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HPLC and GC-MS Profiles for New Potential Sources of Anti-aging & Antioxidant Medicines in *Mentha Piperita* and *Ocimum Basilicum var. Thyrsi Flora*

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Abstract: Phytocompounds of natural origin are not only a potential source for maintaining human health but also the supplement of nutrients for long period survival. In recent years, the essential oils and herbal extracts have attracted a great scientific interest due to its potential as a source of natural antioxidants and biologically active compounds, especially in the last decade with more intensive studies for natural chemotherapies and antioxidant activity. The antioxidant medicine in all over the world is now a day's reviewed by an extensive research on phytochemicals of different plant species and their therapeutic principles. About 80% of the world's population relies that there is a possibility of finding a cure of degenerative diseases from natural antioxidants of herbal origin. In this series we have tried to identify and quantify the standard phytochemicals having antioxidant activity in Mentha Piperita and Ocimum basilicum var Thyrsi flora to find new potential sources of natural antioxidants medicines which check the human cells damage caused by reactive oxygen species (ROS) and harmful radiations for expending the life time (anti-aging) by using HPLC and GC-MS analytical techniques.

Keywords: Standard Phytochemicals, Natural Products, HPLC and GC-MS Techniques, *Mentha Piperita*, *Ocimum Basilicum var. Thyrsi flora*, Phenolic Compounds, Natural. Antioxidants, Reactive Oxygen Species (ROS)

1. Introduction

Since an Ayurvedic period of time, plants and herbs have not been only a valuable source of natural products for maintaining human health but also the supplement of nutrients for long period survival. In recent years, the essential oils and herbal extracts have attracted a great scientific interest due to its potential as a source of natural antioxidants and biologically active compounds [1] especially in the last decade with more intensive studies for natural chemotherapies and antioxidant activity. India has a rich heritage of traditional herbal medicinal systems constituting with its different components like Homeopathy, Ayurveda, Siddha and Unani for curing diseases. Ayurveda and other Indian systems of medicines may be explored to cover various areas like tropical diseases, Cancer, AIDS, Bronchial Asthma etc. With the modern scientific approaches for better leads in the health care [2]. The use of herb parts (leaves stem, root, etc.) as medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history. According to World Health Organization medicinal plants would be the best source to obtain a variety of chemotherapeutic agents [4] for anti-aging, oxidative stress, antioxidants, hyper tension and diseases of aging. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants or herbs. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

The antioxidant medicine all over the world is now a day's reviewed by an extensive research on phytochemicals of different plants species and their therapeutic principles.

About 80% of the world's population relies that there is a possibility of finding a cure of degenerative diseases from natural antioxidants of herbal origin, and governments of third world countries, unable to sustain a complete coverage with Western type anti-aging drugs, have encourages the rational development of traditional treatments [5]. In this sequence there is great need of standardization of percentage of chemical constituents responsible for antioxidant activity and anti-aging, which does not remain uniform as our expectation [6]. The individual plant powders in terms of antioxidant poly phenolic phytochemicals were subjected to various pharmacognostical parameters after determine its antioxidant potential in terms of Radical Scavenging Activity (RSA) and standard phenolic compounds. However, the pharmacological data in Peppermint (*Mentha piperita*) and Thai basil (*Ocimum basilicum var. Thyrsiflora*) are deficient in clearly establishing the scientific rationale for the anti-oxidant medicinal use of these plants; the search for its active constituents is also limited [7]. Hence, there is a continuous and urgent need to discover new potential source of anti-aging compounds with antioxidant potential and novel mechanisms of action for new natural antioxidant medicines.

1.1. Phytochemicals Composition in Mentha Piperita

Peppermint yields 0.1–1% of volatile oil [8] composed primarily of menthol (29–48%), menthone (20–31%), mentho furan (6.8%) and menthyl acetate (3–10%). Other pharmacologically active ingredients include bitter substances, Caffeic acid flavonoids (12%), polymerized polyphenols (19%), carotenes, tocopherols, betadine, choline and tannins [9-11]. Measured low to moderate levels of phenolics with antioxidant activity were reported from pepper mint [12]. The chemistry of peppermint oil is very complex and highly variable. The relative concentrations vary depending on climate, cultivar, and geographic location [13-15]. Peppermint oil and its constituents are commercially used in food, pharmaceutical and cosmetics industries. Menthol is used as a raw material in toothpaste, tooth powder, chewing tobacco, confectionary, mouth fresheners, analgesic balms, cough drops, perfumes, chewing gums, candies and tobacco industry. Tobacco industry constitutes about 40% of the total oil consumption followed by pharmaceutical and confectionary industries. The fresh or dried leaves are the culinary source of mint and are used in breath fresheners, drinks, antiseptic mouth rinses, tooth paste, chewing gum, mint chocolate teas, beverages, jellies, syrups, candies, ice creams and also used as a necessary ingredient in Herbal tea, a popular tea in the northern India and Arab countries. The substances that give the mints their characteristic aromas and flavors are menthol [16].

In Eastern and Western traditional medicine peppermint and its oil have been used as an anti-spasmodic, aromatic, and antiseptic and also in the treatment of cancers, colds, cramps, indigestion, nausea, sore throat and toothaches [17]. Peppermint oil possesses anti-bacterial activity in vitro. Different commercial preparations exhibit various activities [18]. Peppermint oil and menthol have moderate anti-bacterial effects against both Gram-positive and Gram-negative bacteria [19]. Peppermint is also found to possess antiviral and fungicidal activities. Aqueous extracts of the leaves demonstrated significant antiviral activity against Influenza a new castle disease, Herpes simplex, Vaccinia, Semliki Forest and West Nile viruses in egg and cell culture system [20]. It was also found to reduce the incidence and multiplicity of benzopyrene-induced lungs carcinogenicity and mutagenicity [21]. In clinical trials peppermint oil's role in irritable bowel syndrome affirms its effectiveness compared with a placebo with no serious constipation or diarrhea [22-25]. In this paper, the effects of leaves stem, root extracts and essential oil of leaf phytochemical screening of M. Piperita and Thai basil are presented as an anti-ageing and, antioxidant medicines activity.

1.2. Phytochemicals in Thai Basil

Thai basil (Ocimum basilicum var. Thyrsiflora.) is strong aromatic herb and used extensively to add a distinctive aroma and flavour to food. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in development of fragrance & flavour, pharmaceuticals, and cosmetics [26]. Recent scanned literature revealed that no work on phytochemicals investigation, isolation or characterization has been reported yet in Thai basil (*Ocimum basilicum* var. Thyrsiflora.). But lot of work regarding aroma chemicals isolation, characterization as well as pharmaceuticals, and cosmetics application has been published in Basil (Ocimum basilicum) [27]. Traditionally, basil (*Ocimum basilicum*) has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney mal function [28] and also possesses various beneficial effects, e.g., antiseptic, carminative, antimicrobial, and antioxidative properties. [29-30]

2. Materials and Methods

Gallic Acid, Ascorbic Acid, Caffeic Acid, Cumeric Acid and Ellagic Acid were purchased from Sigma-Aldrich, USA. Folin Ciocalteau's phenolic reagent, high performance liquid chromatography (HPLC) solvents and all analytical grade chemicals were from E. Merck, India. 1,1-Dipheny1-2-picryl-hydrazyl (DPPH), Quinol, Resorcinol and Chlorogenic acid were from Sigma-Aldrich, USA. Ellagic acid was obtained from natural remedies, India. Gallic acid and Caffeic acid were from CDH, India. UV spectrophotometers used were Varian Cary50 Spectrophotometer, Systronics smart 2203 double beam spectrophotometer. Plant materials were collected from different districts of Uttar Pradesh viz. Long and Small from Ghazipur district, Jalapeño, Shimla Mirch (Green, Red and Yellow) from Lucknow capital market, further all plant materials were identified by department of botany, university of lucknow, lucknow. Collected samples were washed with distilled water then cut in small pieces and dried in shade at room temperature and grinded with Mixer grinder to a particular mess size then their weight was recorded. (Table 1). The extraction process of all samples were carried out from five different planned

solvent systems. Various experiments (Experiment1-to-10) were performed with extracted samples to study the antioxidant activity in terms of free radical scavenging activities and phytochemicals investigation.

Table 1. *Sample name, their botanical name, Species, abbreviation and weight.*

Sample No.	Name of Sample	Plant Part	Code	Weight (g)
1.	Peppermint (*Mentha piperita*)	Leaves	MP(L)	250.89
2.	Peppermint (*Mentha piperita)*	Stem	MP(S)	280.56
3.	Peppermint (*Mentha piperita*)	Root	MP(R)	210.45
4.	Thai basil (*Ocimum basilicum* var. *thyrsiflora*	Leaves	OB(L)	630.78

Table 2. *Moisture (%) content of fresh weight basis in different plant parts of Mentha piperita and leaves of Ocimum basilicum var. Thyrsiflora.*

S. No.	Sample Name	Plant Part	Moisture (%)
1.	Peppermint (*Mentha piperita*)	Leaves	86.42
2.	Peppermint (*Mentha piperita)*	Stem	81.42
3.	Peppermint (*Mentha piperita*)	Root	79.67
4.	Thai basil (*Ocimum basilicum* var. *Thyrsiflora)*	Leaves	89.74

Table 3. *Absorbance of Standard BHT (at 517 nm).*

SN.	Standard BHT concentration	Absorbance (517nm)
1	50 µg/ml	0.0055
2	100µg/ml	0.0150
3	150 µg/ml	0.0128

2.1. Estimation of Moisture Content [31]

Fresh chopped plant material (1g) was taken, kept in oven at 50-80°C for 12 hrs, weighed (g), moisture% calculated as follows:

$$\text{Moisture\%} = (1\text{-}y)\times 100$$

2.2. Estimation of Antioxidant Activity (AOA) [32]

The antioxidant activity (AOA) in methanol extracts of *Mentha piperita* and *Ocimum basilicum* var. Thyrsiflora plant materials were measured using the1,1-diphenyl-2-picryl hydrazyl (DPPH) and butylated hydroxyl toluene (BHT) as standard at 517 wavelength. The techniques determine the antioxidant activity in terms of the free radical-scavenging capacity.

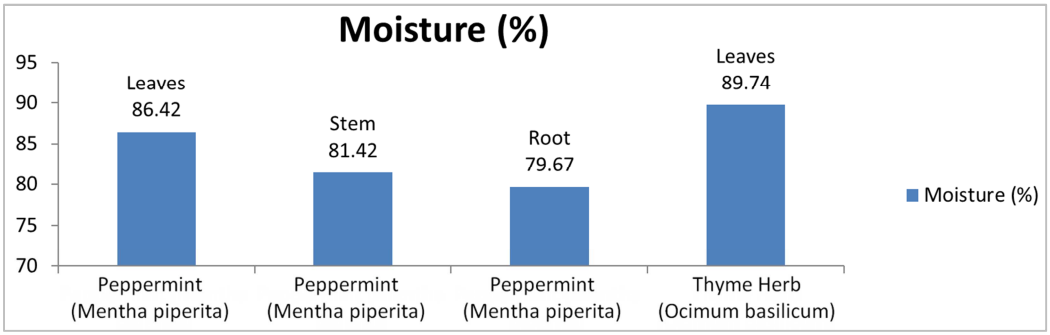


Figure 1. *Moisture (%) content in different plant parts of Mentha piperita and leaves of Ocimum basilicum var. Thyrsiflora.*

2.3. DPPH Method [33]

The scavenging ability of methanol extracts against DPPH free radicals was evaluated as described by Gyamfi *etal*. [34] Briefly, in dried powdered (1.0g) each plant sample of *Mentha Piperita* and *Ocimum basilicum var. Thyrsiflora*, were refluxed separately for 2 hours with methanol (MeOH) (40ml), filtered, then filtrate maintained up to 40 ml by adding fresh MeOH to give extract (A) and the residue was discarded. The extract (A) was diluted with MeOH in three different concentrations as 50µg/ml, 100µg/ml and 150µg/ml. The percentage of inhibition was calculated against a control and compared to BHT (butylated hydroxyl toluene) standard curve. Stock solution of standard BHT was prepared by dissolving 10mg of BHT in10 ml of methanol (1000ppm), then pipette out 0.1 ml from stock solution and diluted it to 10 ml methanol (100 ppm). Similarly 0.001 M DPPH solutions were prepared by dissolving 3.94 mg of DPPH in10 ml methanol. The working standard solution of DPPH was 50µg/ml, 100µg/ml and 150µg/ml. The percentage of DPPH scavenging activities was determined using formula as follows.

$$\%\ of DPPH Scavenging activity = \frac{Absorbance Control - Absorbance sample}{Absorbance Control} \times 100$$

The results are recorded in Tables 3, 4 & 5.

Table 4. *Absorbance at different concentration (517 nm) for free radical scavenging activity by DPPH in various plant samples of Mentha Piperita and Ocimum basilicum var. Thyrsiflora.*

S.NO.	Sample Code	Absorbance at different concentration (517 nm)		
Sample concentration →		**50 µg/ml**	**100 µg/ml**	**150 µg/ml**
1	MP (L) A_1	0.0060	0.0062	0.0064
2	MP (S) A_2	0.0079	0.0065	0.0070
3	MP (R) A_3	0.0066	0.0068	0.0073
4	OB (L) A_4	0.0051	0.0065	0.0074

$$\%\ of DPPH\ Scavenging\ activity = \frac{Absorbance\ Control - Absorbance\ sample}{Absorbance\ Control} \times 100$$

Table 5. *Percentage of DPPH radical scavenging activity in various plant samples of Mentha piperita and Ocimum basilicum var. Thyrsiflora at different concentration.*

% of DPPH Scavenging activity				
S. No.	**Sample Code**	**Sample concentration**		
		50ug/ml	**100ug/ml**	**150ug/ml**
1	MP (L) A_1	61.29	60.00	58.70
2	MP (S) A_2	49.03	58.06	54.83
3	MP (R) A_3	57.41	56.12	52.90
4	OB (L) A_4	67.09	58.06	52.25

Table 6. *HPLC Analysis of standards phenolic compounds present in Mentha piperita and Ocimum basilicum var. Thyrsiflora.*

S. No.	Standard	Concentration	RT	Area	Figure No.
1.	Gallic Acid	30 ppm	2.673	1537839	02
2.	Ascorbic Acid	100 ppm	2.773	542232	03
3.	Caffeic Acid	200 ppm	3.616	12062651	04
4.	Benzoic Acid	200 ppm	6.283	1165350	--
5.	Quinol	200 ppm	6.293	5312450	--
6.	Resorcinol	200 ppm	6.772	3851113	07
7.	Chlorogenic Acid	200 ppm	3.296	5881030	05
8.	Cumeric Acid	200 ppm	4.384	25095596	06
9.	Ellagic Acid	200 ppm	4.409	31616624	--

Table 7. *In plant samples of Mentha piperita and Ocimum basilicum var. Thyrsiflora.*

Standard phytophenolic Compounds	MP(L)	MP(S)	MP(R)	OB(L)
Gallic Acid (ppm)	0.51	0.70	0.47	0.38
Ascorbic Acid (ppm)	6.90	11.59	6.10	4.84
Caffeic Acid (ppm)	4.49	2.41	0.60	0.22
Benzoic Acid (ppm)	---	---	---	---
Quinol (ppm)	---	0.33	0.14	0.18
Resorcinol (ppm)	---	---	---	---
Chlorogenic Acid (ppm)	0.86	1.83	0.95	0.83
Cumeric Acid (ppm)	---	0.86	0.87	0.87
Ellagic Acid (ppm)	---	0.20	0.14	0.12

HPLC Chromatogram of Peppermint (*Mentha piperita*) Leaves

Table 8. *Essential Oil Chemical Constituents of Mentha piperita Leaves.*

Serial No.	Chemical Constituents	KCRI Value	% Abundance
1	Menthol	1173	32.24
2	Cyclohexanone	1282	19.72
3	Menthol Acetate	1279	6.92
4	1,8-Cineole	1030	6.56
5	Cyclohexanol	1403	4.14
6	DL-Limonene	1033	3.98
7	Menthone	1474	3.09
8	Menthofuran	1164	2.18
9	Jasmine	1394	1.50
10	Menthyl isovalerate	793	1.38
11	Isopulegone	1157	1.24
12	Jasmone	1102	0.88
13	Camphene	953	0.34

GC-MS Profile of (*Ocimum basilicum var. Thyrsiflora*) leaves Essential oil

Table 9. *Essential Oil Chemical Constituents of Thai Basil Leaves.*

Serial No.	Chemical Constituents	KCRI Value	% Abundance
1	Sabinene	896	35%
2	1,8-Cineole	1030	11.7%
3	(-)-β-pinene,	628	6.8%
4	Trans caryophyllene	1493	5.95%
5	Trans-α- Bergamotene	1494	2.5%
6	4- Terpeneol	1136	3.6%
7	α- phellandrene	1371	4.8%
8	Limonene	717	4.04%
9	Fenchone	788	2.1%
10	α- Pinene	947	5.55%

2.4. HPLC Analysis [35]

The following methodology was used for obtaining the HPLC chromatogram of each standard phenolic compounds as well as mixture of some standard phenolic compounds using two different gradients of mobile phase in different run times when HPLC analysis was performed. These HPLC fingerprints of standard phenolic compounds could be used as benchmarks for the purpose of comparison when doing the qualitative and quantitative analysis of unknown compounds present in (sample-1-to-sample-9) of selected Mentha species. The multiple chemical constituents were present in the sample could be benchmarked against these standards to assess the identification and quantity estimation of phenolic compounds.

2.4.1. Preparation of Sample Standards

6.0 mg of each standards compound was dissolved in10 ml of HPLC grade methanol resulting in a sample concentration of 600µg/ml. This was sonicated and then passed through what's man nylon membrane filters (0.45µmand 47mm diameter) before injecting it in the column.

2.4.2. HPLC Analysis

The HPLC analysis of 9 standard phenolic compounds and 04 plants samples was used for obtaining the chromatogram of each standard phenolic compound as well as 9 samples. The HPLC analysis was performed using two different gradients of mobile phase in different run times. Total run time was 25 minutes; Gradient elution of two solvents was used–solvent-A (Methanol) and Solvent-B (1%Acetic Acid in water). The gradient programme was started with 60% of A and then change to obtain 35, 10, 60 and 60% of eluent A at 5, 10, 15, and 20 minutes respectively.

2.4.3. Specification of the HPLC Instrument

The analysis of all samples (Sample-1to Sample 4) and standard phenolic compounds (1-9) prepared above was carried out using HPLC Make/ Model SHIMADZU prominence 20 A with Luna 5 u C-18 column. Elution was carried out at a flow rate of 1.0 ml/ min on UV/VIS and PDA at 280 nm with mobile phase MeOH as a solvent A and 1% CH_3COOH in H_2O as solvent B using a gradient elution in 0-5 min with 60% A, 5-10 min with 35% of A, 10-15 min with 10% of A, 15-22 min with 60% of A, and all standard and samples (20µl) were injected.

The retention times (R.T.) and Peak Area (P.A.) under curve of standard phenolic compounds are recorded in Table 6 Figures 2 to 7 and identified compounds, their quantitative estimation of Sample-1to Sample 9 are mentioned in Table 7, Figures 8 to 23.

Table 10. *Identified Compounds and their quantitative estimation in) Leaves sample of Peppermint (Mentha piperita).*

S.No.	Identified Compounds	RT	Peak Area	Quantity (ppm)
1.	Gallic Acid	2.496	196184	0.51
2.	Ascorbic Acid	2.752	939291	6.90
3.	Chlorogenic Acid	3.296	1273866	0.86
4.	Caffeic Acid	3.637	2324283	4.49
5.	Unknown	4.992	189393	-----
6.	Unknown	5.109	303214	-----
7.	Unknown	5.440	337891	-----

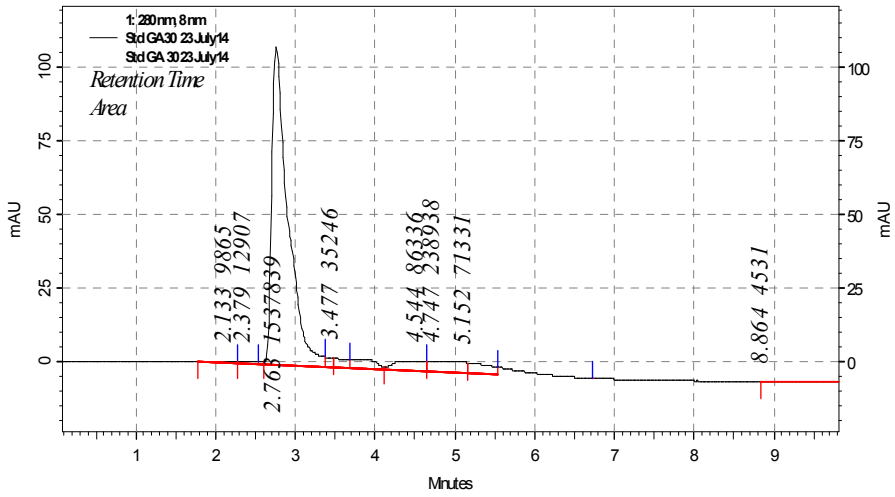


Figure 2. *HPLC Chromatogram of Gallic acid.*

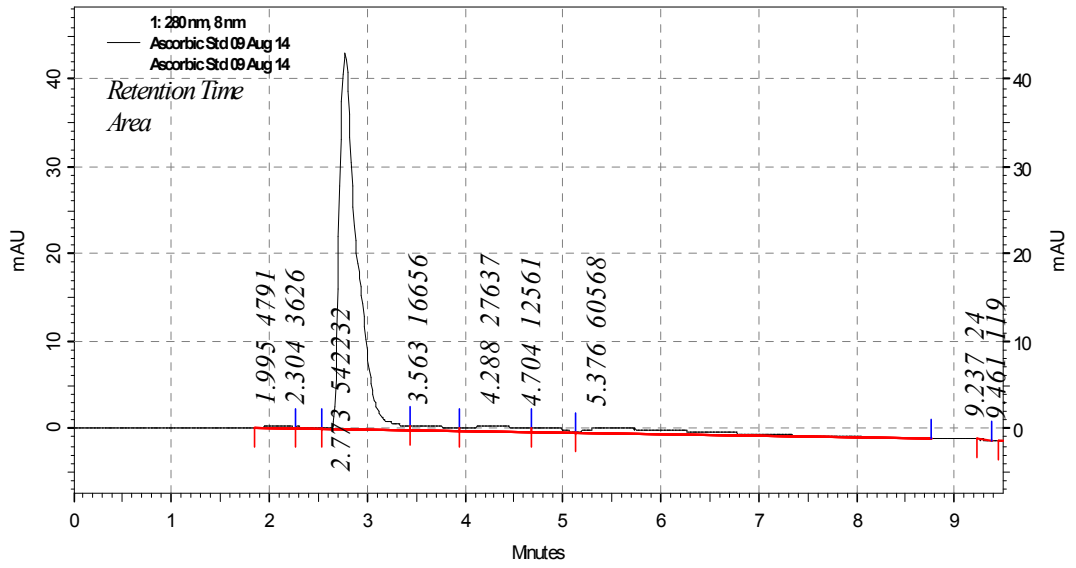


Figure 3. *HPLC Chromatogram of Ascorbic acid.*

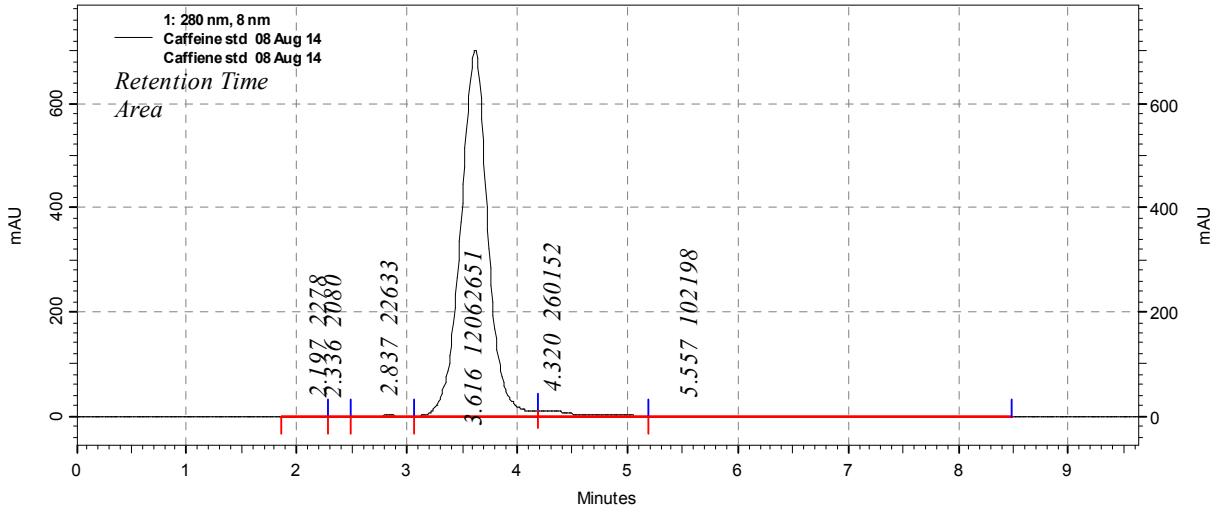
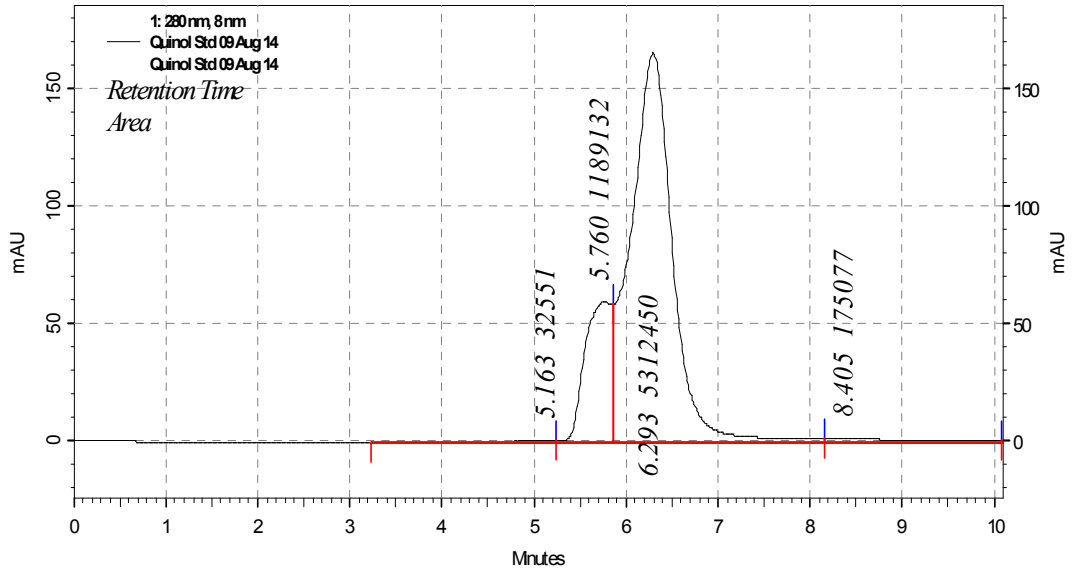


Figure 4. *HPLC Chromatogram of Caffeic acid.*



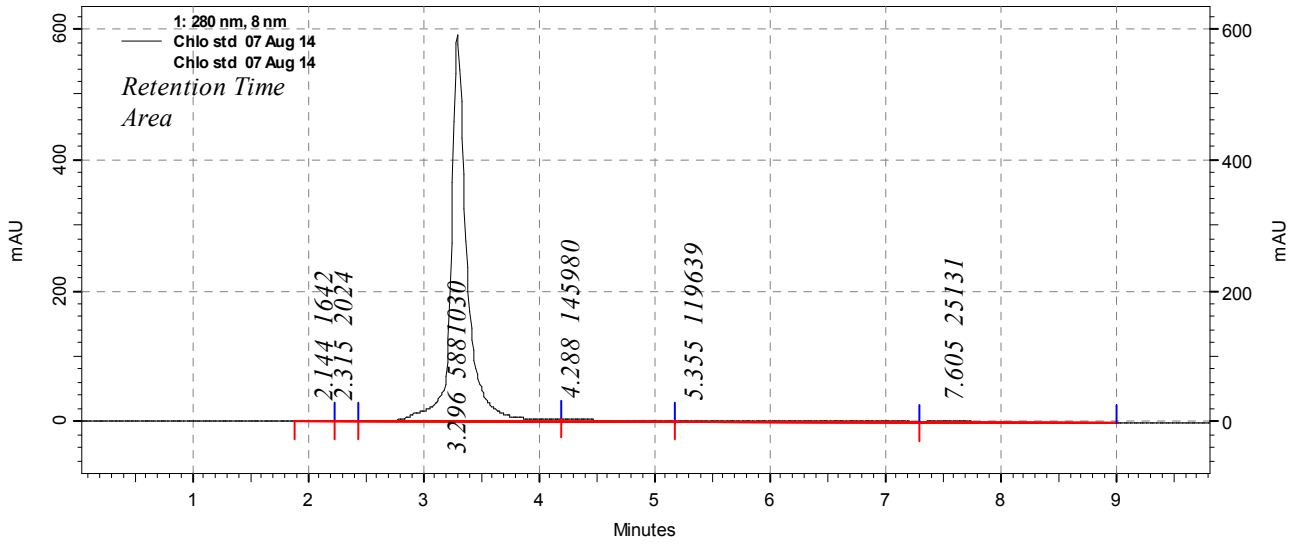


Figure 5. *HPLC Chromatogram of Chlorogenic acid.*

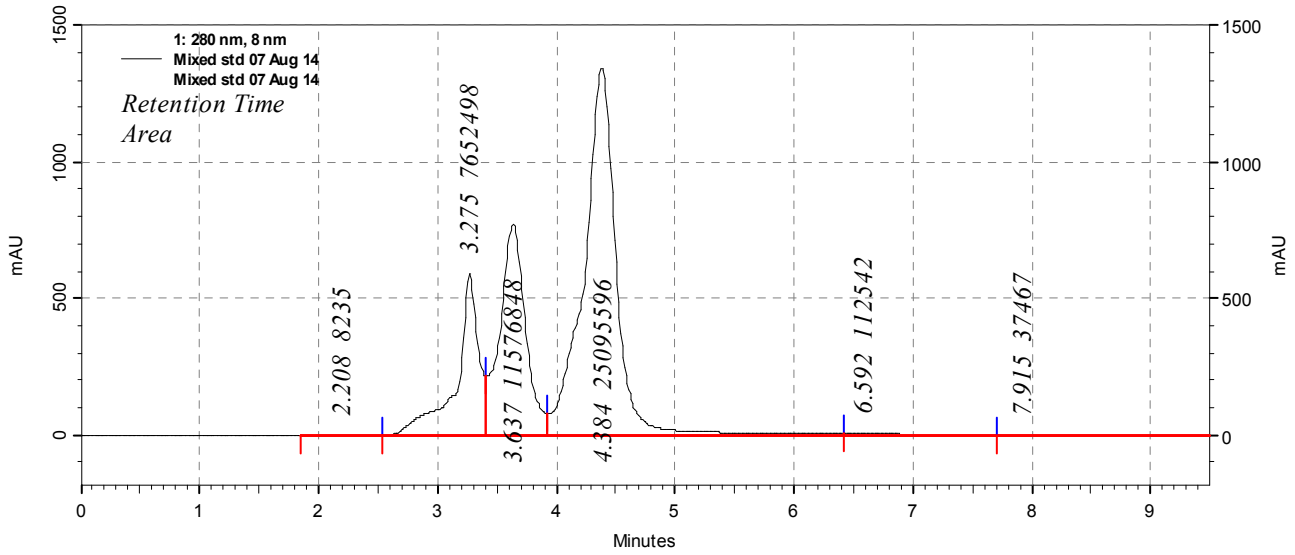


Figure 6. *HPLC Chromatogram of Chlorogenic acid, Caffeic acid, Cumeric acid mixed.*

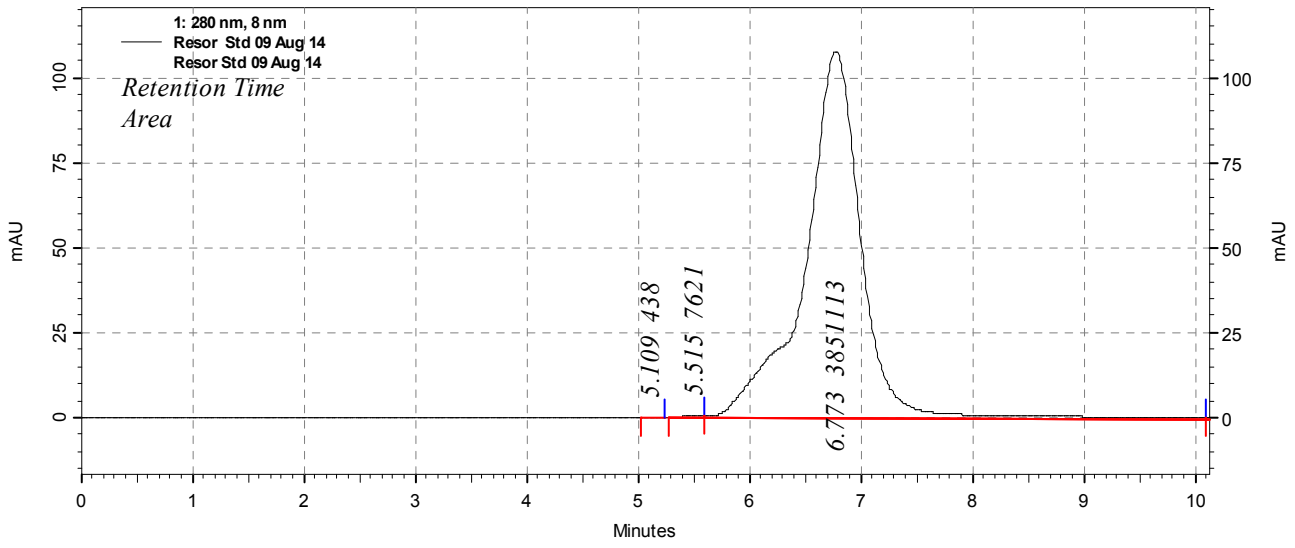


Figure 7. *HPLC Chromatogram of Resorcinol.*

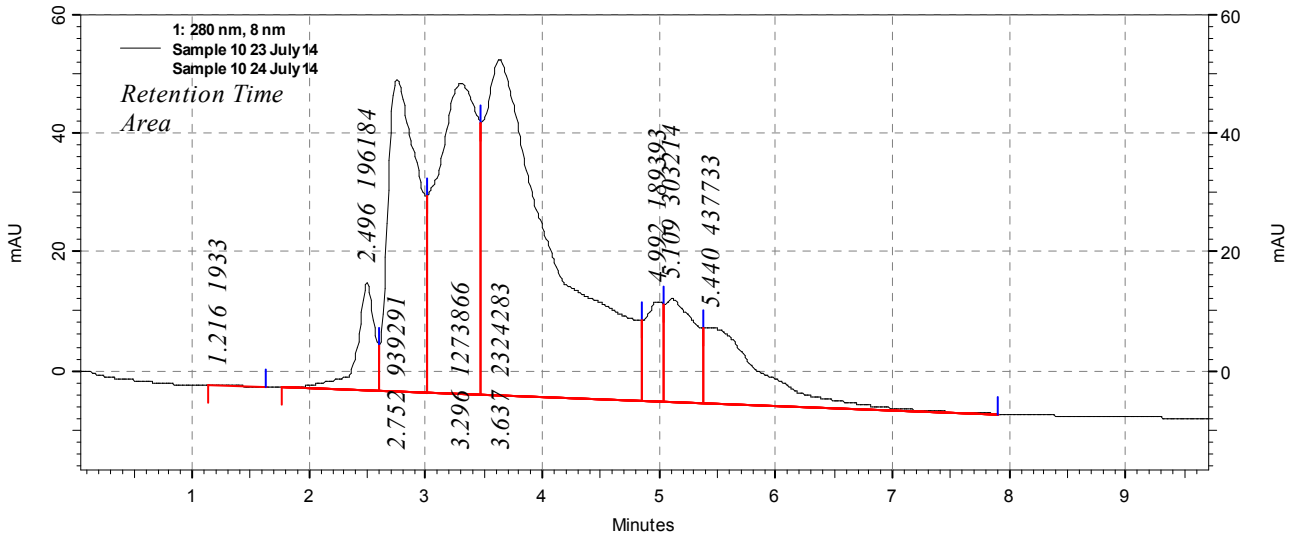


Figure 8. *HPLC Chromatogram of Peppermint (Mentha piperita) Leaves.*

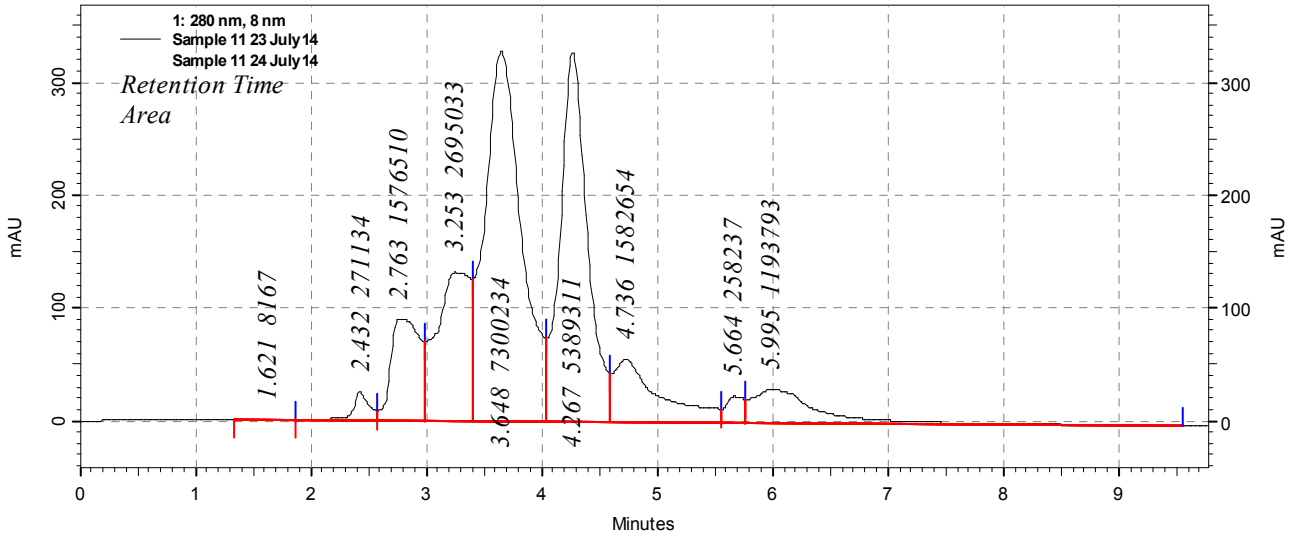


Figure 9. *HPLC Chromatogram of Peppermint (Mentha piperita) Stem.*

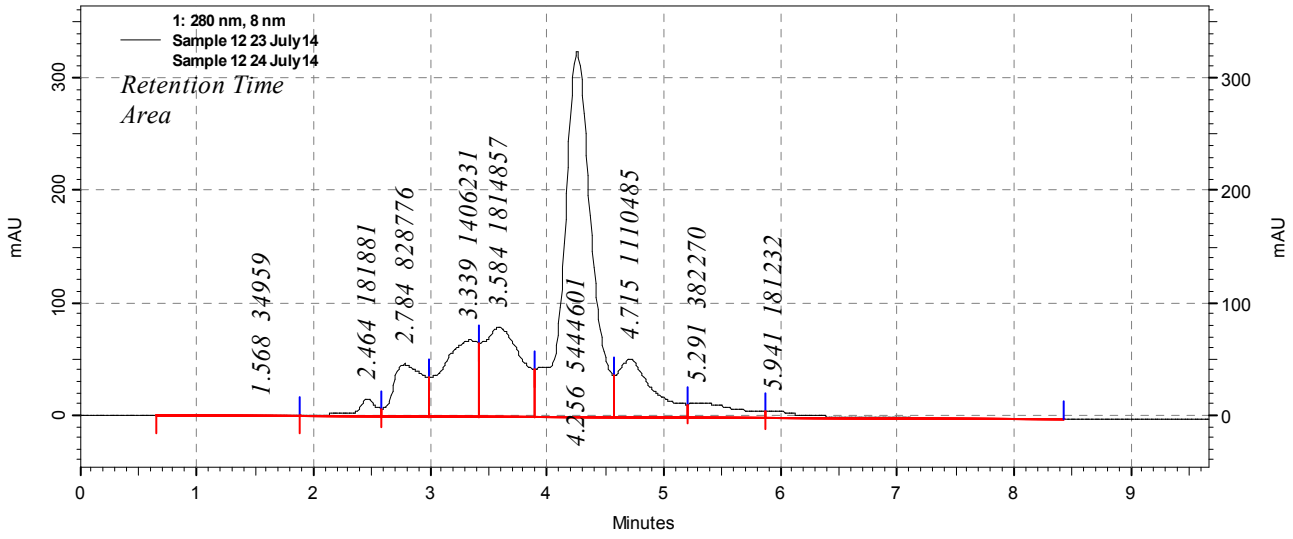


Figure 10. *HPLC Chromatogram of Peppermint (Mentha piperita) Root.*

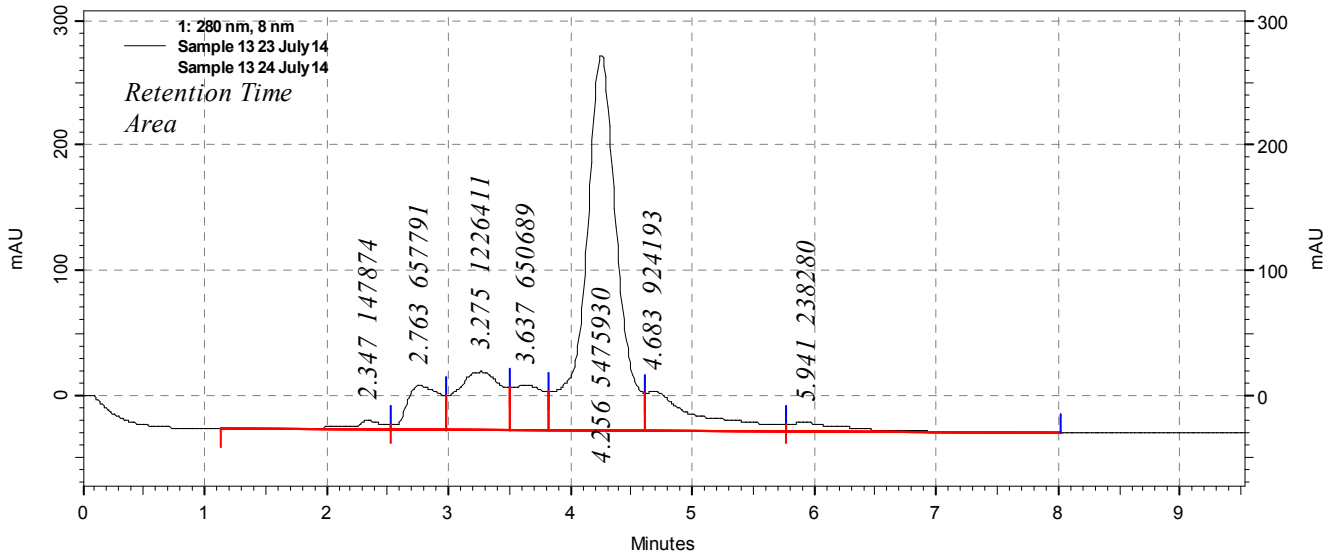


Figure 11. *HPLC Chromatogram of Thai basil (Ocimum basilicum var. Thyrsiflora) Leaves.*

GC-MS of Essential Oil Chemical Constituents in Leaves samples of Mentha piperita and Ocimum basilicum var. Thyrsiflora.

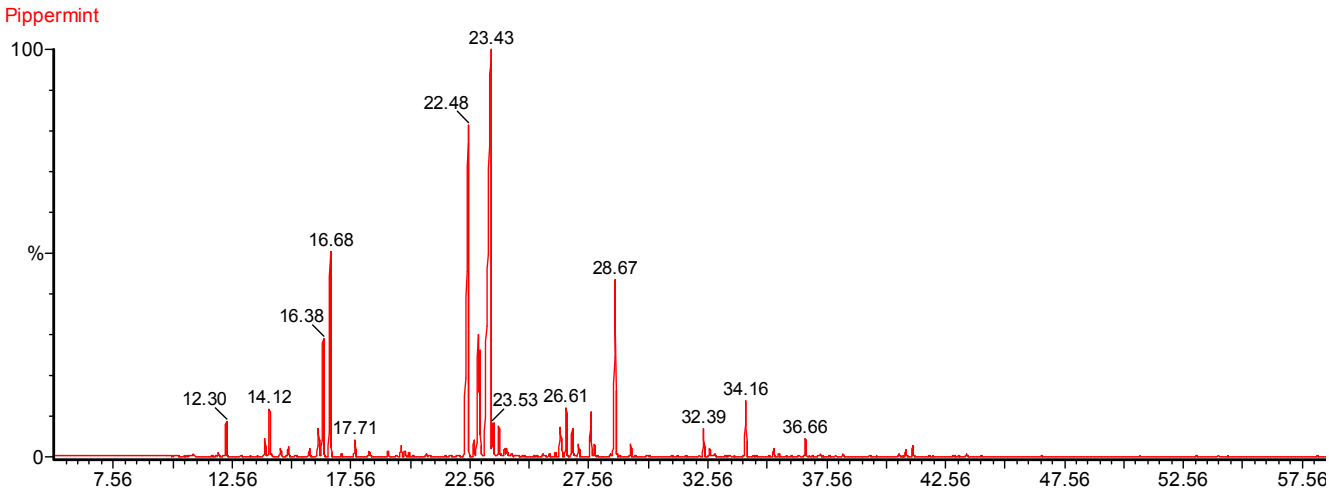


Figure 12. *GC-MS Analysis of Mentha piperita Leaves Essential Oil.*

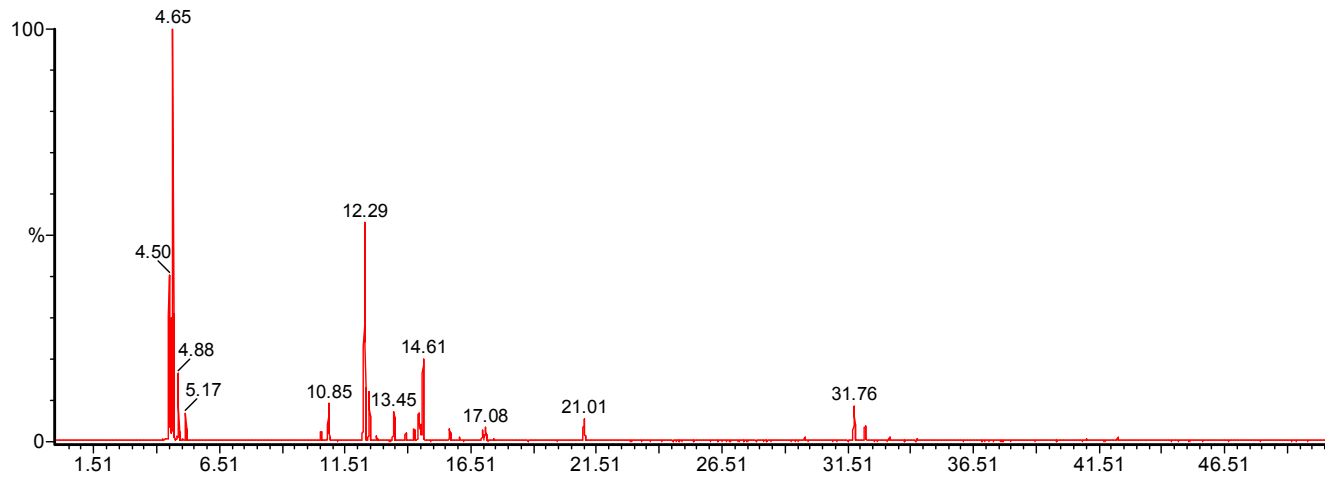


Figure 13. *GC-MS Profile of (Ocimum basilicum var. Thyrsiflora).*

2.5. GC-MS Analysis of Essential Oil of Mentha Piperita and Thai Basil [36]

2.5.1. Experimentation

Both samples *i.e. Mentha piperita* and *Ocimum basilicum var. Thyrsiflora* leaves (20.0g) each was placed in around-bottom flask separately with de-ionized water. The solution was steam distilled at 55°C for 3 hrs under reduced pressure (URP) (95mm Hg). The distillate (900 ml) was extracted with 100 ml dichloromethane (DCM) using a liquid–liquid continuous extract or (LLE) for 6 hrs. After the extract was dried over anhydrous sodium sulphate, the solvent was removed by a rotary flash evaporator. The distillation was stopped when the volume of extract was reduced to 1.0 ml, and then the solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.2 ml and stored at 20°C for subsequent analysis.

Aroma chemicals obtained by URP and LLE were identified by comparison with the Kovat's gas chromatographic retention index (KCRI) [37] and by the MS fragmentation pattern of each component compared with those of authentic chemicals.

2.5.2. Standard Chemicals

Eugenol, thymol, carvacrol,4-allylphenol, 1-octen-3-ol, benzyl alcohol, linalool, methyl-salicylate, estragole,1,8-cineole,4-terpeneol, benzylaldehyde, hexanal, hexanoic acid, undecane, and a-tocopherols (vitamin E) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Butylated hydroxyl toluene (BHT) was bought from Sigma Chemical Co. (St. Louis, MO). Dichloromethane was bought from Gyan Scientific Chemicals Co. Ltd. Lucknow (India).

2.5.3. Specification of the GC-MS Instrument

An HP model 6890 GC interfaced to an HP 5791 a mass selective detector (GC/MS) was used for mass spectral identification of the GC component sat MS ionization voltage of 70eV. A 30m· 0.25 mm i.d. (df=0.25 lm) DB-WAX bonded-phase fused-silica capillary Column was used for GC. The linear velocity of the helium carrier gas was 30cm/s. The injector and the detector temperatures were 250_C. The oven temperature was programmed from 50 to 180_Cat3_C/min and held for 40min. The data are recorded in Tables 8 and 9, Figures 12 and 13.

3. Results and Discussion

The antioxidant activity in terms free radical scavenging activity of various phytochemicals was evaluated for antioxidant medicines as an anti-aging agents in the plant samples of the both Species *Mentha piperita* and *Ocimum basilicumv -ar. Thyrsiflora.* The above studies were carried out for (%) moisture content, HPLC analysis of nine standard phenolic compounds such as Ascorbic Acid, Gallic Acid, Chlorogenic Acid, Caffeic Acid, Quinol, Resorcinol, Cumeric Acid, Ellagic Acid and Benzoic Acid in four samples (1-4) of *Mentha piperita* and *Ocimum basilicum -var. Thyrsiflora* for their quantitative estimation. The results are recorded in Table 7 and also presented graphically (Ghraph-2-7 and 8-11). Additional GC-MS analysis of essential oil of *Ocimum basilicum -var. Thyrsiflora* have been carried out for estimation of aroma chemicals as antioxidant medicines.

The moisture content was found to be maximum (86.42%) in leaves of *M. piperita* as compared to stem and root (79.69%). As light difference was observed in the moisture (%) content of stem and root of *M. piperita* may be because of woody nature (Table 2, Figure 1). The moisture (%) content in the leaves of *O. basilicum* was 89.74%.

The antioxidant activity in terms of free radical scavenging activity was evaluated for antioxidant medicines preparation by DPPH method was determined in each plant material of both *M. piperita* and *O. basilicum*-var. Thyrsiflora. The results are recorded in table 6. The Absorbance at 517 nm for free radical scavenging activity by DPPH was compared with standard BHT at 517 nm at three concentrations (50 ppm, 100 ppm, and 150 ppm). The results are recorded in table 3 and 4. The results demonstrated that the standard BHT has shown maximum (0.015nm) absorbance at 100 ppm, contrary to standard BHT in all the samples prepared. No change was observed in observance e.g. *M. Piperita* leaves had absorbance 0.0060, to 0.0062 and 0.0064 nm at 50 µg/ml, 100 µg/ml and 150 µg/ml respectively. Same observation was made in the leaves of *O. basilicum*. The results thus showed that the concentration does not have any remarkable effect on the percentage DPPH scavenging activity in all the samples of *M. piperita* and *O. basilicum*.

The HPLC analysis of four samples of *M. piperita* and *O. basilicum* when compared with HPLC of standard samples (Table 7), it was inferred that Gallic acid, ascorbic acid, Caffeic acid and Chlorogenic acid were present in all the four samples whereas quinol, Cumeric acid and Ellagic acid were present in these plant samples of *M. Piperita* (stems), M. *Piperita* (roots) and *O. basilicum*. Benzoic acid and resorcinol were absent in all the plant samples studied. Quantitative measurement of the polyphenolic acids was also done by HPLC (Table 10). The ascorbic acid concentration (6.90 ppm) was found to be maximum and Caffeic Acid (4.84ppm) as compared to other acids.

The estimation of aroma chemicals in the leaves of the plant samples of *M. piperita* and *O. basilicum* was carried out by GC-MS analysis (Figures 12 and 13).

Thirteen compounds were identified in *M. piperita* leaves and the compounds were identified in the leaves of *O. basilicum* (Tables 8 and 9) respectively. The maximum percentage of menthol was 32.24% observed in the leaves of *M. piperita*. The maximum abundance (11.7%) of 1,8-cineole was found in the leaves of *O. basilicum* (Figure 13). All the foregoing experiments thus revealed that the leaves of *M. piperita* and *O. basilicum* are the best source of antioxidant. Further, *M. piperita* leaves is a good source of menthol whereas and *O. basilicum* is a good source of 1,8-cineole.

4. Conclusion

1) All the foregoing experiments thus revealed that the

leaves of *M. piperita* and *O. basilicum* are the best source of antioxidant used in formulation of antioxidant and anti-aging medicines. Further, *M. piperita* leaves is a good source of menthol whereas *O. basilicum* is a good source of 1,8-cineole.

2) Thirteen compounds were identified in *M. piperita* leaves and ten compounds were identified in the leaves of *O. Basilicum.* The maximum % of menthol was 32.24% observed in the leaves of *M. piperita*. The maximum abundance (11.7%) of 1,8-cineole was found in the leaves of *O. basilicum,* hence the Sabinene the best source of antioxidant medicines.
3) Quantitative measurement of the polyphenolic acids was also done by HPLC. The ascorbic acid concentration (6.90 ppm) was found to be maximum and Caffeic Acid (4.84ppm) as compared to other acids. Certainly these phytophenolic compounds reduce the reactive oxygen species (ROS) produced during metabolism in the body and acts as anti-aging agents.
4) It was also inferred that gallic acid, ascorbic acid, Caffeic acid and Chlorogenic acid were present in all the four samples studied whereas quinol, Cumeric acid and Ellagic acid were present in these plant samples. All these nutritional organic acids combine with ROS and check the damage of body cells and works as anti-aging agent consequently increase the life time of people who takes in daily diet.

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