International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2019; 7 (4)785-790
ISSN:2320-7817(p) | 2320-964X(o)

Original Article Open Access

Phytochemical screening and evaluation of *In-vitro* antimicrobial properties of *Mentha piperita* L.

Sontakke KS[1*] and Shinde SL[2]

[1]Assistant Professor, Department of Botany, Gopikabai Sitaram Gawande Mahavidyalaya, Umarkhed Dist. Yavatmal, Maharashtra, India.
[2]Professor, Department of Botany, Rajiv Gandhi Mahavidyalaya, Mudkhed Dist-Nanded, Maharashtra, India.
*Corresponding author Email: ikailash442@gmail.com, | [2]sahebshinde4@gmail.com

Manuscript details:

Received: 11.09.2019
Accepted: 21.11.2019
Published: 30.12.2019

Cite this article as:
Sontakke KS and Shinde SL (2019) Phytochemical screening and evaluation of *In-vitro* antimicrobial properties of *Mentha piperita* L.**,** *Int. J. of. Life Sciences*, Volume 7(4): 785-790.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

The present study was aimed to evaluate the phytochemical potential of hexane, acetone, chloroform, ethanol and aqueous extracts of *Mentha piperita* L. leaves. For the experiment, leaves of *Mentha piperita* L. were collected from the region of Nanded (MS), India and authenticated. The leaves of *Mentha piperita* L. showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins and carbohydrates. Acetone and aqueous extracts were observed to be more potent with presence of the entire screened phytochemicals. Acetone and Aqueous extract of plants show maximum content of phenols and flavonoids respectively. Also, the overall extracts showed high antimicrobial response specially acetone and chloroform extract against bacteria while in case of fungi, it showed very least response. These Phytochemical compounds are also known as plant secondary metabolites and are reported to have many biological and medicinal properties. Hence this species is expected to have many therapeutic uses and can be further studied for the production of pharmaceutical drugs.

Keywords: Phytochemical, Antimicrobial, Mint, Extracts.

INTRODUCTION

The Labiatae family is one of the most utilized restorative plants family as an overall wellspring of flavors and furthermore as a solidified wellspring of concentrates with solid antibacterial and cell reinforcement properties. The plant, indigenous to Europe and the Middle East, is presently broad in development in numerous districts of the world. It is discovered wild sporadically with its parent species. Peppermint has a long custom of utilization in people's medication and aromatherapy. *Mentha piperita* L. is a medicinally important plant which belongs to the family Labiatae (Kirethekar, 1985). It is a immigrant herbaceous plant, perennial, and has a four-sided stem while leaves are stalked opposite and toothed and which can reach up to 40 inches in height. The flowers are irregular in shape with pinkish or purplish color (Clark and Menory, 1980).

A large volume of literature is available on the medicinal properties of essential oils present in *Mentha* spp. (Gulluce *et al*., 2007; Rasooli, 2008). Essential oils have been shown to possess antioxidant, insecticidal, antiviral, antifungal and antibacterial properties (Burt, 2004; Kordaly *et al.*, 2005). Antibiotics are the main basis utilized in the treatment of different microbial diseases. On the basis of evidence of the rapid global spread of drug-resistant microbes, the need to find new antimicrobial agents has great importance (Basheer and Abdullah, 2013). Wide varieties of antibiotics are commonly used in the treatment of serious infections caused by some aerobic Gram-negative bacteria (Tumah, 2005).

The widespread use of antibiotics both inside and outside medicine is playing a significant role in the emergence of bacterial resistance (Goossens *et al*., 2005). Although, there were low levels of preexistent antibiotic resistant microorganisms before the widespread use of antibiotics; evolutionary pressure from their use has played a role in increase in multidrug resistance varieties and the spread of resistance between microbial species (Hawkey and Jones, 2009).

In this concern, the present study was aimed to evaluate the phytochemical analysis and antimicrobial potential of *Mentha piperita* L. leaves extracts.

MATERIALS AND METHODS

Collection of plant material:

The fresh leaves of *Mentha piperita* L. was collected from the various localities of Nanded, Maharashtra, India; during August 2020. Leaves were splashed with distilled water to remove soil particles and dirt. 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 the server.

Preparation of plant extracts:

The extracts of selected plant material in different solvents such as aqueous, ethanol, chloroform, hexane and acetone were prepared. The 30 gm of dried powder was extracted with 300 ml solvent using Soxhlet apparatus for 24 hrs. The aqueous, ethanol, chloroform, hexane and acetone extracts were lyophilized and stored in 4^0 C.

Preliminary Qualitative screening of plant extracts:

Standard protocols were used for the phytochemical analysis. Phytochemical screening for the presence of major types of compounds in the extract was done by Harborne (1973) with some modifications.

- **Alkaloids:** 1.36gm of mercuric chloride was dissolved in 60ml distilled water and 5gm of potassium iodide and diluted to 100ml with distilled water. To 1.0ml of an acidic aqueous solution of samples, few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.

- **Flavonoids:** In a test tube containing 0.5ml of various extracts of the samples, 5-10 drops of dilute HCl and a small piece of Zn or Mg were added and then the solution was boiled for a few minutes. In the presence of flavonoids, the reddish-pink or dirty brown colour was produced.

- **Phenols:** To 1.0ml of alcoholic solution of samples, 2.0 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added and the formation of blue or green colour indicates the presence of phenols.

- **Saponins:** In a test tube containing 5ml of various extract of the sample, a few drops of sodium bicarbonate was added. The mixture was shaken vigorously for 3mins. A honeycomb like froth was formed and it showed the presence of saponins.

- **Steroids:** To 2.0ml of various extracts of samples, 1.0 ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. Formation of red colour chloroform layer indicates the presence of steroids.

- **Tannins:** In a test tube containing about 5.0 ml of a various extract, a few drops of 1% solution of lead acetate was added. A yellow or red colour precipitate was formed, indicating the presence of tannins.

- **Terpenoids:** 0.5 ml of extract was mixed with 2 ml of chloroform in a test tube. 3 ml of concentrated sulfuric acid was carefully added to the mixture to form a layer. A reddish-brown colouration was formed for the presence of terpenoids.

- **Carbohydrate:** In a test tube containing 2.0 ml of plant sample, 2 drops of freshly prepared 20% alcoholic solution of a naphthol was added and mixed. To this solution 2.0 ml of concentrated sulphuric acid was added so as to form a layer below the mixture, the formation of the red-violet ring at the junction of the solution and its disappearance on the addition of an excess of alkali solution indicates the presence of carbohydrates.

- **Protein:** 1 part of mercury was digested with 2 parts of HNO_3 and the resulting solution was diluted with 2 volumes of water. To a small quantity of decoction, 5-6 drops of Million's reagent was added. A precipitate which turned red on heating was formed and it indicates the presence of proteins.

Quantitative estimation of plant extracts:

- **Estimation of total phenolic content:** Antioxidant compounds generally contain phenolic group(s) and hence, the amounts of total phenolic compounds in the extracts of the flowers, leaves and seeds were estimated by using Folin–Ciocalteu reagent with Gallic acid as standard. The total phenolic content was estimated according to the method of Singh *et al.* (2011). The aliquot of the extract was taken and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) was added sequentially to the test tube. Soon after overtaxing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm using UV-Vis Spectrophotometer against the reagent blank. A standard curve was prepared using Gallic acid. The linearity obtained was in the range of 1-10 µg/ml. Using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

- **Estimation of total flavonoid content:** The antioxidant activity of medicinal plants could be attributed to its flavonoid content. Flavonoids act as scavengers of various oxidizing species i.e. super-oxide anion, hydroxyl radical or per-oxy-radicals, they also act as quenchers of singlet oxygen (Ratty and Das, 1988). The Aluminium chloride colorimetric method was used to determine the total flavonoids content in plant extract using Quercetin as standard. The total flavonoids content was estimated according to the method of Chang *et al.* (2002). Briefly, 0.5 ml solution of extract in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with the help of a UV-Visible spectrophotometer. The calibration curve was prepared by preparing Quercetin solutions at concentrations 10 to 100 µg/ml in methanol.

Antimicrobial properties:

The antimicrobial activity of the plant extracts was evaluated using Agar Well Diffusion Method of Firdaus *et al.,* (2011) with minor modifications**.** 0.1 ml of diluted inoculum (105 CFU/ml) of the microbial strains was swabbed on the Nutrient agar plates. Wells of 5 mm diameter were punched into the agar plates with the help of sterilized cork borer (5 mm). Using a micropipette, 100 µl of the plant extracts were added to the wells made in the plate. The plates were incubated aerobically in an upright position at 37±2 °C for 24-48 h. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the *Clostridium perfringens, Bacillus cereus, Staphylococcus aureus, Aspergillus niger* and *Rhizopus stolonifer* strains.

RESULTS

Preliminary phytochemical analysis of leaves *Mentha piperita* L. was carried out by using different solvent extracts. The leaves *Mentha piperita* L. showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins and carbohydrates. Alkaloids were found to be absent in chloroform extracts while acetone and aqueous extracts showed the highest presence of alkaloids. Similarly, flavonoids, Terpenoids and proteins were also found to be absent in the chloroform extracts. Most of all phytochemicals showed their presence in ethanol, acetone and aqueous solvent extracts. (Table1).

The antimicrobial activity of different *Mentha piperita* L. leaf extracts were examined against different microbes. Among all the studied extracts, the acetone extract showed the higher zone inhibition. While least inhibition showed by the aqueous and ethanol extract. The overall extracts showed high antimicrobial response in bacteria while in case of fungi, it showed very least response.

Table 1: Phytochemical Analysis of Different solvent extracts of *Mentha piperita* L. leaves.

Phytochemical Test	Acetone	Hexane	Chloroform	Ethanol	Aqueous
Alkaloids	++	++	-	+	+++
Flavonoids	++	++	-	-	++
Phenols	++	-	+	+	++
Saponins	+	++	-	+	+
Steroids	+	+	+	+	+++
Tannins	++	+	++	+	-
Terpenoids	++	++	+	+++	+
Carbohydrate	++	+++	+	++	++
Protein	+	+	++	++	+
Presence: +++ High, ++ Moderate, + Least, - Absent					

Table 2: Total Phenolic and Flavonoid content of Different solvent extract of *Mentha piperita* L. leaves.

Total Content	Acetone	Hexane	Chloroform	Ethanol	Aqueous
Phenolic (mg/g)	7.82±0.20	0.67±0.08	1.64±0.06	6.82±0.16	3.40±0.73
Flavonoids (mg/g)	2.21±0.22	0.19±0.03	3.00±0.05	1.75±0.14	7.34±0.10

Table 3: Antimicrobial activity of *Mentha piperita* L. leaf extracts

Zone of inhibition (mm)					
Extract	***Bacillus cereus***	***Clostridium perfringens***	***Staphylococcus aureus***	***Aspergillus niger***	***Rhizopus stolonifer***
Acetone	16	12	10	R	R
Hexane	13	08	R	R	R
Chloroform	14	13	07	R	00
Ethanol	R	R	R	05	R
Aqueous	R	R	R	R	06

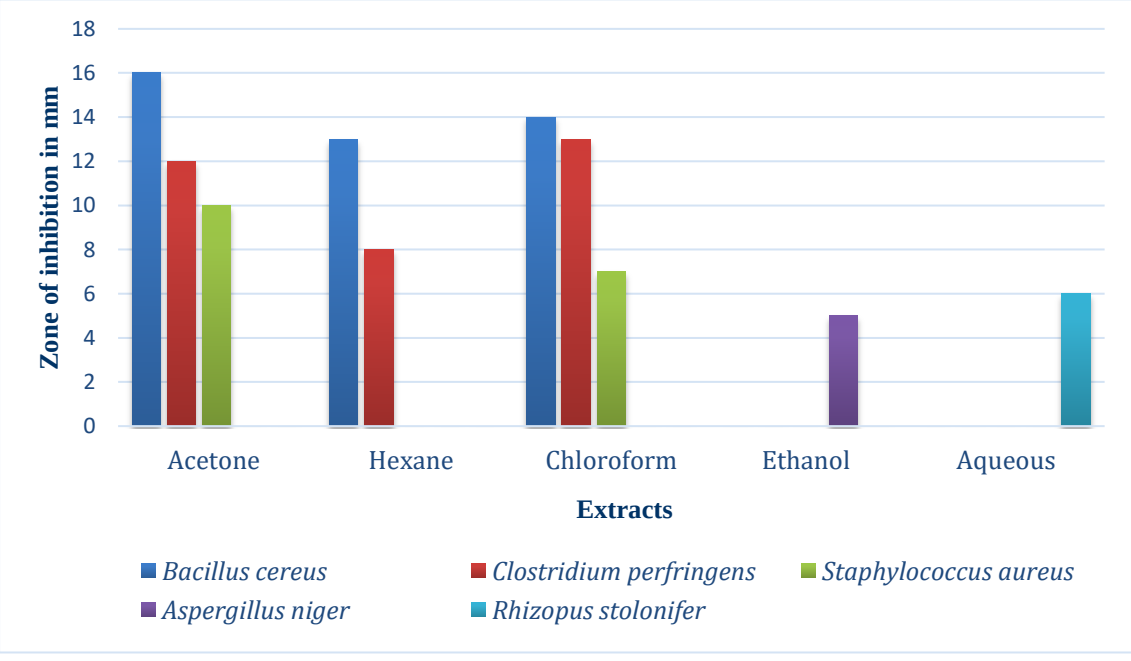

Figure 1: Antimicrobial activity of *Mentha piperita* L. leaf extracts

DISCUSSION:

Preliminary qualitative phytochemical analysis of the different successive crude extracts of seed of *Mentha piperita* L. revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, phenols, saponins, steroids, tannins and terpenoids. These secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses (Kamal *et al*., 2015). The phytochemical properties and antioxidant potential of *Mentha* plants make them the preferred candidates for nutritional and pharmaceutical applications. Due to the presence of these compounds, *Mentha piperita* L. plant show biological activity against various diseases and have been found to be effective in treating various diseases in humans (Lakshmi and Shalini, 2016).

The total phenolic content was estimated according to the method of Singh *et al.* (2011). Among the different crude extracts, the acetone extract showed highest phenolic content of 7.82 mg/g, followed by ethanol extracts of 6.82 mg/g, which is then followed by aqueous extracts of 3.40 mg/g and with chloroform and hexane extracts showed least content which is 1.64 mg/g and 0.67 mg/g respectively. The total flavonoids content was estimated according to the method of Chang *et al.* (2002). Total flavonoids content were found to be highest in aqueous extracts of 7.34 mg/g, followed by chloroform 3.00 mg/g, followed by acetone extract 2.21 mg/g, which is then followed by ethanol extract of 1.75 mg/g and the least flavonoids content was found in hexane extract 0.19 mg/g. They have been reported to have many biological effects on the plants as well as other living organisms. They help in the growth and reproduction of plants and are produced as a response for defense against pathogens (Singh *et al*., 2013; Sameeh *et al*., 2016).

The antimicrobial activity of different *Mentha piperita* L. leaf extracts were examined against different microbes. Among all the studied extracts, the acetone extract showed the higher zone inhibition against *Bacillus cereus*. While least inhibition showed by the aqueous and ethanol extract. The overall extracts showed high antimicrobial response in bacteria while in case of fungi, it showed very least response.

These Phyto-constituents are also known as plant secondary metabolites and are reported to have many biological and medicinal properties. So this species can be expected to have many therapeutic uses and can be further studied for the production of pharmaceutical drugs.

Conflict of interest

The author declares that there is no conflict of interest.

REFERENCES

Basheer A Al-Sum, Abdullah A Al-Arfaj (2013) Antimicrobial activity of the aqueous extract of mint plant. Sci J Clin Med; 2:110-3.

Burt S (2004) Essential oils: their antibacterial properties and potential application in food. Journal of Applied Microbiology 94: 223-253.

Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in *Propolis* by two complementary colorimetric methods. J Food Drug Anal. 10: 178-82.

Clark RK, Menory RC (1980) Environmental effects or peppermint (*Mentha piperita*). Aust J Plant Physiol; 7:685-92.

Firdaus J, Rubina L, Kumar V and M Junaid (2011) Evaluation of antimicrobial activity of plant extracts on antibiotic susceptible and resistant *Staphylococcus aureus* strains. *J. Chem. Pharm. Res*., 3(4): 777-789.

Goossens H, Ferech M, Vander Stichele R, Elseviers M (2005) Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet Group Esac Project 365:579-587.

Gulluce M, Sahin F, Sokmen M, Ozer H, Daferara D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H (2007) Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia.* Food Chem., 104(4): 1449-1456.

Harborne AJ (1973) Phytochemical methods: a guide to modern technique of plant analysis. New York: Chapman Hall.

Hawkey PM, Jones AM (2009) The changing epidemiology of resistance. J. Antimicrob. Chemother. 64:3–10.

Kamal AM, Chowdhury KAA, Shill LK, Hossain MR, Islam N and Anaytulla IA. (2015). Phytochemical screening, cytotoxic and thrombolytic activity of extract of *Brassica oleracea* flower (cauliflower). Global Journal of Pharmacology. 9: 115-20.

Kirethekar Basu I (1985) Indian Medicinal Plants; 1985. p. 714-6.

Kordali S, Kotan R, Mavi A, Cakir A, Ala A and Yildirim A (2005) Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A dracunculus, Artemisia santonicum* and *Artemisia spicigera* essential oils. Journal of Agricultural and Food Chemistry 53: 9452-9458.

Lakshmi DS and Shalini JV (2016) In-vitro antioxidant studies of fresh *Brassica oleracea* and the

characterization of its bioactive compounds using Fourier transform infrared spectroscopy (FTIR). Journal of Chemical and Pharmaceutical Research. 8(5): 900-05.

Rasooli I (2008) Dental Biofilm prevention by *Mentha spicata* and *Eucalyptus camaldulensis* essential oils. Int. J. Infect. Dis., 12(1): 167.

Ratty AK, Das NP (1988) Effects of flavonoids on nonenzymatic lipid peroxidation: structure-activity relationship. Biochem Med Metab Biol. 39(1): 69-79.

Sameeh MY, Mohamed AA and Elazzazy AM (2016) Polyphenolic contents and antimicrobial activity of different extracts of *Padina boryana Thivy* and *Enteromorphasp* marine algae. Journal of Applied Pharmaceutical Science. 6(9): 87-92.

Singh KL, Singh LR, Devi PG, Devi NR, Singh LS and Bag GC (2013) Comparative study of phytochemical constituents and total phenolic content in the extracts of three different species of genus *Hedychium*. Int J Pharm Tech Res. 5(2): 601-06

Singh T, Kasture SB, Mohanty PK, Jaliwala Y, Karchuli MS. (2011) In -Vitro antioxidative activity of phenolic and flavonoid compounds extracted from fruit of *Garcinia indica.* Int J pharma life sci. 2(3): 613-6.

Tumah H (2005) Fourth-generation cephalosporins: *in vitro* activity against nosocomial Gram-negative bacilli compared with beta-lactam antibiotics and ciprofloxacin. Chemotherapy; 51:80-5.