ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Phytochemical Screening of Few Compounds and Analysing the Essential Oil Composition of *Mentha x piperita*

Jinisha Parikh[1]* & Yachana Jha[2]
Department of Biotechnology N. V. Patel College of Pure and Applied Sciences, Vallabh Vidyanagar – 388120, Gujarat, India.
Bioinformatics and Genetics N. V. Patel College of Pure and Applied Sciences, Vallabh Vidyanagar – 388120, Gujarat, India.
***Corresponding author: E-Mail: jinishaparikh@gmail.com, Contact Number: 9825992868**

ABSTRACT

The present study was conducted to screen the phytochemical constituents of *Mentha x piperita* and analyse its essential oil composition. Plant material was subjected to qualitative phytochemical screening for mainly alkaloids, flavanoids, and terpenoids. Phyto-active constituents such as phlobatanin and anthraquinones were found to be absent. The oil was extracted through hydrodistillation and analyzed through gas chromatography mass spectrometry. GC/MS results revealed the main constituents as terpenes and its derivatives. Presence of β-Cubebene, sesquiterpenoids along with other important constituents such as piperitone oxide, monoterpene ketone as well as carvacrol was noted. Piperitone is the essential raw material for the production of synthetic menthol. Each of these constituents has medicinal property such as antioxidant, antibacterial and antifungal providing plant with its medicinal property.

KEY WORDS: *Mentha x piperita*, phytochemical constituents, GC-MS, Terpenoids.

1. INTRODUCTION

Plants have been source of medicinal agents since prehistoric era. Primary and secondary metabolites produced by plants for their growth and development have been used in treatment of various ailments in folk medicines for humans (Leporatti, 2009). These phyto-active constituents are used in modern pharmaceutics as well. Many medicinal plants are used as spices in the culinary industry. According to WHO 80% of world population still relies on medicinal plants for their primary health care. The essential oils are secondary metabolites whose production is associated with primary metabolism and with availability of soil nutrients. They are aromatic compounds produced are used in aromatherapy. Plants synthesize essential oils for a variety of purposes, including protection of the plant against fungi and bacteria, allelopathic activity, defence against insects (terpenoids) and attraction of pollinators and dispersal agents to favour the dispersion of seeds and pollens. The major activities of essential oils are antimicrobial, sedative, anti-inflammatory, bactericidal (Sweetie, 2008), antiviral, antifungal (fungicidal) (Marina, 2009), and preservative for foods. Some substances produced by these plants can be synthesised chemically. Natural products from medicinal plants are known to be chemically balanced, effective and least injurious with none or much reduced side effects as compared to synthetic medicines. Hence, recently there has been more focus on the medicines derived from plants as they are relatively safer to use than the synthetic alternatives. These phyto-active constitutes are complex mixture of chemical compounds and hence techniques such as GC-MS, is used to study such components (Sermakkani and Thangapandian, 2012).

Mentha x piperita is a natural hybrid, formed by cross between *Mentha aquatica* and *Mentha spicata* belonging to family Lamiaceae (Kirethekar and Basu, 1985). It is herbaceous rhizomatous perennial plant which grows around 50 cm tall. The stem is smooth and hairy with square appearance in cross section. The leaves may vary in colour from dark to light green with length of about 4-9 cm and width of about 1.5-4 cm. The leaf has acute apex and margin type is dentate. Peppermint grows rapidly and is invasive. Since peppermint is a hybrid plant it does not produce seeds and is reproduced only through vegetative propagation through runners. The stems and leaves of *mentha* consist of glandular trichomes which produce essential oils which are of great economic importance. The chemical constituents of essential oils of mentha piperita are used in various drugs in pharmaceutical industries, traditional medicines as well as in aromatherapy (Padalia, 2013). The essential oils found in the genus *mentha* have a great importance as a source of bioactive constituents, especially due to its biological properties such as antimicrobial, cytotoxic insecticide, antispasmodic, carminative, antiseptic and anti-inflammatory properties. It is used to treat various ailments such as allergies, common cold, indigestion, irritable bowel syndrome and oral health. Essential oils are also used in fragrance industries (Uphof, 1968). Present study is conducted to investigate phytochemicals present in *Mentha piperita* and also to study the composition of essential oil obtained from its aerial parts using GC-MS.

2. MATERIAL AND METHODS

Several tests for screening the phyto-active components were carried out using powdered samples in aqueous, acidic and alcoholic extracts.

Phytochemical Screening:

Extract preparation: Aerial parts of menthe (leaves and stem) were collected and washed with running tap water to remove the adhering soil and dust particles and then dried at room temperature under shaded conditions for 15 days. The dried sample was then ground to fine powder with the help of mixer grinder. The ground sample was then used for various phytochemical screening.

Acid Extract: For acid extract coarse powder was taken and HCl was added to it and mixed properly. It was allowed to stand for 20 minutes and then filtered. This filtrate was used for further tests.

Alcoholic extract: It was prepared by adding methanol to the coarse powder plant sample and then was allowed it to stand for 30 minutes. Then it was filtered and evaporated. After evaporation the remaining was scrapped using chloroform and used for further tests (Trease & Evans, 1989).

Aqueous extract: The aqueous extract was prepared by adding water to powdered sample and then the mixture was boiled 20 minutes and filtered using Whattman filter paper No 1.

Test for Carbohydrates: In test tube 2 ml of aqueous filtrate was taken and few drops of Molisch reagent was added and mixed thoroughly. Slowly few drops of concentrated H_2SO_4 was added to it through the walls of test tubes. Appearance of violet ring indicated the presence of carbohydrate

Test for tannins: In 2ml of aqueous extract few drops of $FeCl_3$ was added. The appearance of blue colour indicated the presence of hydrolysable tannins and dark green colour indicated the presence of condensed tannins.

Test for saponins (Froth Test): In test tube 2 ml of aqueous extract was taken. It was then shaken vigorously for 15seconds and later it was allowed to stand for 2min. Formation of stable foam (approximately 1cm) indicated the presence of saponins.

Test for Flavonoids: Approximately 2 ml of acid extract was taken and added to two test tubes each. To the first test tube distilled water was added which served as blank. To the second test tube NaOH was added which served as a means to test the presence of flavonoids. Presence of dark yellow colour indicated presence of flavanoids.

Test for Alkaloids: (Dragendroff"s Test): Alcoholic extract was spotted on filter paper using capillary tube and sprayed with Dragendroff's reagent. The formation of pink colour exhibited positive result for alkaloids.

Test for steroids (Liebermann's Test): To the 2 ml of alcoholic extract few drops of acetic anhydride was added. Now add few drops of conc. H_2SO_4slowly along the walls of the test tube. Blue green colour formation at the junction indicated presence of steroid

Test for Terpinoids (Salkowski Test): To 2ml of alcoholic extract few drops of chloroform was added followed by addition of conc. H_2SO_4drops carefully through the walls of test tube. Reddish brown colour indicates the presences of terpinoids.

Test for Anthraquinines Glycosides: In a test tube 2 ml of alcoholic extract was taken and 1 ml of ammonia was added to it. Appearance of rose red colour in the aqueous layer indicated positive test for Anthraquinones Glycoside

Sample Preparation for GC-MS Analysis: For GC-MS analysis fresh aerial parts such as leaves and stems were collected and subjected to hydrodistillation with the help of Clevenger type apparatus for 3 hours and the crude oil extract was collected. Further it was distilled using ethyl acetate and then dried over anhydrous sodium sulphate. The distillate obtained was stored in refrigerator at 4°C for further analysis (Adams, 1991).

GC-MS Analysis: GC-MS analysis was carried out on a GC of Aglient Technologies which comprised of an auto sampler and gas chromatography interface attached to a mass spectrophotometer (GC-MS) instrument which had Column Aligent HP 5 ms (325°C, 30m x 250µm x 250 µm x 0.25 µm) operating in electron impact mode at 70 eV where helium (99.999%) was used as carrier gas at a constant flow of 1 ml/minute and an injector temperature of 250 °C. Mass spectrum was recorded at 70 eV with a scan interval of 0.5 seconds.

3. RESULTS AND DISCUSSION

Biochemical constituents derived from the plants are very much known for its medicinal properties and are used for treatment of various ailments in Ayurveda as well as they serve as base for modern pharmaceutics (Didry, 1998). The present study conducted, screened the major phyto-active constituents of *Mentha x piperita* where most of the test for secondary metabolites including carbohydrates, alkaloids, terpenoids, flavanoids, tannins and saponins, showed positive results except anthraquinies glycosides and phlobatanins. It was also observed that metabolites such as tannins were present in the condensed form. The results for the qualitative analysis are as shown in table 1.

Table.1. Results of Qualitative analysis of *Mentha x piperita*

Phytochemicals	Inference	Phytochemicals	Inference
Carbohydrates	+	Tannins	+ (condensed)
Alkaloids	+	Saponins	+
Terpenoids	+	Flavanoids	+
Anthraquinine Glycosides	-	Phlobatanin	-

(Note: + indicated the presence of the compound, - indicates absence of the compound)

GC-MS Analysis: GC-MS analysis of the extracted oil was carried out and the compounds detected are listed in table 2 below.

Table.2. Composition of essential oil of *Mentha x piperita*)

RT	Compound Name	Area %	M.W
4.077	Carvacrol	12.05	C10H14O
4.651	p-Menthane-1,2,3-triol	13.91	C10H20O3
5.29	1-Acetoxy-p-menth-3-one	60.13	C12H20O3
5.501	Tricyclo[7.2.0.0(2,6)]undecan-5-ol, 2,6,10,10-tetramethyl-	8.21	C15H26O
5.736	4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)butan-2-one	9.61	C13H22O3
6.343	β-Cubebene	100	C15H24
6.75	3,7,11,15-Tetramethyl-2-hexadecen-1-olC15H24	32.75	C20H40O
7.104	p-Cymenene	10.77	C10H12
7.767	Levomenthol	12.71	C10H20O
14.604	Piperitone oxide	12.76	C10H16O2

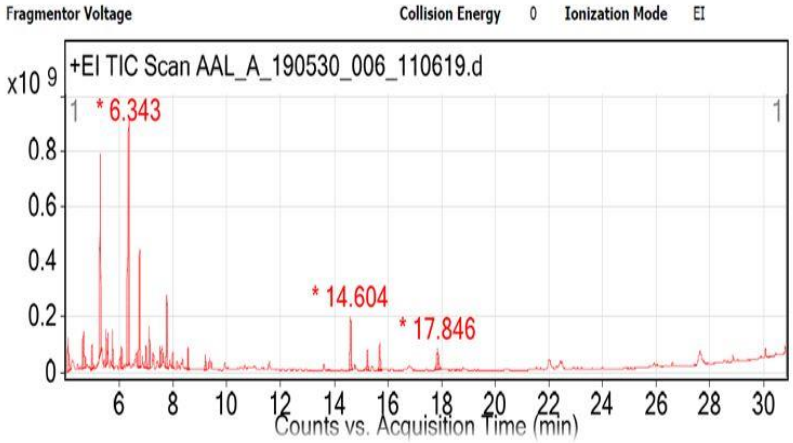

Figure.1. GC-MS of *Mentha x Piperita*

The composition of essential oil in plant varies according to the geographical conditions under which the plant grows. The content of the active constitutes of essential oils may vary depending upon the time of harvest, plant age and atmosphere within the same plant as well as same species grown in different regions. The reports of variation in chemical composition of essential oil have been illustrated earlier. The essential oil of *Mentha arvensis* obtained from Fathepur (Himachal Pradesh) was noted to have major constituents including L- menthone (29.41%), menthol (21.33%) and piperitone oxide (3.62%) and that obtained from and Dhameta (Himachal Pradesh) had L Menthone (29.10%), Menthol (20.25%) and piperitone oxide (6.48%) respectively. This proportion of major constituents was comparatively higher than those samples from Patiala regions. (Sharma, 2009). The major constitutes of essential oils of *Mentha x piperita* studied in Siberia was Menthol and Menthone. β-Cubebene and p-Cymenene presence was studied in the essential oil of *S. buxifolia* which might be considered as potential antioxidant (Boligon, 2012). Generally, terpinoids present in plants are denatured upon oxidation. p-Menthane is one such diverse class of monoterpenes found in plants that is synthesized in specialised structure called glandular trichomes present on stems and leaves of genus Mentha. Production and proportion of menthol and menthofuran depends upon the age of the leaves as well as the day length. The enzyme activity increases with changing climatic conditions such as activity of acetyltransferase that is responsible for rapid conversion of menthol to menthyl acetate in the old leaves of plant subjected to short day-long day inversion (Voirin, 1990). The change in composition might also be affected due to abiotic and biotic stresses. Valladares (2002) observed the change in the composition and concentration of essential oils with respect to herbivore activities. The presence of gall insects decreased the pulegone concentration, indicating the changes in the biosynthesis pathway of essential oil production for *Mintosathchys mollis*, member of Lamiaceae family that is found to have essential oil composition similar to mint oil. The presence of β-Cubebene was recorded in the *M. microphylla* of Gennargentu Mountains of Italy in minor amounts (Tomei, 2003). In the present study the essential oil composition of *Mentha piperita* showed presence of β-Cubebene in maximum percentage area which might be due to environmental conditions. *Mentha piperita* has been used in traditional as well as modern pharmaceutics. The antioxidants and anti-inflammatory compounds are noted to stabilize the free radicals that damage the biological systems (Fale, 2013). Presence of such antioxidant compounds have also been studied in essential oils of some medicinal plants. Earlier study on essential oil of *Mentha piperita* evaluated some major

components such as menthone and menthol (Lawrence, 1997; Gerherman, 2000). Asha (2015) has also reported the antioxidant properties and flavanoids of *Mentha arvensis* along with three other herbs. Piperitone oxide contains allylic and -carbonylic hydrogens present in the essential oils extracted from Himalyan silver mint that is noted to possess high antioxidant properties. When mint oil is applied topically to the skin in form of lotion or ointment it has calming and cooling effect on area affected by the rash or insect bite. Mint plants contain an antioxidant and anti-inflammatory agent called rosmarinic acid. This has been studied for its effectiveness in relieving seasonal allergy symptoms, revealing a promising natural treatment. Compounds such as Limonene consist of antimicrobial properties (Fleming, 1998; Tayarani, 2013) recently has observed and reported that in the patients undergoing chemotherapy treatment who inhaled peppermint essential oil showed a reduction in emetic events which resurged due to chemotherapy treatment. Mint is also a natural anti-microbial agent and is used as breath freshener.

4. CONCLUSION

The present study on aerial parts of *Mentha x piperita* concluded presence of carbohydrates, alkaloids, terpenoids, tannins, saponins and flavonoids in the preliminary phytochemical screening. The GC MS analysis results of essential oils obtained from *Mentha* stem and leaves showed the presence of Levo-menthol, and 1-Aceto-p-menth-3-one and β-Cubebene in higher amounts and piperitone oxide which shows the relative potential of antioxidant activities.

REFERENCES

Adams RP, Cedar wood oil-analysis and properties, In Modern methods of plant analysis oils and waxes Linsking HF, Jackson JE (Eds.) Springer-Verlag, 1991.

Asha D, Mathew L, Rishad KS, Evaluation of HPTLC Fingerprints of Flavonoids and Antioxidant Activity of Selected Medicinal Plants of Lamiaceae Family, International Journal of Pharmacognosy and Phytochemical Research, 7 (2), 2015, 240-245.

Barrosa A, Selene M, Ferreiraa P, Vieirab P, Craveiroc Afranio Aragao, Raquel Oliveira dos Santos Fontenelled, Jane Eire Silva Alencar de Menezesa, Silvaa Afrânio Aragao, Sousa Afranio Aragao, Chemical composition and functional properties of essential oils fromMentha species, Industrial Crops and Products, 76, 2015, 557-564.

Boligon A, Schwanz Thiago Guilherme, de Brum Thiele Faccim, Frohlich Janaína Kieling, Nunes Letícia, Mario Debora Nunes, Hartz Alves Sydney and Athay de Margareth Linde, Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of Scutia buxifolia Reissek Leaves, Pharmaceut Anal Acta, 3 (10), 2012.

Derwich, Benziane E, Taouil E, Senhaji R.O and Touzani M, Aromatic Plants of Morocco, GC/MS Analysis of the Essential Oils of Leaves of Mentha piperita Advances in Environmental Biology, 4 (1), 2010, 80-85.

Didry N, Dubrenil L, Trotin F, Pinkas M, Antimicrobial activity of aerial parts of Drosera Peltata Smith on Oral Bacterial, J. Ethanopharmacol, 60 (1), 1998, 91-96.

Edeoga HO, Okwu DE, Mbaebie BO, Phytochemical constituents of some Nigerian medicinal plants, African Journal of Biotechnology, 4 (7), 2005, 685-688.

Fale P.L, Ferreira C, Rodrigues A.M, Cleto P, Madeira P.J.A, Florencio, Frazao M.H, Serralheiro M.L.M, Antioxidant andante acetylcholinesterase activity of commercially available medicinal infusions after in vitro gastrointestinal digestion, J. Med. Plants Res., 7 (20), 2013, 1370-1378.

Fleming T, PDR for herbal medicines, Medical Economic Company, JNC 1998 Gerherman C, Julea M, Cozar O, Comparative analysis of some active principles of herb plants by GC-MS, Talanta, 53, 2000, 253-262

Golparvar1 Ahmad Reza, Hadipanah Amin, Chemical compositions of the essential oil from peppermint (Mentha piperita L.) cultivated in Isfahan condition Golparvar & Hadipanah, Journal of Herbal Drugs, 4 (2), 2013, 75-80.

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

Lawrence BM, Progress in essential oils, Peppermint oil, PerfumFlav, 22, 1997, 57-66.

Leporatti M.L, Ghedira K, Comparative analysis of medicinal plants used in traditional medicine in Italy and Tunisia, J. Ethnobiol Ethnomed., 5, 2009, 31-39

Marina D. Sokovic, Jelena Vukojevic, Petar D. Marin, Dejan D. Brkic, Vlatka Vajs, and Leo J. L. D. van Griensven, Chemical Composition of Essential Oils of Thymus and Mentha Species and Their Antifungal Activities, Molecules, 14 (1), 2009, 238–249.

Padalia R.C, Verma R.S, Chauhan A, Sundaresan V, Chandan S.C, Essential oil composition of sixteen elite cultivars of Mentha fromwestern Himalayan region, India. Maejo Int. J. Sci. Technol., 7, 2013, 83-93.

Sermakkani M, Thangapandian V, GC-MS Analysis of Cassia italica leaf methanol extract, Asian Journal of Pharmaceutical and Clinical Research, 5 (2), 2012, 90-94.

Sharma V, Sharma N, Singh H, Srivastava D, Pathania V, Singh B, Gupta R, Comparative account on gc-msanalysis of mentha arvensis l. corn mintî from three different locations of north indiaint j. drugdev& res., sep-dec, 1 (1), 2009, 1-9.

Sweetie R. Kanatt, Ramesh Chander, Arun Sharma, Chitosan and Mint Mixture: A new preservative for Meat and Meat products, Food Chemistry, 107, 2008, 845-852.

Tayarani N.Z, Firoozi T.E, Jalali N.N and Hassanzadeh M.K, Antiemetic activity of volatile oil from Mentha spicata and Mentha piperita in chemotherapy-induced nausea and vomiting, *Ecancermedicalscience,* 2013.

Tomei T. E and Manganelli R, Composition of the essential oil of *mentha microphylla* from the gennargentu mountains (sardinia, italy) J. Agric. Food Chem., 51, 2003, 3614-3617

Trease GE and Evans WC, Text Book of Pharmacognosy, 13th edition, Bailliere Tindall, London, 1989, 345-346.

Uphof, J.C.T, Dictionary of Economic Plants, Verlag von Cramer, Germany, 1968, 290.

Valladares G.R, Zapata A, Zygadlo J and Banchio E, Phytochemical Induction by Herbivores Could Affect Quality of Essential Oils from Aromatic Plants J. Agric. Food Chem., 50, 2002, 4059-4061

Voirin Bernard, Brun Nathalie and Bayet Christine, Effects of day length on the monoterpene composition of leaves of *mentha x piperita* Phytochemistry, 29 (3) 1990, 749-755.