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Research Article

Phytochemical Analysis and Study of Antioxidant and Antimicrobial Activities of Two Parts of *Cupressus arizonica* Essential Oils

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The present work aimed to determine the difference in the chemical composition of essential oils isolated from two parts of *Cupressus arizonica* and to evaluate their *in vitro* antimicrobial and antioxidant effects. The yields of the essential oil obtained by hydrodistillation from the leaves and cones were 0.85% and 1.29%, respectively. The chemical analysis of the constituents of the two essential oils of *Cupressus arizonica* was carried out by using the GC and GC/MS techniques. The results of this analysis show that the leaves are dominated by cis-muurola-4 (14), 5-diene (21.27%), umbellulone (19.88%), α-pinene (9.39%), and α-muurolene (7.87%7); on the other hand, the cones are rich in α-pinene (51.07%) accompanied by other variable content constituents, myrcene (17.92%), limonene (9.66%), β-pinene (4.92%), meta-cymenene (2.6%), and α-terpineol (2.38%). The antimicrobial activity against four bacterial strains, four wood decay fungi, and three mould strains were determined using the agar-agar dispersion method. The studied essential oils exhibited moderate antimicrobial properties, which demonstrates the sensibility of all strains tested with the exception of wood rot fungi to which they do not have activity against all concentrations tested. The dosage of antioxidant activity was evaluated using DPPH scavenging and ferric ion reducing power (FRAP). The results indicate that the essential oils from cones of *Cupressus arizonica* possess a strong antioxidant activity (lower IC_{50}) $IC_{50} = 0.098 \pm 0.008/EC_{50} = 0.646 \pm 0.02$) in comparison with those from the leaves ($IC_{50} = 5.297 \pm 0.09/EC_{50} = 2.335 \pm 0.36$). The results suggest that both essential oils could be used as a source of treatment for bacterial infections and also as natural antioxidant substances.

1. Introduction

For several millennia, different civilizations in the world have used medicinal and aromatic plants as a source of medicine to treat several diseases [1–3]. Aromatic and medicinal plants and their extracts have always occupied a considerable place in medicine, culinary preparations, cosmetics, or the food industry [4]. According to the World Health Organization, "the growing interest in traditional medicine is not a return to the past, but an innovative vision of overall patient care. Moreover, traditional medicine covers the primary healthcare needs of 80% of the inhabitants of the planet, particularly in certain Asian and African countries" [5]. Morocco is considered one of the most biodiverse countries in the Mediterranean region [6], with about 4200 taxa including 1280 subspecies [7]. Conventionally, the species of the genus *Cupressus* have been reported to be used for the treatment of styptic problems whooping cough, to improve bladder tone, rheumatism, and to promote venous circulation to the bladder and kidney area [8]. Some of these medicinal plants showed important biological properties including antibacterial, antioxidant, antidiabetic, and anticancer effects. Phytochemical analysis revealed a correlation between bioactive compounds (secondary metabolites) and exhibited biological properties [9]. The genus *Cupressus* contains several medicinal and aromatic plants belonging to the Cupressaceae family. Trees of cypress are generally found native to the warm and temperate climate, with 25 taxa specifically located in Mediterranean regions, North America, and Asia at high altitudes [10]. *Cupressus arizonica* is a medicinal species native to the Southern United States, and it was introduced in Morocco since 1984 and has been cultivated in different parts of the country [11]. The leaves of the tree are evergreen of medium shape, with conical cones of a color varying from dull gray-green to bright blue-green [12, 13]. Essential oils (EOs) of this plant are widely used by people for their biological properties. Indeed, they are used for their antispasmodic [14], antidiabetic [15], diuretic [16], and neuropharmacological activity [17]. There is one paper report on the chemical composition and antimicrobial activity of the two parts of EOs of *C. arizonica* grown in High Atlas, but this is the first research conducted in Middle Atlas, Morocco.

In this context, the main objective of the present research work is to study the difference in the chemical composition of hydrodistilled EOs between *C. arizonica* cones and leaves and to investigate their antioxidant and antimicrobial activities.

2. Materials and Methods

2.1. Plant Material. The aerial parts of *C. arizonica* were collected in the Atlas Mountain of Azrou, Morocco, in March 2017. The authentication of the plant material was done by Dr. Guedira Abdelhamid, a botanist at the Forest Research Center of Morocco, and a voucher of specimens (CPA0317) was added to the herbarium of the Botany Department of Ibn Tofail University, Morocco. The parts of plants are separated and dried at room temperature in the dark.

2.2. Isolation of Essential Oils by Hydrodistillation. The production of essential oils was realized by hydrodistillation using a Clevenger-type device [18]. Briefly, a quantity of 200 g of each part of the plant was ground and mixed with one liter of water for hydrodistillation for three hours using a Clevenger-type apparatus (ISOLAB Laborgeräte, GmbH, Wertheim, Germany) according to the European Pharmacopoeia method (European Pharmacopeia, Council of Europe, Strasbourg, 3rd ed. (1997) 121). Three tests were carried out for the two parts of this species. The essential oils are dried with anhydrous sodium sulfate (Na_2SO_4) and then stored at 4° in the dark.

The yield of EOs (%, v/w) was determined and expressed on a dry weight basis using the following formula:

$$\text{yield of EO\%} = \frac{\text{volume of EO obtained (ml)}}{\text{mass of dry matter (g)}} \times 100. \quad (1)$$

2.3. Chromatographic Analysis/Mass Spectra. The chromatographic analysis was performed at the Hassan-II Agronomic and Veterinary Institute, Morocco. Gas chromatography/mass spectrometry (GC/MS) analysis was performed using a Hewlett–Packard gas chromatographer (HP 6890) coupled with a mass spectrometer (HP 5973). Fragmentation was performed by electron impact at 70 eV. The column used was a HP-5MS capillary column (30 m × 0.25 mm, film thickness: 0.25 m). The column was used at an injector temperature of 250°C with the oven temperature programming ranging from 50 To 250°C with a 4°C/min gradient for 5 min, and then finally kept isothermal for 10 min. The carrier gas is nitrogen, whose flow is fixed at 1.7 mL·min^{-1}. The injection mode of the essential oil (1 μL) was in a split ratio of 1 : 50 using a spectral scan range of 40–450 m/z, and the mass spectra was obtained by electron ionization (EI) at an ionization energy of 70 eV. The ChemStation data analysis software was used to acquire mass spectra and total ion gas chromatography (GC-TIC) profiles.

The identification of essential oil constituents was established by the determination of their Kovats Retention Indices (KI) according to Arov and Dym [19], and by matching the recorded spectra with a computed data library (Wiley 09, Nist 2011), according to a homologous series of *n*-alkanes (C7–C30) [20]. The components of the essential oils have been completed by comparing the fragmentation patterns of mass spectra with those reported in the literature [21].

2.4. Antimicrobial Activity

2.4.1. Microbial Strains. EOs were tested against the following bacteria: *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), and *Micrococcus luteus* (ATCC 9341). They are maintained by subculture in tryptic soy broth (TSB) and keep in the dark for 24 h at 37°C.

The antifungal activity was evaluated against four wood rot fungus (*Coniophora puteana*, *Coriolus versicolor*,

Gloeophyllum trabeum, and *Poria placenta*) obtained from CIRAD's wood preservation laboratory (Montpellier, France), and three molds (*Aspergillus niger*, *Penicillium digitatum*, and *Penicillium expansum*), the latter belonging to the Mycotheque Collection of Microbiology Forestry Centre (Rabat, Morocco) laboratory. All the fungal strains are maintained by transplanting on the nutrient environment PDA (potato dextrose agar) and incubated for seven days at 25°C.

2.4.2. Microbiological Procedure. The minimum inhibitory concentrations (MIC) of EOs were determined according to the method reported by Remmal et al. [22] with some modifications [23], because the essential oil is incapable of being mixed with water and, therefore, to the cultural environment. Emulsification was realized by dispersing EO into a 0.2% agar solution to obtain a homogeneous distribution and make the higher maximum of component/germ contact.

Dilutions of agar solution were prepared at 1/10, 1/25, 1/50, 1/100, 1/200, 1/300, and 1/500 (v/v). A volume of (13.5 mL) of solid TSA (Tryptic Soy Agar) was introduced into each tube for bacteria and PDA for molds, sterilized in an autoclave (20 minutes at 121°C) and cooled to 45°. 1.5 mL of each of the dilutions of agar was added aseptically in order to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000, and 1/5000 (v/v). The tubes are shaken well before being poured into sterile Petri dishes. Negative controls containing the cultural medium and a 0.2% agar solution without essential oils were equally prepared.

The seeding for bacteria is carried out by streaks using a calibrated platinum loop in order to take the same volume of inoculum. This is later presented in the form of a suspension in physiological water of spores resulting from a culture of 7 days in the PDA for fungi, and in the form of a broth culture of 24 h for bacteria. For wood rot fungus, the seeding is done by the fragment's deposition of 1 cm of diameter, taken from the periphery of mycelia resulting from a culture in 7 days in the PDA. Each test is repeated three times to minimize the experimental error.

2.5. Antioxidant Activity

2.5.1. DPPH Radical Scavenging Activity. Radical scavenging activity of EO against the stable 2,2diphenyl-1-picrylhydrazyl (DPPH) was evaluated according to the method reported [24]. 2.5 mL of each essential oil at different concentrations ([0.04–0.8] mg/mL and [0.5–10.5] mg/mL) was added to 1 mL of methanolic solution DPPH (0.3 mM). The mixture was shaken using a vortex, (incubated) and left for 30 min at room temperature in the dark. The absorbance was measured using a spectrophotometer at 517 nm. The obtained data were used to determine the concentration of the sample required to scavenge 50% of the DPPH free radicals (IC_{50}). A negative blank was prepared (1 mL of the DPPH solution + 2.5 mL of methanol). All tests were performed with three replicates for each concentration.

The Inhibition of free radicals by DPPH in percentage (I %) was calculated using the following equation:

$$I\% = \left[\frac{(A\text{blank} - A\text{sample})}{A\text{blank}}\right] \times 10, \quad (2)$$

where Ablank is the absorbance of the control reaction (containing all reagents, except the test sample) and Asample is the absorbance in the presence of essential oil.

The antioxidant power of the EOs tested was estimated by comparison with a natural antioxidant (ascorbic acid).

2.5.2. Ferric Ion Reducing Antioxidant Power (FRAP) Assay. The reducing power of various essential oils was conducted according to Oyaizu [25] and Ferreira et al. [26], with a slight modification. Briefly, 1 mL of each concentration of essential oils diluted in methanol ([0.02–1.2] mg/mL and [1–11] mg/ml) was mixed with 2.5 mL of phosphate buffer (200 Mm, Ph6.6) and 2.5 mL of potassium ferricyanide (1% w/v). After incubation of the samples in a water bath at 50°C for 20 min, 2.5 mL of trichloroacetic acid (10% w/v) was added to stop the reaction and all the tubes were centrifuged at 3000 r/min for 10 minutes. Afterward, 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1% w/v). Finally, the absorbance of the samples was measured at 700 nm using a spectrophotometer.

EC_{50} is the value of oil concentration providing 0.5 of absorbance. Ascorbic acid was used as a reference compound and all tests were carried out in triplicate.

2.6. Statistical Analysis. Each experiment in the study was performed in triplicate, and the data obtained were presented as means ± standard deviation (SD). The OriginPro 2021 software (OriginLab Corporation, Northampton, Massachusetts, USA) was used to process for significant differences between the groups means using Tukey's post hoc test. A probability of $p \leq 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Essential Oil Yield. The average yields of CAEO leaves and cones obtained by hydrodistillation were expressed in ml relative to the 100 g of dry matter of the plant. The yields of EOs from leaves and cones were 0.85% and 1.29%, respectively.

The studies reported by Bouksaim et al. [27], Ismail et al. [28], and Shahin et al. [29] show a high yield from the cones compared to that from the leaves, which is in accordance with the results of our study. However, the difference in the EO yield in aromatic and medicinal plants can be explained by several parameters such as season [30], geographical origin [31], and the part of the treated plant [28].

3.2. Chemical Composition of Essential Oil. Chemical analyses of the constituents of the *Cupressus arizonica* essential oil (CAEO), the percentage content of each compound,

elution order, structural subclass, and retention index are presented in Table 1.

The chemical analysis of EOs isolated from the cones and leaves of *C. arizonica* has identified 20 and 50 chemical compounds, respectively, which represent 99.79% and 99.45% of the total composition of these EOs, respectively.

The monoterpene constitutes the most important fraction of essential oils from cones (monoterpene hydrocarbons 88.27%, oxygenated monoterpenes 11.52%), while the leaves were dominated by the sesquiterpenes fractions (sesquiterpenes hydrocarbons 42.75%, oxygenated sesquiterpenes 11.11%).

The combination of volatile compounds of this species varies in terms of diversity and concentration. The essential oils from the leaves are dominated by cis-muurola-4 (14), 5-diene (21.27%), umbellulone (19.88%), α-pinene (9.39%), and α-muurolene (7.87%), whereas the EO obtained from cones was characterized by a high level of *α-pinene* (51.07%) accompanied by other constituents with variable contents, such as myrcene (17.92%), limonene (9.66%), β-pinene (4.92%), meta-cymenene (2.6%), and α-terpineol (2.38%). In addition, the cones of this species were richer in α-pinene (51.07%). In the comparison of the two studied parts of the plant, the EO from leaves contains a small amount of α-pinene (9.39%) but is rich in other elements such as cis-muurola-4 (14), 5-diene, and umbellulone.

These results are not similar from a quantitative point of view with previous reports on essential oils obtained from Moroccan *C. arizonica* from High Atlas [27], which identified approximately 65 and 30 compounds from the leaves and cones, respectively, with the predominance of the α-Pinene (25.52%), followed by *p*-cymene (10.11%), sabinene (5.81%), myrcene (3.65%), and umbellulone (2.04%). However, the cones are characterized by a high rate of α-pinene (76.45%), myrcene (6.09%), and cadalene (4.57%). It should also be noted that the same species from Western Iran has shown that the essential oil from the leaves presents a significant amount of some chemical constituents such as α-pinene (19.2%), β-phellandrene (9.6%), and sabinene (8.1%), but the percentage of umbullulone, cis-muurola-4(14),5-diene decreased by 14.28% and 11%, respectively, compared to our sample [32], we also noted the presence of sabinene (13.7%), *p*-cymene (3.7%), and camphene (10.1%).

On the other hand, there is a similarity between the quality of our essential oil and that found in Italy [33], and the cones contain the same majority compounds which are α-pinene (72.0%), myrcene (6.5%), and δ-3-carene (5.7%), but with a different amount in terms of the two compounds from which the essential oil extracted from the leaves showed almost a two-time higher content of umbullulone (45.1%) compared to our sample, but cis-muurola-4(14),5-diene presented a less important concentration (3.7% against 21.27%) while α-pinene present the same quantity.

By studying the essential oil of *C. arizonica* from Tunisia, [28] 62 compounds in the samples from leaves were found. The majority of compounds identified from the latter were umbellulone (17.9%), α-pinene (10.3%), and β-cubebene (10.1%), whereas, in the cone oil, 19 compounds were

Table 1: Chemical composition of CAEO.

Compounds	IK (IR)	Content (%)	
		Leaf oil	Cone oil
α-Pinene	932	9.39	51.07
Camphene	950	—	0.61
Sabinene	970	0.41	—
β-Pinene	974	0.32	4.92
Myrcene	984	0.62	17.92
Δ-3-Carene	1006	0.43	2.02
1,4-Cineole	1011	0.23	—
p-cymenene	1019	0.74	0.47
Limonene	1024	2.18	9.66
β-*E*-Cymene	1041	—	1.31
γ-Terpinene	1053	0.43	0.29
Meta-cymenene	1082	0.63	2.6
p-cymenene	1091	0.23	0.33
6-Camphenol	1107	0.22	0.13
trans-Sabinol	1137	0.29	—
cis-Pinene hydrate	1142	0.64	0.58
Camphre	1144	—	0.64
trans-β-Terpineol	1159	—	0.41
Umbellulone	1172	19.88	—
Terpinen-4-ol	1177	3.1	1.37
p-Cymen-8-ol	1180	1.64	—
α-Terpineol	1187	0.68	2.38
cis-Piperitol	1195	0.42	—
trans-Piperitol	1208	0.32	—
cis-Sabinene hydrate acetate	1218	0.25	1.25
Citronellol	1226	0.31	—
Z-Ocimenone	1236	0.38	1.4
trans-Sabinene hydrate acetate	1251	0.29	—
α-Terpinene-7-ol	1281	0.26	—
δ-Terpinene-7-ol	1292	0.65	—
α-terpinylacetate	1342	0.65	0.43
(E)-Caryophyllene	1420	0.24	—
β-Copaene	1429	0.16	—
cis-Muurola-3,5-diene	1449	3.59	—
cis-Muurola-4(14),5-diene	1470	21.27	—
α-Muurolene	1499	7.87	—
β-Curcumene	1515	0.35	—
δ-Cadinene	1525	5.84	—
α-Cadinene	1539	1.32	—
Elemol	1547	0.4	—
Germacrene B	1553	0.75	—
β-Calacorene	1563	0.84	—
Thyjopsan-2α-ol	1586	0.27	—
1,10-Di-epi-cubenol	1617	0.72	—
α-Acorenol	1632	0.24	—
Epi-α-muurolol	1641	4.76	—
α-Cadinol	1652	0.96	—
Dehydroeudesmol	1661	0.53	—
E-Bisabolol-11-ol	1668	0.28	—
epi-α-Bisabolol	1680	0.38	—
α-Bisabolol	1687	0.38	—
Caryophyllene acetate	1701	1.68	—
Curcumenol	1730	0.51	—
2,7(14)-Bisaboladien-12-ol	1759	0.52	—
Total identified %		99.45%	99.79%
Monoterpene hydrocarbons %		14.52	88.27
Oxygenated monoterpenes %		31.07	11.52
Sesquiterpenes hydrocarbons %		42.75	—
Oxygenated sesquiterpenes %		11.11	—

identified; α-pinene was the higher content in this essential oil with an important concentration (79.7%), but it also possess a moderate quantity of δ-3-carene (10.9%) and limonene (3.9%). A different observation was reported by Chéraif et al. [34] on the same species of *Cupressus* collected from the same location, whose leaves were rich in α-pinene (20%), umbellulone (18.4%), and cis-muurola4(14),5-diene (9.4%), but the cones present a similar chemical profile to that founded by Ismail et al. [28].

Several reports investigated the chemical composition of leaf essential oils in different countries, including Iran [35], Greece [36], India [37], and Tunisia [38], and significant chemical variations have been observed in these studies. In the chemical composition of the samples from Iran and India, umbellulone and limonene have been found as major components, while α-pinene was more abundant in oils from *C. arizonica* leaves grown in Greece and Tunisia.

It has been reported that the chemical profile of essential oils might vary with the orientation of biosynthesis toward the preferential formation of specific products under the influence of the seasons, age of the plants, composition of the soil, time of collection, and geographical origin.

3.3. Antimicrobial Activities. The results of the study of antibacterial and antifungal activity of essential oils from the two parts of *C. arizonica* are summarized in Table 2.

The growth of strains in the dishes containing the test oil was judged by the presence or absence of microbial growth compared with the growth in the control tubes without essential oil [39].

The results of biological tests show that CAEO exhibits different antifungal and antibacterial behaviors depending on microorganisms and the part of the plant studied. The EO from the leaves of *C. arizonica* showed an inhibitory power of all the bacterial strains tested at the concentration of 1/100. For *B. subtilis*, *S. aureus* and *M. luteus* were the most sensitive (up to a concentration of 1/500 v/v), but *E. coli* was sensitive to just the first concentration (1/100 v/v). On the other hand, the proliferation of the four bacterial strains was sensitive to the EO of the cones with an inhibitory concentration of up to 1/250 v/v. Indeed, for the EO of the cone, a low concentration (1/1000 v/v) was sufficient to inhibit *P. placenta*, *C. versicolor*, and *G. trabeum*, while *C. puteana* was sensitive up to a concentration of 1//250 v/v, while the essential oil of the leaves inhibited all fungal strains at a concentration of 1/250 v/v. From our results, we can conclude that the fungal strains were more sensitive to the EO extracted from cones than that extracted from leaves.

The essential oils extracted from cones and leaves were found to have no activity against all molds tested even at high concentrations, which corroborates with the studied in [27,40]. The antimicrobial activity observed for the essential oil of *C. arizonica* can be explained by its chemical profile. It is rich in monoterpene hydrocarbons including the major component α-pinene, one of the components that has antibacterial, anti-inflammatory, antiviral, expectorant, sedative, herbicide, and insect repellent properties [41].

In fact, numerous studies have shown that the essential oils of the two parts (leaves and cones) from *C. arizonica* showed modest activities against most of the bacteria tested (*E. coli*, *S. aureus*, *K. pneumoniae*, *S. typhimurium*, *E. faecalis*, and *S. pneumoniae*) [34]. Other studies of the same species from South Carolina found that EOs possess moderate antifungal activity against strawberry anthracnose [42]. Furthermore, a noticeable antifungal activity was also notified between EOs from different parts of other *Cupressus* species [43].

The antibacterial activity of the *C. arizonica* essential oils from leaves was important against Gram-positive than Gram-negative bacteria, which is in accordance with the previous report on essential oils from other species [40,44,45].

Generally, the higher resistance among Gram-negative bacteria could be ascribed to the presence of their outer phospholipidic membrane, almost impermeable to lipophilic compounds [46]. A research on the innovation of new drugs from natural products has led to the identification of a variety of sesquiterpenes characterized by anti-inflammatory, antiparasitic, and anticarcinogenic activities [47]. It can be concluded that EOs of both parts have insignificant activities. Moreover, due to the complexity of the chemical composition of these EOs, several studies have shown that a synergistic effect between different components must be considered [48].

Inhibition of microbe growth, induction of cellular membrane disruptions, interference with particular microbial metabolic activities, or manipulation of signal transduction or gene expression pathways are all plausible mechanisms for phytochemical action. However, due to the synergistic impact of the chemicals, all of these pathways may occur at the same time [49].

3.4. Antioxidant Capacity. In this part, we have examined the two essential oils from *C. arizonica* using two methods of antioxidant assays: DPPH radical scavenging and ferric reducing antioxidant power assay (FRAP).

DPPH is a widely used method to determine the free radical scavenging property from various studied samples [50]. This test is based on the change in color of the DPPH solution from purple to yellow, this change is proportional to the antioxidant power [51]; results are recorded in Table 3. The most potent activity was obtained from the *C. arizonica* cone oil ($IC_{50} = 0.098 \pm 0.008$ mg/mL), followed by the essential oil from leaves ($IC_{50}5.297 \pm 0.09$ mg/mL), whereas, they were all less efficacious than natural antioxidant used (ascorbic acid) with an $IC_{50} = 0.00364 \pm 0.001$ mg/mL.

The reducing power of essential oil is associated with its antioxidant power. This technique was developed to measure the capacity of the essential oil to reduce ferric iron (Fe^{3+}) present in the $K_3Fe(CN)_6$ complex in ferrous iron (Fe^{2+}). In this assay, the intensity of Perl's Prussian blue color was measured at 700 nm, and the intensity of the Prussian color is proportional to the strength of the antioxidant activity [52]. EO isolated from cones had the highest reducing activity (lower EC_{50}) with $EC_{50} = 0.646 \pm 0.02$ mg/mL than that

Table 2: Antibacterial and antifungal activity of essential oil extracted from the leaves and cones of *C. arizonica*.

Concentration	1/100		1/250		1/500		1/1000		1/2000		1/3000		1/5000		*T*
Parts studied	LCa	CCa	LCa	CCa	LCa	CCa	LCa	CCa	LCa	CCa	LCa	CCa	LCa	CCa	
Bacteria															
E. coli	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+
B. subtilis	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
S. aureus	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
M. luteus	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
Fungal strains															
P. placenta	−	−	+	−	+	−	+	−	+	+	+	+	+	+	+
C. puteana	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+
C. versicolor	−	−	+	−	+	−	+	−	+	+	+	+	+	+	+
G. trabeum	−	−	+	−	+	−	+	−	+	+	+	+	+	+	+
Molds															
A. niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. expansum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
P. digitatum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

C: control; −: inhibition; +: growth; LCa: essential oil of leaves of *C. arizonica*; LCc: essential oil of cone of *C. arizonica*.

Table 3: Antioxidant activity of the two EOs evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.

Essential oil	DPPH (IC_{50} = mg/mL)	FRAP (EC_{50} = mg/mL)
Cone EO	0.098 ± 0.008	0.646 ± 0.02
Leaf EO	5.297 ± 0.09	2.335 ± 0.36
Ascorbic acid	0.00364 ± 0.001	0.0190 ± 0.004

Results are average value ± SD of triplicate measurement. The values in the same line with different letters are significantly different ($p < 0.05$) by Tukey's multiple range tests.

from leaves with $EC_{50} = 2.335 \pm 0.36$ mg/mL, this result was also confirmed by the result of the DPPH assay.

In spite of the absence of phenolic compounds in the essence of *C. arizonica*, it could show an important antioxidant power. Indeed, according to the study in [53], there are EOs which are not rich in these phenolic compounds but they endowed with a potential moderate antioxidant. It has been noticed that the essential oil extracted from the cone has a strong antioxidant activity than that extracted from the leaves. This difference can be explained by the antioxidant effect of α-pinene tested individually [54], and by the qualitative and quantitative difference that exists in the chemical profiles of the two parts of the plant. In an earlier report [55], the antioxidant activity of the cone EO showed a strong antioxidant power against the DPPH assay than other parts of the plant. However, the difference observed between the test methods could be explained by the correlation between chemical composition and/or each compound and assay used [56–58].

4. Conclusion

According to our results, there was a signification variation in the chemical composition between essential oils from two parts of *C. arizonica*. Monoterpene constitutes the most important fraction of the essential oils from cones (monoterpene hydrocarbons 88.27%, oxygenated monoterpenes 11.52%), whereas the leaves were dominated by the sesquiterpenes fractions (sesquiterpenes hydrocarbons 42.75%, oxygenated sesquiterpenes 11.11%). The evaluation of the antioxidant activity revealed excellent free radical scavenging activity attributed to the cones than by leaves essential oil. Results of essential oil bioactivities showed that the fungal strains were more sensitive to the EO extracted from cones than that extracted from the leaves, but they do not have an effect on the proliferation of the molds studied. Based on our results, CAEO could be used as an easily accessible source of natural antioxidants in pharmaceutical applications, as well as an initiation to the systematic study of the mechanisms of synergy between the different components. However, further investigations are needed to validate the safety of this EO and its bioactive compounds. Moreover, the mechanisms of actions of the main CAEO compounds should also be determined to validate their pharmacodynamic actions.

Data Availability

All the available data are incorporated in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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