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Original Research

Phytochemical Composition and Biological Activity Evaluation of *Juniperus sabina* Essential Oil

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Abstract

The *Juniperus sabina* L. is considered an endemic plant species in the Kabylia mountains, north-east of Algeria. In order to valorize and provide additional evidence that could promote the conservation of this species, the phytochemical components profile of the *Juniperus sabina* essential oils (EO) extracted from different parts of the plant was characterized, and their biological activities were evaluated. Gas chromatography with flame ionization and mass spectrometry detectors (GC-FID, GC-MS), along with ^{13}C nuclear magnetic resonance (^{13}C NMR), were used to determine the composition of the EO chemical profile. The methods used to analyze the antioxidant properties of EO were DPPH, ABTS, and H_2O_2 scavenging assays, along with TAA and RPA for reducing potential assays. Also, cholinesterase and α-glucosidase inhibitory activities of this EO were tested. The screening

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for phytochemical composition revealed a diverse chemical profile, with major components such as sabinene (24.9%-51.8%) and terpinen-4-ol (11.5%-29.9%). Antioxidant capacity evaluation showed the different responses between plant part EOs and types of antioxidant assays. Therefore, the EO from berries showed a higher activity towards the potential assays of twigs' EO, which exhibited better activity towards radical scavenging assays. The obtained EOs from needles and twigs showed moderate acetylcholinesterase inhibitory activity with lethal inhibitory concentrations of IC_{50} of 28.18 µg/ml and 37.27 µg/ml, respectively. These results suggest that *Juniperus sabina* essential oil (EO) has potential as a natural antioxidant that could be developed for use in food systems, as well as in the treatment of neurodegenerative disorders.

Keywords: antioxidant potential, cholinesterase, *Juniperus sabina*, essential oil, α-glucosidase

Introduction

Natural chemicals are receiving more attention since the synthetic pesticides provide undesirable effects to human health and the environment [1, 2]. Biopesticides, such as plant essential oils (EOs), produced by a number of plant families including Myrtaceae, Lamiaceae, Asteraceae, Apiaceae, and Rutaceae, may be a good alternative for insect pest control [3], since they are biodegradable and show no effects on non-target organisms or the environment [4]. The nature of these chemicals, which includes their rapid degradation, low residual capacity, and different mechanisms of action, is partly responsible for this attitude [2].

The genus *Juniperus* belongs to the Cupressaceae family and is one of the most widespread conifers, present in the Northern Hemisphere and comprising more than 50 species. These evergreen, perennial conifers or small shrubs are found in a wide range of biotopes, from the Arctic tundra to arid Mediterranean regions, and demonstrate considerable ecological adaptability [5]. In North Africa, from the Cupressaceae family, 6 species have been identified with a noticeable random distribution [6]. The updated Algerian flora includes 4 *Juniperus* species: *J. communis* L., *J. oxycedrus* L., *J. phoenicea* Guss., and *J. sabina* L. [5]. The distribution area of *J. sabina* is represented by the Mediterranean region, although it shows considerable taxonomic distinction [7]. In the northeastern part of Algeria, the Kabylia region, all *Juniperus* species are commonly referred to as "Taqa", specifically represented by *J. sabina*, with the local common name "Achalal". The species is classified as endangered, and its occurrence is restricted to areas of the Djurdjura National Park such as Aswel, Issig-Issig, and Azrou Ougougam [6]. Historically, the genus *Juniperus*, especially *J. communis*, has been used for therapeutic, culinary, and cultural purposes [8].

The *Juniperus* species are well known for their extensive chemical composition, which includes terpenes, flavonoids, phenolic acids, resins, tannins, and organic acids. Terpenes such as α-pinene, sabinene, and limonene are the main constituents of juniper essential oils and give them their characteristic aromatic properties [5]. The resin, which is rich in various organic compounds, has long been valued for its antiseptic properties. The tannins contained in the bark and berries are used in traditional medicine and are also important in leather tanning [7]. Essential oils (EOs) extracted from *Juniperus* species have aroused scientific interest due to their diverse properties. These EOs are important in the cosmetics industry, especially for improving the fragrance of perfumes [8]. The endogenous production of free radicals is a potential source of oxidative damage to essential biomolecules such as DNA, lipids, and proteins, which harms cells, tissues, and organs in the human body [9, 10].

Compounds with antioxidant properties, usually found in plants or their extracts, are known for their ability to modulate oxidative stress and exert protective effects on cellular health [11, 12], as well as for scavenging free radicals and preserving food and food products [13]. Juniper EO is also known for its antimicrobial and antioxidant properties, which may have therapeutic effects and present insecticidal properties [14]. In this context, EOs extracted from the aerial parts of *J. sabina* were investigated.

Diabetes, caused by a lack of insulin secretion, is a metabolic disease that leads to elevated blood glucose levels and accompanying complications that seriously threaten human health. Most currently available antidiabetic drugs lower fasting blood glucose levels but have little effect on postprandial glycemic excursions and therefore do not normalize postprandial hyperglycemia. It is known that *α*-glucosidase is directly related to postprandial hyperglycemia, which plays a key role in the development of type 2 diabetes mellitus [15]. Therefore, reducing postprandial hyperglycemia by inhibiting *α*-glucosidase has been shown to be an effective strategy for the treatment of type 2 diabetes mellitus [15].

Alzheimer's disease (AD) is the most common cause of dementia in adults. It is characterized by a progressive deterioration of cognitive function and behavioral changes. The most important class of drugs for the treatment of AD is the cholinesterase inhibitors. The first cholinesterase inhibitor to be approved for the symptomatic treatment of AD was tacrine, followed by donepezil, rivastigmine, and galantamine [16]. Galantamine is a natural alkaloid that was originally

isolated from the plants *Galanthus nivalis* (common snowdrop) and *Galanthus woronowii* (Caucasian snowdrop), members of the Amaryllidaceae family [16]. Research into the inhibitory effects of compounds of natural origin is very important, as their use does not lead to undesirable side effects, as is the case with synthetic drugs [17]. In a normal brain, AChE activity predominates over BChE activity. It has been observed that with the progression of Alzheimer's disease, BChE levels in the brain increase while AChE levels decrease [18]. Since BChE also hydrolyzes acetylcholine (ACh), both enzymes are considered regulators of ACh levels and are important for the treatment of AD [18]. These observations have encouraged scientists to carry out diverse research on the potential bioactive properties of juniper for therapeutic uses and industrial applications. The valorization of *Juniperus* species, due to their diverse chemical composition, is reflected in their applications ranging from traditional medicine to cooking and industrial use. In Algeria, the properties of *J. sabina* are little known or limited, due to its restricted geographical distribution.

The aim of the present study was to characterize the chemical composition and to evaluate the antioxidant and enzyme inhibition properties, including AChE/BChE and α-glucosidase inhibitory activity, of the essential oils isolated from different parts of the *J. sabina* plant: leaves (EOL), twigs (EOT), and berries (EOB), collected from Djurdjura National Park in the Kabylia region, Algeria.

Materials and Methods

Plant Material and Essential Oil Extraction

The vegetal material is represented by the medicinal plant *Juniperus sabina* L. Its identification was carried out using the literature on Algerian flora [6, 7]. A voucher specimen has been preserved and archived in the Collection Herbarium at the Laboratory of Valorization of Natural Biological Resources, Setif 1 University, Algeria.

The aerial parts (leaves, twigs, and berries) of *Juniperus sabina* L. were collected from trees in the Djurdjura National Park boudaries (altitude 1847 m, latitude 04°03'65.57"N, longitude 31°60'62.73"E) in the northern part of Algeria (Fig. 1). The collected material was carefully examined by removing unwanted parts on the spot. Leaves and twigs were cut into small pieces and carefully dried in the shade. For hydrodistillation purposes, 300 g of material from each part was used individually for oil extraction with 5 L of fresh water using a Clevenger apparatus for 3 h. The resulting essential oils (EOs) were dried with anhydrous Na_2SO_4, filtered, and stored at 4ºC in the dark until analysis. The yield of EOs was calculated on the basis of dry weight (w/w).

Gas chromatographic analyses were performed using a Perkin Elmer Clarus 500 gas chromatograph equipped with a flame ionization detector (FID) and two silica gel capillary columns: BP-1 (polydimethylsiloxane, dimensions: 50 m × 0.22 mm i.d., film thickness:

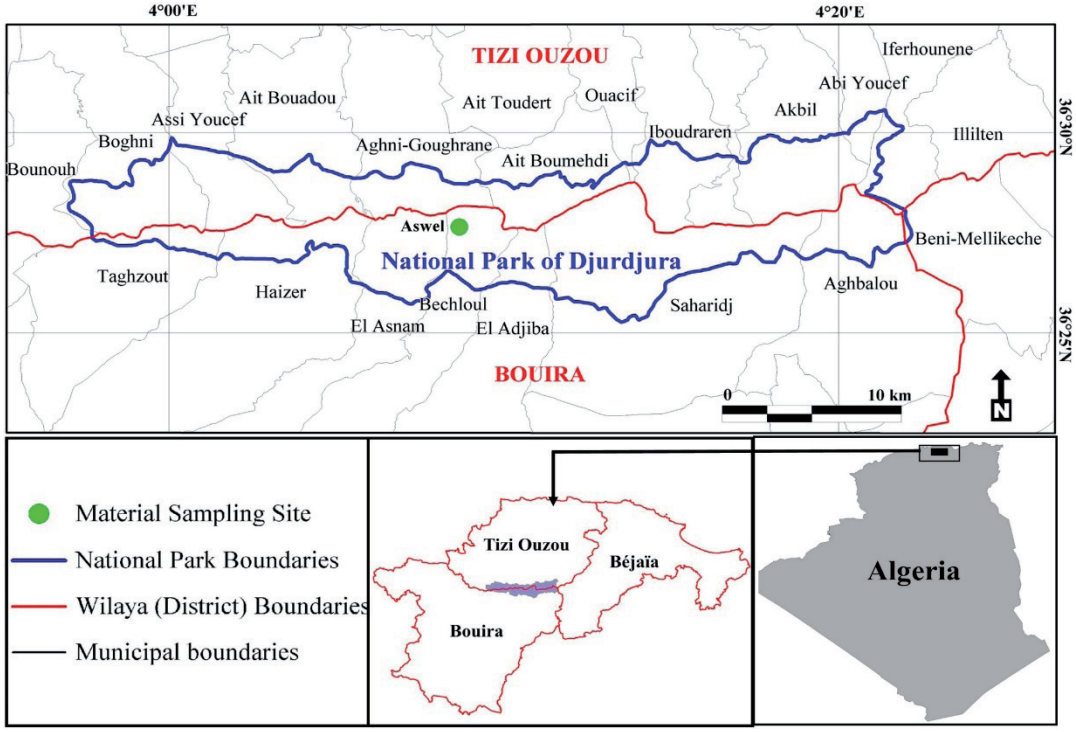

Fig. 1. Geographical situation and location of the *J. sabina* sampling area in the Djurdjura National Park boundaries, north-east of Algeria.

0.25 µm) and BP-20 (polyethylene glycol, dimensions: 50 m × 0.22 mm i.d.). The oven temperature was increased from 60 to 220ºC at a rate of 2ºC/min, followed by an isothermal hold at 220ºC for 20 min. The injector and detector temperatures were set to 250ºC. Hydrogen was used as the carrier gas at a flow rate of 0.8 ml/min and a split ratio of 1:60. The relative proportions of the oil components were expressed as percentages obtained by normalizing the peak areas without using correction factors. The retention indices (RIs) were determined relative to the retention times of an n-alkane series using linear interpolation (software "Target Compounds", Perkin Elmer).

The EOs from the different aerial parts of *J. sabina* were analyzed using a Perkin Elmer Turbo Mass detector (quadrupole) directly coupled with a Perkin Elmer Autosystem XL. This system was equipped with a silica gel capillary column (BP-1 polydimethylsiloxane, dimensions: 50 m x 0.22 mm i.d., film thickness: 0.25 µm). Helium was used as the carrier gas at a rate of 1.0 ml/min and a split ratio of 1:80. The injection volume was set to 0.2 µl, and the temperature of the injector was kept at 250ºC. The oven temperature was programmed to rise from 60 to 230ºC at a rate of 2ºC/min, followed by an isothermal hold at 220 ºC for 20 min. In addition, the ion source temperature was set at 250 ºC, and the ionization energy was maintained at 70 eV. The 13C NMR spectra were recorded with a Bruker AVANCE 400 Fourier transform spectrometer operating at 100.63 MHz for 13C. The spectrometer was equipped with a 5 mm probe, and the measurements were performed in CDCl3. All chemical shifts were referenced to the internal TMS standard. The parameters for the experiment were as follows: a pulse width of 4 µs with a flip angle of 45°, an acquisition time of 2.7 s for a 128 K data table, and a spectral width of 25 kHz (250 ppm). Continuous Pulse Decoupling (CPD) mode was used, and the digital resolution was set to 0.183 Hz/pt. Each sample (40 mg EO in 0.5 ml CDCl3) underwent 3000 accumulated scans.

The identification of the individual components comprised the following steps: (i) evaluation of their gas chromatographic (GC) retention indices (RIs) on polar and nonpolar columns by comparison with reference compounds in a laboratory-prepared library and with literature data [19]; (ii) computer matching with commercially available mass spectral libraries [20, 21]; (iii) comparison of signals in the 13C nuclear magnetic resonance (NMR) spectra of the samples with those in reference spectra from the laboratory spectral library, supported by customized software [22, 23].

Bioactivity Evaluation Assays of *J. sabina* EOs

Antioxidant Activity Assay

Compounds with antioxidant properties showed their ability to modulate oxidative stress and exert protective effects on cellular health, release free radicals, and preserve alimentations. In this context, EOs extracted from the aerial parts of *J. sabina* were investigated. The antioxidant potential of the EOs isolated from different plant parts of *J. sabina* was tested using five different methods: DPPH, ABTS, and H_2O_2 scavenging activity, along with total antioxidant activity assay (phosphomolybdenum method) and reducing power assay, in order to obtain a correct assessment [23]. All spectrophotometric measurements were performed on a UV/Vis spectrophotometer (Specord 50 UV/Vis, Analytic Jena GmbH, Jena, Germany). All experiments were carried out in triplicate, and results were presented as mean±SD.

DPPH Free Radical Scavenging Activity

The evaluation method of EOs for their ability to scavenge DPPH free radicals was adapted with some modifications according to a previous method [24]. Samples of different concentrations of EOs were mixed with 2 ml of an absolute ethanolic solution and DPPH (1 mM). After vigorous shaking, the mixtures were allowed to stand for 30 min in a dark environment. Then the absorbance of these mixtures was measured at a wavelength of 517 nm. The control sample contained only ethanol and was measured in the same way [25]. The ability to scavenge DPPH free radicals was calculated as follows:

$$Inhibition\ \% = \frac{\left(A_{control} - A_{sample}\right)}{\mathrm{A}_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control (containing DPPH solution and ethanol); A_{sample} is the absorbance in the presence of extract/EOs/standards. The results were expressed as inhibitory concentrations (IC_{50}) values.

ABTS Free Radical Scavenging Activity

The method for assessing the ability of EOs to scavenge ABTS radicals was adapted with minor modifications from the method described previously [19]. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was dissolved in water to produce a solution with a concentration of 7 mM. To generate the ABTS radical cation (ABTS•+), the ABTS solution was mixed with potassium persulfate to a final concentration of 2.45 mM. This mixture was then left to stand in the dark at room temperature for 12 to 16 h before use. The ABTS•+ solution was diluted with water until an absorbance of 0.70 (±0.02) at 734 nm was achieved. The reaction mixture consisted of 0.07 ml of the extract and 3 ml of the ABTS solution. After incubation for 6 min, the absorbance was measured at 734 nm [26]. The antioxidant activity of the sample was then calculated using a specific equation based on the change in absorbance due to the interaction of the sample

extract with the ABTS radical. The ability to scavenge ABTS free radicals was calculated as follows:

$$Inhibition\ \% = \frac{(A_{control} - A_{sample})}{\mathrm{A}_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control at the moment of solution preparation; A_{sample} is the absorbance in the presence of extract/EOs/standards after 6 min. The results were expressed as IC_{50} values.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide (H_2O_2) scavenging activity was determined using a modified method [26]. A series of test tubes were prepared by adding 0.25 ml of 1 mM ferrous ammonium sulfate to each. Subsequently, 1.5 ml of different concentrations of EOs or ascorbic acid were added to the tubes, and the solutions were mixed. Then, 62.5 µl of 5 mM hydrogen peroxide was added, and the tubes were incubated for 5 min at room temperature in the dark (to prevent bleaching of the hydrogen peroxide). After incubation, 1.5 ml of 1 mM 1,10-phenanthroline was added to each tube, mixed thoroughly, and incubated for an additional 10 min at room temperature. The absorbance of the mixtures was measured at 510 nm. As a control, a blank solution was prepared containing only 0.25 ml of 1 mM ferrous ammonium sulfate, 1.562 ml of water, and 1.5 ml of 1 mM 1,10-phenanthroline. This control tube showed the maximum absorbance. In addition, a reagent blank containing only 1,10-phenanthroline was prepared, and its absorbance was subtracted from the absorbance values of all other tubes to ensure accurate results [26]. The ability to scavenge hydrogen peroxide was calculated using the following equation:

$$Inhibition\ \% = \frac{A_{sample}}{\mathrm{A}_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control tube without sample(s); A_{sample} is the absorbance in the presence of plant extract/EOs/standards. The results were expressed as IC_{50} values.

Total Antioxidant Activity (TAA) by the Phosphomolybdenum Method

The total antioxidant activity of the EOs was evaluated using the phosphomolybdenum method based on the reduction of Mo(VI) to Mo(V) [27]. 400 µl of the EOs in ethanol was added to 4 ml of a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was incubated at 95ºC for 90 min, and the absorbance was measured at 695 nm in comparison with a blank sample (400 µl ethanol and 4 ml reagent solution) [27]. EOs were tested at a concentration of 0.5 mg/ml, and the antioxidant capacity was expressed as the equivalent of ascorbic acid (AAE µg/ml).

Reducing Power Assay

The reducing power assay was performed according to the previous method [28]. In this analysis, 1 ml of EO or *α*-tocopherol in ethanol was combined with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% solution of $K_3Fe(CN)_6$. After incubation at 50ºC for 20 min, 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000×g for 10 min. Then 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. The absorbance was measured at 700 nm. An increased absorbance of the mixture indicates an increased reducing power [29]. The EOs were tested at 1 mg/ml and compared to *α*-tocopherol at 0.25 mg/ml absorbance.

Enzyme Inhibition Potential Assay

The enzyme inhibitory potential of the EOs isolated from different plant parts of *J. sabina* was tested for cholinesterase inhibitory potential and α-glucosidase inhibitory potential. All enzyme spectrophotometric measurements were performed using a Synergy HTX S1LFA multimode microplate reader (BioTek Instruments, Inc., Winooski, VT). All experiments were carried out in triplicate, and the results were presented as mean±SD.

Cholinesterase Inhibition Potential Assay

The inhibitory effect of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was measured using a slightly modified Ellman assay [28]. A typical run consisted of 180 µl of phosphate buffer (0.1 M, pH 8), 10 µl of DTNB (at a final concentration of 0.3 mM, prepared in 0.1 M phosphate buffer, pH 7, with 0.12 M sodium bicarbonate for stabilization), 10 µl of the sample solution (dissolved in 80% EtOH), and 10 µl of AChE/BChE solution (final 0.03 U/ml). The reagents were mixed in a 96-well plate, and the reaction was started by adding 10 µl of acetylthiocholine iodide or butyrylthiocholine iodide (ATChI/BTChI) to reach a final concentration of 0.5 mM. As a negative control, 80% EtOH was used instead of the sample solution. Non-enzymatic hydrolysis was also monitored by measuring two blank runs for each test. Briefly, in the first blank run, the AChE/BChE was replaced by an appropriate amount of buffer, and in the second blank run, the ATChI/BTChI was replaced by an appropriate amount of buffer. All spectrophotometric measurements were performed at 405 nm and at room temperature for 6 min, and the results were expressed as percent inhibition of enzyme activity or IC_{50} and compared to the standard (tacrine) [28].

α-Glucosidase Inhibition Potential Assay

The enzyme inhibition activity for α-glucosidase was evaluated according to the method previously reported (Priscilla et al., 2014) with minor modifications. The reaction mixture consisted of 50 µl of 0.1 M phosphate buffer (pH 7.0), 25 µl of 0.5 mM 4-nitrophenyl-α-D-glucopyranoside (p-NPG, dissolved in 0.1 M phosphate buffer, pH 7.0), 10 µl of the test sample, and 25 µl of the α-glucosidase solution (a stock solution of 1 mg/ml in 0.01 M phosphate buffer, pH 7.0, was diluted to 0.1 Units/ml using the same buffer immediately before the test). This reaction mixture was then incubated at 37ºC for 30 min. The reaction was terminated by the addition of 100 µl of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffer. The controls were carried out in the same way but using methanol in place of the plant extracts. Acarbose was used as a positive control [15], and the results were expressed as percent inhibition of enzyme activity (%).

Statistical Analysis

For statistical analysis purposes, the activity tests were conducted in triplicate, and the results are expressed as mean±standard deviation (SD). All data were subjected to analysis of variance (ANOVA), and means were compared by least significant difference (LSD) using statistical software (XLStat 2022). Difference at $p<0.05$ was considered to be significant.

Results

The EOs of *J. sabina* extracted from dried plant material have a light yellow color and a strong odor. The yields of the oils from the different parts of the plant were estimated at 1% in the leaves, 0.215% in the twigs, and 0.65% in the berries.

The chemical composition of the EOs isolated from different *J. sabina* plant materials using gas chromatography (GC-FID, GC-MS) revealed the presence of 47 identified compounds. These compounds belong to the groups of monoterpene hydrocarbons (MH), oxygenated monoterpenes (MO), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (SO), and diterpenes (DT). The constituents of *J. sabina* are listed in the order of elution on the BP-1 column, as shown in Table 1.

The dominant components of the analyzed *J. sabina* EOs were monoterpenes. The total percentage of monoterpene hydrocarbons in the EOs from leaves (EOL), twigs (EOT), and berries (EOB) was 71.0%, 59.7% and 52.3%, respectively. The total percentage (%) of oxygenated monoterpenes was 22.5%, 31.8% and 38.7%, respectively. Sesquiterpenes and diterpenes were present in small amounts (less than 4% in total), as mentioned in Table 1.

Among all compounds identified, sabinene showed a higher prevalence in EOL (51.8%) compared to EOT (37.1%) and EOB (24.9%). Terpinen-4-ol, another quantitatively important constituent, showed different concentrations, with 11.5% in EOL, 19.0% in EOT, and a remarkable 29.9% in EOB. The abundance of α-pinene varied, with 2.3% in EOL, 3.0% in EOT, and the highest content found in EOB at 12.5%. p-Cymene, another notable constituent, varied between plant parts, with the highest content found in EOB at 6.8% (Table 1).

The evaluation of the antioxidant capacity of these EOs was carried out using the cited methods (Table 2) in order to obtain a correct assessment [30]. The EOs from all parts of *J. sabina* showed a significantly lower ability to scavenge DPPH radicals than the standards used for comparison (α-tocopherol and thymol). The concentrations of the tested EOs inhibiting 50% of the DPPH radical (IC_{50} values) for EOL, EOT, and EOB were 33.93±0.15 mg/ml, 11.04±0.85 mg/ml, and 41.79±0.098 mg/ml (in the reaction system), respectively (Table 2).

The ability of the tested EOs to scavenge ABTS radicals was much better compared to DPPH radical scavenging, with IC_{50} values (in the reaction system) of 73.6±7.1 µg/ml, 8.5±1.2 µg/ml, and 16.8±0.1 µg/ml for EOL, EOT, and EOB, respectively. This was still weaker when compared to those of *α*-tocopherol and thymol (IC_{50} 0.92±0.07 µg/ml and 0.32±0.02 µg/ml, respectively) (Table 2).

The EOs obtained from different plant parts of *J. sabina* showed a moderate ability to inhibit AChE and a weak ability to inhibit BChE compared to the AChE/BChE inhibitor tacrine (Table 3). The best AChE inhibitory potential was observed for EOL, with a lethal inhibition concentration of IC_{50} = 28.18 µg/ml, while EOT showed a slightly lower inhibitory potential with IC_{50} = 37.27 µg/ml. The inhibitory potential of the tested EOs (concentration in the reaction system 45 µg/ml) against BChE ranged from 22.10% for EOL to 32.33% for EOB. The activity of the tested EOL and EOT could be related to the chemical composition variability of the EOs.

At the tested concentration of 90 µg/ml, the EOs from different plant parts of *J. sabina* showed a low ability to inhibit α-glucosidase compared to acarbose (Table 3). The inhibitory potentials of the *J. sabina* EOs were 9.5% for EOL, 7.24% for EOT, and 5.88% for EOB. The EOs isolated from *J. sabina* contain sabinene and terpinen-4-ol as the most abundant components. EOB also contains a significant amount of α-pinene and p-cymene, but despite the good inhibitory effect of α-pinene, this essential oil showed no inhibitory effect on α-glucosidase.

Table 1. Chemical composition of the essential oils obtained from *Juniperus sabina* leaves, twigs, and berries, collected from the Kabylia region, Algeria.

Compound	RIa	RIa (lit)	RIp	RIp (lit)	Class	Content (%)			Identification mode
						Leaves	Twigs	Berries	
α-Thujene	922	932	1017	1026	MH	2.7	2.8	0.7	GC-RI, GC-MS, ^{13}C-NMR
α-Pinene	929	936	1014	1025	MH	2.3	3.0	12.5	GC-RI, GC-MS, ^{13}C-NMR
Camphene	942	950	1064	1068	MH	0.1	0.1	0.2	GC-RI, GC-MS, ^{13}C-NMR
Thuja-2,4(10)-diene	945	945	1125	1122	MH	-	-	0.2	GC-RI, GC-MS, ^{13}C-NMR
Sabinene	965	973	1024	1022	MH	51.8	37.1	24.9	GC-RI, GC-MS, ^{13}C-NMR
ß-Pinene	969	978	1111	1110	MH	0.2	0.2	1.5	GC-RI, GC-MS, ^{13}C-NMR
Myrcene	980	987	1160	1161	MH	2.8	1.8	2.2	GC-RI, GC-MS, ^{13}C-NMR
δ-2-Carene	994	999[a]	1131	1131[a]	MH	0.2	0.2	-	GC-RI, GC-MS, ^{13}C-NMR
α-Phellandrene	996	998	1165	1167	MH	0.1	0.1	-	GC-RI, GC-MS
α-Terpinene	1008	1010	1180	1169	MH	1.9	2.2	-	GC-RI, GC-MS, ^{13}C-NMR
p-Cymene	1011	1014	1270	1272	MH	2.3	4.3	6.8	GC-RI, GC-MS, ^{13}C-NMR
Limonene*	1020	1025[a]	1201	1200	MH	1.6	1.4	2.4	GC-RI, GC-MS, ^{13}C-NMR
ß-Phellandrene*	1020	1021	1210	1210	MH	-	0.8	0.2	GC-RI, GC-MS, ^{13}C-NMR
1,8-Cineole*	1020	1022	1210	1212	MO	0.7	-	-	GC-RI, GC-MS
(*E*)-ß-Ocimene	1035	1038	1249	1250	MH	0.2	0.1	-	GC-RI, GC-MS, ^{13}C-NMR
γ-Terpinene	1047	1050	1244	1246	MH	3.7	4.4	0.4	GC-RI, GC-MS, ^{13}C-NMR
trans-Sabinene hydrate	1051	1087	1461	1551	MO	1.1	0.7	1.8	GC-RI, GC-MS, ^{13}C-NMR
Terpinolene	1077	1079	1282	1283	MH	1.1	1.2	0.3	GC-RI, GC-MS, ^{13}C-NMR
cis-Sabinene hydrate	1082	1083[b]	1545	1539[b]	MH	1.9	-	1.8	GC-RI, GC-MS, ^{13}C-NMR
Linalool	1082	1086	1545	1547	MO	-	1.8	-	GC-RI, GC-MS
cis-p-Menth-2-en-1-ol	1106	1122	1560	1638	MO	0.9	1.2	1.3	GC-RI, GC-MS
trans-p-Menth-2-en-1-ol	1098	1112	1625	1573	MO	0.6	1.1	1.7	GC-RI, GC-MS, ^{13}C-NMR
Sabina ketone	1125	1132[b]	ND	1606[b]	MO	0.1	0.7	-	GC-RI, GC-MS
Citronellal	1129	1133	1478	1477	MO	0.5	0.2	-	GC-RI, GC-MS, ^{13}C-NMR
neo-Isopulegol	1140	1142	1569	1568	MO	tr	-	-	GC-RI, GC-MS, ^{13}C-NMR
Terpinen-4-ol	1161	1164	1600	1602	MO	11.5	19.0	29.9	GC-RI, GC-MS
α-Terpineol	1172	1175	1693	1697	MO	0.3	0.7	1.1	GC-RI, GC-MS, ^{13}C-NMR
Myrtenol	1174	1178	1784	1796	MO	-	0.5	-	GC-RI, ^{13}C-NMR
cis-Piperitol	1180	1185	1675	-	MO	-	0.3	0.4	GC-RI, GC-MS, ^{13}C-NMR
trans-Piperitol	1191	1192	1750	1695	MO	0.2	0.5	0.7	GC-RI, GC-MS, ^{13}C-NMR
Citronellol	1208	1208[a]	1763	1761[a]	MO	2.6	2.7	-	GC-RI, GC-MS, ^{13}C-NMR
Methyl citronellate	1240	1263[c]	1561	1582	MO	2.1	2.1	-	GC-RI, GC-MS, ^{13}C-NMR
Phellandral	1249	1252[d]	1712	1715[d]	MO	-	tr	-	GC-RI, GC-MS, ^{13}C-NMR
Cuminol	1261	1247	2407	-	MO	-	0.2	-	GC-RI, GC-MS, ^{13}C-NMR
Thymol	1144	1127	1724	1674	MO	-	tr	-	GC-RI, GC-MS, ^{13}C-NMR
Carvacrol	1278	1278	2221	2210	MO	-	0.1	-	GC-RI, GC-MS, ^{13}C-NMR
(*E*)-β-Caryophyllene	1417	1421	1592	1598	SH	0.1	0.1	-	GC-RI, GC-MS, ^{13}C-NMR
Germacrene D	1476	1479	1709	1708	SH	0.2	-	-	GC-RI, GC-MS, ^{13}C-NMR
α-Muurolene	1490	1496	1721	1723	SH	0.2	0.1	-	GC-RI, GC-MS

→

γ-Cadinene	1504	1507	1750	1763	SH	0.4	0.2	-	GC-RI, GC-MS, ^{13}C-NMR
δ-Cadinene	1514	1520	1751	1756	SH	0.8	0.4	0.1	GC-RI, GC-MS
ß-Elemol	1179	1183	1705	1720	SO	1.1	1.5	0.7	GC-RI, GC-MS, ^{13}C-NMR
Caryophyllene oxide	1568	1578	1975	1986	SO	tr	0.2	-	GC-RI, GC-MS
ß-Oplopenone	1587	1592	2063	2092	SO	0.4	1.1	0.3	RI, ^{13}C-NMR
τ-Cadinol	1624	1633	2162	2169[a]	SO	0.1	-	0.4	GC-RI, GC-MS, ^{13}C-NMR
α-Cadinol	1636	1643	2223	2227	SO	0.4	-	0.7	GC-RI, MS, ^{13}C-NMR
Abietadiene	2085	2071	2478	2450	DT	-	-	0.3	GC-RI, MS, ^{13}C-NMR
Total:						97.2	95.1	93.5	
					MH	71.0	59.7	52.3	
					MO	22.5	31.8	38.7	
					SH	1.7	0.8	0.1	
					SO	2.0	2.8	2.1	
					DT	0.0	0.0	0.3	

Components are listed following their order of elution on the apolar column. RIa, Rip – Retention indices on apolar and polar columns, respectively; RIa(lit), RIp (lit) – literature retention indices; MS – mass spectrometry; NMR – nuclear magnetic resonance; MH - monoterpene hydrocarbons; MO – oxygenated monoterpenes; SH – sesquiterpene hydrocarbons; SO – oxygenated sesquiterpenes; DT – diterpenes; tr – traces (0.1%); - – not identified; * – percentage measured on polar column. The values of RIa /RIa (lit) and RIp /RIp (lit) suffixed by the letters (a-d) are obtained from the literature (Gonny et al., 2006; Su and Ho, 2016).

Table 2. Antioxidant potential of *J. sabina* essential oils from different plant parts of *J. sabina*.

Sample	IC_{50} (mg/ml) (M±SD)			TAA (µg/m AAE)* (M±SD)	RPA (A_{700} nm)** (M±SD)
	DPPH	ABTS	H_2O_2		
EOL	33.93±0.15[b]	0.0736±0.0071[a]	1.11±0.018[a]	12.05±1.84[a]	0.0995±0.0025[a]
EOT	11.04±0.85[c]	0.0085±0.0012[b]	n.r.	15.04±1.27[b]	0.0097±0.0005[a]
EOB	41.79±0.98[a]	0.0168±0.00005[b]	0.49±0.018[b]	32.33±1.08[c]	0.376±0.014[b]
α-T	0.007±0.0016[d]	0.00092±0.00007[b]	-	-	0.64±0.02[c]
Thymol	0.0085±0.0001[d]	0.00032±0.00002[b]	-	-	-
AA	-	-	0.013±0.000[c]	n.a.	-

Essential oil from leaves (EOL), essential oil from twigs (EOT), and essential oil from berries (EOB). α-T – α-Tocopherol, AA – Ascorbic acid, DPPH – DPPH radical scavenging; ABTS – ABTS radical scavenging, H_2O_2 – hydrogen peroxide scavenging, TAA – total antioxidant activity (expressed as ascorbic acid equivalents µg/ml); RPA – reducing power assay. Values are given as means of three experiments of three replicates each; values with the same letter within a column are not significantly different at p=0.0001 (Student-Newman-Keuls test). – not tested; n.r. – IC_{50} value not reached; n.a. – not applicable; * – EOs were tested at a concentration of 45 µg/ml; ** – EOs were tested at a concentration of 76 µg/ml, while α-tocopherol was tested at 19 µg/ml.

Discussion

EOs are mainly odorless volatile compounds produced spontaneously by plants as secondary metabolites for purposes other than feeding (i.e., protection or attraction) [31]. In addition to their therapeutic effects, EOs extracted from plants can act as insect growth disruptors [32] or as phagodeterrents [33]. Due to their lipophilic properties, they can enter the organism and interfere with biological, physiological, and neurological systems, resulting in metabolic disorders [34].

Several analyses of the chemical composition of the EOs obtained from *J. sabina* have been carried out. Previous work [35] analyzed the chemical composition of male and female twigs collected in Algeria. The female twig EO comprised sabinene, γ-terpinene, 2-carene, and terpinene-4-ol, while the major components of the male twig EO were represented by sabinene and trans-sabinyl acetate. The present work represents the initial dataset determining the chemical composition of EOs extracted from various parts of *J. sabina* (including needles, twigs, and berries) collected in Algeria. The difference in composition between the EOs obtained from twigs

Table 3. Cholinesterase and α-glucosidase inhibitory activity of *J. sabina* EOs from different plant parts.

Sample	Cholinesterase inhibition (M±SD)			α-glucosidase (%)[b] (M±SD)
	AChE IC_{50}	AChE (%)[a]	BChE (%)[a]	
EOL	28.18±0.71	85.29±0.89	22.10±0.43	9.50±0.11
EOT	37.27±0.67	64.19±0.56	25.26±0.84	7.24±0.13
EOB	n.a.	28.97±0.34	32.33±0.28	5.88±0.05
Acarbose	n.a.	n.a.	n.a.	44.61±0.55
Tacrine	1.21±0.07	97.1±1.20	98.8±1.10	n.a.

J. sabina Essential Oil from leaves (EOL), essential oil from twigs (EOT), and essential oil from berries (EOB): ([a]) – tested concentration for EOs was 45 μg/ml. Acarbose was at 0.9 μg/ml; n.a. – not achieved/applicable. Tacrine was tested at 10 μg/ml; ([b]) and the tested concentration for EOs was 90 μg/ml.

in this study compared to previous work [35] is most likely due to the different procedures of EO extraction.

Other reports have been carried out on *J. sabina* collected in different parts of the world. For example, the EO of *J. sabina* berries collected in Montenegro and obtained by hydrodistillation contained α-pinene (347.06±35.72 mg/g) and sabinene (222.75±20.51 mg/g) as the main constituents [36]. Other work performing GC-MS analysis of EO isolated from air-dried *J. sabina* needles collected in China identified sabinene (19.83%) and terpinen-4-ol (8.96%) as the major constituents [37]. Recent work investigating the chemical composition of EOs isolated from air-dried aerial parts (leaves and cones) of *J. sabina* from Iranian provinces showed sabinene (12.5-58.0%), α-terpineol (0-9.2%), and myrtenyl acetate (0-72.6%) as the dominant components [38]. The major EO constituents from *J. sabina* air-dried leaves collected in Bulgaria and Slovakia were sabinene (16.68-30.98%), terpinen-4-ol (9.25-13.63%), myrtenyl acetate (1.32-23.02%), and elemol (8.45-13.70%) [14].

J. sabina EO from air-dried leaves and cones collected in Kazakhstan contained α-pinene (30.8 and 49.3%) and sabinene (19.4 and 20.4%) as main constituents [39]. Other researchers showed the dominance of sabinene (55.82%) in *J. sabina* EO obtained from the green branches of plants collected in Saudi Arabia [30]. Sabinene was also the major constituent of *J. sabina* EO (up to 80.1%) in the leaves of the plant collected in Bulgaria [14]. Dried leaf EO of *J. sabina* from Iran contained sabinene (12.57%), α-pinene (12.02%), and limonene (9.25%) as main constituents [40, 41]. GC-MS analysis of the EO of *J. sabina* leaves from western Patagonia, Argentina, revealed sabinene (52.3%), limonene (25.1%), and germacrene B (11.5%) as the most abundant components [42]. The main constituents of EOs from the fruits and leaves of male and female plants of *J. sabina* collected in Golestan Province, Iran, were sabinene (48.6%, 21.5%, and 24.3%), α-pinene (8.1%, 14.7%, and 6.2%), and myrcene (10.8%, 6.8%, and 7.6%) [43]. Some works have shown that the EOs from fresh leaves of *J. sabina* contain sabinene (34.8-56.7%) as the major constituent. *J. sabina* fresh leaf material was collected in seven different regions of the following countries: Spain, Switzerland, Kazakhstan, China, and Mongolia [44]. These observed differences in EO profiles can be attributed to factors such as genetic diversity, environmental conditions, and developmental stages that influence the biosynthesis of secondary metabolites [8].

Understanding the chemical composition of *J. sabina* EO is crucial for potential therapeutic application and commercial utilization. Sabinene and terpinen-4-ol, the most abundant in the tested oils in this work, are known for their antimicrobial, anti-inflammatory, and insecticidal activity [45, 46]. The antimicrobial properties of sabinene and its application in agriculture have been extensively studied [47, 48]. Terpinen-4-ol has shown diverse pharmacological potential, including antimicrobial activity, as well as other biological activities [49, 50].

Hydrogen peroxide is an oxidizing agent that inactivates certain enzymes, usually by oxidizing thiol groups (-SH). It diffuses rapidly through the cell membranes and probably reacts with Fe^{2+} or Cu^{2+} ions to form toxic hydroxyl radicals. These radicals are thought to be responsible for the toxic effects of H_2O_2. The ability of the tested EOs of *J. sabina* to scavenge H_2O_2 was weak, since the total antioxidant activity (phosphomolybdate) method revealed that, in the presence of antioxidants (as reducing species), Mo^{6+} was reduced to Mo^{5+} and formed a green-colored complex at acidic pH, which showed maximum absorbance at 695 nm, and this was confirmed by a previous work [51].

The potential reduction of *J. sabina* EOs was tested using the RPA to examine the ability of an antioxidant to reduce Fe^{3+} to a colored Fe^{2+} complex. The reduction potential of EOB was seven times weaker than that of α-tocopherol, while EOL and EOT did not show significant activity. However, EOL showed similar activity to EOB but only at a five times higher concentration. EOB exhibited better antioxidant capacity when examined using TAA and RPA assays, while EOT exhibited better activity toward radical scavenging assays [51].

The DPPH and ABTS scavenging analyses of EOB collected in Montenegro, tested by hydrodistillation,

showed stronger activity toward the ABTS radical than toward DPPH [36]. The reducing activity of this EO was analyzed using the FRAP assay, showing potential activity of the EO isolated from air-dried *J. sabina* needles. This EO showed better scavenging potential toward ABTS (IC_{50} = 49.34±0.95 µmol Trolox/g) when compared to DPPH radicals.

The antioxidant potential of EO is related to its chemical composition. Oxygenated terpene compounds, especially oxygenated monoterpene compounds, are the carriers of this biological activity [52]. Therefore, sesquiterpene compounds show low antioxidant activity. Some compounds present in low concentrations in EOs can contribute strongly to antioxidant potential. These include phenolic derivatives of monoterpenes, such as carvacrol and thymol [53]. Their phenolic groups quench free radicals by donating hydrogen atoms and interrupting free radical chain reactions, and the literature emphasizes that the location and extent of hydroxylation of individual components play a key role in their antioxidant efficacy.

The activity of the tested EOL and EOT could be related to the chemical composition of the *J. sabina* EOs. In fact, quantitatively, the most important compound of these oils, sabinene, has already been shown to have a good inhibitory effect on the AChE enzyme [29].

Several monoterpenes found in essential oils act as inhibitors of acetylcholinesterase, similar to organophosphates and carbamates [54, 55]. Up to now, a small number of pure compounds included in the composition of EOs have been tested for their ability to inhibit this enzyme, such as 1,8-cineole, 1-(S)-α-pinene, and R-(+)-limonene. It was also found that 1,8-cineole competitively inhibited α-glucosidase, while 1-(S)-α-pinene and R-(+)-limonene were uncompetitive inhibitors. In addition, the combination of limonene and linalool showed a significant antidiabetic effect [56]. Molecular docking simulations suggest that 1,8-cineole, geraniol, α-pinene, and β-pinene can produce similar inhibition (binding affinities) to acarbose, known as a good α-glucosidase inhibitor. α-Glucosidase inhibitors alter the absorption of carbohydrates in the intestine by inhibiting their conversion into simple sugars (monosaccharides). These inhibitors therefore significantly reduce the blood glucose level caused by carbohydrates. Inhibitors of this enzyme used in clinical practice are acarbose, voglibose, and miglitol. Their long-term use leads to harmful side effects such as hypoglycemia, diarrhea, and flatulence, as well as impaired absorption of dietary components [57]. For this reason, there is a need to find new natural inhibitors for this enzyme.

Conclusions

It is concluded from the present work that the chemical composition of analyzed EOs from different plant parts of *J. sabina* (needles, twigs, and berries) represents the first report in Algeria. The main components of the analyzed EOs were represented by monoterpenes, more specifically monoterpene hydrocarbons, followed by oxygenated monoterpenes, and the most prominent of the identified compounds were sabinene and terpinen-4-ol, along with a noted presence of α-pinene. The essential oil from leaves (EOL) could be considered a rich source of sabinene since its proportion in the EO exceeds 50%. Assays on the AChE/BChE and α-glucosidase inhibitory activity of *J. sabina* EOs were evaluated, and the obtained results showed moderate reducing antioxidant capacity. In contrast, the radical scavenging assays showed low activity. EOB exhibited the best reducing antioxidant capacity, while EOT exhibited better activity toward radical scavenging assays. EOs obtained from needles and twigs of *J. sabina* showed a moderate ability to inhibit AChE, while EOB showed weak activity toward this enzyme. The three EOs tested expressed low activity toward both BChE and α-glucosidase.

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Conflict of Interest

The authors declare no conflict of interest.

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