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EXTRACTION AND CHARACTERIZATION OF LOCAL *CEDRUS ATLANTICA* ESSENTIAL OIL AND HYDROSOL

BENOUDJIT Fouzia[1*], HAMOUDI Imene[2] and ABOULOUZ Asmaa[2]

1. Unité de Recherche Matériaux, Procédés, Environnement (URMPE), Université M'hamed Bougara, Boumerdès, Algérie
2. Faculté des Sciences, Département de Chimie, Université M'hamed Bougara, Boumerdès, Algérie

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Abstract

Description of the subject: The present work investigated essential oil and hydrosol extracted from local *Cedrus Atlantica* wood.
Objective. The aim of the work was to characterize essential oil and hydrosol extracted by hydrodistillation from local *Cedrus Atlantica* in order to highlight their qualities.
Methods : *Cedrus Atlantica* essential oil and hydrosol physicochemical and spectral (ATR-FTIR) properties were investigated. The essential oil was also analyzed by GC/MS.
Results: Results confirmed the good quality of the essential oil and showed the presence of alkenes and alcohols in both of essential oil and hydrolate. The essential oil has α-Longipinene, Himachalol, α-Himachalene, γ-Himachalene, (E)- α-Atlantone, Allo-Himachalol, and α-Pinene as major compounds.
Conclusion : The chemical composition of *Cedrus Atlantica* essential oil make it very interesting in cosmetic, pharmaceutical and parapharmaceutical industries.
Keywords: *Cedrus Atlantica*; essential oil; hydrosol; physicochemical properties; FTIR spectra; GC/MS analysis.

EXTRACTION ET CARACTÉRISATION DE L'HUILE ESSENTIELLE ET DE L'HYDROLAT DE *CEDRUS ATLANTICA* LOCAL

Résumé

Description du sujet : Le présent travail a porté sur l'étude de l'huile essentielle et de l'hydrolat extraits du bois de *Cedrus Atlantica* local.
Objectifs : L'objectif de ce travail était de caractériser l'huile essentielle et l'hydrolat, extraits par hydrodistillation du bois du *Cedrus Atlantica* local afin de mettre en évidence leurs qualités.
Méthodes : Les propriétés physicochimiques et spectrales (ATR-FTIR) de l'huile essentielle et de l'hydrolat de *Cedrus Atlantica* ont été étudiées. L'huile essentielle a également été analysée par GC/MS.
Résultats : Les résultats obtenues ont confirmé la bonne qualité de l'huile essentielle et ont montré la présence d'alcènes et d'alcools aussi bien dans l'huile essentielle que dans l'hydrolat. Les composés majoritaires de l'huile essentielle sont α-longipinène, himachalol, α-Himachalène, γ-Himachalène, (E)-α-Atlantone, Allo-Himachalol et α-Pinène.
Conclusion. La composition chimique de l'huile essentielle de *Cedrus Atlantica* la rend très intéressante dans les industries cosmétique, pharmaceutique et parapharmaceutique.
Mots clés : *Cedrus Atlantica* ; essential oil ; hydrosol ; physicochemical properties ; FTIR spectra ; GC/MS analysis.

* Auteur correspondant : BENOUDJIT Fouzia, E-mail : f.benoudjit@univ-boumerdes.dz

INTRODUCTION

Essential oils (EOs) are vegetable products which constituents are a complex mixture of volatile molecules. The qualitative properties and quantitative compositions of EOs are different. It can be explained by many factors such as ecological region and field conditions [1-3], genotype [4], maturity stage of plant [5], germination stages [6], plants drying methods [7] and conditions [8], extraction methods [9], extraction stage and conditions [10], harvesting stage [5] and duration of extraction process [11]. Therefore, each EO has specific properties and thus it is important to be characterised in order to evaluate its quality and then to determine its chemical composition. The use of plant extracts is continuously increased and become a part of a competitive market in pharmaceutical, food, cosmetic, and perfumery industries [12 and 13] because of consumers' demand for natural products with less chemicals. Nowadays interest of consumers with natural products is continuously raising. Essential oils (EOs) and hydrosol (HDs) obtained from plant extraction are studied by many researchers [14-20]. In fact, EOs and HDs have relevant chemical constituents which can possess several biological activities and their incorporation in formulations can reduce the use of chemicals. *Cedrus Atlantica* (CA) is belongs to the family of Pinaceae. It is an endemic of the Atlas Mountains of Algeria and Morocco where it occurs in scattered stands at elevations of 1000 to 2500m [21]. The *Cedrus atlantica* essential oil (CAEO) has been shown to possess antihypersensitivity against pain behaviour [22]. It can also have antihyperalgesic [23], insecticidal [24], antimicrobial [25], molluscicidal [26], antibacterial and antifungal activities [27].

EO's chemical composition of different part (i.e. Wood, stems, cones, seeds and flowers) of CA has been studied [6, 25, 28-32]. However, in our knowledge there is no published report on physicochemical properties and spectroscopic analysis of wood EO extracted from CA grown in Algeria, in one hand. In other hand, no study has been done on the characterisation of *Cedrus atlantica* hydrosol CAHD. Thus, the present work focused on organoleptic, physical, chemical, spectroscopic and chromatographic characterization of CA wood extracts (i.e. EO and HD) which tree grown in the Wilaya of Ain Defla situated in Northen Algeria.

The organoleptic and physicochemical properties are significant to assess the quality of the studied oil and thus can be used as basic for its identification. The obtained results were compared with those found in literature from other places to highlight the quality of the local CA wood extracts.

MATERIALS AND METHODES

1. Plant material, EO extraction and obtained HD

CA wood from trees growing on Oued El Had (Wilaya of Ain Defla, Algeria) was collected during the period of dormancy in Decembre 2018. The collected wood was air-dried at room temperature in the shade. The CA wood was subjected to hydrodistillation for 3 h in an alembic, on an industrial scale, of the "Extral-bio" distillatory which located in Chiffa Wilaya of Blida, Algeria. The obtained EO and HD were separated by decantation. The CAEO and CAHD were stored in closed amber coloured bottles and stored at room temperature.

2. Yield of Cedrus Atlantica EO

Yield of CAEO that obtained was calculated as given in equation (1).

$$\%\ \text{Yield}\ (w/w) = \frac{\text{Weight in gram of extracted oil}}{\text{Weight in gram of } \mathit{Cedrus\ Atlantica} \text{ wood sample}} \times 100 \quad (1)$$

3. Organoleptic properties

The different organoleptic characteristics (appearance, colour and odour) of both EO and HD of CA were evaluated. Each EO and HD was placed in a transparent test tube over a white background and the appearance and colour were observed. The characteristic odours were determined by smelling.

4. Physical properties

The pH of CAEO and CAHD were determined using pH paper and approximate value were obtained. The relative density of CAEO and CAHD at 20 ° C were measured using an AP PAAR DMA48 densimeter according to the ASTM standard D4052 – 95. The refractive index values at 20ºC of CAEO and CAHD were obtained by using the digital refractometer Hanna HI 96801. The solubility of the CAEO was determined by mixing in test tube one volume of the EO with specified volumes of ethanol 96%.

5. Chemical properties

The acidic value expresses the number of milligrams of potassium hydroxide (KOH) required for the neutralization of the free acids present in 1 g of EO. The acidic value of CAEO was performed according to ISO 1242 standard. The iodine value is expressed as the number of grams of iodine per 100 g of oil. It is the amount of iodine that can be fixed per 100g of substance by breaking the double bond to the two neighboring carbons. The iodine value of CAEO was performed according to the Algerian legislation [33]. The peroxide value concerned with the number of active oxygen in the organic chains of a fatty substance. The peroxide value of CAEO was expressed as milliequivalents of oxygen per kg of oil (meq O2/1kg) and was accomplished according the Algerian legislation [34].

6. Chromatographic analysis: Gas chromatography-mass spectroscopy analysis (GC-MS)

The chemical composition of the CAEO was determined using Agilent GC 7890B, equipped with Agilent MS 5977A detector. The chromatographic separations were performed on a capillary column HP-5MS (5% Phenyl Methyl Siloxane) (30 m length × 0.32 mm inner diameter × 0.25 µm film thickness). High purity helium was used as the carrier gas at a constant flow rate of 1.3 ml/min. An 1 µl sample was injected in the split mode. The injector temperature was 280 °C. The oven temperature was started at 50 °C. It was raised to 150 °C at a rate of 5 °C/min, to 250 °C at a rate of 14 °C/min, and the to 300 °C at a rate of 10 °C/min. The detector was operating in the electron impact mode (70 eV). The temperatures of the ion source were 250 °C. The amount of each compound was calculated by computing its area against the area of the internal standard in the gas chromatogram. The chemical compositions of the product were identified by Mass Hunter Software. The relative percentages of the constituent compounds were percentages from the GC peak areas based on the total ion chromatogram.

7. Spectral analysis: Fourier transform infrared spectroscopy (FTIR)

CAEO and CAHD Fourier transform infrared spectroscopy characterisations were performed on KBr pellet at various wavenumber infrared rays with a range of frequencies from 4000 to 400 cm-1 with an SP 2000 Saias Monaco FTIR spectrophotometer.

RESULTS

1. Yield and physicochemical properties

The yield of 0.2% (w/w) was obtained by the extraction of CAEO. The organoleptic and physical characteristics of CAEO and CAHD, and chemical properties of CAEO are listed in Table 1. The obtained CAEO was transparent to pale yellow liquid and the CAHD was transparent liquid. Both of CAEO and CAHD had characteristic, warm and woody odour which was accentuated in CAEO. CAEO had a fairly acidic pH of 6. However, pH of CAHD was neutral. pH of CAEO was lower than that of CAHD. The transparency and the neutral pH of CAHD were probably due to its richness with water. Relative densities of CAEO and CAHD were 0.943 and 0.998, respectively. The CAEO was less dense than water as generally expected. Refractive index of CAEO (1.504) was higher than CAHD (1.333). CAHD relative density and refractive index were close to that of water because of the CAHD water richness. CAEO was miscible in ethanol. It showed acid, iodine and peroxide values of 1.122 mg KOH/g, 28.55 mg/100g and 2.80 meq O2/kg, respectively. The CAEO acid and peroxide values were in accordance with international regulations which thresholds are 4 mg KOH/g and 10 meq O2/kg, respectively [39].

2. Gas chromatography-mass spectroscopy analysis

Chemical composition of the CAEO determined using GC/MS analysis are presented in Table 2. Eighteen (18) compounds were identified. Table 2 indicates that CAEO was composed by sesquiterpenes (61.77%), oxygenated sequiterpenes (35.53%) and monoterpenes (2.70%). Thus, CAEO are composed with 64.47% of terpenes and 35.53% of oxygenated trepenes. α-Longipinene (23.35%), Himachalol (23.29%), α-Himachalene (18.21%), γ-Himachalene (17.71%), (E)- α-Atlantone (5.38%) and α-Pinene (2.70%) as major compounds in the composition of CAEO.

Table 1: Organoleptic and physicochemical properties of CAEO and CAHD

Characteristics	CAEO	CAHD
Appearance	Liquid	Liquid
Colour	Transparent to pale yellow	Transparent to pale yellow
Odour	Characteristic, warm and woody	Characteristic, warm and woody
pH	6	7
Relative density at 20ºC	0.943	0.998
Refractive index at 20 ºC	1.504	1.333
Solubility in ethanol 96%	Miscible	/
Acid value (mg KOH/g)	1.122	/
Iodine value (mg/100 g)	28.55	/
Peroxide value (meq O2/kg)	2.80	/

Table 2: CAEO chemical composition identified by GC–MS analysis

N°	RI*	RI [35]	RI [36]	RI [37]	RI [30]	RI [38]	Components	Relative amounts (%)
1	937	936	-	-	-	939	α-Pinene	2.70
2	1353	1359	-	-	-	1352	α-Longipinene	23.35
3	1405	1415	-	-	1387	1407	Longifolene	0.57
4	1449	1461	-	1447	1447	1451	α-Himachalene	18.21
5	1482	1489	-	1476	1476	1482	γ-Himachalene	17.71
6	1516	1523	1515	-	1515	1517	α-dehydro-ar-Himachalene	0.60
7	1542	-	-	-	-	-	aR-Himachalene	0.51
8	1544	-	1530	-	1529	1532	γ-dehydro-ar-Himachalene	0.82
9	1564	-	-	-	-	1563	E-Nerolidol	0.35
10	1581	1586	1572	1573	1574	1579	Himachalene epoxyde	0.35
11	1590	-	-	-	-	-	Calarene epoxide	0.17
12	1592	1610	-	1594	1594	1599	Longiborneol	0.84
13	1615	1625	1608	-	1605	1616	β-Himachalene oxide	0.44
14	1647	1661	1643	1647	1547	1653	Himachalol	23.29
15	1674	1674	-	-	-	1662	Allo-himachalol	3.26
16	1695	-	-	-	-	-	(1R,7S,E)-7-Isopropyl-4,10-dimethylene cyclodec-5-enol	0.74
17	1717	1722	1719	1717	1717	1718	(Z)-α–Atlantone	0.71
18	1773	1783	1782	1773	1773	1778	(E)-α-Atlantone	5.38
Sesquiterpenes hydrocarbons								61.77
Oxygenated sesquiterpenes								35.53
Monoterpenes hydrocarbons								2.70
Total								100.00

*: Retention index for the studied CAEO.

3. Spectral analysis: Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of CAEO and CAHD are shown in Figure 1. The functional groups associated with respective wave numbers of CAEO and CAHD are listed in Table 3. CAEO FTIR spectrum had more peaks than CAHD. The comparison between peaks of CAEO and CAHD indicated the similarities in their FTIR spectra.

In facts, peaks at about 3400 and 1630 cm-1 were present in both CAEO and CAHD spectra. The large band at about 3400 cm-1 corresponded to O-H stretching vibrations which could be due to alcohol groups and/or water content. Peaks at 1624 and 1637 cm-1 in CAEO and CAHD spectrum respectively corresponded to C=C stretching vibrations which could be attributed to alkenes.

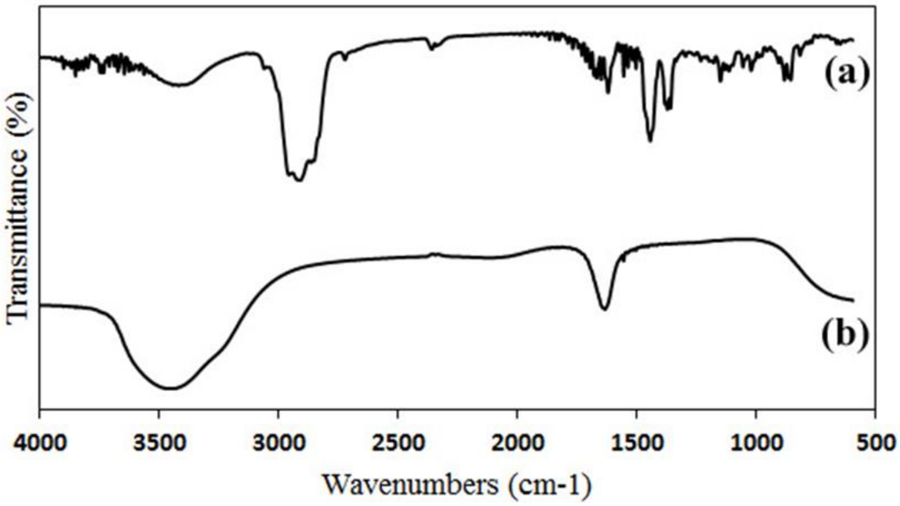

Figure 1: FTIR spectra of the CAEO (a) and CAHD (b).

Table 3: Results of FTIR analysis of CAEO and CAHD

CAEO		
Wavenumbers (cm^{-1})	Bonds	Types of vibration
3419	O-H	Stretch
2918	$-CH_2-$	Stretch
2725	C-H	Stretch
1771	C=O	Stretch
1674	C=C	Stretch
1624	C=C	Stretch
1558	C=C	Stretch
1507	C-C	Stretch
1448	C=C	Stretch
1375	$-CH_3$	Bend
1300	C-O	Stretch
1262	C-O	Stretch
1230	C-O	Stretch
1190	C-O-C	Stretch
1152	C-O	Stretch
1120	C-O	Stretch
1057	C-O	Stretch
1023	C-O	Stretch
868	=C-H	Bend
820	=C-H	Bend
CAHD		
Wavenumbers (cm^{-1})	Bonds	Types of vibration
3463	O-H	Stretch
1637	C=C	Stretch

DISCUSSION

According to Aberchane et *al.* [40], the yields of Cedar wood EO vary greatly depending on the forest source. The EO yield obtained in this study (i.e. 0.2%) was higher than that obtained by hydrodistillation of CA wood which was collected in the Chrea (Wilaya of Blida, Algeria) from September to December 2012 (i.e. Yield: 0.12%) [25]. However, the result of the present study was lower than yield of Moroccan CA wood hydrodistillation obtained by Uwineza et *al.* [28] (1.2%), Uehara et *al.* [35] (8%), Fidah et *al.* [36] (3.35%) and Satrani et al. [37] (2.78%). It was also lower than Moroccan CA wood hydrodistillation and steam distillation (2.5-2.6%) obtained by Aberchane et al. [40], and Lebanon CA wood steam distillation obtained by Saab et *al.* [29] (0.65%). The solubility in ethanol is one of the indicators of oil quality. The oil unfavourable storage conditions (i.e. Air, light, temperature etc.) speeds up polymerization and decreases the solubility of oil in ethanol [41]. As shown in Table 1, CAEO was miscible in ethanol at 1V/1V which indicated its good quality and storage conditions.

The acid value is an index which helps to identify any rancidity, to estimate the oxidation, and to specify the quality of oil [42]. The lower of the acid value of the studied CAEO makes it less exposed to rancidity.

The iodine value gives an idea of the average degree of unsaturation in oil and its stability to oxidation. In fact, according to Obasi et *al.* [43] low iodine value of the oil may be indicative of low susceptibility to oxidative rancidity.

The peroxide value is an index of oxidation progress, quality and shelf-life of oil [44] because of peroxides are the main initial products of oil oxidation [45]. It increases with the storage time, temperature and contact with air of the oil [46]. CAEO presented low peroxide value which could be attributed to the presence of natural antioxidant in the oil [47].

Chemical properties of CAEO indicates its good quality and stability. No data of CAEO and CAHD organoleptic, physical and chemical characteristics are found in the literature to make any comparison.

Benouaklil et *al.* [25], identified 16 volatile compounds in Algerian CAEO (Chréa, Wilaya of Blida) by GC/MS analysis. 69, 41, 23,38 compounds were identified in Moroccan CAEO by Uehara et al. [35], Fidah et *al.* [36], Satrani et *al*. [37], and Aberchane et *al.* [30], respectively.

Himachalol has antispasmodic activity [48] and α-Pinene has a good antibacterial activity [49]. Himachalene is an important product used in the perfume industry and α-Atlantone is one of the basis for important anti-inflammatory pharmaceutical products as well as important anti-cancer additives [29]. Thus CAEO appeared very interesting product which could be valorised in cosmetic, pharmaceutical and parapharmaeutical industries. Morocaan CAEO studied by Benouaklil et *al.* [25] was also composed by terpenes (72.45%) and oxygenated terpenes (3.68%). However, it was principally dominated by β-Himachalene (31.55%), α-Himachalene (15.00%), and Longifolene (11.22%). CAEO analysed by Uehara et *al.* [35] was mainly composed with β-Himachalene (33.45%), α-Himachalene (12.74%), and γ-Himachalene (8.31%). CAEO considered by Fidah et *al.* [36], was principally dominated by E-γ-Atlantone (19.73%), E-α-Atlantone (16.86%), and 5-Isocedranol (11.68%). Chemical composition of CAEO characterized by Satrani et *al*. [37], revealed that it was mainly predominated by E-α-Atlantone (28.75%), β-Himachalene (14.62%), and Himachalol (7.17%). CAEO studied by Aberchane et al. [30] was mostly dominated by β-Himachalene (33.81%), α-Himachalene (10.87%), and E-α- Atlantone (11.24%).

No authors found aR-Himachalene (0.51%), (1R,7S, E)-7-Isopropyl-4,10-dimethylene cyclodec-5-enol (0.74%) and, Clarene epoxide (0.17%) in the composition of their studied CAEOs. Thus, these molecules were characteristics of the studied CAEO.

The huge difference between CAEO of the present study and those previous work in chemical composition qualitatively and quantitatively were observed. These differences can be explained by many factors mentioned in the introduction. This is why, a complete description of the ecological and geographic conditions, pre-treatment and harvesting stage of the plant materials should be presented in order to understand the origin of the chemical composition and quantities components variation in essential oils. The large band at about 3400 cm^{-1} was pronounced in CAHD (Figure 1). It could be explained by CAHD water richness. Functional groups identified by FTIR analysis on CAEO are in the accordance with compounds revealed by GC/MS analysis.

CONCLUSION

The organoleptic and physicochemical characteristics, and chemical composition of *Cedrus Atlantica* essential oil and hydrosol extracted from *Cedrus Atlantica* wood of the Wilaya of Ain Defla were investigated. The essential oil organoleptic and physicochemical characteristics proved its good quality and stability. Organoleptic and physical properties of hydrosol proved its water richness which was confirmed by FTIR analysis. The presence of Himachalol, α-Himachalene, γ-Himachalene, (E)-α-Atlantone and α-Pinene in the essential oil make it very interesting in cosmetic, pharmaceutical and parapharmaeutical industries. aR-Himachalene, (1R,7S, E)-7-Isopropyl-4,10-dimethylene cyclodec-5-enol and, Clarene epoxide were characteristic molecules of the studied *Cedrus Atlantica* essential oil. Further investigations should be made in order to demonstrate the essential oil biologic activities and to determinate the chemical composition of *Cedrus Atlantica* hydrosol in order to compare it with that of the EO.

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