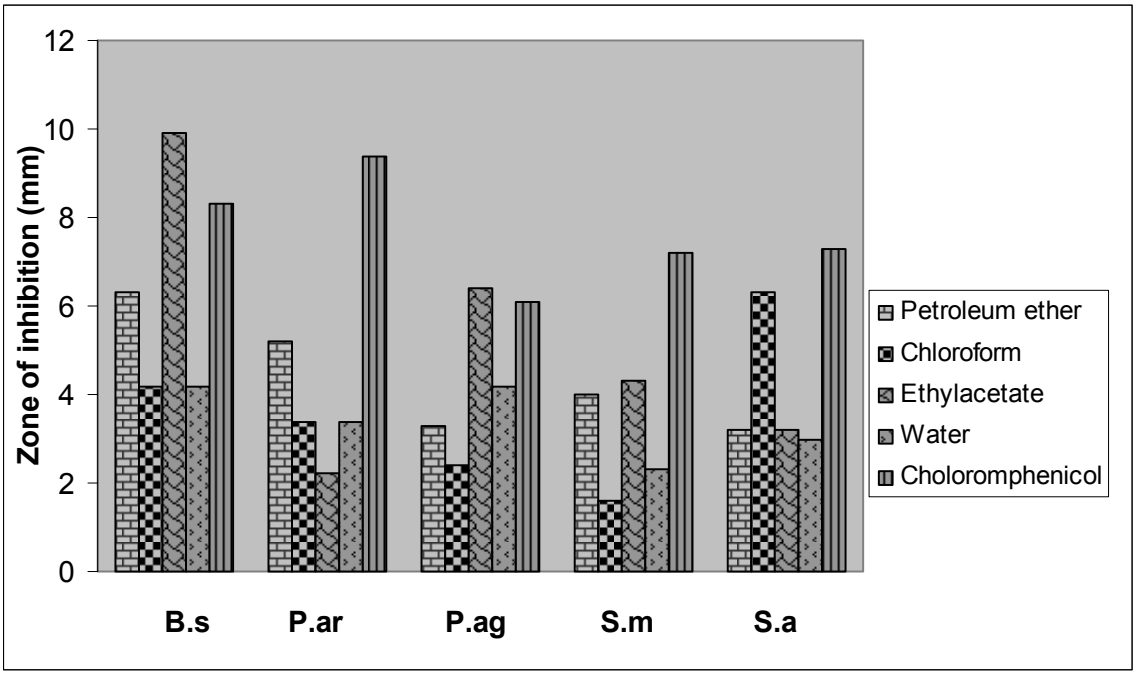
Owais, M. Sharad, K.S. Shebhaz, A. Saleemuddin, M. 2005: Antibacterial efficacy of withania sominifera an indigenous medicinal plant against experimental murine salmonellosis J. Phytomedicine, 12: 229-235.

Perez, C., Pauli., M., Bazerque, P., 1990: An antibiotic assay by agar well diffusion method. Acta Biol. Med. Exp, 15: 113-115.

Stahl, E. 1964: Thin layer chromatography In: A Laboratory Handbook.Vol.2 2nd edition. Allen and unwin, London.. 311-318 pp.

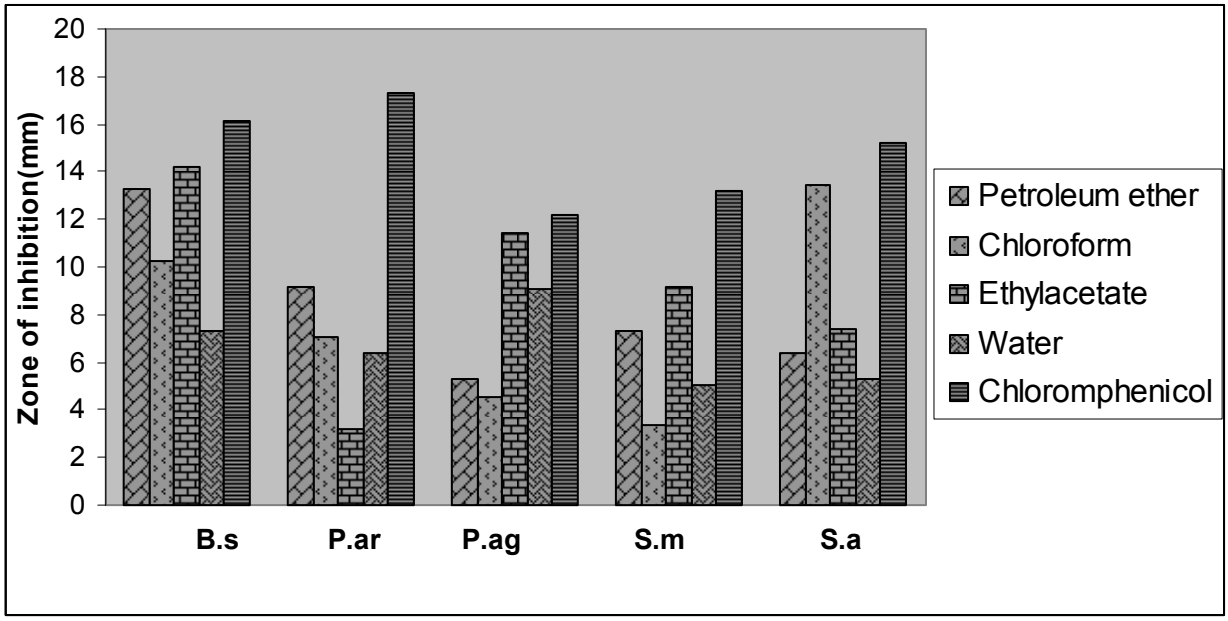
Tomas-Barberan, F., Msonthi, .A., Hostettmann, K. 1988: isolation and identification of the antibacterial compounds form medicinal plants., Phytochemistry. 1: 743-748

Valsaraj, R., Pushpangadan, P., Smith, U.W., Adsersen, A., Nyman, U. 1997: Antimicrobial screening of selected medicinal plants from India. J. of Ethnopharmacology, 58: 75-83.



The antibacterial activity of leaf extracts of menthapiperita was screened at 50 µl concentration against B.s = *Bacillus substillus*, P.ar = *Pseudomonas aureus*, P.ag =*Pseudomonas aerogenosa*, S.m= *Serratia marcesens* and *S.a = Streptococcus aureus.* The leaf extracts were dissolved in different solvents a. Petroleum ether b. Chloroform c. Ethyl acetate d. Water e. Control – Chloromphenicol.

Figure 1: Antibacterial activity of leaf extracts of *Mentha piperita* was screened at 50 µl concentration



The antibacterial activity of leaf extracts of menthapiperita was screened at 100 µl concentration against B.s = *Bacillus substillus,* P.ar = *Pseudomonas aureus* P.ag =*Pseudomonas erogenosa*, S.m= *Serratia marcesens* and *S.a = Streptococcus aureus.*The leaf extracts were dissolved in different solvents Viz a. Petroleum ether b. Chloroform c. Ethyl acetate d. Water e. Control – Chloromphenicol

Figure 2: Antibacterial activity of leaf extracts of *Mentha piperita* was screened at 100 µl concentration

Table 1: Antibacterial activity of the active components present in the leaf extract of *Mentha piperita against various microorganisms*

Extract	Zone of inhibition in (mm) (Mean ± SD)									
	50µl					100 µl				
	B.subtilis	*P.aureus*	*P.aerogenosa*	*S.marscens*	*S.aureus*	*B.subtilis*	*P.aureus*	*P.aerogenosa*	*S.marscens*	*S.aureus*
Pet. Ether	6.3±0.17	5.2±0.21	3.3±0.13	4.0±0.41	3.2±0.31	13.2±0.39	9.2±0.16	5.3±0.33	7.3±.0.33	6.45±0.32
Chloroform	4.2±0.25	3.4±0.23	2.4±0.28	1.6±0.28	6.3±0.32	10.2±0.21	7.1±0.24	4.5±0.32	3.4±.0.30	13.4±0.31
Ethyl acetate	9.9±0.28	2.3±0.27	6.4±0.30	4.3±0.41	3.2±0.25	14.2±0.17	3.2±0.20	11.4±0.29	9.2±0.26	7.4±0.30
Water	4.2±0.25	3.4±0.23	4.2±0.23	2.3±0.35	3.0±0.33	7.3±0.27	6.4±0.28	9.1±0.56	5.08±0.23	5.26±0.28
Chlorompenicol	8.3±0.23	9.4±0.23	6.1±0.14	7.2±0.29	7.3±0.30	16.1±0.18	17.3±0.37	12.2±0.33	13.2±0.37	15.2±0.23

The antibacterial activity of leaf extracts of *Mentha piperita* against various bacterial strain was developed by agar diffusion method. The active component of the leaves of *mentha piperita* were extracted with different solvents. The solid residues in respective solvents at l0mg/ml concentration. The well bored in bacterial culture plates were filled with 100 µl of suspension. (~2mg crude extract). The negative control wells were exposed with the neat solvent. Each value represents mean of five different observations ± S.D.