Research Article

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GC-MS analysis and biological activities of *Thymus vulgaris* and *Mentha arvensis* essential oil

Thymus vulgaris ve *Mentha arvensis* Uçucu Yağlarının GC-MS Analizi ve Biyolojik Aktivite Tayini

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Abstract

Background: Essential oils are chemical products produced by odoriferous glands from a variety of plants. These essential oil have many health benefits i.e. antiseptic, anti-inflammatory and antimicrobial activities. So due to these medicinal properties present study was designed to analyze essential oil of *Thymus vulgaris* and *Mentha arvensis* for their chemical composition and biological activities.

Materials and methods: Essential oil from these plants were extracted by hydrodistillation method, and analyzed by GC-MS. To test the microbial activity of these oil disk diffusion method and micro wells method were used. For free radical scavenging DPPH assay was used. However total phenolic content was measured by colorimetric method.

Results: The GC-MS analysis of *T. vulgaris* oil showed the presence of 47 chemical compounds among which thymol, terpinene, p-cymene and carvacrol were major. However essential oil of *M. arvensis* showed the presence of 28 constituents, among which the Menthone, Menthol, Isomenthone, Eucalyptol, neo-Menthol, cis-Piperitone oxide, Linalool, Thymol, Limonene, and α -Phellandrene were major. Essential oil from both these plant tested for antimicrobial activity showed that the *T. vulgaris* oil was effective against seven bacterial strains and the essential oil of *M. arvensis* was effective against six bacterial strain.

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The antioxidant activity of both samples by DPPH assay which showed positive result.

Conclusion: As both species showed the presence of active components, positive microbial activities, and antioxidant activity so, research should be carried on for further biological activities of these oil for betterment of living beings.

Keywords: GC-MS; Hydrodistillation; Antibacterial; *Thymus vulgaris*; *Mentha arvensis*.

Öz

Amaç: Uçucu yağlar, antiseptik, antiinflamatuvar, antimikrobiyal gibi çeşitli etkileri olan, farklı bitkilerin koku salgı bezleri tarafından üretilen kimyasal ürünlerdir. Bu çalışma, esansiyel yağların bu tıbbi yararları gözönünde bulundurularak *Thymus vulgaris* ve *Mentha arvensis*'in uçucu yağlarının kimyasal bileşimlerinin ve biyolojik aktivitelerinin analizi amacıyla tasarlanmıştır.

Gereç ve yöntem: Bitkilerden elde edilen uçucu yağlar hidrodistilasyon metodu ile ekstrakte edilmiş ve GC-MS ile analiz edilmiştir. Yağların mikrobiyal aktivitesini test etmek için disk difüzyon yöntemi ve mikro kuyucuk metodu kullanılmıştır. Serbest radikal temizleyici etki DPPH yöntemi ile, fenolik içerik ise kolorimetrik metod ile ölçülmüştür.

Bulgular: Her iki uçucu yağın GC-MS analizi sonucunda, *T. vulgaris* yağının yapısında aralarında timol, terpinen, p-simen ve karvakrolün bulunduğu 47 kimyasal bileşimin varlığı; *M. arvensis* yağının içeriğinde ise menton, mentol, izomenton, okaliptol, neo-mentol, cis-piperiton oksit, linalol, timol, limonen ve α -felandren bileşenlerini içeren 28 kimyasal bileşenin varlığı tespit edilmiştir. Antimikrobiyal aktiviteleri incelendiğinde, *T. vulgaris* yağının 7 bakteri suşuna karşı, *M. Arvensis* uçucu yağının ise 6 bakteri suşuna karşı etkili olduğu belirlenmiştir. Her iki uçucu yağın antioksidan aktivitesi, DPPH testi ile pozitif sonuç göstermiştir.

Sonuç: Her iki uçucu yağın aktif bileşenlerinin varlığı, pozitif mikrobiyal ve antioksidan aktiviteleri, bu yağların biyolojik aktiviteleri ile ilgili daha ileri çalışmalara ihtiyaç olduğunu göstermektedir.

Anahtar kelimeler: GC-MS; hidrodistilasyon; antibakteriyal; *Thymus vulgaris*; *Mentha arvensis*.

Introduction

Since ancient times, people used herbs and parts of plants as food and medicine and with the passage of time instead of plant parts, plant products are also utilized for phytotherapy like essential oils from aromatic plants, are used to treat different ailments due to wide array of biological activities i.e. antiseptic, anticancer, spasmolytic, hepatoprotective, antimicrobial, antioxidant, and pesticidal [1]. These essential oils are mostly utilized in aromatherapy to treat different disorders. The essential oils are chemical products with low boiling point, greatly soluble in organic solvents, produced by odoriferous glands in a great variety of plants. These oils accumulate in all parts (leaves, barks, woods, flowers, fruit, rhizomes and seeds) of the plant. These oils contain different chemical compounds due to which they have many important pharmacological properties. Some of the chemical components (d-limonene, d-carvone or geranyl acetate) of these essential oils are used at industrial level as scents, cosmetics, detergents, taste makers, cleansers and as solvents.

Due to different biological activities, a strong interest is developed from last few years in essential oils collected from different odoriferous plants [2]. Until now about 3000 essential oils are observed from different plant species, out of these 300 have commercial importance as medicine, nutriment and cosmetics. One of the famous aromatic plant families is *Lamiaceae*, this family is important due to production of their essential oil from odoriferous plants. This family consists of about 252 genera and more than 6700 species [3]. Essential oils from the members of this family known to possess antiseptic, anti-inflammatory and antimicrobial activities [4]. So due to vast medicinal properties present study was designed to analyze essential oil from two members (*Thymus vulgaris* and *Mentha arvensis*) of this family.

Materials and methods

Collection of sample

Whole plant of *T. vulgaris* L. (Figure 1) at flowering stage were gathered from Ganga Choti, a mountain lying in

Figure 1: *Thymus vulgaris*.

Source: <https://farmyardnurseries.co.uk>.

Figure 2: *Mentha arvensis*.

Bagh, Azad Kashmir, Pakistan and the fresh aerial parts of *M. arvensis* L. (Figure 2) were collected from Muzaffarabad, Azad Kashmir, Pakistan. Plant species were identified with the help of literature and authenticated by taxonomist. Samples of plants are shade dried, converted into fine powder and stored at room temperature for later use.

Hydrodistillation extraction

Extraction of essential oil was done by hydrodistillation method with Clevenger apparatus shown in Figure 3,

Figure 3: Hydrodistillation apparatus.

following the protocol of European Pharmacopoeia [5, 6]. Sixty gram of dried thyme powder with 2.5-L water was poured into the apparatus and extraction was carried out for 2 h until no more oil was left. Thyme oil was decanted after every fraction and collected in Eppendorf tubes. The oil was dried with anhydrous sodium sulfate. After that the oil was stored at refrigerator at 4°C.

For extraction of oil from *M. arvensis* the protocol of Souza et al. [7] was followed, 1 kg of fresh leaves were grind and put into the distillation apparatus. The remaining container was filled with 2.5 L distilled water the processes was carried out for 2 h to obtain maximum oil. Same process was repeated until no more oil was left. *Mentha* oil was decanted after every fraction and collected in Eppendorf tubes. The oil was dried on anhydrous sodium sulfate and stored at 4°C before GC-MS Analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis

Both samples of essential oil were analyzed by GC-MS (Hewlett-Packard model 6890 and Agilent 7890A, USA) by following the protocol of Negahban and Saeedfar [8]. Essential oil were observed by Hewlett-Packard model 6890 (Figure 4) working at ionization energy of 75 eV with a DB-5 capillary tube of 0.25 mm diameter, 0.25 μ m film thickness and 30 m length. The temperature was set to increase with regular interval of time from 60 to 23°C at 5°C/min. Helium was used as a carrier gas at 1.5 mL/min. MS source temperature was retained at 20°C, sample injection volume was 3 μ L, split ratio was 1:60, interface temperature was 230°C and mass scan was 30–655 atomic mass unit.

The analysis was carried on Agilent 7890A attached with FID (Flame Ionization Detector), narrow capillary column (0.25 mm diameter, 0.25 μ m film thickness and

Figure 4: GC-MS apparatus.

30 m length). The temperature was set to increase with regular interval of time from 60 to 230°C at 5°C/min. Nitrogen gas was used as Carrier gas at 2 mL/min. Quantitative data were obtained from FID area percentages.

Identification of components

For identification of compounds after the oil at same conditions *n*-alkanes series was injected to calculate the retention index (RI) of all volatile constituents. The constituents were identified by comparison of their RI with the saved library data such as New York mass spectral library, Wiley Library and by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [9, 10].

Antibacterial activity

Essential oil of *T. vulgaris* was tested on six different strains of bacteria (*Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) by using disk diffusion method. For the assay microorganism suspension (10^6 cells/mL) was poured on the solid media plate (Muller-Hinton agar). Filter paper (Whatman No. 1, pore size 11 μ m) were impregnated with different concentrations (5, 10, 15 and 20 μ L) of essential oil and put on the prepared agar. The plates were then inoculated for 26 h at 38°C. Ciprofloxacin (25 μ g/disk) and cephalixin (15 μ g/disk) were used as positive controls. The diameter of the zone of inhibition after incubation was calculated in millimeters and quantitative data was generated from these results.

The antibacterial activities of *M. arvensis* was tested with the help of broth dilution assay. The assay was performed in 0.2% sterile peptone water. The same bacterial

strains were used for *T. vulgaris* antioxidant activity were suspended in that water with the turbidity visually corresponding to 0.6 Mc Farland. Different dilution (0.09–50.1%) of the oil were made. Fifty microliter of bacterial suspension was inoculated into micro wells with the same amount of essential oil dilution and incubated at 38°C for 26 h. After incubation minimum inhibitory concentration (MIC) was determined. Experiment was performed in triplicates.

Antifungal activities

Antifungal activities was carried out on *Candida albicans* by using Sabouraud agar and Malt Extract-Agar. For toxicity measurement poisoned food technique was used. For the assay inoculum was taken on 5 mm diameter disc and put at the mid of each petri plate containing PDA media. 0.5 mL of different concentration (0, 0.7, 1.30, 2.7, 5.5, 10.6, 100 $\mu\text{L mL}^{-1}$) of both samples were then incubated at 30°C for 6–7 days with these culture. Fluconazole (15 $\mu\text{g/disk}$) was used as positive control. For quantitative data, percentage inhibition of mycelia was measured one by one.

Total phenolic content

To check percentage of phenolic contents, colorimetric method was used by following the protocol of [11]. Gallic acid solution was used as a standard.

Anti-oxidant activity

The essential oil of *T. vulgaris* was checked for anti-oxidant property on 2,2-diphenyl-1-picrylhydrazyl (DPPH) by following the protocol of [12, 13]. For that, 5 mL of DPPH solution were taken with four different concentrations of extract. After incubation for 20 min the absorbance of sample was measured at 517 nm with spectrophotometer. Methanol was taken as blank and quercetin as reference compound.

Percentage inhibition was measured by following formula

$$\text{Percent inhibition (\%)} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

Results and discussion

Chemical composition of essential oil

Oil obtained from *T. vulgaris* is known as yellow oil. That yellow oil was subject for its chemical constituents result

Table 1: Essential oil compositions in *T. vulgaris*.

No.	Compounds	RI ^a	% RA ^b (means $\pm$ SD)
1.	α -Thujene	929	0.86 $\pm$ 0.19
2.	α -Pinene	935	0.59 $\pm$ 0.14
3.	Camphene	950	0.53 $\pm$ 0.13
4.	Sabinene	975	0.04 $\pm$ 0.03
5.	1-Octen-3-ol	981	0.24 $\pm$ 0.09
6.	3-Octanone	987	0.07 $\pm$ 0.03
7.	Myrcene	994	1.23 $\pm$ 0.03
8.	3-Octanol	998	0.19 $\pm$ 0.03
9.	α -Phellandrene	1008	0.26 $\pm$ 0.13
10.	δ -3-Carene	1013	0.09 $\pm$ 0.03
11.	α -Terpinene	1019	1.25 $\pm$ 0.12
12.	p-Cymene	1026	8.55 $\pm$ 0.84
13.	Limonene	1034	0.09 $\pm$ 0.03
14.	1,8-Cineole	1035	0.69 $\pm$ 0.16
15.	(Z)- β -Ocimene	1038	–
16.	Benzene acetaldehyde	1045	–
17.	(E)- β -Ocimene	1049	0.18 $\pm$ 0.05
18.	γ -Terpinene	1065	9.48 $\pm$ 1.84
19.	Cis-Sabinene hydrate	1070	0.85 $\pm$ 0.25
20.	Terpinolene	1090	3.14 $\pm$ 0.59
21.	Linalool	1105	0.10 $\pm$ 0.05
22.	Camphor	1145	0.13 $\pm$ 0.05
23.	Borneol	1167	1.26 $\pm$ 0.33
24.	Terpinene-4-ol	1179	0.23 $\pm$ 0.09
25.	γ -Terpineol	1210	0.26 $\pm$ 0.05
26.	Thymol methyl ether	1237	0.65 $\pm$ 0.15
27.	Carvacrol methyl ether	1245	0.40 $\pm$ 0.13
28.	Thymol	1301	60.55 $\pm$ 2.27
29.	Carvacrol	1315	3.36 $\pm$ 0.76
30.	Eugenol	1361	0.05 $\pm$ 0.03
31.	Isobornyl propionate	1379	0.37 $\pm$ 0.12
32.	β -Bourbonene	1387	0.09 $\pm$ 0.02
33.	(E)-Caryophyllene	1423	1.75 $\pm$ 0.24
34.	Aromadendrene	1440	0.09 $\pm$ 0.03
35.	α -Humulene	1455	0.15 $\pm$ 0.09
36.	Geranyl propanoate	1476	0.15 $\pm$ 0.08
37.	γ -Murolene	1478	0.25 $\pm$ 0.13
38.	Germacrene-D	1482	0.14 $\pm$ 0.05
39.	Valencene	1496	–
40.	γ -Cadinene	1515	0.20 $\pm$ 0.05
41.	δ -Cadinene	1525	0.24 $\pm$ 0.12
42.	α -Cadinene	1538	–
43.	Spathulenol	1579	0.13 $\pm$ 0.05
44.	Caryophyllene oxide	1584	0.35 $\pm$ 0.16
45.	10-epi- γ -Eudesmol	1620	0.13 $\pm$ 0.06
46.	EPI- α -Cadinol	1642	–
47.	α -Cadinol	1655	0.19 $\pm$ 0.05
48.	Oil yield (%w/w)		1.55 $\pm$ 1.27
	Total		99.5

^aRI, retention index.

^bRA, relative area percentage values are means of three experiments $\pm$ SD.

of the study showed the presence of 47 recognized chemical constituents shown in Table 1. The compounds with higher percentage were terpinolene (3.15%), p-cymene (8.53%), γ -terpinene (9.48%), carvacrol (3.35%) and thymol (60.55%). These result were agree with that of Negahban and Saeedfar [8], these researcher also showed the higher percentage of thymol (49%), β -cymene (19.99%), carvacrol (7.63%) and trans-caryophyllene (6.79%) in *T. vulgaris*. Miladi et al. also showed the higher percentage of thymol (52.19%) followed by content of carvacrol (6.72%) [1]. In different studies the percentage of these chemical components are slightly vary from each other one of the research relate these variation to the different climatic conditions of the world which vary in different geographical region of the world. Quantity may also vary due to health and variety of the plant, age of the plant at the time of collection, method of drying and to the method of extraction of the oil from samples [14]. The effect of different geographical zone on percentage composition was also confirmed by the research of Satyal et al. [6] who worked on thyme collected from different regions the result of their study showed that the *T. vulgaris* collected from France showed higher percentage of linalool (76.2%) and linalyl acetate (14.3%) where the thyme obtained from Serbia showed the higher presence of geraniol (59.8%) geranyl acetate (16.7%). In literature study it is also observed that the amount of carvacrol is also higher in other species such as *Thymus palleescens* de Noé an endemic plant of Algeria reported by Sadjia et al. [15]. In our study the percentage of other components was observed less than 2%. A high correlation was observed between γ -terpinene and p-cymene with thymol and carvacrol contents in essential oil of *T. vulgaris* shown in Table 2.

The GC-MS analysis of *M. arvensis* essential oil showed the presence of total 28 chemical components in which most are recognized and cited in previous researcher work these compound are given in Table 3. Components with high percentage were: α -Phellandrene (3.22%), DL-Limonene (1.48%), linalool (2.22%), Linalool (2.22%), cis-Piperitone oxide (3.63%), Menthone (29.42%), neo-Menthol (4.75%), Eucalyptol (6.95%), Isomenthone (10.85%), Menthol (21.35%), and Thymol (1.64%) and some other components with small amount were also identified. Our results agree with that of Souza et al. [7]

they showed the presence of menthol (67.27%) and menthone (13.34%) in highest amount in young leaves of *M. arvensis*. In another study, researcher showed the presence of 25 different components in the essential oil of *M. arvensis* in which the higher concentration was observed of menthol and menthone [7]. Some researcher showed that the percentage of chemical component in essential oil can vary in different cultivar of mint and at different growing stage [16]. However literature study showed that essential oil concentration increases at mature stage of *M. arvensis* [7]. Overall the result of our findings are compatible with those reported in literature.

Antimicrobial activity

The quantitative result of antimicrobial activity of *T. vulgaris* essential oil against different bacterial strain are given in Table 4. Results of our study showed that the antimicrobial potential of *T. vulgaris* essential was concentration dependent by increasing concentration antimicrobial activity also increased. Secondly the effectiveness of essential oil was different against different bacterial strains i.e. high inhibition zone was observed in *K. pneumonia*, *E. coli* and *S. typhimurium*. To compare more thoroughly the effect of *T. vulgaris* on each microorganism (Figure 5 and Table 4) shows the results of multiple comparisons, at each oil amount, was assumed. Essential oil was checked in different pair to compare with each other. All the pairwise differences showed that they are highly significant ($p = 0.00$) with each other except *S. typhimurium* and *E. coli*. The growth of *K. pneumoniae*, *E. faecalis*, *S. aureus*, *P. aeruginosa* and *E. coli* was previously recorded along with the efficacy against *S. typhimurium*, respectively [17]. However, some studies report the inefficiency of thyme essential oil against *E. coli*, *S. aureus* and *K. pneumonia* [18]. Ciprofloxacin and cephalixin was used as positive control. Vast variety of essential oils activity depends on chemical components. Previous studies by the researcher showed a strong relationship of the antimicrobial activities with the presence of phenolic compounds i.e. thymol and γ -terpinene present in essential oil, except p-cymene, which does not show antibacterial efficacy when used alone, but in

Table 2: Thymol, p-cymene, γ -terpinene and carvacrol contents of the essential oil in *T. vulgaris*.

Plant	Thymol (%)	p-cymene (%)	γ -terpinene (%)	Carvacrol (%)	Total (%)
<i>T. vulgaris</i>	60.55 $\pm$ 2.28	8.53 $\pm$ 0.85	9.48 $\pm$ 1.85	3.35 $\pm$ 0.75	82.0

Values are the average of three experiments $\pm$ SD. $p < 0.05$.

Table 3: Essential oil composition in *M. arvensis*.

S. no.	RT ^a	Constituents	RI ^b	RI ^c	RA ^d
1.	3.872	dl-Limonene	–	1155	1.48
2.	4.049	Eucalyptol	–	1207	6.92
3.	4.459	α -Pinene	–	1040	0.69
4.	4.663	β -Pinene	–	1040	1.14
5.	4.789	δ -3-Carene	1202	1148	0.21
6.	5.175	α -Phellandrene	1218	1217	3.22
7.	6.784	Octyl cyclobutane carboxylate	1284	–	0.33
8.	8.368	3-Octanol	1341	1383	1.83
9.	10.21	L-Menthone	1403	1458	29.42
10.	10.368	cis-Sabinene hydrate	1406	1522	0.70
11.	10.963	Isomenthone	1427	1454	3.84
12.	12.917	Linalool	1489	1540	2.22
13.	13.135	neo-Menthol acetate	1496	–	0.30
14.	13.875	trans-Caryophyllene	1519	–	0.52
15.	14.211	neo-Menthol	1530	1598	4.73
16.	14.343	4-Terpineol	1535	1553	0.30
17.	15.545	Menthol	1575	1614	21.35
18.	16.294	trans-Anethole	1598	1810	1.63
19.	16.446	δ -Terpineol	1603	1656	0.23
20.	17.138	2-Acetylfuran	1626	–	1.37
21.	17.228	α -Terpineol	1627	1688	0.43
22.	17.384	cis-Piperitone oxide	1634	1701	3.63
23.	17.974	Isomenthone	1655	1453	6.99
24.	18.209	5-Isopropyl-6,7-epoxy-8-hydroxy-8-methylnon-2-One	1664	–	0.37
25.	22.752	2,6,6-Trimethyl-cyclohex-1-enecarboxylic acid	1670	–	0.42
26.	24.359	3-Methyl-3-(4-methyl-3-pentenyl) oxiranemethanol	1876	–	0.17
27.	24.587	Caryophyllene oxide	1885	1929	0.54
28.	27.784	2,5-Dimethyl-3-hexyne-2,5-diol	2000	–	0.55

^aRT, retention time.^bRI, retention indices on BP-20 polar column.^cRI, actual retention indices of columns (Supelcowax-10, HP-20M, CW-20M and BP-20).^dRA, components percentage.**Table 4:** Antimicrobial activity of *T. vulgaris*.

Test microorganism	Amount of essential oil (5 μ L)	Amount of essential oil (10 μ L)	Amount of essential oil (15 μ L)	Amount of essential oil (20 μ L)
<i>Staphylococcus aureus</i>	24.0 $\pm$ 0.34	29.5 $\pm$ 0.7	30 $\pm$ 0.36	31.6 $\pm$ 0.48
<i>Salmonella typhimurium</i>	14.5 $\pm$ 0.35	20 $\pm$ 0.40	31.5 $\pm$ 0.35	35 $\pm$ 0.25
<i>Pseudomonas aeruginosa</i>	12 $\pm$ 0.27	13.35 $\pm$ 0.34	14 $\pm$ 0.23	15 $\pm$ 0.20
<i>E. coli</i>	14.7 $\pm$ 0.37	20 $\pm$ 0.42	31 $\pm$ 0.32	35 $\pm$ 0.20
<i>Klebsiella pneumoniae</i>	31 $\pm$ 0.13	31.05 $\pm$ 0.32	32 $\pm$ 0.25	33.98 $\pm$ 0.15
<i>Enterococcus faecalis</i>	9 $\pm$ 0.16	16 $\pm$ 0.16	16 $\pm$ 0.19	25 $\pm$ 0.16
<i>Candida albicans</i>	16 $\pm$ 0.39	19.5 $\pm$ 0.56	25.75 $\pm$ 0.25	30.5 $\pm$ 0.18

Effects of thyme oil against bacteria representing by the mean sizes of the inhibitory zones.

synergistic with thymol and γ -terpinene it showed effective antibacterial activity.

The antimicrobial activities of *M. arvensis* revealed that its essential oil is effective against all bacterial strains as shown in (Table 5 and Figure 6). Our study showed

that the menthol essential oil was more effective against microorganism as compared to thyme oil. Even a low concentration of oil was effective to inhibit *S. aureus* and *E. coli*. Minimal bactericidal concentration of *M. arvensis* essential oil, to inhibit growth of different bacteria was,

Figure 5: Essential oil antimicrobial activity of *Thymus vulgaris*. Series 1: *Staphylococcus aureus*; Series 2: *Salmonella typhimurium*; Series 3: *Pseudomonas aeruginosa*; Series 4: *E. coli*; Series 5: *Klebsiella pneumonia*; Series 6: *Enterococcus faecalis*; Series 7: *Candida albicans*.

Table 5: Antibacterial activity of *M. arvensis*.

Series #	Test organism	Amount of essential oil (μL)	% Inhibition (means $\pm$ SD)
Series 1	<i>E. Coli</i>	0.2	(5.8 $\pm$ 5.7)
Series 2	<i>E. facium</i>	0.2	(50.7 $\pm$ 17.8)
Series 3	<i>St. aureus</i>	0.2	(1.20 $\pm$ 1.8)
Series 4	<i>P. aeruginosa</i>	0.2	(38.8 $\pm$ 24.7)
Series 5	<i>P. mirabilis</i>	0.2	(30.0 $\pm$ 18.2)
Series 6	<i>C. albicans</i>	0.2	(69.67 $\pm$ 32.5)

These values are average of three experiments $\pm$ SD. $p < 0.05$.

E. coli (5.8 $\pm$ 5.7) and *S. aureus* (1.20 $\pm$ 1.8). Like thymol, the researcher relate the antibacterial property of menthol to the presence of terpenoids present in mint family. In our study, the essential oil of *M. arvensis* was observed rich in terpenoids due to which the essential oil showed strong activity against different bacterial strains. The result of our study was agreed to that of Mickienė et al. [19] worked

Figure 6: Antibacterial activity of *M. arvensis*.

on different strains of bacteria i.e. *S. aureus*, *Enterococcus faecium*, *P. aeruginosa*, *E. coli*, *Proteus mirabilis*. Horváth and Koščová [20] worked on the essential oil of *M. arvensis* against *S. aureus* also showed that the plant essential oil is effective against microorganisms.

The antifungal activity of essential oil of samples showed different values i.e. for *C. albicans* mycelia inhibition (0%, 10%, 63.50%, 72.30%, 88.60%, 90.25% and 94.6%, 97.1%, 97.75%, 99% and 100%) at concentration of 0, 0.7, 1.30, 2.7, 5.5, 10.6, 23, 32, 42, 55, 100 $\mu\text{L mL}^{-1}$ (Table 6). From our present results it was observed that the antifungal activity was concentration dependent, as with increase concentration the antifungal activity also increases. Our present study are agreed with that of Mickienė et al. [19] worked on the antimicrobial activity of essential oil obtained from *M. arvensis*, showed inhibition of *C. albicans* at higher concentration. These essential oil are not only effective against *C. albicans* but also against other fungal strain. The literature study revealed the effectiveness of *T. vulgaris* against *Rhizopus oryzae* [21]. Quantitative details are shown in Table 6; Figure 7.

Therefore, our study confirm that essential oil of both plants exhibit stronger antimicrobial activity than that of

Table 6: Percent inhibition of mycelia growth on essential oil of *M. arvensis*.

No.	Concentration of oil ($\mu\text{L mL}^{-1}$)	Inoculum size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% Inhibition of mycelial growth
			I	II	III			
1.	0	4	42	42	42	42	38	0
2.	0.7	4	19	18	19	18.67	14.67	10
3.	1.30	4	12	11	12	11.67	7.67	63.5
4.	2.7	4	8	7	8	7.67	3.67	72.30
5.	5.5	4	4	4	4	4.00	0.00	88.60
6.	10.6	4	4	4	4	4.00	0.00	90.25
7.	100.0	4	—	—	—	—	—	100.0

MIC = 5.5 $\mu\text{L mL}^{-1}$.

Figure 7: Antifungal activity of *M. arvensis*.

Table 7: Antioxidant activity and total phenolic content in *T. vulgaris*.

Plant	Total phenolic content ^a (mg GAE/g DW)	Antioxidant activity (IC ₅₀) ^b (µg/mL)
<i>Thymus vulgaris</i>	21.6 ± 0.12	7.8 ± 0.08

^aGallic acid (mg/g) dry weight (DW).

^bIC₅₀: µg/mL.

p < 0.05 values were average of three experiments ± SD.

their major constituents or their mixtures, respectively, which suggests synergistic effects of the minor components, but also the importance of all components in relation to the biological activity of essential oils.

Total phenolic content and antioxidant activity

Many health issues like cancer and heart diseases are caused by free radicals. Literature study showed that plant extracts containing phenolic compounds, exhibit high antioxidants properties. These chemical constituents protect the body against harmful chemicals produce during metabolism of fats [13, 22]. In our present study both samples of essential oil were checked for their total phenolic content. Phenolic contents of both samples of *T. vulgaris* and *M. arvensis* was expressed as gallic acid equivalent by following the standard curve equation: $y = 0.027x + 0.1784$, $R^2 = 0.9966$. In our finding we observed high phenol content in *T. vulgaris* (21.6 mg GAE/g dry weight) as compared to *M. arvensis* values are shown in Table 7.

The free radical scavenging activity of these essential oil were assessed with the help of DPPH assay. During the

study it was observed that the high content of phenol in *T. vulgaris* (7.8 µg/mL) lead to the high antioxidant activity as shown in Table 7. So a correlation was observed between radical scavenging capacity and total phenolic content of essential oil. So we can say that the Phenolic compounds play a very important role in scavenging of free radicals. In our finding it was also observed that with increasing concentration the free radical scavenging also increases. Our findings agree with that of Alizadeh [23] work on the antioxidant activity of *T. vulgaris* by using DPPH assay. However, in literature study it was observed that the antioxidant activity of plants are not only caused by phenolic constituents but also due to the presence of other secondary metabolites like vitamins, flavonoids and carotenoids [24]. From these results we can suggest that *T. vulgaris* is a strong antioxidant can be used as natural antioxidants.

Conclusion

Present study showed that plant essential oil are good in their biological activities. Both essential oil was rich in chemical composition and showed the presence of many important chemical constituents. Essential oil of *M. arvensis* and *T. vulgaris* showed positive results for all biological activities. However, high antioxidant activity was observed in *T. vulgaris* which also show a strong correlation to the presence of high phenolic content. As both species have active components due to which they showed positive antimicrobial activities. So, research should be carried on for further biological activities of these oil for betterment of living beings. It is also a suggestion that instead of common hydrodistillation technique an improved technique such as Solvent-free microwave extraction (SFME) should be utilized for extraction of essential oil.

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