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Chemical Composition, Antioxidant, Antimicrobial and Anti-Proliferative Activities of Essential Oils of *Rosmarinus officinalis* from five Different Sites in Palestine

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Abstract: The chemical profiles of *Rosmarinus officinalis* L. essential oils, collected from five distinct geographical regions in Palestine, were determined using GC-MS. The major phytochemical classes of *R. officinalis* EOs were monoterpene hydrocarbon (24.81–78.75%) and oxygenated monoterpenoids (19.01–73.78%), with 1,8-cineole (4.81–37.83%), α-pinene (13.07–51.36%), and camphor (11.95–24.30%) being the most abundant components of the studied oils. Using the DPPH assay, the antioxidant activity of EOs revealed that EO from the Jenin region had the highest antioxidant activity, with an IC_{50} value of 10.23 ± 0.11 µg/mL, followed by samples from Tulkarm (IC_{50} = 37.15 ± 2.3 µg/mL) and Nablus (IC_{50}= 38.9 ± 0.45 µg/mL). With MICs of 12.5, 12.5, 6.25, 6.25, and 6.25 µg/mL against MRSA, *S. aureus*, *E. coli*, *K. pneumonia*, and *P. vulgaris*, respectively, the EO extracted from the Jenin region of Palestine had the greatest antibacterial activity. Furthermore, EOs from Jenin and Nablus demonstrated stronger anti-candida action than the pharmaceutical formulation Fluconazole, with MICs of 0.781, 0.781, and 1.56 µg/mL, respectively.

Keywords: *Rosmarinus officinalis* L.; essential oils; GC-MS analysis; antioxidant; antibacterial; antifungal

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1. Introduction

Rosmarinus officinalis L. is a shrubby, evergreen, fragrant plant that grows wild across the Mediterranean Sea region and in many Asian countries. It is widely used in cooking and healing across the world [1]. It has been used in traditional medicine as a moderate analgesic, antispasmodic, antidepressant, hypnotic, and to treat emotional disturbance, migraines, rheumatic pain, intercostal neuralgia, and to enhance memory [2]. A great number of in-vivo and in-vitro studies have shown that *R. officinalis* and its components have a broad variety of therapeutic properties, including anti-hysteric, antidepressant, and ameliorative of mental and memory fatigue [3–5], neuroprotective [6], antinociceptive [7], antioxidant [8], and anti-inflammatory [9].

Oxidative stress is induced by an imbalance in the body's redox state; this occurs when the creation of reactive oxygen species outweighs the antioxidants' natural defenses [10]. Oxidative stress is a major contributor to the physiopathology of chronic degenerative illnesses such as diabetes, cancer, and atherosclerosis [11]. Overproduction of free radicals in the human body causes oxidative damage to biomolecules, such as DNA, proteins, and lipids, which can cause or contribute to a variety of chronic diseases and disturbances, such as cancer, septic shock, stroke, chronic inflammation, cardiovascular diseases, myocardial infarction, post-ischemic perfusion injury, aging, rheumatoid arthritis, diabetes, atherosclerosis, and other degenerative diseases [12].

Lung, colon and rectum, liver, stomach, and breast cancers are the leading causes of mortality worldwide, accounting for approximately 10 million fatalities in 2020 [13]. In a multi-stage process, cancer develops when healthy cells are transformed into tumor cells, progressing from a precancerous lesion to a cancerous tumor, in most cases. To produce these changes, a person's genetic background interacts with three types of environmental factors: biological carcinogens, such as parasite, bacterial, or viral infections; chemical carcinogens, such as tobacco smoke and arsenic-contaminated water; and physical carcinogens, such as ultraviolet and ionizing radiation [13]. Furthermore, oxidative stress may lead to neurological and malignant illnesses [14].

The overuse of antimicrobial drugs exacerbates a variety of bacterial and fungal diseases by favoring multidrug-resistant and persistent organisms. In reality, persistent antimicrobial exposure leads to the accumulation of adaptive mutations that improve antibiotic tolerance [15]. Some microbial infections may result in the development of a broad variety of deadly illnesses, including organ malfunctions, autoimmune disorders, diabetes mellitus, and cancer.

Essential oils (EOs) are a mixture of natural compounds, derived from the aerial and subterranean sections of fragrant plants, and have been utilized in traditional medicine since antiquity [16]. They have been shown to possess a broad-spectrum of antibacterial properties, making them intriguing alternatives to antibiotics. The antibacterial action of EOs is ascribed to the damage they inflict to the cell membrane and cell wall of bacterial and fungal infections [17]. Numerous studies have shown that EOs may permeate into the polysaccharide matrix of biofilm and disrupt it [18]. In addition, it was found that EOs may interact with bacterial surface proteins and modify the initial attachment phase to an abiotic surface, indicating their anti-adhesion properties [19]. Over the last two decades, there has been a surge in the research of natural Eos as antibacterial, antioxidant, and anticancer agents for the treatment and prevention of various disorders [1,20,21]. The phytochemical contents of Eos are influenced not only by the plant species, but also by the stocking time, agrochemicals used in preparation, soil type, and climate, as well as the harvested plant portion, variety, and age, among other factors. Due to this variation, essays using Eos should always contain information on the biological characterization of the plant material, as well as the EO's phytochemical profile, to guarantee reproducibility and accuracy of the gathered data [22].

As a result, the present research attempted to evaluate the changes in chemical compositions, antibacterial, antioxidant, and cytotoxic effects of *R. officinalis* EOs collected from five distinct Palestinian governorates against multiple microbial strains and cancer cell lines.

2. Materials and Methods

2.1. Materials

Dimethyl sulfoxide (DMSO) analytical grade, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trollox (97% purity) were purchased from Sigma-Aldrich (Sigma-Aldrich, Darmstadt, Germany).

2.2. Plant Samples Collection, Preparing, and Extraction of Essential Oils

In May 2020, *R. officinalis* leaves were collected from five governorates in Palestine, including Ramallah (coordinates: 31°46′5.9484″ N and 35°12′49.356″ E, altitude 779.46 m), Tulkarm (coordinates: 32°18′37″ N and 35°01′43″ E, altitude 117 m), Jenin (coordinates: 32°27′36.00″ N 35°17′60.00″ E, altitude 250 m), Hebron (coordinates: 31°26′ N 35°0′ E, altitude 1026 m) and Nablus (coordinates: 32°13′15″ N and 35°15′15″ E, altitude 569 m), during the blossoming season. Dr. Nidal Jaradat, a pharmacognosist, was able to identify the plant samples and deposit them in an Herbarium, under the voucher specimen number (Pharm-PCT-2732). The leaves were air-dried in the shade at ambient temperature (26 ± 3 °C) and relative humidity (56 ± 2 RH). The dry materials were crushed into a fine powder and kept in airtight containers, labeled appropriately for future usage. The hydro-

distillation approach was used to extract the *R. officinalis* Eos; 100 g of dried leaf powder was suspended in 1 L of distilled water, and the EOs were extracted using a Clevenger apparatus working at atmospheric pressure for 80 min at 100 °C. The EOs were dried with sodium sulfate and kept at 2 °C until further usage.

2.3. Gas Chromatography/Mass Spectrometry (GC-MS)

A Perkin Elmer Clarus 500 gas chromatograph, connected to a Perkin Elmer Clarus 560 mass spectrometer, was used to analyze the extracted EOs components of the *R. officinalis*. The Perkin Elmer Elite-5 fused-silica capillary column (film thickness 0.25 µm, 30 m × 0.25 mm,) was used to separate the constituents of the EOs. The column temperature was programmed and set to vary between 50 °C for 5 min and 280 °C at a rate of 4 °C/min. The flow rate of Helium as a carrier gas was maintained at a constant throughout all of the chromatographic runs at 1 mL/min. At 250 °C, 0.2 µL of pure EO was injected in split mode with a splitting ratio of 1:50. A full scan mode covering the m/z range of 50–500 was acquired.

The chemical components of the EOs were identified by comparing their MS with the reference spectra in the NIST mass spectrometry data center, as well as by comparing their retention indices to those published in the literature.

2.4. Free Radical Scavenging Activity

The antioxidant properties of the EOs were assessed using the DPPH inhibitory assay published in the literature [23], with Trolox serving as a positive control. A stock solution of 1 mg/mL of each EO was prepared by dissolving 100 mg of each sample in 100 mL of methanol, which was then diluted with methanol to achieve the needed concentrations. The investigated samples' concentrations were 2, 5, 10, 20, 50, and 80 µg/mL. A UV-Vis spectrophotometer (Jenway-7315, Staffordshire, UK), set at 517 nm, was used to measure absorption. The following equation was used to determine the DPPH inhibitory activity of all of the tested EOs.

$$I\ (\%) = [Abs_{blank} - Abs_{test}]/[Abs_{blank}]) * 100\%$$

where I (%), is the percentage of antioxidant activity. The antioxidant half-maximal inhibitory concentration (IC_{50}) for the evaluated samples was calculated using BioDataFit edition 1.02 (data fit for biologist).

2.5. Antimicrobial Activity

The EOs' antimicrobial activity was assessed using one fungal strain, *Candida albicans* (American type culture collection (ATCC) 90028), and six bacterial strains, five of which were ATCC: *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia,* (ATCC 13883), *Proteus vulgaris* (ATCC 8427) and *Staphylococcus aureus* (ATCC 25923), in addition to a diagnostically confirmed Methicillin-Resistant *Staphylococcus aureus* (MRSA). The stock solution was made for each EO at a concentration of 200 µg/mL 20%, dissolved in 20% DMSO, and 60% Muller-Hinton broth. Each of the prepared EO solutions were serially diluted (2-folds) with sterile Muller-Hinton broth to achieve serial dilutions of 50, 25, 12.5, 6.25, 3.125, etc. µg/mL (RPMI medium was used for the *C. albicans* strain); DMSO concentration was 5% at the first well, then was further two-fold serially diluted so its antimicrobial effect was excluded. The dilution process was conducted aseptically in 96-well plates. Micro-well 11 contained EO-free media (inoculated with the microbe), which was used as a positive control for microbial growth in the micro-wells designated to assess the antibacterial activity of the EO. On the other hand, none of the tested microorganisms were inculcated in the EO free micro-well 12. This well served as a negative control for the proliferation of microorganisms. The tested microorganisms were injected aseptically into microwells 1 through 11. Each inoculated plate was incubated at a temperature of 35 °C. Plates inoculated with test bacterial strains were incubated for around 18–24 h, whereas plates inoculated with *C. albicans* were incubated for approximately 48 h. The examined

EO's minimal inhibitory concentration (MIC) was determined by measuring the lowest concentration of EO in the microwell, at which no apparent microbial growth occurred.

In this experiment, Ciprofloxacin and Ampicillin were employed as antibacterial activity controls, whereas Fluconazole was used as an antifungal activity control. Antimicrobial activity of EOs was evaluated in triplicates [24,25].

2.6. Cell Culture and Cytotoxicity Assay

Breast cancer (MCF-7), hepatocellular carcinoma (Hep 3B & Hep G2), skin cancer (B16-F1), colorectal adenocarcinoma (Caco-2), cervical adenocarcinoma (HeLa), human hepatic stellate (LX-2), and Human epithelial kidney (HEK-293T) cells (ATCC, Rockville, MD, USA) were cultured in RPMI-1640 media and supplemented with 1% mixture of Streptomycin and Penicillin, 1% l-glutamine, and 10% fetal bovine serum. Cells were grown at 37 °C in a humidified atmosphere with 5% CO_2. Cells were seeded at 2.6×10^4 cells/well in a 96-well plate. After 48 h, cells were incubated with various concentrations (500, 250, 125, 62.5, 31.25, and 15.62 µg/mL) of the tested EOs for 24 h. Doxorubicin was employed as a positive control for anti-proliferative activity. Cell viability was evaluated by a CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay, according to the manufacturer's instructions (Promega Corporation, Madison, WI, USA). At the end of the treatment, 20 µL of MTS solution per 100 µL of medium was added to each well, incubated at 37 °C for two hours, and the absorbance was determined at 490 nm.

2.7. Statistical Analysis

All the experiments were carried out in triplicates. The results are presented as means ± standard deviation (SD). The results were considered significant only when the p values were less than 0.005.

3. Results and Discussion

3.1. Chemical Composition of Rosmarinus officinalis Essential Oils

Table 1 provides a quantitative and qualitative summary of the essential oil extracted from the aerial parts of *R. officinalis* plants gathered in five West Bank/Palestine locales. All components and their percentage contents are listed in ascending order, based on their retention indices (Kovats indices). The highest EO yields were produced in Ramallah/central region (1.41%), Nablus (1.35%), and Tulkarm (1.31%) in the north, while rosemary collected in Hebron, in the south, afforded the lowest yield (1.01%). The oil yields were lower than those obtained from *R. officinalis* growing in various locations around Tunesia [26]. Variations in oil yield could be ascribed to geographical origin, temperature, relative humidity, soil, genetics, and degree of maturity [26,27].

Table 1. Phytochemical compositions of *Rosmarinus officinalis* essential oils collected from five different Palestinian regions.

		Essential Oil Content (%)					
Name	**$RI_{calculated}$**	**Hebron**	**Jenin**	**Ramallah**	**Nablus**	**Tulkarm**	**m/z of Fragments**
Tricyclene	921	0.09	0.07	0.14	0.13	0.13	136, 121, 105, 93, 77, 67, 55
α-Thujene	926	0.10	0.002	-	-	-	136, 121, 1075, 93, 77, 65, 53
α-Pinene	933	13.07	51.36	26.90	25.29	29.18	136, 121, 105, 93, 77, 67, 53
Camphene	949	6.67	11.62	7.45	9.57	5.81	136, 121, 105, 93, 77, 67, 53
Thuja-2,4(10)-diene	953	-	-	0.11	0.05	0.21	134, 119, 105, 91, 78, 65
Unknown	965	-	1.16	-	-	-	119, 105, 93, 79, 67
β-Pinene	976	2.46	0.30	0.85	2.19	0.58	136, 121, 107, 93, 79, 69, 53
Myrcene	990	0.69	0.56	0.72	0.73	-	136, 93, 79, 69, 53
α-Phellandrene	1005	0.10	-	2.24	1.99	-	136, 93, 77, 65
Unknown	1006	-	0.18	-	-	0.11	136, 121, 91, 77, 63

Table 1. *Cont.*

		Essential Oil Content (%)					
Name	**$RI_{calculated}$**	**Hebron**	**Jenin**	**Ramallah**	**Nablus**	**Tulkarm**	**m/z of Fragments**
α-Terpinene	1016	0.40	1.48	1.01	0.66	0.39	136, 121, 105, 93, 77, 65
p-Cymene	1024	-	10.12	1.39	-	1.82	134, 119, 103, 91, 77, 65
Limonene	1029	-	3.02	-	-	-	136, 121, 107, 93, 79, 68, 53
1,8-Cineole	1033	37.82	4.81	31.09	31.09	33.28	154, 139, 119, 108, 93, 81, 71, 55
Unknown	1058	-	0.11	-	-	-	119, 91, 79, 65
γ-Terpinene	1059	1.23	0.06	0.64	0.90	0.58	136, 121, 105, 93, 77, 65, 53
Terpinolene	1085	0.38	0.04	0.36	0.41	0.36	136, 121, 105, 93, 79, 67, 58
Linalool	1101	0.54	-	0.15	0.16	0.71	136, 121, 107, 93, 79, 71, 55
Chrysanthenone	1122	-	-	0.06	-	-	150, 135, 122, 107,91, 70
cis-Menth-2-en-l-ol	1125	-	-	0.06	0.23	0.02	154, 139, 121, 111, 93, 79, 55
Camphor	1148	24.30	13.86	15.52	18.47	11.95	152, 137, 108, 95, 81, 69, 55
Neoisothujol	1156	-	-	0.05	0.06	0.06	136, 121, 108, 93, 79, 71, 53
Isoborneol	1162	-	0.32	-	0.29	-	136, 121, 110, 95, 67
Pinocarvone	1163	-	0.02	0.04	-	0.66	150, 135, 122, 108, 91, 81, 53
Borneol	1174	4.76	-	3.62	2.25	5.07	154, 139, 121, 110, 95, 79, 67, 55
Terpinene-4-ol	1181	0.94	-	0.65	0.49	0.96	154, 136, 121, 107, 93, 77, 71, 55
Unknown	1188	-	-	-	0.07	0.21	150, 135, 121, 117, 107, 91, 81, 67, 59, 53
α-Terpineol	1196	2.13	-	1.66	1.05	2.69	150, 135, 121, 107, 93, 79, 67, 59, 53
Verbenone	1208	0.12	-	2.23	1.56	2.69	150, 135, 122, 117, 107, 91, 79, 67, 59, 55
Cuminaldehyde	1242	-	-	-	-	0.07	148, 133, 119, 105, 91, 77
Carvone	1245	-	-	-	-	0.04	150, 135, 93, 82, 54
Piperitone	1255	-	-	-	0.02	0.01	152, 137, 110, 95, 82, 67
Isobornyl acetate	1285	3.17	-	0.52	1.06	0.35	154, 136, 121, 108, 95, 79, 67, 55
Piperitenone	1340	-	-	-	-	0.04	150, 135, 121, 107, 91, 79, 67, 53
α-Ylangene	1371	0.02	-	-	-	0.15	204, 189, 161, 119, 105, 93, 79, 67, 55
α-Copaene	1376	-	-	-	-	0.01	204, 161, 119, 105, 91, 81, 67, 55
Methyl eugenol	1401	0.09	0.59	0.06	0.03	0.16	178, 163, 147, 107, 91, 77, 65, 51
β-Caryophellene	1420	0.72	-	2.14	1.06	1.04	204, 189, 175, 161, 119, 105, 91, 79, 69, 55
Linalool butanoate	1426	-	-	-	0.10	-	136, 121, 107, 93, 71, 55
α-Caryopyllene	1457	0.08	-	0.23	-	0.16	204, 147, 135, 121, 107, 93, 80, 67, 53
Germacrene D	1477	0.02	-	-	-	-	204, 161, 147, 133, 119, 105, 91, 79, 67, 55
γ-Muurolene	1476	-	-	-	-	0.13	204, 161, 147, 133, 119, 105, 91, 79, 67, 55
α-Muurolene	1500	0.01	-	–	-	-	204, 189, 161, 147, 133, 119, 105, 91, 81, 67
β-Bisabolene	1507	0.03	-	-	-	-	204, 189, 161, 147, 133, 119, 105, 91, 81, 67
γ -Cadinene	1514	-	-	-	-	0.09	204, 161, 147, 133, 119, 105, 91, 79, 67, 55
δ-Cadinene	1521	0.05	-	-	-	0.24	204, 189, 161, 147, 133, 119, 105, 91, 77, 67
Z-Nerolidol	1525	0.01	-	-	–	0.03	189, 161, 147, 136, 121, 107, 93, 81, 69
Caryophyllene oxide	1586	-	-	0.08	0.10	0.04	177, 161, 149, 135, 121, 107, 91, 79, 69, 55
Oil Yield		1.01	1.22	1.41	1.35	1.31	
Total identified		99.62	98.43	99.97	99.94	99.81	
Monoterpene hydrocarbons		24.81	78.75	41.80	41.92	39.17	
Oxygenated monoterpenes		73.78	19.01	55.66	56.83	58.59	

Table 1. *Cont.*

		Essential Oil Content (%)					
Name	**$RI_{calculated}$**	**Hebron**	**Jenin**	**Ramallah**	**Nablus**	**Tulkarm**	**m/z of Fragments**
Sesquiterpene hydrocarbons		0.93	-	2.37	1.06	1.82	
Oxygenated Sesquiterpenes		0.01	-	0.08	0.10	0.07	
Others		0.9	0.59	0.06	0.03	0.16	

RI = Retention index.

The results of the GC-MS analysis of *R. officinalis* EOs (Figures 1 and 2) enabled the identification and quantification of 26, 17, 27, 26, and 33 different compounds from EOs collected from Hebron, Jenin, Ramallah, Nablus, and Tulkarm, representing 99.62%, 98.43%, 99.97%, 99.94%, and 99.81% of the total oils, respectively. The primary components were as follows: 1,8-cineole (37.82%), camphor (24.30%), α-pinene (13.07%) and camphene (6.67%) for the EO from Hebron; α-pinene (51.36%), camphor (13.86%), camphene (11.62%) and cymene (10.12%) for the EO from Jenin; 1,8-cineole (31.09%), α-pinene (26.69%), camphor (15.52%), and camphene (7.45%) for the EO from Ramallah; 1,8-cineole (31.09%), α-pinene (25.29%), camphor (18.47%) and camphene (9.57%) for the EO from Nablus; and 1,8-cineole (33.28%), α-pinene (29.18%) Camphor (11.95%), and borneol (5.07%) for EO from Tulkarm. Our findings demonstrated that the oil content and compositional profiles of the geographical regions varied. 1,8-Cineole (4.81–37.83%), camphor (11.95–24.30%), and α-pinene (13.07–51.36%) were the most abundant components of the studied oils, with observable quantitative and qualitative variations of these compounds in the chemical profiles of EOs obtained from different Palestinian locations. Endoborneol and verbenone were detected in some of the oils tested with low concentrations (2.97–4.76%). The analysis of *R. officinalis* EOs composition revealed a significant percentage of the monoterpene fraction, amounting to 97–99%, dominated by oxygenated monoterpenes for EOs from Hebron, Ramallah, Tulkarem, and Nablus. Hydrocarbon monoterpenes, on the other hand, was the largest group, accounting for 78.75% of the EOs from Jenin. Sesquiterpene hydrocarbons were detected at modest concentrations (0.93 to 2.37%) in oils from all locations, although oxygenated sesquiterpene was detected in negligible amounts.

Our findings are consistent with prior observations of the *R. officinalis* EO. The most well-known commercial *R. officinalis* EO contains 1,8-cineole (eucalyptol) (19.59%), camphor (18.35%), α-pinene (17.17%), camphene (10.10%), β-pinene (6.08 %), and D-limonene (3.90%) [28]. The EOs of *R. officinalis* collected from different bioclimatic areas in Tunisia, belonging to the upper semi-arid, middle semi-arid, and upper-arid stages, contained 1,8-cineole (29.11–60.44%), camphor (5.88–27.95%), α-pinene (6.76–12.60%), camphene (1.61-12.87%), and borneol (2.61-12.61%), as the most predominant constituents [26].

Socaci et al. [29] identified α-pinene (72.45%), octanone (7.46%), and 1,8-cineole (6.08 %) as the principal constituents of EO fresh of *R. officinalis* fresh leaves. Pintore et al. reported that *R. officinalis* EOs from Sardinia and Corsica included α-pinene (13.7, 24.6%), bornyl acetate (11.3, 17.0%), verbenone (4.4, 24.9%), camphor (2.9, 14.1%), and 1,8-cineole (3.4, 11.3%) as the major compounds, respectively [30]. In addition, Akrout et al. [31] observed three distinct *R. officinalis* chemotypes that identify EOs containing 1,8-cineole/camphor/α-pinene/camphene from Morocco, Tunisia, Turkey, Greece, Yugoslavia, Italy, France, and Algeria. France, Spain, Italy, Greece, Tunisia, Algeria, South Africa EOs containing nearly equal amounts (20–30%) of 1,8-cineole, α-pinene, and camphor. One other chemical composition could be defined according to the comparatively higher amount of myrcene in oils from Argentina and Portugal [31]. This variance in the chemical composition of *R. officinalis* EO could be attributed to geographical differences, bioclimatic variations, plant cultivation, and harvesting techniques.

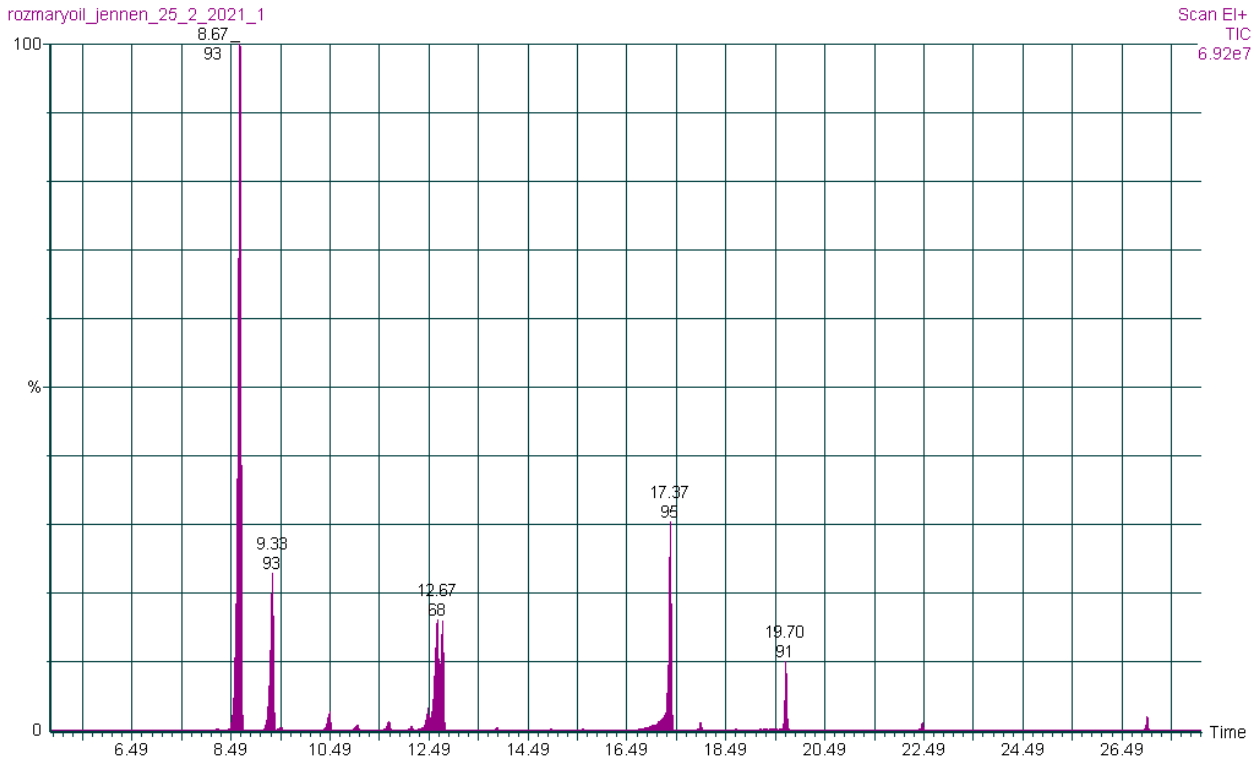

Figure 1. Gas chromatography chromatogram of *R. officinalis* Leaves essential oil collected in Jenin.

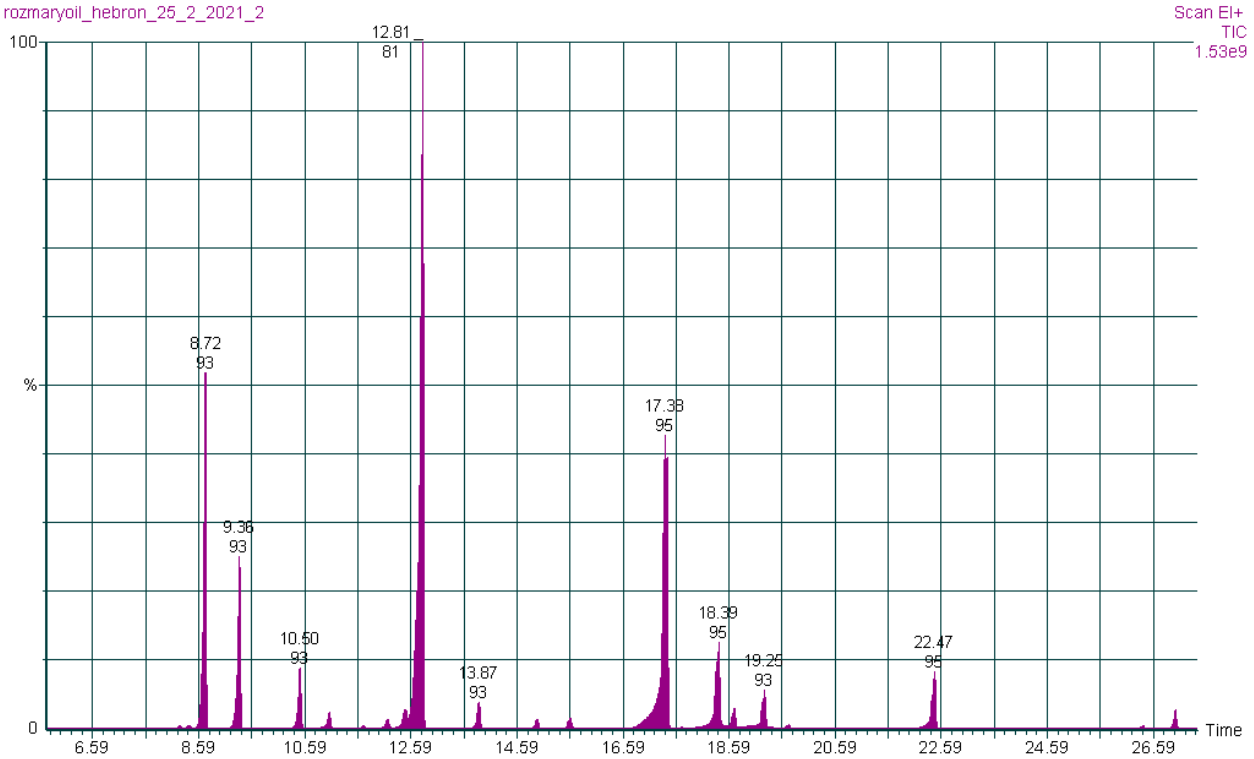

Figure 2. Gas chromatography chromatogram of *R. officinalis* Leaves essential oil collected in Hebron.

3.2. Antioxidant Activity

Plant-based antioxidants have the potential to scavenge free radicals and are considered therapeutically advantageous, and safer, than manufactured chemical antioxidants. Several degenerative diseases, including cardiovascular, diabetes, and Alzheimer's disease,

as well as infections, neurodegeneration, cancer, and chronic renal disease, are associated to the buildup of free radicals, which constitute a hazard to human health. Given the importance of oxidative stress in disease genesis, natural antioxidants are being used to treat a variety of ailments. Due to its antibacterial and antioxidant capabilities, *R. officinalis* EO is being exploited as a bio-preservative in several food sectors. However, studies have shown that it also offers several health benefits [32–34].

The antioxidant activity against the DPPH radical was measured for the five EOs and the positive control (Trolox), and the findings were reported as DPPH scavenging rate and IC_{50} values for Hebron, Ramallah, Tulkarm, Jenin and Nablus were 107.15 ± 0.75, 158.48 ± 0.87, 37.15 ± 2.3, 10.23 ± 0.11, and 38.9 ± 0.45 µg/mL, respectively, in comparison with Trolox (IC_{50} = 3.16 ± 1.03 µg/mL). The inhibition percentage for each concentration of these EOs was depicted in Figure 3. The assessed *R. officinalis* EOs' free radical scavenging capacity improved in a concentration-dependent manner [35].

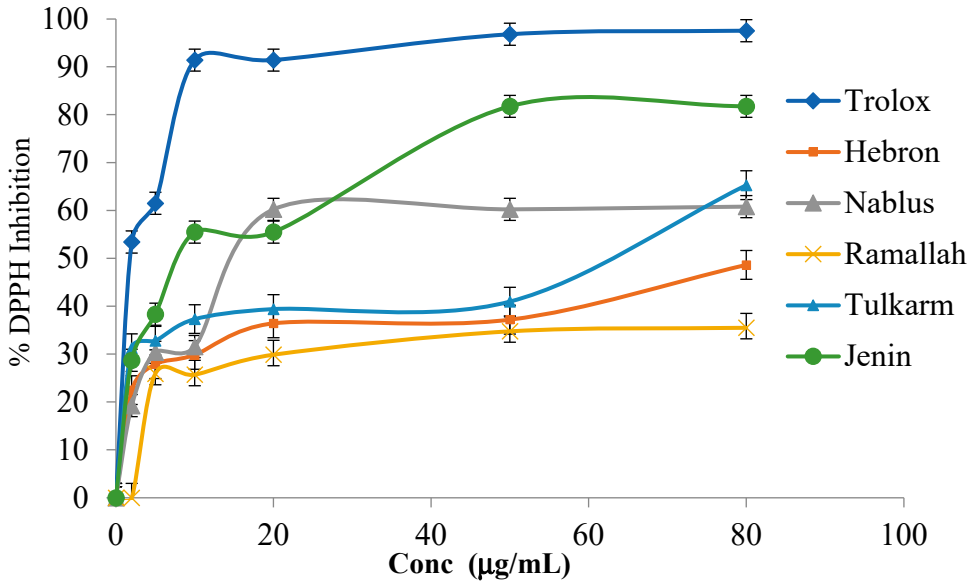

Figure 3. DPPH inhibitory potentials by *Rosmarinus officinalis* essential oils from five Palestinian regions and Trolox.

The free radical scavenging activity test findings revealed that the EO from the Jenin area has the highest antioxidant activity, with IC_{50} values of 10.23 ± 0.11 µg/mL, followed by Tulkarm and Nablus EOs, which have antioxidant potentials with IC_{50} values of 37.15 ± 2.3 and 38.9 ± 0.45 µg/mL, respectively. The high concentration of α-pinene in EO from Jenin may be responsible for its excellent antioxidant action.

Rašković et al. [32] discovered that *R. officinalis* EO from Serbia has a high antioxidant effect (IC_{50} = 77.6 µL/mL) when compared to vitamin E (α-tocopherol), which exhibit a substantial antioxidant property, with an IC_{50} value of 25.3 µg/mL. Hussain et al. [36] discovered that *R. officinalis* EO gathered in Pakistan had a greater antioxidant effect (IC_{50} = 20.9 ± 0.9 µg/mL) than 1,8-cineole (IC_{50} = 45.7 ± 1.5 µg/mL), the main component of *R. officinalis* EO.

3.3. Antimicrobial Activity

The MIC was determined during this study by assessing the EO's inhibitory activity against the selected six bacterial strains and one fungus strain (Table 2), using the broth micro-dilution assay. The EO obtained in Palestine's Jenin area demonstrated the greatest antibacterial activity against MRSA, *S. aureus*, *E. coli*, *K. pneumonia*, and *P. vulgaris*, with MICs of 12.5, 12.5, 6.25, 6.25, and 6.25 µg/mL, respectively. Furthermore, as indicated in Table 2, the EO gathered in the Jenin and Nablus areas showed the most potent anti-candida activity, with MICs of 0.781 µg/mL. *P. aeruginosa* was not affected by any of the EOs tested.

Table 2. Antimicrobial activity MIC values (µg/mL) of *R. officinalis* essential oils collected from five different Palestinian regions.

Samples/Microbe	Bacteria						Fungus
	Gram-Positives		Gram-Negative				Yeast
	Clinical Strain	ATCC 25923	ATCC 25922	ATCC 13883	ATCC 8427	ATCC 9027	ATCC 90028
	MRSA	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*
Hebron EO	50 ± 0.91	50 ± 1.11	R	50 ± 0.88	50 ± 1.09	R	25 ± 1.07
Tulkarm EO	50 ± 1.01	25 ± 0.97	50 ± 1.02	50 ± 1.09	25 ± 0.1	R	12.5 ± 1.01
Ramallah EO	25 ± 0.35	25 ± 0.35	12.5 ± 0.39	12.5 ± 0.92	12.5 ± 88	R	1.56 ± 0.07
Nablus EO	25 ± 0.47	25 ± 0.81	12.5 ± 0.71	12.5 ± 0.2	12.5 ± 0.7	R	0.78 ± 0.01
Jenin EO	12.5 ± 0.2	12.5 ± 0.17	6.25 ± 0.97	6.25 ± 0.33	6.25 ± 0.4	R	0.78 ± 0.01
Ciprofloxacin	12.5 ± 0.15	0.78 ± 0.01	1.56 ± 0.06	0.13 ± 0.04	15 ± 0.97	3.12 ± 0.02	-
Ampicillin	R	25 ± 0.25	3.12 ± 0.59	1.25 ± 0.09	18	R	-
Fluconazole	-	-	-	-	-	-	1.56 ± 0.02

R: resistance.

These data suggest that *R. officinalis* EO from the Jenin area possesses significant antibacterial action when compared to the commercially available antibiotic Ciprofloxacin, particularly against MRSA. Furthermore, EOs from Jenin and Nablus exhibit higher potential anti-candida action than the pharmaceutical formulation Fluconazole, with MICs of 0.781, 0.781, and 1.56 µg/mL, respectively. Several scientific studies have indicated that *R. officinalis* EO exhibits antibacterial activity against *S. aureus*, *P. aeruginosa*, and *E. coli* with MICs of 0.3, 1.26, and 1.52 mg/mL, respectively [36]. In addition, Jardak et al. reported that *R. officinalis* EOs collected in Tunisia possess a strong antibacterial property against *S. aureus* and *S. epidermidis*, with MIC values ranging from 1.25 to 2.5 and 0.312 to 0.625 µL/mL, respectively [37]. In addition, an investigation carried out by Wang et al. found that the *R. officinalis* EO possess potential antibacterial activity against *Bacillus subtilis*, *S. aureus*, *S. epidermidis*, *E. coli,* and *P. aeruginosa* with MIC values of 0.0625, 0.0313, 0.0313, 0.0625, and 0.0625% *v*/*v*, respectively [38].

3.4. Antiproliferative Activity

The MTS assay was used in this study to assess the anti-proliferative effects of Eos on the cell proliferation of breast cancer (MCF-7), hepatocellular carcinoma (Hep 3B & Hep G2), skin cancer (B16-F1), colorectal adenocarcinoma (Caco-2), cervical adenocarcinoma (HeLa), human hepatic stellate cell line (LX-2), and Human epithelial kidney (HEK-293T) cell lines. Cells were subjected to increasing doses of the tested Eos (0, 0.06, 0.125, 0.25, 0.5, 1, 2 mg/mL) for 24 h. The IC_{50} values were calculated from Figure 4A–H and are demonstrated in Table 3. The IC_{50} values of EO gathered in Ramallah region against Heb3B, HeLa, MCF7, HepG2, B16F1, CaCo2, LX2 and Hek293t cells were 0.964, 0.593, 0.532, 1.543, 0.816, 0.792, 1.215 and 1.010 mg/mL, respectively. The IC_{50} values of EO from Tulkarm region against Heb3B, HeLa, MCF7, HepG2, B16F1, CaCo2, LX2 and Hek293t cells were 1.643, 1.060, 0.860, 1.324, 1.159, 0.490, 1.002 and 0.821 mg/mL respectively. The IC_{50} values of EO from Jenin region against Heb3B, HeLa, MCF7, HepG2, B16F1, CaCo2, LX2 and Hek293t cells were 1.348, 1.325, 1.098, >2, 1.65, 0.33, 1.211 and 1.56 mg/mL respectively. The IC_{50} values of EO from Hebron region against Heb3B, HeLa, MCF7, HepG2, B16F1, CaCo2, LX2 and Hek293t cells were 1.332, 0.73, 0.627, 1.47, 1.079, 0.539, 1.123 and 1.722 mg/mL respectively. The IC_{50} values of EO from Nablus region against Heb3B, HeLa, MCF7, HepG2, B16F1, CaCo2, LX2 and Hek293t cells were 1.468, 1.342, 1.125, >2, 0.664, 0.53, 0.54 and 1.17 mg/mL respectively. Doxorubicin medication (a chemotherapeutic drug) had an IC_{50} value of <0.05 mg/mL for all of the examined cell lines, which is regarded very toxic. At 1 mg/mL, the cell viability of each of the EOs was calculated and is presented in Figure 5; at this concentration, all EOs showed a very weak or negligible effect on the Hep3b and HepG2 cancer cell lines, while the lowest cell viability was observed on CaCo2 cancer cell lines. Moreover, Nablus EO showed a significant effect on CaCo2 and B16F1, and the cell viability on these cells was very low in comparison with the other EOs. Our findings

indicate that *R. officinalis* EO has no cytotoxic impact and may therefore be regarded as harmless. This is consistent with the European Medicines Agency evaluation study [39] on the clinical safety of RO and the European Food Safety Authority, which concluded that the margin of safety was wide enough to rule out dietary exposure [40].

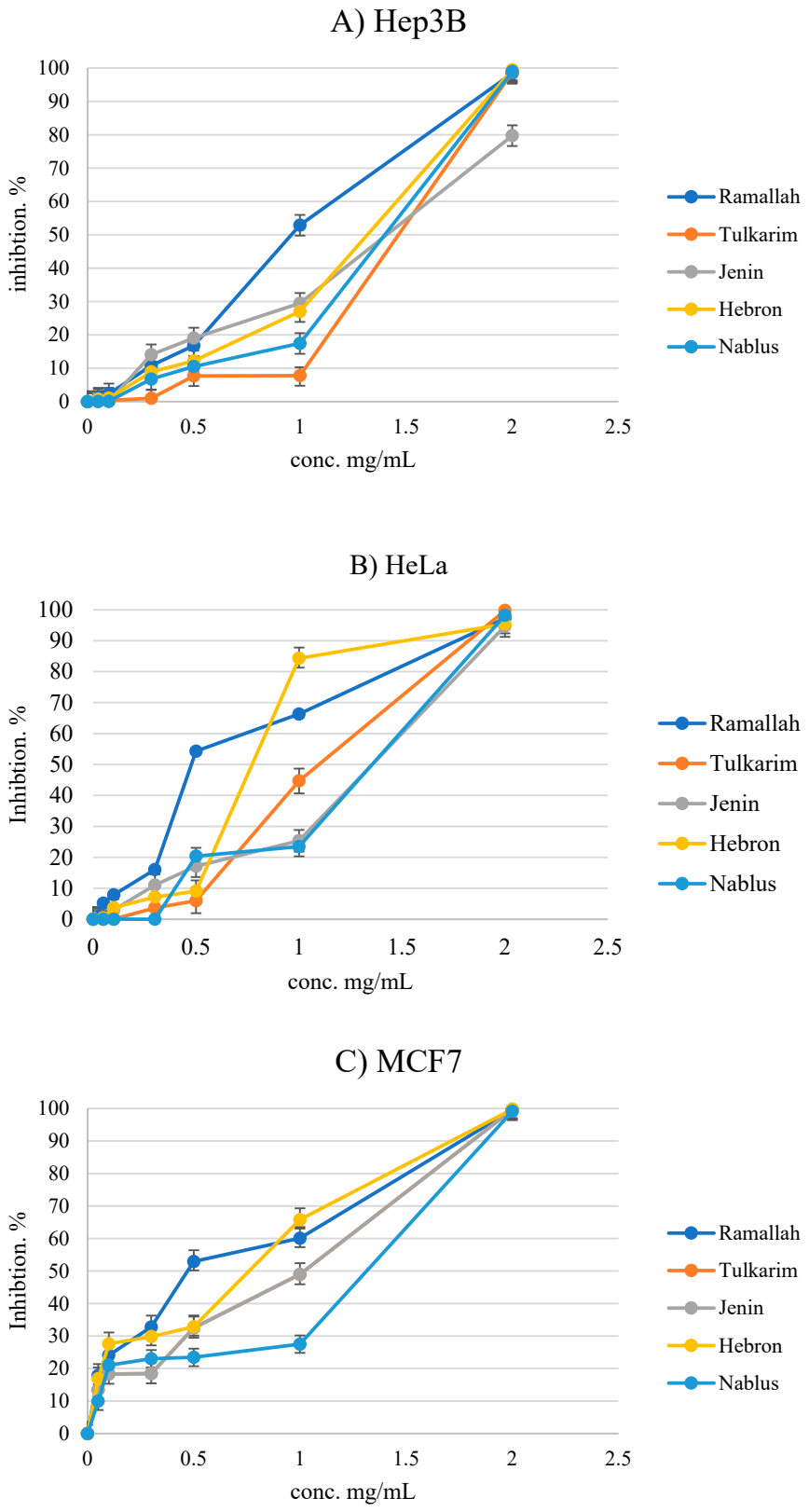

Figure 4. *Cont.*

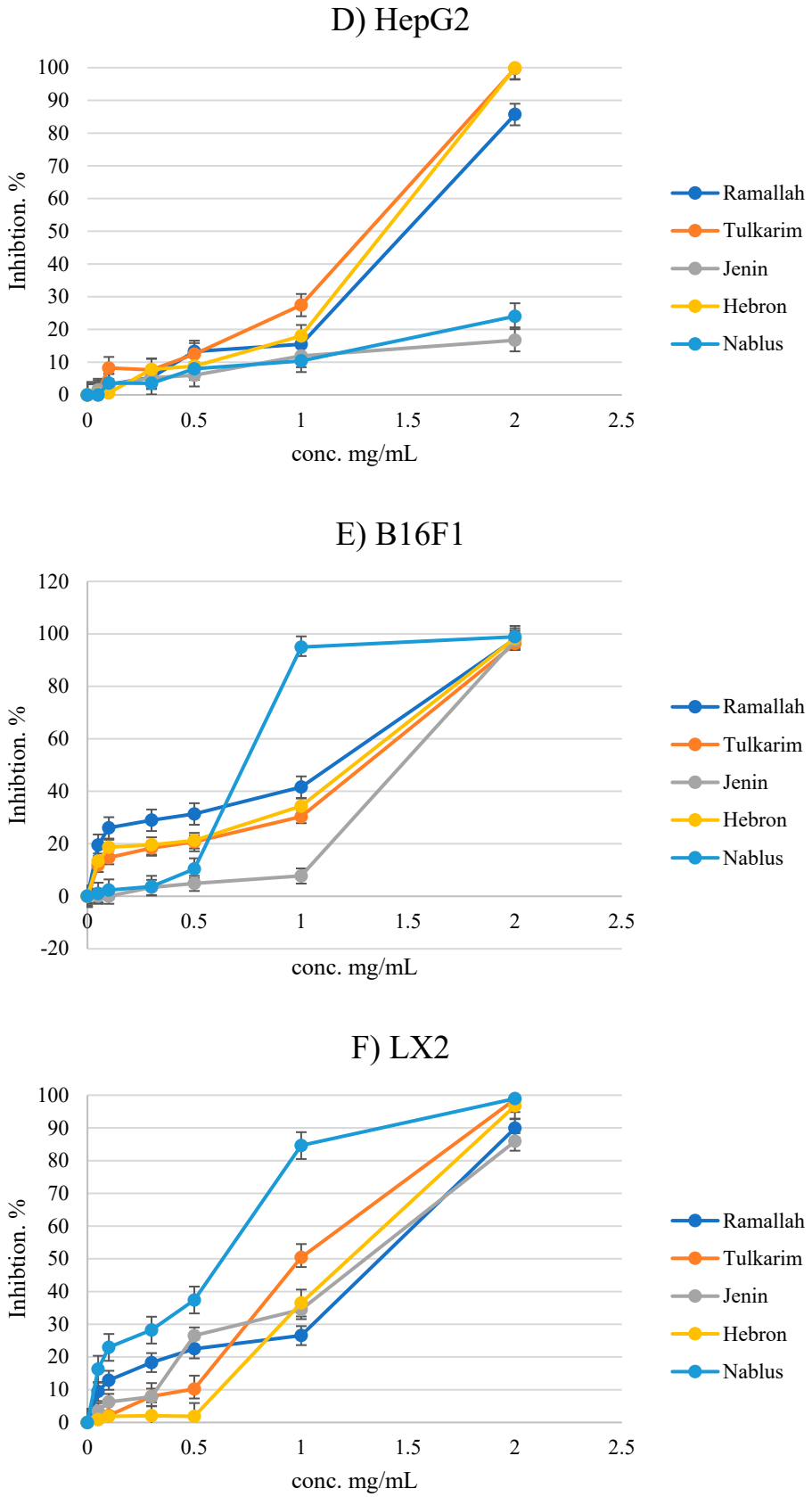

Figure 4. *Cont.*

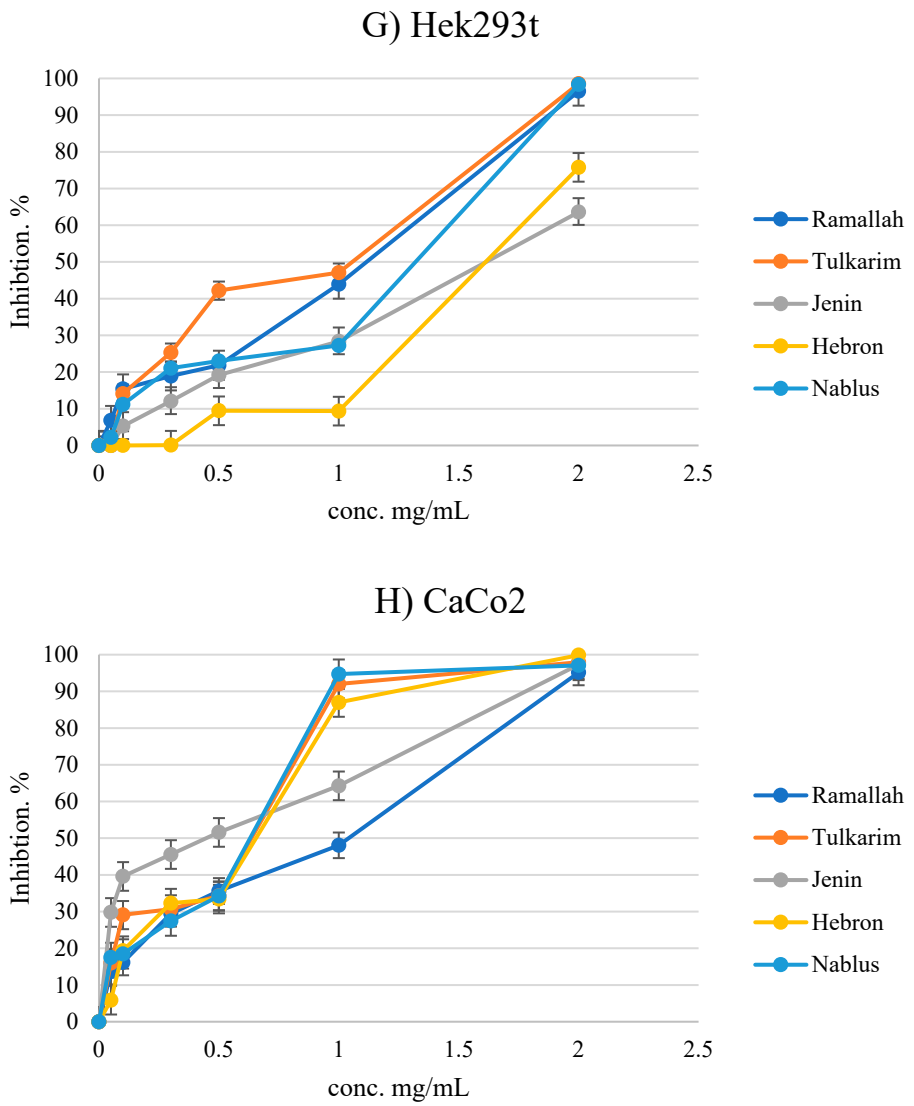

Figure 4. (**A**–**H**). Antiproliferative potential mediated by *Rosmarinus officinalis* essential oils derived from five Palestinian regions.

Table 3. Antiproliferative activity IC_{50} (mg/mL) of *Rosmarinus officinalis* essential oils collected from five different Palestinian regions and doxorubicin as positive control.

	IC_{50}							
	Heb3B	**HeLa**	**MCF7**	**HepG2**	**B16F1**	**CaCo2**	**LX2 Normal**	**Hek293t Normal**
Ramallah	0.96 ± 0.45	0.59 ± 0.18	0.53 ± 0.05	1.54 ± 0.21	0.82 ± 0.05	0.79 ± 0.22	1.21 ± 0.20	1.01 ± 0.28
Tulkarm	1.64 ± 0.17	1.06 ± 0.59	0.86 ± 0.47	1.32 ± 0.24	1.16 ± 0.12	0.49 ± 0.04	1.00 ± 0.23	0.82 ± 0.11
Jenin	1.35 ± 0.18	1.32 ± 0.23	1.09 ± 0.25	>2	1.65 ± 0.35	0.33 ± 0.12	1.21 ± 0.42	1.56 ± 0.02
Hebron	1.33 ± 0.11	0.73 ± 0.35	0.63 ± 0.33	1.47 ± 0.08	1.08 ± 0.42	0.54 ± 0.09	1.12 ± 0.24	1.72 ± 0.17
Nablus	1.47 ± 0.34	1.34 ± 0.20	1.12 ± 0.19	>2	0.66 ± 0.38	0.53 ± 0.05	0.54 ± 0.04	1.17 ± 0.12
Doxorubicin	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

A few oral repeated-dose toxicity investigations in mice and rats have shown that *R. officinalis* EO is safe. For example, dosages as high as 14.1 mg/kg were examined (5 days via gavage), and some trials with doses as high as 400 mg/kg were undertaken for up to 3 months (dietary), with no notable toxicity [40]. Furthermore, *R. officinalis* EO exhibited no harmful impact, according to recent research [41]. On the other hand, Miladi et al. [42]

discovered that *R. officinalis* EO was cytotoxic. Changes to the experimental setup are most likely responsible for this. For example, they used multiple cell lines and time periods. After 24 h, there was no cytotoxic impact, but after 72 h, there was.

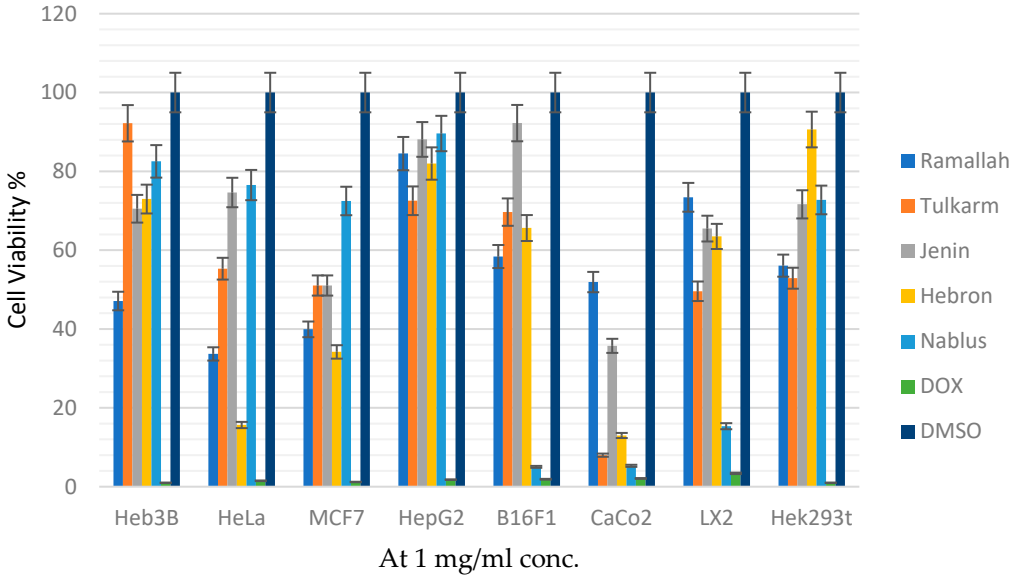

Figure 5. Cell viability % of *R. officinalis* essential oils collected from five different Palestinian regions, Dox and DMSO.

4. Conclusions

This research demonstrated that the molecular profile of the *R. officinalis* EOs cultivated in Palestine differs, depending on where the plant was harvested. The most prevalent component of the EOs extracted from the air-dried leaves was 1,8-cineole, with the exception of the EO gathered in Jenin, where α-pinene and camphor were the most abundant components. The major category in all of the studied EOs was oxygenated monoterpenes, with the exception of the EO collected in Jenin, which was dominated by monoterpene hydrocarbons. The findings of the present study reveal that the *R. officinalis* EO collected from five distinct places in Palestine had strong antibacterial and antioxidant activities. Our research revealed that *R. officinalis* EOs have no cytotoxic properties and are hence safe for usage. To further develop the antibacterial and antioxidant properties of EOs for a variety of practical applications, it is advised that more in-vivo research be conducted. The examined EOs from Palestine may be used as pharmaceutical and natural medicines for the treatment of communicable and non-communicable illnesses, as well as preservatives in the food industry.

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