

GC–MS-based metabolites profiling, in vitro antioxidant, antimicrobial, and anticancer properties of different solvent extracts from leaves, stems, roots, and flowers of *Micromeria fruticosa* (Lamiaceae)

Mohammad Al-Nuri ^{1*,‡}, Ibrahim M. Abu-Reidah ^{2,3*,‡}, Anwar A. alhajeh ¹, Ghadeer Omar ⁴, Ghaleb Adwan ⁴ and Ismail Warad ^{1,5}

¹ Department of Chemistry, An-Najah National University, Nablus, Palestine.

² School of Science and the Environment, Grenfell Campus, Memorial University of Newfoundland, Corner Brook, NL A2H 5G4, Canada.

³ The Functional Foods Sensory Laboratory, Grenfell Campus, Memorial University of Newfoundland, Corner Brook, NL A2H 5G4, Canada

⁴ Department of Biology and Biotechnology, An-Najah National University, Nablus, Palestine.

⁵ Faculty of Pharmacy, Arab American University, P.O. Box 249, Jenin, Palestine.

*Correspondence: maalnuri@najah.edu (M.A.-N.); iabureidah@gmail.com and iabureidah@grenfell.mun.ca (I.M.A.-R.).

[‡] Both authors contributed equally to this work

Table S1. Phytochemical compounds identified in the extracts and plant parts of *Micromeria fruticosa*.

Extract Type	R.T.* (min.)	R.I.**	Identity	Class	%Area	M.F.***	M.W.**** (g/mol)	Biological study
Flower Ethanol	18.44	796	Menthone	Monoterpene	30.05	C ₁₀ H ₁₈ O	154.249	Antimicrobial activity, antibiofilm, and antitumor activities
	19.20	866	Menthol	Monoterpene	21.22	C ₁₀ H ₂₀ O	156.270	Antibacterial, antifungal, antipruritic, anticancer, antioxidant, and cooling effects
	20.82	816	Pulegone	Monoterpene	31.79	C ₁₀ H ₁₆ O	152.233	Antimicrobial, antioxidant, anti-inflammatory, antiulcer, and insecticidal properties
	25.12	614	4,6-Decadiene	-	0.86	C ₁₀ H ₁₈	138.250	Antioxidant, antiasthmatic, and antibacterial
	27.08	794	β-caryophyllene	Sesquiterpene	4.14	C ₁₅ H ₂	204.300	Anti-inflammatory, antioxidant, antinociceptive, neuro-protective, anxiolytic, antidepressant, antimicrobial, antidermatitic, antiacne, antiasthmatic, antiulcer, gastro-protective, sedative, and antispasmodic activities
	29.03	725	β-Cubebene	Sesquiterpene	1.44	C ₁₅ H ₂₄	204.360	Antioxidant activity
	36.71	770	Trans-1,2-diphenylcyclobutane	Cyclobutane	0.56	C ₁₆ H ₁₆	208.298	Antitumor activity
	54.65	558	(2,3-diphenylcyclopropyl)methyl phenyl sulfoxide	Stilbene	0.74	C ₂₂ H ₂₀ OS	332.461	Anticandidal and antioxidant activities
	59.01	559	Oleamide	Fatty amide	9.20	C ₁₈ H ₃₅ NO	281.477	Anti-inflammatory, antibacterial, and antioxidant activities

Extract Type	R.T. (min.)	R.I.	Identity	Class	%Area	M.F.	M.W.	Biological Study
Flower Hexane	18.14	849	Menthone	Monoterpene	5.42	C ₁₀ H ₁₈ O	154.249	Antimicrobial activity, potential antibiofilm, and antitumor activities
	19.14	872	Menthol	Monoterpene	46.40	C ₁₀ H ₂₀ O	156.270	Antibacterial, antifungal, antipruritic, anticancer, and antioxidant activities
	22.63	777	8-Hydroxy-p-menth-4-en-3-one	Terpene-like	8.96	C ₁₀ H ₁₆ O ₂	168.232	No activity was recorded
	25.49	702	4-Pentyl-1-(trans-4-propylcyclohexyl)cyclohexene	-	1.35	C ₂₀ H ₃₆	276.508	No activity was recorded
	29.73	722	Ethyl 14-methyl-hexadecanoate	Fatty acid	1.95	C ₁₈ H ₃₆ O ₂	284.0	-
	32.10	846	1-Formyl-2,2-Dimethyl-3-Trans-(3-Methyl-But-2-Enyl)-6-Methylidene-Cyclohexane	-	1.76	C ₁₅ H ₂₄ O	220.356	Antifungal, insecticidal, and larvicidal agent
	42.12	808	Palmitic acid	Fatty acid	3.63	C ₁₆ H ₃₂ O ₂	256.400	Antioxidant, anticancer, and anti-inflammation
	42.87	890	Ethyl 13-methyl-tetradecanoate	Fatty acid	24.10	C ₁₇ H ₃₄ O ₂	270.457	Antioxidant, β-glucuronidase inhibitory, and anticancer activities
	47.48	867	Ethyl 10-bromodecanoate	Fatty acid derivative	2.43	C ₁₂ H ₂₃ BrO ₂	279.214	Antidiabetic activity
	51.72	841	Methyl 17-methyl-octadecanoate	Fatty acid	0.60	C ₂₀ H ₄₀ O ₂	312.538	Antibacterial agents, antioxidant and anticancer activities
	58.95	781	Oleamide	Fatty amide	3.39	C ₁₈ H ₃₅ NO	281.477	Anti-inflammatory, antibacterial, and antioxidant activities

Extract Type	R.T (min.)	R.I.	Identity	Class	%Area	M.F.	M.W.	Biological Study
Leave Ethanol	18.44	966	Menthone	Monoterpene	14.59	C ₁₀ H ₁₈ O	154.249	Antimicrobial, antibiofilm, and antitumor activities
	20.87	870	Pulegone	Monoterpene	64.11	C ₁₀ H ₁₆ O	152.233	Antimicrobial, antioxidant, anti-inflammatory, antiulcer, and insecticidal properties
	27.08	905	Caryophyllene	Sesquiterpene	11.99	C ₁₅ H ₂₄	204.360	Anti-inflammatory, antioxidant, antinociceptive, neuro-protective, anxiolytic, antidepressant, antimicrobial, antidermatitic, antiacne, antiasthmatic, antiulcer, gastro-protective, sedative, and antispasmodic activities
	29.03	865	β-Cubebene	Sesquiterpene	3.96	C ₁₅ H ₂₄	204.351	Antibacterial activity
	36.70	863	Trans-1,2-diphenylcyclobutane	Cyclobutane	1.03	C ₁₆ H ₁₆	208.304	Agonist activity
	46.26	889	Methyl 8, 11, 14-heptadecatrienoate	Fatty acid derivative	4.32	C ₁₈ H ₃₀ O ₂	278.436	No activity was recorded

Extract Type	R.T (min.)	R.I.	Identity	Class	%Area	M.F	M.W.	Biological Study
Leave Hexane	7.14	898	Styrene	-	5.55	C ₈ H ₈	104.150	Agonist activity
	17.61	717	Terpinolene	Monoterpene	2.56	C ₁₀ H ₁₆	136.234	Antioxidant, antiasthmatic, and antibacterial activities
	18.14	920	Menthone	Monoterpene	9.10	C ₁₀ H ₁₈ O.	154.249	Antimicrobial, antibiofilm, and antitumor activities

	19.19	853	Menthol	Monoterpene	17.82	C ₁₀ H ₂₀ O	156.270	Antibacterial, antifungal, antipruritic, anticancer, and antioxidant activities
	20.88	823	Pulegone	Monoterpene	10.66	C ₁₀ H ₁₆ O	152.233	Antimicrobial, antioxidant, anti-inflammatory, antiulcer, and insecticidal properties.
	22.67	701	8-Hydroxy-p-menth-4-en-3-one	Terpene-like	4.03	C ₁₀ H ₁₆ O ₂	168.232	No activity was recorded
	36.69	858	Trans-1,2-diphenylcyclobutane	Cyclobutane	4.17	C ₁₆ H ₁₆	208.304	Agonist activity
	42.10	585	Palmitic acid	Fatty acid	6.33	C ₁₆ H ₃₂ O ₂	256.400	Antioxidant, anticancer, food additive, and anti-inflammation
	46.25	847	Methyl-hydroxylinolenate	Fatty acid	7.59	C ₁₉ H ₃₂ O ₃	308.462	No activity was recorded
	58.94	771	Oleamide	Fatty amide	32.19	C ₁₈ H ₃₅ NO	281.477	Anti-inflammatory, antibacterial, and antioxidant activities

Extract Type	R.T (min.)	R.I.	Identity	Class	%Area	M.F.	M.W.	Biological Study
Stem Hexane	19.20	880	Menthol	Monoterpene	66.61	C ₁₀ H ₂₀ O	156.270	Antibacterial, antifungal, antipruritic, anticancer, and antioxidant activities
	54.75	887	Hentriacontane	Alkane hydrocarbon	4.40	C ₃₁ H ₆₄	436.850	Antifungal, antioxidant, antitumor, and antibacterial activities
	58.93	766	Oleamide	Fatty amide	28.99	C ₁₈ H ₃₅ NO	281.477	Anti-inflammatory, antibacterial, and antioxidant activities
Stem Ethanol	19.21	864	Menthol	Monoterpene	100	C ₁₀ H ₂₀ O	156.270	Antibacterial, antifungal, antipruritic, anticancer, cooling, and antioxidant effects

Extract Type	R.T. (min.)	R.I.	Identity	Class	%Area	M.F.	M.W. (g/mol)	Biological study
Roots Ethanol	37.902	824	Cis-1-Chloro-9-Octadecene	-	8.30	C ₁₈ H ₃₅ Cl	286.923	-
	42.929	884	Ethyl 14-Methyl-Hexadecanoate	-	26.82	C ₁₈ H ₃₆ O ₂	284.477	-
Roots Hexane	19.204	801	Menthol	Monoterpene	3.61	C ₁₀ H ₂₀ O	156.27	Antibacterial, antifungal, antipruritic, anticancer, and antioxidant activities
	42.159	814	Palmitic acid	Fatty acid	8.24	C ₁₆ H ₃₂ O ₂	256.4	Antioxidant, anticancer, food additive, and anti-inflammation
	42.934	902	Ethyl 13-Methyl-Tetradecanoate	Fatty acid	47.52	C ₁₇ H ₃₄ O ₂	270.457	-
	54.433	823	Diisooctyl phthalate		6.25	C ₂₄ H ₃₈ O ₄	390.556	Antioxidant, antimicrobial, and antifouling
	58.965	750	Oleamide	Fatty amide	14.61	C ₁₈ H ₃₅ NO	281.477	Anti-inflammatory, antibacterial, and antioxidant activities
	61.036	926	Hentriacontane	Alkane hydrocarbon	19.77	C ₃₁ H ₆₄	436.85	Antifungal, antioxidant, antitumor, and antibacterial activities

*RT: retention time, **R.I.: relative index, ***M.F.: molecular formula, ****M.W. molecular weight.

S2. Media and Solutions

1.1. Nutrient Broth

Nutrient broth (ACUMEDIA) was prepared according to the manufacturer's instructions labeled on the bottle. A total of 2.0 g of nutrient broth medium was dissolved in 250 mL of deionized water. The broth was then distributed into tubes with 5-10 mL each and plugged with cotton. The tubes were autoclaved at 121°C for 15 minutes and allowed to cool to room temperature, then stored in a refrigerator.

1.2. Mueller–Hinton Broth

Mueller–Hinton broth (Hi Media Laboratories) was prepared according to the manufacturer's instructions labeled on the bottle. A total of 5.25g of Mueller–Hinton Broth medium was dissolved in 250 mL of deionized water. The broth was then distributed into tubes with 5-10 mL each and plugged with cotton. The tubes were autoclaved at 121°C for 15 minutes, allowed to cool, and then kept in a refrigerator at 4-6°C.

1.3. Mueller–Hinton Agar (MHA)

Mueller–Hinton agar (BD) was prepared according to the manufacturer's instructions labeled on the bottle. A total of 19 g of Mueller–Hinton agar medium was dissolved in 0.5 L of deionized water. The solution was allowed to boil for 1 minute and then autoclaved at 121°C for 15 minutes. After that, it was allowed to cool to about 55°C, and the media was poured into sterile Petri dishes with 25-30 mL each. Then, the plates were left overnight at room temperature. The following morning, the Petri dishes were turned upside down and kept in a refrigerator at 4-6°C.

1.4. Sabouraud Dextrose Agar

Sabouraud dextrose agar (Hi Media Laboratories) was prepared according to the manufacturer's instructions labeled on the bottle. A total of 16.25 g of Sabouraud dextrose agar medium was dissolved in 0.25 L of deionized water. The solution was allowed to boil for 1 minute and then autoclaved at 121°C for 15 minutes. After that, it was allowed to cool to about 55°C, and the medium was poured into sterile Petri dishes with 25-30 mL each;

then, the plates were left overnight at room temperature. The following morning, the Petri dishes were turned upside down and kept in a refrigerator at 4-6°C.

1.5. Normal Saline (0.9% NaCl)

Normal saline solution (0.9% NaCl, MWt 58.44) was prepared by dissolving 2.25 g of NaCl in 250 mL of deionized water. The saline solution was then distributed into tubes with 5-10 mL each and plugged with cotton. The tubes were autoclaved at 121°C for 15 min, allowed to cool, and then kept in a refrigerator at 4-6°C.

1.6. Preparation of McFarland turbidity standard No. 0.5

McFarland 0.5 turbidity standard was prepared by mixing 50 µL of a 1.175% (w/v) barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) solution and 9.95 mL of 1% (v/v) sulfuric acid. The tube, which had the 0.5 McFarland standard, was then sealed with parafilm to prevent evaporation and stored in the dark at room temperature. The 0.5 McFarland standard was vigorously mixed on a vortex mixer before use. As with the barium sulfate standards, a 0.5 McFarland standard is comparable to a bacterial suspension of $1-5 \times 10^8$ colony-forming units (CFU)/mL (Andrews, 2006) [54], or 0.5 McFarland standard is similar to a *Candida albicans* suspension of $1-5 \times 10^6$ yeast cells/mL.

Three to four colonies of each bacterium were transferred into tubes with 5.0-10 mL of sterile normal saline, and the turbidity of the bacterial suspensions was adjusted to have similar turbidity to 0.5 McFarland standard with a bacterial suspension of about 1.5×10^8 cfu/mL. A few colonies from *C. albicans* sub-cultured on Sabouraud dextrose agar were transferred into tubes with 5.0-10 mL of sterile normal saline. The turbidity of the yeast *C. albicans* suspension was about $1-5 \times 10^6$ yeast cells/mL.