Nat. Volatiles & Essent. Oils, 2021; 8(2): 27-36
DOI: 10.37929/nveo.844309

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Chemical characterization and bioactivities assessment of *Artemisia herba-alba* Asso essential oil from South-western Algeria

Nawal Ouguirti[1,*], Fouad Bahri[1], Ahmed Bouyahyaoui[1] and Juergen Wanner[2]

[1]Laboratory of Microbiology and Plant Biology, Faculty of Nature and Life Sciences, Abdelhamid Ibn Badis University, 27000 BP, Mostaganem, ALGERIA

[2]Kurt Kitzing Co., Hinterm Alten Schloss, Wallerstein, GERMANY

*Corresponding author. Email: nawal.ouguirti@univ-mosta.dz

Submitted: 21.12.2020; Accepted: 07.03.2021

Abstract

Artemisia herba-alba Asso is a medicinal plant used widely in Algerian folk medicine for the treatment of several diseases. The present work is aimed to elucidate the chemical composition of essential oil (EO) of *Artemisia herba-alba*. The in vitro antibacterial activity against 11 bacterial strains, and the in vivo acute toxicity and anti-inflammatory activity were evaluated. In this study fifty-four compounds accounting for 94.1% of total oil were identified by GC-FID and GC-MS analysis of the essential oil obtained from leaves. α-thujone (48.0%), β-thujone (13.4%), and camphor (13.1%) are found to be the major compounds. The results of the antibacterial activity using disc diffusion method revealed inhibition zones ranging from 10.00±0.0 to 36.00±0.9mm. Gram-positive was more sensitive to EO compared with Gram-negative bacteria. EO exhibited minimum inhibitory concentrations and bactericidal concentrations of 2.5 mg/ml to 10 mg/ml, and 5 mg/ml to 10 mg/ml respectively. The oil was moderately toxic and belongs to category 4 according to GHS, mortality was achieved at the dose of 2000 mg/kg. EO of *A. herba-alba* reduced significantly the edema induced by carrageenan in rat paw at 44.07% and 44.37% (after five hours) at the doses of 50 mg/kg and 100 mg/kg respectively.

Keywords: *Artemisia herba-alba*, essential oil, antibacterial activity, acute toxicity, anti-inflammatory activity.

Introduction

Since ancient times, humans appreciate the virtues of medicinal plants. The phytotherapy has been used widely as a part of traditional medicine to treat various pathologies, and has now seen an exceptional renewal, especially in the treatment of chronic diseases such arthritis, asthma…etc. (Aati et al., 2020). Several studies have largely focused on the research of natural products particularly in aromatic herbs due to their various pharmacological properties (Zeggwagh et al., 2014; Jakovljević et al., 2020). As a country known for its natural resources, Algeria has a remarkably rich and varied flora that is commonly used in herbal medicine; it covers about 3000 of plant species of which 15% are endemic and belong to different botanical families (Aichour et al., 2018). *Artemisia herba-alba* Asso (Shih in Arabic) is a Greenish-Silver short shrub, it's a medicinal and aromatic plant (Tilaoui et al., 2015), growing in semi-arid and arid areas of the Mediterranean basin. This herb is abundant in large areas, steppes and Sahara Desert of Algeria (Dahmani-Hamzaoui & Baaliouamer, 2010). *Artemisia herba-alba* is one of the most popular medicinal herbs commonly used for the treatment of so many diseases such as colds, coughing, intestinal disturbances, bronchitis, diarrhoea, neuralgias arterial hypertension and/or diabetes, as well as for flavoring tea and coffee (Jouad et al., 2001; Lakehal & A, 2016). Reports about essential oils from this plant revealed various biological activities related to the variation of their particular components such as antimicrobial, antioxidant, antidiabetic, Antileishmanial, anthelmintic and antispasmodic activities (Yashphe et al., 1987; Iriadam et al., 2006; Akrout et al., 2010; Zouari et al., 2010; Adel et al., 2011). Therefore, the aim of this work is to investigate the chemical composition of *A. herba-alba* essential oil from south-western Algeria. Considering also, EOs have a great potential to inhibit growth of diverse microorganisms, and can be explored for antimicrobial drug

DOI: 10.37929/nveo.844309

development, to combat the rapid increase of antibio-resistance, we tested herein the activity of the extracted EO against pathogenic bacteria. In addition, to the best of our knowledge no pharmacological studies on this Algerian chemotype were reported. Thus, the assessment of the *in-vivo* anti-inflammatory activity, and the toxicological effect of this Essential oil was the significant side of our study.

Materials and Methods

Plant material and essential oil extraction

The aerial parts of *A. herba-alba* were collected at the flowering stage in December 2018 in Bechar region Southwestern Algeria (31,512m, 2°35'37.0" W,31°30'43.8" N). Samples were identified by Pr Belhaçaini Hachimi a professor in the university of Sidi Bel-Abbas, Algeria. A voucher specimen was deposited (A.h a ONA 2018) at the laboratory of Microbiology and Plant biology, University of Mostaganem. After harvesting, the plant materials were washed and dried at room temperature in dark conditions for 15 days. After drying, the leaves and flowers (100 g) were used for essential oil extraction using Clevenger-type apparatus for 3h, then the EO was collected dried over anhydrous sodium and stored at 4°C until further uses.

GC-FID and GC-MS analysis

The GC analysis was performed using a Thermo Fisher Scientific Trace GC Ultra fitted with flame ionisation Detector FID, using capillary column (50 m x 0,25 mm x 1,0 mm) SE-52 (95% polydimethyl-, 5% polydiphenylsiloxan) (Grob, 1986). The oven temperature was programmed at 60°C for 1 min, then heated to 230°C at a rate of 3°C/min. Injector and Detector temperature 250°C. The carrier gas was Helium 5.0 with a constant flow rate of 1.5 mL/min; sample (0.1µl of EO) was injected at a split ratio of 1:100. The MS and FID data were obtained in one GC with the help of a MS-FID-splitter consisting of a quartz Y-splitter and a short (ca. 20 cm) 0.1 mm ID fused silica restrictor column as an inlet to the GC-MS interface and a ca. 1 m x 0.25 mm deactivated fused silica column serving as a transfer line to the FID detector. GC-MS analysis was carried out using a Thermo Fisher Scientific ISQ Mass Spectrometer with interface heating at 250°C, ion source 230°C, EI mode at 70 eV, filament 50µA, scan range 40–500 amu. Thermo Xcalibur 2.2 software was used for identifying the compounds by correlating mass spectra to databases of NIST 08, Wiley 8th ed (NIST 17, 2017), Adams library (Adams, 2007), MassFinder terpenoids library (König WA et al., 2001). The retention indices (RI) were established by comparing the calculated ones with literature data or reference compounds. Quantification was attained using normalized peak area calculations without relative FID-response factors.

Antibacterial activity assay

The antibacterial activity of EO was tested against the following strains: *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10876, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 35659, *Klebsiella pneumoniae* ATCC 700603. And four strains obtained from the Microbiological Laboratory of the Military Hospital in Bechar (Algeria): *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus,* these strains were identified with the VITEK system. The antibacterial effect of EO was tested using disc diffusion method according to the CLSI (Abdelli et al., 2018), 100 µl of each inoculum adjusted to 0.5 MacFarland was streaked in petri dishes containing around 15 ml of Muller Hinton Agar (MHA) with a sterile swab. A sterile filter disc of 6mm (Whatman paper No.1) impregnated with 15 µl of EO was deposited in the surface of the agar, treated Petri dishes were stored at room temperature for 1 h before incubating at 37°C for 18-24 h. The antibacterial activity was determined by measuring inhibition diameters.

DOI: 10.37929/nveo.844309

The discs of Gentamycin antibiotic were used as positive growth control. Each experiment was conducted in triplicate and the mean diameter of the inhibition was recorded. Bacterial sensitivity was classified as follow: not sensitive for diameters smaller than 8 mm, between 8 and 14 mm moderately sensitive (+), 14-20 mm sensitive (++), and finally very sensitive for diameter superior than 20 mm (+++) (Sfeir et al., 2013).

Determination of MIC and MBC

The Minimum inhibitory concentration is defined as the lowest concentration of EO inhibits the growth of 90% of microbial population. The MIC and MBC of our EO were evaluated by referring to the method of Chebaibi et al. (2011, 2016), with some modifications. EO sample was dissolved in 10% DMSO to obtain the highest concentration, then serial two-fold dilutions were done in concentration ranges between 1.5% to 0.0029% (V/V%). The microtiter plate of 96 wells were prepared by dispensing 15 µl aliquot from stock solution of EO into the first well, whereas 15 µl from serial dilutions were deposited in the appropriate wells, then 170 µl of MHB inoculated with 15 µl of bacterial strains of 18 h culture adjusted to 0.5 McFarland standard turbidity was added to the corresponding wells, the last well of each strip containing 15 µl of DMSO 10% without EO served for negative control, 170 µL of MHB and 15 µl of inoculum served for the positive control. The final volume in each well was 200 µl. After incubation for 18-24 h at 37°C, the cells viability was determined using the TTC (Triphenyl tetrazolium chloride), prepared extemporarily at 0.4mg/ml in sterile distilled water. 40 µl of this solution was added to each well. The plates were re-incubated at 37°C for around 10 to 30 min, the TTC shows up cell viability by the appearance of red colour. Wells with no visible growth (clear fluid) were streaked onto MHA for revealing the MBC values that correspond to the lowest concentration of the sample showed no visible growth in petri dishes. EO was tested three times against each strain.

Acute toxicity

Healthy male and female rats Wistar, weighing 150-200 g were obtained from animal house of Institute Pasteur of Algeria. The animals were kept in polypropylene cages, allowed to acclimatize to the laboratory conditions for 7 days before experiments under standard conditions (at a room temperature of 22-24°C with a 12h light/dark cycle), they were fed with standard pellet diet, with water access *ad libitum.* All experimental procedures were performed according to the Animal Ethics Committee. The Acute Toxic Category method (ATC) of the acute oral toxicity test was adapted from OECD guidelines 423 (OECD, 2002). Animals were fasted for 16 h with free access to water before the experiment day, they were weighed before the dose administration. EO was diluted in 1% Tween 80 and administered orally by gavage to groups of rats (n=3) at doses of 50, 100, 200, 300, 500, 2000 mg/kg, respectively. The control group received only the vehicle, all animals kept under continuous observation after treatment to detect changes in behaviour or physical appearance, injury, pain and illness or mortality. After 24 h observation was conducted daily for 7 days.

Anti-inflammatory activity

In this part of experiment, the anti-inflammatory activity of EO was investigated on carrageenan-induced inflammatory paw edema (Abdel Jaleel et al., 2016; Abdelli et al., 2017)**.** The rats were divided into 4 groups of 6 animals each, they were fasted for 16 h prior to the experiment with free access to water. Group A (control) were treated with the vehicle (1% of tween 80, 10 ml/kg), Group B treated with the standard anti-inflammatory drug (Diclofenac at 10 mg/kg), Group C and D received EO at concentrations of 50, and 100 mg/kg respectively. One hour after the oral administration of EO, edema was induced by injecting 100 µl of carrageenan solution at 1% (w/v) in saline subcutaneously at the sub-plantar region of the right hind paw.

DOI: 10.37929/nveo.844309

The volume of each rat's paw was measured before and after carrageenan injection, and then hourly up to 5h post carrageenan injection. Inhibition percentage of edema was calculated as follows:

$$\boldsymbol{inhibition}\ \% = \frac{(\boldsymbol{Vt} - \boldsymbol{V0})\boldsymbol{control} - (\boldsymbol{Vt} - \boldsymbol{V0})\boldsymbol{tested}}{(\boldsymbol{Vt} - \boldsymbol{V0})\boldsymbol{control}} \times \mathbf{100}$$

Where Vt is the paw volume at t time after carrageenan injection and V0 is the paw volume before carrageenan injection (mm).

Statistical analysis

Results were statistically analysed using SPSS software. Through unpaired t-Student test and data are expressed as the mean ±SD for inhibition diameters; and by one-way ANOVA followed by tuckey post hoc test and data were expressed as mean ±SEM for paw edema. The *P* values less than 0.05 were considered to be significant.

Results and discussion

Chemical composition of essential oil

The EO extraction yielded 1.73% (w/w), yellow liquid oil with a strong herbaceous smell characteristic of *A. herba-alba* species. Fifty-four compounds were identified by GC-FID and GC-MS representing 94.1% of the total composition, listed in Table 1 sorted by their retention indices. The EO is mainly constituted by oxygenated monoterpenes (80.3%), followed by monoterpene hydrocarbons (10.8%), and a very low quantity of oxygenated sesquiterpenes (0.2 %). The major compounds are α-thujone (48.0%), β-thujone (13.4%) and camphor (13.1%). Other components found in a small amount are camphene (3.6%), γ-terpinene (1.4%), borneol (1.3%), and *p*-cymene (1.0%). This chemotype is similar of those reported by Said et al. (2016), with a percentage of 72.7%, 11.8%, 15.5% and 45.4%, 32.8%, 21.8% of α-thujone, β-thujone, and camphor respectively in Beni-Ouenif (Bechar, Algeria) and the Atlas Mountains (Merrakech, Morocco). However, α-thujone as the major compound was also reported by Belhattab et al. (2014) in Bougaa, Bousaada and Boutalleb, instead of camphor and chrysanthenone. The thujone chemotype was found also in some samples of southern Tunisia (Akrout et al., 2010; Mighri et al., 2010). For further comparison camphor represent the major component with high levels in samples collected from Batna (Bertella et al., 2018), Msila, Bousaada, Bourj Bou Arrerridj (Dahmani-Hamzaoui & Baaliouamer, 2010), Tunisia (Bachrouch et al., 2015), Morroco (Paolini et al., 2010). An interesting fact is that these species represent different EO chemotypes and polymorphism revealing that the chemical composition of EO is influenced by the harvesting period, geographical region, environmental and morphological parameters.

Antibacterial activity

The antibacterial activity of EO was evaluated in vitro using disc diffusion method, the data revealed variable activity against all the tested strains (Table2). Inhibition zones ranged from 10.00±0.0 to 36.00±0.9mm. Gram-positive was more sensitive to EO compared with Gram-negative bacteria. An important effect was noticed against *B. cereus (BC1), S. aureus (SA1), E. coli (EC1), K. pneumoniae (KP1)* and *K. pneumoniae (KP2)* which were very sensitive with inhibition zones of 36.00±0.9, 31.17±0.8, 23.33±1.5, 20.67±1.5, and 21.00±1.0 mm respectively compared with the positive control (discs of gentamycin). *P. aeruginosa (PA2) and S. aureus (SA2)* were less sensitive. More precise data on antibacterial activity was acquired by the determination of the MIC and MBC values are shown in Table 3. It indicates that oil exhibited the lowest MIC 2.5mg/ml

DOI: 10.37929/nveo.844309

(3.75µl/ml) against the strain *K. pneumoniae (KP2)* whereas all the other strains have a higher MIC and MBC of 5 and 10 mg/ml.

Table 1: Chemical composition of *A. herba-alba* essential oil determined by GC-FID and GC-MS.

N°	Compounds	RI	%Area	N°	Compounds	RI	%Area
1	1-octene	791	0.1	29	1-decen-3-ol	1178	0.2
2	*cis*-salvene	851	0.1	30	terpinen-4-ol	1185	0.3
3	tricyclene	929	0.3	31	cryptone	1192	0.1
4	α-thujene	931	0.5	32	α-terpineol	1196	0.1
5	α-pinene	940	0.3	33	myrtenal	1204	0.4
6	camphene	957	3.6	34	*cis-p*-menth-2-en-7-ol	1210	0.3
7	thuja-2,4(10)-diene	961	0.1	35	verbenone	1216	0.1
8	1-octen-3-ol	975	tr.	36	3-isopropylphenol	1224	0.4
9	sabinene	979	0.9	37	*n*-hexyl-2-methylbutanoate	1235	tr.
10	β-pinene	985	0.2	38	isobornyl formate	1238	0.1
11	myrcene	991	0.16	39	cumin aldehyde	1247	0.3
12	verbenene	997	tr.	40	carvone	1249	0.1
13	α-phellandrene	1010	0.4	41	piperitone	1261	0.9
14	α-terpinene	1022	0.9	42	6-camphenol	1284	0.3
15	*p*-cymene	1028	1.0	43	thymol	1289	0.1
16	limonene	1034	0.1	44	carvacrol	1300	0.4
17	β-phellandrene	1036	0.5	45	(*Z*)-jasmone	1404	0.6
18	1,8-cineole	1037	0.5	46	(*E*)-β-caryophyllene	1441	0.1
19	γ-terpinene	1063	1.4	47	*allo*-aromadendrene	1492	0.5
20	terpinolene	1094	0.5	48	bornyl isovalerate	1520	0.1
21	α-thujone	1114	48.0	50	isobornyl isovalerate	1529	0.1
22	β-thujone	1123	13.4	51	δ-cadinene	1538	0.1
23	*trans*-sabinol	1146	0.1	52	bornyl angelate	1616	0.1
24	*trans*-pinocarveol	1149	0.1	53	isobornyl angelate	1623	tr.
25	camphor	1154	13.1	54	xanthoxylin	1682	0.2
26	sabina ketone	1162	0.6		Oxygenated monoterpenes		80.3
27	pinocarvone	1170	0.3		Monoterpene hydrocarbons		10.9
28	borneol	1175	1.3		Oxygenated sesquiterpenes		0.2
					Total identified		94.1

tr. = trace (<0.05%)

These concentrations are nearly similar to those found by Bertella et al. (2018) with another chemotype of *Artemisia herba-alba* EO against strains of *S. aureus* (MRSAA1, MRSAB1) and *B. cereus* with MIC and MBC of 5 and 10 mg/ml respectively. It's less important compared with results of Mighri et al. (2010). However, our essential oil showed a bactericidal activity referred to the ratio MBC/MIC ≤2. Our results corroborated so many studies that reported that essential oils are slightly more active against Gram-positive than Gram-negative bacteria (Zouari et al., 2010), the resistance of these last ones can be due to their hydrophilic outer membrane which can block the penetration of hydrophobic compounds (Akrout et al., 2010). The antibacterial activity of *A. herba-alba* EO can be attributed to its hydrocarbons and oxygenated monoterpenes that can destroy cellular integrity resulting in permeability alteration (Goudjil et al., 2016). In addition, EOs rich in phenolic components show a great antimicrobial activity. However, in fact it's difficult to ascribe the activity to a single component, because the biological effectiveness of EO is due to the involvement of both major and minor constituents acting either synergistically or antagonistically with major components.

DOI: 10.37929/nveo.844309

Table 2. Antibacterial activity of *Artemisia herba-alba* essential oil against pathogenic bacteria

Microorganisms	**Inhibition zones (mm±SD)**	
	Essential oil (15µl)	**Gentamycin (30µg)**
Escherichia coli ATCC 25922 (EC1)	23.33±1.5*	19.33±1.15*
Klebsiella pneumoniae ATCC700603 (KP1)	20.67±1.5**	14.00±1.00**
Pseudomonas aeruginosa ATCC 27853 (PA1)	10.00±0.0***	29.66±1.15***
Proteus mirabilis ATCC 35659(PM1)	21.33±1.5^{ns}	22.00±1.00^{ns}
Staphylococcus aureus ATCC6538 (SA1)	31.17±0.8^{ns}	29.00±1.73^{ns}
Bacillus cereus ATCC10876 (BC1)	36.00± 0.9***	18.44±1.39***
Escherichia coli 2 (EC2)	19.92±1.0***	27.67±0.58***
Klebsiella pneumoniae 2 (KP2)	21.00± 1.0^{ns}	18.33±0.58^{ns}
Pseudomonas aeruginosa 2 (PA2)	19.92±1.9^{ns}	22.67±1.53^{ns}
Staphylococcus aureus 2 (SA2)	13.67±1.2**	20.89±0.84**

All values are expressed as means± standard deviation of three experiments. Data were analyzed through unpaired t-Student test. *p* values <0.05 were considered to be significant (*<0.05,**<0.001,***<0.0001), ns: no significant differences

Table 3: Minimum inhibitory and bactericidal concentration values of *A. herba-alba* essential oil

Microorganisms	**MIC (mg/ml)**	**MBC (mg/ml)**
Escherichia coli ATCC 25922(EC1)	5	5
Klebsiella pneumoniae ATCC700603(KP1)	5	5
Pseudomonas aeruginosa ATCC 27853(PA1)	>10	>10
Proteus mirabilis ATCC 35659(PM1)	5	5
Staphylococcus aureus ATCC6538(SA1)	5	10
Bacillus cereus ATCC10876(BC1)	10	10
Escherichia coli 2(EC2)	10	10
Klebsiella pneumoniae 2(KP2)	2.5	5
Pseudomonas aeruginosa 2(PA2)	>10	>10
Staphylococcus aureus 2(SA2)	10	10

Acute toxicity

Results of acute toxicity revealed that EO of *A. herba-alba* is classified to category 4 (>300-2000 mg/kg) with its LD_{50} of 500mg/kg according to the Globally Harmonized Classification System (GHS) OECD423 guidelines (OECD, 2002). This EO is moderately toxic with its median lethal dose between 50 and 500 mg/kg based on Hodge and Sterner toxicity scale 2005 (Ahmed & Azmat, 2014). Mortality was achieved in the tested animals at the dose of 2000 mg/kg after 30 min. An abnormal behaviour such convulsion, sedation, dyspnea and bleeding were observed before death.

Anti-inflammatory activity

The results of anti-inflammatory assay established by the edema model induced by carrageenan injection are given in Table 4 and Figure 1. It was noticed that the pre-treatment of rats with different concentrations of the plant EO reduces progressively the volume of paw edema starting from the second hour with a high

significant difference (*P<0.001*) up to four hours as compared to the control group. EO exhibited a percentage inhibition of 44.07%, and 44.37% at doses of 50, and 100 mg/kg BW respectively after five hours. However, those percentages were less than that observed with the standard drug (Diclofenac 66.05%). these results demonstrate clearly the anti-inflammatory effect of *A. herba-alba* EO in a dose-dependent manner on edema formation in rat's paw.

The acute inflammatory response is characterized by biphasic response, early or first phase associated with the rapid production of histamines and serotonin (about 1h), the bradykinins (1.5-3 h) causing the vasodilatation. Whereas the late or second phase (up to 3 h) is mainly due to the release of prostaglandins, proteases, lysosomes, nitric oxide (NO) and by locally infiltration by neutrophils producing free oxygen radicals (Ouédraogo et al., 2012, Abdel Jaleel et al., 2016; Aichour et al., 2018). This last one is very sensitive to steroidal and non-steroidal anti-inflammatory drugs (El Hachimi et al., 2017). *A. herba-alba* essential oil exhibited a rapid anti-inflammatory effect one hour after the injection of the phlogistic agent. A high inhibition was observed at the third hour with percentages of 54.16%, 64.91% for both concentrations 50 mg/kg, and 100 mg/kg respectively compared with Diclofenac. The obtained data demonstrate that the EO possesses an effect on both phases of inflammation and reduce significantly the edema induced by carrageenan injection. Those results go along with a previous study that showed the NO and PGE2 inhibition effect of *A. herba-alba* EO, and the suppression of the total number of leukocytes using the subcutaneous air pouch (SAP) model (Abu-Darwish et al., 2015; Qnais et al., 2016).

Table 4. Effect of essential oil of *A. herba-alba* on carrageenan induced paw edema in rats.

	1H	2H	3H	4H	5H
Control	6.70±0.18	6.55±0.1	6.52±0.07	6.72±0.26	6.39±0.25
Standard 10mg/kg	7.08±0.19	6.11±0.17	5.67±0.16*	5.24±0.13***	4.35±0.03***
EO 50mg/kg	6.40±0.17	5.49±0.17**	5.19±0.22***	5.73±0.16***	5.42±0.04**
EO 100mg/kg	5.95±0.09	5.51±0.03**	4.64±0.13***	5.16±0.13***	5.24±0.12***

Values are expressed in means ±SEM (n=6), control: (vehicule: Tween80 ,1%), standard: Diclofenac. Statistical analysis was carried out by one-way ANOVA followed by tuckey post hoc test (*P<0.05, **P<0.01, ***P<0.001)

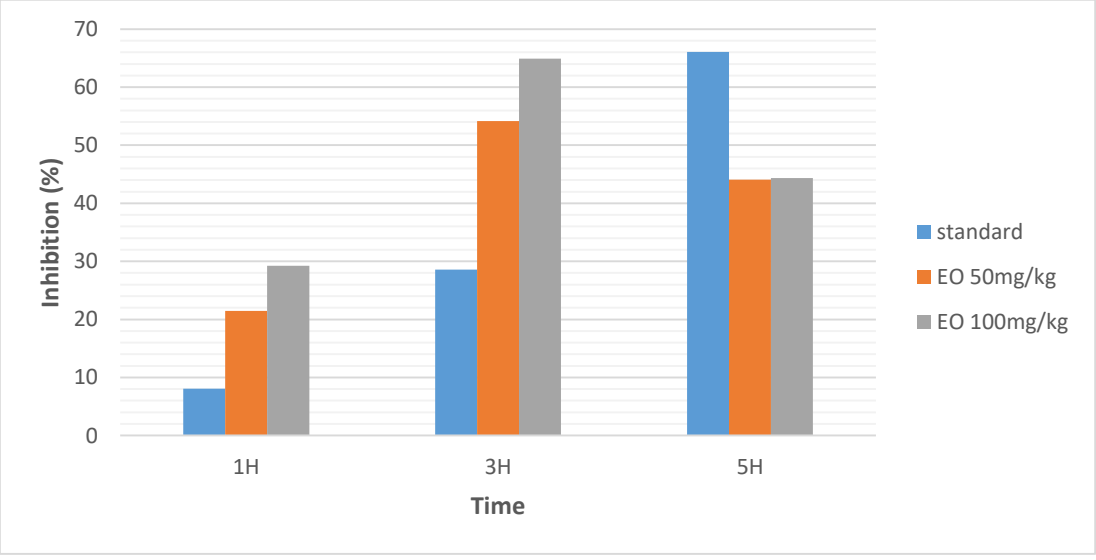

Figure 1: Percentage inhibition of paw edema by *A. herba-alba* essential oil and Diclofenac during 5 hours after carrageenan injection

Conclusion

The present work showed that *A. herba-alba* EO possesses a significant antibacterial activity especially against Gram-positive bacteria which confirms previous studies. The chemotype we reported reinforce the fact that the geographical region can influence the chemical composition of EO. To the best of our knowledge, there are few reports about the *in-vivo* anti-inflammatory properties of this essential oil. This last one afforded a good effect on both phases of inflammation at low concentrations, and it seems to be moderately toxic with its LD_{50} of 500mg/kg according to GHS (category 4). These findings add a valuable information to the pharmaceutical activity of this oil. However, it requires further *in-vivo* studies and clinical trials to confirm the safety and its possible uses.

ACKNOWLEDGEMENT

The project was supported by the General Directorate of Scientific Research and Technical Development of Algeria (GDRSTD). We would like to thank Miss Amraoui Amira; Misses Tahlaiti amina and Hamed Djahira laboratory engineers in the university of Abdel Hamid Ibn Badis, for the facilities given for the realization of this work.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

References

Aati, H. Y., Perveen, S., Orfali, R., Al-Taweel, A. M., Aati, S., Wanner, J., Khan, A., & Mehmood, R. (2020). Chemical composition and antimicrobial activity of the essential oils of *Artemisia absinthium*, *Artemisia scoparia*, and *Artemisia sieberi* grown in Saudi Arabia. *Arabian Journal of Chemistry*.

Abdel Jaleel, G. A. R., Abdallah, H. M. I., & Gomaa, N. E. L. S. (2016). Pharmacological effects of ethanol extract of Egyptian *Artemisia herba-alba* in rats and mice. *Asian Pacific Journal of Tropical Biomedicine*, *6*(1), 44–49.

Abdelli, W., Bahri, F., Höferl, M., Wanner, J., Schmidt, E., & Jirovetz, L. (2018). Chemical Composition, Antimicrobial and Anti-inflammatory Activity of Algerian *Juniperus phoenicea* Essential Oils. *Natural Product Communications*, *13*(2).

Abdelli, W., Bahri, F., Romane, A., Höferl, M., Wanner, J., Schmidt, E., & Jirovetz, L. (2017). Chemical Composition and Anti-inflammatory Activity of Algerian *Thymus vulgaris* Essential Oil. *Natural Product Communications*, *12*(4).

Abu-Darwish, M. S., Cabral, C., Gonçalves, M. J., Cavaleiro, C., Cruz, M. T., Efferth, T., & Salgueiro, L. (2015). *Artemisia herba-alba* essential oil from Buseirah (South Jordan): Chemical characterization and assessment of safe antifungal and anti-inflammatory doses. *Journal of Ethnopharmacology*, *174*, 153–160.

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectorscopy* (4th ed). Allured Pub. Corp.

Adel, K., Zied, Z., Ahmed, B. kir, Neacute ji, G., Mohamed, D., & Radhouane, G. (2011). Chemical constituents and antioxidant activity of the essential oil from aerial parts of *Artemisia herba-alba* grown in Tunisian semi-arid region. *African Journal of Biotechnology*, *10*(15), 2923–2929.

Ahmed, M., & Azmat, A. (2014). acute toxicity (lethal dose 50 calculation) and histopathological effects of methanolic extract of *berberis vulgaris* in mice. *World Journal of Pharmaceutical Research*, *3*(9), 10.

Aichour, R., Benzidane, N., Arrar, L., Charef, N., & Baghiani, A. (2018). Hepatoprotective and Anti-inflammatory Activities of Algerian Capparis spinosa. L. *Annual Research & Review in Biology*, *25*(3), 1–12.

Akrout, A., Jani, H. E., Amouri, S., & Neffati, M. (2010). *screening of antiradical and antibacterial activities of essential oils of Artemisia campestris l., Artemisia herba-alba asso, & Thymus capitatus hoff. et link. growing wild in the southern of tunisia. Recent Research in Science and Technologie*, *2(1)*, 29-39.

Bachrouch, O., Ferjani, N., Haouel, S., & Jemâa, J. M. B. (2015). Major compounds and insecticidal activities of two Tunisian Artemisia essential oils toward two major coleopteran pests. *Industrial Crops and Products*, *65*, 127–133.

Belhattab, R., Amor, L., Barroso, J. G., Pedro, L. G., & Cristina Figueiredo, A. (2014). Essential oil from *Artemisia herba-alba* Asso grown wild in Algeria: Variability assessment and comparison with an updated literature survey. *Arabian Journal of Chemistry*, *7*(2), 243–251.

Bertella, A., Benlahcen, K., Abouamama, S., Pinto, D. C. G. A., Maamar, K., Kihal, M., & Silva, A. M. S. (2018). Artemisia herba-alba Asso. Essential oil antibacterial activity and acute toxicity. *Industrial Crops and Products*, *116*, 137–143.

Chebaibi, A., Marouf, Z., Rhazi-Filali, F., Fahim, M., & Ed-Dra, A. (2016). Évaluation du pouvoir antimicrobien des huiles essentielles de sept plantes médicinales récoltées au Maroc. *Phytothérapie*, *14*(6), 355–362.

Chebaibi, A., Rhazi Filali, F., Amine, A., & Zerhouni, M. (2011). Effet bactéricide (in vitro) des extraits aqueux des feuilles du grenadier marocain (*Punica granatum* L.) sur des bactéries multirésistantes aux antibiotiques. *Phytothérapie*, *9*(3), 158–164.

Dahmani-Hamzaoui, N., & Baaliouamer, A. (2010). Chemical Composition of Algerian *Artemisia herba-alba* Essential Oils Isolated by Microwave and Hydrodistillation. *Journal of Essential Oil Research*, *22*(6), 514–517.

El Hachimi, F., Alfaiz, C., Bendriss, A., Cherrah, Y., & Alaoui, K. (2017). Activité anti-inflammatoire de l'huile des graines de Zizyphus lotus (L.) Desf. *Phytothérapie*, *15*(3), 147–154.

Goudjil, M. B., Ladjel, S., Bencheikh, S. E., Hammoya, F., Bensaci, M. B., Zighmi, S., & Mehani, M. (2016). *Bioactivity of Artemisia Herba-alba essential oil against plant pathogenic fungi*. *Der Pharma Chemica*, *8(3),*46-52.

Grob, K. (1986). *Making and manipulating capillary columns for gas chromatography*. Dr. Alfred Hüthig Verlag.

Iriadam, M., Musa, D., Gümüshan, H., & Baba, F. (2006). *Effects of two Turkish medicinal plants Artemisia herba-alba and Teucrium polium on blood glucose levels and other biochemical parameters in rabbits*. *J Cell Mol Biol, 6(5*), 19-24.

Jakovljević, M. R., Grujičić, D., Vukajlović, J. T., Marković, A., Milutinović, M., Stanković, M., Vuković, N., Vukić, M., & Milošević-Djordjević, O. (2020). In vitro study of genotoxic and cytotoxic activities of methanol extracts of *Artemisia vulgaris* L. and *Artemisia alba Turra*. *South African Journal of Botany*, *132*, 117–126.

Jouad, H., Haloui, M., Rhiouani, H., El Hilaly, J., & Eddouks, M. (2001). Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). *Journal of Ethnopharmacology*, *77*(2–3), 175–182.

König WA, Joulain D, & Hochmuth DH. (2001). *GC/MS Library: Terpenoids and Related Constituents of Essential Oils*.

Lakehal, S., & A, M. (2016). Essential Oil Composition and Antimicrobial Activity of *Artemisia herba-alba* Asso Grown in Algeria. *Medicinal Chemistry*, *6*(6).

Mighri, H., Hajlaoui, H., Akrout, A., Najjaa, H., & Neffati, M. (2010). Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. *Comptes Rendus Chimie*, *13*(3), 380–386.

NIST 17 / NIST 20 Mass Spectral Library, NIST 2020/2017 Database, Agilent Format Available. (2017).

OECD. (2002). *Guidance Document on Acute Oral Toxicity Testing*. OECD.

Ouédraogo, N., Lompo, M., Sawadogo, R. W., Tibiri, A., Hay, A.-E., Koudou, J., Dijoux, M.-G., & Guissou, I. P. (2012). Étude des activités anti-inflammatoire, analgésique et antipyrétique des décoctés aqueux des feuilles et des racines de Pterocarpus erinaceus Poir. (Fabaceae). *Phytothérapie*.

Paolini, J., Ouariachi, E., Bouyanzer, A., Hammouti, B., Desjobert, J.-M., Costa, J., & Muselli, A. (2010). Chemical variability of *Artemisia herba-alba* Asso essential oils from East Morocco. *Chemical Papers*, *64*(5).

Qnais, E. Y., Alatshan, A. Z., & Bseiso, Y. G. (2016). Chemical composition, antinociceptive and anti-inflammatory effects of Artemisia herba-alba essential oil. *J Food Agric Environ*, *14*, 20-7.

Said, M. E.-A., Vanloot, P., Bombarda, I., Naubron, J.-V., Dahmane, E. M., Aamouche, A., Jean, M., Vanthuyne, N., Dupuy, N., & Roussel, C. (2016). Analysis of the major chiral compounds of *Artemisia herba-alba* essential oils (EOs) using reconstructed vibrational circular dichroism (VCD) spectra: En route to a VCD chiral signature of EOs. *Analytica Chimica Acta*, *903*, 121–130.

Sfeir, J., Lefrançois, C., Baudoux, D., Derbré, S., & Licznar, P. (2013). In Vitro Antibacterial Activity of Essential Oils against Streptococcus pyogenes. *Evidence-Based Complementary and Alternative Medicine: ECAM*, *2013*.

Tilaoui, M., Ait Mouse, H., Jaafari, A., & Zyad, A. (2015). Comparative Phytochemical Analysis of Essential Oils from Different Biological Parts of *Artemisia herba-alba* and Their Cytotoxic Effect on Cancer Cells. *PLOS ONE*, *10*(7), (1-15).

Yashphe, J., Feuerstein, I., Barel, S., & Segal, R. (1987). The Antibacterial and Antispasmodic Activity of *Artemisia herba-alba Asso. II*. Examination of Essential Oils from Various Chemotypes. *International Journal of Crude Drug Research*, *25*(2), 89–96.

Zeggwagh, N. A., Michel, J. B., & Eddouks, M. (2014). Acute hypotensive and diuretic activities of *Artemisia herba-alba* aqueous extract in normal rats. *Asian Pacific Journal of Tropical Biomedicine*, *4*.

Zouari, S., Zouari, N., Fakhfakh, N., Bougatef, A., & Ayadi, M. A. (2010). *Chemical composition and biological activities of a new essential oil chemotype of Tunisian Artemisia herba-alba Asso*. *Journal of Medicinal Plants Research*, *4(10),* 871-880.