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Research Article

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Chemical composition and antimicrobial activity of the essential oil from *Artemisia herbaalba Asso* growing in the north west of Algeria

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ABSTRACT

The study was carried out on essential oil of Artemisia herbaalba. CG/SM qualitative analysis of white wormwood essential oil allowed us to identify 99.61% of the total peak areas. The main one is Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R)- (29.82%). Other main components are determined as 1,3-Cyclopentadiene, 1,2,5,5-tetramethyl- (15.58), Eucalyptol (6,51%) in the oil. On a biological level, evaluation of the essential oil revealed an interesting antimicrobial activity towards Escherichia coli, Staphylococcus aureus, Candida albicans, Fusarium oxysporum f. sp. ciceris(Chickpea Fusarium Wilt), Fusarium solani (Chickpea root-rot) and Globisporangium ultimum (Aleppo pine damping off and root-rot diseases).Results are also interesting for the pharmaceutical and agro-food industry fields using essential oil and this oil can be used in disease management against these soil-borne pathogens.

Key words: Antimicrobial activity, Essential oil, CG/SM, White wormwood.

INTRODUCTION

Genetic diversity of many steppe plants species is decreasing, and for some of them in a frightening way. Therefore, as a contribution to promote steppe plants genetic inheritance, we have focused our attention on *Artemisia herbaalba* (from the Asteraceae family), a medicinal steppe plant well known by the Algerian population as “white worm wood”. *Artemisia herbaalba Asso* is interesting for many fields: it is a unique source of feeding for nomad herd rearing and its essential oil is mainly used in perfumery industry. Medicinal and aromatic plants as well as their essences have several biological activities. They are used in phytotherapy for their antiseptic properties towards bacteria infectious diseases. In the phytosanitary and agro-alimentary fields, essential oils or their active substances could be employed like agents of protection against fungi and micro-organisms who invade the foodstuffs**.** Chickpea *Fusarium* wilt and Chickpea root-rot caused, respectively, by *Fusarium oxysporum* f. sp. *ciceris* (FOC) and *Fusarium solani*(FS), represented the most widespread and destructive diseases of this legume crop. *Globisporangium ultimum* (Trow) Uzuhashi, Tojo& Kakish. (syn. *Pythium ultimum Trow, syn. P. ultimum Trow* var. *ultimum*) (GU) is a known oomycetal species from Pythiums. l. causing damping-off and/or root rot on Aleppo pine (*Pinushalepensis Mill*). Our purpose is to study chemical composition of *Artemisia herbaalba* essential oil obtained through hydro-distillation as well as to evaluate its antimicrobial effect.

EXPERIMENTAL SECTION

2.1. Plant material

Artemisia herbaalba belongs to the Asteraceae family. White wormwood is a forage, medicinal and aromatic plant. The aerial part of the plant was collected in Sidi Ahmed station (Saidaprovince) in the Tell steppe interface area North West Algeria.

2.2. Isolation of essential oil

We obtained *Artemisia herbaalba* essential oil through a hydrodistillation process from the plant material using a Clevenger –type apparatus for 3hours. Before dark stored at 4°C, essential oil was dried up using anhydrous sodium sulfate. The essential oil yield was calculated on a dry weight by gravimetric method.

2.3. Essential oil analysis

We analyzed *A. herbaalba* essential oil through chromatography coupled with mass spectrometry.

The oil was investigated using a chromatograph HP (Agilent Technologies) MDS 5973 coupled to a 6890 plus mass spectrometer, equipped with fused-silica capillary column HP-5MS (30m; 0.25mm i.d.; film thickness 0.25µm).

2.4. Antimicrobial activity

2.4.1. Microbial strains

Antimicrobial activity of *A. herbaalba* essential oil was tested against: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25213), *Candida albicans* (ATCC 10231), *Fusarium oxysporum* f.sp. *ciceris* (Chickpea Fusarium Wilt) (FOC), *Fusarium solani* (Chickpea root-rot) (FS) and *Globisporangium ultimum*(Aleppo pine damping off and root-rot diseases) (GU)..

2.4.2. Disk Diffusion method

The agar diffusion method was used to evaluate the antimicrobial activity of essential oil as described previously**[1]**. The Microbial suspension - *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25213)-was spread on the MH agar medium. Then, absorbent disk (6 mm diameter) containing 5µl of essential oil were placed on the culture medium surface inoculated with different microbial strains (the bacterial suspension was adjusted to 0.5Mc Farland).

*Candida albicans*was first grown on Sabouraudchlorenphenical plate at 30°C for 18-24 h prior to inoculation onto the nutrient agar. The fungal suspension was adjusted to 2 Mac Farland. The inoculum was streaked on to Sabouraud chlorenphenical agar. A sterile filter disc, diameter 6 mm was placed. 5 µL of essential oil was dropped on each paper disc. The treated Petri dishes were placed at 4°C for 1-2h. After that, petri dishes were incubated at 37°C for 18-24h. Finally antimicrobial activity was evaluated by measuring inhibition zone diameter around every disk. Experiment was conducted three times for every tested strain. To determine the minimum inhibitory concentration (MIC), we used micro-dilution on liquid medium method with a 96 micro well plate.

2.4.3. Antifungal activity

2.4.3.1. Mycelia growth

Fungal isolates –*Fusarium oxysporum* f. sp. *ciceris*(FOC), *Fusarium solani*(FS) and *Globisporangium ultimum* (GU)-are cultivated on PDA medium during seven days at 25°C in darkness. The choice of the concentrations of the essential oil is carried out on the basis of preliminary test. The mycelia growth was carried out by direct contact method, which consists in adding essential oil in PDA medium at 45°C to obtain concentrations of 1000, 500 and 250 ppm. After obtaining these various dilutions and their solidification in Petri dishes (15ml/dish), mycelia discs of 1cm of diameter, issue of 7 days old culture of each isolate, was deposited in the center of each Petri plate. Control plates containing the culture medium and the mycelia disc of each isolate without essential oil were realized. Each test is repeated three times. After incubation at 25°C during 7 days, by taking account of the growth of control, the percentage of inhibition (PI) is calculated by the following formula: $PI\% = dT - dE/dT \times 100$; with; PI (%): percentage of inhibition, dT: Average diameter of the growth zone of the control and dE: Average diameter of the growth zone of the test**[2, 3].**

2.4.3.2. Evaluation of the sporulation

The evaluation of the sporulation was estimated from plates having been used to study the mycelia growth. Four discs of 5 mm in diameter were cut out with a perforating punch (cookie cutter) along the diameter of the same colony and were put in a tube containing 1 ml of distilled water. The spores suspension is then agitated using a vortex in order to release the spores of the conidiophores. These experiments are repeated three times. After counting the full number of the spores using a cell of Malassez at a rate of 10 counting by suspension, the values are

expressed of number of the spores per unit of area (mm^2). The percentage of inhibition of the sporulation (IS) is calculated by the following formula: $IS\% = [(N_0 - Nc) / N_0] \times 100$; With N_0: The mean number of the spores estimated for the control and Nc: The mean number of the spores estimated in the presence of essential oil**[3]**.

2.4.3.3. Evaluation of Germination

The collected suspension of the spores is adjusted at a rate of 105 spores/ml of distilled water using a cell of Malassez. We spread out 0.1 ml of this suspension on Petri dishes containing PDA medium with essential oil at the same concentrations previously mentioned. Three repetitions are carried out simultaneously for each concentration. The counting of the spores germinated or not was carried out on a total of 200 spores after 5 to 18 hours of incubation at 25°C in darkness. A spore is considered germinated if the length of the germinatif tube is higher than its smaller diameter. Percentage of inhibition of the germination of the spores Ig is given according to the following formula: $Ig\% = (N_0 - Nc) / N_0 \times 100$; with$N_0$: The number of the spores having germinated in the culture medium without essential oil (control) Nc: The number of the spores germinated in the presence of essential oil**[3]**.

RESULTS AND DISCUSSION

3.1. Chemical composition of the essential oil

Chemical composition of white Artemisia aerial part essential oil obtained through hydrodistillation was analyzed with GC and GC-MS.31 components were identified on HP5-MS column from the essential oil representing 99.61% of the total (majority compounds are shown in table 1).The hydrodistillation yielded 0.93% of oil (yield (w/w)). This output is comparable to those obtained from 5 oils *Artemisia herbaalba* recollected from different regions in Algeria (0.2% to 0.95%)**[4]**. It is worth mentioning that the same variation was recorded with sagebrush in Spain (0.41% to 2.30%)**[5].***A. herbaalba* extraction yield is related to plant origin: Tunisia (0.68% to1.93%) and Morocco (0.86%). It has to be mentioned that this is an average output compared to certain plants used in industry as essential oil source: superior to rose yield (0.10% - 0.35%) and inferior to thyme yield (2.0- 2.5%)**[6]**.The main components of essential oil of Artemisia from Saida region are different than those reported by several authors with *Artemisia herba alba* from other regions: α and β-thujone (7.85% to 30%)**[7, 8, 9, 10],** Cis-chrystanthenyl acetate (10.60% à 25.12 %)**[4, 8],** being the most abundant components. Essential oil chemical composition of plants collected in different regions of Algeria is characterized by presence of high thujone percentage**[4, 9]**. It is worth mentioning that in our study these compounds were found in small quantities (1.41%).Therefore, *A. herba alba* essential oil chemical composition variation may be due to exogenous factors such as soil types and their components, temperature, altitude and also due to endogenous factors such as plant genetic inheritance**[6].**

Table 1: Essential oil of *Artemisiaherba-alba*

N°	Majority Compounds	%
1	Camphene	0.663
2	Eucalyptol	6.513
3	Bicyclo [3.2.0] hept-2-ene, 2-methyl	4.311
4	α Thujone	1.409
5	β Thujone	1.089
6	1,3-Cyclopentadiene, 1,2,5,5- tetramethyl-	15.582
7	Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R)-	29.816
8	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	1.093
9	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl	3.803
10	Chrysanthenone	8.216
11	2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	2.659
12	1-Penten-3-one, 2-methyl-	2.381
Total		99.61
Yield		0.93

3.3.2. Antimicrobial activity

Experimental results show that *Artemisia herbaalba* essential oil has an antimicrobial activity on all tested strains (as presented in table 2 and 3).The antimicrobial activity varies from strain to strain and can be noticed in inhibition zones showing that certain are more sensitive than others. According to the classification made by**[11]**, the tested essential oil showed strong activity (inhibition zone ≥ 20mm) against *Candida albicans* and moderate activity (inhibition < 20-12mm) against *E. coli* and *Staphylococcus aureus.* Several compounds are cited as responsible for the antiseptic properties of essential oils: thymol, carvacrol, camphor…**[8]**. This activity of *A. herbaalba* essential oil would be related to its oxygenated monoterpenes compounds. Indeed, in essential oils it was shown that monoterpenes are able to destroy cellular integrity resulting in respiration inhibition and permeability alteration**[12, 8]**.

Table 2: inhibition zone (mm) and minimal inhibitory concentration (µl/ml) for essential oil of *Artemisia herbaalba*

Microorganism	Diameter of inhibition zones (mm)	MIC (µl/ml)
Escherichia coli (ATCC 25922)	12.83 ± 0.76	12.5
Staphylococcus aureus (ATCC 25213)	14.33 ± 0.57	6.25
Candida albicans (ATCC 10231)	30 ± 3.60	3.12

The antifungal activity of essential oil was tested by the method of diffusion and the appreciation of the diameters of the inhibition zones *in vitro*. Obtained results of the direct contacts method show that the mycelial growth of the various strains is affected by the various concentrations of essential oil. Indeed, more the concentration of essential oil increases more the rate of inhibition increases. A maximum of effect is obtained with the amount of 1000 ppm, this oil reduced the development of *FS*, *GU* and *FOC* with a rate of 78.33, 58.88 and 50.61% respectively (as shown in Table 3).Differences of sensitivity or resistance are noted according to the concentrations and to the various strains tested since it is observed that the *GU* strain is more resistant compared to *FS* and *FOC* at a low concentration (250 ppm).Therefore, for the test of the mycelia growth, the most sensitive strain to essential oil is *FS* (I% › à 75%). The analysis of the results indicates that the three strains tested are sensitive to the essential oil. This sensitivity results in a reduction in the spores with the increase in the concentration. The rates of inhibition are higher than 50% for *FOC* (91,87), *GU* (90.40%) and *FS* (86,25%) with the concentration of 1000ppm. The three percentages of sporulation of each studied strain are higher than CI_{50} (*FOC,* 58.53%; *GU,* 63.46% and FS, 75.31%).For the low concentration (250 ppm), we find that the percentage of inhibition of *FOC* and *FS* is more or less equal to CI50, contrary in *GU* which has a percentage lower than the value CI_{50}.Therefore, this essential oil has an inhibiting effect on the sporulation of the strains studied with concentrations of 500 and 1000 ppm. For the germination of the spores, it appears that the most important effect was observed for *GU*. An inhibiting activity of 100% was noticed by the application of the two concentrations (500ppm and 1000ppm) on the germination of *GU*, on the other hand the percentage of germination at the concentration of 250 ppm is lower than the value of CI_{50}.On the basis of percentage of germination of the conidia, essential oil appeared to be active on all strains tested and at all the concentration of essential oil used. The antifungal activity of essential oil of *A. herbaalba* can be allotted the presence of camphor (29.816%) and thujone (1.409%). Our results are in conformity with other research, who declared that there is a relation between the antifungal capacity and the presence oxygenated monoterpenes**[13, 8, 6]**.

Table 3: Inhibition (%) of *Artemisia herba-alba* essential oil on conidia germination, mycelia growth and sporulation of *Fusariumsolani, Fusariumoxysporum and Pythiumultimum*

		F. solani	***F. oxysporum***	***P. ultimum***
Conidia Germination	Control	0	0	0
	250ppm	44.44 ± 9.36	52.05 ± 9.77	64.28 ± 6.68
	500ppm	55.55 ± 17.54	61.64 ± 6.51	100 ± 0
	1000ppm	88.88 ± 8.93	78.08 ± 1.82	100 ± 0
Croissance mycélienne	Control	0	0	0
	250ppm	50 ± 13.75	6.1 ± 2.13	0
	500ppm	51.25 ± 1.25	19.12 ± 5.65	54.81 ± 9.7
	1000ppm	78.33 ± 1.44	50.61 ± 9.5	58.88 ± 8.88
sporulation	Control	0	0	0
	250ppm	45.39 ± 29.18	58.53 ± 24.07	34.31 ± 7.38
	500ppm	75.51 ± 10.97	58.92 ± 13.74	63.46 ± 4.88
	1000ppm	86.25 ± 22.11	91.86 ± 4.13	90.46 ± 2.81

CONCLUSION

Medicinal and aromatic plants are an important and inexhaustible source of natural bioactive substances and compounds. The study was conceived to assess antimicrobial activity of *A. herbaalba* aerial part essential oil. The main compounds of essential oil of *A. herbaalba*from Saidaare different than those reported by several authors from other regions. The antimicrobial activity of the essential oil of *A. herbaalba* was determined. The results showed that examined oil has an important antimicrobial activity; it was found that it was active against all strains tested. Finally, if white wormwood is regarded as having substances for human medicine and animal feeding, it has also substances with important effects on a biological level. Whatever efforts are made in research works in this field, they will always remain insufficient to interpret, understand and take profit of all virtues and qualities embodied by steppe medicinal plants.

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