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GC/MS analysis and *in vitro* antioxidant and antibacterial activity of essential oil of *Artemisia herba-alba* Asso of Algeria

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Abstract

The main objective of this study is to investigate the chemical composition, antioxidant and antimicrobial activities of the essential oil of *Artemisia herba-alba Asso.* of southern Algeria. Essential oil of *Artemisia herba-alba Asso* was extracted by hydrodistillation, and their chemical composition was identified by GC/MS Antioxidant activity of essential oil, has been done by using DPPH assay. The antimicrobial activity of essential oil was realised by the agar disc diffusion method. The essential oil extracted from the aerial parts by hydrodistillation was analysed by GC/MS. 39 constituents, representing 99.3% of the oil, were identified, of which the major ones, Thujone (12,759 %), Camphor (7,751%), Eacalyptol (4,525%), Isoborneol (1,119%). IC_{50} values observed for DPPH essay were 20,27 ±0,767 mg/ml. On the other hand, this oil was found effective against all tested strains; this activity ranged from 15.67±1.53 mm with *Listeria innocua CIP 74915*. These results provided evidence that the studied plant might indeed be a potential source of natural antioxidant and antimicrobial agents.

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Introduction

Since ancient times, Aromatic plants have been used as well as in therapy in preserving and flavoring food; in the last decade, scientific research has focused its interest on their essential oils and natural extracts as sources of antimicrobial compounds and antioxidants (costa *et al*., 2015) Traditional plants have emerged as potential sources of antioxidants, antimicrobials, and secondary metabolites for therapeutic interventions, which has opened doors for the development of novel plant-based antibacterial agents. A number of such compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens (Aqil *et al*., 2007).

In the Sahara of Algeria, the flora is very rich in medicinal plants which produce valuable natural substances such as essential oil. Actually, essential oil and its components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multipurpose functional use (Boukhris *et al*., 2012). Many essential oils also have been confirmed to possess antioxidant activity (Zhang *et al*., 2006). As part of the study evaluation of the biological effectiveness of the medicinal plants, *Artemisia herba-alba* Asso (Asteraceae family) is widespread in semi-arid and arid steppes of North Africa, Spain, the Middle East, and the Northwest of Himalaya (Wang, 2004).

This plant is used as aromatisant for tea and in folk medicine for the treatment of colds coughing, intestinal disturbances and as an antidiabetic agent (Jouad *et al*., 2001), hypertension and cold (Singh *et al*., 2016). This plant is used for the treatment of gastric disturbances such as diarrhea, abdominal pain and for healing external wounds. The terpenoid sesquiterpene lactone dehydroleucodine mainly found in the aerial parts of *Artimissia herba-alba*, is responsible for its medicinal properties (Abood *et al*., 2017). The essential oil of this species was known for its therapeutic disinfectant, anthelminthic and antispasmodic virtues (Hatimi *et al*., 2001) .This study deals with the valorization of medicinal and aromatic plants of the Algerian flora in order to find new bioactive natural products Information concerning in vitro antioxidant activities of the essential oil from the *Artemisia herba-alba* has not been reported earlier.

The aim of this work is to provide more information on the chemical composition of the essential oil obtained from aerial part of *Artemisia herba-alba* originated from southern Algerian and investigate their antimicrobial and antioxidant activities.

Materials and methods

Plant material

Aerial parts of *Artemisia herba-alba* were collected during the flowering phase (November 2018) from Ghardaia is located within the Sahara Desert in northern-central of south Algeria (32° 29′ 0″ N, 3° 40′ 0″ E) (Site1)(Fig. 1). The plant material was cleaned, chopped into pieces and derided in air.

The climate of Ghardaia city is a hot desert climate characterised by summers with torrid heats reaching 35°C (Fig. 2) and mild winters with an average minimum just above freezing point. The relative humidity is very low except for the winter months, where 22 % is common (Fig. 3). Precipitation is low and less than 18 mm throughout the year (Fig. 4).

Extraction of the essential oil

The essential oil was extracted from air-dried parts of *Artemisia herba-alba* by hydrodistillation for (3h) using a Clevenger apparatus type. The yield of each essential oil was determined on average over the three replicates. These oils were kept at 4 °C until analysis (Bruneton, 1999).

Antioxidant activity

The radical scavenging activity of the essential oil was measured using 2,2-diphenyl-1-picrylhydrazyl, which was evaluated with the methodology described by Blois (1958) as elaborated by Elmastas (2007). A volume of 200 µl of the different dilutions of essential oils (methanolic solutions of EO) was mixed with 800

µl of the 0.004% (w / v) DPPH ethanol solution in dry test tubes. The reaction mixture was stirred vigorously and incubated for 30 min at room temperature and in the dark. Absorbance was measured at 517 nm. The negative control is composed of 200 µl of methanol and 800 µl of the DPPH.

The scavenging capacity of DPPH radical was calculated using the following formula. Where, A control (Ac) is the absorbance of the control reaction and A sample (AS) is the absorbance in the presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged. Ascorbic acid was used as standard.

Fig. 1. Location of the Wilaya of Ghardaïa.

$$\% \ inhibition = \frac{Ac - As}{Ac} \times 100$$

The oil concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extracts concentrations (Shimada *et al.*, 1992).

Gas chromatography-mass spectrometry (GC-MS)

Analysis by GC / MS was carried out using a Varian GC 3800 equipped with an SPB1 capillary column (30 mm, 0.25 mm, 0.25 mm) and a "Mass Selective MS Saturn Series 2200, column SPB-1. The temperature of the detector was 250 °C, and the injector 210 °C, the oven temperature was programmed as before and the transfer line temperature was 280 °C and operating under the GC condition programmed heating at 55 °C for 1 min to 150 °C for 3 min to 250°C for 8 min. The injector temperature was 250 °C. Helium was the GC carrier gas.

Phytochemical test

The methanol extracts were subjected to phytochemical tests for secondary metabolites, tannins, saponins, steroids, alkaloids and glycosides in accordance with Trease and Evans (1987) and Harborne (1998) with little modification.

Evaluation of antibacterial activity

In this study, four strains Gram-negative bacteria *Pseudomonas aeruginosa ATCC 25922, Salmonella*

enterica, Listeria innocua Cip 74915 and *Escherichia coli ATCC 27853* and one strains Gram-positive bacteria *Klebsiella pneumonia ATCC7000603* provided from hospital Elhakim Saadan Biskra (Algeria).

This assay was carried out using the disc agar diffusion method with a little modification (Phaiphan, 2014). Tested strains grown on Müller-Hinton agar at 37 °C for 18 h for bacteria were suspended in a saline solution (0.9% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/mL). The suspension was used to inoculate 90 mm diameter Petri plates containing the medium cited above. Sterile paper discs No. 1 (6 mm diameter) was impregnated with 10µL of essential oil; after sterilisation the disc was laded on the surface of agar plates. Before incubation, the incubation conditions were at 37 °C for 24 h for bacteria. Antimicrobial activities were evaluated by measuring the inhibition zone diameters. The work was achieved in aseptic conditions. Chloramphenicol (C30), Céfixime (CFM), Gentamycin (GEN), Ofloxacine (OF) and Co-Trimoxazole Sulfamethoxazole (COT) were used as a positive control to determine the sensitivity of Gram-negative and Gram-positive bacteria, respectively (Schinor, 2007). All tests were performed in triplicate for each microorganism's strain and the final results of the inhibition zone measured in millimetres were presented as the average.

Statistical analysis

Analysis of variance (ANOVA) was performed on the data obtained using Co Stat-Statistics Software version 6.4.

The significance of the differences among treated samples was evaluated using the LSD test for comparisons the means ± standard deviation (SD) of the diameter of inhibition. Each experiment has three replicates and three determinations were conducted and the significance level for all measurements was considered at $p<0.05$.

Results and discussion

Air-dried parts of *Artemisia herba-alba* were subjected to hydrodistillation using a Clevenger-type apparatus. Liquid, gilded yellow and penetrating strong odour essential oil was obtained with a yield of 1.50 ± 0.0930% (w/w), based on the dry weight of the plants, but in the other study was obtained with a yield of 0,65 % (Akrout, 2004) in Tunisia, 1,2 % (Zaim *et al*., 2012) in Maroc, 0,95%(Belhattab *et al*., 2014) in Algeria.

Table 1. Chemical composition of the essential oil of *Artemisia herba-alba* in the aerial part.

Peak	TR (min)	Peak Name	%	Peak	TR (min)	Peak Name	%
1	5,031	Camphene	0.41	21	12,859	Benzenemethanol	0.198
2	5,419	β -Phellandrene	0.455	22	13,018	Thymol	0.282
3	6,659	Eucalyptol	4.525	23	14,116	Phenol	0.069
4	7,195	3-Carene	0.265	24	14,261	α -Cadinol	0.104
5	7,756	5-heptadien-4-ol	1.072	25	14,754	5 α -Pregnane	0.017
6	8,305	Thujone	12.759	26	15,092	1H-Indene	0.025
7	9,389	Camphor	7.751	27	15,211	Caryophyllene	0.045
8	9,581	Bicycloheptan-2-one	0.011	28	15,401	1-Butyn-3-one	0.025
9	9,653	Cis-3-ethyl-endo-tricyclo5	0.124	29	15,993	α -Caryophyllene	0.014
10	9,716	Pinen-3-one	0.188	30	16,810	ɣ -Elemene	0.417
11	18,597	(-)-Spathulenol	0.686	31	18,139	Santalol, cis,.alpha.-	0.116
12	10,026	Isoborneol	1.119	32	18,463	Longipinocarvone	0.07
13	10,443	(1R)-(-)-Myrtenal	0.232	33	18,919	Cubenol	0.226
14	10,798	trans-Shisool	0.524	34	19,040	Adamantane-1-carboxylic acid	0.116
15	11,114	Isobornyl formate	0.215	35	19,586	Isoaromadendrene epoxide	0.017
16	11,419	Acetic acid	0.2	36	20,087	2-Naphthalenemethanol	0.035
17	11,821	2-Cyclohexen-1-one	0.838	37	20,472	1,2-Longidione	0.031
18	12,043	p-Menth-2-en-7-ol	0.076	38	21,593	Benzenepropanoic acid, penty	0.019
19	12,309	Bornyl acetate	0.29	39	24,665	Acrylic acid	0.015
20	12,483	Artemiseole	0.16			NI	55.266

Chemical composition of essential oils

Chemical analysis of Essential oil extracted from the aerial parts of *Artemisia herba-alba* by hydrodistillation was analysed by GC/MS. 39 constituents, representing 99.3% of the oil, were identified, of which the major ones, Thujone (12,759 %), Camphor (7,751%), Eacalyptol (4,525%), Isoborneol (1,119%) (Table 1). Zaim *et al.* (2012). Were obtained the major compounds are constituted by the chrysanthenone (28,10%) and Dahmani-Hamzaoui et Baaliouamer (2010) is observed that the camphre with (49,3%), in the other the major compounds are constituted by the cischrysanthenyl acétate (25,12%) (Bezza *et al.*, 2010).

Table 2. Phytochemicals found in methanolic extract of *Artemisia herba-alba*

Phytochemicals	Aerial part
Flavonoids	+
Saponin	-
Steroids	+
Reducing sugars	+
Tannins	++
Alkaloids-wagnersreagents	+
Alkaloids-Draghandroff-reagents	+
Volatile oils	+
Key: + = present, - = absent	

The difference Chemical analysis of essential oils of Artemisia herba-alba might be attributed to the age of the plant and plant part studied (El-massry *et al.*, 2002). This specific variability existing within the *Artemisia herba-alba* species can be of geographical, genetic (Karousou *et al.*, 2005), seasonal origin, or even ecological (soil, humidity, etc.) (Ghanmi *et al.*, 2010).

Phytochemical screening

Investigations on the phytochemical screening of *Artemisia herba-alba* plant extracts revealed the presence of alkaloids, flavonoids, steroid and triterpene, tannins and Reducing sugars; these compounds are known to be biologically active (Table 2). Our results are in close agreement with that reported by Mohamed *et al.* (2010).

Table 3. Zone of Inhibition of bacterial strains against of essential oil *Artemisia herba-alba* and positive control.

Bacteria strain	Zone inhibition(mm)						
	Essential oil	C30	GEN	CFM	OF	COT	P
E.coli	10.77 ± 0.46^{d}	20 ± 1^{c}	18 ± 1^{c}	24 ± 0.5^{b}	30 ± 1^{a}	25 ± 0.79^{b}	0.000
L. innocua	15.67 ± 1.53^{c}	26 ± 0.7^{b}	30 ± 3.46^{ab}	20^{c}	28 ± 0.23^{ab}	32 ± 0.6^{a}	0.000
K. pneumoniae	6^{c}	30 ± 2.46^{a}	30 ± 1.78^{a}	20 ± 1^{b}	27 ± 0.4^{a}	28^{a}	0.000
P .aeruginosa	6^{d}	12 ± 0.56^{c}	26 ± 0.66^{b}	8^{d}	30 ± 1.77^{a}	6^{d}	0.000
S. enterica	6^{e}	20 ± 0.87^{b}	18^{c}	20 ± 0.4^{b}	15.30 ± 1.13^{d}	29 ± 0.50^{a}	0.000

Means of three replicates ± SD (standard deviation) followed by at least one same letter are not significantly different according to LSD test at $p < 0.05$.ND = not detected.

Antioxidant activity

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Shirwaikar *et al.*, 2006). Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. DPPH free radical scavenging is an accepted mechanism by which antioxidants act to inhibit lipid peroxidation.

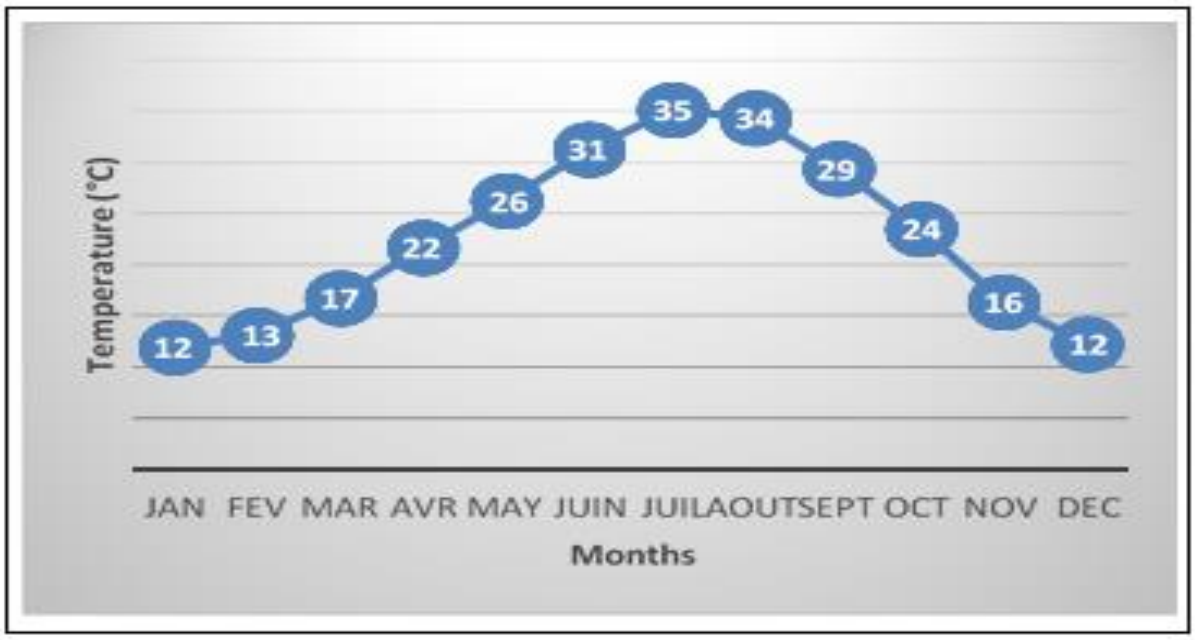

Fig. 2. Annual monthly change in average temperature during the period (2007-2016).

This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis.

Our results revealed that the oil the Artemisia herba-alba had a similar free radical scavenging activity when compared with standard ascorbic acid (Fig. 5). The results indicated the proton donating ability of the extractives, which could serve as free radical inhibitors or scavengers and can also be served as primary antioxidants. IC_{50} for DPPH radical-scavenging activity was 20,27 ±0,767 mg/ml.

Fig. 3. Annual monthly change in average humidity during the period (2007-2016).

The IC_{50} values for Ascorbic acid, 2.895± 0.063µg/ml. Indeed, Akrout *et al.* (2011) found that the anti-free radical activity of essential oils of *Artemisia campestris*, which is of the Thujone (α + B) type, is relatively weak if it is compared to that of Thymuscapitatus. Mighri *et al.* (2009), for their

part, carried out a study on four species of Artemisia using DPPH, ABTS and linoleic acid and they observed the activity of all the essential oils studied remained lower than that of controls and that *Artemisia herba-alba* has low activity. This is confirmed in a study by Lopes-Lutz *et al.* (2008) on some species of Artemisia. On the other hand, several studies have confirmed that the antioxidant activity of essential oils is linked to their major compounds where essential oils rich in oxygenated compounds (linalool, eugenol, geraniol, borneol and α-terpineol, etc.) have an antioxidant activity more marked than that with a hydrocarbon terpene (Falleh *et al.*, 2008).

Based on this principle, we can attribute the variability of the antioxidant activity to that of the chemical composition, which itself can be attributed to several factors: edaphic, climatic, etc.

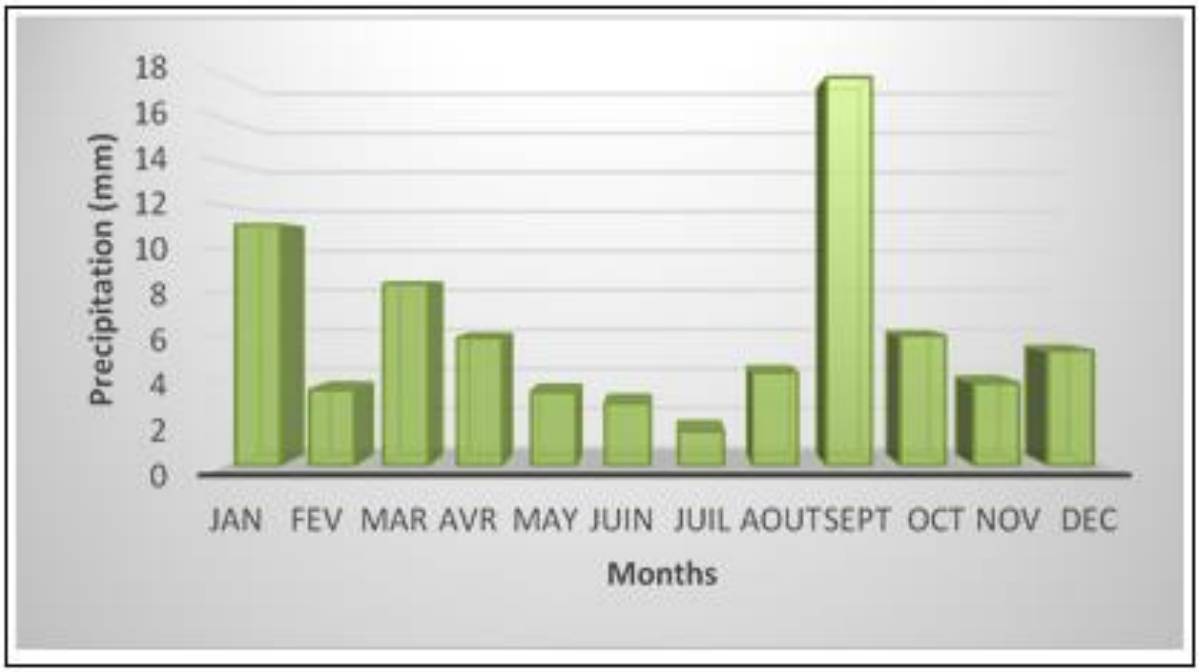

Fig. 4. Annual monthly change in average Precipitation during the period (2007-2016).

Antibacterial activity

The lipophilic character of the hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of essential oil components (Griffin *et al.*, 1999). Due to these data, we were interested in studying the antimicrobial activity of the essential oil. The results were summarized in (Table 3), which showed that essential oil extracted from *Artemisia herba-alba* prevented the growth of some tested microorganisms with an inhibition zone medium diameter. The highest inhibition zone was recorded for *Listeria innocua at 15.67±1.53 mm*.

It should be mentioned that there are no background antibacterial studies on *Artemisia herba-alba*, while in *Artemisia herba-alba* some studies have been reported as the essential oil exhibited much higher antibacterial activity with 31,3mm against *Klebsiella oxytoca* (Bertella, 2019).

This low activity of the essential oils tested could be related to their chemical composition; indeed, the study of the antibacterial activity of certain constituents of Essential oils has distinguished: phenolic compounds with high antimicrobial activity, such as Thymol and carvacrol (Cosentino *et al.*, 1999).

The constituents with low antibacterial activity are pulegone, Menthone, 1,8-cineole, p-cymene, isomenthone, myrcene, pinene, piperitone, Liminene, linalool, terpinene, sesquiterpenes and Terpenic (Lattaoui and Tantaoui, 1994; Carson *et al.*, 1995; Chalchat *et al.*,1995).

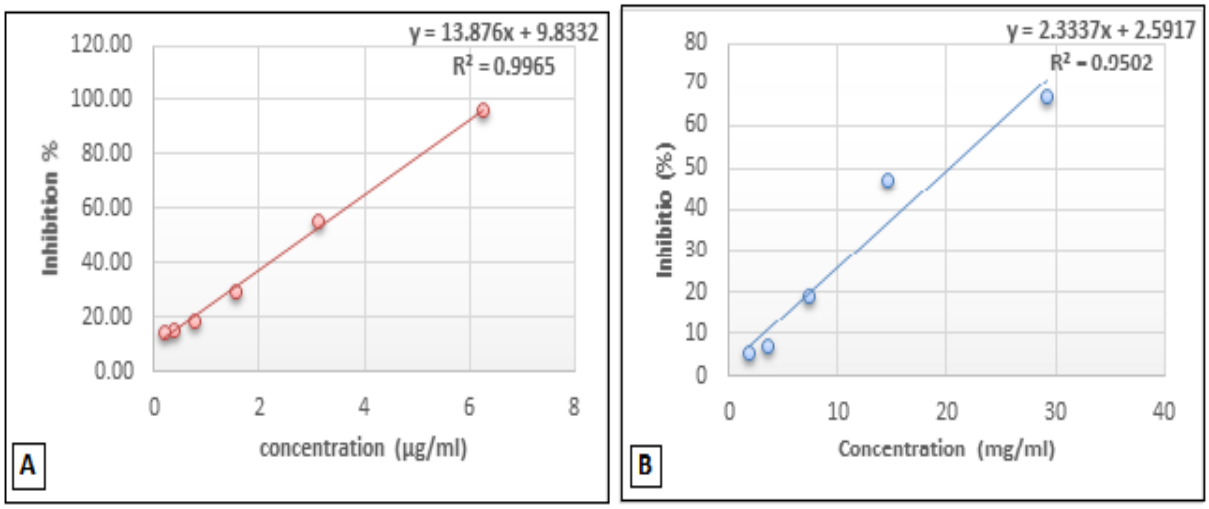

Fig. 5. (A, B). Reducing power of antioxidant activity of Ascorbic acid (A) and essential oil of *Artemisia herba-alba* (B).

In particular, the modes of action of essential oils and their main constituents described so far all seem to affect the cytoplasmic wall or membrane. However, the chemical variability of essential oils suggests the existence of molecules that can act through new cellular mechanisms (Guinoiseau, 2010). The main characteristic of the molecules present in essential oils is their hydrophobicity. It allows their solubilization in the membranes, which causes a destabilization of the structure and an increase in the membrane permeability (Sikkema *et al.*, 1994). So, we can attribute the variation of the antibacterial activity according to the type of Essential oils, which in themselves can be varied according to the edapho-climatic factors (Kadri *et al.*, 2017).

Conclusion

Artemisia herba-alba Asso, known also as desert wormwood (known in Arabic as shih, Armoise blanche. The phytochemical screening of *Artemisia herba-alba Asso* plant extracts revealed the presence of alkaloids, flavonoids, steroid and triterpene, tannins, and Reducing sugars. Air-dried parts of *Artemisia herba-alba* were subjected to hydrodistillation using a Clevenger-type apparatus. Liquid, gilded yellow and penetrating strong odour essential oil was obtained with a yield of 1.50 ± 0.0930% (w/w), based on the dry weight of the plants. Chemical analysis of Essential oil extracted from the aerial parts of *Artemisia herba-alba* by hydrodistillation was analysed by GC/MS. 39 constituents, representing 99.3% of the oil, were identified, of which the major ones, Thujone (12,759 %), Camphor (7,751%), Eacalyptol (4,525%), Isoborneol (1,119%). For the antioxidant activity, IC_{50} for DPPH radical-scavenging activity was 20,27 ±0,767 mg/ml. The IC_{50} values for Ascorbic acid, 2.895± 0.063µg/ml. On the other hand, this oil was found effective against all tested strains; this activity ranged from 15.67±1.53 mm with *Listeria innocua CIP 74915*. These interesting results show that the *Artemisia herba-alba* grown in the desert of Algeria has an important antioxidant and antimicrobial activity, which encourages further in-depth investigations on their pharmacological proprieties.

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