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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF ARTEMISIA HERBA-ALBA ASSO., ASTERACEAE

Nasser Hatem*[1], Lara Hanna Wakim[1], Nabil Nemer[1], Baydoun Safa[2] & Arnold Apostolides Nelly[1]

[1]Faculty of Agriculture and Food Sciences, USEK, Kaslik, Lebanon
[2]Research Center for Environment and Development, Beirut Arab University, Lebanon

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ABSTRACT

Essential oil (E.O.) of *Artemisia herba-alba Asso*. (Asteraceae) known as Shih, growing wild in Hermel (Lebanon) was obtained by hydrodistillation and analyzed by GC and GC-Mass spectrometry (MS). The results were α-Pinene (45.89%) followed by Borneol (11.3%), 1,8-Cineole (10.8%), Terpineol (6.45%), Camphene (3.94%), γ-Terpinene (3.2%), α-Terpinene (2.72%), (+)-4-Carene (2.2%).

The antimicrobial activities of E.O. from *Artemisia herba-alba Asso* were evaluated against five bacteria and two fungi strains by disc diffusion method and microdilution method.
Among Gram positive bacteria growths, *S. aureus* was shown to be more sensitive (90 mm) *E. faecalis* (25 mm). On the other hand, three different Gram negative bacterial strains were tested,*S. enteritidis* showed maximum zone of inhibition (40 mm), followed by *E. coli* (26 mm) and *P. aeruginosa* (25 mm) respectively. Theinhibition zones displayed by fungalstrains tested were40 mm and 30 mm for *C. albicansand A. fumigatus*, respectively.
The results of the study showed an interesting antimicrobial profile which could provide promising pharmaceutical and economic benefits of the potential use of the plant essential oils.
The present investigation supported the traditional use of *Artemisia herba-alba* in the Lebanese folk medicine as an antimicrobial active representative of the genus Artemisia. *Artemisia herba-alba*extracts should be further studied for their potential use in preventing / treating diseases cited in the traditional Lebanese medicine.

KEYWORDS: Artemisia herba-alba; Asteraceae; Essential Oil; Chemical Composition; Antimicrobial Activity; Hermel-Lebanon.

I. INTRODUCTION

Medicinal plants continue to play an essential role in traditional healthcare systems in all cultures across the world. In recent years, the use of medicinal plants as a source of natural therapies, phytonutrients or nutraceuticals has gained a tremendous increase and has become an integral part of modern societies. Considering the antibiotic resistance of some bacteria, increasing body of scientific evidence has shown that medicinal plants area very promising source for effective antimicrobial agents, either alone or in combination with conventional antibiotics.

Artemisia herba-alba Asso. (*A.h.a*), commonly known as "white wormwood or desert wormwood" in English and "Shih" in Arabic (Segal et al., 1987) is one of the most popularly used plants in Lebanon. This plant species grows wild in arid areas of the Mediterranean basin and the steppes of the Middle East, West Asia and North Africa (Mohamed et al., 2010) and is abundantly found in the steppe of North East Lebanon (Qamou'at al Hermel, Ras Baalbeck, Ma'arra Ras Baalbeck). It has a characteristically whitish appearance; hence the common name *A.h.a*.. It has been used extensively as a traditional medicine to cure a variety of conditions, including tooth ache, colds coughing, intestinal and respiratory diseases (Bailey and Danin, 1981; Jouad et al., 2001), bronchitis, diarrhea, diabetes mellitus (Wright, 2002), neuralgia and hypertension. (Tahraoui et al., 2007; Tantaoui and Elaraki et al, 1994), cardiac and renal diseases (Zeggwagh et al. 2014; Zeggwagh et al. 2008), insecticidal activity (Derwich et al., 2009). Other traditional uses include administration as antibacterial, analgesic, antispasmodic herbal tea (Laid et al., 2008), to treat arterial hypertension (Ziyyat et al., 1997) and as

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anthelmintic (Mohamed et al., 2010). Considerable research has recently shown a range of biological and or pharmacological activities of *A.h.a*. as an antibacterial and antifungal (Imelouane et al., 2010) antileishmanial (Essid et al., 2015) anthelmintic and antispasmodic agent (Yashphe et al., 1987) and neurological disorders as Alzheimer's disease, epilepsy and other disorders (Salah and Jager, 2005).

Considerable work on the chemical composition of the essential oil of *A.h.a*. from Jordan, Morocco, Egypt and several other countries is cited in literature. Generally, the essential oil is largely reported to be composed by monoterpenes, mainly oxygenated, such as 1,8-Cineole, Chrysanthenone, Chrysanthenol (and its acetate), α/β-Thujones, and Camphor as major components (Mohamed et al., 2010; Belhattab et al., 2014). Nevertheless, the chemical ethanolic and ethyl acetate extracts of the dried plant obtained from a local herbal store has been characterized and shown to obtain flavonoids, Terpenoids and Cinnamic acid derivatives.

Despite the huge number of published works concerning the chemical characterization of *A.h.a*. essential oil from various countries, no studies have to date been conducted on the chemical characterization and antimicrobial activities of *A.h.a.* of the essential oil from Lebanon.

The aim of the present investigation was to evaluate the essential oil composition and antimicrobial activity of *A.h.a*. *Asso.* growing wild in the steppe Qamouet Hermel in the north Bekaa in Lebanon.

II. MATERIALS AND METHODS

Essential oil extraction and chemical composition

Plant material

Artemisia herba-alba Asso was collected in the steppe of Hermel–Bekaa Lebanon (Latitude 34.426765 N, Longitude 36.412766 E and Altitude 680 m) (Figure 1 and 2).

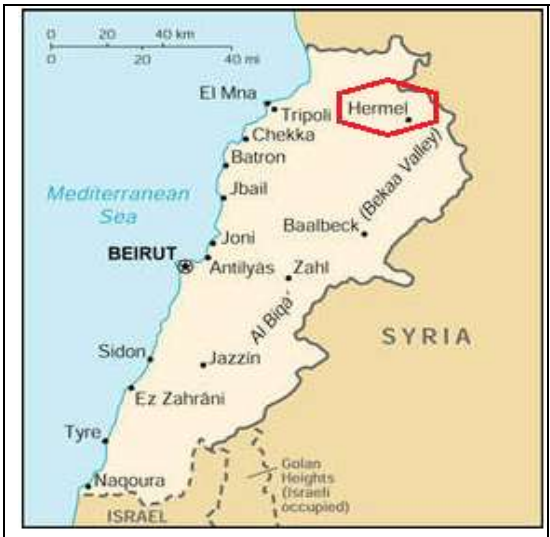

Figure 1- Artemisia herba-alba Asso. growing in the steppe (semi-arid) of Hermel region

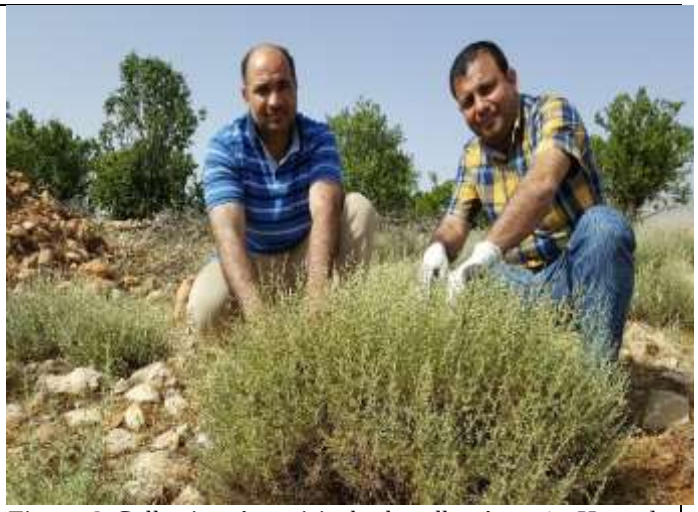

Figure 2-Collecting Artemisia herba-alba Asso, in Hermel-24 July 2016

The species identification was performed by Prof. Arnold N. using the determination keys of the new Flora of Lebanon and Syria (Mouterde, 1983). At a random sample of flowering aerial parts were collected in July 2016. Certified voucher specimens were deposited at the Herbarium of the department of botany and Medicinal plants, Faculty of the Agriculture, University Holy Spirit, Kaslik, Lebanon.

Clevenger type apparatus

The Artemisia herba-alba collected plant material was fragmented and essential oil was isolated by hydrodistillation for 4 hours using a Clevenger type apparatus following to the standard procedure of the 6th Edition of European Pharmacopeia (2007).

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GC and GC/MS analysis

GC and GC/MS analyses 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 e^-V 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 60°C programed 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 potency of *Artemisia herba-alba* essential oils. Among the tested bacterial strains, *Staphylococcus aureus* ATCC BAA-1026 and *Enterococcus faecalis* ATCC29212 were Gram positive and *Escherichia coli* ATCC 11303, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 9027were Gram negative, and two pathogenic fungal strains *Aspergillus fumigatus* ATCC 204305, *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. 100 µL of suspension containing 10^6 CFU/mL of microorganisms were spread on cultures of Müller Hinton agar medium (Merck). Sterile 6 mm diameter filter paper discs (Whatman N◦ 3) were impregnated with 10 µL of essential oil and 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, and Nystatine was used as standard antifungal. A blank disc was used as a negative control. The bacterial cultures were incubated at 37°C for 24 h, whereas *Candida albicans* and *Aspergillus 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 SD were computed (Bonev et al., 2008).

Determination of minimum inhibitory concentration (MIC) by microdilution method

Minimum inhibition concentration of *A.h.a*. defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation was determined using the agar 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 MHB nutrient broth. Microdilution wells containing 100 µL of standardized suspension of tested microorganisms added to 100 µL of a suspension of MHB and *A.h.a*. essential oil of different concentrations. The microplates were incubated overnight at 37°C. All the tests were performed in triplicates. Average values were determined.

IV. RESULTS

Chemical composition of A.h.a. essential oil

The extraction of the essential oil using Clevenger apparatus yielded 2% volume/mass of a yellow colored oil of a strong aromatic odor. Table 1 presents the qualitative and quantitative composition of the GC and GC/MS analysis of this oil where the percentages and the retention indices of the identified components are listed in order of their elution on the HP-5MS column. From the data obtained, 30 compounds were identified accounting for 98.65% of the total oil including 59.29% of monoterpene hydrocarbons and 33.03% of oxygenated monoterpenes as the main chemical classes of the oil**.** While sesquiterpenes constituted 4.59%, hydrocarbons formed 1.41% and other trace components accounted 0.33%.

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The major components of the oil were α-Pinene (45.89%) followed by Borneol (11.3%), Eucalyptol (10.8%), Terpineol (6.45%), Camphene (3.94%), γ-Terpinene (3.2%), α-Terpinene (2.72%), (+)-4-Carene (2.2%).

Table 1: Chemical Composition of Artemisia herba-alba Asso.essential oil.

N	RT	Library/ID	%
1	7.1841	α-Pinene	45.89
2	7.659	Camphene	3.94
3	9.6331	α-Terpinene	2.72
4	9.8562	p-Cymene	1.34
5	10.1252	Eucalyptol	10.8
6	11.0178	γ-Terpinene	3.2
7	11.9791	(+)-4-Carene	2.2
8	14.1935	Hotrienol	0.49
9	16.425	Borneol	11.3
10	17.5637	Terpineol	6.45
11	18.1473	Terpinolene	0.7
12	18.3705	Propellane	1.41
13	18.5479	Myrtenol	1.12
14	34.4321	Methyl Cinnamate	0.5
15	34.9928	Jasmone	0.51
16	35.4162	Germacrene D	1.08
17	35.6966	β-Cubebene	0.16
18	36.904	Limonene	0.63
19	37.1386	Rosefuran	0.62
20	37.8252	γ-Cadinene	0.35
21	38.0426	2,3,4,5-Tetramethylthiophene	0.33
22	38.4718	Anisole	0.39
23	38.5519	Nerolidol	0.46
24	38.7464	(+) spathulenol	0.4
25	38.9811	1,5-Dibromo-3-methyl-pentane	0.24
26	39.0726	Eucarvone	0.26
27	39.2156	Verbenol	0.35
28	39.6448	Caryophyllene oxide	0.45
29	40.1369	Naphthalenone	0.2
30	40.3143	Sativene	0.16
		Total	98.65
Monoterpenes hydrocarbons			59.29 %
Oxygenated monoterpenes			33.03 %
Sesquiterpenes hydrocarbons			3.08 %
Oxygenated Sesquiterpenes			1.51 %
Hydrocarbons			1.41 %
Others compounds			0.33 %
Total			98.65 %

V. ANTIMICROBIAL ACTIVITY

Growth inhibition by disc diffusion assays

The results of the antimicrobial activity of the oil evaluated against five bacterial strains and two fungal strains are presented Table 2. It is evident that the strains displayed variable degree of susceptibility against investigated oil with inhibition zones ranging between 20 mm with *P. aeruginosa* and 90 mm *S. aureus*. Among Gram positive bacteria growths, *S. aureus* was shown to be more sensitive (90 mm) *E. faecalis* (25 mm). On the other hand, three different Gram negative bacterial strains were tested and among these microorganisms, *S.*

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enteritidis showed maximum zone of inhibition (40 mm), followed by *E. coli* (26 mm) and *P. aeruginosa* (25 mm) respectively. In regards to fungal strains tested, the inhibition zones displayedwere40 mm and 30 mm for *C. albicansand A. fumigatus*, respectively.

Table 2. Mean ± SD growth inhibition zones (mm) of A.h.a and a group of antibiotics*.

Antibiotics / Microorganism	A.h.a	OX 1µg /disc	TI 75µg /disc	CB 100µg /disc	CL 25µg /disc	PI 100µg /disc	E 15µg /disc	TE µg /disc	Nys
E.coli	26 ± 0.1	0	33 ± 0.1	36 ± 0.5	18 ± 0.2	25 ± 0.1	12 ± 0.2	27 ± 0.1	-
S.enteritidis	40 ± 0.4	0	30 ± 0.8	30 ± 0.1	18 ± 0.2	25 ± 0.1	19 ± 0.1	25 ± 0.2	-
P.aeruginosa	20 ± 0.3	0.0	20 ± 0.5	32 ± 0.6	19 ± 0.1	29 ± 0.4	15 ± 0.1	7 ± 0.3	-
S.aureus	90 ± 0.0	0.0	0.0	15 ± 0.5	0	0	0	40 ± 0.2	-
E.faecalis	25 ± 0.2	0	25 ± 0.3	35 ± 0.1	16 ± 0.3	32 ± 0.3	30 ± 0.1	20 ± 0.1	-
C.albicans	40 ± 0.1	-	-	-	-	-	-	-	31 ± 0.2
A.fumigatus	30 ± 0.2	-	-	-	-	-	-	-	10 ± 0.1

* Oxacillin(1µg), Ticarcillin (75µg), Carbenicillin (100µg), Colistin(25µg), Piperacillin(100µg), Erythromycin(15µg), Tetracycline(30µg) and Nystatine

Minimum Inhibitory Concentration

The MIC values of *A.h.a.* using broth microdilution method are presented in Table 3. Maximum activity was observed against *S. aureus* with MIC value of 0.1 mg/ml, 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) and *P. aeruginosa* whichseemed to be resistant to the investigated oil with a MIC of 7.5 mg/ml.

Table 3.MIC values of the *Ach.frg* essential oil

Microorganisms	*S. aureus*	*E. faecalis*	*S. enteritidis*	*P. aeruginosa*	*E. coli*	*A. fumigatus*	*C. albicans*
MIC mg/ml	0.1	2.5	2.5	7.5	2	2	1.5

VI. DISCUSSION

The results of Table 1 indicate that the composition of the essential oil of *A.h.a*. under this study has a unique chemical profile in spite of some similarities in the major groups of compounds of essential oils reported from other countries (Table 4). As such, α-Pinene (45.89%), Bornéol (11.3%), Eucalyptol (10.8%), Terpineol (6.45%) were the major compounds of this oil, whereas, Camphre, β-Thujone, α-Thujone, Eucalyptol, Chrysanthenone, Trans-Pinocarveol were reported as dominant in some chemotypes in other studies (Table 4). On the contrary, with the exception to Eucalyptol, these compounds were not detected in this study. Specifically, in Jordan, Hudaib and Aburjai (2006) reported α- and β-Thujones (27.27%), Caryophyllene acetate (5.7%), Sabinyl acetate (5.4%), Germacrene-D (4.6%) and α-Eudesmol (4.2%) as the principal components. In a more recent study, Abou-Darwich and coauthors (2015) reported β-Thujones (25.1%), α-Thujones (22.9%), Eucalyptol (20.1%) and Camphor (10%) as the main composition. In Iran, the main compound were β-Thujone (35.66%), Camphor 34.94%), 1,8-Cineole (7.42%) and α-Thujone (4.12%)(Sharifian et al., 2012). In an Algerian oil, the major components were camphor (39.5%), chrysanthenone (10.38%), 1,8-cineole (8.6%), α-thujone (7.03%), Borneol (3.35%) (Lakehal et al. 2016). This prominent chemodiversity of the oils is not only limited to the plant from different counties but also is reported in different localities of the same country. Major differences in composition are in the oils from Morocco: Camphor (40–70 %), α- or β-Thujone (32–82 % and 43–93 %, respectively), Chrysanthenone (51.4 %), Chrysanthenyl acetate (32– 71 %), or Davanone (20–70 %) (Paolini et al., 2010). Similar wide variations are also reported in Tunisian chemotypes: Cineole 1.5 – 26.99%),

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Thujones (1 – 64.67%), Chrysanthenone (1 – 17.37%), Camphor (0.56 – 16.73%), Borneol (0.72 – 10.75%), Chrysanthenyl acetate (0.52 – 7.37%), Sabinyl acetate (0.53 – 22.46%), Davana ethers (0.65 – 6.23%) and Davanone (2.37 – 20.14%) (Haouari and Ferchichi , 2009) and Spain: Davanone (0.5 – 39.1%), 1,8-Cineole (0.8 – 25.8%), Chrysanthenone (0.1 – 36.4 %), Cis-Chrysanthenol (0.2 – 27.8 %), Cis-Chrysanthenyl acetate (0.2 – 18.4%), p-Cymene (0.6 – 20.6%), α-Pinene (0.2 – 17.2 %). Totally different main compositions of two different localities from Egyptwere reported (Abou El-Hamdet al., 2010). This wide chemical variability may be a result of the genetic characteristics of the plant combined with the influences of geographical locations and climatic conditions as well as the difference of the developmental stages of the plant and method used to obtain the essential oil (Mighri et al., 2010; Belhattab et al., 2014; Lakehal et al., 2016; Singh and Guleria, 2013). Actually, plant biosynthetic pathways and consequently the relative proportion of the main characteristic compounds are known to be influenced by these factors (**Ben Ghnaya 2013)**

Table 4. Main chemical composition of fresh aerial parts of A.H.A of some countries of the Middle East and Mediterranean.

Country	Main chemical composition	Citation
A.H.A under study (Lebanon)	α-Pinene (45.89%) ; Borneol (11.3%) ; Eucalyptol (10.8%) ; Terpineol (6.45%) ; Camphene (3.94%) ;	Our research
Spain	Davanone(0.5 – 39.1%), 1,8-Cineole (0.8 – 25.8%), Chrysanthenone (0.1 – 36.4 %), Cis-Chrysanthenol (0.2 – 27.8 %), Cis-Chrysanthenyl acetate (0.2 – 18.4%), p-Cymene (0.6 – 20.6%), α-Pinene (0.2 – 17.2 %)	Salido et al. 2004
Jordan	β-Thujones (25.1%), α-Thujones (22.9%), Eucalyptol (20.1%) and Camphre (10%)	Abou-Darwish et al., 2015
Jordan	α- and β-Thujones (27.7%), Sabinyl acetate (5.4%), Germacrene D (4.6%), α -Eudesmol (4.2%) and Caryophyllene acetate (5.7%)	M. Hudaib, T. Aburjai (2006)
Algeria	Camphor (39.5%), Chrysanthenone (10.38%), 1,8-Cineole (8.6%), α-Thujone (7.03%), Borneol (3.35%)	Lakehal et al. 2016
Algeria	Camphor (17–33%), α-Thujone (7–28%) and Chrysanthenone (4–19%)	Belhattab et al., (2012)
Morocco	Camphor (40–70 %), α- or β-Thujone (32–82 % and 43–93 %, respectively), Chrysanthenone (51.4 %), Chrysanthenyl acetate (32– 71 %), or Davanone (20–70 %)	Paolini et al., 2010
Tunisia	Cineole (1.5 – 26.99%), Thujones (1 – 64.67%), Chrysanthenone (1 – 17.37%), Camphor (0.56 – 16.73%), Borneol (0.72 – 10.75%), Chrysanthenyl acetate (0.52 – 7.37%), Sabinyl acetate (0.53 – 22.46%), Davana ethers (0.65 – 6.23%) and Davanone (2.37 – 20.14%)	Haouari and Ferchichi 2009
Egypt	Chrysanthenyl acetate as major component (31%) and Chrysanthenol (6.4%)	Abou El-Hamdet al. 2010
	1,8-Cineole (50%), Thujone (27%), Terpinen-4-ol (3.3%), Camphor (3%) and Borneol (3%)	
Iran	β-Thujone (35.66%), Camphor 34.94%), 1,8-Cineole (7.42%), α-Thujone (4.12%)	Sharifian et al. 2012

Research into the antimicrobial actions of monoterpenes suggests that they diffuse into bacteria and damage cell membrane structures (Sikkema et al., 1995). Indeed, in essential oils, it was shown that monoterpenes hydrocarbons and oxygenated monoterpenes in essential oil are able to destroy cellular integrity resulting in respiration inhibition and permeability alteration (Cox et al., 2000). Besides, the most abundant component in essential oil of A.h.a, Camphor has been reported to exhibit bacteriostatic activity against *P.aeruginosa* (Imelouane et al. 2010) and this compound is a major constituent in a number of antibacterial essential oils (Magiatis et al., 2002; Tabanca et al., 2001). According to Burt (Burt, 2004), given the large number of different groups of compounds present in essential oils, the antibacterial activity of essential oils is most likely not attributable to a specific mechanism but to several mechanisms related to various targets in the cell.

This generally higher resistance in the Gram-negative bacterium *P.aeruginosa*could be ascribed to the structure of the cell wall of Gram-negative bacteria primarily made up of a liposaccharide that blocks the penetration of

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hydrophobic compounds and prevents the accumulation of essential oils in the membrane of target cells. The absence of this barrier in Gram-positive bacteria allows the direct contact of the essential oil hydrophobic constituents with the phospholipid bilayer of her cell membrane, where they bring about their effect, causing an increase of ion permeability and leakage of vital intracellular constituents or impairment of the bacterial enzymes systems (Cowan, 1999; Wendakoon and Sakaguchi, 1995) .

The antibacterial activity and consequently the minimum inhibition concentration of essential oils can be influenced by the growing region of the plant, the extraction method used, the plant used (leaf or whole plant) the method of preparation of the raw material (fresh or dry), the type of organism, the cultivation conditions (incubation time, temperature, oxygen), the culture medium, the concentration of the test substance and the solvents used to dilute the oil, among other factors (Burt, 2004; Dellacassa et al. 1999; Celiktas et al. 2007).The essential oil evaluated has a great variety of phytochemicals that could be responsible for a larger or smaller part of the antimicrobial activity.

In addition, other minor components such as Borneol (11.3 %) have been also reported to have antimicrobial potential (Mighri et al., 2010). In other studies, α-pinene has been known to exhibit antimicrobial activity against the bacterial strains *P.aeruginosa*, *E.coli* and *S. aureus* (Wang et al., 2012). In fact, the biological effectiveness of essential oils is related to their different constituents (major, minor and their mutual ratios) acting either synergically or antagonistically with major components (Hummelbrunner and Isman, 2001; Pavela, 2014). In general, the antimicrobial activity of the essential oils tested was more pronounced against Gram positive than Gram negative bacteria (Nedorostova and Kloucek, 2009).

VII. CONCLUSIONS

The composition of the essential oil of *A.h.a. Asso* growing wild in Hermel, Lebanon has been analyzed and its antimicrobial activity investigated in this study. The results indicate a unique chemical composition and a very high susceptibility of the tested microorganisms to the oil. This is used in the treatment of diseases caused by the microorganism tested. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine. Considering the increase development of resistance of bacteria, fungi and yeast to antibiotics, the present investigation provide support to the antibacterial properties of this plant oil. This study is the first report on essential oil composition and antimicrobial activity of the *A.h.a*. and calls for further investigations to elucidate the effects of the oil and extracts on other biological activities. The present investigation supports the traditional use of this plant in Lebanese folk medicine as antimicrobial in the treatment of a wide range of diseases and its potential as a good source for novel drug discovery.

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