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Research Article

Chemical and Functional Characterization of Tunisian *Artemisia absinthium* Volatiles and Non-volatile Extracts Obtained by Supercritical Fluid Procedure

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ABSTRACT
The main objective of the present work was to study the chemical composition of antioxidants (polyphenol, flavonoids), antimicrobial and antioxidant activities of *Artemisia absinthium* L. essential oil obtained by maceration and by supercritical carbon dioxide (SC-CO2). The SC-CO_2 was explored at various pressure (10, 12, 18 and 20 MPa), and various size particle (0.25, 0.31, 0.56 and 1 mm) and at temperature of 40°C. The antioxidant activity of extracts was evaluated using 2, 2-diphenyl-l-picrylhydrazyl (DPPH), 2, 2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The tested plants were extracted with maceration in methanol and with supercritical CO_2. Biologically, very sensitive effects of *A. absinthium* essential oil are found against Gram-positive bacteria (*Listeria monocytogenes*, *Bacillus subtilis* and *Staphylococcus aureus*), three Gram-negative bacteria (*Campylobacter coli*, *campylobacter jejuni* and *campylobacter fetus*). In all SFE assay, the highest yield (2.5%) was reached at 40°C, 18 MPa and 0.25 mm particle size. A total of 25 chemical compounds were successfully identified by GC-MS with β-thujone (8.307%) and α-Farnesene (7.537%) are the most abundant compounds. the major subclasses are monoterpenes oxygenated (19.02%) and hydrocarbures (33.755%) respectively. The results show that the leaves extracts obtained by maceration contain the highest antioxidant capacity with radical DPPH (5.26 µg/ml of Trolox). On the basis of the obtained results of antioxidant activity (IC50), Artemisia has a potential use as natural antioxidants due to their significant antioxidant activity. Their antiradical scavenging activity revealed a good relationship with the total phenolics content (r^2 = 0.884) and with flavonoids content (r^2 =0.999). The essential oils extracts of plants exhibited good activity against all microorganisms tested.

Keywords: *Artemisia essential oils,Supercritical fluid extraction, Antimicrobial activity, Antioxidant activity,phenolic content, flavonoids content.*

INTRODUCTION

For a long time, a great number of medicinal plants are widely used in several fields such as folk medicine, pharmaceuticals as well as in cosmetics and food-stuffs (food flavoring, preservation and in the fragrance)[1]. Theirs essential oils present a strong antimicrobial and antioxidant potential against various pathogenic bacteria, fungi and viruses[2]. Studies are ongoing throughout the world in search for the bioactive compounds derived from plant volatile oils with a low profile of side effects. Many researchers show that the beneficial effects are the consequences of several compounds such as diterpenoids, triterpenoids, fatty acids, flavonoids and phenolic compounds. In Tunisia, in order to promote local phyto-aromatherapy for modern therapeutic applications against current pathologies many conventional studies on medicinal plants and essential oil bearing aromatic plants are being intensively carried. Artemisia commonly, growing naturally, called wormwood is one of these medicinal plants species. It is native to warm Mediterranean countries. Wormwood has been naturalized in northeastern North America, North and West Asia and Africa. Artemisia is a perennial small shrub distributed in Europe and Asia[3]. In traditional medicine, it is recommended to relieve digestive problems and stomach pains. In pharmaceutical industry, absinthe returned in the manufacture of many drugs (antiseptic, digestive, emmenagogue, tonic and vermifuge). It is also used in

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Table 1: Yield obtained in different extraction conditions

	P(MPa)	*PS (mm)*	*A. absinthium (Y %)*
SFE1	10	1	0.75
		0.56	1.19
		0.31	1.27
		0.25	-
SFE2	12	0.31	1.80
		0.25	-
		0.56	-
SFE3	18	0.56	1.83
		0.31	2.25
		0.25	2.50
SFE4	20	0.31	1.80
ME	1 (atm)	0.25	10

P : Pressure, Y : Yield of extraction, PS : Particle size

indigenous systems of medicine as a vermifuge, an insecticide, in the treatment of chronic fevers and for inflammation of the liver, as an antispasmodic and antiseptic[4]. Their essential oil has a good antimicrobial and antifungal activity[4-6]. This species known to possess ethnomedical and biological properties related to anthelmintic activity[6]. Since the chemical composition of plant materials depend on the extraction method and the separation techniques a lot of attention to the development of new procedures has received. However, the extractions methods of plant volatile oils and their constituents have progressed with time from conventional liquid-solid extraction such as steam distillation, percolation, maceration or organic solvent extraction using Soxhlet to others innovative methods friendly to the environment and human health. Supercritical fluid extraction, a clean process which enables to achieve high extraction yields and hence important degrees of purity of the desired compounds, is regarded as an alternative to the cited classical methods which are characterized by numbers of inconvenient, particularly the solvent toxicity and the impact on the environment[1]. In the context to resolve the increased demand for safe and natural plant medicinal oils, we are interested to apply two different extraction methods as maceration and the supercritical carbon dioxide model extraction of essential oil from Tunisian Artemisia. The choice of this model was mainly guided by its fiability as reported in the literature[7-9]. In the second hand we evaluate the chemical and functional characterization of the obtained oils. The antimicrobial activity was investigated by microdilution assay, while antioxidants activities were determined with DPPH and ABTS assays, in order to receive a reliable picture of the differences between the two radicals.

MATERIALS AND METHODS

Plant material

Absinthe (*Artemisia absinthium* L.) leaves (2 kg) were collected in September 2013 from Kairouan (9°47'56.41"E Longitude, 35°37'40.86"N Latitude) located in the middle of Tunisia on the Mediterranean coast of North Africa. Plant materials (leaves) were immediately transported to laboratory (LACReSNE). Leaves have been air-dried. Before any manipulation *Artemisia absinthium* L. leaves were ground using a crushing Moulinex (Masterchef Delicio) in order to obtain a fine and homogenous powder and kept in desiccators until analysis.

Chemicals reagents

Folin –Ciocalteu reagent, ABTS ($C_{18}H_{18}O_6S_4.2NH_3$, M = 548.7 g.mol^{-1}, 98 %), persulfate of potassium ($K_2S_2O_8$), Trolox (acid 6-hydroxy-2, 5, 7, 8-tetramethylechroman-2-carboxylic) (98 % purity), sodium nitrite (≥ 99.0 % purity), gallic acid and catechin were purchased from Sigma Aldrich (St. Louis, MO, USA). β-carotene and sodium carbonate were from Fluka Biochemika (Switzerland), concentrated hydrochloric acid (37%) and absolute methanol were purchased from Panereac Quimica Sa (Barcelone). Reagents were furnished by Fluka, DPPH radical ($C_{18}H_{12}N_5O_6$, 90 % purity), $AlCl_3 6H_2O$ and all other chemical reagents used for extract were obtained from Merck.

Extraction methods

Maceration extraction (ME)

20 mg of dried leaves of each plant were added to a mixture of 10 ml of methanol-water (80/20: w/w). After soaking overnight, the mixture was filtered through Whatman filter n° 2 and centrifuged (2500 rpp/min) and the solvent was evaporated in a vacuum evaporator (Buchi Rotavapor R-205) at 40°C and 17.5 MPa. The obtained extracts were stored in a freeze for subsequent analysis.

Supercritical fluid extraction (SC-CO_2)

Supercritical Fluid extraction SCF is an alternative method to the maceration using organic solvents and it is very slow. For these reasons we are interested to use the supercritical carbon dioxide this method is conceptually simple to perform. The used basic pilot SCFE unit has been conceived and assembled in the Laboratory of Reactions and Process Engineering (LRGP, Nancy, French) it consists of a cylinder of liquid carbon dioxide, an extractor column (300 x 23 mm) contained 200 mL within a temperature controlled zone, three separators equipment, a syringe pump that allows the pressure to be adjusted to a known value above the critical temperature of 31°C and critical pressure of 74 bar[1]. To obtain higher yield several experimental variables such as pressure, temperature, extraction time and sample size must be optimized. The adopted parameters and experimental conditions are summarized in Table 1. Approximately, 20 g of plant powder (0.25 to 1 mm for particle size) was introduced in

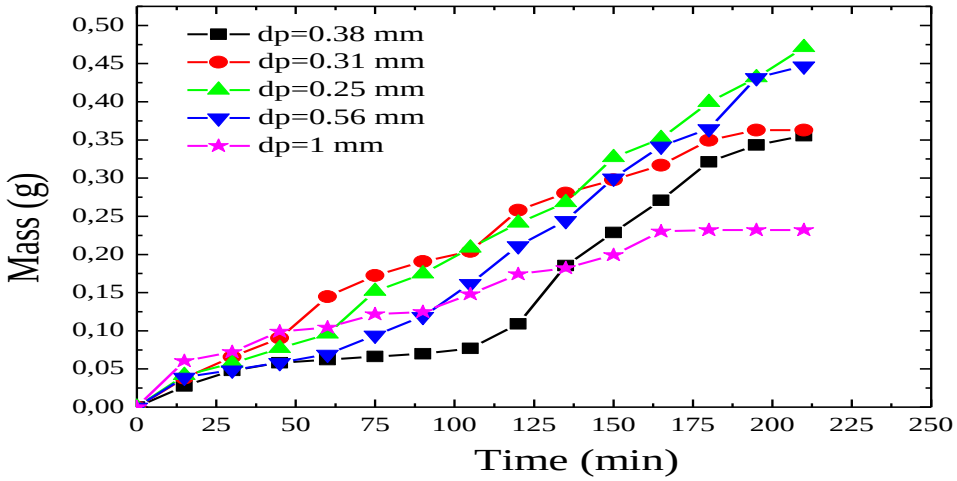

Figure 1: Mass of oil of *A. absinthium* at different particle size at 18MPa

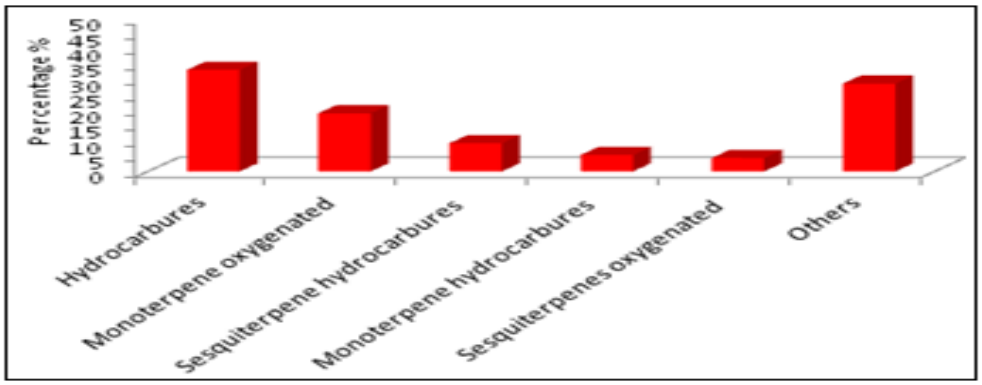

Figure 2: Evolution of percentages of different volatile compounds subclasses in *Artemisia* essential oil.

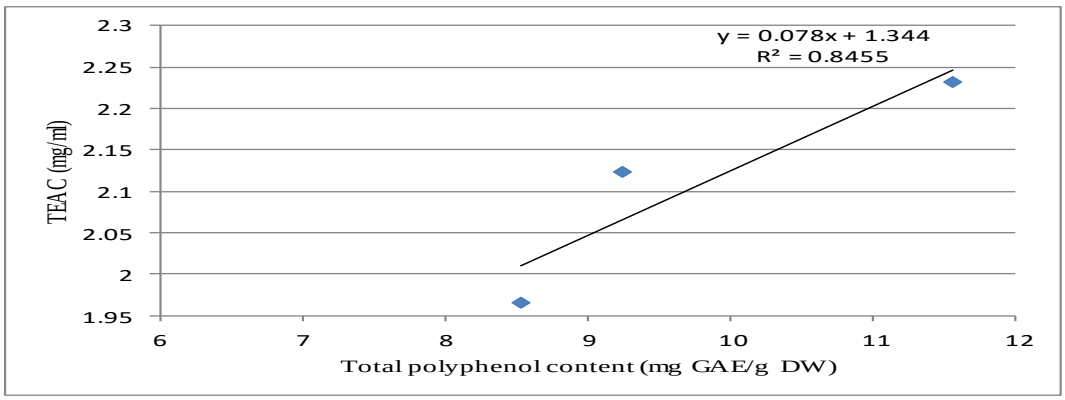

Figure 3: Relationship between total polyphenol content with ABTS

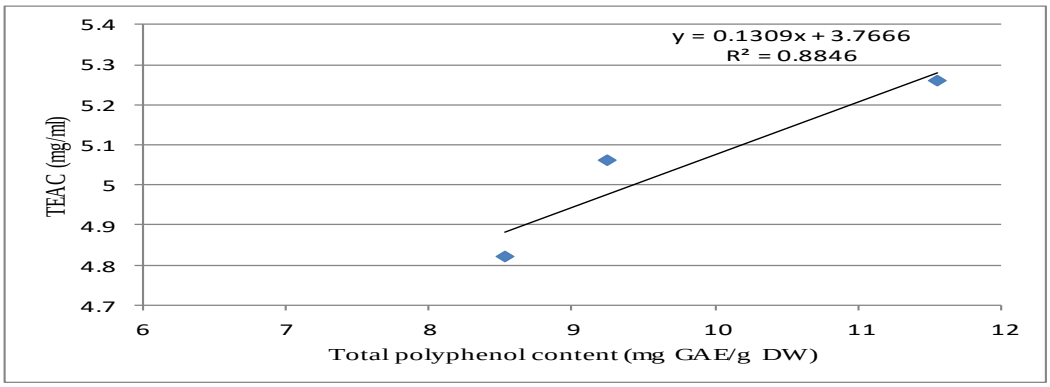

Figure 4: Relationship between total polyphenol content with DPPH

stainless steel cylinder, (200 cm^3 capacity), connected with three cyclonic separators. The sample is filled into the extractor vessel during 30 minutes under supercritical CO_2 (without flow of supercritical CO_2) and extracted at adjusted temperature (40°C) and pressure (250 bar) controlled by an individual electric heater and by valves, respectively. The CO_2 is liquefied at 3.2 °C using a pumped cold exchange (Dosapro Milton Roy - MILROYALD) and heated with another exchanger to obtain the supercritical state. The dried leaves of absinthe were extracted at 10, 12, 18 and 20 MPa for pressure, 40°C for temperature and 0.25 to 1 mm for particle size. The extraction time is between 2 and 3h. The temperature in the separators was 20°C and theirs pressures were 1, 2 and 4 MPa, respectively. The supercritical CO_2 flow rate was comprised between 7.5 and 13.5 $g.min^{-1}$. The extraction was performed in triplicate, the extracts were collected in one tube and the yield was calculated.

Phytochemical composition of volatile and non-volatile Artemisia extracts

Total polyphenol content

Total polyphenol content in Artemisia leaves was quantified using the Folin–Ciocalteu reagent, according to the modified method of Singleton and Rossi[10]. The extraction was made using different solvents polarity such as methanol/water (80:20, V/V), ethanol/water (50:50, V/V). Briefly, to 50 µL of each sample 750 µL Folin Ciocalteu reagent/water (1:14) were added. The mixture was left for 3 minutes and mixed with 15 % sodium carbonate Na_2CO_3 solution. The solution was rigorously vortex, homogenized and heated at 100 °C for 2 min, and kept in the dark room for 30 min for incubation. Absorbance was read at 700 nm using the Shimadzu UV-VIS spectrophotometer. All assays were carried out at least in triplicate and MeOH was used as blank (50μL instead of the extract). Methanolic dilutions of gallic acid were used as standard; results were expressed as equivalents of gallic acid (mg GAE.g^{-1} DW).

Total flavonoid content

Total flavonoid content was measured according to the modified colorimetric method of Zhishen[11]. Briefly, 125 µl of Artemisia leaves extract were added to 75 µl of $NaNO_2$ (5%). The mixture was incubated for 6 minutes. 150 µl of $AlCl_3$, $6H_2O$ freshly prepared are added, after 5 minutes of incubation, 500 µl of NaOH (1M) were added to the mixture, the final volume was adjusted to 2500 ml with distilled water. The absorbance was measured at 510 nm in a spectrophotometer (Shimadzu UV-VIS spectrophotometer). The result calculated using a pre-prepared catechin calibration curve (0–300 mg/L). The blank was prepared using the same procedure with 1 mL of ultra-pure water without extract; flavonoid amounts in extract were expressed in mg cathechin /g dry matter (mg CE. g^{-1} of DM).

Volatile composition of oil with GC-MS

Quantitative and qualitative data of Artemisia essential oils were determined using a Hewlett Packard 5890 (Hewlett-Packard HP 5890, Agilent Technologies Inc Palo Alto, CA, USA). The compounds were separated by gas chromatograph coupled with mass spectrometer Hewlett Packard 5972, equipped with a capillary column of fused-silica DB-5 (30 m x 0.25 mm ID, film thickness 0.25 µm). The carrier gas was helium (99.99%) at a flow rate 1.2 ml/min. The furnace temperature is maintained at 50°C for one minute and increases to 280°C (5 minutes) at a rate of 9 °C/min, the injector and detector temperatures were 240°C and 250°C respectively. Approximately, 0.2 µL of *Arthemisia* essential oil were injected with split ratio of 1:50. The concentration of the injection oil is 1% in hexane. The ionization energy is 70 eV. The order of the masses is 40-300 m/Z. The peaks were tentatively identified based on library search using database HP chemstation: HP Wiley or by comparison with the kovalts Retention Index. The identities of some components were confirmed by their mass spectral and their retention data of the authentic chemicals obtained under identical GC-MS conditions.

Evaluation of radical scavenging capacity (RSC)

It was also shown that the measure of antioxidant capacity in natural products by only one assay is often not reliable; therefore, in this investigation, we used two complementary assays such as DPPH radical scavenging assay and $ABTS^{\cdot+}$ radical to check the antioxidant activity of Artemisia leaves.

DPPH radical scavenging capacity

Radical scavenging activity of *A. absinthium* leaves extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was conducted spectrophotometrically as described by some researchers. The method is firstly introduced by Blois (1958)[12], developed by Brand-Williams[13] and criticized by Molyneux[14]. The principle of the assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant[15]. Briefly, 20 µL of methanol *Artemisia absinthium* leaves extract was added to 2 mL of 410^{-3} mM of $DPPH^{\bullet}$ (prepared in methanol). After incubation in a water bath at 37 °C for 30 min, the free radical scavenging capacity using DPPH radical was evaluated by measuring the decrease of absorbance at 517 nm every 2 min until the reaction reached its state. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve. The percentage inhibition I (%) was calculated as follows:

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

(Where A_1 is the absorbance at 517 nm; A_0 refers to the absorbance of DPPH without sample added at 517 nm).

The percentage inhibition was calculated against a control and compared to a Trolox standard curve 10–100 mM and expressed as Trolox equivalent antioxidant capacity. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage versus extracts concentration.

ABTS radical scavenging capacity

Antioxidant activities of Artemisia leaves samples were also analyzed by testing their ability to scavenge the $ABTS^{+}$ free radical using a modified methodology previously reported by Ozgen[16]. 10 mg ABTS radical and potassium persulfate solution (2.45 mM) were dissolved in distilled water to a final concentration of 7 mM and 2.45

Table 2: Phenolic, flavonoid and antioxidants activity of *Artemisia absinthium* L. extracts by maceration and supercritical fluid extraction

Artemisia absinthium L.						
EM	PC	FC	ABTS	DPPH	IC50 (ABTS)	IC50 (DPPH)
SFE3	8.53 ± 2.30	5.99 ± 0.20	1.965	4.82	0.057	0.129
SFE4	9.25 ± 1.30	7.33 ± 0.15	2.124	5.06	0.052	0.124
ME	11. 56 ± 1.20	8.49 ± 0.50	2.232	5.26	0.048	0.118

EM: Extraction Method, PC: Polyphenol Content (mg GAE.100g^{-1} DW), FC: Flavonoid Content (mgCE.g^{-1} DW), DPPH and ABTS: (mM TE/g extract) IC50: (mg. mL^{-1}). SFE3: extract at 18MPa, SFE4: extract at 20MPa

Table 3: Components of essential oils of *A. absinthium* extracted with SFE

A. Absinthium		
Compounds	RT (min)	Area %
Thyjanol	4.44	2.459
β-thyjone	4.83	8.307
Camphor	5.08	2.690
Borneole	5.26	5.564
Bicyclo-(6.1.0)-nonene	8.34	2.804
β-Selinenol	8.67	4.392
Azulene	9.24	4.775
5-Amino-1,4-dihydro-quinoxoline-2,3-dione	10.77	5.768
Arborescin	11.22	1.690
α-Farnesene	11.27	7.537
Pyridine, pentamethyle	11.32	1.190
2,2'-Dihydroxy-4'-methyl-5-methoxybiphenyl	11.73	4.663
Ethanone-1-(3,4-dihydro-4-hydroxy-6,7 dimethyl-2 naphthalene)	12.01	4.565
Acetamide-2,2-dipheny-N(-2-benzyloxy-ethyl)	12.07	2.509
1,3,4-Thiadiazole,2-(4-methoxyphenyl)-5-phenyl	12.09	2.292
Cis-Salvene	12.43	5.701
Pyrolidinone	12.93	2.324
6-Hydroxy-7-methyl-oct-3-enedithioic"isopropyl-ester"	13.25	3.781
β-Carboline	13.60	3.301
Tetratricontane	14.89	4.951
4-Cyclohexylidene-1-butanol	15.32	4.128
Heptacosane	18.14	6.903
Heneicosane	18.04	2.106
1-Chloro-heptacosane	19.69	2.888
Tetracosane	21.75	5.101
Total terpenes identified		99.777%

RT : Retention Time

mM respectively. These two solutions were mixed and the mixture allowed to stand in the dark at room temperature for 16 h before use in order to produce ABTS radical ($ABTS^{\bullet+}$). The solution was then diluted to an absorbance of 0.7±0.01 at 734 nm to form the test reagent. Reaction mixtures containing 20 mg of sample and 20 ml of reagent were incubated in a water bath at 30 °C during 60 min. The percentage inhibition was calculated against a control and compared to a Trolox standard curve 10–100 mM and the scavenging capacity was calculated as the percentage of inhibition (%I) of free radical ABTS by extract samples using the formula given below:

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

(Where A_1 is the absorbance at 734 nm; A_0 refers to the absorbance of $ABTS^{+}$ without sample added at 734 nm.

In vitro antimicrobial activity

Antimicrobial activities of essential oils extract by SFE were tested against 3 Gram-negative bacteria: Campylobacter jejuni MB_5195_05 THL, Campylobacter coli CCUG 11283T NVU) and Campylobacter fetus CCUG 6823T NVU. Gentamycin, Erythnomycin and Augmentin were used as the contole antibiotic. All pathogenic strains were supplied from the Committee Antibiogram of French Society for Microbiology (CA-SFM, 2010). The microbial strains were suspended in a normal saline solution to a turbidity of 0.5 Mac Farland standards (10^6 CFU (colony Forming Units)/mL of bacterial cells. The inoculums were stored at 4°C until use.

Disc diffusion method

Antimicrobial activity was determined by agar disc diffusion method[17]. Filter paper disks (6 mm in diameter) were impregnated with 15 µL of each extract and placed on the agar surface, after staying at 4°C for 1 hours, were incubated under anaerobic conditions at 37°C for 24 h for bacteria. A disk with only 15 µL of DMSO (without leaves oil) was used as a negative control. Antimicrobial activity was evaluated by measuring, (in triplicate), the diameter of the growth inhibition zones in millimeters with an accuracy of 0.5 mm. The initial test concentration of essential oil of samples obtained with SFE extraction was diluted in the proportion of (5%, 2.5%, 1.25%, 0.66%, 0.33% and 0.15%).

Statistical analysis

All assays were performed in triplicate for each extracting condition. An analysis of variances (ANOVA) for each experiment (yield and quality evaluation) was carried out. The results are reported as standard deviation ±SD (standard deviation) obtained from the three measurements.

RESULTS AND DISCUSSION

Extraction yield

The results of extraction yield obtained by both maceration and supercritical carbon dioxide extraction at different particles size and pressures are giving in Table 1. As seen to results a greet variability of yield are shown by varying the extraction method. Many studies also show the

Table 4: Antibacterial activity of essential oils of *Artemisia absinthium*

	Inhibition zone diameter (mm)					
Concentration (%)	5	2.5	1.25	0.66	0.33	0.15
Campylobacter jejuni	12±1	10.33±0.5	7±1	8.66±1	7±1	-
Campylobacter fetus	13±1	11.16±0.7	10.66±1.15	8.16±1	8±1.25	-
Campylobacter coli	17.16±1.7	11.66±0.5	9.5±0.5	8.66±2.0	7.66±1.0	-

	Antibiotic (Inhibition zone: mm)		
	Gentamycin	Augmentin	Erythromycin
C. jejuni	25	30	22
C. fetus	<16	<14	30
C. coli	30	30	25

C: Campylobacter; ± values are mean inhibition zone (mm) ± S.D of three replicates; - : no inhibition

influence of the extraction methods on yields and oils quality[18]. In the SFE the yield results are influenced by many parameters such as pressure and particle size. However, it increases directly by pressure, particle size and time extraction. The highest extraction yield (2.50%, w/w) was obtained at 40°C, 18 MPa and 0.25 mm (Fig.1). The effect of extraction condition on the yield is presented in the Table 1. In the temperature, we show the increasing of pressure increases the extraction yield. Indeed, at 40°C after 200 minutes the yield increases from 1.25% at 10 MPa and 1 mm of particle size to 1.85% at 18 MPa (Table 1). This behavior is due to increased density of the supercritical CO_2 under pressure, which leads to an increase in the solvent power and the solubility, therefore a greater extraction yield. It is also observed that the addition of absinthe leaves at 0.25 mm particle size significantly increased yields from 1.25% at 10 MPa to 2.50% at 18 MPa, the highest yield was observed at 18 MPa (0.25 mm) (Fig.1), there is evidence that presence of fine particle (0.25 mm) and pressure increases the contact surface between solvent and the plant matrix, which increases the solubility.

Phenols contents

Total phenolic contents of *A. absinthium* leaves measured according to the Folin Ciocalteu method are shown in Table 2. The highest amount of phenolic content is obtained by maceration method (11.56 ± 1.20 mg GAE.g^{-1} DW) while in SCF-CO_2 extraction TP is 8.53 mg GAE.g^{-1} DW. Total flavonoid contents (TF) can be determined in the sample extracts/fractions by reaction with sodium nitrite, followed by the development of colored flavonoid-aluminum complex formation using aluminum. TF of the extracts expressed by catechin equivalent ranged from 5.99 ± 0.20 mg CE/g DM with SFE extraction to 8.49 ± 0.50 mg CE. g^{-1} DW with maceration (Table 2). Our results are lower than the results given by Mahmoudi (12.4 ± 0.6 mg CE. g^{-1} of DW)[19] and higher than the results given by Mohamed Ali (1.89 mg QE/g DW)[20].

Chemical composition of essential oil

The chemical composition is presented in Table 3. In total, 25 components covering more than 99% of total oil composition were identified from *A. absinthium*. The main components were β-thujone (8.307%), α-Farnesene (7.537%), Cis-Salvene (5.701%), Borneole (5.564%) and Azulene (4.775%). The major contribution was attributed to the hydrocarbures (33.755%) of all compounds followed by monoterpene oxygenated (19.02%) and sesquiterpene hydrocarbures (9.227%), monoterpene hydrocarbures group which represents 5.965% of all compounds finally the sesquiterpenes oxygenated present the lowest percentages (4.392%) (Fig. 2). The chemical composition of essential oils of *A. absinthium* growing in USA shows that β-thujone (17.5 to 42.3 %) and cis-sabinyl acetate (15.1 to 53.4%) are the major compounds[21]. In addition, in the absinthe collected from different European countries (Greece, spain, Ukraine, France and Italy), α-thujone and β-thujone were detected as major components but with different percentages ranging from 4.5 to 38.7%[22]. The essential oil obtained from *A. absinthium* grown in Brazil contain a significant amount of β-thujone (10.10%)[23].

Antioxidant capacity and reducing power of Artemisia absinthium L. extracts

Two in vitro assays based on DPPH and $ABTS^+$ radical scavenging capacity, respectively, were used to evaluate antioxidant capacity of Artemisia extract. The results of antioxidant activity were shown in Table 2. Total antioxidant activity, measured by the DPPH method, ranged from 4.82 with SFE extraction (IC_{50}= 0.129 mg. mL^{-1}) to 5.26 mM. g^{-1} DW (IC_{50}= 0.118 mg. mL^{-1}) with maceration extraction. The maceration extract exhibited the greatest antioxidant activity TEAC (5.26 mM TE/g of dry extract) (TE: Trolox Equivalent) with DPPH method. The results show that all extract of absinthe leaves had low antioxidant activity (IC_{50} = 0.048 mg. mL^{-1}) compared to standard control antioxidants such as BHT (10.77 ± µg. mL^{-1}). There was a significant variation in the percentage inhibition of the $DPPH^{\bullet}$ radical for the methanol extractions (IC_{50} = 118 µg/ml) in again leaves exhibited the highest antioxidant capacity with SFE of *Artemisia absinthium* L. (IC_{50} = 129 µg/ml), but this value was lower than those cited by Harminder[24] (IC_{50} = 146.3 µg/mL in *Artemisia scoparia* and lower than those cited by Mahmoudi[19,20] (IC_{50} = 612 µg/mL). The results of antioxidant capacity are expressed in terms of Trolox equivalent antioxidant capacity (TEAC) and shown in Table 2. The free radical scavenging activity determined by ABTS ranged from 1.965 mMTE/g of dry mater of *A. absinthium* with SFE to 2.232 mM/g of dry mater with ME

Table 5: Lineal correlation coefficients between composition and antioxidant capacity

	DPPH	ABTS
TP	0.884	0.845
TF	0.999	0.995

TP: Total Polyphenol, TF : Total Flavonoid

extraction. The ME extract which contains the highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity TEAC (2.232 mM TE/g of dry extract). The highest capacity antioxidant showed that Artemisia species was a good source of phenolic and therefore it could be a good source of natural antioxidants.

Antibacterial activity

Antimicrobial activity of obtained essential oils from *A. absinthium* against the employed micro-organisms is summarized in Table 4. As seen to these results, the plant extract showed the huge potential to be used as antimicrobial agent where it was found to inhibit the growth of all tested bacteria. *A. absinthium* oil was the most active against *Campylobacter coli* (CCUG 11283T) followed by *Campylobacter fetus* (CCUG 6823T) and *Campylobacter Jejuni* (MB_9515_05), the measured inhibition zone ranged from 7±1 to 17.16±1.7 mm. The activity of these oils varies depending on the concentration and the nature of the strains tested. Most of the extracts have antibacterial activity, but at the concentration of 0.15% of essential oils, all strains have no inhibition. The diameter of inhibition produced by essential oils of *A. absinthium* is significantly lower than those produced by three antibiotics (Gentamycin, Augmentin and Erythromycin). The results of the strain of *Campylobacter fetus* show that this strain is resistant to Gentamycin and Augmentin. Our results are lower with those reported by Lopes-Lutz[23] how reported that essential oil of wormwood aerial parts growing in Canada has a high activity against Staphylococcus aureus (inhibition zone = 25 ± 1.4 mm). The results reported by Kamel Msaada [25] show that the inhibition zone of antimicrobial activity of aerial parts of *A. absinthium* ranged from 16 to 25 mm. showed less susceptibility to the essential oils (inhibition zone ranged from 6 to 11 mm). The results show that *A. absinthium* essential oil was endowed with a significant antibacterial activity that is closely related to the organoleptic quality of the oil, which in turn depends strongly on the collection country of plant material.

Correlation between total polyphenol content and trolox equivalent antioxidant capacity

Correlation analysis was used to explore the relationships amongst the different antioxidant variables measured for Artemisia extracts. Results revealed that total polyphenols and flavonoids content were highly correlated with DPPH $0.884<r<0.999$ and ABTS $0.845<r<0.995$ (Table 5). We show also that total flavonoids where higher correlated than total polyphenols based on ABTS and DPPH. As shown in Table 5, the ABTS assay showed higher linear relationship with TP and TF contents than DPPH thus due to that $ABTS^{+}$ solution was more stable and suitable for determination of water-soluble phenolics in *Artemisia* which had been defatted. Recently many studies have been described a positive correlation between the determination of phenolic compounds and the correlation between composition and activity by DPPH, FRAP, ORAC and ABTS assays[15,26]. Results revealed that polyphenols from *Artemisia absinthium* L. extract had high antioxidant activities. Free radical scavenging of phenolic compounds is an important property underlying their antioxidant activity.

CONCLUSION

Extraction of Artemisia leaves was investigated by two different methods. Comparing the experimental results, the next attributes were established: The maceration extraction presents the best yield of essential oil, In SC-CO_2 the highest yield value was observed at 18 MPa, 40°C and 0.25 mm of particle size combining extraction yield and product quality (antioxidant activity, TP, TF). We found a considerable correlation between phenolic compounds with ABTS and DPPH assays (Fig.3, Fig.4). The good correlation between the two methods of the characterization of antioxidant capacity (ABTS and DPPH) suggests that the antioxidant compounds from analyzed medicinal herbs present both reducing power and radical scavenging capacity. The essential oil of *Artemisia absinthium* showed an interesting antibacterial activity. The antibacterial activity is economically important considering the availability of herbal material.

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