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Original Article

Isolation, Purification and Characterization of L-Carvone from *Mentha longifolia* Using Fractional Distillation and Quantified by Gas Chromatography

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ABSTRACT

Menta longifolia is an aromatic herb consists of dried aerial parts of *Mentha longifolia* belongs to family Lamiaceae. Overground parts of the plant yield 2-4% of an essential oil, of which the chief component appears to be the monoterpene ketone, carvone. *Mentha longifolia* thus approaches spearmint (*Mentha spicata*) rather than peppermint (*M. piperita*) in oil composition. Other major constituents of the essential oil, according to various studies, include piperitenone and its oxide, piperitone and its oxide and pulegone. The present study involves isolation of *l*-Carvone from *Mentha longifolia* using fractional distillation as a tool for separation which was quantified using Gas chromatography. Characterization of purified L-Carvone was done using NMR spectroscopy (^{1}HNMR and ^{13}C-NMR). Fractions collected using fractional distillation was evaluated for anti-microbial activity for further studies.

Keywords: *Mentha longifolia*, L-Carvone, Gas chromatography, NMR.

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INTRODUCTION

The Lamiaceae family includes the well-known genus Mentha, which produces an essential oil with significant medicinal and fragrant properties. This genus may provide a high yield and can be grown in locations with a temperate climate all over the globe.[1] The vast majority of species, on the other hand, are farmed in every agro-climatic area because of their rapid rate of growth and their capacity to tolerate the harsh environmental circumstances that exist today.[2] Mentha oil, often known as menthol, is the primary byproduct of the genus Mentha and has found widespread use in the medicinal, culinary, and cosmetics sectors.[3] Farmers who were involved in the growing of mentha herbs believed this crop to be their primary source of revenue. In 2014, the value of the worldwide trade in metha oil was 205 million USD, and it is expected that this value would reach 300 million USD by 2024. With a share of 80 percent of the world's total output, India is by far the most important producer of mentha oil.[4]

Mentha longifolia, commonly known as wild mint or horsemint, is a perennial herb that belongs to the Lamiaceae family. It is widely distributed throughout Europe, Asia, Africa, and North America and is used for various medicinal and culinary purposes. It is rich in bioactive compounds such as menthol, rosmarinic acid, and flavonoids, which have potent antioxidant, antimicrobial, and anti-inflammatory properties.[5] These compounds make it a valuable ingredient in traditional medicine for treating various ailments such as gastrointestinal disorders, respiratory infections, and skin diseases. Furthermore, *Mentha longifolia* has also been found to have potential as a natural insecticide, as it contains insect-repellent compounds such as pulegone and menthofuran. Studies have shown that it can be effective against mosquito larvae, houseflies, and stored-product insects.[6] In addition to its medicinal and insecticidal properties, *Mentha longifolia* is also used in culinary practices. Its leaves are commonly used as a flavoring agent in tea, salads, and various dishes. Moreover, *Mentha longifolia* is easy to grow and maintain, making it a popular herb for home gardens and commercial cultivation.[7] Overall, *Mentha longifolia* is a valuable herb with various potential uses. Its bioactive compounds make it a valuable natural resource for medicinal and practical purposes. However, it is important to use it with caution and consult a healthcare provider before using it for medicinal purposes.

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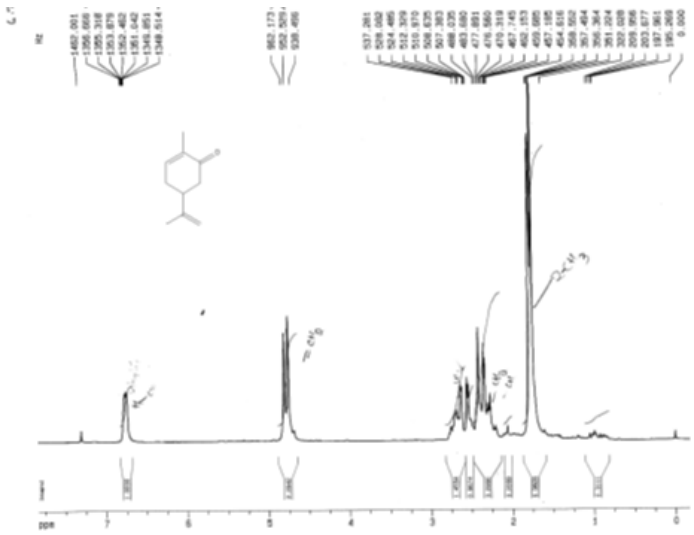
MATERIALS AND METHODS

The leaves of *Mentha longifolia* L. (spearmint) were collected from botanical garden of Indian Institute of Integrative Medicine, Jammu in November. Fresh plant material 500 g, was subjected to simultaneous extraction and isolation of essential oil by following hydro-distillation method. The essential oil was separated and collected after 4-5 hr. The oil was treated with anhydrous sodium sulphate to remove the traces of moisture in it.

Experimental

Extraction, fractionation and isolation of l-carvone from the essential oil

The extracted oil was evaluated for different parameters such as optical rotation, refractive index and specific gravity. Completely dried extracted oil was allowed to fractionate to get different types of fractions as specified in Table 1. The most enriched fraction was carefully subjected to column chromatography packed with silica gel for the isolation of L-carvone. Gradient elution was initiated with hexane-ethylacetate, gradually increasing the proportion of ethyl acetate to 5% in hexane. Various fractions of approximately 10-15 mL were collected with varying polarity of solvent system. The entire separation process was screened by carrying out Thin Layer Chromatography (TLC) of each fraction using Hexane: ethylacetate (8:2 %v/v) solvent system and anisaldehyde sulphuric acid as detector.[8] Fractions collected with 5% ethylacetate in hexane showed single spot in TLC and represented as L-carvone. The chemical struture of L-carvone was confirmed by comparing its ^{1}H-NMR and ^{13}C-NMR data with the preliminary data reported in literature.[9]



Gas Chromatography

The essential oil and its different fractions were analysed by gas chromatography using silica gel as stationary phase. Nitrogen was used as a carrier gas at the flow rate of 30 mL/min, whereas hydrogen was used as fuel at the same flow rate as that of carrier gas and oxygen is used as oxidant at the flow rate of 300 mL/min. The injector temperature was 250°C whereas that of detector was 25°C. The oven temperature was kept in between 150-200°C. The column used was SS having (1/8) inch in height. Packing was OV-17 and the detector was FID.[10]

RESULTS

Evaluation of physical properties of the extracted oil

Optical rotation $[\alpha]_{D}^{+25}$: 52 to -53.95°C.

Table 1: Fractions of the Extracted oil.

Sl. No.	Fraction number	Pressure	Colum temperature in °C	Jacket Temperature	Fraction Volume in mL
1	Fraction I	37-38	76-83	55-60	130
2	Fraction II	29-34	83-80	55-60	57
3	Fraction III	29-34	80-86	55-60	53
4	Fraction IV	26-21	86-100	55-60	250
5	Fraction V	21-18	110	55-60	225
6	Fraction VI	18-16	120	55-60	100

Table 2: GC report for the fraction-I.

Peak No.	RT min:sec	Height mV	Area mV-sec	Amount % area
1	02:21	3.642	35.163	0.311
2	02:33	1.674	14.643	0.130
3	02:57	42.510	736.758	6.526
4	04:01	721.326	10496.509	92.972
5	05:00	0.531	6.881	0.061

Total Area: 11289.954 mV-Secs.The major component in fraction No. I containing 92.972% is l-carvone.

Refractive Index Nd: 1.4858.

Specific gravity d: 0.0331

DISCUSSION

The use of essential oils has garnered substantial popularity and acknowledgement due to its diverse range of advantages and uses. The significance of essential oils stems from their multifaceted capabilities, rendering them advantageous in several domains of existence. They are often used in aromatherapy, as natural fragrances, for skincare purposes, as cleaning and disinfecting agents, and to maintain human health and well-being. *Mentha longifolia*, often referred to as Wild Mint, has essential oil inside its foliage and stems, hence imparting its distinctive fragrance and taste. The essential oil derived from *Mentha longifolia* has many uses and advantages, since it contains several pharmacologically active constituents such as menthol, menthone, isomenthone, 1,8-Cineole, Piperitenone oxide, carvone, among others. L-Carvone, a significant component derived from *Mentha longifolia*, is classified as a monoterpene ketone according to its chemical structure. This compound plays a crucial role in imparting the distinctive fragrance and taste found in the essential oil of *Mentha longifolia*. The pharmacological qualities attributed to *Mentha longifolia*, including as anti-inflammatory, anti-bacterial, respiratory advantages, and carminative effects, are purportedly related to the presence of L-carvone in its essential oil.

Table 3: GC report for the fraction-II.

Peak No.	RT min: sec	Height mV	Area mV-sec	Amount % area
1	03:00	7.526	153.860	2.599
2	03:55	476.252	5765.213	97.401

Total Area: 5919.073 mV-Sec.The major component in fraction No. II containing 97.401% is l-Carvone.

Table 4: GC report for the fraction-III.

Peak No.	RT min:sec	Height mV	Area mV-sec	Amount % area
1	03:06	3.846	61.769	1.393
2	03:53	379.175	4352.954	98.164
3	05:13	0.590	19.656	0.443

Total Area: 4434.379 mV-sec.The major component in fraction no III containing 98.164% is l-Carvone.

Table 5: GC Report of Fraction –IV.

Peak No.	RT min: sec	Height mV	Area mV-Sec	Amount % area
1	02:31	0.063	0.814	0.018
2	02:58	0.200	1.966	0.043
3	03:12	1.916	21.386	0.463
4	03:40	14.774	179.566	3.890
5	04:13	0.155	2.259	0.049
6	04:47	0.891	13.573	0.294
7	05:03	6.316	97.520	2.113
8	05:55	4.158	105.132	2.277
9	06:17	2.810	44.981	0.974
10	06:41	6.491	104.708	2.268
11	06:58	4.666	64.031	1.387
12	07:19	35.161	721.308	15.625
13	08:48	157.744	3161.251	68.481
14	10:22	0.635	23.777	0.515
15	11:18	1.076	18.467	0.400
16	13:13	2.055	40.430	0.876
17	14:12	0.797	15.088	0.327

Total Area: 4616.257mV-sec.The major component in fraction no IV containing 68.481% is l-Carvone.

Table 6: GC Report of Fraction–V.

Peak No.	RT min:sec	Height mV	Height mV	Amount % Area
1	00:40	0.286	2.345	0.040
2	03:20	0.745	2.345	0.121
3	03:46	2.773	7.161	0.610
4	04:55	0.194	36.017	0.047
5	05:15	0.727	2.797	0.212
6	06:16	0.442	12.553	0.197
7	06:30	0.504	11.658	0.111
8	06:48	4.015	6.536	1.588
9	07:25	24.075	93.811	9.237
10	09:01	217.388	545.771	85.288
11	10:38	0.739	5039.233	0.430
12	11:22	1.929	25.426	0.552
13	13:17	3.255	32.612	1.082
14	14:15	1.486	63.956	0.484

Total Area: 5908.460 mV-Sec.The major component in fraction no V containing 85.288 % is l-Carvone.

Table 7: GC Report for Fraction–VI.

Peak No.	RT min: sec	Height mV	Area mV-Sec	Amount Area
1	00:41	0.244	1.846	0.033
2	03:19	1.058	12.174	0.218
3	03:45	0.941	14.109	0.253
4	04:53	0.229	2.769	0.050
5	06:51	0.521	10.598	0.190
6	07:31	5.838	138.286	2.482
7	09:01	216.637	5060.303	90.809
8	09:38	1.616	29.067	0.522
9	10:10	0.648	15.986	0.286
10	10:40	0.587	15.556	0.279
11	11:20	4.711	79.834	1.433
12	12:22	0.838	19.342	0.347
13	13:18	2.798	58.322	1.047
14	14:17	4.991	98.319	1.764
15	15:16	0.410	15.950	0.286

Characterisation of l-Carvone.^{1}H NMR interpretation of l-Carvone: 6.7 (m, 1H), 4.8-4.7 (m, 2H), 2.6-2.56 (m, 1H), 2.54-2.5 (m, 1H), 2.43-2.40 (m, 3H), 1.75-1.73 (m, 6H).^{13}C NMR of l-carvone: 143.6, 109.6, 42.6, 41.8, 34.7, 20.4, 15.1.

The steam distillation process was used to extract the essential oil from the leaves of *Mentha longifolia*. The separated essential oil was first assessed for a range of physical parameters, including optical rotation, refractive index, and specific gravity. The aforementioned findings are shown above. Following the completion of the physical examination, the extracted oil underwent column chromatography in order to isolate L-carvone. Gas chromatography was used to separate L-carvone from six distinct fractions obtained from column chromatography. Based on the results of gas chromatography analysis shown in Tables 2-7, it was determined that the essential oil obtained from the leaves of *Mentha longifolia* exhibited a substantial concentration of L-carvone. The isolated L-carvone is further characterized by ^{13}C and ^{1}H NMR. Which is an important parameter for the proper characterization of isolated constituent.

CONCLUSION

The present study was intended as a contribution to confirm the better knowledge of the essential oil of the leaves of *Mentha longifolia*. Mentha oil was extracted from *Mentha longifolia* using hydrodistillation and then enriched by Fractional Distillation. The enriched constituents were analysed using Gas Chromatography. L-Carvone was isolated using Column Chromatography and characterized using ^{1}H-NMR and ^{13}N-CMR. However, it is obvious that further investigations are needed to elucidate the entire chemical composition and to determine the exact contribution of each component to the biological activities. So this essential oil also used in folk medicine in treatment of many infections and its possible commercial exploitation as sustainable development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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