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Myrtus communis L.: essential oil chemical composition, total phenols and flavonoids contents, antimicrobial, antioxidant, anticancer, and *α*-amylase inhibitory activity

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Abstract

Background *Myrtus communis* L. leaves, due to their tonic and antiseptic properties, have been used as folk medicine in many communities to treat a variety of conditions such as inflammation, peptic ulcers, diarrhea, leucorrhoea, headaches, excessive perspiration, and skin diseases. In this study, we examined the chemical makeup and biological properties of *M. communis* essential oils (EOs) from two locations in Palestine, including Jericho, the world's deepest site and Jenin. The plant's methanol and ethyl acetate extracts' biological efficacy were also assessed.

Results The GC–MS analysis revealed that the EO of *M. communis* leaves from Jenin included 39 components, the majority were 1,8-cineole (31.98%), linalool (21.94%), linalool acetate (11.42%), α-pinene (10.22%), and myrtenol (6.87%). While 33 compounds, were discovered in *M. communis* EO from Jericho, with *cis*-4-thujanol (27.37%), 1,8-cineole (24.32%), myrtenol (12.97%), and myrtenal (12.46%) being the main constituents. The EO, ethyl acetate, and methanol extracts were tested (in vitro) for antibacterial, anticancer, antioxidant, and α-amylase inhibitory properties. The EO from Jericho demonstrated greater antibacterial efficacy against *Escherichia coli*, *Proteus vulgaris, Klebsiella pneumoniae*, *Staphylococcus aureus*, and, methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC values of 0.27, 0.135, 0.135, 0.27, and 0.135 mg/mL, respectively. MRSA, *S. aureus* and *C. albicans* were all susceptible to the antimicrobial efficiency of methanol and ethyl acetate extracts (MIC = 0.097–0.195, 0.097–0.195, and 0.049 mg/mL, respectively). Methanol and ethyl acetate extracts, in contrast to EOs, showed high DPPH activity, with IC_{50} values ranging from 3.60 ± 0.35 to 25.70 ± 0.48 µg/mL. Both oils showed moderate cytotoxic activity against HeLa, MCF7, 3T3, and LX-2 cell lines, with IC_{50} values ranging from 202.02 ± 2.27 to 592.40 ± 2.55 µg/mL for Jenin and 199.80 ± 3.41 to 914.54 ± 3.05 µg/mL for EO from Jericho. Furthermore, EO and methanol extracts from Jenin inhibited α-amylase with IC_{50} values of 950.48 ± 2.54 and 795.43 ± 1.88 µg/mL, respectively.

Conclusions Our findings indicate that *M. communis* extracts and EOs contain a vast array of pharmacologically active compounds with potent antioxidant, antibacterial, and antifungal activities that can be exploited to develop new types of natural pharmaceuticals and warrant further in vivo investigation for their therapeutic potential.

Keywords *Myrtus communis*, Essential oil, Total phenols, Flavonoids, Antimicrobial, Antioxidant, Anticancer, *α*-Amylase

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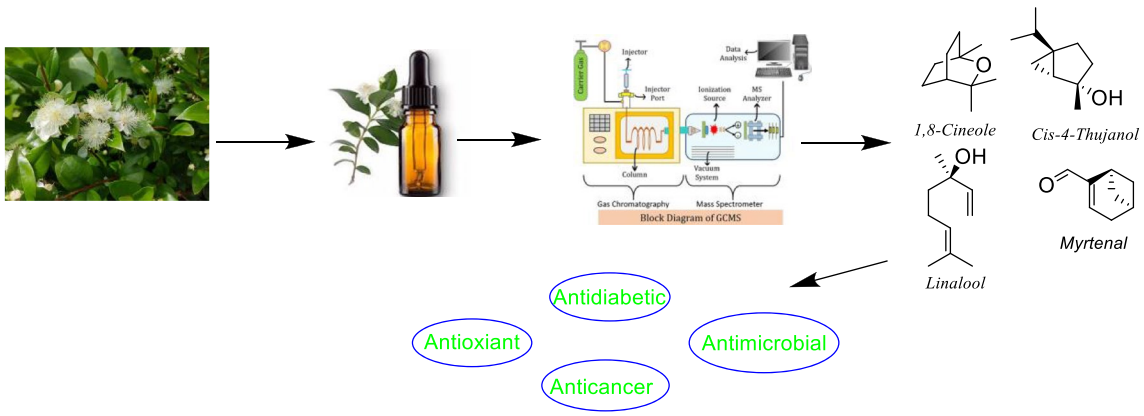
Graphical Abstract



Introduction

Aromatic plants have been exploited as food, food additives, cosmetics, and medicines since antiquity. As natural goods are inherently safer and less expensive, the general population has become more interested in using herbal medications rather than synthetic drugs over the past three decades [1, 2]. The public's familiarity with the advantages and possible uses of medicinal and aromatic herbs, as well as their general knowledge about food, health, and nutrition, is growing. Essential oils are one of the several secondary metabolites that these plants produce [1, 2]. Essential oils (EOs) are complex combinations of up to 300 different organic volatile compounds with molecular weights of less than 300, including but not limited to alcohols, phenols, aldehydes, ketones, esters, ethers or oxides, amines, amides, heterocycles, and, most importantly, terpenes [2, 3]. EOs are widely utilized in the cosmetics and perfume industries due to their rich and varied composition [2]. In order to uncover novel and beneficial applications in human health, agriculture, and the environment, a greater understanding of the chemical and biological characteristics of these extracts and their constituents is required. Essential oils have the potential to serve as effective replacements for or supplements to, man-made chemical compounds without the harmful consequences of the latter. Antifungal, antiviral, antibacterial, insecticidal, cancer chemoprotective, and antioxidant capabilities are only a few of the many biological effects of EOs [1–3].

Myrtus communis L., a member of the Myrtaceae family, is a famous essential oil plant that has been utilized in traditional medicine for ages. There are over 3000 different species in the Myrtaceae family, and they are split up among 100 different genera. The evergreen *M. communis* bush can reach a height of three meters; it has aromatic, evergreen leaves, and it produces tiny black fruits along the stems and branches. Its natural range includes North Africa, western Asia, and southern Europe, but it has since expanded to include Australia, the northwestern Himalayas, and South America as well [4, 5].

Due to its tonic and antiseptic properties, the leaves of *M. communis* have been utilized as a folk medicine in numerous communities for treating a variety of conditions, including inflammation, peptic ulcers, diarrhea, bleeding, urethritis, conjunctivitis, leucorrhoea, headaches, excessive perspiration, and skin diseases [4–6]. *M. communis* extensive use in folk medicine, as well as the pharmaceutical industry's acknowledgment of its products, emphasizes the need for a greater understanding of the herb's different characteristics, such as its phytochemical, toxicological and pharmacological capabilities. The phytochemical makeup of the yellow or greenish-yellow EO derived from *M. communis* leaves, flowers, and berries was discovered to be dependent on production region, harvesting season, and extraction method [4–6]. The principal constituents of EO obtained from the leaves were discovered to be 1,8-cineole, limonene, linalool, α-pinene, myrtenyl acetate, and α-terpinolene in the majority of locations [5–9]. *M. communis* essential oils (EOs) were analyzed to determine their chemical makeup in a variety of countries, including Greece, Cyprus, Montenegro, Croatia, Italy, Sardinia, Tunisia, Algeria, and Iran [5].

To our knowledge, the phytochemical components of Palestine-grown *M. communis* have never been examined. Due to its significance in traditional medicine and

the perfume and flavor industries, this investigation's goal was to look at the phytochemical constituents EO of *M. communis* growing in two different locations, namely Jericho and Jenin, and to evaluate its in vitro antioxidative, metabolic enzyme inhibitory, and antimicrobial effects.

Experimental

Chemical and reagents

The majority of the chemicals utilized in this study were bought from Sigma-Aldrich and were of analytical quality (Darmstadt, Germany). The Folin–Ciocalteu reagent, aluminum chloride hexahydrate, 2,4,6-tripyridyl-s-triazine (TPTZ), 1,1′-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, vanillin, catechin, methanol, sodium carbonate, and sodium nitrite were acquired from Sigma-Aldrich (Boston, USA).

Plant materials and essential oils distillation

M. communis leaves were collected in April 2021 in two locations in Palestine: Jericho (latitude: 31°52′00″N, longitude: 35°27′00″E, elevation above sea level: − 233 m = − 764 ft), the world's deepest place, and Jenin (latitude: 32°27′33″N, longitude: 35°18′03″E, elevation above sea level: 161 m = 528 ft). The plant was identified, and voucher specimens were referred to the An Najah National University Herbarium with the code Pharm-PCT-1621. Before being dried in the shade at room temperature (25 ± 3 °C) and humidity (55 ± 4 RH), the leaves were repeatedly rinsed with water. To repeat the studies, one kilogram of dried leaves from each cultivar was collected and divided into three portions. The dried leaves were roughly powdered, and EO samples were extracted from the crushed leaves using a Clevenger-type device and hydrodistillation for 3 h (Merck, USA). The oils were deposited in airtight vials at 4 °C until use.

Extraction procedure

M. communis dried leaves (200 g) were macerated in 0.5 L of methanol (MeOH) for 48 h at room temperature while stirring. After filtration, the filtrate was concentrated to dryness to yield 12.5 g of dark oil. The extracts were kept at − 20 °C until use. The MeOH extract (12.5 g) was suspended uniformly in water, placed in a round bottom flask attached to a Teflon stirrer, and fractionated with hexane (200 mL × 3) and ethyl acetate (EtOAc, 200 mL × 3), in that order. All EtOAc layers were combined and concentrated under a vacuum to provide 4.23 g of sticky mixture.

Chromatographic analyses

A Hewlett-Packard Model 5890 Series II GC equipped with a fused-silica capillary column (0.25 mm × 30 m, film thickness of 0.25 mm) and coupled to a Perkin Elmer Elite-5-MS (Perkin Elmer, USA). was used to analyze two replicates of each sample. Helium was used at a flow rate of 1.1 mL/min. The injection port and detector temperatures were kept at 250 °C. The oven temperature was set to 50 °C for 5 min before being increased by 4.0 °C each minute to 280 °C. In split mode, 0.2 µL of EO with a splitting ratio of 1:50 was injected. The overall running time was 62.50 min, with a solvent delay ranging from 0 to 4.0 min. Components were recognized by associating mass spectra of the components with authentic samples and/or the data from NIST, by interpreting EI-fragmentation of the molecules, and by relating retention indices (RIs) computed relative to a reference mixture of *n*-alkanes (C_6–C_{30}).

Determination of phenols in extracts

Using the Folin–Ciocalteu technique, the total phenolic content of *M. communis* MeOH extract and EtOAc fraction was determined [10]. Each extract fraction was diluted in MeOH at a concentration of 0.5 mg/mL. 0.5 mL of the solution of the MeOH extract, 2.5 mL of 10% aqueous Folin–Ciocalteu's reagent, and 2.5 mL of a 7.5% aqueous $NaHCO_3$ solution were mixed. After that, the samples were thermostatically incubated for 45 min at 45 °C. The absorbance was determined using a spectrophotometer at 765 nm. For each test, three copies of each sample were made, and the mean absorbance was found. Using the gallic acid standard solution, the technique was repeated, and a calibration line was generated by serially diluting the sample (10, 40, 50, 70, and 100 µg/ml). Based on the absorbance that was measured, the amount of gallic acid equivalent in each fraction was given as mg of GAE/g.

Determination of flavonoids in extracts

Total flavonoid concentration in MeOH and EtOAc extracts was estimated using the aluminum chloride colorimetric technique [11]. To sum up, a total of 200 mg of each extract was dissolved in 40 mL of MeOH (5 mg/mL), and then 1 mL of the MeOH solution was added to 4 mL of water in a test tube. The solution was adjusted by adding 0.5 ml of 5% $NaNO_2$ and 0.5 ml of 10% $AlCl_3$. After waiting for 10 min, 2 mL of 1 M NaOH was added to the mixture. Distilled water was used to get the total volume up to 10 mL. At room temperature, the samples were left to incubate for 30 min. The absorbance was determined by using a spectrophotometer at 510 nm. In terms of total flavonoid content, extracts were expressed as Quercetin Equivalents (mg of QUE/g).

1,1-Diphenyl-1-picrylhydrazyl (DPPH) assay

A modified DPPH (1,1-diphenyl-2-picrylhydrazyl radical) assay (12), which gauges antioxidants' capacity to lower the DPPH radical, was employed to evaluate the antioxidant activity of *M. communis* EOs and extracts [12]. A DPPH stock solution (0.5×10^{-4} M) in MeOH was prepared. Extracts (1 mg/mL) were made by dissolving 50 mg of each EO in 50 mL of MeOH. Methanol was employed to dilute the solutions, resulting in concentrations ranging from 1 to 100 µg/mL (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, and 100 µg/mL). One mL of stock solution, one mL of MeOH, and one mL of extract solution were combined, agitated, and incubated for 30 min at room temperature in the dark. A UV/VIS spectrophotometer was utilized to quantify the decline in absorbance at 517 nm in comparison to a blank (MeOH solution) (Jenway-7315, Staffordshire, UK). The control consisted of 1 mL of MeOH and 3 mL of DPPH solution. Trolox (Sigma-Aldrich, USA) was employed as a reference for comparison. The antioxidant activities of the samples were estimated using the following formula: % DPPH reacted = (AB − AA)/AB × 100, where AB and AA are the absorbance values of the control and test samples, respectively. BioDataFit edition 1.02 was employed to calculate the antioxidant half-maximal inhibitory concentration (IC_{50}) for the tested substances (data fit for biologists). The tests were repeated three times.

Antimicrobial activity

The antimicrobial activity of *M. communis* EOs and extracts was evaluated employing a fungal strain, *Candida albicans* (American type culture collection (ATCC 90028), and six bacterial strains, namely *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), *Proteus vulgaris* (ATCC 8427), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 25923), and the diagnostically confirmed methicillin-resistant *Staphylococcus aureus* (MRSA). To make a 200 µg/mL stock solution, each EO was mixed with 20% DMSO and 60% Muller–Hinton broth. Using sterile Muller–Hinton broth, the produced EO solutions were serially diluted by a factor of two to achieve final concentrations of 50, 25, 12.5, 6.25, 3.125, etc., µg/mL (RPMI medium was utilized for the *C. albicans* strain); DMSO concentration was 5% in the first well and was further diluted twofold to exclude its antimicrobial effect. On 96-well plates, the dilution technique was performed aseptically. The bacteria under investigation were aseptically injected into micro-wells 1–11, whereas the EO was placed in micro-wells 1–10. Micro-well 11 was employed as positive microbial growth control, while micro-well 12 (without EO and microbe), was used as a negative control. Plates injected with test bacterial strains were nurtured at 35 °C for 18–24 h, whereas plates inoculated with *C. albicans* were incubated for 48 h at 35 °C. The minimum inhibitory concentration (MIC) of the tested EO was obtained by determining the lowest concentration of EO in the micro-well at which no visible microbial growth occurred [13]. In this study, ciprofloxacin and ampicillin were utilized as positive antibacterial activity controls. Fluconazole was used as a positive control for antifungal activity. While all the tests without plant material were considered negative controls. The antimicrobial activity of the samples was determined in triplicate [14].

Cell culture and cytotoxicity assay

HeLa, MCF-7, and Hep3B human cancer cell lines were cultivated in RPMI-1640 media supplemented with 1% L-glutamine (Sigma-Norwich, UK), 1% penicillin/streptomycin antibiotics (BI, India), and 10% fetal bovine serum. At 37 °C, in a humidified 5% CO_2 atmosphere, cancer cells were cultured. In a 96-well plate, cells were planted at 2.6×10^4 cells/well. After 48 h, cancer cells were cultured for 24 h at several concentrations (500, 120, 60, 30, and 10 µg/mL) of the tested EO. Cell viability was determined using the CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay, as directed by the manufacturer (Promega Corporation, Madison, USA). After the treatment, 20 µL of MTS solution per 100 µL of media was added to each well, and the well plates were incubated at 37 °C for 2 h. At 490 nm, the absorbance was measured [15].

α-Amylase inhibitory activity

To prepare plant extract solutions of varying concentrations (10, 50, 70, 100, and 500 µg/mL), 100 mg of the extract was dissolved in 5 mL of 10% DMSO, and the resulting volume was brought up to 100 mL with a buffer solution (0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl, pH 6.9). Thereafter, a stock solution of porcine pancreatic α-amylase enzyme (Sigma-Aldrich, USA) (2 units/mL) was prepared by dissolving 12.5 mg of α-amylase with a minimum of 10% DMSO and then bringing the volume to 100 mL with a buffer solution. The solution of corn starch (Alzahraa Firm, Palestine) was made by dissolving 1 g of starch in 100 mL of distilled water. 200 µL of the extract solution was combined with 200 µL of the *α*-amylase stock solution and incubated at 30 °C for 10 min.

After that, 200 µL of the starch solution was added, and the mixture was incubated for 3 min. at 30 °C. Then, 3,5-dinitro salicylic acid (Sigma, India) was added, and the mixture was heated in a water bath at 85–90 °C for 10 min, cooled to room temperature, and 5 mL of distilled water was added. The blank solution was made by replacing the plant EO with 200 µL of a buffer solution. Acarbose was employed as a positive reference

chemical, and absorbance was measured at 540 nm with a UV–Vis spectrophotometer. The α-amylase inhibitory potential was computed as follows: I% α-amylase inhibitory = $[ABS_{blank} - ABS_{test}]/[ABS_{blank}]) \times 100\%$ [12].

Data analysis

All conducted tests on *M. communis* EO and extracts were performed in triplicate. The results were expressed as means ± the standard deviation (SD), while the outcomes were considered significant when the *p*-values were < 0.05.

Results and discussion

GC–MS characterization of *M. communis* essential oil

Table 1 indicates the chemical composition of the investigated EOs collected from two regions in Palestine, with the identified components itemized in the order of their elution on the DB-5 column, along with their retention indices and percentages. *M. communis* from Jericho yielded 1.31% pale yellow EO, while air-dried leaves from Jenin yielded 1.15% EO. The yield is higher than what was achieved from *M. communis* in Lebanon (1%) [16], and it is also considered to be superior to the yields obtained in Algeria at (0.32%) [17] and Morocco (0.68%) [18]. 35 of the 36 detectable constituents were recognized in the EO of *M. communis* from Jenin accounting for 98.84% of the total oil, with 1,8 cineole (31.98%), linalool (21.94%), linalool acetate (11.42%), α-pinene, (−)-myrtenol (6.87%), and α-terpineol (4.41%) being the most abundant. Only 33 of the 41 detected components from the EO of *M. communis* from Jericho, totaling 99.9%, were recognized, with the most prevalent compounds being *cis*-4-thujanol (27.37%), 1,8-cineole (24.32%), myrtenol (12.97%), myrtenal (12.46%), and trans-4-thujanol acetate (9.48%). Table 1 shows that the chemical makeup of the two analyzed EOs gathered from the two locations differs significantly in terms of both quality and amount of components as well as in the EOs yields. Differences in ecological factors, environmental conditions such as water, nutritional stress, temperature, and geographical source may be partially linked to this variability in essential oil content and ratios of various components [7, 9, 19, 20]. Reviewing the literature showed that *M. communis* leaves EO has been the subject of numerous previous studies from around the world, and the EO makeup appears to be heavily affected by geographical, seasonal, climatic, or genetic differences [7, 9]. Our findings on the constituents of EO from the Jenin region are reasonably comparable with those reported in published research [7, 9], which suggested that 1,8-cineole was the predominant constituent of *M. communis* EO. The order of abundant components recorded in the literature, on the other hand, is different. Thus, myrtenyl acetate, which was

Table 1 The chemical constituents of *M. communis* leaves EOs from Jericho and Jenin governorates

No.	Name	RT	RI	Percentage	
				Jericho	Jenin
1	*Trans*-2-hexenal	5.73	856	–	0.30
2	Isobutyl isobutyrate	7.99	889	0.14	0.50
3	*α*-Pinene	8.7	933	3.95	10.22
4	*β*-Pinene	10.49	976	0.05	0.09
5	Myrcene	11.07	990	0.21	0.38
6	Pseudolimonene	11.72	1006	–	0.23
7	*δ*-3-Carene	11.81	1008	0.04	0.21
8	*p*-Cymene	12.17	1017	–	0.05
9	*o*-Cymene	12.5	1025	0.17	1.34
10	1,8-Cineole	12.82	1033	24.32	31.55
11	Z-β-Ocimene	13.42	1047	0.28	0.71
12	δ-Terpinene	13.88	1059	–	0.36
13	Terpinolene	14.98	1085	–	0.92
14	Linalool	15.65	1102	–	21.65
15	*cis*-4-Thujanol	15.65	1102	27.37	–
16	*n*-Amyl isovalerate	15.75	1105	–	0.46
17	Terpinen-4-ol	18.69	1181	0.24	0.32
18	α-Terpineol	19.28	1196	–	4.35
19	Myrtenol	19.29	1197	12.97	–
20	Myrtenal	19.36	1198	12.46	6.78
21	*Trans*-4-Thujanol acetate	21.26	1251	9.48	11.26
22	Linalyl acetate	21.33	1253	1.04	0.42
23	*Trans*-Sabinyl acetate	21.87	1296	0.01	0.10
24	nd	22.00	1271	0.01	–
25	nd	22.33	1281	0.01	–
26	Thymol	22.47	1285	0.02	–
27	Methyl myrtenate	22.78	1294	0.10	–
28	Sabinyl acetate	22.87	1296	0.17	0.10
29	Carvacrol	23.06	1302	0.11	–
30	nd	23.19	1305	0.01	–
31	nd	23.50	1315	0.01	–
32	nd	23.63	1319	0.02	–
33	Myrtenyl acetate	23.82	1324	0.62	2.89
34	nd	23.92	1327	0.01	–
35	nd	24.15	1334	0.02	
36	Linalool propanoate	24.38	1341	0.01	–
37	α-Terpinyl acetate	24.61	1348	1.77	1.30
38	Neryl acetate	25.00	1360	0.48	0.21
39	nd	25.49	1374	–	0.01
40	Geranyl acetate	25.65	1379	0.92	0.55
41	*β*-Elemene	26.03	1390	0.05	0.03
42	Methyl eugenol	26.41	1402	0.70	0.37
43	*β*-Caryophyllene	27.01	1421	0.71	0.70
44	*α*-Caryophyllene	28.15	1458	0.40	0.22
45	Fumaric acid dimyrtenyl ester	28.37	1463	0.12	0.04
46	Cyclopropane, 1-(2-methylene-3-butenyl)-1-(1-methylenepropyl)	28.73	1476	0.02	–

Table 1 (continued)

No.	Name	RT	RI	Percentage	
				Jericho	Jenin
47	*β*-Selinene	29.22	1491	0.34	0.07
48	(E,E)-α-Farnesene	29.43	1498	0.33	0.07
49	*β*-Bisabolene	29.77	1509	–	0.7
50	Geranyl isobutanoate	29.98	1516	0.02	0.01
51	Caryophyllene oxide	32.03	1585	0.22	0.07
Oil yield				1.31	1.15
Total identified				99.93	99.54
Phytochemical groups					
	Monoterpene hydrocarbons			4.72	14.57
	Oxygenated monoterpenes			92.99	82.74
	Sesquiterpene hydrocarbons			1.83	1.79
	Oxygenated sesquiterpenes			0.22	0.07
	Others			0.17	0.37

RT retention time, *RI* retention index, *nd* not identified

detected in high concentrations in *M. communis* leaves EOs from Tunisia [21], Morocco [22], and Spain [23], was found in only 2.86% and 0.62% of Jenin and Jericho EOs, respectively. Furthermore, 1,8-cineole was regarded as the second most abundant component in certain studies [6, 24, 25], whereas α-pinene was found to be the most significant constituent [26–30], which we detected in Jericho EO at 3.95% and Jenin oil at 10.08%. Jericho *M. communis* leaf oil, on the other hand, is unlike any other oil recorded in the literature, and this can be attributed to Jericho's peculiar environment and soil, which is located close to the Dead Sea (Figs. 1, 2).

It has been demonstrated that plant polyphenols possess positive pharmacological effects, such as vasodilation, antibacterial, antioxidant, and anti-inflammatory characteristics. They can inhibit the production of new radicals by supplying hydrogen to highly reactive species, as a result of their antioxidant properties [31]. Several studies have investigated and validated the antioxidant properties of phenolic substances found in nature [31–34]. Using the Folin-Ciocalteu reagent to quantify the total phenolic content of *M. communis* extracts in terms of mg GAE/g, Table 2 demonstrates that the EtOAc fraction (133.6 mg GAE/g for Jenin, and 129.9 mg GAE/g for Jericho) contained twice as many total phenols as the MeOH extract (62.65 mg GAE/g for Jenin, and 53.43 mg GAE/g for Jericho). Nevertheless, flavonoid concentration was identical in MeOH extract (14.05 and 20.40 mg QE/g) and EtOAc (14.58 and 19.56 mg QE/g) fraction from both locations, with Jericho extracts having higher flavonoid levels. According to Bouaziz et al. [32], the total phenolic and flavonoid contents of the EtOAc

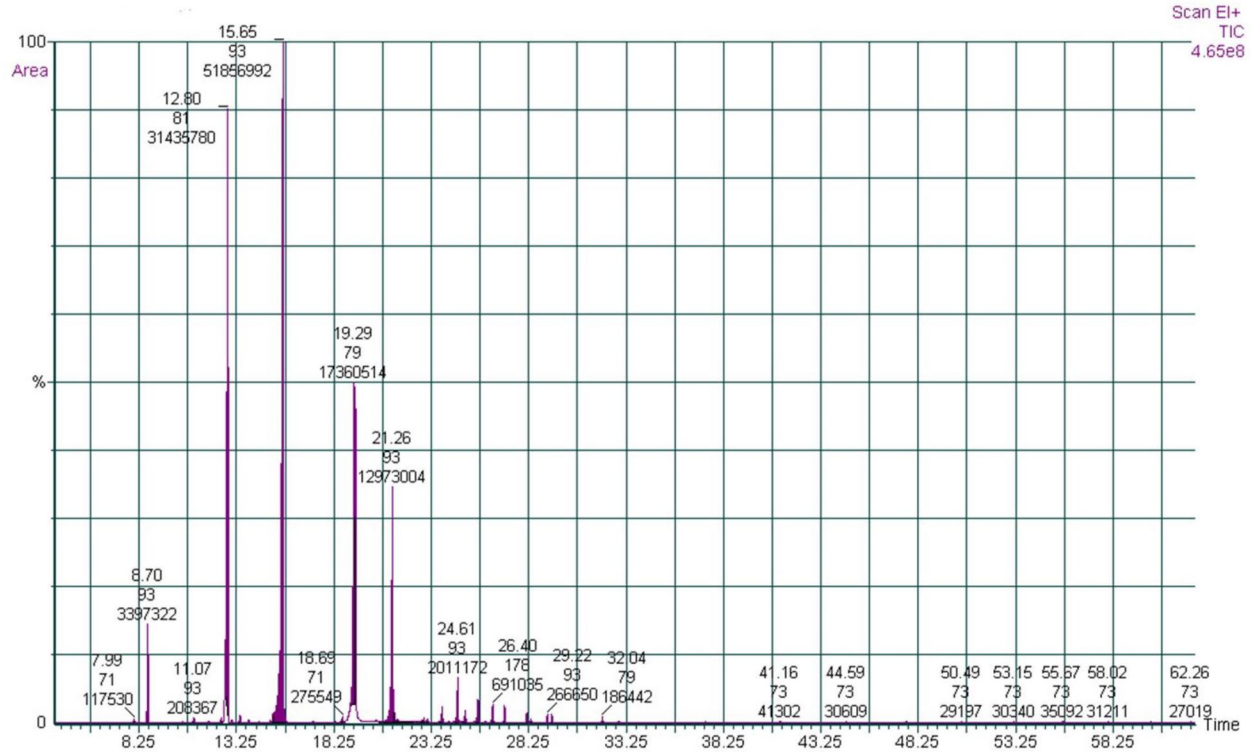


Fig. 1 *M. communis* EO GC chromatogram from Jericho

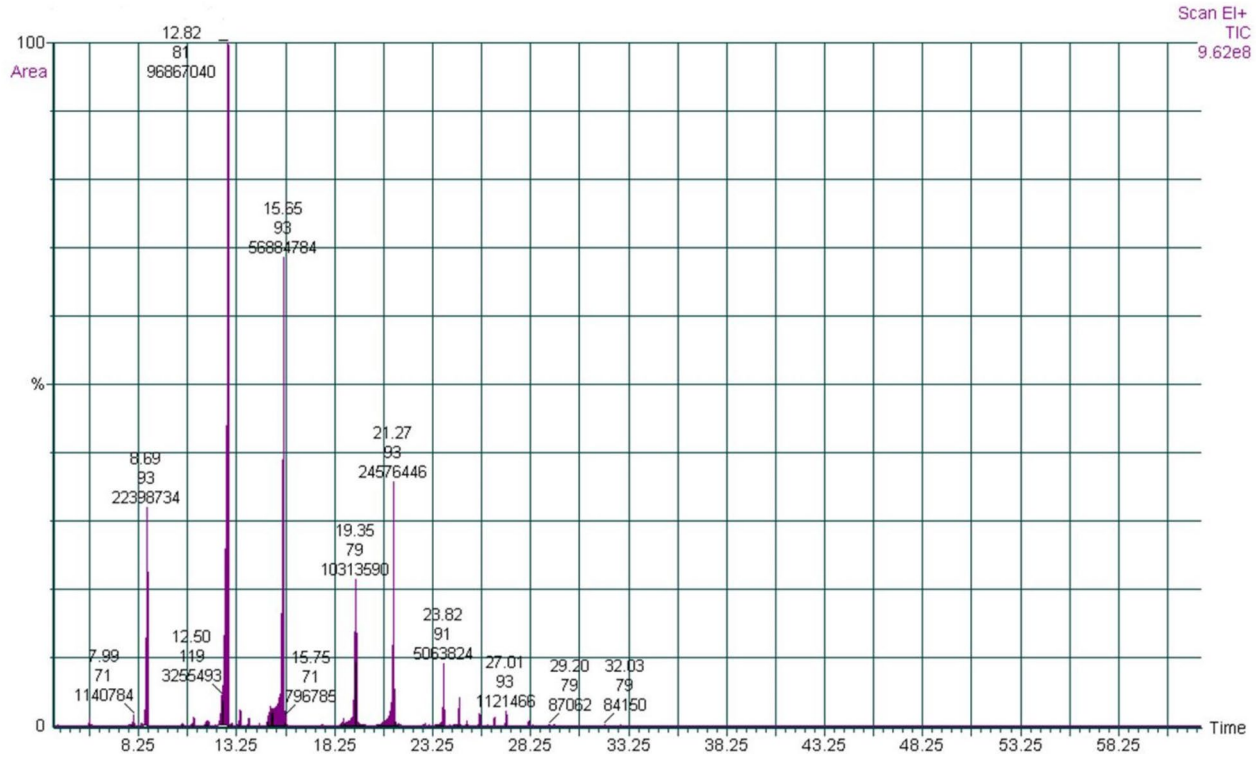


Fig. 2 *M. communis* EO GC chromatogram from Jenin

Table 2 Contents of total phenols and flavonoids of methanol extract and EtOAc fraction of *M. communis* from Jenin and Jericho

	Jenin		Jericho	
	Methanol extract	Ethyl acetate fraction	Methanol extract	Ethyl acetate fraction
Total phenolic content (mg GAE/g)	62.65	133.6	53.43	129.9
Total flavonoids content (mg QE/g)	14.05	14.58	20.40	19.56

fraction of *M. communis* leaves were greater than those of the MeOH extract, with values of 435.37 mg GAE/g and 130.75 mg QE, respectively. Total phenolics in extracts of Moroccan *M. communis* leaves prepared with water, methanol, and ethyl acetate varied from 10.81 to 35.56 mg GAE/g of extract, with the EtOAc fraction containing the lowest concentration [33]. Also, the total flavonoids in the MeOH extract were found to be six times higher than those in the EtOAc fraction, with values of 129.96 QE/g and 21.97 QE/g, respectively [33]. Total phenolic and flavonoid contents of ethanolic *M. communis* leaf extract were reported by Bouaoudia-Madi et al. to be 63.11 ± 0.35 mg GAE/g, and 13.65 ± 0.09 mg RE/g, respectively [34]. Total phenolic content in methanolic and EtOAc extracts of Egyptian *M. communis* leaves was reported by Nassar et al. to be 472.47 ± 3.73 mg GAE/g and 714.33 ± 4.69 mg GAE/g, respectively [35]. Total flavonoid content in *M. communis* leaves was reported to be 281.15 ± 21.88, and 53.62 ± 13.27 mg RE/g, respectively.

Antimicrobial activity

By using the broth microdilution method on Mueller–Hinton agar, the antimicrobial activity of the EOs and four extracts was evaluated against six bacterial strains (the most common pathogenic species) and one fungal strain. Results revealed that the EOs and extracts possessed antimicrobial properties (Table 3). Essential oil of the *M. communis* from Jericho demonstrated a higher level of antimicrobial activity against all of the tested bacterial strains in comparison to EO from Jenin, with the exception of *P. aeruginosa*, where both showed comparable levels of activity (Table 3). With a MIC value of 0.135 mg/mL, the Jericho EO demonstrated the highest antibacterial activity against MRSA, *P. vulgaris*, and *K. pneumonia*. On the other hand, the Gram-negative bacteria *P. vulgaris* was most susceptible to the EO of *M. communis* from Jenin (MIC = 0.56 mg/

Table 3 Minimum inhibitory concentration values (mg/mL) for different *M. communis* extracts against selected pathogens (bacteria and fungi)

Microbe	EOJen	EOJer	MeJen	EtOAcJen	MeJer	EtOAcJer	Ciprofloxacin	Ampicillin	Fluconazole
MRSA	1.13	0.135	0.78	0.195	0.78	0.097	1.56	NA	NA
S. aureus	1.13	0.27	1.56	0.195	0.78	0.097	0.78	3.12	NA
K. pneumoniae	1.13	0.135	12.5	12.5	25	1.56	0.125	12.5	NA
E. coli	1.13	0.27	12.5	12.5	25	1.56	1.56	3.12	NA
P. vulgaris	0.56	0.135	12.5	12.5	25	3.125	15	18	NA
P. aeruginosa	18.1	17.3	12.5	25	25	3.125	3.12	NA	NA
C. albicans	1.13	1.08	0.049	0.049	0.049	0.049	NA	NA	1.56

EOJen EO from Jenin; *EOJer*: EO from Jericho, *MeJen* methanol extract from Jenin, *EtOAcJen* ethyl acetate extract from Jenin, *MeJer* methanol extract from Jericho, *EtOAcJer* ethyl acetate extract from Jericho, *NA* no antimicrobial activity

mL). When compared to the positive controls, ciprofloxacin and ampicillin, EO from Jericho was more effective against all bacteria tested (excluding *P. aeruginosa*), while EO from Jenin was more effective against ampicillin only (Table 3). The antifungal effectiveness of the tested EOs was marginally higher than that of fluconazole, the positive control (MIC = 1.56 mg/mL). The Gram-negative bacteria, *P. aeruginosa*, was the most resistant to *M. communis* extracts and EOs, which is in agreement with the literature data [21, 36, 37]. Several works around the world reported the evaluation of *M. communis* EO and extracts against Gram-positive and Gram-negative bacteria and fungi. Ghasemi et al. reported that *M. communis* EO from Iran displayed better activity against *C. albicans* (MIC = 0.036 mg/mL) than against *E. coli* (MIC = 10 mg/mL) [36]. Tunisia's *M. communis* leaves EO [37], of which 1,8-cineole makes up half of the EO, exhibited moderate antibacterial activity against all tested bacterial strains including *E. coli* and *K. pneumoniae*, with MIC values ranging from 12.5 to 25 mg/mL. On the other hand, Hsouna et al. [21], reported that Tunisia's *M. communis* leaves EO, of which myrtenyl acetate (20.75%), 1,8-cineole (16.55%), and α-pinene were the main components, exhibited high antibacterial action against *E. coli*, *K. pneumoniae*, and *P. aeruginosa* with MIC values of 2.5, 2.5 and 1.25 mg/mL, respectively. Touaibia et al. [38] stated that *M. communis* leaves EO from Algeria, in which limonene (12.93%), octadienol (12.85%) and α-pinene (10.01%) were the main components, displayed antibacterial activity against *E. coli*, *K. pneumonia*, and *P. aeruginosa* with MIC values of 1.125, 4.50, and 18.00 mg/mL, respectively. Randrianarivelo et al. [39] demonstrated that the oxygenated terpenes found in *M. communis* oil, such as 1,8-cineole, linalool, and α-terpineol, possess substantial antibacterial action. *M. communis* leaves EO, with α-pinene (35.6%), 1,8-cineole (29.6%), linalool (6.87%), and α-terpineol (7.07%) as the major components, from Tunisia, demonstrated interesting antimicrobial activity against Gram-negative bacteria such as *P. aeruginosa*, *E. coli* with inhibition zones between 18 and 20 mm, and moderate antibacterial activity against Gram-positive bacteria but no antifungal activity against *C albicans* [40]. Mahboubi et al., on the other hand, discovered that leaves EO from Iran with 1,8-cineole (36.1%), α-pinene (22.5%), linalool (8.4%), bornyl acetate (5.2%), and α-terpineol (4.4%) had good antifungal action against fungus with a MIC value of 8 µL/mL [41]. Yadegarinia et al. [42], showed that *M. communis* EO from Iran, which contains α-pinene, limonene, 1,8-cineole, and linalool, was more effective against *C. albicans* than *E. coli* or *S. aureus*, with MIC values of 2 µL/mL for *C. albicans* and 4 and 8 µL/mL for bacteria, respectively. Ebrahimabadi et al. [43] found that EO from *M. communis* leaves containing a high concentration of α-pinene, 1,8-cineole, and linalool had a strong inhibitory effect against *C. albicans*, with MIC values ranging from 1.0 to 2.0 mg/mL. Aboutabl et al. [44] discovered that *M. communis* leaves EO from Egypt, which contains eugenol (35.5%), 1,8-cineole (27.2%), and limonene (21.8%), has antifungal activity against *C. albicans* with a MIC value of 100 µL/mL.

The mode of EOs' activity is determined by their chemical makeup, and their antibacterial action is not due to a single mechanism but rather to a cascade of reactions concerning the whole bacterial cell; these features are together referred to as "EOs flexibility" [40]. Essential oils are known to inhibit bacterial development and prevent the assembly of "toxic metabolites" by bacteria. Most EOs have a larger effect on Gram-positive bacteria than on Gram-negative species, which is probably due to the differing compositions of the cell membranes of the two types of bacteria [40].

With a MIC value of 0.78 mg/mL, the MeOH extracts of *M. communis* from Jericho demonstrated potent

bactericidal activity against MRSA and *S. aureus*, while EtOAc extracts from both regions demonstrated significantly greater bactericidal activity against MRSA and *S. aureus* (Table 3). The fungicidal activity of *M. communis* extracts in MeOH and EtOAc from both localities was stronger against *C. albicans*, with a MIC value of 0.049 mg/mL, while EOs from Jericho and Jenin had lower activity, with MIC values of 1.08 and 1.13 mg/mL, respectively. In contrast to other extracts, the EtOAc extract from Jericho demonstrated higher antibacterial activity against *K. pneumonia*, *E. coli*, *P. vulgaris,* and *P. aeruginosa* with MIC values of 1.56, 1.56, 3.125, and 3.125 mg/mL, respectively. In addition, all of the studied extracts had stronger antifungal activity against *C. albicans* than the two EOs with MIC values of 0.049 mg/mL. Methanol and ethyl acetate extracts from both regions outperformed the positive controls with regard to antimicrobial activity against MRSA, *S. aureus*, and *C. albicans* (Table 3). Each extract's antifungal activity against *C. albicans* (MIC = 0.049 mg/mL) exceeded that of the positive control medication fluconazole (MIC = 1.56 mg/mL). Our results are in agreement with some of the data reported previously in the literature. MeOH and aqueous plant leaves extract displayed antibacterial activity against MRSA, *S. aureus*, and *P. aeruginosa* with MIC values of 0.781 mg/mL [45]. Mansouri et al. [46] tested the antibacterial activity of a MeOH extract of *M. communis* on ten bacterial strains, including *S. aureus*, *P. vulgaris*, *E. coli*, and *P. aeruginosa*, and found that the MIC values ranged from 0.1 to 2.0 mg/mL. With MIC values of 0.1, 0.1, 0.8, and 1.5 mg/mL, respectively, for *S. aureus*, *E. coli*, *P. vulgaris*, and *P. aeruginosa*, the soluble fraction of MeOH extract in EtOH was able to kill these bacteria [46]. Pathogens can be killed by *M. communis* extract because it has active components that work as antimicrobials. In fact, polyphenolic compounds, tannins, and flavonoids are known to be in the leaves of *M. communis* [45]. Messaoud et al. [47] reported that MeOH plant leaves extract from Iran showed an antibacterial effect against different microorganisms including *S aureus* and *K. pneumoniae* with MIC values ranging from 12.5 to 25.0 mg/mL. These results displayed the robust antibacterial action of the *M. communis* plant.

Antioxidant activity of *M. communis*

The antioxidant activities of both EOs as well as for the methanol extract and ethyl acetate fraction of *M. communis* were determined by DPPH assay, which is one of the most preferred methods for the determination of antiradical potential [48]. As shown in Fig. 3 and Table 4, methanol extracts of *M. communis* gathered from Jenin and Jericho presented high antioxidant potential with IC_{50} values of 25.70 ± 0.45 and 8.550 ± 0.31 µg/mL,

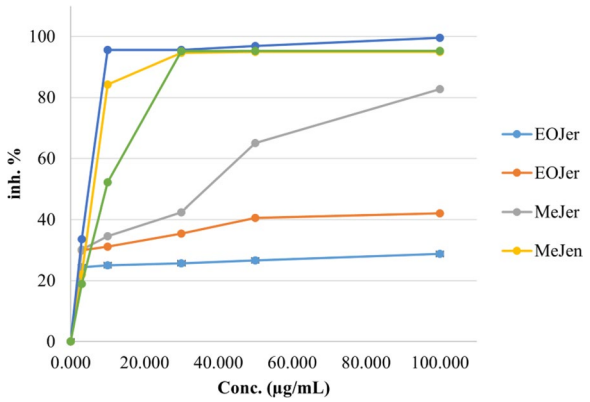


Fig. 3 Inhibition% of DPPH by Trolox and *M. communis* essential oil and extracts

respectively, which are comparable to those of the positive control (Trolox, IC_{50} = 10.25 ± 1.02 µg/mL). Jericho and Jenin ethyl acetate fractions have dose-dependent free radical scavenging activities, with IC_{50} values of 3.60 ± 0.35 and 4.86 ± 0.48 µg/mL, respectively, which outperformed the positive control Trolox. The EOs of *M. communis* lowered the concentration of the free radical DPPH just marginally. Methanol extract and ethyl acetate fraction showed much higher antioxidant activity than *M. communis* EOs, which could be due to a minor phenolic content of the EOs. Our EOs antioxidant properties are consistent with a previous study conducted on *M. communis* EOs from Italia, Morocco, and Yemen which displayed weak activity with IC_{50} values ranging from 0.80–4.50 mg/mL [17, 22, 47–50]. EOs from Tunisia [47] and Algeria [17] displayed better activity for DPPH free radical scavenging with IC_{50} values ranging from 200 to 693 µg/mL. Furthermore, our antioxidant results for methanol extract and ethyl acetate fraction were consistent with prior findings for plant leaves ethyl acetate, methanol, and aqueous extracts, which demonstrated significant antioxidant activity with IC_{50} values of 3.5, 9, and 11 µg/mL, respectively [51]. Wannes et al. [52] reported significant free radical scavenging properties of a methanolic extract of *M. communis* leaves, stems, flowers, and seeds in Tunisia with IC_{50} values of 3, 90, 3, and 10 µg/mL, respectively, with greater scavenging ability on DPPH radicals than EOs of leaf (IC_{50} = 600 µg/mL), stem (IC_{50} = 2,000 µg/mL), flower (IC_{50} = 550 µg/mL), and seeds ((IC_{50} = 10 mg/mL) [52]. The neutralization of free radicals (DPPH) by the methanol extracts, either by the transfer of a hydrogen atom or an electron, may be the cause of the observed antioxidant action [52]. Accordingly, it has been stated that the antioxidant activity depends not only on the total phenolics but also on the

Table 4 The inhibitory activity of *M. communis* essential oil, extracts, and Trolox against DPPH

Concentration µg/mL	EOJen	EOJer	MeJen	EtOAcJen	MeJer	EtOAcJer	Trolox
3.00	24.41	29.92	30.19	21.98	18.94	33.59	0.01
10.00	25.07	31.10	34.59	84.23	52.14	95.59	40.44
30.00	25.72	35.43	42.45	94.62	95.20	95.59	95.90
50.00	26.64	40.55	65.09	94.98	95.33	96.89	93.03
100.00	28.74	42.13	82.70	94.98	95.33	99.61	94.26
IC_{50} (µg/mL)	Ni	Ni	25.70 ± 0.45	4.86 ± 0.48	8.55 ± 0.31	3.60 ± 0.35	10.25 ± 1.02

IC_{50} half-maximal inhibitory concentration, *DPPH* 1,1-diphenyl-2-picrylhydrazyl, *EOJen* EO from Jenin, *EOJen* EO from Jenin, *EtOAcJer* EO from Jericho, *MeJen* methanol extract from Jenin, *EAJen* ethyl acetate extract from Jenin, *MeJer* methanol extract from Jericho, *EtOAcJer* ethyl acetate extract from Jericho

Table 5 The IC_{50} (µg/mL) for Jericho and Jenin EOs against cells lines

Cancer cell lines	IC_{50} (µg/mL)		
	Jericho EO	Jenin EO	Dox
HeLa	914.54 ± 3.05	592.40 ± 2.55	0.84 ± 1.1
MCF7	762.45 ± 2.25	597.01 ± 3.11	0.37 ± 0.22
3T3	644.47 ± 2.89	215.25 ± 1.07	1.21 ± 1.0
LX-2	199.80 ± 3.41	202.02 ± 2.27	5.72 ± 0.09

phenolic compounds' type and the presence of hydrolyzable tannins [53].

Anti-cancer activity

Several studies on the possible cytotoxic action of *M. communis* EO on cancer cell lines [30, 54, 55], and none on 3T3 and LX-2 cell lines, have been published. EOs from *M. communis* were evaluated in vitro against cervical adenocarcinoma (HeLa), breast cancer (MCF-7), fibroblast (3T3), and hepatic stellate cell lines (LX-2). For 24 h, cells were exposed to zero, 15.65, 31.25, 62.5, 125, 250, and 500 µg/mL of each of the EOs examined. Cell viability was inhibited by EOs by between 75 and 91% at a concentration of 500 mg/mL. As shown in Table 5, Jenin EO inhibited the proliferation of 3T3, MCF-7, and HeLa cell lines with IC_{50} values of 215.25 ± 1.07, 597.01 ± 3.11 and 592.01 ± 2.55 µg/mL, respectively, as compared to Jericho EO against the same cell lines (IC_{50} values ranging from 644.47 ± 2.89, 762.45 ± 2.25, and 914.54 ± 3.05 µg/mL), with 3T3 cell line was the most sensitive. Higher concentrations of oxygenated monoterpenes, such as 1,8-cineole are likely responsible for the higher cytotoxicity of the Jenin EO compared to the EO from Jericho. Scazzocchio et al. found no toxicity of a commercial *M. communis* EO on HeLa cells after a 24 h treatment [56]. *M. communis* EO from Yemen showed an IC_{50} of 110 µg/mL after 72 h on the HT29 cell line [54]. Harassi et al. found considerable cytotoxicity of two Moroccan *M. communis* EOs against MCF7 and P815 cells after 48 h, with IC_{50} ranging from 4.0 to 6.25 mg/mL for MCF7 and from 53.9 to 260 µg/mL for P815 [55]. *M. communis* EO from Salerno, Italy, demonstrated significant cytotoxicity against SH-SY5Y cells, with an IC_{50} of 209.1 µg/mL, according to Caputoi et al. [30]. According to the National Cancer Institute's criteria, only natural compounds with an IC_{50} value below 20 µg/mL are considered to be cytotoxic; however, our IC_{50} result was > 20 µg/mL, suggesting that the EO was not cytotoxic [30].

α-Amylase activity

Diabetes is a metabolic disorder that raises blood sugar levels. In addition to long-term problems like kidney failure, stroke, heart disease, eye damage, foot ulcers, and diabetes can also cause ketoacidosis and nonketotic hyperosmolar coma [57]. Antioxidants and diabetes are closely related; antioxidants protect and maintain the beta-cells functionality against oxidative stress [58]. Since diet-rich antioxidants are crucial for the deterrence and treatment of numerous diseases, it has been shown that there is a high correlation between dietary antioxidant ingestion and fortification against diabetes [59]. α-Amylase is the main enzyme in saliva that breaks down 1,4-glycosidic bonds in starch. Its suppression with the synthetic α-amylase inhibitor acarbose, which has negative effects, is frequently used to control postprandial hyperglycemia [59, 60]. It was previously established that the presence of monoterpenes, such as thymol, limonene, and α-pinene exert powerful inhibiting properties against the activities of α-amylase and α-glucosidase [59]. The screening of *M. communis* EOs and extracts on the carbohydrate hydrolyzing activity of α-amylase in comparison to acarbose revealed that α-amylase inhibition appeared to be ineffective. Table 6 and Fig. 4 show that the best activity was seen for plant EO and methanol extract obtained in Jenin, with IC_{50} values of 950.48 ± 2.54 and 795.43 ± 1.88 µg/mL, respectively, while EO and extract from Jericho showed no activity.

Table 6 *α*-Amylase inhibitory effects of *M. communis* essential oil and extracts

Concentration (µg/mL)	EOJen	EOJer	MeJen	MeJer
50.00	3.33	7.78	34.68	No inhibition
100.00	3.06	18.89	36.02	
400.00	3.33	20.28	38.93	
900.00	40.56	23.89	54.36	
1000.00	60.28	25.56	55.31	
IC_{50} (µg/mL)	950.48 ± 2.54	> 1000	795.43 ± 1.88	

EOJen EO from Jenin, *EOJer* EO from Jericho, *MeJen* methanol extract from Jenin, *MeJer* methanol extract from Jericho

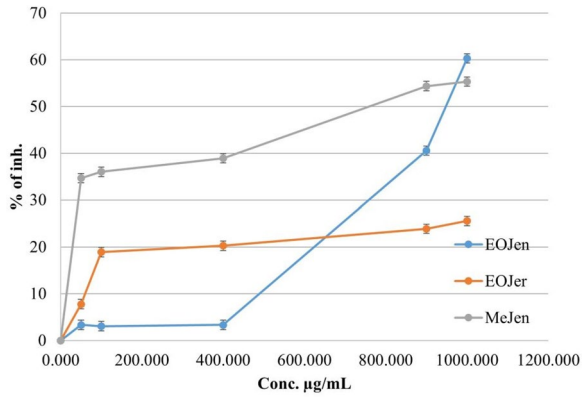


Fig. 4 *α*-Amylase inhibition of *M. communis* essential oil and Jenin MeOH extract

Accordingly, Ibrahim et al. [61] reported that *M. communis* EO from Egypt exhibited dose-dependent α-amylase inhibition activity, with the greatest inhibiting activity, reported at 1000, 750, and 125 µg/mL with IC_{50} values of 96.22 ± 0.140, 83.20 ± 0.162, and 36.24 ± 0.146 µg/mL, respectively, compared to acarbose standard 88.81 ± 0.69, 78.95 ± 0.917, and 23.16 ± 0.190 µg/mL.

Conclusions

Plant extracts, particularly EOs, are gaining popularity in the pharmaceutical, food, cosmetic, and perfumery industries. Their widespread use as antioxidants, antimicrobials, antifungals, anti-anxiety agents, and pain relievers makes them a viable herbal medicinal option. The EO of *M. communis* as well as methanol and ethyl acetate extracts offered considerable antifungal activity against *C. albicans* in comparison to the standard. Methanol extract as well as ethyl acetate fraction also depicted an appreciable free radical scavenger effect using DPPH assay. Additionally, EOs displayed moderate anticancer activity. More research is required on the methanol extract and ethyl acetate fraction to identify and isolate additional bioactive components for assessing in vitro and in vivo biological activity.

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Author contributions
Conceptualization (NA-M and NJ); methodology (NA-M and NJ; software, (SJ and NA-M); validation (NA-M, NJ and SJ); formal analysis (NA-M, NA and SJ); investigation (NA-M, and SJ); resources (NA and NJ); data curation (NA-M, NA, NJ); writing—original draft preparation (NA-M); writing—review and editing (NA-M, NJ, NA); visualization (NA-M); supervision (NA-M and NJ) project administration (NA-M). All authors read and approved the final manuscript.

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