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Identification of volatile components and antioxidant assessment of the aerial part extracts from an Algerian *Cistus albidus* L. of the Aures region

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Abstract

The present study was aimed to investigate the essential oil composition and the antioxidant properties of an Algerian Cistus albidus L. from the Aures region. The essential oil was extracted by steam distillation technique and analyzed by GC-MS and GC-FID methods and the antioxidant evaluation was determined by the DPPH radical scavenging assay. Total phenol and flavonoid contents were measured using Foline-Ciocalteau and aluminium trichloride methods. The results revealed that the main compounds were respectively 15,16-dinorlabd-8(20)-en-13-one (22.3%), methacrylic acid (17.3%) and manool (12.4%). The highest antioxidant capacity was reported for ethyl acetate extract (IC_{50} 0.445 ± 0.112 mg/mL) which had also the highest total phenol (298.8 ± 115.8 µgAGE/mg) and flavonoid contents (262.9 ± 1.6 µgRuE/mg, respectively).

Keywords: Cistus albidus L; essential oil; 15,16-dinorlabd-8(20)-en-13-one; manool; antioxidant properties; GC-MS; GC-FID.

1. Introduction

The Cistaceae is a large family of perennial herbaceous plants consisting of 8 genera, with about 175 species [1] including the genus *Cistus* [2]. It is frequently found distributed in the Mediterranean region [3-5]. The aromatic genus *Cistus* consisting of around 25 species [6] used in traditional folk medicine to treat several diseases [7-12]. *C. albidus* (called Rock rose) is a small evergreen shrub distributed especially in the arid places (0 to 1400 m) of Mediterranean regions including Algeria [13]. In fact, many biological proprieties have been reported for *C. albidus* such as antioxidant, anti-inflammatory and antiviral activities [14-16] as well as its richness in essential oils [17-22]. Therefore, the Algerian *Cistus albidus* L. collected from the Aures area and used in traditional medicine was chosen in this study to identify its essential oil constituents and to evaluate antioxidant properties of its organic extracts.

2. Experimental part

2.1. Collection and identification of plant material and extraction

Aerial part (leaves and stems) of *C. albidus* were collected in June 2015 from Foum-Toub region of Batna (35° 24′ 18″ nord, 6° 32′ 59″ est, 57 km south east from Batna, Altitude: 1164 m). The identification of the species was confirmed by Professor Mohamed Kaabeche, Setif 1 University, Algeria, and a voucher specimen (CA/55/VAR/05-15) was deposited in the herbarium of

VARENBIOMOL Research Unit, Université des Frères Mentouri, Constantine, Algeria.

The air-dried materiel of *C. albidus* (3500 g) was extracted three times with 70% ethanol (3 × 10 L) at room temperature. The obtained alcoholic extract was then filtered through cotton and concentrated under reduced pressure, yielding the correspondent ethanolic dry extract (670 g, R=19.14%). The dry residue was further suspended in water and submitted to liquid-liquid extraction with chloroform (3 × 300 mL), ethyl acetate (3 × 300 mL) and *n*-butanol (3 × 300 mL). Extracts were concentrated by a rotary vacuum evaporator to yield the $CHCl_3$ (6.50 g), EtOAc (21.17 g) and *n*-BuOH (136.4 g) extracts.

2.2. Extraction and analysis of essential oils

The plant material of *C. albidus* (250 g) was subjected to steam distillation in a Kaiser Lang apparatus for three hours. The obtained oil (27.7 mg) was collected and dried over anhydrous sodium sulphate and kept at 4°C until analysis. The yield of the oil was calculated in relation of the plant weight.

2.3. GC-FID Analysis

The obtained essential oil was analyzed on an Agilent gas chromatograph (GC-FID) Model 6890, equipped with a HP-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane, 25 m x 0.25 mm, film thickness 0.25 µm), programmed from 50°C (5 min) to 250 °C at 3°/min and held for 10 min. Injector and flame ionization detector temperatures were 280 and 300 °C, respectively. The oil was diluted in acetone (3.5%, *v/v*) and injected in split mode (1/60), helium was used as a carrier gas (1.0 mL/min). Solutions of standard alkanes (C_8-C_{20}) were analyzed under the same conditions to calculate retention indices (RI) with Van del Dool and Kratz equation [23-24].

2.4. GC-MS Analysis

Mass spectrometry was performed on an Agilent gas chromatograph-mass spectrometer (GC-MS) Model 7890/5975, equipped with HP-5MS capillary column (25 m x 0.25 mm, film thickness 0.25 µm) programmed with the same conditions as for GC-FID. The mass spectrometer (MS) ionization was set in positive electron impact mode at 70 eV and electron multiplier was set at 2200 V. Ion source and MS quadrupole temperatures were 230 °C and 180 °C, respectively. Mass spectral data were acquired in the scan mode in the *m/z* range 33-450. The essential oil constituents were identified by matching their mass spectra and retention indices (RI) with those of reference compounds from libraries [25-26]. The proportions of the identified compounds were calculated by internal normalization.

2.5. Phytochemical screening (initial tests)

A few milligrams of *C. albidus* extracts were dissolved in distilled water in test tubes. After stirring for a few minutes, the appearance of persistent foam indicates the presence of saponins [27].

The appearance of a red color after addition of HCl and magnesium pieces indicates the presence of flavonoids [28].

The addition of iron trichloride ($FeCl_3$, 1%) led to appear of a blue-black color in the presence of gallic tannins and a blue-green color in the presence of catechic tannins [29].

2.6. DPPH radical scavenging assay

The antioxidant property of the extracts was assessed by DPPH method (2,2-diphenyl-1-picrylhydrazyl) using the protocol described by Ozturk et al. [30]. Extracts were prepared in methanol (8.0 mg/mL), then, an aliquot of 30 µL of sample solutions at different concentrations was added to 3.0 mL of a DPPH methanolic solution (0.04 mg/mL). The mixture was incubated for 30 in the dark at room temperature. The absorbance of the samples was measured at 517 nm wavelength with UV-Vis spectrophotometer (Thermo Scientific™ Evolution™ 300). The ability to scavenge the DPPH radical was calculated by using the following equation: % inhibition = [(Ac – As)/Ac] x 100; where Ac is the blank control absorbance and As is the sample absorbance at 517 nm. Ascorbic acid was used as a positive control. All determinations were carried out in duplicates, and the results were expressed as IC_{50} values from a calibration curves using Microsoft Excel.

2.7. Determination of total phenolic content

The total phenol content (TPC) of the extracts was assayed measured by the Folin-Ciocalteu method [31]. Briefly, the extracts were prepared at a concentration of 1.0 mg/mL in distilled water. 500 µL aliquot were transferred into a test tube and 1.0 mL of Folin-Ciocalteu reagent (1 N) was added, then, the mixture was allowed to stand for 4 min. After that, 5.0 ml of sodium carbonate solution (20%) were added to the mixture and the absorbance was read at 765 nm with a UV-Vis spectrophotometer after 2.0 hours of incubation in dark. All determinations were carried out in duplicates, and the TPC was expressed from a calibration curve as µgGAE/mg [32].

2.8. Determination of total flavonoid content

The total flavonoid content (TFC) of the extracts was determined by the aluminium trichloride method described by Ordonez et al. [33] using rutin as the reference compound. A volume of 2.0 mL of ethanolic solution of extracts (1.0 mg/mL) was mixed with 2.0 mL of $AlCl_3$ (2.0%). After incubation at room temperature for 1.0 hours, the absorbance was measured at 420 nm. All determinations were carried out in duplicates, and the TFC was expressed from a calibration curve as µgRuE/mg [32].

3. Results and discussion

The obtained essential oil from our *C. albidus* had a yellow color and a disagreeable odor with a low yield of 0.01% (*w/w*). The results of analyzes by GC-MS (Table 1) and GC-FID (Figure 1) led to identify eleven (11) constituents to represent seven (07) oxygenated compounds (42.3%) and four (04) hydrocarbon compounds (6.6%), all representing 61.5% of the essential oil composition. The main compounds were 15,16-dinorlabd-8(20)-en-13-one (22.3%) followed respectively by methacrylic acid (17.3%), diterpene oxygenated manool (12.4%), while, the minor constituents were ledol, selin-11-en-4-α-ol and monoterpene α-terpineol with 0.8; 0.6 and 0.4%, respectively. The results revealed also the presence of three major unknown compounds with 4.9; 4.9 and 4.2%.

According to the previously published works, the chemical composition of our sample was different, where the main constituents 15,16-dinorlabd-8(20)-en-13-one, methacrylic acid and manool were not present in the same sample studied in other regions. Indeed, the two predominant components of our oil 15,16-dinorlabd-8(20)-en-13-one and manool have a labdanic skeleton, while, the same sample from France [21] was characterized by the predominance of sesquiterpenes with bisabolan skeleton α-zingiberene (12.8%) and α-curcumene (7.7%), whereas, o-guaiacol (20.7%), E-caryophyllene (19.3%) and β-bourbonene (13.3%) were the main constituents in a Spanish sample [22]. Thus, the main compounds of an Italian *C. albidus* flowering tops and flowers oil [20] were α-zingiberene (13.7 to 20.7%), α-cadinol (7.7 to 11.4%) and *ar*-curcumene (11.2 to 13.2%), while, α-cadinol (11.1%), juniper camphor (8.7%), germacrene D (7.9%) and spathulenol (7.8%) were the major constituents of the leaves oil. Furthermore, the labdanic skeleton of two major components of our species 15,16-dinorlabd-8(20)-en-13-one and manool was also reported in some *Cistus* species such as *C. monspeliensis*, *C. ladaniferus*, *C. parviflorus* and *C. creticus* [21, 34-35], thus, these main constituents were specific in our *C. albidus* oil. It has also shown that *C. monspeliensis* oil and *C. palinhae* extracts have compounds with cistane and clerodan skeletons [36-38] those were not present in our *C. albidus* oil. To our knowledge, it is the first time that diterpenes with labdanic skeleton have described in our species oil and with high contents, as we noticed the novelty of these molecules in the *Cistus* genus. Moreover, this variability could be attributed to several factors such climate, altitude, soil conditions, part studied of the plant, time of collection and extraction methods of the oil [1, 24, 40-41].

The phytochemical screening of *C. albidus* aerial part extracts (Table 2) revealed the presence of saponins in the *n*-butanol extract, gallic tannins and flavonoids in both ethyl acetate and *n*-butanol extracts. The results of the antioxidant evaluation of *C. albidus* extracts (Table 3, Figure 2) by DPPH radical scavenging assay revealed a high antioxidant proprieties for the ethyl acetate and *n*-butanol extracts (IC_{50} = 0.445 ± 0.112 and 0.461 ± 0.055 mg/mL, respectively) compared to ascorbic acid (0.142 ± 0.019 mg/mL). The total phenol content of the extracts measured by Foline-Ciocalteau method revealed that the ethyl acetate and *n*-butanol extracts had the highest TPC (298.80 ± 115.86 and 253.70 ± 24. 69 µgGAE/mg, respectively) compared to the chloroform extract (47.89 ± 9.78 µgGAE/mg). The high total flavonoid content measured by aluminium trichloride assay (Table 3 and Figure 4) was reported for the ethyl acetate and *n*-butanol extracts (262.87 ± 1.65 and 254.72 ± 7.68 µgRuE/mg, respectively) compared to the chloroform extract (96.54 ± 2.90 µgRuE/mg). These results showed a good positive correlation between the TPC, TFCs and the antioxidant proprieties of the extracts. In fact, several published works have reported the high antioxidant potential of *C. albidus* extracts [15-16, 42], indeed, Bouyahya et al. [43] reported the high content of TPC in *C. albidus* extracts, in addition, Goncalves et al. [14] reported a positive correlation between TPC and the antioxidant activity measured by different methods. Moreover, due to these important results, *C. albidus* could be in the future a source for new antioxidant ingredients to improve the diversity in modern food.

Table 1: Chemical composition of *C. albidus* essential oil

No.	RT	[b]RI	[a]Compounds	Percentage %
1	9.848	1198	α-Terpineol	0.4
2	10.906	1271	Nonanoic acid	1.1
3	12.205	1366	Unknown	0.5
4	12.958	1424	Unknown	0.4
5	13.831	1494	Unknown	0.6
6	13.917	1501	Unknown	0.5
7	14.070	1514	Unknown	0.5
8	14.645	1563	Unknown	1.3
9	14.728	1570	Unknown	0.5
10	14.946	1588	Unknown	0.5
11	15.091	1600	Ledol	0.8
12	15.811	1665	Selin-11-en-4-α-ol	0.6
13	15.973	1679	Unknown	0.7
14	16.386	1717	Unknown	0.4
15	16.852	1761	Unknown	2.5
16	17.341	1808	Unknown	0.9
17	17.694	1842	Unknown	0.7
18	17.880	1861	Unknown	0.9
19	18.470	1920	Unknown	0.8
20	**18.610**	1934	**Unknown**	**4.2**
21	18.679	1941	Unknown	0.8
22	**18.830**	1957	**15,16-Dinorlabd-8(20)-en-13-one**	**22.3**
23	**18.873**	1961	**Unknown**	**4.9**
24	19.105	1985	Unknown	0.5
25	19.145	1989	Unknown	1.0
26	19.290	2004	Unknown	0.4
27	19.352	2010	Unknown	0.7
28	19.388	2014	Unknown	1.6
29	19.543	2029	Unknown	0.8
30	19.702	2044	Unknown	0.8
31	19.746	2049	Unknown	0.6
32	**19.795**	**2053**	**Manool**	**12.4**
33	20.021	2075	Unknown	1.0
34	20.177	2091	Unknown	0.5
35	20.439	2121	Unknown	0.7
36	20.492	2128	Unknown	1.2
37	20.712	2155	Unknown	0.6
38	**21.379**	2236	**Methacrylic acid (M 292)**	**17.3**
39	21.677	2271	Unknown	1.0

40	21.919	2299	Tricosane	1.9
41	**22.171**	2330	**Unknown**	**4.9**
42	23.530	2499	Pentacosane	1.2
43	25.028	2700	Heptacosane	1.6
44	26.428	2900	Nonacosane	1.9
Oil yield				0.01
Total content				99.4
Total identified				61.5
Oxygenated compounds				42.3
Hydrocarbon compounds				6.6

[a]Compounds are listed in order of their RI
[b]RI (retention index) measured relative to *n*-alkanes (C8-C20) using HP-5MS column.
RT (retention time)

Table 2: Results of percentage yields and phytochemical screening of *C. albidus* aerial part extracts

Extracts	Yield (%)	Saponins	Flavonoids	Gallic Tannins	Catechic tannins
$CHCl_3$	0.18	-	-	-	+
AcOEt	0.63	-	++	+++	-
n-BuOH	3.90	+++	++	+++	-
Essential oil	0.01				

Keys: (-) not detected (absent); (+) slightly present; (++) moderately present; (+++) highly present.

The percentage yields of the extracts were calculated based on dry material weight as: Yield (%) = $(W_1 \times 100) / W_2$; Where; W_1 = weight of extract after solvent evaporation; W_2 = Weight of the air-dried material.

Table 3: Phenol and flavonoid contents and IC_{50}-values of antioxidant properties of *C. albidus* aerial part extracts

Extracts	Total phenol content (µgGAE/mg)	Total flavonoid content (µgRuE/mg)	DPPH radical scavenging (IC_{50}, mg/mL)
$CHCl_3$	47.89 ± 9.78	96.54 ± 2.90	1.369 ± 0.378
AcOEt	298.80 ± 115.86	262.87 ± 1.65	0.445 ± 0.112
n-BuOH	253.70 ± 24.69	254.72 ± 7.68	0.461 ± 0.055
Ascorbic acid			0.142 ± 0.019

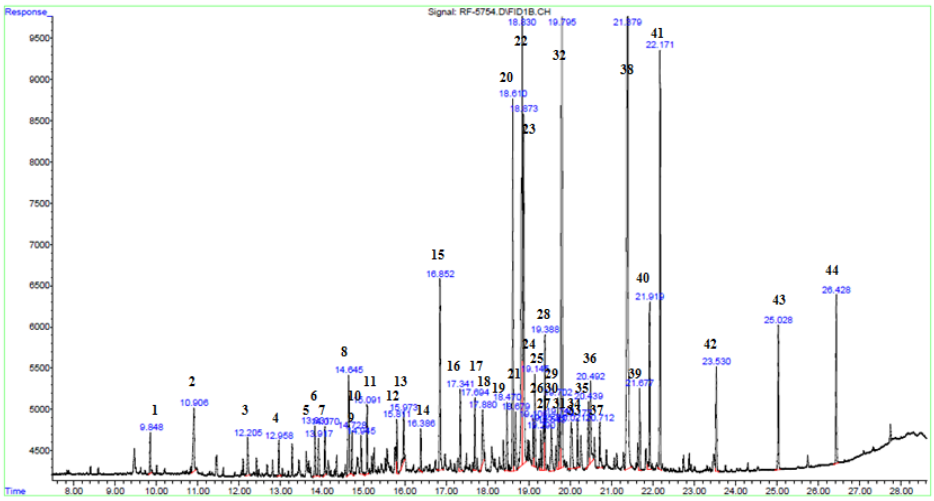

Figure 1. GC-FID Chromatogram of *C. albidus* essential oil.

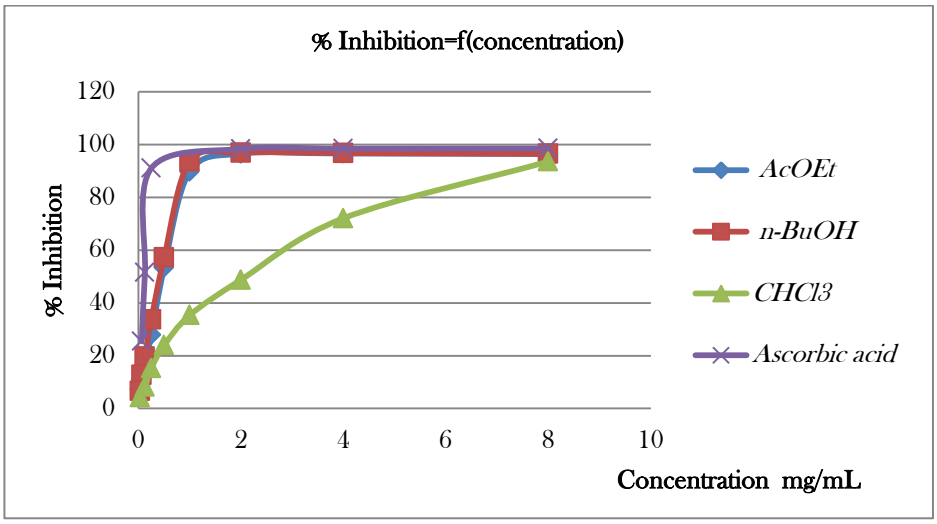

Figure 2. DPPH radical-scavenging activity of *Cistus albidus* L. aerial part extracts (chloroform, ethyl acetate and *n*-butanol extracts, data are expressed as mean ± standard deviation, n = 2)

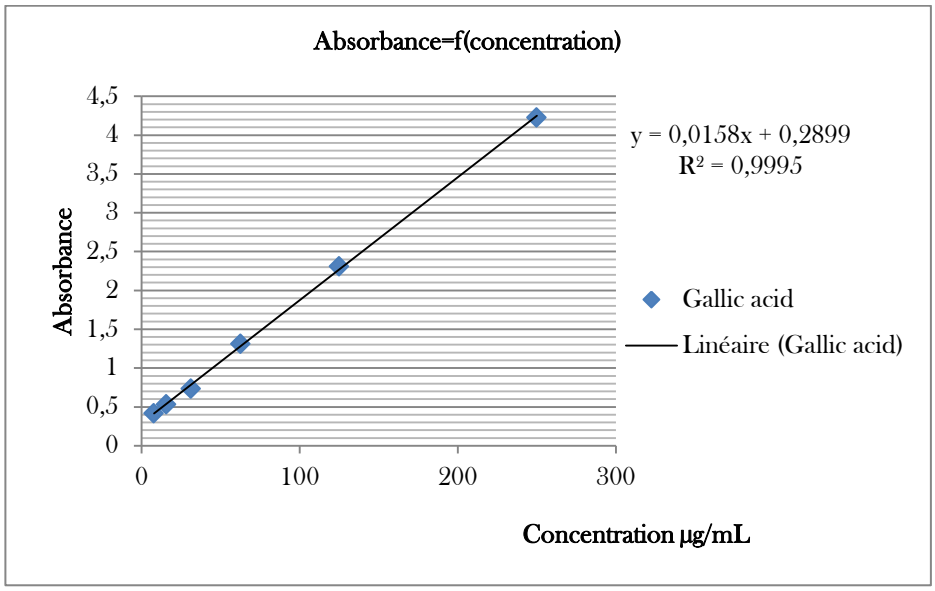

Figure 3. Calibration graphs for total phenol content (µg/mL)

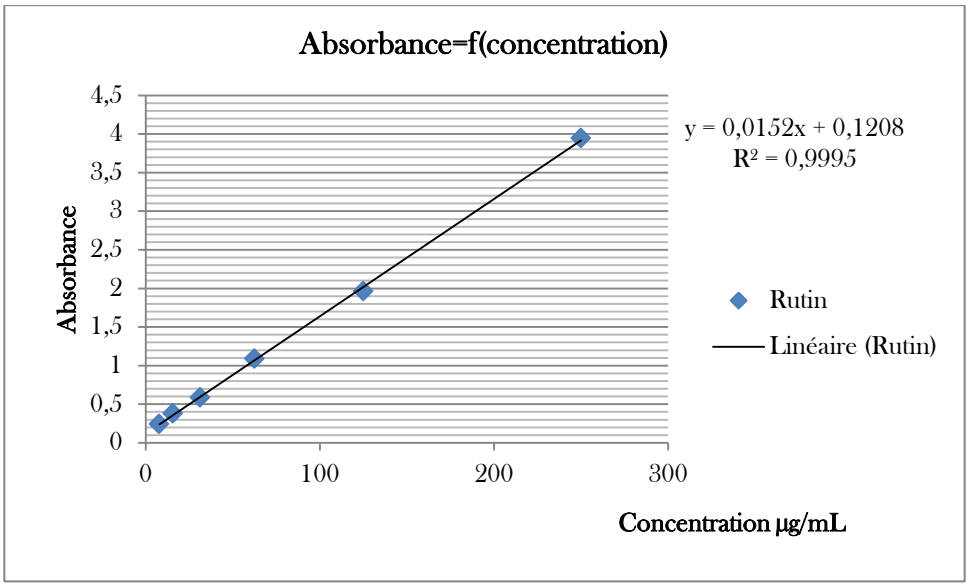

Figure 4. Calibration graph for total flavonoid content (µg/mL)

4. Conclusion

In this study, we reported the volatiles constituents by GC-MS and GC-FID methods of the Algerian *C. albidus* aerial part oil from the Aures area. The main identified compounds were respectively 15,16-dinorlabd-8(20)-en-13-one (22.3%), methacrylic acid (17.3%) and manool (12.4%). *Indeed,* it is the first time where we reported the presence of diterpenes with labdanic skeleton and with high contents. Moreover, this novelty could be attributed to several factors such changing climatic conditions, altitude, soil, part studied, material nature (fresh or dry), and time of collection or extraction methods. However, the results of antioxidant assessment revealed that ethyl acetate and *n*-butanol extracts exhibited high antioxidant proprieties in a good correlation with of the high total phenol and flavonoid contents. Thus, the obtained results underline the economic interest of the Algerian *C. albidus* of the Aures area which can be in the future a good source for natural antioxidant agents or as nutritional supplements in functional foods. In addition, our perspective in the future are to continue the *in vivo* and *in vitro* studies on the *C. albidus* oil to determine the relation essential oil composition-activity, and further phytochemical and

biological studies will be conducted on *C. albidus* extracts to identify the secondary metabolites responsible for its activity.

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