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Original Article

Modulatory effects of *Mentha longifolia* and *Mentha spicata* essential oils on *Candida albicans* biofilm formation

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Abstract

Mentha longifolia (horsemint) and *Mentha spicata* (spearmint) are economically valuable aromatic plants widely utilized in food, cosmetic, and pharmaceutical industries due to their rich reservoir of bioactive compounds. This study investigated the antifungal and antibiofilm properties of essential oils extracted from these species against various Candida strains, particularly *Candida albicans*, a major opportunistic pathogen responsible for oral and systemic infections. Essential oils were extracted via hydrodistillation using a Clevenger apparatus and chemically characterized through GC-MS, revealing that *M. longifolia* oil was predominantly composed of pulegone (29.7%), menthone (26%), and eucalyptol (17.8%), while *M. spicata* oil was mainly enriched in pulegone (68.5%), eucalyptol (5.2%), and thymol (3.5%). The antifungal activity, evaluated by agar-well diffusion and broth microdilution assays, showed inhibition zones ranging from 9–14 mm for *M. longifolia* and 9–12 mm for *M. spicata*, with corresponding minimal inhibitory concentrations (MICs) of 0.39–6.25 mg/mL and fungicidal concentrations (MFCs) of 12.5–100 mg/mL. Both oils markedly reduced biofilm biomass in a concentration-dependent manner, with up to 90% inhibition observed at 4× MIC. The potent biofilm disruption was attributed to the high terpenoid content, capable of altering fungal membrane integrity. Overall, these findings demonstrate that the essential oils of *M. longifolia* and *M. spicata* possess significant antifungal and antibiofilm potential, highlighting their possible application as natural, plant-derived therapeutic agents for controlling Candida-associated oral and biomedical infections.

Keywords: *Mentha longifolia*, *Mentha spicata*, Essential oils, *Candida*, Biofilm

1. Introduction

According to the World Health Organization (WHO), 80% of the population in developing nations depends on traditional herbal treatments as their primary source of healthcare. Herbal medicines constitute a minimum of 25% of all pharmaceutical medications. Plants utilized for their fragrant or medicinal qualities frequently possess active pharmacological compounds. A recent study has demonstrated that specific medicinal and aromatic herbs possess antiseptic, antiviral, anti-allergic, anti-inflammatory, analgesic, estrogenic, and immune-stimulating properties [1, 2].

Lamiaceae comprises about 6,000 species, many of which possess medicinal properties and are utilized in medicine. Lamiaceae, consisting of 45 genera and 574 species, is Turkey's most extensive family of indigenous plants. Mentha L. species is notable for its fragrant properties and ability to operate as an antiseptic, anticarcinogenic, expectorant, relaxant, diuretic, and treatment for common cold, indigestion, nausea, and sore throat. Assessing these plants' medicinal properties and oxidative stress indicators according to their geographical origins is essential before utilizing them for medical purposes [3,4].

Various kinds of Mentha are used to produce spices and herbal teas. All parts of the Mentha plant, including the leaves, stems, and roots, have been used in tribal and traditional medicines [5]. Mentha aquatica, Mentha longifolia, Mentha piperita, Mentha spicata, and Mentha arvensis L. are economically important species. These species can generate phytochemicals with practical applications in various industries, including pharmaceuticals, food, flavors, ointments, and related sectors. Some examples of these phytochemicals are iso-menthol, iso-menthone, cineol, limonene, piperine, carvacrol, dipentene, linalool, and thujone [6, 7].

Recent investigations have shown that Mentha sp. has an essential impact on candidal species. This study examines the chemical content of Mentha longifolia and Mentha spicata plants on the candidal biofilm found in gynecological samples.

The study aims to examine the phytochemical composition of Mentha species and assess the impact of the phy-

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tochemical composition of Mentha longifolia and Mentha spicata on candidal biofilm.

2. Materials and methods

2.1. Origin of Mentha Species and Essential Oil Extraction

The two Mentha kinds investigated, M. longifolia and M. spicata, were obtained fresh from a local market in the Hail region in December 2021. Powdered Mentha (100 g) was extracted for 3 hours with 500 mL of distilled water using a Clevenger-type device, as described in the European Pharmacopoeia [8]. Before analysis, the resulting oil was dried over anhydrous sodium sulfate and stored in sealed glass vials in a refrigerator at four °C.

2.2. Chemical properties of Mentha essential oils

The chemical composition of the volatile oil extracted from the aerial sections of M. longifolia and M. spicata was investigated using a Hewlett-Packard 6890 chromatograph equipped with a flame ionization detector (FID) and an electronic pressure control injector. A gas chromatography apparatus coupled to mass spectrometry (GC-MS) was used on a gas chromatograph HP 7890 (II) and an HP 5975 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with an electron impact ionization of 70 eV. We used an HP-5MS capillary column (30 m x 0.25 mm, Agilent Technologies, Hewlett-Packard, CA, USA) with a 0.25 µm film thickness.

We raised the temperature by five °C/min from 40°C to 280°C. Helium served as the carrier gas, with a 1.2 mL/min flow rate, a split ratio of 60:1, a scan time of 1 second, and a mass range of 40-300 m/z. The bioactive compounds in M. longifolia and M. spicata volatile oils were found using retention index (RI) estimates for (C8-C22) n-alkanes and comparisons to genuine molecules. By comparing recorded mass spectra of substances to those in the Wiley/NBS mass spectral library of the GC-MS data system, to other published mass spectra, and to data reported as a relative proportion of the overall peak area, as previously described by Essid et al. [9].

2.3. Various strains of Candida

This study was carried out by the Ethics Committee of Hail Affairs, with reference number H-08-L-074. Anonymity was ensured to safeguard patient privacy and data confidentiality in compliance with The Declaration of Helsinki. The study participants were individuals from the Hail region of Saudi Arabia experiencing cutaneous infections. This study utilized a compilation of reference and clinical Candida strains. Five Candida strains were obtained from the American Type Culture Collection: Candida utilis ATCC 9255 (A1), Candida guillermondii ATCC 6260 (A4), Candida tropicalis ATCC 1362 (A8), Candida albicans ATCC 10231 (A14), Candida albicans ATCC 20402 (A15), and Saccharomyces cerevisiae ATCC 20407 (A9). These strains were used as controls in the study.

2.4. Evaluation of the inhibitory effects of the substance on Candida growth.

2.4.1. Disk Diffusion Assay

The antifungal activity against Candida species was evaluated using the agar well diffusion method. The inoculum density of all Candida strains was adjusted to approximately 1×10^7 cells/mL (corresponding to an optical density of 0.5 at 540 nm), and the standardized suspensions were uniformly spread onto the surface of Sabouraud dextrose agar plates using a sterile cotton 10 µL of the tested EO was applied to Whatman disc number 3, which had a diameter of 6 mm. The impregnated discs were then placed on the surface of inoculation plates. Positive control was established using the standard antifungal drug itraconazole discs. After being kept in a controlled environment at a temperature of 37 °C for 24 hours, the region that prevented the proliferation of microbes (the zone of inhibition, in millimeters) was identified. The trials were conducted thrice [10].

2.4.2. Determination of the Minimum Inhibitory and Minimum Fungicidal Concentrations

The minimum inhibitory concentration (MIC) values for the essential oils (EOs) of M. spicata and *M. longifolia* against all strains of Candida were measured using the broth dilution method, following the standard methods outlined by [10]. To determine the MFC values, 10µL samples were obtained from every well with a medium that showed no growth signs. These samples were subsequently transferred onto Sabouraud dextrose agar plates. The number of organisms that remained alive after being incubated at 37 °C for 24 hours was calculated as CFU/mL. The minimum fungicidal concentration (MFC) was defined as the lowest concentration of the essential oil capable of killing 99% of the fungal cells. Itraconazole served as the positive control, tested within a concentration range of 12.5–0.003 mg/mL.

2.5. Biofilm formation on polystyrene

In this study, biofilms were cultivated on pre-sterilized polystyrene flat-bottom 96-well microtiter plates (Iwaki, Tokyo, Japan) for 48 hours using yeast nitrogen-base media. Medium batches were inoculated with yeast cultures that had been incubated overnight and placed in an orbital shaker at 150 rpm, with a temperature of 37°C. After 24 hours in the stationary growth phase, the cells were collected, rinsed with phosphate-buffered saline (PBS) with a pH of 7.2, and adjusted to a density of 107 cells per milliliter. Each microtiter plate well received 100 microliters of a standardized cell solution containing 10^7 cells per milliliter. The plate was then incubated at 37 °C for 48 hours to allow the yeasts to attach to the wells' surfaces for 90 minutes. Three wells of each plate were used as negative controls, using the same procedure but without adding Candida suspensions. After the adhesion phase, cells that did not adhere were eliminated from the wells by gently washing them twice with 200 µL of PBS. Each well received a transfer of 100 microliters of yeast nitrogen base medium. The plates were then incubated at 37°C in a shaker operating at 75 rpm. The adherent biofilm was immobilized using 95% ethanol and then treated with 100 µL of a 1% solution of crystal violet (Merck, Lyon, France) for 5 minutes. Next, the unbound crystal violet was eliminated, and the wells were rinsed three times with sterile distilled water. Subsequently, the water was assimilated, and the microtiter plate was left to dry in the air for 30 minutes. Next, the biofilm was dissolved using a solution of acetic acid (33%). Subsequently, a volume of 125 µL was extracted from each well and transferred to a 96-well microtiter plate. The optical density at a wavelength of 570 nm was then calculated. Biofilm production was classified

as strongly positive (OD570 ≥ 1), weakly positive (0.1 ≤ OD570 < 1), or negative (OD570 < 0.1) [11].

2.6. Evaluation of the antimicrobial properties of Mentha essential oils against biofilm formation

The antibiofilm activity of the essential oil was determined against Candida strains with a strong propensity to generate biofilms. The inhibitory effect of Mentha essential oils (EOs) on establishing and developing biofilms was assessed using the methodology outlined by Aneja and Sharma [12]. The biofilms were cultured for 48 hours at 37°C on 96-well microtiter plates. Subsequently, various concentrations of E. cardamom EO (1/2× MIC, 1× MIC, 2× MIC, and 4× MIC) were added. Subsequently, 100 µL of the EO was dissolved in DMSO and combined with Sabouraud broth to achieve concentrations of 1/2× MIC, 1× MIC, 2× MIC, and 4× MIC per well. The plates were incubated for 24 hours, after which the biofilm biomass was evaluated using CV staining. The CV-stained biofilm cells were measured at a wavelength of 570 nm using a microplate reader. The percentage of biofilm eradication was calculated using the following formula: The growth inhibition rate is calculated by subtracting the optical density (OD) of the sample from the OD of the growth control, dividing the result by the OD of the growth control, and multiplying by 100.

2.7. Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA to compare the effects of different concentrations of essential oils on biofilm formation and antifungal activities across Candida strains. Differences between groups were considered statistically significant at $p<0.05$.

3. Results

3.1. Antifungal activity of *Mentha* Eos

Candida is the causative agent of the fungal infection referred to as candidiasis. Certain strains of Candida can infect individuals, with Candida albicans being the most widespread species. Since fungi are an inherent element of the ecosystem, it becomes difficult to evade their presence. Externally, fungi inhabit the soil, plants, trees, and many forms of vegetation. Furthermore, they can be found on the skin and numerous internal surfaces. This fungus has gained significant attention due to its high resistance to commonly used antifungal drugs. In pursuit of this objective, scientists actively seek novel chemicals and molecules with antifungal properties.

Essential oils are natural compounds used in the food, cosmetic, and medicinal sectors. Essential oils derived from Mentha species possess potent antibacterial and antifungal activities, making them practical as natural preservatives and fungicides. The biological activity of natural products is influenced by their chemical composition, which can vary with the method of collection, plant part, climatic conditions, and extraction method.

The antifungal properties of the essential oils of M. longifolia and M. spicata, as well as carvone, were examined. The results (Table 1) indicate that the essential oils exhibited significant antifungal properties against all

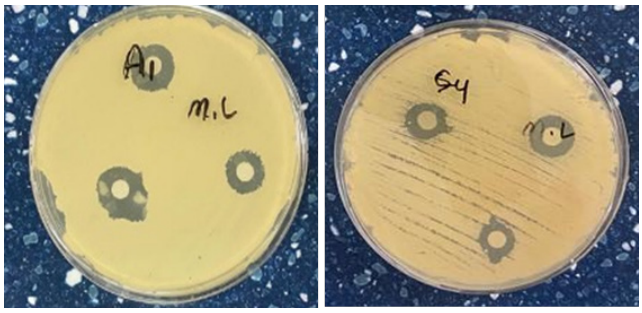

Fig. 1. Antifungal effect of *M. longifolia* EO against clinical *C. albicans* (C4) and reference *C. utilis* (A1) strains using disc diffusion method.

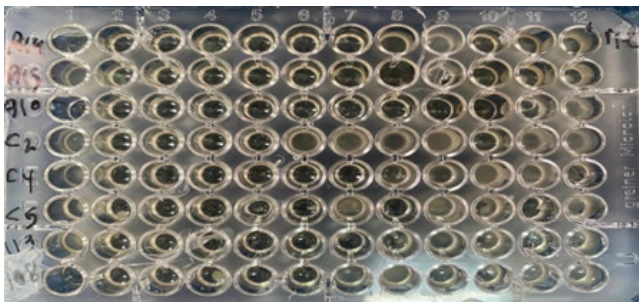

Fig. 2. Result of MIC and MFC of *M. longifolia* EO.

Table 1. Antifungal activity of *Mentha longifolia.*

Strains	Species	*Mentha spicata* EO (10 mg/ml)		
		IZ±SD (mm)	MIC (mg/ml)	MFC (mg/ml)
A1	*C. utilis* ATCC 9255	14.66±0.57	0.39	12.5
A4	*C. guillermondii* ATCC 6260	11±1	0.39	50
A8	*C. tropicalis* ATCC 1362	11±0	3.125	100
A9	*S. cerevisiae* ATCC 20407	11.66±0.57	3.125	12.5
A10	*C. albicans*	11.66±0.57	1.562	100
A14	*C. albicans* ATCC 10231	10.66±0.57	6.25	25
A15	*C. albicans* ATCC 20402	12±1	6.25	100
C2	*C. albicans*	12.66±1.15	3.125	100
113	*C. albicans*	9.66±0.57	0.39	50
C4	*C. albicans*	13.33±1.52	0.78	100
C5	*C. albicans*	13.33±0.57	6.25	100
108	*C. albicans*	11.66±0.57	0.39	100

IZ: Inhibition zone; SD: Standard deviation; MIC: Minimal inhibitory concentration; MFC: Minimal fungicidal concentration

12 Candida strains. The susceptibility of Candida strains to M. longifolia essential oil resulted in inhibitory zones ranging from 9 to 14 mm (Figure 1). The MIC and MFC values ranged from 0.39 to 6.25 mg/ml and 12.5 to 100 mg/ml, respectively (Figure 2).

3.2. Antifungal activity of *Mentha spicata*

The Candida strains sensitive to Mentha spicata essential oil exhibited inhibitory zones, MIC values ranging from 0.39 to 6.25 mg/ml, and MFC values ranging from 12.5 to 100 mg/ml. Carvone extract showed larger inhibitory zones (9–28 mm) and more potent antifungal activity against four of the twelve Candida strains. For example, the inhibition zone diameter against C. albicans strain C5 was 17.66±0.57 mm, and against C. albicans ATCC 10231 (A14) was 25±0 mm (Figure 3, Table 2).

3.3. Analysis of the chemical makeup of Mentha essential oils

GC-MS analysis identified 41 compounds in M. spicata essential oil, representing 97.1% of total components. Pulegone (68.5%) was the predominant constituent, followed by eucalyptol (5.2%), thymol (3.5%), isoborneol (2.0%), cis-dihydrocarvone (1.7%), β-bourbonene (1.7%), and longifolene (1.6%) (Table 4, Figure 5).

For M. longifolia, nineteen compounds were identified, accounting for 96.8% of total constituents. Pulegone (29.7%), menthone (26.0%), and eucalyptol (17.8%) were major components (Table 5, Figure 6).

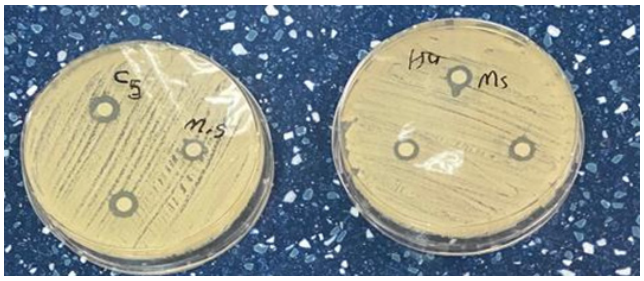

Fig. 3. Antifungal effect of *M. longifolia* EO against clinic *C. albicans* (C5) and reference *C. guillermondii* (A4) strains using disc diffusion method.

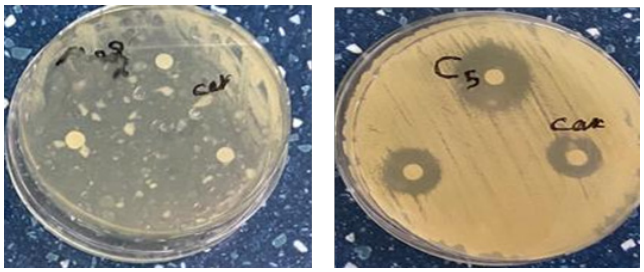

Fig. 4. Antifungal effect of carvone against clinic *C. albicans* (C5) and reference *C. tropicalis* (A8) strains using disc diffusion method.

3.4. Anti-Biofilm Activity

The anti-biofilm effect of M. longifolia EO against clinical Candida strains (Table 6) revealed that increasing EO concentration reduced optical density and biofilm

Table 2. Antifungal activity of Mentha *spicata.*

Strains	Species	*Mentha spicata* EO (10 mg/ml)		
		IZ±SD (mm)	MIC (mg/ml)	MFC (mg/ml)
A1	*C. utilis* ATCC 9255	10.66±0.57	0.78	50
A4	*C. guillermondii* ATCC 6260	9.66±0.57	0.39	50
A8	*C. tropicalis* ATCC 1362	9.33±0.57	3.125	100
A9	*S. cerevisiae* ATCC 20407	9.33±0.57	3.125	12.5
A10	*C. albicans*	15±1	1.562	100
A14	*C. albicans* ATCC 10231	12±0	6.25	25
A15	*C. albicans* ATCC 20402	10.66±0.57	6.25	100
C2	*C. albicans*	11.33±0.57	3.125	100
113	*C. albicans*	10±0	0.39	50
C4	*C. albicans*	11±0	0.78	100
C5	*C. albicans*	11.66±0.57	6.25	100
108	*C. albicans*	12.66±1.15	0.39	100

IZ: Inhibition zone; SD: Standard deviation; MIC: Minimal inhibitory concentration; MFC: Minimal fungicidal concentration.

Table 3. Antifungal activity of carvone and itraconazole.

Strains	Species	Carvone (10 mg/ml)	*Mentha spicata* EO (10 mg/ml)		
		IZ±SD (mm)	IZ±SD (mm)	MIC (mg/ml)	MFC (mg/ml)
A1	*C. utilis* ATCC 9255	9±1	6±0	0.156	2.5
A4	*C. guillermondii* ATCC 6260	14.66±1.15	13±1.73	0.625	5
A8	*C. tropicalis* ATCC 1362	9.33±0.57	14.33±0.57	0.625	2.5
A9	*S. cerevisiae* ATCC 20407	28±2	12.66±0.57	0.078	1.25
A10	*C. albicans*	10.66±1.15	12±0	0.078	10
A14	*C. albicans* ATCC 10231	25±0	14±1.73	0.156	2.5
A15	*C. albicans* ATCC 20402	10.66±1.15	14.66±0.57	0.078	10
C2	*C. albicans*	15±1	10±0	0.156	2.5
113	*C. albicans*	28.66±1.15	6±0	0.312	10
C4	*C. albicans*	13.33±1.15	6±0	0.312	10
C5	*C. albicans*	17.66±0.57	7.33±0.57	0.078	10
108	*C. albicans*	26±0	7±0	0.039	10

Table 4. Chemical composition of *M. spicata* EO using GC-MS.

Compound Name	Amount	KI Calc	Ki Adams	Ki nist	RT	%
α-Pinene	0.231	856.9876	939	1012	12.708	0.2
Sabinene	0.524	892.5207	975	1115	15.454	0.5
1-Octen-3-ol	0.066	905.5642	979	1452	16.462	0.1
β-Pinene	0.194	911.633	979	1120	16.931	0.2
3-Octanol	0.107	918.4524	991	1386	17.458	0.1
Carveol (fr.1)	0.748	921.0792	1024	1146	17.661	0.7
α-Terpinene	0.381	932.6087	1017	1170	18.552	0.4
o-Cymene	0.201	939.2081	1026	1250	19.062	0.2
Eucalyptol	5.278	943.5041	1031	1253	19.394	5.2
γ-Terpinene	0.357	971.4027	1059	1254	21.55	0.3
cis-Sabinene hydrate	0.167	979.2702	1070	/	22.158	0.2
Terpinolene	0.319	998.5895	1088	1267	23.651	0.3
Pinocarveol trans	0.275	1010.734	1139	/	24.827	0.3
Cis-Verbenol	0.085	1027.459	1141	1668	26.075	0.1
Ocimene <neo-allo->	0.124	1038.046	1144	/	26.865	0.1
Thujanol <neoiso-3->	0.213	1042.241	1151	/	27.178	0.2
Menthone	0.448	1055.897	1152	1474	28.197	0.4
Isopulegol >iso->	0.868	1068.011	1159	1533	29.101	0.9
Isoborneol	2.072	1079.188	1160	1642	29.935	2.0
Dihydro carvone <cis->	1.732	1097.252	1192	1645	31.771	1.7
Dihydro carveol <neo->	0.791	1117.461	1194	1755	33.183	0.8
Pulegone	69.861	1173.222	1237	1662	37.079	68.5
Carvone oxide. trans-	0.144	1176.614	1276	1800	37.316	0.1
β-Ionone	0.384	1187.677	/	/	38.089	0.4
Thymol	3.61	1204.211	1290	2172	39.613	3.5
Piperitenone	0.884	1220.751	1343	/	40.705	0.9
Carvyl acetate <cis->	0.418	1253.196	1367	/	42.847	0.4
β-Bourbonene	1.691	1268.6	1388	1498	43.864	1.7
β-Element	0.923	1276.886	1390	/	44.411	0.9
β-Longipinene	0.296	1287.367	1400	/	45.103	0.3
Longifolene-(V4)	1.641	1299.318	1407	1574	45.892	1.6
β-Copaene	0.35	1302.049	1432	1477	46.469	0.3
Aromadendrene	0.351	1316.669	1441	1631	47.375	0.3
Muurola-3.5-diene	0.379	1319.251	1453	/	47.535	0.4
Muurola-4(14).5-diene	0.825	1335.033	1466	/	48.513	0.8
γ-Muurolene	0.866	1370.986	1479	1750	50.741	0.8
Calamenene <trans->	0.542	1392.658	1522	/	52.084	0.5
δ-Cadinene	0.132	1402.324	1523	/	52.978	0.1
Caryophyllene oxide	0.233	1443.461	1583	2008	55.403	0.2
Cubenol	0.214	1476.285	1646	2080	57.338	0.2
α-Cadinol	0.193	1509.598	1654	2224	59.658	0.2
						TOT
	100					**97.1**

RT: Retention time

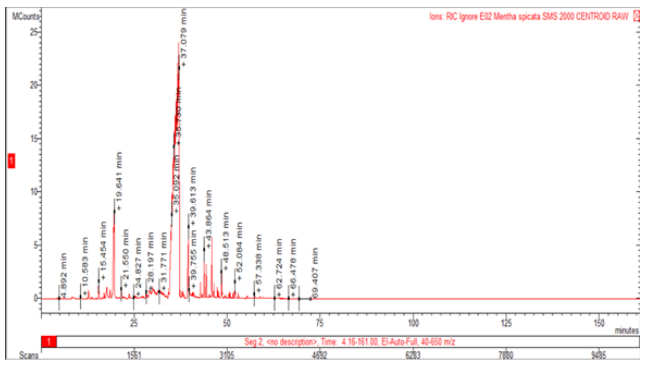

Fig. 5. Chemical composition of *Mentha spicata* EO.

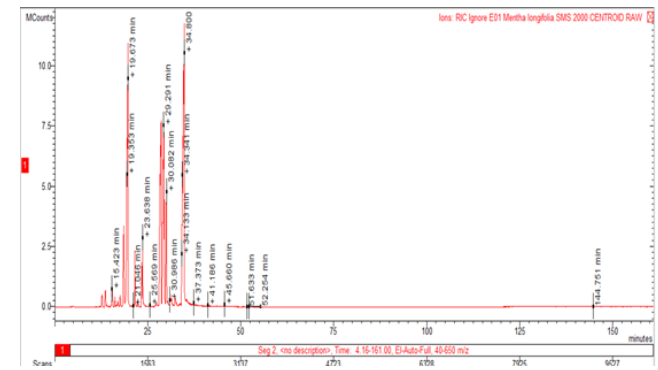

Fig. 6. Chemical composition of *M. longifolia* EO.

formation. Similarly, M. spicata EO (Table 7) demonstrated biofilm inhibition, especially at higher concentrations (2×MIC and 4×MIC), confirming its effectiveness in resisting Candida biofilm development

4. Discussion

Essential oils obtained from Mentha species have garnered heightened scientific and industrial attention owing to their robust antibacterial and antifungal characteristics. The current investigation demonstrated that both Mentha

Table 5. Chemical composition of *M. longifolia* EO using GC-MS.

Compound Name	Amount	Ki calc	Ki Adams	Ki NIST	RT	%
α-Pinene	0.671	856.1206	939	1012	12.641	0.7
Camphene	0.97	867.7924	954	1075	13.543	1.0
Cyclohexanone 3-methyl-	0.119	872.2955	952	/	13.891	0.1
β-Pinene	1.07	892.1196	979	1110	15.423	1.0
3-Menthene	0.336	902.2386	987	/	16.205	0.3
β-Myrcene	0.238	911.8659	990	1145	16.949	0.2
α-Phellandrene	0.568	920.3028	1002	1177	17.601	0.6
α-Terpinene	4.384	932.0393	1017	1187	18.508	4.3
Eucalyptol	18.147	942.9736	1031	1210	19.353	17.8
γ-Terpinene	1.713	971.118	1059	1221	21.528	1.7
Mentha-3.8-diene	0.274	981.5735	1072	/	22.336	0.3
Terpinolene	3.272	998.4213	1088	1267	23.638	3.2
Menthone	26.491	1070.557	1152	1474	29.291	26.0
Borneol	3.069	1072.246	1169	1715	29.417	3.0
Isocitral <(Z)->	5.849	1081.158	1164	/	30.082	5.7
α-Terpineol	0.816	1098.177	1188	1662	31.352	0.8
Verbenone	0.425	1106.698	1205	1726	32.431	0.4
Pulegone	30.293	1132.074	1237	1661	34.204	29.7
Caryophyllene	0.075	1295.804	1419	1612	45.66	0.1
	100					**TOT 96.8**

Table 6. Anti-biofilm activity of *M. longifolia* EO against *Candida* strains.

Strains	Concentration	OD_{570nm} ± SD	Biofilm potency
	Blank	0.81±0.05	Moderate biofilm
	1/2x MIC	0.7±0.01	Moderate biofilm
A1	MIC	0.43±0.03	Moderate biofilm
	2*MIC	0.09±0.004	Low biofilm
	4*MIC	0.07±0.009	Low biofilm
	Blank	1.17±0.06	High biofilm
	1/2* MIC	0.92±0.04	Moderate biofilm
A15	MIC	0.82±0.04	Moderate biofilm
	2*MIC	0.09±0.004	Low biofilm
	4*MIC	0.04±0.008	Low biofilm
	Blank	0.66±0.1	Moderate biofilm
	1/2* MIC	0.55±0.09	Moderate biofilm
C4	MIC	0.08±0.005	Low biofilm
	2*MIC	0.06±0.004	Low biofilm
	4*MIC	0.03±0.006	Low biofilm
	Blank	2.58±0.17	High biofilm
	1/2* MIC	2.18±0.08	High biofilm
C5	MIC	1.92±0.04	High biofilm
	2*MIC	1.44±0.09	High biofilm
	4*MIC	0.92±0.04	Moderate biofilm
	Blank	1.97±0.06	High biofilm
	1/2* MIC	1.98±0.015	High biofilm
113	MIC	0.94±0.05	Moderate biofilm
	2*MIC	0.78±0.08	Moderate biofilm
	4*MIC	0.58±0.07	Moderate biofilm

OD: Optical density; SD: Standard deviation; MIC: Minimal inhibitory concentration.

longifolia and Mentha spicata essential oils shown considerable antifungal efficacy against twelve Candida strains, including clinical isolates of Candida albicans. These findings underscore the extensive potential of Mentha-derived bioactives as natural antifungal agents, in accordance with current trends advocating for the substitution of synthetic fungicides with environmentally benign and plant-based alternatives [13, 14].

4.1. Antifungal Efficacy

Both of these essential oils have been shown to have powerful fungistatic and fungicidal properties, as evidenced by their low MIC/MFC values and their extensive inhibition zones. In particular, the significant inhibition that was recorded for M. spicata and the carvone extract implies that monoterpenes, oxygenated hydrocarbons, and other volatile chemicals have a synergistic role in the process of disturbing the growth of fungi. Through the use of GC-MS analysis, the presence of carvone, pulegone, menthone, and eucalyptol was determined to be among the most important elements. Furthermore, the existence of these constituents is associated with the antifungal effects that have been documented prior to [15]. In particular, carvone and pulegone have the ability to disrupt the fungal

Table 7. Anti-biofilm activity of *M. spicata* EO against clinical *Candida* strains.

Strains	Concentration	OD_{570nm} ± SD	Biofilm potency
	Blank	0.81±0.05	Moderate biofilm
	1/2* MIC	0.55±0.01	Moderate biofilm
A1	MIC	0.42±0.07	Moderate biofilm
	2*MIC	0.06±0.02	Low biofilm
	4*MIC	0.05±0.03	Low biofilm
	Blank	1.17±0.06	High biofilm
	1/2* MIC	1.89±0.04	High biofilm
A15	MIC	1.12±0.07	High biofilm
	2*MIC	0.98±0.01	Moderate biofilm
	4*MIC	0.2±0.03	Moderate biofilm
	Blank	0.66±0.1	Moderate biofilm
	1/2* MIC	0.24±0.06	Moderate biofilm
C4	MIC	0.08±0.01	Low biofilm
	2*MIC	0.07±0.05	Low biofilm
	4*MIC	0.03±0.07	Low biofilm
	Blank	2.58±0.17	High biofilm
	1/2* MIC	1.98±0.08	High biofilm
C5	MIC	1.67±0.02	High biofilm
	2*MIC	1.12±0.2	High biofilm
	4*MIC	0.77±0.03	Moderate biofilm
	Blank	1.97±0.06	High biofilm
	1/2* MIC	1.55±0.01	High biofilm
113	MIC	1.12±0.04	Moderate biofilm
	2*MIC	0.66±0.06	Moderate biofilm
	4*MIC	0.23±0.01	Moderate biofilm

OD: Optical density; SD: Standard deviation; MIC: Minimal inhibitory concentration.

plasma membrane by their interaction with ergosterol, which results in an increase in permeability and increases the amount of cellular components that are lost (16).

4.2. Mechanisms of Antifungal Action

The antifungal activity of Mentha essential oils can be linked to the combined action of monoterpenes and sesquiterpenes, which effect many fungal targets concurrently. This is the mechanism by which the oils exert their antifungal properties. Terpenoids are lipophilic compounds that can integrate into the membranes of fungi, at which point they change the fluidity of the membranes and disturb the proton gradients that are necessary for the synthesis of ATP [17].

These disruptions can lead to mitochondrial malfunction and suppression of cell wall formation in Candida species, which ultimately results in cell lysis due to the destruction of the cell. It has been demonstrated that the compounds pulegone and menthone, which are typically found in M. longifolia, have the ability to control lipid peroxidation and create reactive oxygen species (ROS), hence generating oxidative stress within fungal cells [18].

4.3. Chemical Composition and Biological Correlations

When it comes to establishing the bioactivity of essential oils, the chemical diversity of these oils is an extremely important factor. The results of the GC–MS analysis showed that monoterpenes, in particular pulegone (29.7–68.5%), were the predominant component of both of the Mentha oils. Pulegone was supported by lower levels of oxygenated derivatives, such as eucalyptol and menthone. These chemicals not only have antifungal activities, but they also have antioxidant capabilities, which may improve their stability and the biological function they perform [19].

It is possible for ecological and environmental factors, such as the composition of the soil, the time of harvest, and the weather conditions, to have an effect on the variations in composition that occur among Mentha species [20]. These factors might be responsible for the reported differences in antifungal efficacy between M. spicata and M. longifolia, as well as other Mentha taxa that have been examined all over the world.

4.4. Comparative Analysis with Previous Studies

The inhibition zones that were recorded for M. spicata and M. longifolia are compatible with those that were reported by [21, 22], who revealed similar antifungal performance against Candida albicans and Candida tropicalis. This was determined by comparing our experiments with the previous research that had been conducted.

Furthermore, it has been demonstrated that carvone is a powerful antifungal monoterpene that is able to prevent the production of ergosterol in fungal membranes [23]. Enhanced membrane penetration and disruption of fungal metabolism are two of the potential outcomes that can result from the synergistic effects of Mentha essential oil components like menthol, limonene, and thymol. As a result, the powerful antifungal activity that was reported in this investigation might be the result of interactions between various bioactive chemicals that are either additive or synergically effective.

4.5. Biofilm Inhibition and Resistance Mitigation

It is well known that certain species of Candida are capable of forming biofilms that are resistant to traditional antifungal medications and display improved resistance. The results of our research showed that the essential oils of M. longifolia and M. spicata greatly inhibited the pro-

duction of biofilms at increasing concentrations. This was accompanied by a discernible reduction in both the optical density and the mass of the biofilm over time. This anti-biofilm action is most likely the result of interference with quorum sensing and extracellular polymeric substance (EPS) formation, both of which are processes that are essential to the stability of biofilms [24].

Terpenoid molecules have the potential to impede the formation of hyphae and disrupt the adhesion proteins of fungi, hence limiting the maturation of biofilm. A similar set of data was reported by *Dahuja* et al. [17], who found that plant-derived terpenes exhibited a powerful antibiofilm action against Candida spp. isolates.

4.6. Pharmaceutical and Clinical Relevance

The antifungal and antibiofilm properties of Mentha essential oils make them good candidates for drug development. Their natural origin, capacity to break down in the environment, and low toxicity make them good ingredients for topical treatments, mouthwashes, and inhalable medicines that fight fungal infections [25]. Adding oils from Mentha to food packaging films and disinfection formulas could also make products safer and last longer [19].

Also, adopting encapsulation or nanoemulsion technology can make oils more stable and help them release their antifungal properties in a regulated way [26].

4.7. Limitations and Future Perspectives

Both antifungal and antibiofilm properties have been identified in Mentha essential oils, which places them in a position to be considered as potentially useful candidates for pharmaceutical development. These ingredients are appealing for use in the formulation of topical creams, mouthwashes, and inhalable treatments that are aimed against fungal infections [25]. Their natural origin, biodegradability, and low toxicity make them appealing. Additionally, the integration of oils derived from mentha into food packaging films and disinfection compositions has the potential to improve both the safety of the product and its stability over time [19].

In addition, the utilization of encapsulation or nanoemulsion technology has the potential to enhance oil stability and controlled release, hence further enhancing the antifungal activity of these substances [26].

5. Conclusions

Horsemint (Mentha longifolia) and spearmint (Mentha spicata) are economically and medically useful Mentha species. While M. longifolia is mostly wild, M. spicata is mostly cultivated but can be naturalized. Phenolic acids, flavonoids, and essential oils in the Lamiaceae family have antispasmodic, anti-inflammatory, analgesic, stimulant, and antioxidant activities. Mentha species have been used in traditional medicine to treat nausea, ulcerative colitis, pneumonia, and liver illnesses.

The chemical composition of Mentha essential oils depends on genetic, environmental, and ecological factors such soil composition, altitude, temperature, and provenance. These variables affect bioactive component quantity and variety, especially terpenoids, which give plants their scent and therapeutic properties.

Essential oils from Mentha species have been widely investigated for their antibacterial properties, particularly against Candida species. The current study confirms that M. longifolia and M. spicata essential oils are strong antifungal and anti-biofilm.

Carvone, pulegone, and eucalyptol dominate their terpene-rich chemical profiles, which explain their biological potential. Combining these chemicals inhibits fungal growth and biofilm formation, which is crucial to fungal persistence and treatment resistance. These essential oils have antifungal and anti-biofilm properties, making them promising industrial applications.

They are safe, natural alternatives to synthetic fungicides and preservatives in pharmaceutical, food, and cosmetic sectors. Clinical trials, nanoencapsulation, and molecular studies should be used to determine the mechanisms of action.

For repeatability and bioactivity, extraction and chemical profiling must be standardized. The findings support the use of Mentha essential oils in modern clinical and biotechnological applications as sustainable, eco-friendly fungal infection treatments and product safety enhancers.

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Author contributions

Ayshah Alrashidi: Conceptualization, Funding acquisition, Project administration, Writing – original draft. Badria Abd El-Rahman:Investigation, Methodology, Writing – review & editing. Meshari Alazmi: Conceptualization, , Resources, Supervision, software, Writing – review & editing. Safa Mustafa: Resources Writing – original draft, Writing – review & editing.

Conflict of Interest

The author declares no conflict of interest.

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