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CHEMICAL COMPOSITION AND ANTIOXIDANT POTENTIAL OF BRANCH, NEEDLE, CONE OILS OF *CEDRUS ATLANTICA*, ANTIOXIDANT PROPERTIES FROM AURES REGION (ALGERIA)

BENSAID S O[1], BAALI N[2], FADEL H[1], BENSOUICI C[3], AKKAL S[1], BICHA S[1*]

1: Research Unit: Valorization of Natural resources, Bioactive molecules and Analyses Physicochemical and Biological (VARENBIOMOL), Department of Chemistry, Faculty of Science, University Mentouri-Constantine 1, 25000 Constantine, Algeria.

2: Laboratory of Biology and Environement, Faculty of Sciences of Nature and life University of Brothers Mentouri, Constantine1 Algeria

3: Biotechnology Research Center, Ali Mendjli Nouvelle Ville UV 03, BP E73, Constantine, Algeria

***Corresponding Author: Dr. Bicha Sabrina: E Mail: bichasabrina2016@gmail.com; Tel: +213 540898581**

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ABSTRACT

The aim of the present work is to characterize the phytochemistry and antioxidant activity of the cone, branch and needle essential oils of *Cedrus atlantica* growing in Khenchla, east montain region of Algeria. The chemical constituents of the essential oils obtained from the cones, branches and needles of the species were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). Cis-α-Bisabolene (19.57%) was the main component of branch and needle oils followed by Perhydrofarnesyl acetone (4.74%) and Spathuleno (4.54%). Cone oils contained α-pinene (46.613%) besides β-pinene (9.698%) and D-limonene (4.351%). Moreover, the antioxidant activity was assessed using DPPH and Hydroxyl radical scavenging assays. It was found that the essential oils have a significant antioxidant effect when tested by the two methods but it is less effective than vitamin C.

Keywords: Branch and needle oils, cone oils, GC-MS, *Cedrus atlantica*, Antioxidant Properties

INTRODUCTION

Cedrus is one of the most important genus of the Pinaceae family, there are only four *Cedrus* species classified by their morphological diversities, *C. atlantica* (Endl.) Carr, *C. libani* A., *C. brevifolia* (Hook. fil.) Dole, and *C. deodara* (D. Don) **[1]**. The four species are widely used in the popular medicine, the Atlas cedar (*C. atlantica*) is an endemic species of Algeria and Morocco **[2-3]**. Various studies have shown many important pharmacological properties of *Cedrus atlantica* such as antiseptic, diuretic, astringent, fungicidal, sedative **[4-5]** as well as antimicrobial, anti-inflammatory and antiviral activities **[6-7]**. Several previous studies reported the chemical composition of different parts of this plant **[8]** nevertheless, few studies have been performed on essential oils and their antioxidant and antibacterial activities **[9-10]**. We report herein, our results concerning the chemical composition of branch, needle and cone oils of *Cedrus atlantica* collected from the area of Khenchla revealed some differences from those previously reported on this species **[11-12]**. Furthermore, we investigate the anti-oxidant activity of essential oils of plant using both DPPH and Hydroxyl radical scavenging assays.

MATERIALS AND METHODS

Plant material

The Aerial parts Branch, Needle and Cone of *C. atlantica* were collected on December 2018 in Aures region, in Yabous (El Kantina) near Khenchla (61 km south-west from Khenchla, 35°24′29″ nord, 6°38′31″ East, Altitude: 1167 m). A Voucher specimen (CA/114/VAR/12-18) was identified by Professor Mohamed Kaabeche, Setif 1 University, Algeria, and deposited in the Herbarium of the Varenbiomol Research Unit, University of Mentouri Brothers, Constantine, Algeria.

Extraction

The fresh aerial parts branches and needles (963 g) and cones (719g) of *C. atlantica* were subjected to steam distillation for three hours in a Clevenger apparatus. Each obtained oil was collected, and kept at +4 °C until analysis **[13]**.

GC–MS analysis

GC analysis was performed using an Agilent Technologies GC 17. A gas chromatograph equipped with a gross-linked HP 5MS column (30mX0.25mm, film thickness 0.25µm). The oven temperature was programmed as isothermal at 60 °C for 8 min, helium was used as the carrier gas at a rate of 0.5 ml/min. GC/MS was performed using a HP Agilent technologies 6800 plus mass selective detector, the operating conditions were the same as for the analytical GC. The MS operating parameters were as follows: ionization potential,70 eV; ionization current, 2 A; ion source temperature, 280 °C; resolution, 1000 scan time,5 s; scan mass

range, 34–450 u; spit ratio, 50:1; injected volume, 1.0 µL. The identification of compounds of the essential oil was based on their retention times in comparison with matching spectral peaks available with NIST and Wiley mass spectral libraries, as well as by comparison of the fragmentation patterns of the mass spectra and their retention indices with those reported in the literature **[14]**. The retention indices were calculated for all constituents, using a series of n-alkanes.

Determination of antioxidant activity

DPPH radical scavenging assay

The hydrogen atom-or-electron donation ability of branches, needles and cones essential oils was measured from the bleaching of the purple coloured methanol solution of DPPH radical. This spectrophotometric assay uses the stable radical, 2,2-diphenylpicrylhydrazyl (DPPH), as a reagent **[15]**. Briefly, 1ml of various concentrations of the each essential oil sample in methanol was added to 1mL of a methanol solution of DPPH (0.135mM). The mixture was vigorously shaken and then allowed to stand at room temperature for 30 min in the dark. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The commercial known antioxidant, vitamin C was used for comparison. The tests were carried out in triplicate. The ability of the branch, needle and cone essential oils to scavenge DPPH radical was calculated as % inhibition by the following equation:

Inhibition (%) = [(Absorbance of control – Absorbance of sample) / Absorbance of control] × 100

Hydroxyl radical scavenging assay

Hydroxyl scavenger ability was performed by generating the hydroxyl radicals from $FeSO_4$ and hydrogen peroxide. The radical scavenging activity of branch, needle and coneoils was determined by their ability to hydroxylate salicylate **[16]**. To 1 ml of different samples concentrations, 300 µl of FeSO4 (8mM) solution and 250 µl of H_2O_2 (20 mM) were added and mixed. To initiate the reaction, 250 µl of salicylic acid in ethanol (3mM) was added. The reaction mixture was allowed to stand for 30 min in a water bath at 37 °C, after which 450 µl of distilled water was added and the mixture was centrifuged. The absorbance of the supernatant was measured at 510 nm. Vitamin C was used for comparison. The tests were carried out in triplicate. The percentage scavenging activity was calculated by the same equation as given for the DPPH assay.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The yield of steam distillation was based on percent (w/w) in relation to the dry weight of the plant. Our result showed that the yield of cone oils (0.051%) was better than that of branches and needles (0.017%). 88 constituents were determined representing

74.11% of branch and needle oils. The identified components **(Table 1 and Table 2)** are listed in order of their experimental retention times and retention indices. The major components were characterized withc is-α-Bisabolene (19.58%), Perhydrofarnesyl acetone (4.78%), Spathulenol (4.54%), 7-dimethyl-4(E), 6-octadien-2-ol (3.51%),α-Cedrene (2.31), Caryophyllene oxide (2.26%), Neryl acetate (2.14%) and n-Hexadecanoic acid(2.02%). Moreover, this investigation allowed the identification of 37 constituents corresponding to 99.33%.of the cone essential oils. The major components wereα-pinene(46.61%), β-pinene (9.70%), Borneol, acetate, (7.33%), D-Limonene (4.35%),D-Verbenone (2.61%)and β-Myrcene(2.28%).There was a signifcant difference between the two oils. The comparison of our results with previous studies shows important qualitative and quantitative differences in compositions. Indeed, *C.atlantica* growing in Algiers contained mainly α-pinene (17.7%), caryophyllene oxide (10.3%), β-caryophyllene (9.1%) and α-terpineol (6.7%) **[11]**. The studies done by Dahoun *et al*. **[17]**; Benouaklil *et al*. **[12]**, Aberchane *et al*. **[18]** ; Boudarene *et al*. **[11]**, Satrani *et al*. **[19]** and Derwich *et al*. **[20]** on essential oils of *Cedrus atlantica* wood were dominated by β-Himachalene and α-Himachalene. Furthermore, the seed oil of *C. atlantica* collected from north-eastern Algeria also exhibited α-pinene (37.1%) as major component, while the seed oil from central Algeria species contained the diterpene manool (20.7%) as major component **[21]**. However cis-α-Bisaboleneis was reported for the first time from *Cedrus atlantica* essential oil. Moreover, a high percent of α -pinene is obtained. In summary, these results indicate significant differences in the composition of the branch, needle and cone oils of *Cedrus atlantica* compared with previous studies, the variations found in the chemical composition of essential oils, qualitatively and quantitatively depend on certain environmental factors, the plant part used, the age, the period of its growth cycle, Moreover, differences in the harvest time, or even genetic factors **[12, 22]**. Also, the observed difference between the chemical composition of the essential oils of the Moroccan Atlas cedar and those of Algeria could be due to the difference of both climatic and geographical factors, such as the altitude and the soil type **[23]**.

Antioxidant properties

The scavenging effect of branch, needle and cone oils on DPPH radical was concentration-dependent as shown in **Figure 1**. At 200µg/ml, branch and needle oils displayed noticeable DPPH radical scavenging activity with percentage of inhibition value of 41.4%, while the value of cone oils was 30.5%. In hydroxyl radical assay, the percentage inhibitions pattern of branch, needle and cone oils was similar to DPPH as shown in **Figure 2**. At 200µg/ml,

the percentage scavenging activity of each oil against hydroxyl radical was 53.5% and 29.7% for branch, needle and cone oils respectively. The scavenging potential of vitamin C toward DPPH and hydroxyl radicals was better than branch, needle and cone oils.

Free radicals play a key role in pathological manifestations. These plants derived constituents perform their role either by quenching the ROS or by acting as a defense shield to protect the antioxidant defense mechanism **[24-27]**. DPPH is one of the most common antioxidant assays to analyze the free radical scavenging activity of the test compound antioxidants transfer either electrons or hydrogen atoms to DPPH and thus reduce a number of DPPH radical equal to their number of available hydroxyl groups **[28]**. In addition, the stable yellow-colored DPPH-H simultaneously formed, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidants **[29]**. Our findings revealed that the branch, needle and cone essential oils scavenged DPPH radicals in a concentration dependent manner, indicating the ability of tested essential oils to act as hydrogen donors. Furthermore, our results revealed that branch, needle and cone essential oils were able to scavenge hydroxyl radical produced from $FeSO_4$ and hydrogen peroxide system in a concentration dependent manner. This observation suggests that the tested essential oils can be used as an alternative remedy to synthetic antioxidants in combating the oxidative activity of hydroxyl radical. The hydroxyl radical is the most active in the reactive oxygen species, and it can cause most damage to the organism **[30]**. The antioxidant ability of branch, needle and cone essential oils may be due to the mixture of dozens of compounds of different functional groups, polarity and chemical behavior which produces either synergistic or antagonistic effect on antioxidant activity. The findings in this study are in agreement with previous essential oil researchers, that some essential oils are strong natural antioxidants **[31-33]**.

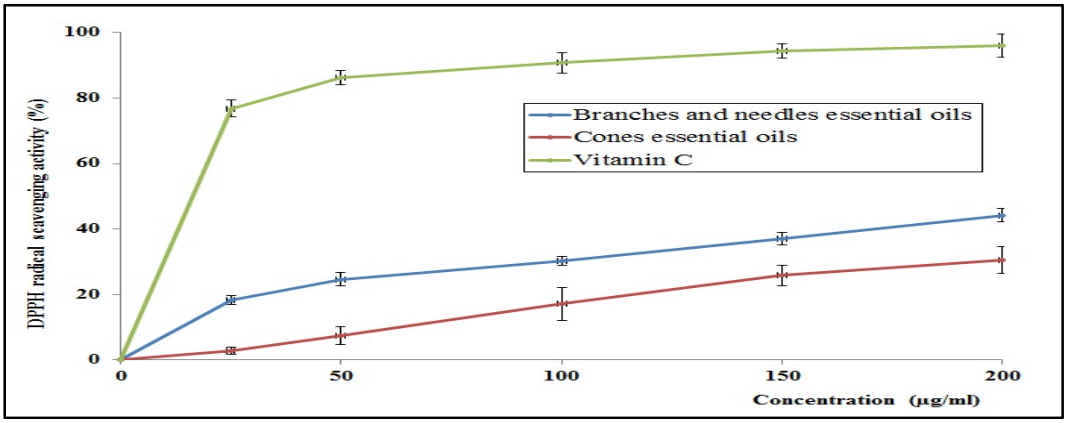

Figure 1: DPPH radical scavenging ability of branch, needle and cone essential oils (n=3)

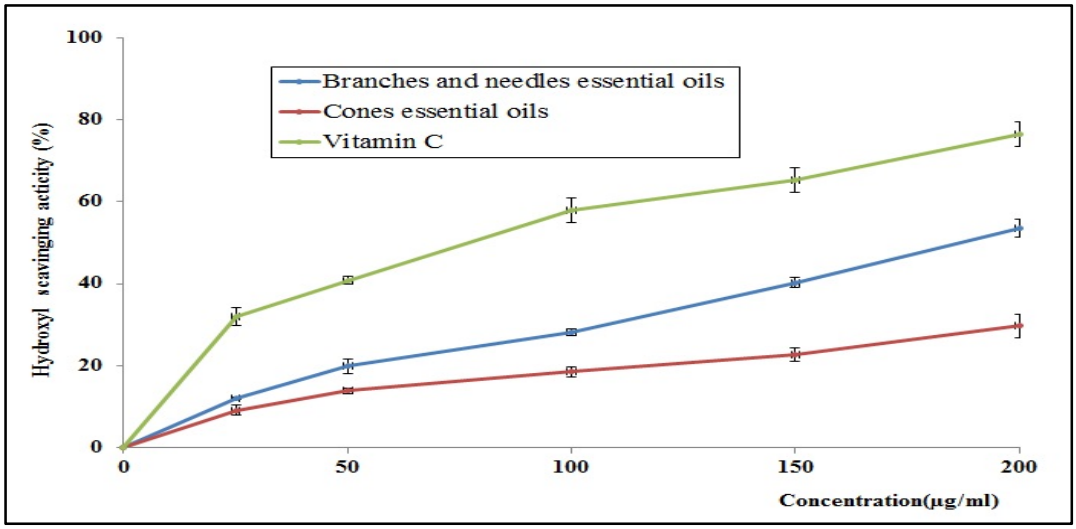

Figure 2: Hydroxyl radical scavenging ability of branch, needle and cone essential oils (n=3)

Table 1: Chemical composition, retention indices and percentage composition of cones essential oil of *Cedrus atlantica*

Pic	Compounds[a]	RI[b]	(%)
1	α-pinene	952	46.61
2	Camphene	936	1.22
3	verbenene	969	0.26
4	β-pinene	993	9.70
5	β-Myrcene	1008	2.28
6	O-Cymene	1039	0.78
7	D-Limonene	1043	4.35
8	3,4-Dimethyl-4-penten-1-yn-3-ol	1102	0.47
9	α-Pinene oxide	1112	2.19
10	Linalool L	1116	0.68
11	δ- Cyclogeraniolene	1120	1.12
12	trans,cis-2-methyl-1,3-cyclooctadiene	1130	0.15
13	α-Campholenal	1140	0.30
14	trans-Pinocarveol	1152	1.29
15	trans-Verbenol	1159	1.74
16	Pinocarvone	1176	0.83
17	L-Borneol	1179	1.04
18	Terpinene-4-ol	1191	0.60
19	Cryptone	1102	1.09
20	α-terpineol	1206	2.17
21	Myrtenal	1210	1.36
22	D Verbenone	1224	2.62
23	Trans carveol	1234	0.37
24	Propanal, 2-methyl-3-phenyl-	1253	0.17
25	l-Carvone	1258	0.25
26	2-1-Methylcyclopentyloxy tetrahydropyran	1272	0.30
27	2-Methyl-2-cyclopenten-1-ol	1290	0.20
28	Bornyl acetate	1302	7.33
29	cis-Sabinol	1322	0.21
30	E,Z-4-Ethylidenecyclohexene	1338	0.16
31	Geranyl acetate	1401	0.40
32	trans-β-Farnesene	1471	1.66
33	13-Epimanoyl oxide	2005	0.17
34	Epimanool	2073	4.35
35	Abieta-8,11,13-trien-7-one	2310	0.49
36	Tricosane	2321	0.27
37	Pentacosane	2521	0.15
	Identified compounds(%)	Total	99.33%

Table 2: Chemical composition, retention indices and percentage composition of branch and needle essential oils of *Cedrus atlantica*

Pic	Compound[a]	RI [b]		(%)
1	α-Pinene	946		0.31
2	β-Myrcene	1006		0.17
3	Yomogi alcohol	1015		1.07
4	D-Limonene	1041		0.40
5	1,8-Cineole	1043		0.15
6	2,7-dimethyl-4(E),6-octadien-2-ol	1052		3.51
7	3,3,6-Trimethyl-1,5-heptadien-4-ol	1099		1.72
8	Furfuranol	1119		0.11
9	Camphor	1156		0.12
10	Epoxy artemisia ketone	1164		0.70
11	Borneol	1178		0.08
12	Carvomenthene	1187		0.18
13	α-terpineol	1204		0.07
14	Dicyclopropylmethanone	1213		0.24
15	Dipropylamine	1228		0.47
16	Cyclohexene	1238		0.31
17	Z-3-Hexenyl hexanoate	1261		1.36
18	L-bornyl acetate	1300		0.12
19	3-Cyclohexene-1-methanol	1314		1.12
20	3-Dodecenal	1379		0.82
21	α-Copaene	1390		0.20
22	Neryl acetate	1402		2.14
23	trans-Caryophyllene	1438		0.44
24	α-Himachalene	1462		0.43
25	α-Humulene	1469		1.02
26	trans-.beta.-Farnesene	1475		0.07
27	α-Amorphene	1491		0.22
28	δ -Germacrene	1496		0.91
29	γ-Cadinene	1509		0.21
30	α-Cadinene	1516		0.71
31	δ-Cadinene	1539		0.11
32	cis-α-Bisabolene	1559		19.58
33	Caryophyllene oxide	1566		1.09
34	Ledene oxide-(II)	1569		0.11
35	Dodecanoic acid	1587		0.19
36	Spathulenol	1593		4.54
37	Caryophyllene oxide	1598		2.26
38	Humulene epoxide II	1623		0.26
39	Geranyl acetate	1627		0.94
40	α-Cedrene	1641		2.31
42	τ-Muurolol	1658		0.23
43	γ-Curcumene	1663		1.36
44	α-Cadinol	1671		0.13
45	Aromadendrene	1673		0.33
46	3-n-Hexylthiane, S,S-dioxide	1694		0.19
48	Tetradecanal	1732		0.23
49	(2E,6E)-famesol	1741		0.24
50	2-hydroxy cyclopentadecanone	1743		0.19
51	Myristic acid	1784		0.17
52	(E)-alpha-Atlantone	1792		0.35
53	Farnesyl acetate	1861		0.73
54	Perhydrofarnesyl acetone	1864		4.78
55	6-Phenyl-2-tetralone	1894		0.66
56	Ethyl linoleate	1905		0.74
57	Olealdehyde	1912		0.22
59	Oxacycloheptadecan-2-one	1945		0.20
60	α-Terpinolene	1952		0.32
62	n-Hexadecanoic acid	1991		2.02
63	Isopimaradiene	2006		1.05
65	Juvabione	2036		0.28
66	Abieta-8,11,13-triene	2072		0.30
69	n-Heneicosane	2120		0.46
70	Phytol	2133		0.16
72	n-docosane	2221		0.18
74	1,19-Eicosadiene	2245		1.07

Table 2 continued…

pic	compound[a]	RI[b]		(%)
75	Dehydroabietinal	2284		0.10
76	Sesquirosefuran	2308		0.46
77	Methyl isopimarate	2311		0.29
78	n-Tricosane	2321		0.11
79	Dehydroabietic acid	2356		0.09
80	Benzenamine, 2,4,6-trimethyl-	2383		0.11
81	n-Tetracosane	2420		0.15
82	1,21-Docosadiene	2448		0.26
83	n-Pentacosane	2521		0.40
84	Octadecanal	2653		0.14
85	n-Heptacosane	2719		0.11
86	Nonacosane	2900		0.16
	Identified compounds (%)	Total		74.11
[a]compounds listed in order to their RI [b]RI (retention index) measured relative to n-alkanes (C8-C20) using HP5MS				

CONCLUSION

The steam distillation of cone essential oils of *Cedrus atlantica*, collected from Khenchla (Eastern Algeria) were characterized by α - pinene (46.61%). Furthermore, branch and needle oils were characterized by cis-α-Bisabolene. There was a significant difference between the two oils. It is interesting to note that the chemical composition of the present essential oil differs from those reported in the literature. Furthermore cone, branch and needle essential oils of *C. atlantica* possess good antioxidant properties. The cone oil, branch and needle oil could be used as antioxidant agent.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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