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Achillea fragrantissima Essential Oil, Wild Grown in Saudi Arabia and Egypt: Detailed Comparative Chemical Profiling, and Evaluation of Allelopathic, Antioxidant, and Antibacterial Activities

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Abstract: One of the biologically beneficial oils against many ailments is *Achillea fragrantissima* essential oil (EO). The current study focused on the comprehensive comparative chemical characterization of *A. fragrantissima* EOs, which were gathered from Saudi Arabia and Egypt, as well as evaluation of their allelopathic, antioxidant, and antibacterial functions. With a respective total oil mass of 96.9% and 96.1%, 40 compounds were found in the EOs from Saudi Arabia (38 compounds) and Egypt (26 compounds). Terpenes represented the main constituents including mono- (52.6% and 75.4% from Saudi Arabia and Egypt, respectively) and sesquiterpenoids (42.1% and 19.7%, respectively). The α-thujone (12.0%), myrcenyl acetate (10.3%), alloaromadendrene oxide-(1) (5.9%), artemisia ketone (4.9%), β-thujone (4.7%), lavandulol (4.2%), and santolina alcohol (4.0%) represented the main components of the overall oil of the Saudi Arabian plant-derived EO. However, the main constituents of the EO of the Egyptian plant were 4-terpineol (17.4%), myrcenyl acetate (9.1%), artemisia ketone (9.0%), α-thujone (8.6%), yomogi alcohol (6.2%), santolina alcohol (6.2%), and β-thujone (5.8%). The chemometric analysis exhibited a strong association between the two EOs from Saudi Arabia and Egypt in addition to the samples collected from Jordan. The Saudi and Egyptian *A. fragrantissima*' EOs were found to have significant allelopathic potencies against the weed *C. murale*. The seed germination, seedling shoot growth, and root growth of *C. murale* were all reduced by the EO of the Saudi ecospecies by 79.9, 56.7, and 68.6%, respectively, with IC_{50} values of 66.5, 68.0, and 69.2 μL L^{-1}, respectively. The two oils from Saudi Arabia and Egypt exhibited potent antioxidant activity against the DPPH free radicals, with IC_{50} values of 30.94 and 28.72 mg/L, respectively. In addition, the two oils from Saudi Arabia and Egypt exhibited strong abilities to scavenge ABTS radicals with respective IC_{50} values of 39.02 and 37.13 mg/L. Additionally, the two EOs showed a much higher antibacterial activity than the antibiotics tested against all bacterial strains, with the exception of *Enterobacter cloacae*. The two oils exhibited antibacterial activity against the examined strains, except *Bacillus subtilis* and *Salmonella typhimurium*, for which the Egyptian species shown greater inhibition. The results revealed that *Escherichia coli* and *Staphylococcus epidermidis* were more sensitive, while *Enterobacter cloacae* was more resistant.

Keywords: *Santolina fragrantissima*; volatile constituents; phytotoxicity; free radical scavenging; bacterial inhibition

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1. Introduction

The essential oils (EOs) derived from different parts of medicinal herbs have become very widely used in health care, folk medicine, and the food and cosmetics industries. Plant EOs have a complex mechanism of action because of their complex chemical diversity [1]. Several EOs are very significant for the treatment of numerous diseases and are recorded in the European Pharmacopoeia [2]. Chemically, EOs presents rich resources of a complicated mixture of volatile constituents including mono-, sesqui- and diterpenes, phenylpropanoids, hydrocarbons, and others with characteristic odors and commercial significance [3].

Plants belonging to the *Achille* genus (Asteraceae) are common and widely distributed in the countries of the Middle East [4]. Many *Achille* plants are widely used in traditional medicine around the world for the treatment of several ailments such as stomach pain and spasms, rheumatism, hemorrhoids, pneumonia, allergic rhinitis, and inflammation [2,5,6].

A. fragrantissima is one of the common plants in different areas around the world and especially in the Arabian region, with several traditional uses as diuretic and for the treatment of stomach complaints, skin conditions, hepatic disease, inflammation, diabetes, dysmenorrhea, respiratory complaints, as well as for the treatment of eye infections and wound healing [7–11]. Additionally, the extracts of different parts of this plant are well known for having significant antioxidant, anti-tumor, antitrypanosomal, antimicrobial, analgesic, antidiabetic, neuroinflammatory and neuroprotective, and anti-inflammatory potentialities due to their chemical constituents [8,9]. Several bioactive metabolites were characterized from different extracts of *A. fragrantissima* including phenolic acids, flavonoids, lignans, terpenes, and alkamides [9,12].

Different reports have described the chemical composition of EOs derived from *A. fragrantissima* collected from different countries around the world, especially the Arabian countries, including Egypt [5,13–15], Saudi Arabia [2,14,16], Jordan [17,18], Yemen [19], and others. All of these studies revealed that terpenes, especially mono and sesquiterpenes, are the main components of these EOs derived from different plant parts with an abundance of yomogi alcohol, santolina alcohol, artemisia ketone, artemisia alcohol, α-thujone, and β-thujone [5,9,19]. The EOs derived from *A. fragrantissima* collected from different locations exerted many biological potentialities, which were documented, such as anticancer [13], xanthine oxidase and tyrosinase inhibitory [19], antioxidant [14], anticholinesterase [17,18], antimicrobial [5,15], and phytotoxic activities [5].

Herein, the present document summarizes the results of the (i) chemical profiling based upon the GC-FID and GC–MS analyses, (ii) construction of a comparative study between the EOs included in this study and previously studied EOs via chemometric analysis, (iii) assessment of the allelopathic activity against seed germination and seedling growth of the weed, *Chenopodium murale*, (iv) evaluation of DPPH and ABTS radical scavenging activities, and (v) estimation of the antibacterial effects on the Gram-negative and Gram-positive bacteria of the EOs derived from the aerial parts of *A. fragrantissima* collected from Saudi Arabia and Egypt.

2. Materials and Methods

2.1. Collection and Preparation of the Plant Samples

The aerial parts of *A. fragrantissima* were collected during the flowering stage from wadi habitat in Saudi Arabia and Egypt. The Saudi samples of *A. fragrantissima* were collected from Wadi Harqan, Alqareenah, Riyadh Region, Saudi Arabia, and the voucher plant samples (KSU-AGRIC-0010113002) were saved in the Plant Production Department Herbarium, King Saud University. The Egyptian plant samples were collected from Wadi Hagool, Suez, Egypt, and the voucher samples (Mans-0010106007) were placed in the Herbarium Botany Department, Faculty of Science, Mansoura University, Egypt. The plant samples were collected in paper bags and immediately transferred to the laboratories. The plant samples were dried under shade at 25 ± 2 °C in the open air. Then, all of the samples were crushed and prepared for EO isolation.

2.2. Isolation of EO Samples via Hydrodistillation

The above-ground parts of *P. equisetiformes* (150 g) were hydrodistilled for three hours using a Clevenger apparatus in a round glass flask (5 L). Next, the extracted EO layer was separated using 3 drops of *n*-hexane, before being immediately dried with 0.5 g of anhydrous Na_2SO_4. The same procedure was used to obtain three EO samples from three plant samples (weighing 200 g each). For the GC–MS analysis and biological evaluations, three distinctive dark-brown glass vials containing the three EO samples were placed into a refrigerator set at 4 °C.

2.3. EOs Analysis via the GC Flame Ionization Detector (GC-FID)

The GC flame ionization detector (GC-FID) analysis of the isolated EO samples from the two plant samples was performed using the same procedures and conditions reported previously [20]. Briefly, an HP-5890 series II apparatus (Hewlett-Packard, Palo Alto, CA, USA) configured with two silica capillary columns (30 m, 0.25 mm; film thickness: 0.25 m), an HP-Wax, and a DB-5 (Agilent, Santa Clara, CA, USA) were used to conduct the GC analysis. Injector and detector temperatures were set at 250 °C, and the oven temperature was programmed to increase from 60 °C to 220 °C at a rate of 5 °C per minute (carrier gas N_2 at 2 mL/min; spitless injection).

2.4. Gas Chromatography Coupled to Mass Spectrometry (GC–MS) Analysis of EOs

All of the extracted EO samples were then subjected to gas chromatography coupled to mass spectrometry (GC–MS) analysis with the typical conditions described in documented protocols [21,22]. In brief, the GC–MS analysis of the EOs was performed by the TRACE Ultra-GC (THERMO Scientific™ Corporate, Waltham, MA, USA) along with an ISQ™ mass spectrometer (EC-Thermo Scientific single quadrupole-MS). The column of TR-5 MS, characterized by a film thickness and internal diameter of 0.25 µm, and 30 m × 0.32 mm, respectively, was used in the GC–MS system. Helium (as a carrier gas) flowed at 1.0 mL min^{-1} with a divided ratio of 1:10. The temperature program was regulated at 60 °C for one minute, followed by an increase of 4.0 °C min^{-1} up to 240 °C. Each EO sample, in a 1 µL n-hexane aliquot, 1:10 (*v*/*v*, ratio), was injected via the injector along with a detector at 210 °C. The measurement of the mass spectroscopic data was carried out using electron ionization (EI) at the spectral range of *m*/*z* 40–450 and 70 eV.

The chemical compounds of the EOs were characterized depending upon the (i) Automated Mass Spectral Deconvolution and Identification (AMDIS), (ii) Wiley Library of Spectral Collection, the (iii) Library of NIST (Gaithersburg, MD, USA; Wiley, Hoboken, NJ, USA) used for the fixing the retention indices relative to the C8–C22 n-alkanes, and/or (iv) authentic and reference compounds.

2.5. Biological Assays

2.5.1. Allelopathic Activity against the Weed, *Chenopodium murale*

The phytotoxic effects of the two extracted EO samples of *A. fragrantissima* were evaluated against the weed *C. murale* with the same documented protocol [23]. The weed's mature germinated seeds were gathered from contaminated agricultural fields. Until further inspection, the seeds with equal size were chosen and stored in paper bags at 25–27 °C. The seeds were surface sterilized using sodium hypochlorite (0.3%) for three minutes prior to setting up the allelopathic bioassay experiment. They were then thoroughly rinsed three times with distilled water and dried under sterilized conditions. A series of concentrations (0, 25, 50, 75, and 100 µL L^{-1}) of the surfactant Tween 80® (Sigma-Aldrich, Darmstadt, Germany) were created by dilution in order to investigate the allelopathic activity of the isolated EOs. Filter paper (Whatman Grade 1) was used to line the inside of the Petri plate (90 mm), which had been moistened with either 4 mL of each concentration or Tween (control). Twenty sterilized weed seeds were then placed on the filter paper in an even layer. To prevent EO leakage from the plates, three plates were used for every concentration and covered with Parafilm® (Sigma, St. Louis, MO, USA). The experiment was run three

times with a total of 90 plates [5 treatments (4 EO concentrations + 1 control), 3 replication plates, 3 experimental replicates, 2 plant species]. The growth chamber used to incubate the plates was set to 25 ± 2 °C and a 12 h light/12 h dark cycle was used. Daily counts of seeds that germinated and had radicles longer than 2 mm were carried out, and on the tenth day of incubation, length measurements were conducted of the roots and shoots of the seedlings. The inhibition of germination and seedling growth was computed via the following equation:

$$Inhibition\ \% = 100 \times \frac{[(Germination/length\ (root/shoot)_{Control} - (Germination/length\ (root/shoot)_{Treatment})]}{(Germination/length\ (root/shoot)_{Control})}$$

The IC_{50} values—the concentration of EO required to achieve 50% inhibition—were determined on the basis of the data of germination and seedling growth inhibition.

2.5.2. Antioxidant Assessments

Using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and the free radical 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, both were procured from Sigma-Aldrich, Taufkirchen, Germany), the two derived EOs samples from the two *A. fragrantissima* samples were evaluated for their antioxidant abilities.

For the DPPH experiment, the radical originated in methanol at a concentration of 0.3 mM. The EOs were developed with an array of concentrations (5, 10, 20, 30, 40, and 50 mg L^{-1} for each). The mixture used for the reaction contained an equal proportion of the EO and the radical [24]. The absorbance at 517 nm was measured with a spectrophotometer (Spectronic 21D, Milton Roy, East Lyme, New London, CA, USA) after 30 min of incubation in the dark.

The ABTS radical scavenging, on the other hand, was carried out in accordance with the method outlined by Re et al. In a nutshell, the radical was made using identical concentrations as those used for DPPH. A total of 2 mL of the radical and 2 mL of the EO sample were combined and rapidly shaken before being incubated for 6 min in the dark. At 734 nm, the absorbance was measured. As a positive control, ascorbic acid was utilized. The following equation was used to calculate the inhibition of scavenging:

$$\text{Inhibition of radical scavenging \%} = (A_{Cont} - A_{Sample})/A_{Cont} \times 100.$$

where A_{Cont} and A_{Sample} are the control and EO sample absorbances, respectively.

By graphing an exponential curve of concentration and scavenging %, the IC_{50} value was determined. Data were expressed as mean values and standard deviation (±SD) after the experiment was conducted with three replications.

2.5.3. Antibacterial Effects on the Gram-Negative and Gram-Positive Bacteria

The Gram-positive Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC29213, Staphylococcus epidermidis ATCC12228, and Enterobacter cloacae ATCC13047, and Gram-negative Escherichia coli ATCC 25922, Klebsiella pneumonia ATCC 13883, Pseudomonas aeruginosa ATCC15442, and Salmonella typhimurium ATCC1408 bacterial strains used in the present study were sourced from the Mycology Laboratory's culture collection at the Faculty of Medicine, Mansoura University, Mansoura, Egypt. The antibacterial assays were performed using the method of agar diffusion as described in the previous documented protocol [5].

2.6. Data Treatment and Chemometric Analysis

A dataset of the EO compounds with a concentration >2.5% was performed using the EO of the presently studied samples as well as 15 reported samples of *A. fragrantissima*. The dataset was subjected to multivariate analysis including principal component analysis (PCA) and hierarchal cluster analysis. The analysis was performed using JMP® Pro 16.0.0 (512257) software (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussions

3.1. Composition of EOs Derived from Saudi Arabian and Egyptian A. fragrantissima

The aerial parts of *A. fragrantissima* were collected from Saudi Arabia and Egypt and air dried. Each plant sample was subjected to hydrodistillation to produce pale-yellow EOs with a yielding ratio of 0.96 and 0.84% (*w*/*v*) from Saudi Arabia and Egypt, respectively. *A. fragrantissima* was already well known as being a rich resource of essential oil with a high yield [5,9,19]. All of the EO samples were analyzed via GC–MS and the identified compounds are listed in Table 1.

Table 1. Chemical composition of the essential oil derived from *Achillea fragrantissima* collected from Saudi Arabia and Egypt.

No	Compound Name	KI		Collected from		Type	Identification
		Publ.	Exp.	Saudi Arabia	Egypt		
1	Santolinatriene	905	905	0.3 ± 0.01 *	2.0 ± 0.06	MH	MS, KI
2	*α*-Pinene	936	938	ND	0.3 ± 0.01	MH	MS, KI
3	Yomogi alcohol	996	994	0.6 ± 0.02	6.2 ± 0.11	OM	MS, KI
4	*p*-Cymene	1026	1025	0.3 ± 0.01	0.1 ± 0.00	MH	MS, KI
5	Santolina alcohol	1038	1040	4.0 ± 0.15	6.2 ± 0.21	OM	MS, KI
6	Artemisia ketone	1062	1061	4.9 ± 0.14	9.00 ± 0.23	OM	MS, KI
7	Artemesia alcohol	1083	1084	0.7 ± 0.01	3.3 ± 0.09	OM	MS, KI
8	α-Thujone	1105	1103	12.0 ± 0.26	8.6 ± 0.22	OM	MS, KI
9	*β*-Thujone	1115	1116	4.7 ± 0.13	5.8 ± 0.18	OM	MS, KI
10	3-Thujanone	1124	1126	3.0 ± 0.10	1.8 ± 0.06	OM	MS, KI
11	*cis*-Sabinol	1140	1143	0.3 ± 0.01	0.5 ± 0.02	OM	MS, KI
12	Lavandulol	1171	1173	4.2 ± 0.17	3.6 ± 0.08	OM	MS, KI
13	4-Terpineol	1198	1197	2.6 ± 0.05	17.4 ± 0.31	OM	MS, KI
14	*cis*-Geraniol	1255	1253	0.8 ± 0.03	-	OM	MS, KI
15	Myrcenyl acetate	1261	1260	10.3 ± 0.29	9.1 ± 0.23	OM	MS, KI
16	Sabinyl acetate	1291	1293	0.2 ± 0.01	-	OM	MS, KI
17	*p*-Cymen-7-ol (Cumic alcohol)	1287	1289	2.3 ± 0.06	1.5 ± 0.08	OM	MS, KI
18	Benzene acetic acid, ethyl ester	1246	1244	ND	0.3 ± 0.01	OH	MS, KI
19	Nerol acetate	1365	1367	1.4 ± 0.04	-	OM	MS, KI
20	Germacrene D	1480	1483	2.8 ± 0.07	1.1 ± 0.03	SH	MS, KI
21	Valencene	1493	1491	1.4 ± 0.04	1.1 ± 0.03	SH	MS, KI
22	Spathulenol	1516	1514	2.0 ± 0.05	-	OS	MS, KI
23	*α*-Sesquiphellandrene (Zingiberene)	1526	1525	11.6 ± 0.10	7.0 ± 0.12	SH	MS, KI
24	Caryophyllene oxide	1573	1573	3.4 ± 0.07	2.4 ± 0.05	OS	MS, KI
25	*trans*-Sesquisabinene hydrate	1577	1575	0.8 ± 0.03	ND	OS	MS, KI
26	Isoaromadendrene epoxide	1594	1596	0.2 ± 0.01	ND	OS	MS, KI
27	Neoclovenoxid-alcohol	1608	1609	1.2 ± 0.05	ND	OS	MS, KI
28	Salvial-4(14)-en-1-one	1612	1611	1.6 ± 0.05	0.3 ± 0.01	OS	MS, KI
29	Aromadendrene oxide-(2)	1631	1630	0.4 ± 0.01	ND	OS	MS, KI
30	*.tau.*-Cadinol	1640	1638	1.9 ± 0.07	1.0 ± 0.03	OS	MS, KI
31	Alloaromadendrene oxide-(1)	1641	1640	5.9 ± 0.14	3.3 ± 0.08	OS	MS, KI
32	α-Eudesmol	1653	1652	0.6 ± 0.02	ND	OS	MS, KI
33	α-Bisabolol	1685	1686	1.4 ± 0.06	0.6 ± 0.01	OS	MS, KI
34	Ledene oxide	1682	1681	5.9 ± 0.21	2.9 ± 0.15	OS	MS, KI
35	Davanol acetate	1689	1691	0.3 ± 0.01	ND	OS	MS, KI
36	Hexahydrofarnesyl acetone	1845	1845	0.4 ± 0.02	ND	OS	MS, KI
37	*n*-Docosane	2200	2201	0.7 ± 0.02	ND	OH	MS, KI
38	*n*-Heptacosane	2700	2700	1.0 ± 0.04	1.0 ± 0.02	OH	MS, KI
39	*n*-Nonacosane	2900	2900	0.4 ± 0.01	ND	OH	MS, KI
40	*cis*-Vaccenic acid	2141	2140	0.4 ± 0.01	ND	OH	MS, KI
Monoterpenes		Hydrocarbons (MH)		0.6	2.4		
		Oxygenated (OM)		52.0	73.0		
Sesquiterpenes		Hydrocarbons (SH)		15.8	9.2		
		Oxygenated (OS)		26.3	10.5		
Oxygenated hydrocarbons (OH)				2.1	1.0		
Total				96.9	96.1		

* Relative area concentration, KI: Kovats index (published values of KI (publi) and experimental values of KI (Exp.), ND: not detected.

From the presented data in Table 1, forty compounds, including terpenes as the main constituents, were assigned as being identified from both EOs of Saudi Arabia and Egypt with a respective total oil mass of 96.9% and 96.1%. All of the characterized terpenoids were categorized into only two classes including mono- (52.6% and 75.4% from Saudi Arabia and Egypt, respectively) and sesquiterpenoids (42.1% and 19.7%, respectively). The abundance of terpenoids, especially mono- and sesquiterpenoids, was in agreement with all of the published data of EOs of this plant around the world [2,5,9,13–15,17–19]. The data listed in

Table 1 revealed that monoterpenes are dominated in both EOs by two types, oxygenated monoterpenes and hydrocarbons, with predominantly the oxygenated type (52.0% and 73.0% from Saudi Arabia and Egypt, respectively). Oxygenated sesquiterpenes were found in considerable concentrations in the Saudi Arabian and Egyptian EOs with concentrations of 26.3% and 10.5%, respectively, along with a low concentration of the sesquiterpene hydrocarbons (15.8% and 9.2%, respectively). The amplitude of monoterpenes in both EOs, especially the oxygenated types, corresponded with the documented chemistry of the oils from different parts of *A. fragrantissima* collected from different locations around the world [2,5,9,13–15,17–19,25–30].

Furthermore, α-Thujone (12.0%), myrcenyl acetate (10.3%), artemisia ketone (4.9%), β-thujone (4.7%), lavandulol (4.2%), santolina alcohol (4.0%), and 3-thujanone (3.0%) represented the main components of the overall oil, especially the oxygenated terpenoids of the Saudi Arabian plant EO. On the other hand, caryophyllene oxide (3.4%) and alloaromadendrene oxide-(1) (5.9%) were the major oxygenated sesquiterpenes. However, the main constituents of the EO of the Egyptian plant were 4-terpineol (17.4%), myrcenyl acetate (9.1%), artemisia ketone (9.0%), α-thujone (8.6%), yomogi alcohol (6.2%), santolina alcohol (6.2%), β-thujone (5.8%), lavandulol (3.6%), and artemisia alcohol (3.3%). Meanwhile, α-sesquiphellandrene (7.0%) represented the major sesquiterpene hydrocarbons and alloaromadendrene oxide-(1) (3.3%) was the main oxygenated sesquiterpene. These compounds were found to be the main constituents of most studied EOs of this plant, collected from several countries [2,5,9,13–15,17–19,25–30].

These data exhibited that the EO derived from the Egyptian plant was richer than the Saudi Arabian plant in the mono- and sesquiterpene contents, especially the oxygenated forms. This quantitative and qualitative variation between the locations could be ascribed to the environmental conditions between the Egyptian and the Arabian peninsula deserts [16,31,32]. Also, some reports have described that the terpene hydrocarbons are the constituents that are most affected by humidity, temperature has a direct effect on the oxygenated forms of terpenes [16,33]. All of these data demonstrated the low concentration of the terpene hydrocarbons and the abundance of oxygenated terpenes.

3.2. Multivariate Chemometric Analysis

The chemometric assessment was built based on the main compounds of the *A. fragrantissima* EO (>2.5% of total composition) and those of the other documented ecospecies of *A. fragrantissima* pursuant to various conditions, including (i) collection location: Jordan, Saudi Arabia, Egypt, and Yemen; (ii) plant parts: flowers, leaves, and aerial plants; (iii) plant part condition: dry and fresh; and (iv) isolation technique: hydro-distillation (HD) and microwave (Micro). The multivariate analysis of the main components of the different Polygonum plants, including principal component analysis (PCA) (Figure 1A–C), hierarchical clustering (Figure 2A), and a constellation plot (Figure 2B), served as the basis for this research.

All of the results of these cometric analyses, including PCA (Figure 1A–C), hierarchical clustering (Figure 2A), and a constellation plot (Figure 2B), revealed that the *A. fragrantissima* EOs of the 15 different ecospecies under consideration have a weak relationship (+15.1%) with each other. These results exhibited a strong correlation between the two ecospecies collected from Egypt and SA, in the current investigation, via the presence of artemisia ketone, α-thujone, β-thujone, lavandulol, 4-terpineol, myrcenyl acetate, α-sesquiphellandrene (Zingiberene), caryophyllene oxide, and alloaromadendrene oxide-(I) as abundant compounds with relative concentrations of 2.43–12.01%. Furthermore, the Egyptian and SA ecospecies exhibited a good association with the flower samples collected from the Al Azraq region, Jordan, in terms of the majority of the following: artemisia ketone, artemisia alcohol, santolina alcohol, α-thujone, and β-thujone [17] (Figures 1 and 2). The Egyptian samples collected from Sainia and Sharkia exhibited a strong relationship between each other, but they showed a low association with the two samples under study [5,13–15]. Also, the *A. fragrantissima* samples collected from Madina and Rhafa, SA, exhibited a sig-

nificant correlation with all of the samples collected from the Al Azraq region, Jordan, in terms of the presence of artemisia ketone, santolina alcohol, α-thujone, and β-thujone as the main components [2,9,14,17] (Figures 1 and 2). But, the Egyptian sample collected from Alexanderia showed a very weak association with both samples under investigation [14,15]. On the other hand, the collected samples from Yemen exhibited a moderate correlation with the present study's samples via the presence of high relative concentrations of artemisia ketone and santolina alcohol [19]. Based on all of these findings, it can be concluded that changes in environmental factors, such as the climate, humidity, temperature, and soil conditions, as well as the age and type of the plants that were employed, directly correlated with changes in the EO composition [23,34–36]. One of the primary factors affecting the variety of EO compositions was also found to be the procedure of isolation [5]. This effects were clearly seen in the weak association between the *A. Fragrantissima* isolated EO, observed via microwave and hydrodistillation techniques [5,37].

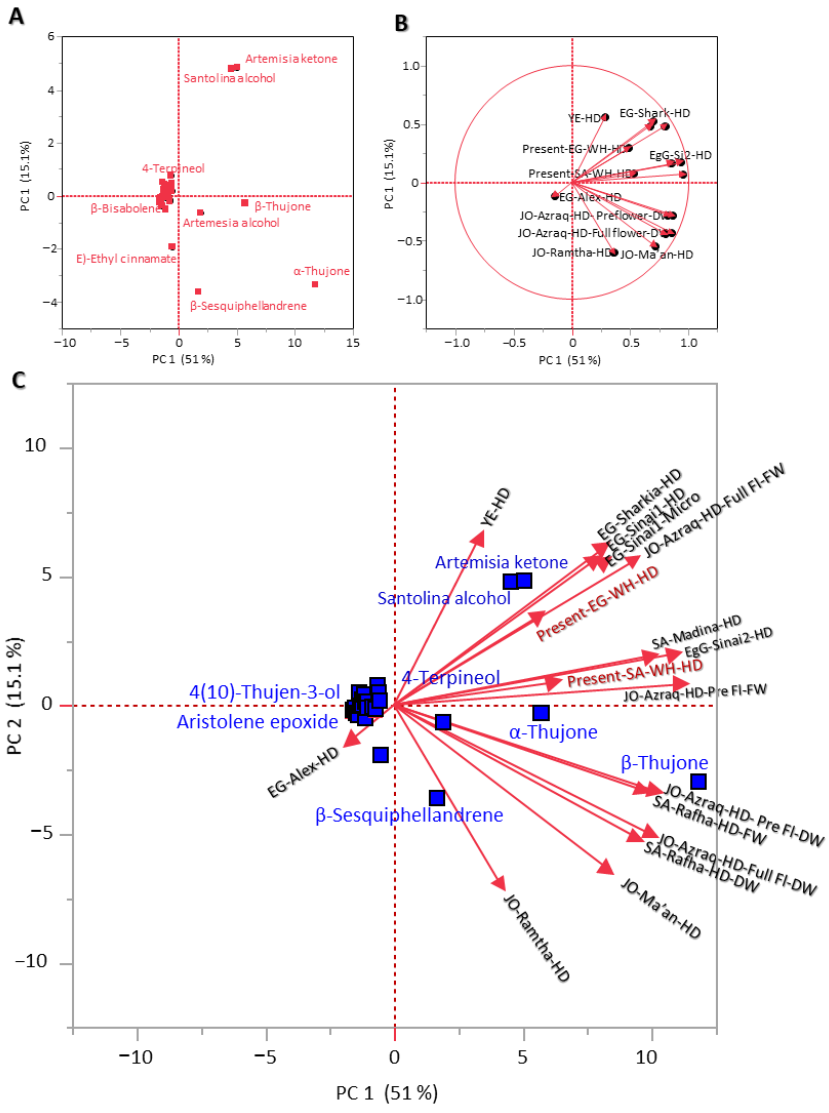

Figure 1. Principal component analysis (PCA) and cluster analysis of *Achillea fragrantissima* and 15 other reported samples of *A. fragrantissima* in different locations around the world depending upon the EOs' main components (>2.5%). (**A**) PCA compounds score plot, (**B**), PCA variables and (**C**) PCA biplot.

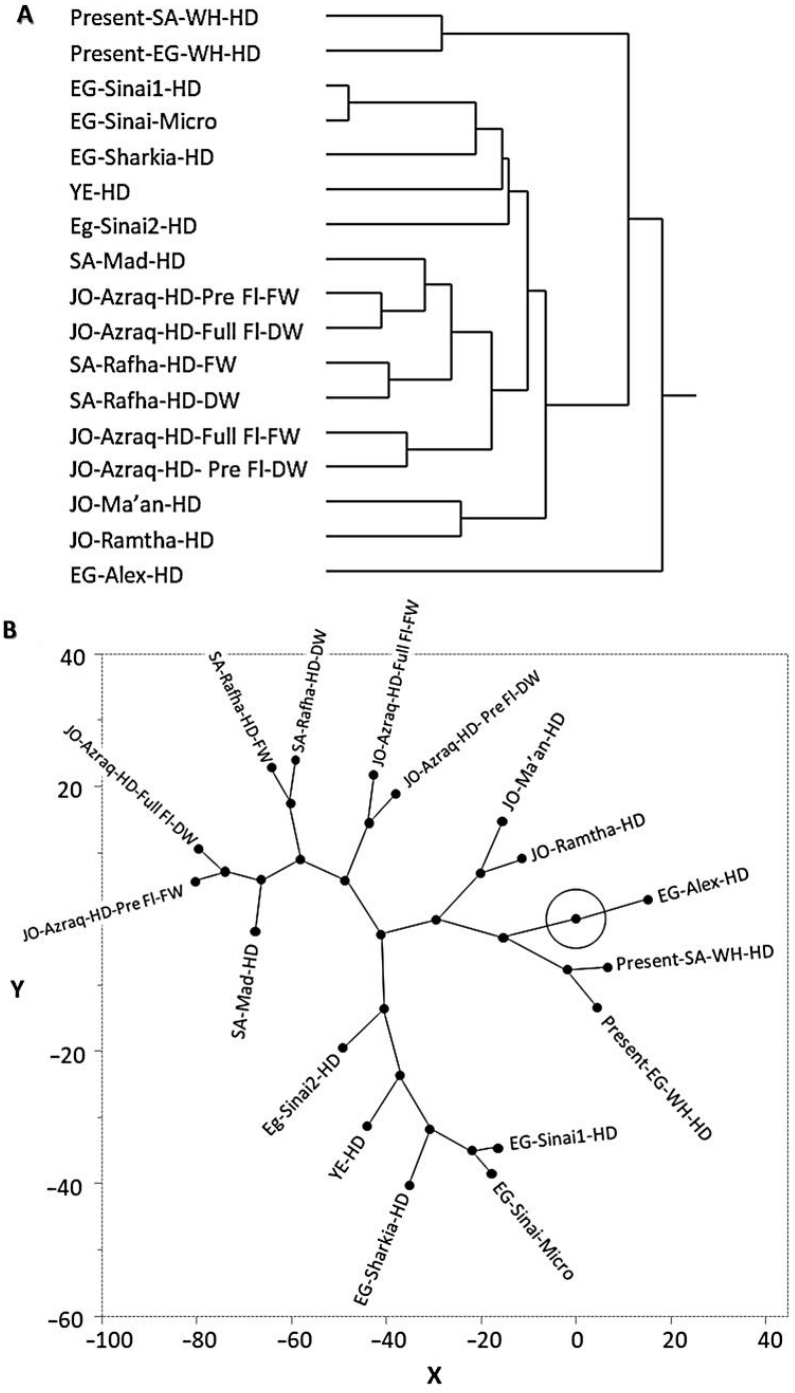

Figure 2. Cluster analysis including hierarchical clustering (**A**) and constellation plot (**B**) of *A. fragrantissima* along with 15 reported *A. fragrantissima* ecospecies collected from several locations around the world, based upon the main EO components' concentration.

3.3. Biological Activities of A. fragrantissima EOs

3.3.1. Allelopathic Activity

The isolated EOs of Saudi and Egyptian ecospecies of *A. fragrantissima* were tested for their allelopathic activity against seed germination and seedling growth of the weed *C. murale*. For the EO of the Saudi ecospecies, the seed germination of *C. murale* was diminished by 79.9% at the concentration of 100 µL L^{-1}, while the seedling shoot growth and root growth were decreased by 56.7% and 68.6%, respectively (Figure 3A). Based on the data of IC50 values, the roots showed the lowest IC_{50} value (66.5 µL L^{-1}),

i.e., they were more inhibited, compared to shoot growth and seed germination of *C. murale*, with IC_{50} values of 68.0 and 69.2 µL L^{-1}, respectively (Figure 3B). The root system has been reported to be more sensitive to allelochemicals compared to the shoot growth due to the direct contact with the isolated EO as well as the higher permeability of root cell membranes [20,38,39]. Also, the root's main functions are the absorption of water and nutrients from the medium and then translocating them to the shoots; therefore, it is the first area to be affected by the absorbed solutions [40]. Additionally, the allelochemicals can alter the membrane permeability, retard cell division, and increase oxidative stress, which leads to disruption of the membrane structure and function [34,41].

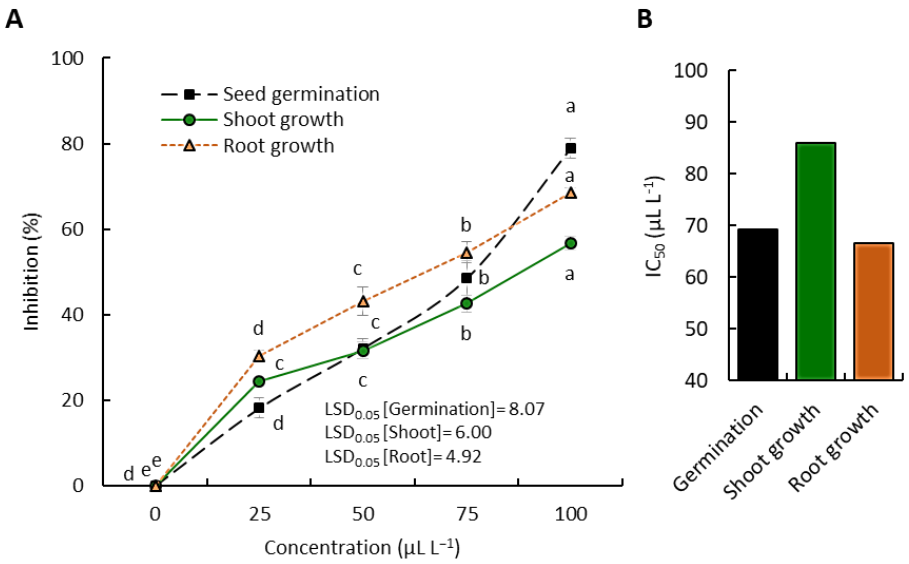

Figure 3. Allelopathic activity of the essential oils isolated from Saudi ecospecies of *Achillea fragrantissima*. (**A**) Effect of different concentrations on the seed germination, seedling shoot growth, and seedling root growth of *C. murale*, and (**B**) the IC_{50}. Different letters within each parameter mean values significant at $p \leq 0.05$.

On the other hand, the EO of the Egyptian ecospecies of *A. fragrantissima* showed considerable allelopathic activity on the seed germination and seedling growth of *C. murale*. At the highest concentration of the EO (100 µL L^{-1}), the seed germination of *C. murale* was retarded by 53.2%, while the seedling shoot growth was decreased by 51.9%, and finally, the seedling root growth was diminished by 58.8% (Figure 4A). Regarding the IC_{50}, the root growth attained the lowest IC_{50} value (86.5 µL L^{-1}), while the shoot growth and seed germination showed IC_{50} values of 95.0 and 90.3 µL L^{-1}, respectively (Figure 4A).

Comparing the allelopathic activity of both ecospecies of *A. fragrantissima* (Saudi and Egyptian), the EO of the Saudi ecospecies showed more allelopathic activity against the weed *C. murale*, compared to the Egyptian ecospecies. These findings could be attributed to the variation in the composition of the compounds within their EO profile (Table 1). The Saudi ecospecies has a higher number of chemical compounds (38 compounds) than the Egyptian ecospecies (26 compounds). Also, the content of the oxygenated compounds within the both Saudi and Egyptian ecospecies are high.

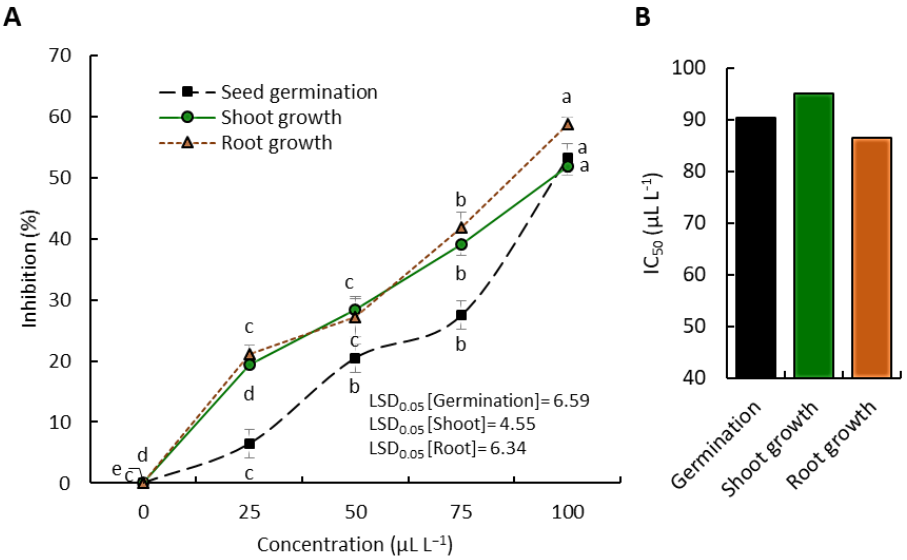

Figure 4. Allelopathic activity of the essential oils isolated from Egyptian ecospecies of *Achillea fragrantissima*. (**A**) Effect of different concentration on the seed germination, seedling shoot growth, and seedling root growth of *C. murale*, and (**B**) the IC_{50}. Different letters within each parameter mean values significant at $p \leq 0.05$.

The determined allelopathic activity could be attributed to the activity of the major compounds within the EO, such as α-thujone, myrcenyl acetate, and zingiberene. These compounds can act together, i.e., in synergism, or in a singular manner as allelochemicals that interfere with the physiological and/or biochemical processes in the cells of *C. murale* [42]. The major compound, α-thujone, has been reported to possess various biological activities such as genotoxicity, carcinogenic properties, and immune-modulatory and antimutagenic effects [43]. Also, the sesquiterpene, zingiberene has been reported within the chemical profile of *Chrysoma pauciflosculosa*, which showed considerable allelopathic activity [44]. These compounds were found in high concentrations within the EO of the Saudi ecospecies compared to the Egyptian ecospecies (Table 1). However, in the Egyptian ecospecies, the major compounds of 4-terpineol, santolina alcohol, and artemisia ketone were determined in higher concentrations compared to the Saudi ecospecies. These compounds were reported in higher concentrations in EOs with potential allelopathic activity [45–47].

3.3.2. Antioxidant Potencies

The EOs of both *A. fragrantissima* samples were assessed using the DPPH and ABTS procedures in comparison with ascorbic acid, a widely used antioxidant medication (Table 2). It came to light that the scavenging of DPPH radicals increased directly as the concentrations of both EOs increased. For the SA sample, the DPPH colors were diminished by 62.35% and 76.37% with the EO concentrations at 40 and 50 mg mL^{-1}, respectively. Likewise, the Egyptian sample's EO concentrations at 40 and 50 mg mL^{-1} reduced the DPPH hues by 67.41% and 79.02%, respectively. Also, it was found that the scavenging of ABTS radicals increased in direct proportion to the concentrations of the two EOs. Within the SA sample, at EO concentrations of 40 and 50 mg mL^{-1}, the ABTS colors were reduced by 53.39% and 61.05%, respectively. In the same way, for the Egyptian sample, the EO at concentrations of 40 and 50 mg mL^{-1} decreased the DPPH colors by 56.08% and 63.20%, respectively. Thus, the two EOs exhibit a dose-dependent antioxidant behavior.

Table 2. DPPH and ABTS radicals scavenging activity percentage and IC_{50} values by the EO of the *Achillea fragrantissima* and ascorbic acid as standard.

Treatment	Conc. (mg/L)	Scavenging Activity (%)			
		DPPH	IC_{50} (mg/L)	ABTS	IC_{50} (mg/L)
Egyptian A. fragrantissima	5	10.93 ± 0.29 ^{HI}	30.94	9.58 ± 0.21 ^{I}	39.02
	10	19.27 ± 0.38 ^{G}		14.74 ± 0.34 ^{H}	
	20	32.13 ± 0.91 ^{E}		26.21 ± 0.78 ^{F}	
	30	53.91 ± 1.57 ^{C}		40.68 ± 1.06 ^{D}	
	40	62.35 ± 1.89 ^{B}		53.39 ± 1.62 ^{C}	
	50	76.37 ± 2.16 ^{A}		61.05 ± 2.05 ^{B}	
	$LSD_{0.05}$	4.09 ***			
	F-value	263.54			
Saudi Arabian A. fragrantissima	5	13.58 ± 0.41 ^{HI}	28.72	11.727 ± 0.36 ^{I}	37.13
	10	22.67 ± 0.69 ^{G}		16.34 ± 0.50 ^{H}	
	20	35.08 ± 1.06 ^{E}		27.92 ± 0.85 ^{F}	
	30	56.56 ± 1.71 ^{C}		42.97 ± 1.30 ^{D}	
	40	67.41 ± 2.04 ^{B}		56.08 ± 1.70 ^{C}	
	50	79.02 ± 2.39 ^{A}		63.20 ± 1.92 ^{B}	
	$LSD_{0.05}$	4.32 ***			
	F-value	243.94			
Ascorbic acid	1	4.80 ± 0.11 ^{EF}	11.78	2.14 ± 0.09 ^{F}	13.03
	2.5	14.02 ± 0.42 ^{E}		10.36 ± 0.32 ^{E}	
	5	40.74 ± 1.23 ^{D}		37.08 ± 1.04 ^{D}	
	10	53.28 ± 1.65 ^{BC}		45.62 ± 1.36 ^{CD}	
	15	59.57 ± 1.84 ^{A}		55.91 ± 1.82 ^{B}	
	20	72.72 ± 2.17 ^{A}		69.06 ± 2.35 ^{A}	
	$LSD_{0.05}$	9.98 ***			
	F-value	57.92			

Different letters within each row mean values significant at $p \leq 0.05$. *** Significant at $p \leq 0.001$.

The overall findings showed that the EOs from the SA sample of *A. fragrantissima* samples have a potent ability to scavenge DPPH free radicals, with an IC_{50} value of 30.94 mg/L. This oil also demonstrated a significant capacity to scavenge ABTS radicals, with an IC_{50} value of 39.02 mg/L. In addition, the EO derived from the Egyptian *A. fragrantissima* sample has a substantial ability to neutralize DPPH and ABTS free radicals, with IC_{50} values of 28.72 mg/L and 37.13 mg/L, respectively. As a reference antioxidant, ascorbic acid has been shown to have IC_{50} values of 11.78 mg/L for DPPH and 13.03 mg/L for ABTS.

The biological potencies of any natural extracted and/or isolated materials such as EOs and solvent extracts were directly associated with its chemical constituents [48]. Several EOs have been described as having potent antioxidant activities because some of the EO's constituents, especially those that are oxygenated and/or hydroxylated, have the innate capacity to inhibit or postpone the aerobic oxidation process of organic matter [49]. Based upon these theories, present chemical analysis exhibited that both EOs have the preponderance of the oxygenated constituents (80.48 and 84.48% from Saudi Arabia and Egypt, respectively) of the total oil mass, including 78.34% and 83.52% of oxygenated terpenes, respectively. As a consequence of their significant interaction with peroxyl free radicals, which are eliminated through formal hydrogen atom transfer, the oxygenated and/or phenolic constituents of the EOs behaved as antioxidants [50]. Thus, components including the free hydroxylated groups were found to be major components, such as 4-terpineol, lavandulol, artemisia alcohol, and santolina alcohol, along with the other oxygenated components. The other main oxygenated components such as artemisia ketone, thujone (α-

and β-), alloaromadendrene oxide-(1), and ledene oxide supported the abilities of these oils to scavenge the radicals. On the other hand, some EOs exhibit antioxidant behavior even though they lack phenol [49,51]. Therefore, some nonoxygenated compounds were also reported to provide a contribution as antioxidant agents such as α-sesquiphellandrene (Zingiberene). This compound has been described as the main component of the strong active antioxidant EO derived from *Guarea kunthiana* [52]. As described above, the main oxygenated compounds acted as in a synergetic and/or singular manner with each other as well as with the minor components to exhibit the potent antioxidant potencies of the two oils.

3.3.3. Antibacterial Activity

To test the antibacterial efficacy of EOs isolated from both Saudi and Egyptian ecotypes of *A. fragrantissima*, the agar well diffusion experiment was used. The results showed significant ($p \leq 0.05$) antimicrobial activity against some Gram-positive and Gram-negative human pathogenic bacteria (Table 3). Except for *Enterobacter cloacae*, the findings showed that the two EOs had potent antibacterial potentialities, in comparison with three conventional antibiotics, chloramphenicol, gentamicin, and tetracycline (Table 3). Furthermore, the EOs of the Saudi ecospecies had more antibacterial efficacy against the majority of examined bacterial strains, with the exception of *Bacillus subtilis* and *Salmonella typhimurium*, for which the Egyptian species shown greater inhibition. In all tested EOs, *Escherichia coli* and *Staphylococcus epidermidis* bacteria were more sensitive, whereas *Enterobacter cloacae* strains were more resistant.

Table 3. Antibacterial activity of the *Achillea fragrantissima* essential oils and some selected reference antibiotics (10 mg mL^{-1}).

Microbes	EO (10 mg mL^{-1})				Standard Antibiotic IZ (10 mg L^{-1})			
	Egyptian		Saudi		Ampicillin	Chloramphenicol	Gentamicin	Tetracycline
	IZ	MIC	IZ	MIC				
Gram-negative bacteria								
Escherichia coli	25.01 ± 0.75 ^{A}	0.052	26.13 ± 0.71 ^{A}	0.049	21.08 ± 0.55 ^{C}	11.31 ± 0.31 ^{D}	25.93 ± 0.76 ^{A}	22.10 ± 0.58 ^{A}
Klebsiella pneumonia	21.07 ± 0.59 ^{B}	0.062	21.67 ± 0.64 ^{B}	0.061	7.73 ± 0.15 ^{DE}	11.07 ± 0.28 ^{D}	21.13 ± 0.51 ^{C}	21.64 ± 0.61 ^{A}
Pseudomonas aeruginosa	17.86 ± 0.51 ^{C}	0.092	18.52 ± 0.57 ^{C}	0.085	6.52 ± 0.13 ^{E}	10.64 ± 0.23 ^{D}	11.38 ± 0.36 ^{D}	0.00 ^{D}
Salmonella typhimurium	13.84 ± 0.36 ^{D}	0.126	13.09 ± 0.41 ^{D}	0.127	0.00 ^{F}	0.00 ^{E}	0.00 ^{E}	10.90 ± 0.32 ^{C}
Gram-positive bacteria								
Bacillus subtilis	22.81 ± 0.62 ^{AB}	0.051	21.45 ± 0.58 ^{B}	0.053	8.55 ± 0.17 ^{D}	20.15 ± 0.61 ^{B}	20.68 ± 0.52 ^{C}	11.36 ± 0.28 ^{C}
Staphylococcus aureus	20.50 ± 0.58 ^{B}	0.052	22.06 ± 0.63 ^{B}	0.048	28.98 ± 0.92 ^{A}	15.09 ± 0.45 ^{C}	24.07 ± 0.63 ^{B}	18.67 ± 0.48 ^{B}
Staphylococcus epidermidis	24.14 ± 0.76 ^{A}	1.410	25.38 ± 0.82 ^{A}	0.051	22.09 ± 0.64 ^{C}	24.76 ± 0.73 ^{A}	24.50 ± 0.58 ^{B}	20.53 ± 0.52 ^{AB}
Enterobacter cloacae	9.92 ± 0.21 ^{D}	0.051	10.23 ± 0.09 ^{E}	0.049	24.66 ± 0.81 ^{B}	19.41 ± 0.52 ^{B}	20.05 ± 0.61 ^{C}	18.06 ± 0.37 ^{B}
$LSD_{0.05}$	2.54 ***		1.11 ***		1.22 ***	1.32 ***	1.09 ***	2.54 ***
F-value	42.94		284.86		667.73	354.30	460.54	84.79

Value is an average of three replicas of the inhibition zone (IZ) diameter expressed as mm ± standard error. $^{A–F}$: significant variations after the Tukey's test at a probability level of 0.05. LSD: least significant difference. *** $p < 0.001$.

Hydrophobicity, that allows them to divide with the lipids found within the cell membranes of bacteria and mitochondria, and makes them more permeable by upsetting the cell structures, is a crucial characteristic of EOs and their constituents [53]. This ultimately leads to the death of the bacterial cell because a significant amount of crucial molecules and ions are lost from the bacterial cell. Certain compounds target the efflux systems of various kinds of Gram-negative bacteria to regulate antibiotic resistance [54]. Based upon these facts, the present EOs, that include 80–84% oxygenated components, acted to attack the cell membranes of both bacterial strains used, including positive and negative strains. The functional groups found in the active components of the EO and their interactions worked together to establish the efficacy of the oils [53]. Based upon the present chemical components, the oxygenated monoterpenes and sesquiterpenes are very important antibacterial agents that act with synergetic and/or singular effects. The oxygenated terpenoids have been documented to have significant antibacterial functions as

the main constituents of the active antibacterial EOs of the leaves of *Eucalyptus teretecornis* and *Zanthoxylum alatum* [55–57]. As the primary ingredient in the potent antibacterial EO obtained from *Guarea kunthiana*, the nonoxygenated sesquiterpene α-sesquiphellandrene (Zingiberene) was also discovered to have a high antibacterial effect [52].

4. Conclusions

The chemical constituents of EOs derived from *A. fragrantissima* collected from various locations has been described in various papers. The EOs of the A. fragrantissima ecospecies collected in Saudi Arabia and Egypt were isolated and analyzed using GC–MS and multivariate chemometric techniques. According to the chemical data, both ecospecies are exceptionally rich in terpenoids, particularly sesquiterpenes and monoterpenes. The results of the multivariate analysis revealed many associations with other ecospecies collected from Yemen, Jordan, (Sainia and Sharkia), Egypt, and Madina (Saudi Arabia), and similarities in the chemical profile of the EOs of these two ecospecies. Additionally, these analyses showed that the chemical composition of the oils varied depending on the method of isolation. In comparison to the standard antioxidant drug ascorbic acid, both oils demonstrated a potent antioxidant defense against DPPH and ABTS free radicals. The seed germination, seedling shoot growth, and root growth of the weed *C. murale* were all severely suppressed by the two EOs. Additionally, both oils showed potent bacterial growth inhibitory effects against several Gram +ve and Gram −ve bacteria, especially *E. coli* and *S. epidermidis*. The oxygenated compounds that make up the two oils' main constituents can be directly credited with all of these biological activities. These components may work independently or in synergy with each other and the other minor compounds.

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