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Chemical Compounds Profile, Antibacterial and Antioxidant Activities of the Essential Oil Extracted from the *Artemisia herba-alba* of Southern Algeria

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ABSTRACT

Extraction of essential oils from locally available plant *Artemisia Herba-alba* was carried out using steam-distillation method. Extracted oils were screened for their chemical composition, antibacterial and antioxidant activities in order to find new metabolite products, which are characterized by a biological activity. Thirty-three constituents, representing 97.54% of the essential oil of *Artemisia herba-alba* was determined by GC-MS analysis. The main compounds identified are: davanone (42.8%), camphor (15.96%) and thujone (9.63%). The antimicrobial activity of the oil was tested using the agar disc diffusion method, by determining the inhibition zone and the minimum inhibitory concentration. The results have shown a great potential of the antimicrobial activity against the tested strains with an enhanced sensitivity towards the gram-negative strains of *Salmonella enteric*, *Klebsiella* and the gram-positive strains of *Listeria monocytogenes* and *Staphylococcus* sp. in comparison with the other tested bacteria. The test's results of the essential oil's antioxidant activity, obtained with the anti-radical method 2,2-diphenyl-1-1-picrylhydrazil (DPPH) and the iron-reducing power (FRAP), were compared with those of the ascorbic acid; the usual synthetic antioxidant. The comparison have demonstrated, firstly with the DPPH method a considerable level of antioxidant activity of the essential oil (IC_{50} = 17.73±0.29 μg mL^{-1}) but still lower than that found for the synthetic antioxidant that is the ascorbic acid while the opposite occurred with the iron reduction method, with an higher obtained value of EC_{50} =12.53±0.25 μg mL^{-1}, for the essential oil's antioxidant activity.

Key words: Antibacterial activity, antioxidant activity, *Artemisia herba-alba*, chemical composition, essential oil, GC/MS

INTRODUCTION

Known since antiquity, aromatic plants are used as all plants in medicine, perfumery, cosmetic and culinary flavoring, they are part of our daily life without us knowing.

In our days, progress in biochemistry and organic and pharmacological analysis has revealed that a plant species is a factory that can synthesize thousands of different chemical constituents

and begin a rational sort through the mass actions assigned to plants. Indeed, these constituents are classified into two types of primary and secondary metabolism. Secondary metabolites are products with complex chemical structures often widely dispersed and very different depending on the species. There are more than 200,000 secondary metabolites classified according to their chemical affiliations (Bouheroum, 2007). We cite for example, essential oils and phenolic compounds more particularly flavonoids.

Essential oils of plants are beginning to have a lot of interest as a potential source of natural bioactive molecules. They are the subject of study for their possible use as an alternative for the treatment in infectious diseases and to protect food against oxidation. These oils are used in aromatherapy, pharmacy, perfumery and cosmetics (Kanko *et al*., 2004) because of their wealth through active ingredients which are loaded with vital energy naturally.

Artemisia herba-alba Asso (chih), belonging to the family "Asteraseae"; this is among the most medicinal plants used by the local population because of their medicinal properties as well as a flavoring in tea and coffee (Bezza *et al*., 2010). The plant known as the "wormwood" is characteristic of the steppes of the Middle East and North Africa. It is extensively used to treat stomach disorders, hepatic, in addition to a wide variety of ailments and against certain forms of poisoning, also as antitumor agent, antispasmodic, antiseptic, antigenotoxic, antidiabetic and antibacterial (Abu-Irmaileh and Afifi, 2003; Bezza *et al*., 2010; Mighri *et al*., 2010).

Because of these ethno-botanical surveys, the most interesting of this study was to be able to determine the chemical composition of the essential oils extracted from the arial parts of a plant, to explore the effect of these oils on some pathogenic bacterial strains and to use their antioxidant power as a natural alternative.

MATERIALS AND METHODS

Vegetal material: Arial parts of *Artemisia herba-alba* were collected in April 2013 from mount of Boukhil in Djelfa region (Algeria) coordinates (N 36°52'18.011"E 6°53'14.786') and then dried in the shade for 10 days. The plant was identified by Dr. Abdelmadjid Chahma a botanist in Department of Biology, Ouargla University, Algeria, a specimen was deposited at the herbarium of the University under the number GO2013-3.

Extraction of essential oil: The extraction of essential oil was carried by steam distillation, in a Clevenger apparatus by immersing 100 g of dry leaves in a flask of 1000 mL of water for 3 h. The essential oil obtained was dried with $MgSO_4$ and kept in the refrigerator at 4°C.

Gas chromatography-mass spectrometry essential oil analysis: The essential oil was analyzed in INRAP (National Institute of Research and Physico-chemical analysis) Tunisia, using an Agilent 6890 gas chromatograph coupled to an Agilent 5975B mass selective detector with electron impact ionization (70 eV) and an Agilent Chemstation software (Agilent Technologies, Palo Alto, USA). Separation of oil constituents was performed on HP-5MS; 5% phenyl methyl siloxane capillary column (30 m×0.25 mm, film thickness 0.25 μm) in the split mode (1:50) at 250°C. The oven temperature was programmed at 50°C for 1 min, raised to 300°C at 2°C min^{-1} and finally held at this temperature for 10 min. Helium was used as carrier gas at a flow of 0.8 mL min^{-1}. Linear Retention Indices (RI) for all compounds were determined using n-alkanes as standards. Identification of individual compounds was performed by matching their patterns mass spectral fragmentation with corresponding data (NIST 05 and Wiley7 libraries) and by the laboratory database.

Antibacterial activity

Bacterial strains used: The microbiological material consists of eight bacterial pathogens strains, responsible of some serious infectious diseases. These bacteria are *Escherichia coli*, *Klebsiella pneumoniae*, *Leisteria monocytogenes*, *Proteus*, *Pseudomonas aeruginosa*, *Salmonella enterica, Staphyloccous aureus* and *Staphyloccous* sp. They come from the microbiology laboratory of the hospital Mohamed BOUDIAF-Ouargla-and microbiology laboratory of the University Kasdi Merbah, Ouargla.

Disc diffusion method: The evaluation of the antibacterial activity of *A. herba-alba* essential oil was performed using the disc diffusion method according to the NCCLS recommendations (NCCLS., 2000).

The disc method is a technique of distributing products to test from a paper disc that can qualitatively measure the sensitivity of antimicrobial effects (Aouni *et al*., 2013).

This method has been chosen in this study for its reliability and simplicity. It will provide preliminary results on the sensitivity of the strains and antibacterial activities of the product through the diameters of inhibition appearing around the discs.

The bacterial strains were spread on the Mueller Hinton Agar (MHA). Discs (6 mm, Whatman #3) impregnated in the essential oil were placed on the surface of such media and incubated at 37°C for 24 h. All assays were performed three times.

Determination of the Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of oil was determined for microbial strains by disk diffusion in Mueller-Hinton according to Benabderrahmane *et al*. (2009) with some modification. Because of the non-miscibility of essential oils in water, it was diluted in DMSO (dimethyl sulfoxide) to get a concentration range of 1-0.01 mg mL^{-1} and then incorporated into discs of 6.0 mm diameter by 0.01 mL. The same volume of DMSO was used as control. The microbial suspensions were calibrated according to the standards (0.5 McFarland equivalent that are 10^8 CFU mL^{-1}) (Cavallo *et al*., 2005), 0.1 mL of inoculum then inoculated into the agar immediately.

The discs containing various concentrations of oil were placed directly on the surface of the agar. Under 37°C during 24 h. The MIC was the lowest concentration of essential oil required for completely inhibit the growth of the tested microorganisms around the disc.

Antioxidant activity

Free radical scavenging effect: Antiradical activity of *Artemisia herba-alba* essential oil was evaluated by measuring the scavenging activity on the 2,2-diphenyl-l-1-picrylhydrazil (DPPH) radical, using the method described by Braca *et al*. (2002) and Mighri *et al*. (2010), with slight modification. A various dilutions of essential oil and DPPH radical solution were prepared in absolute ethanol. One milliliter of each sample concentration was mixed with a same volume of 0.1 mM DPPH. The reaction was carried out at room temperature in the dark for 30 min and after that, the absorbance was recorded at 517 nm. Mixture of 1 mL of DPPH solution and 1 mL of ethanol was taken as a blank. Ascorbic acid was used as a positive control. Inhibition of DPPH free radical in percent (I%) was estimated in following way:

$$I(\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}} \right) \times 100$$

where, A_{blank} is the absorbance of the control reaction, A_{sample} is the absorbance of the test essential oil or Ascorbic acid. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate.

Ferric reducing power: According to the method described by Oyaizu (1986), we determined iron reducing activity of our essential oil. Sample of 1.0 mL of various dilutions was mixed with 2.50 mL of phosphate buffer (0.2 mol L^{-1}, pH 6.6) and 2.5 mL of 1% potassium-ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to it, then later was centrifuged at 3000 rpm for 10 min. After that, 2.5 mL of this mixture was added to 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%), vigorously mixed, finally the absorbance was measured at 700 nm. Essential oils were diluted in ethanol. Blank sample similarly prepared by replacing extracted with ethanol, to calibrate the instrument (UV-VIS spectrophotometer). Ascorbic acid (in the 10-100 μg mL^{-1} range) was the positive control where the absorbance was measured in the same conditions as samples. EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power and obtained by interpolation from linear regression analysis (Piaru *et al*., 2012). Increased absorbance of mixture reaction indicate increasing power reduce (Singh *et al*., 2011).

RESULTS AND DISCUSSION

Chromatographic analysis: *Artemisia herba-alba Asso (chih),* volatile oil gave yield of 0.54%, with yellowish color and a very strong and persistent odor of chih.

The chromatographic analysis of the essential oil extracted from the Djelfa's region has allowed the identification of Thirty-three compounds, representing about 97.54% of the whole harvested plant HE (Table 1).

In recent years, the *Artemisia herba-alba* essential oil has been carefully studied; its composition diversity is caused by plants growth in different countries; even those from different localities in the same country have led to many chemotypes. The essential oil of *Artemisia herba-alba* from South Algeria is mainly composed of Davanone (42.8%), Camphor (15.96%) and Thujone (9.63%), which represent 68.39% of our oil's total composition. According to the previous reports concerning this species, this major compounds combination of the *Artemisia herba-alba* was not reported in any other parts of the world.

The Davanone was reported as the main component of 11 samples from the southern region of spain followed by the 1, 8-cineole or chrysanthenone and the cis-chrysanthenol (Salido *et al*., 2004).

Most of the time, the oil has been widely reported to be mainly composed of oxygenated monoterpenoids, such as 1,8-cineole, chrysanthenone, chrysanthenol (and its acetate), α/β-thujone and camphor (Dob and Benabdelkader, 2006; Bezza *et al*., 2010; Mighri *et al*., 2010; Paolini *et al*., 2010).

The change in the chemical composition of the essential oils could be attributed to the geographical origin of the plant, the extraction technique, time of harvest and the climatic factors (Smith *et al*., 2005; Figueiredo *et al*., 2008).

Antibacterial activity: During our investigations, the antimicrobial activity was evaluated by observing the inhibitory power of our essential oil sample of *Artemisia herba-alba* at different concentrations on the bacteria to be tested. The results are summarized in Table 2. The values

Table 1: Chemical composition of the essential oil of the algerian's *Artemisia herba-alba*'s aerial part

Compounds	RT	Aera (%)
Tricyclene	715	0.12
1R-α-pinene	762	0.45
Camphene	823	2.91
Sabinene	929	0.37
L-β-pinene	939	0.21
β-myrcene	1020	0.94
β-cymene	1184	0.25
Eucalyptol	1219	6.50
γ-terpinene	1365	0.17
Thujone	1650	9.63
Trans-8-hydroxylinalool	1681	0.35
β-Thujone	1708	1.52
1, 2, 5, 5-tetramethyl-1,3-cyclopentadiene	1780	6.56
Camphor	1893	15.96
Pinocarvone	1983	0.30
2-Nonyne	2013	1.36
L-4-terpineol	2085	0.53
trans-chrisanthenyl acetate	2617	0.64
L-bornyl acetate	2764	0.24
α-terpinene	3172	0.29
cis-jasmone	3483	0.35
β-cubebene	3946	1.24
Bicyclogermacrene	4036	0.35
Davana ether	4176	0.94
Caryophyllene oxide	4524	0.65
Davanone	4664	42.80
Nerolidol	4684	0.17
β-dihydroagarofuran	4698	0.39
Cyclohexane ketone	4767	0.21
α-pinene oxide	4828	0.33
α-himachalene	4884	0.28
Lilac alcohol	4944	0.53
Total		97.54

RT: Retention index

Table 2: Antibacterial activity of the essential oil of the algerian *Artemisia herba-alba*'s aerial part

Micro-organisms	Disc diffusion assay (inhibition zone mm)	MIC (mg mL^{-1})
Gram negative		
Escherichia coli	12.20±0.52	0.83
Salmonella enterica	18.43±0.51	0.25
Proteus	11.13±0.23	-
Klebsiella pneumoniae	22.13±0.64	0.12
Pseudomonas aeruginosa	12.03±0.45	0.71
Gram positive		
Staphylococcus aureus	10.17±0.76	-
Listeria monocytogenes	19.37±0.40	0.2
Staphylococcus sp.	23.10±0.85	0.16

Values are expressed as Mean±SD (n = 3)

shown are the average of three measurements. The inhibitory action results in the appearance of an inhibition zone around the paper disc impregnated with crude studied extract. The diameter of the inhibition zone varies from one bacterium to another. As it has been reported in the literature, we considered that an extract posses a bacteriostatic action if its inhibitory diameter is larger than 8 mm (Moreira *et al*., 2005).

Indeed, the essential oil of *Artemisia herba-alba* showed a significant inhibitory effect against the studied microorganisms.

The most susceptible microorganisms to this essential oil were the *Salmonella enterica*, the *Klebsiella pneumoniae* of gram negative, the *Listeria monocytogenes* and the

Staphylococcus sp. of gram positive whose growth was stopped at a minimal inhibitory concentration, respectively, of 0.25, 0.12, 0.2 and 0.16 mg mL^{-1}.

Following these results, the essential oil is considered moderately active against the *Escherichia coli* and *Pseudomonas aeruginosa* strains, with an inhibitory diameter of 12.2, 12.03 mm and a minimal inhibitory concentration, respectively, of 0.83 and 0.71 mg mL^{-1}.

Only the strains of *Staphylococcus aureus* are the most resistant, this justifies why our essential oil's composition has no power over that kind of microbial strains.

According to Oussalah *et al*. (2007), the biological activity of an essential oil is to be put in relation with its chemical composition, the functional groups of the main components (alcohols, phenols and aldehydes) and the synergistic effects between the components.

Most of the works that had for object the study of the compounds action mechanism of the active essential oils, affirm that their main zone of action is the bacterial plasma membrane (Shunying *et al*., 2005). They are able to disintegrate the bacteria cell membrane (Silva and Junior, 2010). The membrane loses its structure and becomes more permeable to ions. The lesion on the cell membrane can also allow the dissipation of the pH gradient and the decrease of the membrane's potential (Lambert *et al*., 2001).

The antimicrobial action of our oil can be related to its richness in three main compounds (Davanone, Camphor and Thujone), which are known for their antibacterial activity against several bacterial strains tested (Juteau *et al*., 2002; Delamare *et al*., 2007; Lopes-Lutz *et al*., 2008). The combined action (synergy) of different compounds at the origin of this extract may explain the variation in results between same species from different regions of the world. According to Oussou *et al*. (2004), these molecules would act mostly by a synergistic action, or alone in the essential oil. In addition, these minor compounds can significantly contribute to the essential oils activity (Lahlou, 2004; Kordali *et al*., 2005).

Antioxidant activity: The essential oil's antioxidant activity was determined by two different methods. The results are summarized in Table 3.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable radical, purple in solution and having a maximum absorption characteristic at 517 nm. The routine protocol applied is based on the disappearance of this maximum when the DPPH is reduced by an antiradical property compound, causing the color change to yellow. The IC_{50} value (otherwise known as the inhibitory concentration at 50%) is determined for our oil and represents the standard used. It is defined as the concentration of the required sample to cause a 50% decrease in the absorbance of the initial solution of DPPH. The IC_{50} are inversely proportional to the scavenger effect whose low values reflect a significant anti-radical effect (Villano *et al*., 2007).

From Table 3, t he IC_{50} values obtained reveal that the ascorbic acid showed the lowest IC_{50}; with an IC_{50} value of (IC_{50} = 6.42±0.36 μg mL^{-1}) compared to our essential oil (IC_{50} = 17.73±0.29 μg mL^{-1}), which reflects a significant anti-radical potential. It appears from these results that the vitamin C (ascorbic acid) is the most effective antioxidant compared with the essential oil studied.

Table 3: Antioxidant activities of the *Artemisia herba-alba*'s essential oils

	Scavenging activity (μg mL^{-1})	
Antioxidants	DPPH IC_{50}	FRAP EC_{50}
Artemisia herba alba essential oil	17.73±0.29	12.53±0.25
Ascorbic acid	6.42±0.36	66.73±0.37

Values are expressed as Mean±SD (n = 3)

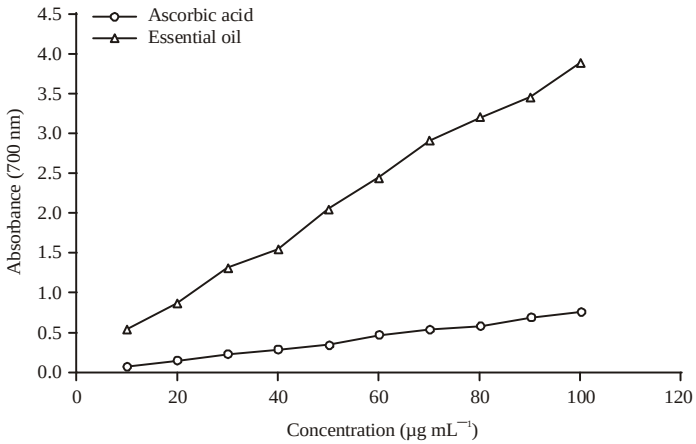

Fig. 1: Reducing power of the *Artemisia herba alba's* essential oil

For the reducing power, the antioxidant activity of the sample is based on the reduction of the ferric ion (Fe^{3+}), present in the $[K_3Fe(CN)_6]$ complex, of ferrous ion (Fe^{+2}) which causes the transformation of the potassium ferricyanide's yellow color to a blue one in a reaction medium at 700 nm, which intensity depends on the essential oil's reducing power. The essential oil of *Artemisia herba-alba* showed an antioxidant activity with an EC_{50} value of 12.53±0.25 µg mL^{-1}, while the ascorbic acid as a witness gives a value of 66.73±0.37 µg mL^{-1}. Figure 1 shows that, the reducing power increases with the concentration of samples and has clearly demonstrated that the essential oil possess a more effective antioxidant activity compared with the witness.

In several reports, the antioxidant activity of the essential oils can be linked to the phenolic content. Indeed, the comparative study on the DPPH radical's reducing ability by different chemotypes has proved that the phenolic chemotypes had showed *in-vitro*, more expressed and much stronger antioxidant capacities than those of the non-phenolic chemotypes (Jukie and Milos, 2005). In our case, despite the low content of phenolic compounds in the essential oil of *Artemisia herba-alba* (Table 1), this oil has showed an antioxidant activity which sparks an interest for its therapeutic use and cosmetic applications. This result is probably due to a synergistic or antagonistic interaction between the different essential oil constituents that can create an effective system towards the free radicals which could explain this tendency. It has been reported by several authors that the synthetic antioxidants have more ability to trap the DPPH radical than the essential oils (Tepe *et al*., 2005; Kizil *et al*., 2010; Nanasombat and Wimuttigosol, 2011).

CONCLUSION

In order to search for new antibacterial natural agents and antioxidants with therapeutic advantages, the essential oil from a very abundant medicinal plant in the Algerian flora, *Artemisia herba-alba* was taken as the subject of a phytochemical study.

The chemical profiles of the investigated oil were highlighted by Davanone, Camphor and Thujone as major compounds. A considerable degree of antibacterial and antioxidant activities were found in the essential oil evaluated in this study. Our results clearly demonstrate that the essential oils of *Artemisia herba-alba* can be an interesting natural alternative that can be useful for food preservation and pharmaceutical treatment.

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