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ESSENTIAL OIL OF *ARTEMISIA HERBA ALBA* FROM MOROCCAN SAHARA: CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES

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Abstract

Essential oils are widely used in aromatherapy, pharmacy, perfumery and cosmetics. Their use is linked to their various recognized biological activities. After extracting the essential oils from the aerial part of *Artemisia herba alba* by hydrodistillation using an " Alambic" pilot extractor, the chromatography analysis was carried out by gas chromatography coupled with mass spectrometry (GC-MS) for the determination chemical compositions and chemotype identification. The results showed that the essential oil of *Artemisia herba alba* is characterized by the presence of α-thujone (28.92%), (+) - 2-Bornanone (22.27%), and Pulegone (14.42%). These three majority compounds were obtained with a percentage of (65.61%). The antimicrobial power has been studied *in vitro* against Gram-positive bacteria: *Streptococcus pneumoniae* (CECT 8737), *Listeria monocytogenes* (CECT 911), *Staphylococcus aureus* (CECT 86), and *Enterococcus faecalis* (CECT 4176), bacteria Gram negative: *Pseudomonas aeruginosae* (CECT 108), *Yersinia enterocolitica* (CECT 4315), *Klebsiella pneumoniae* (CECT 7787), *Escherichia coli* (CECT 516). The results of the antimicrobial tests of the essential oil of *Artemisia herba alba* showed a very high activity during the treatments, this activity probably due to the major constituent.

Keywords: *Artemisia herba alba; essential oils; physico-chemical properties; GC-MS; antimicrobial activities.*

Introduction

In Morocco, physiographic and bioclimatic diversity constitutes an explanatory variable for the richness and floristic diversity, particularly in terms of medicinal and aromatic plants (MAPs) **[1]**. Medicinal flora is widely used by people who have expertise in its use, cultivation and conservation. In fact, most of the rural populations use MAPs as remedies for their health problems, and use them in cosmetology, perfumery and food **[2-3]**. Essential oils are secondary metabolites, are also of scientific interest in addition to economic interest; This is evidenced by the number of publications encountered on this subject in the literature and the number of plant species whose essences are marketed **[4-6]**. The scientific and economic interest of natural essences resides, moreover, in the fact that the systematic investigation of aromatic plants often provides new molecules, which serve as models for the industrial synthesis of structural analogues **[7-9]**. Essential oils, with their broad spectrum of action against a large number of applications, constitute a very promising alternative, without being a source of danger to human health or of pollution to the environment. Essential oils are widely used in aromatherapy, pharmacy, perfumery, cosmetics and the food industry **[10-11]**. Their use is linked to their various biological activities recognized, for example, as antifungals, antibacterials, anti-insects or antivirals **[12]**. They have been used in alternative medicine for a very long time for their antimicrobial and especially antifungal properties **[13]**. The chemical composition of essential oil is strongly influenced by biotic and abiotic factors. It depends on climatic, seasonal and geographical conditions as well as on the harvest period of the plant or even on the extraction technique **[14]**.

The genus *Artemisia* belongs to the Asteraceae family, with over 350 different species found mainly in arid and semi-arid areas of Europe, America, North Africa and Asia **[15]**. *Artemisia herba-alba* or white mugwort is a species often called steppe thyme or desert wormwood, it is known in Morocco under the Arabic name of "chih" **[16]**. *Artemisia herba-alba* is a small perennial bush 20 to 40cm tall. It can be recognized by its stems bristling with small, fleshy, pubescent leaves, but above all by its smell, so strong that on a hot day it is blown by the wind into the passenger compartment of a passing car allows it to grow spontaneously in arid and steppe regions. When grown, it pairs well with other plants **[17]**. White grass mugwort makes an excellent border plant. It is also used as a background plant to showcase perennials with brightly colored flowers. It is drought and salinity resistant and very easy to grow. She likes well-drained soils and full sun. It is an essentially fodder plant, very appreciated by the cattle. The flowering tops of white mugwort contain essential oil which gives it softening and purifying properties. This oil is mainly used in cosmetics. In Morocco, nomads use it for its purgative, febrifuge (drug that helps fight fever), deworming and antidiabetic properties. The decocted of the aerial parts is effective for intestinal bloating and aerocolies (increase in the size of the colon). In traditional medicine, the macerate of the leaves is used to treat bronchitis, coughs, diabetes, hypertension and diarrhea **[18-20]**. The dried leaves are used as a decoction, while the essential oil is used for rubbing or massaging against rheumatism, such as on painful parts of the body **[21]**.

The studies carried out during this thematic focus mainly on the valorization of Moroccan aromatic and medicinal plants and essential oils, in particular the chemical composition and the biological powers of *Artemisia herba alba.*

Material and methods

Plant Material

In this study *Artemisia herba alba* was collected from the region of Guelmim (Moroccan Sahara), these species were verified by a botanist at the Forestry Research Center of Khenifra (Morocco).

Essential oil extraction

The extraction of the essential oil was carried out by hydrodistillation using a pilot extractor « mini Alambic » for 2 hours, in a laboratory at the École Supérieure des Technologies khénifra-Maroc. The amount of 10 kg of dry plant material was used with a volume of 50 liters of water. The temperature of the device is set at 100 ° ± 5 ° C, to promote maximum evaporation of water and essential oil without destroying it. This method is recommended by the European Pharmacopoeia **[22]**. The essential

oil is collected and weighed to calculate the yield, then stored in opaque and hermetically sealed bottles at 4 ° C ± 1. This essential oil will be studied and analyzed. The yield was calculated according to the formula: R% = (MHE / MVS) x 100. MHE: mass of the essential oil and MVS: mass of the dry plant material. The two masses are expressed in the same unit.

chemical compositions of essential oils

Chromatographic analyzes were performed on a Varian type gas chromatograph (model 3380) equipped with a fused silica capillary column: HP-5 J&W Agilent (5% phenyl 95% methyl polysiloxane; 30 m × 0.25 mm di × 0.25 µm) and a flame ionization detector (FID) set at 250 ° C. Injector temperature 200 ° C; split mode injection (leakage ratio: 1: 100) of 1 µl of a 10% solution of HE in hexane; the carrier gas used is nitrogen with a flow rate of 0.8 ml / min; oven temperature programmed from 50 to 200 ° C at a rate of 5 ° C / min, then maintained at 200 ° C for 20 minutes. In the case of EO analysis, the dichloromethane extract is analyzed without prior dilution. The GC / MS analyzes were carried out on a gas chromatograph of the Hewlett-Packard type (GC 5890, series II) equipped with an HP-5 fused silica capillary column (5% phenyl 95% methyl polysiloxane; 30 m × 0.25 mm id × 0.25 µm). The mass detector is of the quadrupole type (Model 5972), the ionization energy used is equal to 70 eV. The temperature of the column is programmed from 70 to 200 ° C at a rate of 10 ° C / min. Injection in split mode 1:10; quantity injected: 1 µl of an EO solution diluted to 10% in hexane. The injector temperature is set at 220 ° C and the transfer temperature is 180 ° C. The carrier gas is helium, the flow rate of which is set at 0.6 ml / min; ionization energy 70 eV; electron multiplier 1400 eV; scanning area [35–300]; scanning speed 3 scans / s. The identification of the constituents was carried out on the basis of their retention indices calculated by comparing the retention times with those of a series of alkanes (C_9 – C_{20}) and by comparing their mass spectra with those of the NBS libraries. 75K / NIST 98, from the literature and from the laboratory library (constituted from isolates of formally identified natural raw materials). The relative percentages of the different constituents are obtained by electronic integration of the signals obtained by CG-DIF (assuming that all the constituents are characterized by the same response coefficient with respect to the detector) **[23]**.

Antibacterial test

The evaluations of the properties of essential oil of *Artemisia herba* were carried out against Gram-positive bacteria such as: *Streptococcus pneumoniae* (CECT 8737), *Listeria monocytogenes* (CECT 911), *Staphylococcus aureus* (CECT 86), and *Enterococcus faecalis* (CECT 4176), Gram-negative bacteria: *Pseudomonas aeruginosae* (CECT 108), *Yersinia enterocolitica* (CECT 4315), *Klebsiella pneumoniae* (CECT 7787), *Escherichia coli* (CECT 516).

In the first test, the diameters of the zones of inhibition of EO were determined by the method of Ainane et al. (2014) **[24]** from 24-hour cultures (10^5 - 10^6 CFU / mL). Flood seeding was done from the obtained inoculum, solubilizing the colonies in sterile distilled water and agar. The obtained inoculum (1 mL) is poured into Petri dishes containing Mueller Hinton's agar. The excess inoculum was subsequently aspirated, and the dishes were dried in an oven (37 ° C). After 15 min of drying, the wells were cut out using Pasteur pipettes (6 mm thick end). Then, each EO fraction (50 µL) and gentamycin (50 mL) (control) were distributed into each well. After diffusion, the cultures were incubated in incubators at 37 ° C for 24 h. The inhibition halos were measured with a caliper. The activity is considered zero for a diameter of the inhibition zone less than or equal to 8 mm; low for a diameter of the inhibition zone between 8 and 14 mm, medium for a diameter of the inhibition zone between 14 and 20 mm; strong for a diameter of the inhibition zone greater than or equal to 20 mm.

For the determination of the minimum inhibitory concentration (MIC), a medium consisting of sterile Mueller Hinton broth and a sterile solution of tween 80 was prepared in order to obtain a homogeneous distribution of the EO fractions in the medium, and to maximize their miscibility in the medium. For the determination of the MIC, 10 test tubes were used. The first eight were used to prepare concentration ranges (80; 40; 20; 10; 5; 2.5; 1.25 and 0.625 mg / mL) of each EO fraction; the last two containing the positive and negative controls. In the test tubes containing each fraction of EO and the positive

control, 10 µL of the inoculum was introduced. After 24 h incubation of the tubes at 37 ° C, the MIC corresponding to the lowest concentration of essential oil capable of inhibiting bacterial growth after 18 to 24 h of contact, was determined **[25].** The concentration ranges used for the determination of the MIC were used to measure the CMB. Samples were taken from the control tube and from each of the tubes without a bacterial pellet, then deposited "streaked" on MHA agar. The inoculated dishes were incubated for 24 h at 37 °C **[25]**.

Results and discussion

Essential oil analysis

The average yield of *Artemisia herba alba* essential oil was calculated based on the dry plant material obtained from the aerial parts (stems, leaves and flowers). The essential oil yield obtained is given in Table 1.

The average essential oil yield of *Artemisia herba alba* was calculated based on the dry plant matter of the above-ground part of the plant. The average yield obtained from essential oils extracted from the studied *Artemisia herba-alba* plant is of the order of 1.08%. This rate is relatively higher than that of essential oils extracted from the same species collected in Tunisia in the region of Matmata (0.65%) **[26]**, and the same species collected in Algeria in the region of M'sila (1.02%) **[27]**, and in the Biskra region (0.95%) **[28]**. On the other hand, our results are significantly lower than that carried out in Jordan (1.3%) **[29]**. On the other hand, the rate of return of essential oil of the species *Artemisia herba-alba* varies according to the harvest period, in which in Spain it varies according to the provenance, from 0.41% to 2.30% **[30]**, and in the Guerçif region in Morocco; it varies between 0.56% and 1.23% **[31]**. We can therefore conclude that there are several factors that influence the yield among these factors the harvest season the origin of the plant the altitude it is the part used and we do not forget the initial process of extraction.

The analysis of the results of the chemical composition carried out by gas chromatography coupled with mass spectrometry of the essential oil of *Artemisia herba alba* studied is listed in Table 2. Analysis of the results given in Table 2 showed that *Artemisia herba alba* contains 36 biologically active compounds. The main major chemical compounds of essential oil identified are: α-thujone (28.92%), (+) - 2-Bornanone (22.27%), and Pulegone (14.42%). These three majority compounds were obtained with a percentage of (65.61%).

The results obtained by Touil *et al.*, 2012 **[32]**, carried out in Algeria show that the essential oil of *Artemisia herba alba* from Djelfa is mainly composed of davanone (62.20%) accompanied by other constituents at relatively low levels: carvacrol (4.88%), davana ether (3.62%), camphor (3.48%), eucalyptol (2.24%). The essential oil of *Artemisia herba alba* from Djelfa is widely different from that of the region of M'sila which is dominated by camphor (19.4%), trans-pinocarveol (16.9%), chrysanthenone (15.8%) and β-thujone (15%) **[27]**. It is also different from the essential oil of *Artemisia herba alba* from Biskra which mainly contains cis-chrysanthenyl acetate (25.12%), 2E, 3Z-2-ethyliden-6-methyl-3, 5- heptadienal (8.39%), α-thujone (7.85%), myrtenyl acetate (7.39%), verbenone (7.19%), chrysanthenone (4.98%) **[28]**. This is also the case for the essential oil of *Artemisia herba alba* from Tunisia **[26]**, for which have shown that the essential oil of this species from Matmata is dominated by α-thujone (43.85%), sabinyl trans-acetate (17.46%) and β-thujone (10.10%) accompanied by 1,8-cineole (3.30%), chrysanthenone (2.32%) and chrysanthenyl acetate (3.93%). While Davanone has been found to be the major constituent of the essential oil of *Artemisia herba alba* from Morocco **[31]** and Spain **[30]**. The variations encountered in the chemical composition of essential oils, from a qualitative and quantitative point of view, can be due to certain ecological factors, to the part of the plant used, to the age of the plant and to the period of the vegetative cycle, or even genetic factors.

Antimicrobial activities

The results of the antibacterial evaluations of all the methods applied to the essential oil of *Artemisia herba alba* are expressed in terms of diameters (Φ) of zones of inhibition measured around the discs, of the minimum inhibitory concentration (MIC), of the minimum bactericidal concentration (CMB) and ratio (CMB / MIC). All the values obtained are mentioned in (Table 3). From the results obtained, it can be concluded that the essential oil of *Artemisia herba alba* possesses interesting antimicrobial activities

against the strains studied. The diameters of zones of inhibition Φ obtained from the disk diffusion method vary from 12.48 to 16.02 mm, all the strains show a very important and strong activity. The minimum MIC inhibitory concentrations vary from 25.50 to 183.41 µL / mL and the minimum bactericidal CMB concentrations vary from 25.96 to 376.82 µL / mL. So in general, the essential oil of *Artemisia herba alba* shows remarkable activities against all the strains studied. According to the CMB / CMI ratio, the essential oil of *Artemisia herba alba* has bactericidal effects against strains: *Streptococcus pneumoniae* (CECT 8737), *Listeria monocytogenes* (CECT 911), *Staphylococcus aureus* (CECT 86), *Pseudomonas aeruginosae* (CECT 108), *Klebsiella pneumoniae* (CECT 7787), *Escherichia coli* (CECT 516), While *Enterococcus faecalis* (CECT 4176) and *Yersinia enterocolitica* (CECT 4315) has bacteriostatic effects (Table 4). In general, it has also been established in numerous studies that the activity of an essential oil is related to the majority compounds and the possible synergistic effects between the constituents.

Conclusion

Medicinal plants represent an inexhaustible source of natural bioactive substances and compounds. This work is part of the promotion of Moroccan aromatic and medicinal plants. *Artemisia herba Alba* was harvested from Moroccan Sahara, precisely in the Guelmim region. Chromatographic analyzes using GC-MS identified 36 constituents, including 3 major chemotypes: α-thujone (28.92%), (+) - 2-Bornanone (22.27%), and Pulegone (14.42%). The chemical composition of essential oil is strongly influenced by biotic and abiotic factors. It depends on climatic, seasonal and geographical conditions as well as on the harvest period of the plant or even on the extraction technique. The *in vitro* antibacterial tests carried out revealed significant bacterial activity against all the strains studied. The results obtained in this *in vitro* study are obviously only a first step in the search for new biologically active molecules. Additional studies would open a door wide open to the discovery and characterization of the active ingredients contributing to these biological activities.

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Table 1. Yield of essential oil.

Species	Yield
Artemisia herba alba	1.08 %

Table 2. Chemical composition of the essential oil of *Artemisia herba alba.*

Pic	RT	Components	(%)
1	8.49	Camphene	1.92
2	9.19	β-Terpinene	0.18
3	9.84	Mesitylene	0.22
4	10.84	meta-Cymene	0.67
5	11.00	3-Carene	0.32
6	**11.52**	**Eucalyptol**	**2.97**
7	14.02	3,3-Dimethyl-6-methylenecyclohexene	1.67
8	14.21	Z,Z,Z-8,9-Epoxyeicosa-5,11,14-trienoic acid, methyl ester	0.21
9	14.55	Thujone	1.42
10	**15.24**	**α-thujone**	**28.92**
11	**16.09**	**(+)-2-Bornanone**	**22.27**
12	16.28	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-	0.19
13	16.44	5H-Inden-5-one, 1,2,3,3a,4,7a-hexahydro-7a-methyl-, trans-	0.35
14	**16.57**	**endo-Borneol**	**2.07**
15	16.85	L-4-terpineneol	0.83
16	18.60	Chrysanthenone	0.14
17	**19.42**	**Pulegone**	**14.42**
18	19.78	verbenyl	0.37
19	19.92	Piperitone	0.73
20	20.54	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	0.21
21	20.71	Borneol	0.52
22	21.20	Carvacrol	0.33
23	22.21	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-	0.90
24	**23.13**	**cis,cis-Photocitral A**	**3.57**
25	23.42	1(2H)-Naphthalenone, octahydro-4-hydroxy-, trans-	0.21
26	23.60	α-Copaene	0.27
27	25.18	Caryophyllene	0.86
28	26.33	Humulene	0.19
29	26.57	Alloaromadendrene	0.15
30	**27.25**	**(+)epi-Bicyclosesquiphellandrene**	**2.17**
31	27.75	γ-Muurolene	0.72
32	28.49	β-Cadinene	0.35
33	30.23	Butane-1,1-dicarbonitrile, 1-cyclohexyl-3-methyl-	0.17
34	30.81	3-Methyl-2-butenoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	0.57
35	31.08	Guaia-1(10),11-diene	0.19
36	**45.62**	**i-Propyl 14-methyl-pentadecanoate**	**2.54**
Total			**93.79**

Table 3. Parameters of antibacterial activity of essential oil of *Artemisia herba alba.*

Strains	Applied methods	*Artemisia herba alba*
***Streptococcus pneumoniae* (CECT 8737)**	**Φ (mm)**	12.76 ± 0.23
	CMI (µL/mL)	61.57
	CMB (µL/mL)	61.85
	CMB/CMI	1.00
***Listeria monocytogenes* (CECT 911)**	**Φ (mm)**	14.65 ± 0.45
	CMI (µL/mL)	57.59
	CMB (µL/mL)	57.05
	CMB/CMI	0.99
***Staphylococcus aureus* (CECT 86)**	**Φ (mm)**	15.52 ± 0.85
	CMI (µL/mL)	25.50
	CMB (µL/mL)	25.96
	CMB/CMI	1.01
***Enterococcus faecalis* (CECT 4176)**	**Φ (mm)**	13.35 ± 0.21
	CMI (µL/mL)	67.54
	CMB (µL/mL)	135.9
	CMB/CMI	2.01
***Pseudomonas aeruginosae* (CECT 108)**	**Φ (mm)**	15.97 ± 0.10
	CMI (µL/mL)	166.45
	CMB (µL/mL)	167.71
	CMB/CMI	1.00
***Yersinia enterocolitica* (CECT 4315)**	**Φ (mm)**	12.48 ± 0.76
	CMI (µL/mL)	183.41
	CMB (µL/mL)	376.82
	CMB/CMI	2.05
***Klebsiella pneumoniae* (CECT 7787)**	**Φ (mm)**	13.19 ± 0.44
	CMI (µL/mL)	86.16
	CMB (µL/mL)	86.28
	CMB/CMI	1.00
***Escherichia coli* (CECT 516)**	**Φ (mm)**	16.02 ± 0.37
	CMI (µL/mL)	34.89
	CMB (µL/mL)	35.09
	CMB/CMI	1.00

Table 4. Bactericidal or bacteriostatic character of essential oils.

Strains	*Artemisia herba alba*
***Streptococcus pneumoniae* (CECT 8737)**	Bactericidal
***Listeria monocytogenes* (CECT 911)**	Bactericidal
***Staphylococcus aureus* (CECT 86)**	Bactericidal
***Enterococcus faecalis* (CECT 4176)**	Bacteriostatic
***Pseudomonas aeruginosae* (CECT 108)**	Bactericidal
***Yersinia enterocolitica* (CECT 4315)**	Bacteriostatic
***Klebsiella pneumoniae* (CECT 7787)**	Bactericidal
***Escherichia coli* (CECT 516)**	Bactericidal