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Research Article

Reinvestigation of Chemical Composition, Pharmacological, Antibacterial and Fungicidal Activity of Essential oil from *Mentha longifolia* (L.) Huds

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Abstract

Background and Objective: *Mentha longifolia* is an extremely flavorful and aromatic herb of the mint family native to many parts of Europe, Asia and Northern and Southern Africa also known as horse mint. Extracts of *M. longifolia* have been used for centuries as a food flavoring and a medicine. The present study was aimed to reinvestigate the essential oil of *Mentha longifolia* (L.) Huds. for its chemical composition and concomitantly anti-inflammatory, analgesic, antipyretic, antibacterial and fungicidal activity. **Materials and Methods:** The essential oil was extracted using hydrodistillation method and analyzed by GC-MS. Anti-inflammatory, anti-nociceptive and antipyretic activities of essential oil were experimentally determined using mice model. Antibacterial activity and antifungal activity were determined by using disc diffusion and agar dilution method, respectively. Results were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. **Results:** The major constituents identified in the essential oil of Chamoli collection were piperitenone oxide (60.44%), 1,8-Cineole (6.16%), piperitenone (3.46%), dl-limonene (2.72%) and piperitone (1.69%). The Joshimath collection showed the presence of piperitenone oxide (36.89%), cis-piperitone oxide (17.89%), camphor (3.11%), isophorone (2.52%), thymol (2.41%) and coumarin (1.41%) and the Badrinath collection contains cis-piperitone oxide (42.74%), piperitenone oxide (33.48%), 4-Hydroxypiperitone (3.26%), thymol (2.52%), 2-Isobutylcyclopent-3-en-1-one (1.57%) and piperitenone (1.02%). The other constituents were present in minor quantities (less than 1.0%) in all three collections. The essential oil of *Mentha longifolia* show insignificant anti-inflammatory and analgesic activity but shows significant antipyretic, antibacterial and antifungal activity. **Conclusion:** The study indicated the wide chemical diversity in the essential oil of *Mentha longifolia* (L.) Huds collected from different geographical regions. The variation in chemical constituents may also effect the biological activities of the samples collected from different locations.

Key words: *Mentha longifolia* (L.) Huds, piperitenone oxide, cis-piperitone oxide, pharmacological activity, lamiaceae

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A number of *Mentha* species, indigenous to Himalayan region grow wild in India which include *Mentha longifolia*, *Mentha piperata*, *Mentha spicata* and *Mentha arvensis*^{1,2}. *Mentha longifolia* has the widest natural geographical distribution of any *Mentha* species from Central Asia to Western Europe and in Southern Africa with 22 subspecies. In India its distribution is limited to Western Himalayas from Kashmir to Garhwal and Ladakh, coming down to the sub-Himalayan ranges in Jammu. Four varieties of *Mentha longifolia* (L.) Huds viz. *M. longifolia* (L.) Huds var. *longifolia*, *M. longifolia* (L.) Huds incana variety with slender interrupted spikes and *Mentha longifolia* (L.) Huds var. *royleana* with stout continuous spikes have been reported from Uttarakhand².

The plant *Mentha longifolia* (Labiatae) is generally known for its multiple pharmacological activities like, antipyretic, anti-inflammatory activities^{3,4}, antioxidant^{5,6}, hepatoprotective⁷, spasmodic, choleric, CNS stimulative effects⁸, calcium channel blocking activity⁹, antimutagenicity¹⁰ as well as antimicrobial activities^{5,11-16}. Several chemotype of *Mentha longifolia* are reported from different countries. The differences in their chemical composition may be because of both biotic (genetic, ontogeny, morphogenic) and abiotic (soil, climate, altitude and temperature) factors which may affect biosynthesis and growth.

Mentha longifolia have been reported to contain a range of components, including ceramides¹⁷, cerebrosides¹⁷, glycosides¹⁸, flavonoids¹⁰. The main active component of *Mentha longifolia* was its essential oil, which was reported to govern its various properties. Among different chemotypes reported from different country. Piperitone oxide rich chemotype has been reported from Greece¹⁹, Italy²⁰, Lithuania²¹, Tajakistan²² and Turkey²³.

Piperitone oxide and piperidinone oxide rich varieties has been reported from Bosnia²⁴, Egypt²⁵, Tajakistan²², Greece²⁶, India²⁷⁻³⁰, Iran³¹, Jordan³², Pakistan³³, Turkey²³, Uzbekistan³⁴, Lithuania²¹ and Morocco³⁵. While carvone rich chemotype has been reported from Crete³⁶, Croatia³⁷, Greece²⁶, India³⁸, Iran³⁹, Sudan⁴⁰.

Linalool rich with piperitone oxide and/or has been reported from Turkey²³. Carveol rich chemotype has been reported from Iran⁴¹, dihydrocarvone rich from Serbia⁴², Turkey²³, Mentha furane rich chemotype from South Africa⁴³, Menthone rich from South Africa⁴⁴, puligone/menthone/isomenthone rich chemotype from Tunisia⁵ and Israel⁴⁵ and 1,8 cineole rich chemotype from Iran⁴⁶.

Keeping in mind the above chemotypic diversity, in present communication *M. longifolia* from three different

regions of Uttarakhand was taken to investigate its chemical diversity and various pharmacological activities like anti-inflammatory, antinociceptive, antipyretic, antibacterial and antifungal activity of the essential oil.

MATERIALS AND METHODS

Plant source: Fresh plant material was collected from three different locations (Badrinath, Chamoli and Joshimath) in mountainous region of Uttarakhand, India and was identified by plant taxonomist Prof. Y.P.S. Pangty. The specimens have been deposited in the Department of Chemistry, Pantnagar for future reference.

Isolation of essential oil: About 1000 g of the fresh collected aerial parts of the herbs were subjected to hydro distillation for 8 h using Clevenger-type apparatus according to the European Pharmacopoeia⁴⁷. The oil was extracted in dichloromethane (Merk, AR-grade) and dried over anhydrous sodium sulfate. The yield of oil in different collections ranged from 0.1-0.2% w/v and the oil was stored at 4°C for further analysis.

GC/MS analysis: GC-MS data were recorded on gas chromatograph HP 6890 with mass selective detector MS 5973 (Agilent technologies, USA) fitted with a HP-5 msec fused silica column (30 m × 0.25, 0.25 µm film thickness). The carrier gas was helium and initial column temperature was 50°C rising 250°C at a rate 5°C min⁻¹ the MS detector acquisition parameters. Transfer line held at 260°C and detector was held at 280°C. Detection was performed in the full scan mode from m/z 41-450. Components were identified with the aid of an automatic system of processing data of GC-MS supplied by NIST mass spectra library. Further confirmation was done by referring to Kovats index data generated from a series of alkanes (C9-C28)⁴⁸.

Experimental animals: Animals [Swiss albino mice (R)] were procured from Lab animal division, Central Drug Research Institute, Lucknow. The mice were divided into four groups of six mice each for the experiments. They were housed in standard cages at a constant temperature of 22 ± 1°C, relative humidity 55 ± 5% with 12 h light-dark cycle (08:00-20:00) for one week at least before the experiment. The experimental protocol was approved by the Committee on Animal Research (ethical committee) with Registration No. 330/CPCSEA. All tests were conducted under the guidelines of the ethical

committee for the study. The essential oil collected from Badrinath was used for evaluation of pharmacological activities.

Anti-inflammatory activity

Carrageenan-induced mice paw edema: The anti-inflammatory activity of *Mentha longifolia* essential oil (MLEO) was determined by the carrageenan-induced edema test in the right hind paw of mice. Male ICR mice (six per each group) were fasted for 24 h before the experiment with free access to water. Fifty microliter of 1% carrageenan suspension (Sigma Co., USA) in saline was injected into the plantar side of right hind paw of the mice⁴⁹. Percentage reduction of edema was measured immediately at 1st, 3rd and 24th h after the administration of the carrageenan, using a plethysmometer. Indomethacin (10 mg kg⁻¹ b.wt.) was administered intraperitoneally 30 min before carrageenan injection. MLEO (50 and 100 mg kg⁻¹ b.wt.) was orally administered 60 min before carrageenan injection. The control was given in an equal volume of saline.

Formaldehyde induced paw edema: The formaldehyde induced arthritis essential oil was determined by following the method used⁵⁰. One percent solution of formaldehyde (0.10 mL) was injection in the right hind paw of the mice on day 1 of the experiment. Doses of *M. longifolia* essential oil (50 and 100 mg kg⁻¹ b.wt.) were administered orally daily in the morning till the end of the study period i.e., for 10 days. Ibuprofen at a dose of 10 mg kg⁻¹ b.wt., orally given used as the standard drug. Saline water was used as negative control. In the evening hours, plethysmometrically, paw volume of all the mice was measured for 10 days.

Analgesic activity

Acetic acid-induced writhing response: The writhing test in mice was carried out by following the reported method⁵¹. In the brief the writhes were induced by intraperitoneal injection of 1.0% acetic acid (v/v, 0.1 mL/10 g b.wt.). There are two different doses (50 and 100 mg kg⁻¹ b.wt.) of MLEO administered orally to each groups of mice, 60 min before chemical stimulus. Ibuprofen as a positive control was administered 30 min prior to acetic acid injection. The number of muscular contractions was counted over a period of 30 min after acetic acid injection. The data represented the total numbers of writhes observed during 10 min.

Hot plate method: It is a simple and sensitive method for studying analgesic and hyper analgesic reaction in mice and was performed to measure analgesic response latencies⁵².

Antipyretic activity: To evaluate the antipyretic activity of essential oils, pyrexia was developed by using yeast by method described by Rao *et al.*⁵³. Pyrexia was induced by subcutaneous injection of 10 mL kg⁻¹ of 20% suspension of Brewer's yeast (*Sacchromyces cerevisiae*). The mice were allowed to remain quiet in the cage for 18 h for a rise in body temperature. At 19th h, the rates were again restrained to record their rectal temperature. Immediately, 0.1 mL/10 g of the MLEO in the doses of 50 and 100 mg kg⁻¹ b.wt. and paracetamol (33 mg kg⁻¹ b.wt.) was administered orally. Control group received 0.2 mL normal saline. Temperature was recorded at hourly interval in all the mice upto 3 h.

Toxicity: The acute toxicity test in mice and rats was carried out. Female mice (R) (20-30 g) were divided into test and control groups comprising of 6 animals in each group. The test was performed by using increasing oral doses of MLEO (500, 800 and 1000 mg kg⁻¹ b.wt.), in 10 mL kg⁻¹ volume to different test groups. Control group received saline solution (10 mL kg⁻¹). The experimental mice were allowed for food, were all kept under regular observation for 48 h, for any mortality or behavioral changes.

Antibacterial activity: The essential oils were screened against pathogenic bacteria's including one gram-negative (*Salmonella enterica enterica*) and 1 gram-positive (*Staphylococcus aureus*). These were procured from Department of Pharmacology, College of Veterinary and Animal sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S.Nagar, U.K., India. Essential oil was dissolved in 10% Tween 20 which was also used as control. Antibacterial activity was determined by using the disc diffusion method⁵⁴.

Fungicidal bioassay: The tested phytopathogenic fungi including *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* were procured from Department of Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S.Nagar, U.K., India. The antifungal activity was measured by agar dilution method and expressed as percentage inhibition against the mycelia growth diameter⁵⁵.

Statistical analysis: Data were expressed as Mean \pm SE. Results were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test and $p < 0.05$ was considered to be statically significant.

RESULTS AND DISCUSSION

The results of GC-MS analysis of *Mentha longifolia* collected from different locations viz: Chamoli, Joshimath and Badrinath of Garhwal region (Uttarakhand) in the month of October during flowering stage are recorded in Table 1. The GC-MS analysis of the essential oil collected from Chamoli, revealed the presence of 20 compounds contributing to 82.11% of the oil. The major constituents identified in the oil were piperitenone oxide, 1,8-Cineol, piperitenone, dl-limonene and piperitone. The other constituents were present in minor quantities (less than 1.00%) which include α -pinene, β -pinene, 3-octanol, p-cymene, β -phellandrene (trans) decahydronaphthalene, p-cresol, linalool oxide, α -terpinolene, p-cymene-8-ol, α -copaene, caryophyllene oxide, m-ethyl cumene and (t) muurolol.

In the essential oil of *M. longifolia* collected from Joshimath. sample, 13 compounds were identified which contributed to (76.75%) of the total oil. The major identified compounds were piperitenone oxide, cis-piperitone oxide, camphor, isophorone, thymol and coumarin. The other minor components present were piperitenone, resorcinol, sesamol, α -thujone, thymol and spathulenol.

In the essential oil of *M. longifolia* collected from Badrinath, 34 components were identified which contributed to 99.67% of the total oil. cis-piperitone oxide, piperitenone oxide, 4-Hydroxypiperitone, thymol, 2-Isobutylcyclopent-3-en-1-one and piperitenone, were identified as major components. The other minor components (less than 1.0%) were 3-Octanol, p-cymene, dl-limonene, trans (β) ocimene, linalool, 3-octanyl acetate, isophorone, borneol, 4-Terpineol, p-cymene-8-ol, α -terpineol, piperitone, β -bourbonene, β -elemene, β -caryophyllene (E) β -farnesene, dehydroaromadendrene, germacrene-D, δ -cadinene, Spathulenol, caryophyllene oxide, widdrol, muurolol, α -cadinol, trans-piperitenone oxide, resorcinol, cis-iso piperitenone and Z-cinrolone.

Comparison of the results recorded in Table 1 revealed a significant difference in the chemical make up of the essential oils of *Mentha longifolia* collected from different locations. Particularly, cis-piperitone oxide (42.47%) was the major component in Badrinath collection and higher in amount collected from Joshimath (25.47%) and absent in Chamoli⁵⁶.

This compound was also present (7.04%) in Sirmaur, Kashmir oil²⁸, 23.20% from Harsil, India²⁹ and was absent in Mana³⁰ and Iran collection⁴⁶. Piperitenone oxide (60.44%) present in essential oil from Chamoli was close to the earlier reported 58.90%⁵⁶ but greater than in other samples of Badrinath (33.48%), Joshimath (36.89%), Harsil (18.60%)²⁹ and Sirmaur (54.23%)²⁸. This was found to be present (21.20%) in Mana sample^{30,45} and (18.90%) in Israel sample⁴⁷. The species growing in lower elevations was different than *M. longifolia* (L.) Huds. Subsp. *himalaiensis* growing in higher elevations. Carvone earlier reported (9.80%) and (0.09%)^{28,56}, it was found to be absent in other samples^{30,45}. Similarly 4-Hydroxy piperitone oxide (1.50%) reported earlier⁵⁶ was not present in any of the collections. Camphor (3.11%), coumarin (1.41%), nonen-2-one (2.37%) sesamol (0.52%) and α -thujone (0.35%) present in the samples from Joshimath, were absents in other samples reported earlier. Sample from Joshimath contained Isophorone (2.52%) greater than that present in the sample from Badrinath (0.74%) but was absent in the oil from Chamoli. This compound was not present in *M. longifolia* analysed earlier. Furthermore, there was great variation in the minor components of the essential oil. The difference in the chemical makeup of the oils from different locations may be due to difference in ecological conditions altitudes and temperature of the collection sites. Many *M. longifolia* sp. growing in mediterranean region are piperitenone oxiderich chemotypes^{20,57,58} and piperitone oxide type. Samples contained cis-piperitone oxide but not trans-piperitone oxide in all the collections whereas sample from Sirmaur contained (24.06%) and Mana (48.70%) contains trans-piperitone oxide but not cis isomer. However, could not find any collection which is Carvone type growing in higher elevations. Though Carvone type are reported from Iran³⁹ and Sudan⁴⁰ and pulegone type from Jordan²⁵ and Tunisia⁵. The results are significant from chemical diversity point of view.

Anti-inflammatory activity

Carrageenan-induced mice paw edema: The anti-inflammatory effects of the essential oil on carrageenan-induced edema in the mice right hind paw are presented in Table 2. There was a gradual increase in edema paw volume of mice in the control and sample group. However, in the standard drug treated group a significant reduction in edema was observed at 24th h (37.19%). The essential oil could not produce significant reduction in paw volume compared to control. The inhibitory effect of the oil recorded with a dose level of 50 and 100 mg kg⁻¹ in 24 h were 3.57 and 7.22%, respectively in 24 h.

Table 1: Variation in the essential oil composition (% in oil) of *Mentha longifolia* from different regions

Compounds	KI	Present investigation							
		Badrinath	Chamoli	Joshimath	Mathela <i>et al.</i> ⁵⁶	Kharakwal <i>et al.</i> ²⁹	Singh <i>et al.</i> ²⁸	Verma <i>et al.</i> ³⁰	Golparvar <i>et al.</i> ⁴⁶
2-Pentanone	682	t	3.18	3.00	-	-	-	-	-
α -pinene	932	t	0.23	t	-	0.40	-	0.60	8.92
Camphene	954	-	-	-	-	-	-	t	1.27
Sabinene	969	-	-	-	-	0.20	-	0.30	13.94
β -pinene	974	t	0.11	t	-	0.20	-	0.70	-
3-Octanone	983	-	-	-	-	-	-	t	-
3-Octanol	988	0.97	0.92	t	-	1.60	0.36	0.10	-
Myrcene	990	-	-	-	-	-	0.32	t	0.89
α -phellandrene	1002	-	-	-	-	-	-	t	-
α -terpinene	1017	-	-	-	-	-	0.06	t	-
p-cymene	1020	0.73	0.13	-	-	-	0.20	t	-
dL-limonene	1024	0.31	2.72	0.10	-	1.10	1.42	0.90	-
β -phellandrene	1025	-	0.12	-	-	-	-	-	-
1,8-Cineol	1026	-	6.16	-	-	0.30	1.25	0.10	37.16
Trans- β -ocimene	1050	0.25	-	-	-	0.10	0.20	0.10	-
Trans-decahydronaphthalene	1053	-	0.42	-	-	-	-	-	-
γ -terpinene	1063	-	-	-	-	-	0.08	-	0.65
p-cresol	1071	-	0.13	-	-	-	-	-	-
Linalool oxide	1084	-	0.11	-	-	-	-	-	-
α -terpinolene	1086	-	0.62	-	-	-	0.04	t	0.64
Linalool	1096	0.81	-	-	-	1.40	0.25	0.30	0.29
Z-cinerolone	1097	0.20	-	-	-	-	-	-	-
m-ethyl cumene	1098	-	0.94	-	-	-	-	-	-
2-Methyl butyl 2-methylbutyrate	1100	-	-	-	-	0.80	-	-	-
α -thujone	1102	-	-	0.35	-	-	-	-	-
3-octanyl acetate	1110	0.18	-	-	-	0.90	-	-	-
Isophorone	1118	0.74	-	2.52	-	-	-	-	-
3-Octanol acetate	1123	-	-	-	-	-	-	0.10	-
2-Octanol acetate	1127	-	-	-	-	-	-	-	0.34
Cis-allo-ocimene	1130	-	-	-	-	-	-	-	2.87
Dihydro linalool	1139	-	-	-	-	-	-	t	-
Camphor	1141	-	-	3.11	-	-	-	-	-
Eucarvone	1146	-	-	0.20	-	0.70	-	-	-
3-Nonanol	1163	-	-	-	-	0.20	-	-	-
Borneol	1165	0.69	-	-	-	-	-	-	-
Menthofuran	1168	-	-	-	-	-	-	-	0.19
Borneol	1170	-	-	-	-	-	-	-	0.88
4-Terpineol	1174	0.21	-	-	-	0.10	0.20	0.60	-
Cis-pinocamphone	1175	-	-	-	-	-	-	t	-
p-cymene-8-ol	1179	0.68	0.92	-	-	0.30	0.12	0.40	-
Isopulegone	1185	-	-	-	-	-	-	-	0.75
α -terpineol	1186	0.37	-	-	-	-	0.75	0.20	-
Myrtenol	1194	-	-	-	-	0.40	-	-	-
Myrtenal	1195	-	-	-	-	-	-	0.10	-
Octanol acetate	1213	-	-	-	-	-	-	0.40	-
Cis-carveol	1226	-	-	-	-	-	-	-	2.67
Pulegone	1235	-	-	-	-	-	-	-	6.14
Carvone	1239	-	-	-	9.80	0.80	0.09	-	0.49
Piperitone	1249	0.72	1.69	-	-	1.10	-	-	-
Cis-piperitone oxide	1254	42.47	-	25.47	25.70	23.20	7.04	t	-
Trans-piperitone epoxide	1256	-	-	-	-	-	-	48.70	-
Cis-chrysanthenyl acetate	1265	-	-	-	-	-	-	0.10	-
p-menth-1-en-7-al	1275	-	-	-	-	-	-	0.10	-
Thymol	1289	2.52	-	2.68	-	0.80	0.46	1.40	-
Naphthalene,1-isocyano	1294	-	-	-	-	-	-	-	0.41
2-Hydroxy piperitone	1305	-	-	-	-	-	-	1.60	-
Sesamol	1311	-	-	0.52	-	-	-	-	-
Citronellic acid	1312	-	-	-	-	1.80	-	-	-
Trans-carvyl acetate	1339	-	-	-	-	-	-	-	-
Piperitenone	1340	1.02	3.46	0.20	-	-	0.25	0.40	-

Table 1: Continue

Compounds	KI	Present investigation							
		Badrinath	Chamoli	Joshimath	Mathela <i>et al.</i> ⁵⁶	Kharakwal <i>et al.</i> ²⁹	Singh <i>et al.</i> ²⁸	Verma <i>et al.</i> ³⁰	Golparvar <i>et al.</i> ⁴⁶
α-cubebene	1345	-	-	-	-	0.50	-	-	-
α-longipinene	1349	-	-	-	-	-	-	1.00	-
α-terpinolene	1349	-	-	-	-	-	-	-	0.63
Eugenol 1358	-	-	-	-	-	-	t	-	-
Piperitenone oxide	1366	33.48	60.44	36.89	58.90	18.60	54.23	21.20	18.97
α-copaene	1374	-	0.23	-	-	-	-	-	-
Daucene	1381	-	-	-	-	-	-	0.20	-
β-bourbonene	1387	0.54	-	-	-	-	-	t	0.32
β-elemene	1389	0.36	-	-	-	0.60	-	0.30	-
4a-α,7-β,7a-α-nepetalactone	1392	-	-	-	-	-	-	0.70	-
Cis Jasnone	1394	-	-	-	-	0.40	-	-	-
β-caryophyllene	1417	0.60	-	-	-	4.30	3.02	2.30	1.55
Coumarin	1432	-	-	1.41	-	-	-	-	-
β-copaene	1432	-	-	-	-	-	-	0.60	-
Trans-farnesene	1445	-	-	-	-	2.20	-	-	-
α-humulene	1452	-	-	-	-	0.70	-	1.50	-
(E) β-farnesene	1454	0.93	-	-	-	-	-	0.40	-
Dehydroaromadendrene	1460	0.20	-	-	-	-	-	-	-
γ-gurjunene	1477	-	-	-	-	-	-	0.30	-
Germacrene-D	1484	0.23	-	-	-	5.40	-	9.80	-
Cis iso piperitenone	1493	0.74	-	-	-	-	0.41	-	-
Bicyclogermacrene	1500	-	-	-	-	-	-	0.60	-
α-Murolene	1500	-	-	-	-	-	-	0.30	-
β-bisabolene	1505	-	-	-	-	-	-	t	-
Germacrene A	1512	-	-	-	-	-	-	t	-
γ-cadinene	1513	-	-	-	-	-	-	0.20	-
α-cadinene	1522	-	-	-	-	-	-	t	-
δ-cadinene	1522	0.19	-	-	-	0.20	-	0.50	-
Germacrene-D-4-ol	1575	-	-	-	-	-	-	0.10	-
Spathulenol	1577	0.75	-	0.21	-	0.40	-	0.20	-
Caryophyllene oxide	1582	0.73	0.26	-	-	0.80	1.09	t	-
Widdrol 1599	0.24	-	-	-	-	-	-	-	-
Resorcinol	1622	0.99	-	0.60	-	-	0.09	-	-
α-murolol	1633	0.41	0.18	-	-	-	-	0.10	-
α-Cadinol	1652	0.59	-	-	-	-	-	t	-
Trans-piperitenone oxide	1724	0.99	-	-	0.80	21.10	24.06	-	-
Diosphenol	1789	-	-	-	-	0.70	-	-	-
4-Hydroxy piperitone	1904	3.26	-	-	2.80	1.60	0.34	-	-
1, 2-Benzenediol	-	-	-	0.12	-	-	-	-	-
2-Isobutylcyclopent-3-en-1-one	-	1.57	-	-	-	-	-	-	-
Nonen-2-one	-	-	-	2.37	-	-	-	-	-
4-Hdroxy piperitone oxide	-	-	-	-	1.50	-	-	-	-
3,5,5-Trimethyl-2-cyclohexenone	-	-	2.66	-	-	-	-	-	-
α-bourbonene	-	-	-	-	-	1.10	-	-	-
Total		99.67	82.11	76.75	99.50	94.40	96.83	97.50	99.97

t: Trace (less than 1%)

Table 2: Acute anti-inflammatory activity of essential oils of, *Mentha longifolia* (Mean ± SE, n = 6)

Groups	Treatments	Dose (mg kg ⁻¹)	Change in paw thickness			Inhibition	
			0 h	4 h	24 h	4 h	24 h
1	Control	0.20 mL	2.40 ± 0.02	2.33 ± 0.01	2.32 ± 0.01	2.92	3.06
2	Ibuprofen	40	2.34 ± 0.01	1.73 ± 0.02 ^a	1.47 ± 0.02 ^a	25.77	37.19
3	MLEO	50	2.33 ± 0.01	2.25 ± 0.02 ^{ab}	2.25 ± 0.02 ^{ab}	3.57	3.57
4	MLEO	100	2.33 ± 0.02	2.24 ± 0.02 ^{ab}	2.16 ± 0.02 ^{ab}	4.00	7.22

One-way ANOVA followed by Dunnett's multiple comparison test, ^aSignificant (p < 0.05) as compared to control, ^bSignificant (p < 0.05) as compared to drug, MLEO: *Mentha longifolia* essential oil

Sub-acute anti-inflammatory activity: No significant inhibition was generated at doses of 50 and 100 mg kg⁻¹ MLEO as compared to the control (Table 3) in sub-acute anti-inflammatory activity, where arthritis was induced by

Table 3: Effect of essential oil of *Mentha longifolia* on formalin induced sub acute inflammation (Mean \pm SE, n = 6)

Groups	Treatments	Dose (mg kg ⁻¹)	Volume of inflammation										
			Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
1	Control	0.2 mL	2.13 \pm 0.02	2.27 \pm 0.04	2.36 \pm 0.03	2.52 \pm 0.04	2.38 \pm 0.03	2.30 \pm 0.02	2.30 \pm 0.02	2.28 \pm 0.01	2.27 \pm 0.01	2.21 \pm 0.01	2.26 \pm 0.01
2	Ibuprofen	10	2.11 \pm 0.02	2.13 \pm 0.02 ^a	2.19 \pm 0.01 ^a	2.27 \pm 0.01 ^a	2.21 \pm 0.02 ^a	2.19 \pm 0.01 ^a	2.15 \pm 0.01 ^a	2.26 \pm 0.01 ^a	2.17 \pm 0.01 ^a	2.19 \pm 0.01 ^a	2.15 \pm 0.01 ^a
3	MLEO	50	2.14 \pm 0.02	2.34 \pm 0.03 ^b	2.32 \pm 0.01 ^b	2.48 \pm 0.03 ^b	2.44 \pm 0.02 ^b	2.33 \pm 0.02 ^b	2.32 \pm 0.02	2.29 \pm 0.02	2.29 \pm 0.01	2.27 \pm 0.01	2.28 \pm 0.01
4	MLEO	100	2.15 \pm 0.02	2.19 \pm 0.01 ^b	2.25 \pm 0.01 ^b	2.44 \pm 0.01 ^b	2.37 \pm 0.01 ^b	2.34 \pm 0.01 ^b	2.37 \pm 0.01	2.33 \pm 0.01	2.29 \pm 0.01	2.28 \pm 0.01	2.28 \pm 0.01

One-way ANOVA followed by Dunnett's multiple comparison test, ^aSignificant (p<0.05) as compared to control, ^bSignificant (p<0.05) as compared to drug, MLEO: *Mentha longifolia* essential oil

Table 4: Anti-nociceptive activity of essential oils of *Mentha longifolia* (Hot Plate Method) (Mean \pm SE, n = 6)

Groups	Treatments	Dose (mg kg ⁻¹)	Hot plate reaction time (min)					
			0	30	60	90	120	150
1	Control (Saline water)	0.02 mL	3.00 \pm 0.04	2.99 \pm 0.03	2.97 \pm 0.04	2.89 \pm 0.03	2.88 \pm 0.05	2.87 \pm 0.02
2	Indomethacin	05	3.25 \pm 0.04 ^a	3.84 \pm 0.04 ^a	4.96 \pm 0.05 ^a	4.21 \pm 0.05 ^a	4.06 \pm 0.05 ^a	3.84 \pm 0.04 ^a
3	MLEO	50	2.91 \pm 0.03	2.92 \pm 0.04	2.98 \pm 0.04	2.99 \pm 0.03	2.87 \pm 0.03	2.93 \pm 0.04
4	MELO	100	3.00 \pm 0.03	2.94 \pm 0.04	2.99 \pm 0.04	2.87 \pm 0.03	2.89 \pm 0.04	2.93 \pm 0.06

One-way ANOVA followed by Dunnett's multiple comparison test, ^aSignificant (p<0.05) as compared to control, ^bSignificant (p<0.05) as compared to drug, MLEO: *Mentha longifolia* essential oil

Table 5: Anti-nociceptive activity of essential oils of *Mentha longifolia* (Writhing effect) (Mean \pm SE, n = 6)

Group	Treatments	Dose (mg kg ⁻¹)	Numbers of writhings	Writhings (%)	Inhibition (%)
1	Control	0.20 mL	140.50 \pm 1.88	100.00	
2	Ibuprofen	40	79.33 \pm 2.11 ^a	56.46	43.54
3	MLEO	50	134.33 \pm 0.88 ^{a,b}	95.61	4.41
4	MELO	100	124.33 \pm 0.88 ^{a,b}	88.49	1.15

One-way ANOVA followed by Dunnett's multiple comparison test, ^aSignificant (p<0.05) as compared to control, ^bSignificant (p<0.05) as compared to drug, MLEO: *Mentha longifolia* essential oil

formaldehyde injection on day zero and the samples were administered orally daily for 10 days. During the investigation the essential oil was found to be insignificant comparatively standard drug, ibuprofen.

Analgesic activity

Hot plate test: The hot plate test was useful for the evaluation of centrally acting analgesics which are known to elevate the pain threshold of mice towards heat⁵⁹. The low reaction time shown by the mice treated with the plant oil suggests that it is not a centrally acting analgesic and thus is ineffective (Table 4).

Acetic acid-induced abdominal writhing test: Data recorded in Table 5 on the acetic acid-induced writhing responses in mice are indicative of no analgesic activity of essential oil. Ibuprofen significantly reduced writhing responses induced by acetic acid when compared with the control group. There was no significant effect of MLEO at 50 and 100 mg kg⁻¹ in decreasing writhing responses in mice and showed inhibition of only 1.15 and 4.41% compared to control (Table 5).

Antipyretic activity: The results of the antipyretic activity of the essential oil are presented in Table 6. Administration of the yeast to the rats produced significant increase in rectal temperature 18 h after yeast injection. The essential oil produced a moderate antipyretic activity. Paracetamol and MLEO showed significant antipyretic activity throughout the test period of 3 h. The essential oil showed maximum

inhibition (37.61 \pm 0.01) at 100 mg kg⁻¹ in 3 h and didn't showed instant effect as the standard drug paracetamol (Table 6).

Acute toxicity: The sample was administered intraperitoneally and orally at doses of 150, 300, 450 and 600 mg kg⁻¹ of body weight. The animals were observed during the first two hours for toxic signs and then mortality was recorded for each group at 24, 48 and 72 h after dose administration. Essential oil did not cause any behavioral changes and no death was observed. Thus it was considered to be a practically non-toxic substance.

Antibacterial activity: It has been reported that the essential oil components act on outer membrane permeability in gram-negative bacteria⁶⁰. As recorded in Table 7, essential oil of *Mentha longifolia* (MLEO) showed moderate antibacterial activity against the tested pathogenic bacterial strains. The activity of essential oil showed a zone of inhibition of 18.70 \pm 0.6 mm against *S. aureus* and 17.13 \pm 0.3 mm against *S. enterica enterica*.

Antifungal activity: The results of antifungal activity assays against the three phytopathogenic fungi showed that the essential oil has inhibitory effect on the growth of fungi. At 250 ppm essential oil inhibited the growth of *Fusarium oxysporum* above 92% followed by *Sclerotium rolfsii* (70.66%). The essential oil was found to be least effective against *R. solani* (Table 8).

Although leaf extract of *Mentha longifolia* was reported to show anti-inflammatory, analgesic and antipyretic activity^{3,4} but the essential oil of *Mentha longifolia* show insignificant

Table 6: Effect of essential oils of *Mentha longifolia* on yeast induced pyrexia in mice (Mean±SE, n = 6)

Groups	Treatments	Dose (mg kg ⁻¹)	Body Temp. before administration of drug (°C)				Body Temp. after administration of drug (°C)			
			-18 h	0 h	1 h	2 h	3 h	6 h		
1	Control	0.20 mL	37.31±0.02	38.43±0.05	38.64±0.05	38.46±0.12	38.61±0.06	38.40±0.07		
2	Paracetamol	33	37.26±0.01	38.42±0.05	37.42±0.08 ^a (86.31)	37.39±0.02 ^a (88.76)	37.40±0.02 ^a (87.90)	37.30±0.02 ^a (96.69)		
3	MLEO	50	37.27±0.01	38.45±0.03	38.40±0.02 ^{ab} (4.79)	37.88±0.02 ^{ab} (48.31)	37.66±0.02 ^{ab} (67.46)	37.61±0.02 ^a (70.99)		
4	MELO	100	37.27±0.01	38.48±0.02	38.29±0.01 ^{ab} (16.39)	37.86±0.03 ^{ab} (51.79)	37.61±0.01 ^{ab} (72.18)	37.56±0.01 ^{ab} (76.45)		

One-way ANOVA followed by Dunnett's multiple comparison test, ^aSignificant (p<0.05) as compared to control, ^{ab}Significant (p<0.05) as compared to drug, MLEO: *Mentha longifolia* essential oil

Table 7: Antibacterial activities of the essential oils of *Mentha longifolia*

Samples (400 µg/disc)	Test bacteria [Zone of inhibition (mm)]	
	<i>Staphylococcus aureus</i>	<i>Salmonella enterica enterica</i>
Control	0.00±0.0	0.00±0.0
Ampicilline (30 µg/disc)	33.30±0.2 ^a	28.30±0.4 ^a
MLEO	18.70±0.6 ^d	17.13±0.3 ^e

Values are means of three replications±SE, Means with the same letter are not significantly different at $p \leq 0.05$, MLEO: *Mentha longifolia* essential oil

Table 8: Antifungal activities of essential oils of *Mentha longifolia*

Sample	Concentration (ppm)	Growth diameter (mm)			Inhibition (%)		
		<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>	<i>Sclerotium rolfsii</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>	<i>Sclerotium rolfsii</i>
Control	100	35.00±0.2	35.00±0.3	35.33±0.6	00.00	00.00	00.00
MLEO	100	07.53±0.3*	18.87±0.4*	13.20±0.4*	78.48	46.09	62.64
	250	02.67±0.4*	15.03±0.2*	10.37±0.6*	92.38	57.04	70.66

Values are means of three replications±SE, one-way analysis of variance (*Multiple comparisons versus control group (Dunnett's Method): $p < 0.050$, MLEO: *Mentha longifolia* essential oil

anti-inflammatory and analgesic activity but shows significant antipyretic activity. It can be concluded that the activity of *Mentha longifolia* leaf extract was reported earlier are not due to essential oil constituents.

Since the essential oil consists of complex mixture of numerous components. The major or trace components may be responsible for the antimicrobial activities. Piperitone, cis-piperitone oxide, thymol, piperitenone oxide present in the oils investigated are also reported to be highly effective against various phytopathogens and bacterial strains⁶¹⁻⁶³.

CONCLUSION AND FUTURE RECOMMENDATIONS

The study indicate the wide chemical diversity in the essential oils of *Mentha longifolia* (L.) Huds. collected from different geographical regions both qualitatively and quantitatively. It is reported that various biological activities in essential oils are associated with the synergetic effects of minor compounds. Based on these facts the diversified chemical composition of *M. longifolia* can be used for various biological activities, however, no report on pharmacological activity of *M. longifolia* essential oil exist, although work on leaf extracts is reported.

These results recommended that further work is required to check the genetic variability among the different populations using DNA and protein profiling.

SIGNIFICANCE STATEMENTS

The present study reveals the significant chemotypic diversity in the chemical composition of essential oils of *Mentha longifolia* (L.) Huds. with the population distributed in Mediterranean region and in Western Himalayan regions

reported so far. The chemical variation may lead to variation in pharmacological activities. The present study will help to take up further studies on pharmacological activities with other chemovariants of *Mentha longifolia* (L.) Huds. to correlate the activities since this plant has many ethnobotanical uses. The study is of significant academic importance to study the biodiversity of medicinal and aromatic plants.

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