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Comparative study of chemistry compositions and antimicrobial potentials of essential oils and oleoresins from dried and fresh *Mentha longifolia* L.

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ABSTRACT

Objective: To investigate the chemical compositions and antimicrobial potentials of the essential oils and oleoresins obtained from fresh and dried *Mentha longifolia* L.

Methods: Gas chromatography and gas chromatography-mass spectrometer techniques were used to determine the profiling of the essential oils and oleoresins. In order to determine the antimicrobial efficacy of the volatile oil and oleoresins, the pathogenic fungus *Aspergillus niger* (1884), *Aspergillus flavus* (2479), *Fusarium monoliforme* (1893), *Fusarium graminearum* (2088) and *Penicillium viridicatum* (2007) were undertaken whereas four pathogenic bacteria *Bacillus subtilis* (1790), *Staphylococcus aureus* (3103) (Gram-positive), *Escherichia coli* (1672), *Pseudomonas aeruginosa* (1942) (Gram-negative) were selected for the present study. Food poisoned, inverted Petri plate, agar well diffusion and disk diffusion methods were employed for investigating antimicrobial potentials.

Results: Piperitenone oxide, an oxygenated monoterpene, dominated the chemical compositions of essential oils and oleoresins whose compositions varied from 23.5%–87.8%. Both essential oils showed good antifungal activities against *Aspergillus* and *Fusarium* species. The antibacterial investigations revealed that Gram-positive bacteria were more sensitive to the essential oils.

Conclusions: Drying the fresh herbal materials influences the chemical contents and the biological activities of the essential oils and oleoresins. Such results indicate that essential oils of *Mentha longifolia* L. can be possible candidates for further investigations to isolate and characterize their active principles as possible new natural preservatives.

1. Introduction

The genus *Mentha* L. (Lamiaceae) is comprised of more than 25 species, mainly perennial herbs growing wildly in damp or wet places throughout temperate regions of Eurasia, Australia and South Africa. Leaves, flowers and the stems of *Mentha* spp. are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor. In addition, *Mentha* spp. have been used as a folk remedy for the treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints due to its anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anti-catarrhal activities[1]. Recently, investigations of natural products for the discovery of active compounds with antimicrobial and antioxidant properties from plant origin which can be applied to the food industry have gained interests. Natural active compounds could be an alternative to the employ of synthetic chemicals. Such compounds can be used to prolong the storage stability of food by inhibiting the growth of foodborne spoilage or pathogenic microorganisms and protect food from oxidative stress damage[2]. The antimicrobial activity of plant-derived essential oils is the basis of many applications, especially in food preservation, aromatherapy and medicine. Antimicrobial activities of mint essential oils are revealed in several species such as *Mentha suaveolens*[3], *Mentha rotundifolia*[4], *Mentha pulegium*[5], *Mentha aquatica* and *Mentha longifolia* (*M. longifolia*)[6,7].

Based on these considerations, the present study deals with the

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investigation of chemical compositions and antimicrobial efficacies of fresh and dried leaves of *M. longifolia*. In this work, our aim is to define the variation in chemical compositions and antimicrobial potencies of essential oils and oleoresins due to the drying process.

2. Materials and methods

2.1. Essential oils and oleoresins extraction

The extraction of essential oils were in accordance with the European Pharmacopoeia[8] by using Clevenger-type apparatus for 6 h. The fresh mint essential oil (FMEO) was obtained from washed fresh *M. longifolia* L. (aerial parts, 250 g) whereas it was sun-dried and powdered for extracting the dried mint essential oil (DMEO). The light green and light yellow colored oils were dried over anhydrous sodium sulfate to remove traces of moisture.

Oleoresins were extracted from fresh and dried aerial parts of *M. longifolia* L. with ethanol and n-hexane by using Soxhlet apparatus. The solvent was removed by distillation to produce different viscous oleoresins, namely fresh mint ethanol (FMET), dry mint ethanol (DMET), fresh mint *n*-hexane (FMNH) and dry mint n-hexane (DMNH) oleoresins which were stored under cold condition until further use.

2.2. Chemical compositions analysis

Chromatographic determinations using gas chromatography-mass spectrometer (GC-MS) (Analytical Technologies SA, Buenos Aires, Argentina) were run on PerkinElmer Elite-5MS (5% phenyl methyl siloxane) by using capillary column (30 m × 0.25 mm × 0.25 μm). The carrier gas was helium with a flow rate of 1 mL/min. GC-MS interphase, ion source and selective mass detector temperatures were maintained at 280, 230 and 150 ℃, respectively.

The temperature programs was fixed at 60 ℃ (1 min), 60–185 ℃ at a rate of 1.5 ℃/min, 185 ℃ (1 min), 185–275 ℃ at a rate of 9 ℃/min, 275 ℃ (2 min) with split ratio 80:1 for essential oils and oleoresins. Comparison of their retention indices and mass spectra with the published data and computer matching was done with the Wiley 275 and National Institute of Standards and Technology libraries provided with computer controlling GC-MS systems[9,10]. The retention indices were calculated by using a homologous series of *n*-alkanes C8–C18.

2.3. Antimicrobial investigations

All the fungal and bacterial strains for antimicrobial investigations were procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India.

2.3.1. Antifungal investigations

In order to determine the antifungal efficacy of the volatile oil and oleoresins, the pathogenic fungus *Aspergillus niger* (*A. niger*) (1884), *Aspergillus flavus* (*A. flavus*) (2479), *Fusarium monoliforme* (*F. monoliforme*) (1893), *Fusarium graminearum* (*F. graminearum*) (2088) and *Penicillium viridicatum* (*P. viridicatum*) (2007) were undertaken. Cultures of each of the fungi were maintained on Czapek Dox Agar media with adjusting pH 6.0–6.5 and slants were stored at 4 ℃. The antifungal activities of the volatile oil and oleoresins against fungi were undertaken by using inverted petri plate and poisoned food techniques[11,12].

2.3.2. Antibacterial investigations

Four pathogenic bacteria *Bacillus subtilis* (*B. subtilis*) (1790), *Staphylococcus aureus* (*S. aureus*) (3103) (Gram-positive), *Escherichia coli* (*E. coli*) (1672), *Pseudomonas aeruginosa* (*P. aeruginosa*) (1942) (Gram-negative) were selected for the present study. They were subcultured on nutrient agar broth (Hi-media) and stored at 4 ℃. Active cultures for experiments were prepared by transferring one loopful of cells from stock cultures to the flask of nutrient agar broth, which were incubated without agitation for 24 h at 37 ℃. To determine the antibacterial activity of the essential oil and oleoresins, agar well and disk diffusion method were followed as recommended by Ramdas *et al.*[12]. For standard, 0.2 mL of aqueous solution of streptomycin (1 mg/ mL) was used. After incubation for 24 h at 37 ℃, all plates were examined for any zones of growth inhibition.

2.4. Statistical analyses

Experimental results were presented as means ± SD of three parallel measurements (data were not shown). Significant differences between means were determined by student's *t*-test by using a Microsoft Excel (Microsoft Office, India) statistical analysis program and $P < 0.05$ was considered as significant.

3. Results

3.1. Phytochemistry

A careful interpretation of the experimental data obtained from GC-MS analysis resulted in the identification of a large number of components in the essential oils and oleoresins of fresh and dried leaves of *M. longifolia* (Tables 1 and 2). Table 1 shows identifications of 23 components in FMEO and DMEO representing about 94.0% and 98.4%, respectively, of the total amount. Table 2 shows the chemical compositions of ethanol and *n*-hexane oleoresins extracted from fresh and dried *M. longifolia*. A total of 21 and 23 components were identified in FMET and FMNH representing about 85.9% and 83.5%, respectively, of the total amount whereas in DMET and DMNH, a total of 24 and 20 components were identified representing about 72.6% and 83.1%, respectively, of the total amount. The dominant components identified in both essential oils were piperitenone oxide with percentages of 79.1 in FMEO and 87.8 in DMEO followed by small fractions of piperitenone, terpinen-4-ol, lippiaphenol and caryophyllene oxide. The major components found in all oleoresins were pipertenone oxide along with phytosterols such as stigmastan-3,5-diene, hentriacontane, stigma-5-en-3β-ol and fatty acids (palmitic, oleic and stearic).

Table 1
GC-MS analysis of fresh and dried mint (*M. longifolia* L.) leaves essential oils.

Compounds	FMEO (% FID)	DMEO (% FID)	AI#	AI (lit.)	Identification*
α-Pinene	0.2	tr	928	932	MS, RI, Co-GC
β-Pinene	0.3	tr	973	974	MS, RI, Co-GC
3-Octanol	1.1	0.3	985	988	MS, RI, Co-GC
p-Cymene	0.1	tr	1 019	1 020	MS, RI, Co-GC
Limonene	2.2	0.4	1 024	1 024	MS, RI, Co-GC
1,8-Cineole	0.3	tr	1 025	1 026	MS, RI, Co-GC
Linalool	0.3	0.2	1 096	1 095	MS, RI, Co-GC
Borneol	1.3	0.5	1 166	1 165	MS, RI, Co-GC
Terpinen-4-ol	0.4	0.1	1 172	1 174	MS, RI, Co-GC
p-Cymen-8-ol	0.2	0.6	1 179	1 179	MS, RI
α-Terpineol	0.5	0.3	1 188	1 186	MS, RI, Co-GC
Shisofuran	0.1	0.6	1 199	1 198	MS, RI
Carvone	0.2	tr	1 241	1 239	MS, RI, Co-GC
Piperitone	0.3	tr	1 248	1 249	MS, RI, Co-GC
cis-Piperitone epoxide	0.6	tr	1 251	1 250	MS, RI
Isopiperitenone	1.1	0.6	1 272	---	MS, RI
Piperitenone	2.9	1.5	1 338	1 340	MS, RI
Piperitenone oxide	79.9	88.5	1 369	1 366	MS, RI
Lippiaphenol (diosphenolene)	1.0	1.8	1 401	---	MS, RI
β-Caryophyllene	tr	1.0	1 415	1 417	MS, RI, Co-GC
Germacrene-D	0.3	0.5	1 487	1 484	MS, RI
Caryophyllene oxide	0.7	0.8	1 579	1 582	MS, RI
Dill apiole	tr	0.4	1 615	1 620	MS, RI
Total identified (%)	94.0	98.1			
Monoterpene hydrocarbons	2.8	0.4			
Oxygenated monoterpenes	89.1	94.7			
Total monoterpenoids (%)	91.9	95.1			
Sesquiterpene hydrocarbons	0.3	1.5			
Oxygenated sesquiterpenes	0.7	0.8			
Total sesquiterpenoids (%)	1.0	2.3			
Others	1.1	0.7			

tr: Trace < 0.05%; AI#: Arithmetic index, calculated on an HP-5 capillary column using a homologous series of *n*-alkanes C8-C18; *Co-GC: Co-injection with an authentic sample; AI (lit.): values reported by Adams[24]; % FID: Percentages obtained from electronic integration measurements by using flame ionization detection.

Table 2
GC-MS analysis of fresh and dried mint (*M. longifolia* L.) leaves oleoresins in different solvents.

Compounds	FMET (% FID)	FMNH (% FID)	DMET (% FID)	DMNH (% FID)	AI#	AI (lit.)	Identification*
p-Cymene	tr	0.1	tr	---	1 019	1 020	MS, RI, Co-GC
Limonene	tr	0.1	tr	---	1 024	1 024	MS, RI, Co-GC
1,8-Cineole	tr	tr	tr	---	1 025	1 026	MS, RI, Co-GC
p-Cymenene	4.3	tr	3.0	tr	1 091	1 089	MS, RI, Co-GC
Isopiperitenone	tr	0.5	tr	tr	1 272	---	MS Ref 1
Piperitenone	1.5	0.7	1.1	0.1	1 338	1 340	MS, RI
Piperitenone oxide	53.3	30.2	47.7	23.2	1 369	1 366	MS, RI
Germacrene-D	tr	tr	1.2	---	1 490	1 484	MS, RI
Neophytadiene	---	---	1.3	3.5	1 830	---	MS, RI
Palmitic acid	3.0	1.6	5.1	4.8	1 967	---	MS, RI, Co-GC
Phytol	tr	tr	2.2	2.5	2012	1 942	MS, RI
Stearic acid	tr	1.7	tr	1.9	2 157	---	MS, RI, Co-GC
Oleic aid	---	---	---	15.7	2 128	---	MS, RI, Co-GC
Monoolein	---	---	---	4.4	---	---	MS, RI, Co-GC
Pentacosane	tr	0.6	tr	---	---	2 500	MS
Hexacosane	tr	1.3	tr	---	---	2 600	MS
Heptacosane	tr	2.5	tr	---	---	2 700	MS
Octacosane	tr	3.9	tr	tr	---	2 800	MS
Nonacosane	tr	6.0	0.1	tr	---	2 900	MS
Triacontane	tr	6.3	0.1	tr	---	3 000	MS
Hentriacontane	---	12.1	0.1	3.7	---	3 100	MS
Stigmastan-3,5-diene	13.5	---	1.9	---	---	---	MS
Vitamin E	---	---	0.5	1.1	---	---	MS
Tritriacontane	---	---	---	3.3	---	3 300	MS
Pentatriacontane	---	5.9	----	0.9	---	3 500	MS
Ergost-5-en-3β-ol	2.7	0.4	1.3	0.1	---	---	MS
Hexatriacontane	---	7.9	----	---	---	3 600	MS
Stigma-5-en-3β-ol	7.5	1.6	6.9	15.0	---	---	MS, Co-GC
Stigmast-5,22-dien-3β-ol	0.1	0.1	0.1	2.9	---	---	MS, Co-GC
Total	85.9	83.5	72.6	83.1			

tr: Trace < 0.05%; AI#: Arithmetic index, calculated on an HP-5 capillary column using a homologous series of *n*-alkanes C8-C22; *Co-GC: Co-injection with an authentic sample; AI (lit.): values reported by Adams[24]; % FID: Percentages obtained from electronic integration measurements by using flame ionization detection.

3.2. Antimicrobial investigations

The antimicrobial activities of essential oils and oleoresins against the microorganisms were examined in the present study and their potency was qualitatively assessed by the presence or absence of inhibition zones. In both Petri plate and food poisoned method, both oils, namely FMEO and DMEO, showed good activities (> 50% zone of inhibition) against *A. niger* at 10 µL dose (Tables 3 and 4). These oils were also found to be effective in controlling the growth of *A. flavus*, *A. oryzae* and *F. graminearum* (> 40% zone of inhibition) in the food poisoned method whereas the zones of inhibition were in the range from 10%–20% in inverted petri plate method. These oils were ineffective against *P. viridicatum* in both methods. The oleoresins, namely DMET, DMNH, FMNH, FMET, showed very weak or negligible antifungal activity due to their viscous nature. The results obtained for oleoresins in the both methods were not very satisfactory. The antibacterial investigations results are given using agar well diffusion and disc diffusion method, in which tested bacterial strains showed different levels of sensitivity towards both essential oils and their oleoresins at different doses (Tables 5 and 6). The zone of inhibition for Gram-positive bacteria in both methods lies in between 5–16 mm whereas for Gram-negative bacteria it is in between 5–10 mm at 10 µL. Streptomycin was used as the standard drug. In both agar well diffusion and disc diffusion methods, Gram-positive bacteria including *B. subtilis* and *S. aureus* showed moderate level of sensitivity towards DMEO and FMEO whereas the level of sensitivity of Gram-negative bacteria including *E. coli* and *P. aeruginosa* is very less for both essential oils. The resistance of Gram-negative bacteria towards tested samples is related to lipopolysaccharides in their outer membrane. The investigations revealed the inefficiency of oleoresins towards the tested bacterial strains.

Table 3
Antifungal investigations by using inverted Petri plate method.

Samples	Doses (µL)	% Zone inhibition*					
		A. niger	*A. flavus*	*A. oryzae*	*F. monoliforme*	*F. graminearum*	*P. viridicatum*
FMEO	5	25.70 ± 0.20	15.40 ± 0.30	19.40 ± 0.56	12.70 ± 0.50	15.40 ± 0.14	-
	10	37.30 ± 0.26	27.30 ± 0.36	30.60 ± 0.52	20.10 ± 0.20	21.30 ± 0.17	-
FMET	5	5.10 ± 0.20	1.30 ± 0.20	2.80 ± 0.36	-	7.30 ± 0.20	-
	10	9.40 ± 0.30	2.60 ± 0.30	6.30 ± 0.30	12.70 ± 0.30	12.40 ± 0.14	-
FMNH	5	-	-	-	-	-	-
	10	4.70 ± 0.46	3.70 ± 0.14	2.60 ± 0.40	5.80 ± 0.10	-	-
DMEO	5	23.20 ± 1.20	14.50 ± 1.50	13.40 ± 3.20	12.30 ± 1.60	14.30 ± 1.40	-
	10	35.70 ± 1.50	27.60 ± 2.10	20.90 ± 1.50	25.60 ± 1.50	23.50 ± 1.60	-
DMET	5	-			-	-	-
	10	-	-		-	-	-
DMNH	5	-	-		-	-	-
	10	-	-	-	-	-	-

-: No inhibition.

Table 4

Antifungal investigations by using food poisoned method.

Samples	Doses (μL)	% Zone inhibition*					
		A. niger	*A. flavus*	*A. oryzae*	*F. monoliforme*	*F. graminearum*	*P. viridicatum*
FMEO	5	35.70 ± 0.20	35.40 ± 0.30	19.40 ± 0.56	12.70 ± 0.50	35.40 ± 0.14	-
	10	57.30 ± 0.26	47.30 ± 0.36	40.60 ± 0.52	41.10 ± 0.20	43.30 ± 0.17	-
FMET	5	7.10 ± 0.20	2.30 ± 0.20	2.80 ± 0.36	-	7.30 ± 0.20	-
	10	11.40 ± 0.30	4.60 ± 0.30	6.30 ± 0.30	12.70 ± 0.30	9.40 ± 0.14	-
FMNH	5	3.40 ± 0.44	4.30 ± 0.17	-	-	-	-
	10	4.70 ± 0.46	9.70 ± 0.14	2.60 ± 0.40	10.80 ± 0.10	-	-
DMEO	5	33.20 ± 1.20	31.20 ± 2.10	21.30 ± 0.20	15.60 ± 0.30	21.50 ± 0.90	-
	10	54.50 ± 1.40	42.30 ± 1.40	40.80 ± 0.90	40.50 ± 1.50	41.20 ± 1.40	-
DMET	5	5.60 ± 0.90	-	-	-	-	-
	10	6.70 ± 0.30	-	-	-	-	-
DMNH	5	2.80 ± 1.30	-	-	-	-	-
	10	3.60 ± 1.50	-	-	-	-	-

-: No inhibition.

Table 5

Antibacterial activities of essential oils and oleoresins of dried and fresh *M. longifolia* L. against a few bacterial species by the agar well diffusion method.

Samples	Doses (μL)	Diameter of inhibition zone (mm[a])			
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
FMEO	5	5.40 ± 0.37	6.50 ± 0.40	2.60 ± 0.31	3.20 ± 0.17
	10	13.70 ± 0.57	16.40 ± 0.44	7.80 ± 0.14	6.70 ± 0.15
FMET	5	-	-	-	-
	10	-	-	-	-
FMNH	5	-	-	-	-
	10	-	-	-	-
DMEO	5	4.50 ± 0.32	4.30 ± 0.36	-	-
	10	12.30 ± 1.20	14.50 ± 1.40	-	-
DMET	5	-	-	-	-
	10	-	-	-	-
DMNH	5	-	-	-	-
	10	-	-	-	-
Streptomycin	5	-	-	-	-
	10	13.50 ± 0.20	14.30 ± 0.47	-	-

-: No inhibition; [a]: Average of three replicates.

Table 6

Antibacterial activities of essential oils and oleoresins of dried and fresh *M. longifolia* L. against a few bacterial species by the disc diffusion method.

Samples	Doses (μL)	Diameter of inhibition zone (mm[a])			
		B.subtilis	*S.aureus*	*E.coli*	*P. aeruginosa*
FMEO	5	4.60 ± 0.32	3.60 ± 0.40	2.40 ± 0.20	1.20 ± 0.17
	10	9.80 ± 0.42	8.90 ± 0.20	5.60 ± 0.52	4.30 ± 0.15
FMET	5	-	-	-	-
	10	-	-	-	-
FMNH	5	-	-	-	-
	10	-	-	-	-
DMEO	5	3.90 ± 0.70	4.40 ± 1.40	-	-
	10	7.80 ± 1.30	6.70 ± 1.50	-	-
DMET	5	-	-	-	-
	10	-	-	-	-
DMNH	5	-	-	-	-
	10	-	-	-	-
Streptomycin	5	13.60 ± 0.44	12.30 ± 1.20	-	-
	10	21.30 ± 1.20	17.80 ± 1.60	-	-

-: No inhibition; [a]: Average of three replicates.

4. Discussion

According to recent investigations, dominant compounds in essential oils from *M. longifoila* flowers were piperitone oxide, piperitenone oxide, β-caryophyllene, thymol, cis- and trans-dihydrocarvone and menthofuran[1,13]. Variations of chemotypes in other *Mentha* spp. were reported by different studies[7,14]. Differences in chemical compositions observed for essential oils are likely related to abiotic factors such as climate specific regions of provenance samples and geographical factors such as altitude and soil types[15]. The variations in the chemistry of different species of *Mentha* essential oils across the world might be due to the varied agroclimatic (climatic, seasonal, geographical) conditions of the regions, isolation regimes and adaptive metabolism of plants. Plants of Lamiaceae family are known to store their oils on or near the leaf surfaces. There is also an impact of harvesting regime, drying mode and storage period on the quantity of essential oils in the leaves[16].

Previous results indicate that essential oils of *M. longifolia* showed higher antimicrobial and antifungal activities than tested commercial substances[6]. Marinokovié *et al.* showed that piperitenone oxide has strong antifungal activity against *Aspergillus* and *Fusarium* species which are in accordance with our results[17,18]. In general, oxygenated monoterpenes are significantly more active than hydrocarbon monoterpenes. Many studies reported the antibacterial efficacy of extracts obtained from *M. longifolia* against a series of pathogens including *S. aureus, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenis, Lactobacillus acidophilus, P. aeruginosa*[19,20]. Results obtained by Gullece *et al.* showed that essential oils of *M. longifolia* ssp. *longifolia* did not have selective antimicrobial activities on the basis of the cell as well as differences of bacterial microorganisms[7]. This result may be explained by the high content of cis-piperitone epoxide (18.4%), pulegone (15.5%) and piperitenone oxide (14.7%) in the essential oils of *M. longifolia* ssp. *longifolia* analyzed in the study[21].

The antimicrobial activities of essential oils depend on their chemical compositions[22]. Their biological activities are often attributed to their major components[23]. In previous reports, the antibacterial activity of *Mentha pulegium* has been attributed to its major compounds[7,24]. However, minor components appear to play a significant role and it is not well known that constituents or mixtures of them are mainly responsible for their antimicrobial activity. According to Mahboubi and Haghi[14], the synergistic effects of other constituents can explain antimicrobial activities of *Mentha* essential oils than major compounds.

Essential oils could make their way from the traditional into the modern medical domain. The results introduce essential oils extracted from fresh and dried *M. longifolia* as natural food preservatives with fewer side effects. Plant extracts appear as a promising alternative and have more beneficial effects than their synthetic counterparts through being safer, more acceptable, affordable and culturally compatible. These extracts can represent a step forward in the search for novel antimicrobial agents when there is an urgent need for novel drugs. The active extracts of the aforementioned medicinal plants justify further studies to isolate

and characterize the active principles. It is important to develop a better understanding of their mode of the biological action for new applications as antimicrobial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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