

Essential oil content and chemical composition of *Mentha Longifolia* growing wild in district Panjgur Balochistan.

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ABSTRACT

This study aimed to characterize the essential oil content and chemical composition of *Mentha longifolia* (plant) species that grows in the wild (District Panjgur, Balochistan, Pakistan). Essential oil was extracted by steam distillation from the leaves of the plant, and its GC-MS analysis was performed. The main constituents of essential oil were 3-cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butanyl)-, cyclohexene, 1-methyl-4-(1-methylethylidene)-, eucalyptol, l-menthone and caryophyllene oxide. Eucalyptol and cyclohexene derivatives which contribute to the

woody, minty and herbaceous aroma of the plant were particularly important as they have been reported to have antibacterial, antioxidant and anti-inflammatory activities. The essential oil composition marked differences from the previous studies in Punjab and Khyber Pakhtunkhwa (KP), where the compounds present in the extract were similar to those reported in this study. Caryophyllene oxide was identified as one of the compounds responsible for stress/anxiety reduction which further supports the medicinal value of *M. longifolia* oil. The oil also had insecticidal activity. It was used for pest control as well as mosquito repellent. This study highlights the biological and therapeutic importance of *M. longifolia* and suggests potential industrial applications in agriculture, medicine, and personal care. It also offers insights into the chemical makeup of the plant and opens up the way to further exploration of its different potential uses in order to obtain the safest and sustainable application of the plant in both scientific and practical contexts.

Key Words: Chemical composition, Essential oil, *Mentha longifolia*, Therapeutic properties

Introduction

For at least hundreds of years, indigenous peoples of varying ethnic backgrounds have relied on the medicinal properties of various plants in order to treat human and animal illnesses. However, the traditional culture of medicinal plants and the plant itself in general are threatened by various environmental and anthropogenic sources (Lone and Bhardwaj, 2013). Medicinal herbs have been used as natural remedies for many centuries, especially when they are used natively as healthful preparations or as infusions and essential oils (Rezaei *et al.*, 2021). India (and China in particular) is one of the countries with a large biodiversity and extensive use of traditional medicines. For instance, almost 46% of the higher plant species found in India are in the Himalayas (Lone and Bhardwaj, 2013). The demand for medicinal plants has increased dramatically over the last few decades, as it is estimated that 8–9% of people living in developing countries relies on herbal medicines for their primary healthcare needs (Rasul, 2018). *M. longifolia* is a fragrant herb that is part of the Lamiaceae family. It grows widely in Europe, Asia, and North Africa. The herb has a

peppermint-like smell and is often used in traditional medicine. Its long spikes produce flowers that are lilac-white in color. The oil from *M. longifolia* contains many compounds. The main one is pulegone, and others include piperitenone, 1,8-cineole, isomenthone, menthol, and methone (Salihila *et al.*, 2019). These compounds are what give the herb its powerful properties: it can fight bacteria, reduce spasms, act as an antioxidant, and even help protect nerves or work against cancer. It is also known to repel insects. People often use the plant to ease digestive issues, soothe sore throats, help with missed periods, and treat respiratory problems. In cooking, especially in regions like Iran and the Middle East, the young leaves of *M. longifolia* are popular for flavoring. They are used to add taste to foods and drinks such as yogurt-based beverages, as well as candies and chewing gum. Even in traditional medicine, crushed leaves are applied to relieve headache by being used as a nasal spray or in herbal medicines (Bonokwane *et al.*, 2022). Currently available research indicates that the bioactivity of the plant against arthropod pests would be evident with the high fumigant activity of the essential oil from *M. longifolia* against *Varroa destructor* mites (Kavallieratos *et al.*, 2022). Moreover, the application of *M. longifolia* essential oil as aromatherapy and scented candles in accordance with the increasing demand for natural and environmentally friendly cosmetic products requires caution because the indoor air pollution that may be caused by burning the scented candles is a concern (Afriani *et al.*, 2024; Halligudi and Al Ojaili, 2013; Chandrasekaran *et al.*, 2021). This present study aims at extraction and chemical characterization of essential oil of wild *M. longifolia* collected from the District Panjgur, Balochistan.

Material and Methods

Study period and location

The experiments were carried out between November 2023 and September 2024 at the Biochemistry Laboratory, University of Balochistan, Quetta.

Plant collection

M. longifolia Plants Fresh leaves were collected in flowering season at different places in District Panjgur Balochistan. Samples were collected in areas near Newan

River in Khudabadan, Washboodh, Zandi Dhaaz and Saduk. Identification of the plant was done by Dr. Shaista Anjum, Department of Botany, University of Balochistan.

Sample preparation

The plant material was carefully taken to the laboratory for processing. Mud and other surface debris was washed off about 100 grams of *Mentha* leaves with tap water. The leaves were separated from the stems and left to dry at room temperature for 2 weeks. After 2 weeks the dry leaves were crushed into the powder using a mortar and pestle. The powdered material was wrapped in clean plastic bags and stored in a cool place for further use.

Essential oil extraction

The essential oil (EO) was hydro-distilled using a portable hybrid solar essential oil distillation attached with Clevenger apparatus. The dried / powdered leaf material was mixed with 250 mL of tap water in a 250 mL round bottom flask and the solution was distilled for 2–3 hours. A yellow-colored essential oil was obtained with a yield ranging from 0.5% to 0.7%. The extracted oil was collected in triplicate. To remove residual moisture, it was dried over anhydrous sodium sulfate in a desiccator for a few days. Finally, the oil was stored in sealed dark glass containers at 4°C until further analysis.

Chemical characterization using GC-MS

Gas Chromatography-Mass Spectrometry (GC-MS) was employed to analyze the chemical composition of the essential oil using an Agilent Technologies 6890N GC system equipped with a 7683B Series auto-injector and a 5975 inert XL Mass Selective Detector (Agilent Technologies, CA, USA). A HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm) was used for compound separation.

The sample (1.0 µL) was injected in split mode with a ratio of 1:100. Helium served as the carrier gas at a flow rate of 1.5 mL/min. The oven temperature was programmed to rise from 80°C to 220°C at a rate of 4°C per minute, holding for 3 minutes initially and 10 minutes at the final temperature. Injector and transfer line temperatures were set at 220°C and 290°C, respectively. Detection was carried out using electron ionization at 70 eV, and mass spectra were recorded over a scan range of 50–550 m/z. The chemical constituents were identified and quantified using the

instrument's built-in data analysis software, based on peak area percentage (Iqbal *et al.*, 2013).

Results and discussion

M. longifolia (wild mint), abundantly found in the Panjgur district, is widely recognized for its medicinal, culinary, and insect-repellent properties. To explore its bioactive potential, essential oil was extracted from 100 g of dried leaves using hydro-distillation, yielding 0.18 ml of oil. The chemical composition was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), which identified 14 constituents, accounting for 79.9% of the total oil content (Table 1).

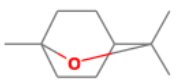
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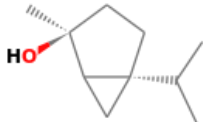
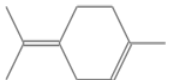
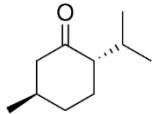
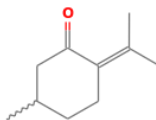
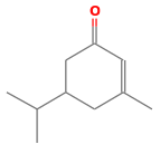
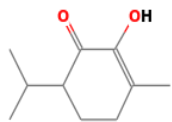
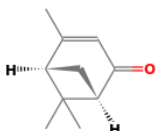
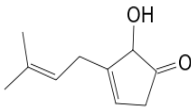
- **3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butanyl)-** (21.45%)
- **Cyclohexanone, 5-methyl-2-(1-methylethylidene)-** (19.20%)
- **Eucalyptol (1,8-cineole)** (16.17%)
- **Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-** (14.74%)

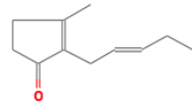
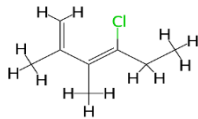
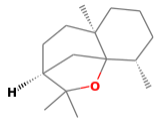
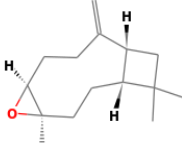
Eucalyptol (Retention time: 3.416, 16.17%)

Eucalyptol is a monoterpene with a minty, camphorous aroma and multiple applications across medicinal, cosmetic, and food industries. It is known for its antimicrobial, antifungal, and insect-repelling properties, making it valuable in pharmaceuticals and aromatherapy (Kazak, 2022; Shiekh *et al.*, 2024). The concentration of eucalyptol in this study (16.17%) is higher than reported in South Algeria (5.72%) (Saifi *et al.*, 2023), Saudi Arabia (1.98–10.38%) (Desam *et al.*, 2017; Abdel-Hameed *et al.*, 2024), Sudan (2.36%) (Balla *et al.*, 2017), and Kenya (0.14%) (Bett *et al.*, 2022), but lower than that reported in Egypt (24.4%) (Hassanein *et al.*, 2020).

Table 1. Chemical composition of the essential oil of *M. Longifolia* extracted from leaves

Sr. No.	Compound name	Molecular Mass	Molecular formula	RT (mins)	Area %	Structure
01.	Eucalyptol	154.2493	C ₁₀ H ₁₈ O	3.416	16.17	

02.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1 α ,2 β ,5 α)-	154.2493	C ₁₀ H ₁₈ O	3.697	0.04	
03.	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	136.2340	C ₁₀ H ₁₆	3.865	0.08	
04.	l-Menthone	154.25	C ₁₀ H ₁₈ O	4.828	4.26	
05.	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	152.2334	C ₁₀ H ₁₆ O	6.316	19.20	
06.	2-Cyclohexen-1-one, 3-methyl-5-(1-methylethyl)-, (S)-	152.2334	C ₁₀ H ₁₆ O	6.984	1.44	
07.	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)-	168.2328	C ₁₀ H ₁₆ O ₂	7.045	0.51	
08.	2-Oxabicyclo[2.2.2]octan-6-one, 1,3,3-trimethyl-	170.2487	C ₁₀ H ₁₈ O ₂	7.130	0.32	
09.	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	150.2176	C ₁₀ H ₁₄ O	7.951	14.74	
10.	3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butanyl)-	166.22	C ₁₀ H ₁₄ O ₂	8.249	21.45	

11.	3-Methyl-2-pent-2-ethyl-cyclopent-2-enone	164.2441	C ₁₁ H ₁₆ O	8.340	1.11	
12.	(Z)-4-Chloro-2,3-dimethyl-1,3-hexadiene	144.642	C ₈ H ₁₃ Cl	9.002	0.23	
13.	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a, 9-tetramethyl-, [3R-(3α,5α,9α,9α)]-	222.3663	C ₁₅ H ₂₆ O	9.278	0.12	
14.	Caryophyllene oxide	220.3505	C ₁₅ H ₂₄ O	10.033	0.23	

Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)- (1α,2β,5α) (Retention Time: 3.697, 0.04%)

This compound, structurally similar to menthol, is a monoterpene alcohol contributing to the cooling sensation associated with mint. Though present in small amounts (0.04%), it is significant due to its therapeutic and aromatic properties (Mikaili *et al.*, 2013; Kamatou *et al.*, 2013). Higher concentrations have been reported in China (0.69%) (Wang *et al.*, 2020), Nigeria (7.61%) Ugbabe *et al.*, (Ugbabe *et al.*, 2023), and Iran (0.50%) (Nasiri *et al.*, 20240).

Cyclohexene, 1-methyl-4-(1methylethylidene)-, (Retention Time: 3.865, 0.08 %)

A monoterpene ketone, pulegone is appreciated for its minty scent and used in traditional medicine as well as flavors and perfumes (Sousa *et al.*, 2011). This plant *M. longifolia* collected from the studied district Panjgur, can repel insects and fight bacteria. It contains these special qualities at a concentration of (0.08%). Other places have higher concentrations: Iran with (2.49%) (Nasiri *et al.*, 20240), Turkey with (2.28%) (Kalin, 2013), and Algeria with (0.18–0.46%) (Saidi *et al.*, 2024; Abdelali *et*

al., 2024). Because of these properties, this plant shows a lot of potential for making natural insect repellents, medicines, and for industrial applications.

l-Menthone (retention time 4.828, area % 4.26)

M. longifolia essential oils often contain a chemical called l-Menthone, which is a type of monoterpene ketone with the chemical formula $C_{10}H_{18}O$. This substance appears as a clear, pale-yellow liquid and is known for its strong minty smell. Plants produce l-Menthone by combining isoprene units, a process important for creating flavors and fragrances (Dong *et al.*, 2010). The carbonyl group in l-Menthone is likely key to its ability to act as an insecticide, playing a significant role in its biological activity. Due to its calming, antibacterial, and antioxidant traits, l-Menthone is commonly found on food, beverages, medicines, and topically used preparations. The present content (4.26%) is less than studies from China (41.6 %) (Pang *et al.*, 2020), Iran (13.4 %) (Shafaie *et al.*, 2019), Tunisia (10.66 %) (Zouari-Bouassida *et al.*, 2018), and Saudi Arabia (6.62%) (Al-Sarar *et al.*, 2014), however it is higher than Egypt (1.0%) (Hassanein *et al.*, 2020). Compared to other research, the present content is lower.

Cyclohexanone, 5-methy-1-2-(1methylethylidene)- (Retention time: 6.316, area %: 19.20)

One of the most common compounds in the present research is this monoterpenoid ketone. It is minty and herbaceous in smell and has a layout that improves its bioactivity and reactivity. Furthermore, showing possible medicinal effects like stress reduction and mild analgesic impact, it is widely used in fragrance and personal care items (Abdoul-Latif *et al.*, 2024). Furthermore, it might act as a natural insect repellent, have antimicrobial and antifungal properties, and present with others (Ainane *et al.*, 2019; Hussien *et al.*, 2021).

The current concentration is 19.20%, much higher than in other countries. For example, in Sudan, it is only 0.19% according to a study reported by Balla *et al.* (2017). Turkey has a concentration of 2.08% based on research reported by Aksoy *et al.* (2019). In Iran, the range is between 2.05% and 7.98%, as found in studies reported by Verdi *et al.* (2017) and Baygar, (2019). Saudi Arabia has a concentration of 0.75% according to research reported by Kiki and Ibrahim, (2020). In Morocco,

the numbers range from 0.24% to 4.17%, based on findings of Ainane *et al.* (2019) and Said *et al.*, (2013). However, this concentration is lower than in Egypt, where it is between 27.71% and 33.46%, according to studies reported by Abou-Dobara *et al.* (2023) and Hussien *et al.* (2021).

2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-(S)- (Retention Time: 6.984, Area %: 1.44)

With methyl and isopropyl groups, this compound has a cyclohexenone skeleton, thereby having both polar and hydrophobic features. Having a strong organic scent, it is found in fragrances and aromatherapy. Its organization points to possible antimicrobial, anti-inflammatory, and antioxidant activity, therefore it would be most helpful in respiratory and skin treatment formulations (Mammadova, 2021; Nowak *et al.*, 2022; Yildirim *et al.*, 2019). Although lower than in Kazakhstan (2.09%), (Nowak *et al.*, 2022), the concentration discovered in this analysis (1.44%) is higher than that from Turkey (0.15%), (Yildirim *et al.*, 2019), India (0.50%), (Sandhu *et al.*, 2021), Mexico (0.22%), (Zavala-Gómez *et al.*, 2021), and France (0.18%), (Badache *et al.*, 2019).

2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methylethyl)- (Retention Time: 7.045, Area %: 0.51)

This compound contains both hydroxyl and isopropyl groups, which influence its functional properties. It has a fresh herbal scent and is used in perfumery and aromatherapy. Reported biological effects include antioxidant and antimicrobial activity, making it suitable for cosmetic and skin protection applications (Mbatha *et al.*, 2022; DŽambiĆ *et al.*, 2020; Dean *et al.*, 2011; Kim, 2021). In this study, the content (0.51%) is lower than previous reports from Turkey (1.63%), reported by Temel *et al.* (2023), Nigeria (0.66%), reported by Bassey *et al.* (2024), Thailand (2.09%), reported by Kakanopas, (2021), India (0.67%), reported by Namasudra *et al.* (2021), and Egypt (3.44%), reported by Salman *et al.* (2020), but higher than Libya (0.3%), reported by Janacković *et al.* (2015).

2-Oxabicyclo [2.2.2]octan-6-one, 1,3,3-trimethyl- (Retention Time: 7.130, Area %: 0.32)

1,8-Cineole, a bicyclic ether with a ketone group, contributes a fresh, slightly

carbonaceous aroma commonly found in mint and eucalyptus oils. It has therapeutic benefits such as enhancing respiratory clarity, anti-inflammatory, and mild analgesic effects, making it valuable in respiratory and muscle relief formulations. It also exhibits antimicrobial and insect repellent properties, useful in eco-friendly products (Janacković *et al.*, 2015). In this study, the concentration of 1,8-cineole (0.32%) is lower than reported in China (2.07%), by Chitra *et al.* (2020), Egypt (1.54%), by Abou-Dobara *et al.* (2023), and Mexico (1.18%), by Zavala-Gómez *et al.* (2021), but higher than Sudan (0.25%), by Adam *et al.* (2021).

Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- (Retention Time: 7.951, Area %: 14.74)

Used in aromatherapy and highly regarded for its medicinal properties in perfumes and colognes, this ketonic compound has a camphor like fragrance. It could relieve suffering similar camphor and menthone, so helping to break up congestion. Furthermore, possible antibacterial and insect repellent effects are possible, therefore increasing its applicability in personal care and herbal products (Bett *et al.*, 2022). Although lower than Egypt (18.7%), (Adam *et al.*, 2021) and Korea (17.55%), (El-Kelany, 2020), the concentration of camphorone in this study (14.74%) is higher than reported from Kenya (10.91%), [17] and China (7.32%), (Wang, 2020).

3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butanyl)- (Retention Time: 8.249, Area %: 21.45)

Used in aromatherapy and perfumes for its calming and grounding properties, this hydroxy ketone has a unique earthy, sweet, and herbal fragrance. It also shows anti-inflammatory and antioxidant effects, further supporting its hypothetical medical uses (Marko *et al.*, 2002). The insecticidal activity of this compound suggests its utility in pest control formulations (Leblalta *et al.*, 2020). The concentration of this compound (21.45%) is higher than reports from Brazil (2.58%), (Leblalta *et al.*, 2020; Calgaro *et al.*, 2023), Pakistan (10.07%), (Aihetasham *et al.*, 2015; Sonam *et al.*, 2021), and India (7.76–56.72%), (Neri *et al.*, 2024), but lower than Algeria (89.09%), (Marko *et al.*, 2002).

3-Methyl-2-pent-2-ethyl-cyclopent-2-enone (Retention Time: 8.340, Area %: 1.11)

This cyclic ketone is known for its earthy, woody, and sweet aroma, adding depth to mint-based essential oils. It is used in aromatherapy for stress relief and has potential antimicrobial and anti-inflammatory properties (Fedotova and Konovalov, 2023). The concentration of this compound (1.11%) is higher than studies from Algeria (0.77%), (Marko *et al.*, 2002) and Turkey (0.19%), (Kim, 2021), but lower than reports from Russia (1.14%), (Lui *et al.*, 2015) and China (1.31%), (Rushendran *et al.*, 2023).

(Z)-4-Chloro-2,3-dimethyl-1,3-hexadiene (Retention Time: 9.002, Area %: 0.23)

(Z)-4-Chloro-2,3-dimethyl-1,3-hexadiene is an organic compound with a six-carbon chain, containing two double bonds and a chlorine atom in the rare (Z) configuration. The compound's configuration affects its molecular stability and interactions, making it a unique component in essential oils, such as those from *M. longifolia*. Chlorinated hydrocarbons are rare in natural extracts, and their presence may be due to environmental factors or plant metabolic processes (Lo *et al.*, 2020). Although its use in the fragrance industry is limited due to its reactive nature, it could be valuable as a chemical intermediate in synthetic processes. *M. longifolia* essential oil contains a special compound that we can study to understand how it is produced in different ways or how environmental factors affect its composition (Ihsan *et al.*, 2017). In the United States, this essential oil is being researched. Studies from Algeria by researchers Ihsan *et al.* (2017) and Yakhlef *et al.* (2020) found high amounts of this compound: (0.443% and 0.38%), which are higher than the amounts found in this study (0.23%).

2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3a,5a,9a,9a)]- (Retention Time: 9.278, Area %: 0.12)

A ring-fused compound, the polycyclic sesquiterpenoid contributes earthy, woody, and perhaps hot aromas to essential oils. Used in aromatherapy, it is said to be soothing, anti-inflammatory, antimicrobial, and antioxidant (Diaz *et al.*, 2008). Its occurrence in *M. longifolia* essential oil suggests grounding and medicinal properties. The stereochemistry of the compound being complicated renders it significant in research of biosynthetic processes of aromatic plants (CP, 2023). In the present

study, the concentration of the compound in *M. longifolia* essential oil is (0.12%), which is higher than the study occurred in India was lower (0.11%), (Chen et al., 2022), whereas China (1.30%), (Fagbemi *et al.*, 2022) and Nigeria (0.42%), (Macedo *et al.*, 2019) were higher. Perhaps due to differences in climate, soil content, type of plant, and method of extraction, Brazil and Nigeria studies discover even higher levels (6.02% and 1.642%, respectively), (Ohiri *et al.*, 2023; Kamal, 2022).

Caryophyllene oxide (Retention Time: 10.033, Area %: 0.23)

Caryophyllene oxide is a chemical known for its spicy and woody smell. It is commonly used in perfumes due to its ability to remain stable and enhance the lasting power of scents. Beyond its fragrance, caryophyllene oxide has other useful properties: it can reduce inflammation, kill bacteria, and repel insects (Sing *et al.*, 2023). These qualities make it valuable in consumer products and also as a natural substitute for chemical insect repellents. The essential oil from the plant *M. longifolia* contains caryophyllene oxide, which may provide health benefits like nerve protection, anxiety relief, and inflammation reduction. The amount of this chemical in *M. longifolia* essential oil varies around the world (Sharopov *et al.*, 2012). As the present study indicated that the content of chemical compound was about (0.23%), that is very lower than the study occurred in India, it is about (0.48%), (Sharopov *et al.*, 2012) whereas in Taiwan (11.33%), (Wang *et al.*, 2020). In Tajikistan, the concentration ranges from (0.9 to 2.5%) (Bertoli *et al.*, 2011) and in Poland, it measures around (0.5%) (Bertoli *et al.*, 2011).

Conclusion

The obtained result of the present study reveals the key molecules uniquely present in the *M. longifolia* leaf essential oil (EO) including 3-cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butanyl)-, cyclohexene, 1-methyl-4-(1-methylethylidene)-, eucalyptol, menthone, and caryophyllene oxide. GC-MS analysis reveals a mixture of alcohols, ketones, and terpenes that contributes to the woody, minty, and herbaceous aroma of the oil. Their antibacterial, antioxidative, and anti-inflammatory qualities make these substances promising for medicinal and medicinal applications. When compared to earlier research in other Pakistani provinces; Punjab and KPK, the study findings

also showed the variation in the range of these compounds' contents. Notably, caryophyllene oxide helps to lower stress and anxiety, therefore underpinning the therapeutic potential of *M. longifolia* of the area studied. The essential oil also seems to work well as a mosquito repellant and as a pest control insecticide for agriculture. By highlighting the biological and medicinal value of *M. longifolia*, the results provide knowledge about its possible applications in several fields, including agriculture and medicine. Among scientists and the younger generation, this study seeks to highlight the plant's many uses as well as its safety for use.

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Conflict of Interest

Author declares no conflict of interest

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