ANTIBACTERIAL ACTIVITY OF THE *Artemisia herba-alba* Asso ESSENTIAL OIL (SOUK AHRAS, ALGERIA) AGAINST FOURTEEN BACTERIAL STRAINS

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Abstract. *Artemisia herba-alba* Asso (*Asteraceae*) essential oil from Fedj el dib region (Souk Ahras, Algeria) was obtained by hydro-distillation of aerial parts (stems, leaves and flowers) and analyzed by gas chromatography-mass spectrometry (GC/MS) to determine its chemical composition. The main compounds obtained are: 5,6-Dicarbadecaborane (68,33%), 1,5,5-Trimethyl-6-methylene-cyclohexene (7.00%), 4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyle (5.13%), Bicyclo-[3.1.1]heptane-2-methanol (4.39%) and Cyclo-pentane carboxylic acid 3-methylene (4.19%), *Artemisia* essential oil also contains Phenol, 2,3,5-trimethyl-(3.15%), Phosphoric acid tribornyl ester (0.61%) and beta.-Pinene (0.29%) and the Thujone (0.16%). Further to the analytic study of essential oil, a biological study was carried out; the purpose of this biological study was to assess the antibacterial properties of *Artemisia herba-alba* Asso essential oil against fourteen Gram-positive and Gram-negative bacterial strains using disk diffusion method. The results obtained reveal that the *Artemisia herba alba* Asso essential oil exerts a strong antimicrobial activity against all tested pathogenic bacteria except *P. aeruginosa* and *Proteus vulgaris* which were resistant even to the highest concentration of essential oil.

Keywords: essential oil; *Artemisia herba-alba* Asso; hydro-distillation; GC/MS; antibacterial activity.

INTRODUCTION

In order to specify the tasks to be developed for medicinal plants research, it is necessary to make an inventory and analyze the current state of this field at all levels.

According to Yaniv and Bachrach (2005) [35], both phases find themselves in a race to develop new medicines, with fewer on no side-effects, for therapeutic and preventive application in illnesses for which causality-based treatment has been non-existent or imperfect.

Essential oils are very interesting natural products that have been used since ancient times, in many different traditional healing systems all over the world, because of their biological properties [33]; the term "biological" includes all activities that these mixtures of volatile compounds (mainly mono- and sesquiterpenoid, benzenoids, phenylpropanoids, etc.) exert on macro-organisms; humans, animals, plants and micro-organisms; bacteria, viruses, etc. [21]. Currently the resistance of pathogenic microorganisms to antibiotics poses a particularly serious public health problem. Indeed, this resistance renders treatment ineffective some times and requires the search for new antimicrobial agents. It is estimated that without an effective response, resistance to antibiotics might cause common infections to once again become fatal, and by 2050 lead to approximately 10 million deaths annually worldwide [25]. In this case and in recent years there has been a growing interest in research and development of new antimicrobial agents from various biological sources such as medicinal plants to combat microbial resistance [14, 32]. Especially that the essential oils have a very complex chemical structure. This explains its antimicrobial effectiveness; and the maladjustment of the bacteria in the medium containing the essential oil. In the present study, an antimicrobial activity of *Artemisia herba alba* Asso essential oil was investigated.

MATERIAL AND METHODS

Plant material

The aerial part; stems, leaves and flowers of the *Artemisia herba-alba* Asso. (*Asteraceae*) was harvested during the flowering period in July 2017 at Fedj el dib region (Souk Ahras, Algeria). The plant material was taxonomically identified by Pr. Azzedine chefrour (Department of Biology, Faculty of Sciences of Nature and Life, Mohamed Cherif Messaadia University Souk Ahras-Algeria), Subsequently, the Drug was dried at room temperature for twenty days so as to ultimately obtain a biological product ready for extraction of the essential oil with the hydro-distillation method.

Essential oil extraction

Artemisia herba-alba Asso essential oil was extracted by hydro-distillation, this method is described as used by BHUIYAN *et al*. (2009) [10], The aerial part was harvested and dried at room temperature then placed in a Clevenger device and subjected to distillation process for 3 to 4 hour [27]. The oil samples were then transferred to a dark glass bottle and stored at a temperature of 4°C [4] before analysis and bioassays tests, and the picture below (Figure 1) shows the essential oil extraction process.

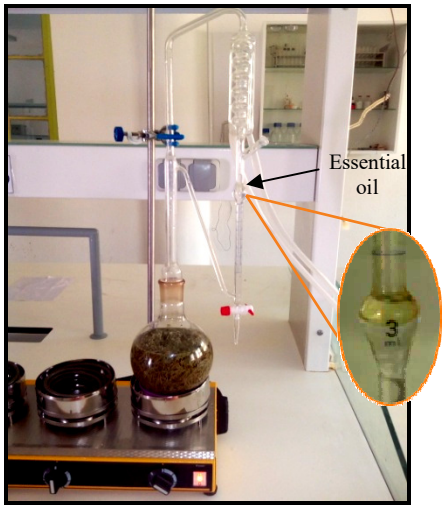

Figure 1. Extraction of essential oils by hydrodistillation method

Chromatographic analysis of the *Artemisia herba alba* essential oil

Essential oils are a very complex mixture of naturally occurring aromatic chemicals, known as its components. Identifying which chemicals exist in any one essential oil is not an easy task because as well as the approximately 30000 known aromatic molecules, there are many as yet unidentified components. On average, each essential oil is made up of between 100 and 300 components, although some contain fewer and some contain more [36]. To know the therapeutic properties of the essential oil, its components are usually separated and identified. Thus, the chromatographic separation phase of the primary oil is more than necessary before undertaking any biological study.

In our study, the composition of the essential oil hydro-distilled from the aerial part of *Artemisia herba alba* Asso was determined by GC/MS analysis; Gas chromatography-mass spectrometry analysis was performed using a GC Varian CP-3800 instrument coupled to a Saturn 2200 MS / MS mass spectrophotometer, the separation was carried out at using a capillary column (25 mx 0.2 mm x 0.11 µm) fed with helium as a carrier gas [29].

Method for evaluating antibacterial activity

Target microorganisms

The choice of strains is tested according to the use of traditional medicine of wormwood; fourteen bacterial strains were chosen: Nine Gram-negative bacteria were *Klebsiella pneumonia*, *Klebsiella pneumonia*$^{+}$ *(Klebsiella pneumoniae carbapenemase* positive*), Pseudomonas aeruginosa* (ATCC 27853 and VIN2), *Escherichia coli* (ATCC 25922 and BLSE), *Acinetobacter* (OXA-23 and *A. baumannii* (BMR)) isolates-resistant to all antibiotics and *Proteus vulgaris*, Five Gram-positive strains were *Staphylococcus aureus* (ATCC 43300, ATCC 29213, ATCC 25923 and NSSA), *Enterococcus faecalis* (ATCC 29212). The bacteria were acquired the laboratory of Development and Control of Hospital Pharmaceutical Preparations, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar-Annaba University (Algeria).

Measurement of antimicrobial activity of essential oils

An antimicrobial activity of *Artemisia herba alba* Asso essential oil was investigated using disk diffusion method (aromatogram method); in this method, as the name suggests, Sterilized paper discs (φ6 mm) were impregnated with known quantities (07µl) and various concentrations (1:1, 1:2 and 1:4) of essential oil placed on Petri dish containing a Mueller-Hinton agar that has been previously inoculated with a culture of the bacterium strain to be tested accustomed to 0.5 McFarland standard with sterile saline, then the samples are incubated at 37°C for 24 hours. After diffusion, the antibacterial activity was evaluated by measuring zone of inhibition (diameter mm) [7, 12]. The antibacterial test was performed in triplicate to avoid mistakes. The negative control in this experience was 10% DMSO [30].

Generally, microorganisms are classified as susceptible, intermediate or resistant, depending on the diameter of the inhibition zone [35].

Determinations of the minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of microorganism after 24h of incubation [8, 17, 28], MIC was determined only with micro-organisms that displayed inhibitory zones. In this context, the *Artemisia herba alba* essential oil was decimally diluted with Dimethyl Sulfoxide (DMSO) and pipetting 10 µl of each dilution into a sterilized paper disc (φ6 mm) placed on Petri dish containing a Mueller-Hinton agar that has been previously inoculated with a culture of the bacterium to be tested, A negative control was also included in the test using a paperdisc saturated with DMSO to check possible activity of this solvent against the bacteria assayed. Finally the Petri dishes are incubated at 37°C for 24 hours [16].

RESULTS

Yield of essential oil

The rate of essential oil of *Artemisia herba alba* harvested from the Fedj el dib region (Souk Ahras, Algeria) is 1.80 ±0.03%.

Organoleptic characteristics

The essential oil of *Artemisia herba alba* obtained by hydro distillation has a limpid liquid appearance, a very pale-yellow color, very aromatic odor and bitter flavor.

Organoleptic properties are a means of checking and controlling the quality of the essential oil. To this end, the results obtained are compatible those reported by AFNOR (1986) [1] having analyzed the essential oils of wormwood studied.

Chromatographic analysis of the *Artemisia herba alba* essential oil

Chemical composition of the extracted essential oil of *Artemisia herba alba* aerial part, grown in Fedj el dib (Souk Ahras, Algeria) was determined by gas chromatography-mass spectrometry (GC/MS); the constituents of this essential oil are listed in order of their appearance in Table 1 below. According to the result, twenty volatile compounds were identified; predominantly 5,6-Dicarbadecaborane (68,33%), 1,5,5-Trimethyl-6-methylene-cyclohexene (7.00%), 4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyle (5.13%), Bicyclo-[3.1.1]heptane-2-methanol (4.39%) and Cyclo-pentane carboxylic acid 3-methylene (4.19%), *Artemisia* essential oil also contains the Phenol, 2,3,5-trimethyl- (3.15%) and beta-Pinene (0.29%).

Antibacterial activity of *Artemisia herba alba* essential oil from souk ahras

The aromatogram test of the essential oil of *Arteùisia herba alba* from the Fedj el dib (Souk Ahras, Algeria) region is shown in Table 2.

The antimicrobial activity of the *Artemisia herba alba* essential oil against the microorganisms employed and its activity potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, its diameters and MIC values. Consistent with the result, essential oil is endowed with important antibacterial activity with all bacterial strains; except *Pseudomonas aeruginosa* and *Proteus vulgaris* which were resistant even to the highest concentration of essential oil. The maximum inhibition zone was observed at the level of *Acinetobacter* OXA-23 bacteria with (21 mm). On the other hand, the lowest inhibition zone was observed in *Klebsiella pneumoniae carbapenemase*(+) with 9mm, Disk diffusion test result of essential oil against the microorganisms employed is shown in Table 2. Based on the obtained results, we can conclude that the antimicrobial activity of the extracted essential oil is linked to its main components determined by Gas chromatography-Mass spectrometry.

Table 1. Chemical composition of *Artemisia herba-alba* Asso. essential oils from Souk Ahras (Algeria)

Peak	Constituents	*RT (min)	Air (%)
1	1,5,5-Trimethyl-6-methylene-cyclohexene	5 036	7.00
2	Camphene	5 334	2.12
3	3-Carene	5 720	0.16
4	beta-Pinene	5 857	0.29
5	Bicyclo[3.1.1]heptane-2-methanol, 6,6-di	6 009	4.39
6	4,8,12-Tetradecatrien-1-ol, 5,9,13-trime	6 154	5.13
7	Carbamic acid, N-phenyl-, 1,5-dimethyl-1	6 388	3.09
8	Phenol, 2,3,5-trimethyl-	6 593	3.15
9	5,6-Dicarbadecaborane(12)	7 375	68.33
10	Phosphoric acid, tribornyl ester	8 254	0.61
11	Phenol, 2,4,6-trimethyl-	8 360	0.42
12	Benzene, 4-ethenyl-1,2-dimethyl-	8 453	0.38
13	Cyclopentanecarboxylic acid, 3-methylene	8 609	4.19
14	3-Cyclohexen-1-ol, 1-methyl-4-(1-methyle	8 693	0.38
15	Thujone	8 759	0.16
16	2,6-Dimethyl-1,3,5,7-octatetraene, E,E	8 801	0.00
17	2-Cyclohexen-1-ol, 4-ethyl-1,4-dimethyl-	9 014	0.00
18	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]h	9 080	0.00
19	1,3,8-p-Menthatriene	9 189	0.18
20	Bicyclo[2.2.1]hept-2-en-7-ol	9 324	0.00

*RT: Retention time obtained by chromatogram.

Table 2. Inhibition diameters of *Artemisia herba alba* essential oil against fourteen bacterial strains

Bacterial strains	Single disc	DMSO disk	Inhibition zone (mm) with different essential oil concentrations			MIC
			Pure oil	Dilution 1:2	Dilution 1:4	
Pseudomonas aeruginosa ATCC 27853	6	6	6	6	6	/
Pseudomonas aeruginosa VIN2	6	6	6	6	6	/
Escherichia coli ATCC 25922	6	6	13	9	8	1:4
Escherichia coli BLSE	6	6	12	9	7	1:4
Acinetobacter OXA-23	6	6	21	15	14	1:10
Acinetobacterspp	6	6	13	11	10	1:10
Proteus vulgaris	6	6	6	6	6	/
Klebsiella pneumoniae	6	6	11	10	8	1:4
Klebsiella pneumoniae carbapenemase(+)	6	6	9	7	6	1:2
Staphylococcus aureus ATCC 43300	6	6	13	13	12	1:10
Staphylococcus aureus ATCC 29213	6	6	14	13	10	1:10
Staphylococcus aureus ATCC 25923	6	6	15	13	10	1:10
Staphylococcus aureus NSSA	6	6	11	9	8	1:4
Enterococcus faecalis ATCC 29212	6	6	10	8	8	1:10

DISCUSSION

This study aimed at determining the chemical composition and antibacterial activity of *Artemisia herba alba* essential oil extracted by hydrodistillation method. The essential oil yield obtained was more or less 1.80 ±0.03%, it is relatively higher than other medicinal plants as for example *Salvadora persica* (0.6 %) [3], *Matricaria recutita* gave 0.55% ± 0.045 [13] and even the same species like the *Artemisia herba alba* grown in Matmata Tunisia (0.65%) [2], Djelfa in Algeria (0.80) [24], Libya (0.90) [23], Batoum-Souk Ahras in North-east of Algéria (1.48%) [15], Boussada in North-west of Algeria (0.76%) [9] and also that of Taforalt in Morroco (1.00 %) [22].

Like most species of the genus, the essential oil of *Artemisia herba alba* also contain the Thujone (0.16%), after analysis of other studies conducted on this very plant, it was found that the chemical compositions obtained in this study were generally similar to those obtained by Akrout in Tunisia 2004 [2]. While other researchers have found that camphor is the leader with a variable rate limited between 17% and 45% according to the work of Delimi (2018) [15], Bouzidi (2016) [11], Amri *et al*. (2013) [6], Belhattab *et al*. (2012) [9] and Ghanmi *et al*. (2010) [18] carried out in the various regions of North Africa.

Then, after analytical study the essential oil was tested for its antibacterial activity against fourteen Gram-negative and Gram-positive bacterial strains. The obtained results proved that the *Artemisia herba alba* essential oil have a significant antibacterial activity with all bacterial strains; except *Pseudomonas aeruginosa* and *Proteus vulgaris* were resistant even to the highest concentration of essential oil. These results are consistent with those obtained in several studies conducted on *Artemisia herba alba* in Algeria [15], Lebanon [19], Morocco [5] and also Tunisia [39].

Based on these results, *Artemisia herba alba* essential oil can be used as a natural antimicrobial agent for the treatment of many infectious diseases caused by these pathogenic germs resistant to antibiotics.

The antibacterial activity of the essential oil was tested by the disc diffusion assay revealed that the gram positive bacteria more susceptible to essential oils than Gram negative bacteria [31]. Gram-negative strains (*Escherichia coli* and *Klebsiella pneumoniae, Pseudomonas, Acinetobacter ssp, Proteus vulgaris)* are less susceptible because, according to Vaara (1992) [34] they possess an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering [34]. While gram positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*) with a simple membrane structure are less protected against the diffusion of fine essential oils particles [20]. However, the weak antibacterial activity of Gram negative bacteria observed in this investigation is in accord with other studies [5, 15, 19, 39].

Germs from *Pseudomonas aeruginosa* group 'ATCC 27853 and VIN2' and *Proteus vulgaris*, were shown to be completely resistant to the essential oil studied. These strains are known for their great ability to develop resistance to many antimicrobial agents, hence the frequent involvement of all of the *Pseudoomonas aeruginosa* germ in hospital infections (Mann *et al*., 2000) [26].

According to Zouari *et al*. (2010; 2014) [38, 39], the resistance of the *P. aeruginosa* strain to essential oils is not surprising. this bacterium has an intrinsic resistance to biocidal agents, which is related to the nature of its membrane. The latter is composed of lipopolysaccharides which form an impermeable barrier to hydrophobic compounds.

These differences in the sensitivity of microorganisms against the essential oil of *A. herba alba*, can be explained by the quantity and quality of bioactive molecules or the nature and composition of the cell wall as well as the potency of the enzymatic system of the cell which controls its metabolism (Bouzidi, 2016) [11].

In conclusion, it can be said that the secondary metabolic substances, such as essential oils, of white mugwort can be used as adjuvants or in combination with antibiotics to achieve a synergistic effect especially against gram negative bacteria having develop an antibioresistance.

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