

ABSTRACT

Essential oil of fresh aerial parts of *Achillea fragrantissima* growing wild in Lebanese steppe was obtained by hydrodistillation and analyzed using Gas Chromatography and GC/Mass spectrometry.

Antimicrobial activity of the essential oil against 5 bacteria and 2 fungi was determined by diffusion and microdilution methods.

The results revealed a yield of 1.25 % v/w fresh weight of essential oil that had a yellowish color and pleasant fragrant aroma. Fifty one compounds were identified. Artemisia ketone (29.97%), α -Thujone (13.34%), Germacrene (11.5%) followed by α -Cubebene (6.25%), Spathulenol (3.63%), β -Sesquiphellandrene (3.52%) and γ -Muurolene (3.27%) were the main components.

The oil displayed a high degree of inhibitory activity against the tested five bacteria: *S. aureus*, *E. coli*, *E. faecalis*, *S. enteritidis*, *P. aeruginosa* that was equal or greater than that of several commonly prescribed antibiotics. The oil also showed high antifungal activity against both *C.albicans* and *A.fumigatus* with the latter exhibiting higher susceptibility to the oil than that against the antifungal Nystatine.

This study presents the first report on the chemical composition of the essential oil of Lebanese *A.fragrantissima*, and confirms the traditional therapeutic use of the plant. Further pharmacological research is needed to exploit this potential either alone or in combination with existing antibiotics as a promising contribution to the discovery of novel drugs against infectious diseases.

Keywords: Achillea fragrantissima; Asteraceae; GC-MS, Essential Oil, Antimicrobial Activity, Lebanon.

I. INTRODUCTION

Medicinal plants have for millennia served as a valuable source for therapeutic agents in traditional medicine. Numerous historical records as well as modern ethnobotanical and pharmacological studies have presented a good evidence on their importance in the treatment of a wide range of diseases. The genus *Achillea* L. (Asteraceae), commonly known as Yarrow, is represented by more than 140 fragrant perennial herbaceous species widespread in Southern Europe, Mediterranean and Middle East regions (Nemeth and Bernath, 2008). The name of the genus was designated after the hero Achilles of the Greek *mythology* Iliad for his use of the plant for wound healing during the Trojan War (Benedek et al., 2007). In Arabic countries, *Achillea* species locally named as *Qaysoon*, *Gesoom* or *Bu'eithraan* (Ahmed et al., 1990; Elmann et al., 2011 and Gamil et al., 2014) are widely used in traditional medicine. Specifically, *A. fragrantissima* is indicated in the treatment of many different ailments (Palombo and Semple, 2001). Decoctions and infusions of the aerial part of this species are used against fever, common cold, inflammations and high blood pressure. It is also used as an anthelmintic, carminative and urinary tract antiseptic. Chronic diseases such as rheumatism, arthritis and other inflammatory disorders, (El-Ashmawy, 2017), diabetes mellitus, skin inflammations, wounds are also treated by these preparations (Said et al., 2002). Furthermore, the inhalation of fumigation of *A. fragrantissima* is reported to have a calmative effect to cure bronchitis and spasms (Oran and Al-Eisawi 1998) and to have insecticidal as well as rodenticidal activities (Hifnawi et al. 2001). Recent pharmacological studies have confirmed the anti-inflammatory activities of the plant and demonstrated its antioxidant, antiproliferative capacity and oxidative stress (Eissa et al., 2014; Hammad et al 2013). Moreover, this plant is also shown to be effective against protozoal disease such as *Trypanosoma evansi* (El-Ashmawy et al., 2017). In 2013, a US patent application

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was obtained by Alenad and Al-Jaber for the use of its extract in the treatment of chronic myeloid leukemia cells (US 2014/0314886 A1). More recently, the extract of aerial plant was also reported for its cancer preventive activity (Hamed *et al.*, 2016)

A. fragrantissima is rich in polyphenolics, tannins and flavonoids, and chemical composition of the essential oils from different origins has shown that Santolina alcohol, Artemisia ketone, cis-Thujone and trans-Thujone constitute the main compounds (El Deeb, 1985; Fleisher and Fleisher, 1993; Hifnawy *et al.*, 2001; El Shazly *et al.*, 2004 and Goswamy *et al.*, 2016).

In Lebanon, *A. fragrantissima* is reported as most abundant Achillea species in the steppe region of Hermel in the North East part of the country (Mouterde, 1983). Extracts, infusion, decoction and essential oils are traditionally used through oral and external applications in the treatment of several diseases, particularly, diabetes, cancer, bronchitis and stomach ailments and wound healings.

To the best of our knowledge there has been no studies in Lebanon on the chemical composition of the essential oil nor on the antimicrobial activities of *A. fragrantissima*. Hence, the objective of the present study was to investigate the essential oils yield percent and the composition of the fresh aerial parts of the plant collected from Hermel-North Bekaa, Lebanon, as well as to screen the antimicrobial efficacy of the oil.

II. MATERIAL AND METHODS

Plant material

Fresh aerial parts (400g) of the wild growing *A. fragrantissima* (Figure-1) were collected at random from the steppe of Hermel in North Bekaa-Lebanon in June 2017 at Latitude 34.426765 N, Longitude 36.412766 E and Altitude 700 m (Figure-2).

The species identification was performed based on the taxonomic keys of the “New Flora of Lebanon and Syria” (Mouterde, 1983). Voucher specimen was deposited at the Herbarium of the Department of Botany and Medicinal plants, Faculty of the Agriculture, University Holy Spirit, Kaslik, Lebanon.



Figure 1. *Achillea fragrantissima* (Forssk.) Sch.Bip. (Asteraceae) Growing wild in North Bekaa, Lebanon
Hydrodistillation of essential oil

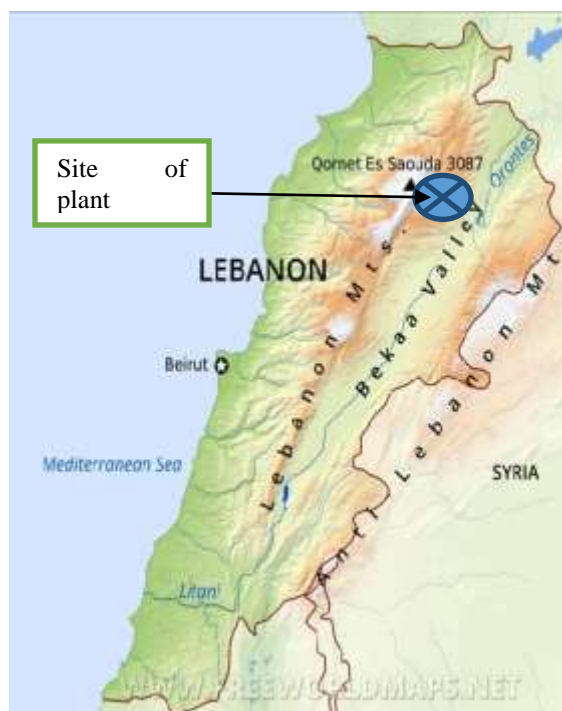


Figure 2. Site of plant collection, Source: Free World Maps.

Fragmented plant material was used for essential oil isolation by hydrodistillation using Clevenger type apparatus for 4 hours according to the standard procedure of the 6th Edition of European Pharmacopeia (2008).

Gas Chromatography (GC) and GC/Mass spectrometry (MS) analysis

GC and GC/MS analysis of the oils were performed by Agilent Technologies 7890 gas chromatography equipped with a Flame Ionization Detector (FID) and a HP-5MS 5% capillary column (30m x 0.25mm x 0.25 µm film thickness). Mass spectra were recorded at 70 eV of electron energy and a mass range of 50-550 m/z. The carrier gas was Helium at a flow of 0.8 mL/min.

The initial column temperature was set at 60°C for 1 minute programmed to increase to 280°C at a rate of 4°C/min. The split ratio was 1:40. The injection temperature was set at 300°C. The purity of Helium gas was 99.99 %. A sample of 1 µL was injected manually in the split mode. Components identification was based on retention indices and comparison with mass spectral data of authentic standards and computer matching with Wiley 229, Nist 107, Nist 21 libraries as well as by comparing the fragmentation patterns of the mass spectra with those reported in the literature.

III. ASSESSMENT OF ANTIMICROBIAL ACTIVITY

Bacterial and fungal strains

Certified bacterial and fungal strains (Medi Mark, Europe) were used in screening the antimicrobial effect of *Achillea. fragrantissima* essential oil. Among the tested bacterial strains, *Staphylococcus aureus* (ATCC BAA-1026) and *Enterococcus faecalis* (ATCC 29212) were Gram positive, *Escherichia coli* (ATCC 11303), *Salmonella enteritidis* (ATCC 13076) and *Pseudomonas aeruginosa* (ATCC 9027) were Gram negative, and two pathogenic fungal strains *Aspergillus fumigatus* (ATCC 204305) and *Candida albicans* (ATCC 10231).

Assessment of growth inhibition zone by disc diffusion method

The antimicrobial and antifungal activity of essential oil was carried out by disc diffusion method. Amount of 100 µL of suspension containing 10⁶ CFU/mL of microorganisms were spread on cultures of Müller Hinton agar medium (Merck). Sterile 6 mm diameter filter paper discs (Whatman N° 3) impregnated with 10 µL of essential oil were placed on the agar.

Standard reference discs of the antibiotics Oxacillin (1µg), Ticarcillin (75µg), Carbenicillin (100µg), Colistin (25µg), Piperacillin (100µg), Erythromycin (15µg) and Tetracycline (30µg) were used as standard antimicrobial positive controls while Nystatine (100µg) was used as standard antifungal positive control. A blank disc was used as a negative control. The bacterial cultures were incubated at 37°C for 24 h, whereas *C.albicans* and *A.fumigatus* were incubated at 27°C for 48 h and 5 days, respectively. The diameter of growth inhibition zones around discs were measured using a Caliper. The test was run in triplicate and the mean values and Standard deviation SD were computed (Bonev et al., 2008).

Determination of minimum inhibitory concentration (MIC) by microdilution method

Minimum inhibition concentration of *A. fragrantissima* defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation was determined using the microdilution dilution method (CLSI, 2012). This test was performed based on five stock concentrations of the oil (50 mg/mL, 25 mg/mL, 10 mg/mL, 5 mg/mL and 2.5 mg/mL) employing doubling serial dilutions of the oil in 5 ml Muller Hinton Broth (MHB). Microdilution wells containing 100 µL of standardized suspension of tested microorganisms added to 100 µL of a suspension of MHB and *A. fragrantissima* essential oil of different concentrations. The microplates were incubated overnight at 37°C. All the tests were performed in triplicates and average values were determined.

IV. RESULTS AND DISCUSSION

Composition of essential oil

The fresh aerial parts of *A.fragrantissima* yielded 1.25 % v/w fresh weight of essential oil that had a yellowish color and pleasant fragrant aroma. The GC and GC-MS analysis of the oil revealed the identification of 51 compounds representing 99.91 % of total oil (Table 1). Oxygenated monoterpenes (56.66%) including Artemisia ketone (29.97%) and α-Thujone (13.34%) constituted the main compounds of the oil followed by

sesquiterpene hydrocarbons (30.58%) represented by Germacrene-D (11.5%), α -Cubebene (6.25%), β -Sesquiphellandrene (3.52%), γ -Muurolene (3.27%) and β -Eudesmol (2.45%). Whereas oxygenated sesquiterpenes formed 10.23% with Spathulenol (3.63%) being the major component. Monoterpene hydrocarbons contributing to only 1.23% of the oil was mainly constituted of Santolina-triene (0.32%) and Trans-Isolimonene (0.28%).

In spite of the expected variability usually noted in the essential oils of a specific species from different origins, striking similarities between this oil and that obtained by steam distillation of the fresh aerial parts of the plant from the North Badia in Jordan (Al-Sohaili and Al Fawwaz, 2014) and two regions in Egypt (Negev desert and the Sinai) (Fleisher and Fleisher, 2011), were observed. Artemisia ketone and α -Thujone constituted 19.87%, and 12.36%, respectively, of the oil from Jordan. The values of these compounds ranged between 13.2 – 23.8% for Artemisia ketone and 25.5 – 36.5% for α -Thujone of the plant from Egypt (Negev desert and the Sinai) (Fleisher and Fleisher, 2011). Such resemblance may be attributed to the similarities in the physiological responses of the plant to the similar semiarid Mediterranean-Steppe climate and soil textures characterizing the origins of the plants in this and the aforementioned studies (Table 2). On the contrary, considerable qualitative and quantitative variabilities between the oils in the herein study and other studies from different origins are evident in Table 2. Such variations may be further highlighted by the results obtained from different ecoregions in Egypt illustrating the influence of changing ecological niches on the oil composition (Sanli and Karadogan, 2017). In Egypt, Caryophyllene oxide (23.50%) and 1-Terpinen-4-Ol (11.15%) were the main components of the plant from Alexandria (Choucry, 2016), while cis-Thujone (28.4%), Santolina alcohol (16.1%), Artemisia ketone (14.8%) and trans-Thujone (12.5%) were the main constituents from Allamain desert and Sinai Peninsula (Almadiy *et al.*, 2016). Whereas, Thujone (33.97%), Trans-2,7-dimethyl- 4,6-octadien-2-ol (24.40%) were the major components in Sinai (Zeedan *et al.*, 2014). Additionally, the differences noted between the oils of the wild and cultivated forms from North Jordan also illustrate the important influence of the growing conditions even under the same ecological conditions (Hazem *et al.*, 2012). The observed similarities or variabilities emphasize the general consensus that chemotype, genetic variations, nutritional status of the plant, nature of soil, collection timing, and the extraction method as well as the ecological differences (climatic, seasonal and geographical) have influences on the composition of plant essential oils (Arumugam *et al.*, 2016).

Table 1. Chemical composition of fresh areal parts of *Achillea fragrantissima* (Forssk.) Sch.Bip. (Asteraceae) from North Bekaa, Lebanon (June 2016).

RT	Library/ID	Area%
6.5446	Santolina-triene	0.32
9.5428	Yomogi alcohol	0.4
10.0063	Eucalyptol	0.01
12.6842	Artemisia ketone	29.97
14.87	α-Thujone	13.34
15.7111	Thujone	2.1
15.8885	α -Pinene	0.08
16.0258	Sabinol	0.34
16.3635	Camphene	0.55
16.8555	Pinen-3-one	0.53
17.5422	Cis-Geraniol	1.84
17.6566	Terpinen-4-ol	0.21
18.0743	3-Cyclohexene-1-methanol	1.32
18.349	4-Methoxyacetyl-1,4-Hexadiene	1.26
18.8067	Amylene hydrate	0.60
18.9784	Estragole	0.58
19.0814	Dicyclopropyl Methanone	0.31
22.1198	3-Methyl-2-butenic acid, 3-methylbut-2-enyl ester	0.68

28.4425	Sabinyl acetate	0.62
32.5108	1,5,5-Trimethyl-6-methylene-cyclohexene	2.22
33.2089	Benzenepropanoic acid, ethyl ester	0.22
34.113	α-Cubebene	6.25
36.2301	Germacrene D	11.5
36.4533	γ-Muurolene	3.27
36.6822	Epiglobulol	1.23
37.6377	β -Cadinene	0.58
38.0554	β-Sesquiphellandrene	3.52
38.4502	Isomethylionone	0.39
38.6391	2-Phenylanisole	0.59
38.8851	Spathulenol	3.63
39.074	Salvial-4(14)-en-1-one	0.59
39.2113	Ethyl hydrocinnamate	0.87
39.3543	L-Calamenene	0.69
39.423	Cyclopropylidene-3,3-dimethylcyclohex-5-ene diepoxide	0.31
39.7663	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	1.32
39.9666	β -Eudesmol	2.45
40.1096	(1R)-(+)-Trans-Isolimonene	0.28
40.1726	Isoaromadendrene epoxide	0.30
40.2985	Endo-8-hydroxy-cycloisolongifolene	0.63
40.3957	Limonene diepoxide	1.21
40.5617	Vitispirane	0.35
41.0538	Methyl hydrocinnamate	0.16
41.2082	Copaenol	0.24
41.3456	α -Caryophylladienol	0.39
41.7232	Isoeugenol	0.17
42.118	Perhydrofarnesyl acetone	0.24
42.6673	Nonadecane	0.07
42.8733	E-2-Tetradecen-1-ol	0.15
43.1308	(S)-valine	0.70
45.0877	o-Tolyl methylcarbinol	0.10
48.4294	Palatinol	0.22
Monoterpenes hydrocarbons		1.23
Oxygenated monoterpenes		56.66
Sesquiterpenes hydrocarbons		30.58
Oxygenated Sesquiterpenes		10.23
Others compounds		1.21
Total		99.91

Table 2. Content of the principal essential oil (%) of aerial parts of *A. fragrantissima* from different countries of the Middle East and this study.

Country	Main Chemical Composition	Citation
Lebanon (North Bekaa)	Artemisia ketone (29.97%); α -Thujone (13.34%); Germacrene D (11.5%); α -Cubebene (6.25%); Spathulenol (3.63%); β -Sesquiphellandrene (3.52%).	This study
Jordan (North Badia)	Artemisia ketone (19.87%) ; β -Sesquiphellandrene (14.57%); Carvacrol (13.44%); α -Thujone (12.36%); Artemisyl acetate (6.06%); β -Thujone (3.91%) ; trans-Sabinyl acetate (3.62%).	Al-Sohaili and Abdullah, 2014
Jordan (cultivated in Hashemite University, Zarqa)	4-Terpeneol (15.65%); Linalool (11.0%); Carvone (9.42%); β -Phellandrene (6.2%); γ -Terpinene (5.6%) ; β -Pinene (4.55%); Verbenone (4.42%) ; Cedrol (3.0%) ; p-Cymene (2.95%); α -Thujone (2.4%).	Hazem et al., 2012
Egypt (North coast of Alexandria)	Caryophyllene oxide (23.5%); 1-Terpinen-4-ol (11.15%) ; Viridifloral (9.84%); Guaienol (9.84%); p-Cymen-3-ol (8.21%); β -Bisabolene (6.88%); Yomogi alcohol (6.43%).	Choucry, 2017
Egypt (Allamain desert and Sinai Peninsula)	Cis-Thujone (28.4%); Santolina alcohol (13.1%), Artemisia Ketone (16.8%); trans-Thujone (12.5%); Camphor (4.7%); Yomogi alcohol (3.2%); 1,8-Cineole (2.7%).	Almadiy et al. 2016
Egypt (Sinai Peninsula)	α -Thujone (33.97%); trans 2,7-dimethyl 4,6-octadiene-2-ol (24.4%); 2,5,5-Trimethyl 3,6-heptadien-2-ol (8.23%); Eucalyptol (8.17%); Artemisia alcohol (3.49%).	Zeedan et al. 2014
Egypt (Sinai desert- vicinity of Sader Hetan)	cis-Thujone (29.48%); Santolina alcohol (18.29%); Artemisia ketone (15.24%); trans-Thujone (10.83%); trans-Pinocarveol (6.83%); Yomogi alcohol (4.35%).	El Shazly et al., 2004
(Egypt) Negev desert and the Sinai	α -Thujone (25.5 – 36.5%); Artemisia ketone (13.2 – 23.8%); Santolina alcohol (12.5 – 21.2%).	Fleisher and Fleisher, 2011
Jordan (Al Jubeiha- suburb of Amman)	Artemisia ketone (32.46%) ; β -Sesquiphellandrene (15.05%); α -Thujone (9.92%); Carvacrol (6.28%); β -Thujone (6.26%); Artemisyl acetate (6.05%).	Hamad et al., 2014
Iraq (Karbala)	Thujone (57.5%); Santolina alcohol (31.4%); Eugenol (5.4%); Santolinatriene (2.6%).	Abaas et al., 2013
Egypt (Saint Catherine, South Sinai)	Thujone (33.97%);trans-2,7-dimethyl- 4,6octadien-2-ol (24.40%); 2,5,5-trimethyl-3,6-heptadien-2-ol (8.23%); Eucalyptol (8.17%); 1,5-Heptadien-4-one-3,6-trimethyl (7.65%); Artemisia alcohol (3.49%); Santolina triene (1.97%).	Zeedan et al., 2014

Antimicrobial Activity

The results of the antimicrobial activity of the oil against five bacterial strains and two fungal strains using the disc diffusion assay are presented in Table 3. It is evident that the tested strains displayed high degree of susceptibility against the investigated oil with *S. aureus* being the most sensitive (45 ± 0.1 mm), followed by *E. coli* (40 ± 0.2 mm), *E. faecalis* (38 ± 0.2 mm), followed by *S. enteritidis* (31 ± 0.3 mm) and *P. aeruginosa* (30 ± 0.2 mm), respectively. Table 3 also shows that the oil generally exhibited equal or greater antibacterial activity when compared with the tested antibiotics namely Oxacillin, Ticarcillin, Carbenicillin, Colistin, Piperacillin, Erythromycin, and Tetracycline. This is highly apparent in the case of the antibiotic Oxacillin, against which all the tested bacterial strains were completely resistant. In accordance with these results *S. aureus* displayed a MIC value of 0.1 mg/ml confirming its highest susceptibility to the oil. This was followed by *C. albicans* (1.5 mg/ml), *E. coli* and *A. fumigatus* (2.0 mg/ml), *E. faecalis* and *S. enteritidis* (2.5 mg/ml). Nevertheless, in spite of the high susceptibility of *P. aeruginosa* observed by the disc diffusion assay, the respective MIC value reached 7.5 mg/ml (Table 4). This may be a result of the attributes of the experimental

conditions, particularly the medium chosen for growth of this specific bacteria on the antimicrobial activity of the oil (Friedman *et al.*, 2004; Gomez-Lopez *et al.*, 2005 and Rodriguez-Tudela *et al.*, 2003).

With regards to the yeast *C.albicans* and the fungus *A. fumigatus*, the inhibition zones displayed similar diameters (15mm) which were around half of that of Nystatine (31 ± 0.2 mm) with *C.albicans*, but higher than of the same antibiotic with *A.fumigatus* (10 ± 0.1 mm).

Table 3. Mean \pm SD growth inhibition zones (mm) of *A. fragrantissima* and a group of antibiotics* against the tested microorganisms.

Oil and Antibiotics Microorg.	<i>A. frag.</i> oil	OX. 1 μ g /disc	TI. 75 μ g /disc	CB. 100 μ g /disc	CL. 25 μ g /disc	PI. 100 μ g /disc	E. 15 μ g /disc	TE. 30 μ g /disc	Nys. 100 μ g /disc
<i>E.coli</i>	40 \pm 0.2	0.0	33 \pm 0.1	36 \pm 0.5	18 \pm 0.2	25 \pm 0.1	12 \pm 0.2	27 \pm 0.1	-
<i>S.enteritidis</i>	31 \pm 0.3	0.0	30 \pm 0.8	30 \pm 0.1	18 \pm 0.2	25 \pm 0.1	19 \pm 0.1	25 \pm 0.2	-
<i>P.aeruginosa</i>	30 \pm 0.2	0.0	20 \pm 0.5	32 \pm 0.6	19 \pm 0.1	29 \pm 0.4	15 \pm 0.1	7 \pm 0.3	-
<i>S.aureus</i>	45 \pm 0.1	0.0	0.0	15 \pm 0.5	0	0	0	40 \pm 0.2	-
<i>E.faecalis</i>	38 \pm 0.2	0.0	25 \pm 0.3	35 \pm 0.1	16 \pm 0.3	32 \pm 0.3	30 \pm 0.1	20 \pm 0.1	-
<i>C.albicans</i>	15 \pm 0.1	-	-	-	-	-	-	-	31 \pm 0.2
<i>A.fumigatus</i>	15 \pm 0.2	-	-	-	-	-	-	-	10 \pm 0.1

* OX: Oxacillin, TI: Ticarcillin, CB: Carbenicillin, CL: Colistin, PI: Piperacillin, E: Erythromycin, TE: Tetracycline and Nys: Nystatine.

Table 4. MIC mean \pm SD values of the *A. fragrantissima* essential oil against the tested microorganisms.

Microorganisms	<i>E. coli</i>	<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>C. albicans</i>	<i>A. fumigatus</i>
MIC mg/ml	2 \pm 0.1	2.5 \pm 0.1	7.5 \pm 0.1	0.1 \pm 0.01	2.5 \pm 0.1	1.5 \pm 0.1	2 \pm 0.1

The findings clearly indicate that the essential oil of *A. fragrantissima* possesses a powerful wide spectrum of antimicrobial inhibitory potential exhibiting more efficacy than some commonly prescribed antibiotics (Table 3). Although the comparison with the previously reported antimicrobial properties of the oil may not be absolutely legitimated due to the high variability in composition and assessment assays, similar high efficacy was reported against several pathogenic (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*), food spoilage microorganism including (cheese, meat, milk, and tomato) and the ORF virus (a parapox virus) (Barel *et al.*, 1991; Hazem *et al.*, 2012; Hammad, 2014; Zeedan *et al.*, 2014; Almadiy *et al.*, 2016; Al-Sohaili and Al-Fawwaz, 2014). These antimicrobial properties may be mostly attributed to the major components of the essential oil. Nevertheless, the interactive functions of minor components may also have an important role. This potential has been indicated in a recent study that has shown that the essential oil exerts a greater antibacterial activity when compared to some of its main components such as Artemisia ketone or cisthujone alone (Almadiy *et al.*, 2016). It has been postulated that antimicrobial activities of the essential oil of *A. fragrantissima* may be attributed to the synergistic effect of both main and minor components indicating that several mechanisms possibly acting on several targets potentiate the antimicrobial influence. Moreover, the growth inhibition diameters and MIC values indicated in Tables 3 and 4, clearly illustrate that the oil had similar efficacy against Gram-positive and Gram-negative bacteria. Similar results have been recently reported by Almadiy and co-investigators on the plant from Egypt (Almadiy *et al.* 2016). This antimicrobial effect may be primarily associated with the oil destabilization effect of the cellular architecture leading to the breakdown of integrity and increased permeability of cell membrane, thus, disrupting many cellular activities including energy production, membrane transport, and other metabolic regulatory functions (Swamy *et al.*, 2016). At this end, the release of five polypeptides, leakage of K⁺ ions into the incubation medium and the inhibition of respiration as

well as the reduction of ATP content were reported in *E. coli* cells treated with the essential oil of *A. fragrantissima* (Barel and Yashphe, 1989).

V. CONCLUSION

This study presents first report on the chemical composition and antimicrobial activity of the essential oil of the aerial parts of wild *A. fragrantissima* from Lebanon. The reported findings confirm the traditional therapeutic value of the plant and indicate the high potential of its essential oil as a source for antimicrobial agents. Comprehensive research aiming at fully exploiting this potential either alone or in combination with existing antibiotics and other therapies, might contribute to the discovery of novel drug of antimicrobial natural components in combating infectious diseases. The identification of active components and relevant mechanisms of action also stand for another set of ambitious requirements.

VI. REFERENCES

- [1] Abbas, I.S., Hamzah, M.J., Majeed, A.H. (2013). Analysis with evaluation of drying temperature on essential oil content of *Achillea fragrantissima* L. and *artemisia herba alba* L. *International Journal of Pharmacy and Pharmaceutical Sciences*. Vol 5, Issue 3, ISSN-0975-1491.
- [2] Abdulrhman A. Almadiy, Gomah E. Nenaah, Basma A. Al Assiuty, Eman A. Moussa, Nabila M. Mira, (2016).
- [3] Ahmed AA, Jakupov k J, Seif El-din AA, Melek FR (1990). Irregular oxygenated monoterpenes from *Achillea fragrantissima*. *Phytochemistry* 29: 1322-1324.
- [4] Al Sohaili and Al Fawwaz, 2014. Composition and antimicrobial activity of *Achillea fragrantissima* essential oil using food model media. *European Scientific Journal* October 2014 edition vol.10, No.30 ISSN: 1857 – 7881 (Print) e - ISSN 1857- 7431.
- [5] Alenad, A.M., Al-Jaber, N.A., Krishnaswamy, S., Yakout S.M., Al-Daghri, N.M. and Alokail, M.S. (2013). *Achillea fragrantissima* extract exerts its anticancer effect via induction of differentiation, cell cycle arrest and apoptosis in chronic myeloid leukemia (CML) cell line K562. *Journal of Medicinal Plants Research*, 7, 1561-1567.
- [6] Almadiy, A.A., Nenaah, G.E., Al Assiuty. B.A., Moussa, E.A., Mira, N.M., 2016. Chemical composition and antibacterial activity of essential oils and major fractions of four *Achillea* species and their nanoemulsions against foodborne bacteria. *LWT - Food Science and Technology* Volume 69, June 2016, Pages 529-537
- [7] Hazem, A., Al-Charchafchi, F. and Ghazzawi, D. (2012). Biochemical, antibacterial and antifungal activity of extracts from *Achillea fragrantissima* and evaluation of volatile oil composition. *Pelagia Research Library Der Pharmacia Sinica*, 3 (3):349-356.
- [8] Arumugam G., Swamy M. K., Sinniah U. R. 2016. *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. *Molecules*. ;21(4):p. 369. doi: 10.3390/molecules21040369. [PubMed] [Cross Ref].
- [9] Barel, S., Segal, R. and Yashphe, J. (1991). The antimicrobial activity of the essential oil from *Achillea fragrantissima*. *Journal of Ethnopharmacology*, 33, 187-191. [http://dx.doi.org/10.1016/0378-8741\(91\)90177-F](http://dx.doi.org/10.1016/0378-8741(91)90177-F)
- [10] Benedek, B., Kopp, B., and Melzig, M. F. (2007). *Achillea millefolium* L. the anti-inflammatory activity mediated by protease inhibition. *Journal of Ethnopharmacology*, 113(2): 312-317.
- [11] Bonev, B., J. Hooper, and J. Parisot. 2008. "Principles of Assessing Bacterial Susceptibility to Antibiotics Using the Agar Diffusion Method." *Journal of Antimicrobial Chemotherapy* 61.6: 1295-301.
- [12] Chemical composition and antibacterial activity of essential oils and major fractions of four *Achillea* species and their nanoemulsions against foodborne bacteria. *LWT - Food Science and Technology*. journal ISSN :0023-6438.
- [13] Choucry, M.A. 2017. Chemical composition and anticancer activity of *Achillea fragrantissima* (Forssk.) Sch. Bip. (Asteraceae) essential oil from Egypt. *Journal of Pharmacognosy and Phytotherapy*, Vol. 9(1), pp. 1-5, January 2017 DOI: 10.5897/JPP2015.0399 Article Number: 2D7E7B862099).
- [14] Choucry, M.A., 2016. Chemical composition and anticancer activity of *Achillea fragrantissima* (Forssk.) Sch. Bip. (Asteraceae) essential oil from Egypt. *Journal of Pharmacognosy and Phytotherapy*. Vol. 9(1), pp. 1-5, January 2017.
- [15] Eissa TF, González-Burgos E, Carretero ME, Gómez-Serranillos MP, 2014. Compositional analysis and in vitro protective activity against oxidative stress of essential oils from Egyptian plants used in

- traditional medicine. *Nat Prod Commun.* 2014 Sep;9(9):1377-82.
- [16] El-Ashmawy, I. M. 2017. Anti-Inflammatory and cyclooxygenases inhibitory effects of asteraceae, rich in flavonoids and tannins. *ejpmr*, 2017,4(12), 96-102.
- [17] El-Ashmawy, I.M., Al-Wabe11, N.A., Bayad, A.E. (2016). *Achillea fragrantissima*, rich in flavonoids and tannins, potentiates the activity of diminazine aceturate against *Trypanosoma evansi* in rats. *Asian Pacific Journal of Tropical Medicine* 2016; 9(3): 228–234.
- [18] El-Deeb K (1985) Ph. D. Thesis: Chromatographic and thermal analysis of certain volatile oil containing plants, Faculty of Pharmacy, Cairo University.
- [19] Elmann, A., Mordechay, S., Erlank, H., Telerman, A., Rindner, M. and Ofir, R. (2011) Anti-neuroinflammatory effects of the extract of *Achillea fragrantissima*. *BMC Complementary and Alternative Medicine*, 11, 98. <http://dx.doi.org/10.1186/1472-6882-11-98>. [Citation Time(s):2].
- [20] El-Shazly, A. M. Hafez, S. S. & Wink M. (2004) Comparative study of the essential oils and extracts of *Achillea fragrantissima* (Forssk.) Sch. Bip. And *Achillea santolina* L. (Asteraceae) from Egypt. *Pharmazie* 59: 226–230
- [21] Euro+Med Plant Base - the information resource for Euro-Mediterranean plant diversity. Published on the Internet <http://ww2.bgbm.org/EuroPlusMed/> (September 2015).
- [22] European Pharmacopoeia 6th Edition (2008). Council of Europe (COE) – European Directorate for the Quality of Medicines.
- [23] Fleisher Z. and Fleisher A., 1993. Volatiles of *Achillea fragrantissima* (Forssk.) Sch.Bip. Aromatic Plants of the Holy Land and the Sinai. Part XI. Florasynth Inc. 300 North Street, Teterboro, NJ, 07608. *Journal of Essential Oil Research* Volume 5, 1993- Issue 2.
- [24] Fleisher Z. and Fleisher A., 2011. Volatiles of *Achillea fragrantissima* (Forssk.) Sch. Bip. Aromatic Plants of the Holy Land and the Sinai. Part XI. *Journal of Essential Oil Research* . Volume 5, 1993 – Issue 2. pages 211-214 | Received 01 Sep 1992, Published online: 28 Nov 2011.
- [25] Friedman, M., Henika, P.R., Levin, C.E., and Mandrell, R.E. 2004. Antibacterial Activities of Plant Essential Oils and Their Components against *Escherichia coli* O157:H7 and *Salmonella enterica* in Apple Juice. *J. Agric. Food Chem.*, 2004, 52 (19), pp 6042–6048. DOI: 10.1021/jf0495340
- [26] Gamil, S.G., Zeedan, A.M., Abdalhamed, M.E., Abdelshafy, O.S. and Abdeen, E. (2014). Antimicrobial, Antiviral Activity and GC-MS Analysis of Essential Oil Extracted from *Achillea fragrantissima* Plant Growing In Sinai Peninsula, Egypt
- [27] Gomez-Lopez, A., Aberkane, A., Petrikkou, E. 2005. Analysis of the influence of tween concentration, inoculum size, assay medium, and reading time on susceptibility testing of *Aspergillus* spp, *J. Clin. Microbiol.* 43 - 1251–1255.
- [28] Goswami, P., Chauhan, A., Ram S. Verma, Rajendra, V., Sajendra C.P., Mahendra, K.V., Darokar, P. & show all, 2016. Composition and antibacterial activity of the essential oil of *Artemisia nilagirica* var. *septentrionalis* from India. *Journal of Essential Oil Research* . Volume 28, - Issue 1
- [29] Hamed, A. R., Hegazy, M-E. F., Higgins, M., Mohamed, T. A., Abdel-Azim, N. S., Pare, P. W., & DinkovaKostova, A. T. (2016). Potency of extracts from selected Egyptian plants as inducers of the Nrf2-dependent chemopreventive enzyme NQO1. *Journal of Natural Medicines*, 70(3), 683-688. DOI: 10.1007/s11418-016-0994- 0.
- [30] Hammad, H.M., Abu, C., Matar, S.A., Litescu, S.C., Abuhamdah, S., Al-Jaber, H.I., Afifi, F.U. (2014). Biological activities of the hydro-alcoholic and aqueous extracts of *Achillea fragrantissima* (Forssk.) grown in Jordan. *Natural Science*, Vol.6, No.1, 23-30 <http://dx.doi.org/10.4236/ns.2014.61005>
- [31] Hammad, H.M., Abu, C., Matar, S.A., Litescu, S.C., Al Jaber, H.I., Abualraghib, A.S. and Afifi, F.U. (2013) Biological activities of the hydro-alcoholic and aqueous extracts of *Achillea biebersteinii* Afan. (Asteraceae) grown in Jordan. *African Journal of Pharmacy and Pharmacology*, 7, 1686-1694
- [32] Hifnawy MS, Rashwan OA, Rabeh MA (2001). Comparative chemical and biological investigations of certain essential oils belonging to families Asteraceae, Lamiaceae and Graminae. *Bull Fac Pharm Cairo Univ* 39: 35-53.
- [33] Mouterde P. (1983). *Nouvelle Flore du Liban et de la Syrie*. El Machreq. Editeurs :Beyrouth, distribution Librairie orientale.
- [34] Nemeth, E. and Bernath, J. (2008). Biological activities of yarrow species (*Achillea* spp.). *Current Pharmaceutical Design*, 14, 3151-3167. <http://dx.doi.org/10.2174/138161208786404281>
- [35] Oran, S.A. and EL-Eisawi, D.M. (1998). Check-list of medicinal plants in Jordan. *Dirasat*, 25, 84-112.
- [36] Palombo EA, Semple SJ (2001). Antibacterial activity of traditional Australian medicinal plants. *J Ethnopharmacol* 77: 151-157.

- [37] Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition (2012). This standard contains the current Clinical and Laboratory Standards Institute-recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.
- [38] Rodriguez-Tudela, J.L., Chryssanthou, E., Petrikkou, E. 2003. Interlaboratory evaluation of hemacytometer method of inoculum preparation for testing antifungal susceptibilities of filamentous fungi, *J. Clin. Microbiol.* 41- 5236–5237.
- [39] Said O, Khalil K, Fulder S, Azaizeh H. 2002. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the WestBank region. *J. of Ethnopharmacology* 83, 251 – 265.
- [40] Şanlı A. and Karadogan T. (2017). Geographical Impact on essential oil composition of endemic *Kundmannia anatolica* Hub.-Mor. (Apiacea). *Afr J Tradit Complement Altern Med.* 14(1): 131–137.
- [41] Shimon Barel and Jacob Yashphe 1989. Effect of the essential oil from *Achillea fragrantissima* on *Escherichia coli* cells. *Current Microbiology* 19(6):337-341.
- [42] Swamy, M.K., Akhtar, M.S. and Sinniah, U.R. (2016). Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. *Evidence-Based Complementary and Alternative Medicine*, Volume 2016, Article ID 3012462, 21 pages. Hindawi Publishing Corporation. <http://dx.doi.org/10.1155/2016/3012462>
- [43] US 2014 / 0314886A1- *Achillea fragrantissima* extract, method for preparing achillea fragrantissima extract and method for treating chronic myeloid leukemia. <https://patents.google.com/patent/US20140314886>.
- [44] Zeedan G. S.G. , Abdalhamed A. M. ,Ottai M. E., Abdelshafy S., and Abdeen E. , 2014.“Antimicrobial, antiviral activity and GC-MS analysis of essential oil extracted from *Achillea fragrantissima* plant growing in Sinai Peninsula, Egypt,” *Journal of Microbiology and Biochemical Technology*, vol. 8, article 006. doi: 10.4172/1948-5948.S8-006)..

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