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Chemical composition and antibacterial activity of essential oil of *Launaea lanifera* Pau grown in Algerian arid steppes

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ABSTRACT

Objective: To evaluate the essential oil composition and the antibacterial activity of an Algerian endemic plant, *Launaea lanifera* Pau (*L. lanifera*), grown in arid steppe regions.
Methods: *L. lanifera* essential oil was isolated from aerial parts by steam distillation and its chemical composition was evaluated by gas chromatography-flame ionization detector and gas chromatography with electron impact mass spectrometry. Furthermore, its *in vitro* antibacterial activity against four bacterial strains was tested following the agar disk diffusion method.
Results: This species had a very low essential oil yield (0.005%). Twenty-four (92.6%) individual components were identified. The main constituents were hexahydrofarnesyl acetone (31.6%), (E)-β-ionone (8.5%), (E)-β-damascenone (7.0%), 2-methyltetradecane (3.8%), *n*-heptadecane (3.8%), limonene (2.8%) and β-caryophyllene (2.8%). No noteworthy antimicrobial activity was observed on the tested bacteria, neither Gram negative nor Gram positive.
Conclusions: This is the first report on the volatile constituents and antibacterial activity of *L. lanifera*. The studied essential oil does not possess significant activity against the tested microorganisms.

1. Introduction

Launaea (Asteraceae family) is one of the most common genus in the arid and Saharan regions of North Africa [1]. *Launaea lanifera* Pau (*L. lanifera*) (synonym: *Launaea acanthoclada* Maire [2]) is a yellow flowered perennial herb up to 40 cm high [3]. It grows in Algerian superior arid steppes and in some regions of Morocco [4,5]. This species grows also in the arid regions of Southeast Spain [6]. Nine other species of the genus *Launaea* are also present in Algeria, namely, *Launaea pumila* (Cav.) Kuntze, *Launaea arborescens* (Batt.) Murb. (*L. arborescens*), *Launaea capitata* (Spreng.) Dandy, *Launaea angustifolia* (Desf.) Kuntze, *Launaea nudicaulis* (L.) Hook.f. (*L. nudicaulis*), *Launaea quercifolia* (Desf.) Pamp., *Launaea mucronata* (Forssk.) Muschl., *Launaea amal-aminae* N.Kilian, and *Launaea fragilis* (Asso) Pau (*L. fragilis*), and six subspecies, *Launaea fragilis* subsp. *fragilis, Launaea mucronata* subsp. *mucronata, Launaea mucronata* subsp. *cassiana*, *Launaea angustifolia* subsp. *angustifolia*, *Launaea angustifolia* subsp. *arabica* [7].

Traditionally, *Launaea* species have been used in North Africa for the treatment of several diseases, especially those of liver, lungs and stomach, as well as to heal infected wounds [8]. In Saharan regions, some plants in this genus are called Marar, derived from the word Murr, which means “bitter” because of the bitterness that it imparts to the milk of camels that graze on it [9]. Many studies have been previously conducted to investigate the chemical composition of the various *Launaea* species. However, most of these investigations were focused on crude solvent extracts [10–16]. To the best of our

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knowledge, only three researches studied the essential oils of the Algerian Saharan species: *L. arborescens* [17], *Launaea resedifolia* [*L. resedifolia* (*L. fragilis*)] [18] and *L. nudicaulis* [19]. Moreover, another research work about the essential oil composition of *L. nudicaulis* grown in Oman has also been published [20]. Crude extracts, including water extracts, of *Launaea* species were also tested for their antibacterial [13,15,21], antifungal [22], antiparasitic [23], nephroprotective [24], antiurolithiatic [25], antioxidant [26] and allelopathic [27] activities. This work deals with the essential oil composition and antibacterial activity of *L. lanifera* grown in Algeria, never previously studied.

Secondary metabolites of plants are a potential source of new types of natural products. The essential oils may be an alternative way to fight against pathogenic microorganisms, especially resistant bacteria [28]. In this context, the present work focuses on the valorization of Algerian native plants, particularly those from arid steppe regions.

2. Materials and methods

2.1. Plant material

L. lanifera Pau has been identified according to published material [2,4,7] and was authenticated by Dr Norbert Kilian, Head of the Research Group Asterales, Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universitaet Berlin (private communication). It is a chamaephyte, chasmophile and lithophil plant that prefers thin and very stony soils [7,29]. Aerial parts (flowers, synflorescence branches and leaves) were collected in a rocky ground habitat at the flowering stage, during April 2014, in El Kattar (400 m of altitude), Department of Biskra, located 450 km south of Algiers in the Aures region. Here, the plant is known as "Agherramou". The collected plant material consists mainly of flowers and synflorescence branches. Dry synflorescence branches of the previous years were eliminated, then the plant was dried at room temperature for 1 month. A voucher specimen is deposited in the herbarium of the Department of Nature and Life Sciences, University of Biskra, Algeria, under the code AST-010-4-2014.

2.2. Essential oil extraction

The essential oil was extracted by steam distillation for 5 h using a modified Clevenger apparatus. In brief, the steam from a boiling flask (2000 mL of water) passes through a modified separatory funnel (2000 mL), containing 1000 g of the plant material, prior to reach the Clevenger apparatus. About 5000 g of the dry plant were used and the operation was repeated five times. Each time, the oil, including that remaining on the walls of the glassware, was recovered by decantation after addition of diethyl ether (Biochem Chemopharma, Cosne-Cours-sur-Loire, France). After evaporation of the solvent, the oil was stored at 4 °C in dark glass vial.

2.3. Gas chromatography analysis

GC analyses were performed on an HP-5890 Series II instrument (Hewlett-Packard Company, Wilmington, USA) equipped with DB-WAX and DB-5 capillary columns (30 m × 0.25 mm, 0.25 µm film thickness). The parameters of the analysis were as follows: oven temperature programmed from 60 °C to 240 °C at 3 °C/min, injector and detector temperatures 220 °C; helium was used as the carrier gas at a constant flow rate of 2 mL/min, detector dual FID, 0.5 µL injection of a 10% hexane solution of the oil, split ratio 1:30.

Identification of components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRIs) relative to the *n*-hydrocarbons series (C8–C25) (Fluka, Buchs, SG, Switzerland).

2.4. Gas chromatography with electron impact mass spectrometry analysis

Gas chromatography with electron impact mass spectrometry analyses were performed with a Varian CP-3800 gas chromatograph (Varian, Walnut Creek, CA, USA) equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. The analytical conditions were: injector and transfer line temperatures 220 and 240 °C respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 µL (10% hexane solution); split ratio 1:30.

Identification of the constituents was based on comparison of their retention time with those of authentic samples, comparing their LRIs relative to the series of *n*-hydrocarbons (C8–C25) (Fluka, Buchs SG, Switzerland), and on computer matching against commercial (NIST 02 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and mass spectra literature data [30–35].

2.5. Bacterial strains

Four bacterial strains were tested in this study: two Gram-negative [*Escherichia coli* (*E. coli*) ATCC 25922 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853] and two Gram-positive [*Staphylococcus aureus* (*S. aureus*) ATCC 25923 and *S. aureus* ATCC 43300]. The bacteria were obtained from the collection of bacterial clinical isolates of the Bacteriology Laboratory of Benbadis Hospital University Centre, Constantine, Algeria.

2.6. Antibacterial activity

Mueller-Hinton (MH) agar medium (Biochem Chemopharma, Cosne-Cours-sur-Loire, France) was used to test the *in-vitro* antibacterial activity of the essential oil following the agar disk diffusion method [36]. First, a suspension in physiologic sterile water of each bacterium was prepared from MH fresh culture plates to a final concentration of approximately 10^6 colony forming unit/mL measured by the turbidimetric method [37].

The suspensions were incubated for 1 h at 37 °C, then spread on the MH medium, while the oil was diluted in dimethyl sulfoxide (DMSO) (Biochem Chemopharma, Cosne-Cours-sur-Loire, France). Three dilutions (1/2, 1/20 and 1/100), corresponding to vol (oil)/vol (DMSO) ratios of 1:1, 1:19 and 1:99, were used. A volume of 5 µL of each dilution was dropped on Whatman paper disc (5 mm in diameter) with the respective oil

content per disc of 2.5 µL, 0.25 µL and 0.05 µL. An additional disk (negative control) was dropped with 5 µL of DMSO and then all the discs were deposited on the agar. The experience was repeated three times. The plates were incubated for 24 h at 37 °C and the inhibition zone diameters (zone around the disks plus diameter of the disk) were measured in millimeters.

3. Results

A very low yield of about 0.005%, calculated from the dry weight and corresponding to about 250 µL of orange-yellow oil was obtained, with no characteristic odor.

All the identified compounds of *L. lanifera* essential oil were listed in Table 1 in order of their elution from the DB-5 capillary column, along with their relative percentages, as well as their LRIs relative to the *n*-hydrocarbons series. Twenty-four components, representing 92.6% of the whole volatile compounds have been identified in the present study. As can be noted from Table 1, the oil is rich in apocarotenoids (56.7%), mainly hexahydrofarnesyl acetone (31.6%), followed by (E)-β-ionone (8.5%) and (E)-β-damascenone (7.0%). Limonene and β-caryophyllene were the only identified compounds among monoterpene hydrocarbons and sesquiterpene hydrocarbons, respectively. They occurred in low amount and at the same percentage (2.8%). The other compounds constituting the remaining 28.5% of the total composition were to a large extent non-terpene hydrocarbons, with 2-methyltetradecane and *n*-heptadecane (3.8% each) as the main ones.

Table 1
Chemical composition of the essential oil of *L. lanifera* grown in Algerian arid steppes.

No.	Constituents	LRI[a]	% Peak area
1	Limonene	1 032	2.8
2	Nonanal	1 102	2.2
3	Decanal	1 205	2.9
4	Undecanal	1 307	1.2
5	(E)-β-damascenone	1 382	7.0
6	Tetrahydrogeranylacetone	1 407	1.6
7	Dodecanal	1 409	2.2
8	β-Caryophyllene	1 419	2.8
9	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	1 429	1.5
10	(E)-geranylacetone	1 455	1.7
11	2-Methyltetradecane	1 462	3.8
12	3,4-Dehydro-β-ionone	1 486	3.2
13	(E)-β-ionone	1 487	8.5
14	*n*-Pentadecane	1 500	1.3
15	Tridecanal	1 510	2.2
16	Dihydroactinidiolide	1 536	1.6
17	Caryophyllene oxide	1 582	1.8
18	*n*-Hexadecane	1 600	2.0
19	Tetradecanal	1 614	1.2
20	*n*-Heptadecane	1 700	3.8
21	Pentadecanal	1 716	2.0
22	Benzyl benzoate	1 763	1.2
23	*n*-Octadecane	1 800	2.5
24	Hexahydrofarnesyl acetone	1 845	31.6
	Monoterpene hydrocarbons		2.8
	Sesquiterpene hydrocarbons		2.8
	Oxygenated sesquiterpenes		1.8
	Apocarotenoids		56.7
	Others		28.5
	Total identified		92.6

[a]: Relative to the *n*-hydrocarbons series (DB-5 column).

Table 2
Antibacterial activity, expressed in mean diameters (mm) of growth inhibition zones [disc diameter (5 mm) included], of the essential oil of *L. lanifera* grown in Algerian arid steppes.

Strains tested		Oil content per disc (µL)		
		2.5	0.25	0.05
Gram positive	*S. aureus* ATCC 25923	7	NI	NI
	S. aureus ATCC 43300	6	NI	NI
Gram negative	*P. aeruginosa* ATCC 27853	NI	NI	NI
	E. coli ATCC 25922	NI	NI	NI

NI: No inhibition zone.

The antibacterial properties of the characterized essential oil were evaluated by measuring the inhibition zones on MH agar, as shown in Table 2. Limited inhibition zones were obtained even at the highest concentration (1/2) of the oil. This was only the case against *S. aureus* ATCC 25923 and ATCC 43300, with diameters not exceeding 7 mm. For the other tested strains, the essential oil showed no activity at all the concentrations.

4. Discussion

The essential oil yield obtained in the current study is very low compared to those obtained from *L. resedifolia* (*L. fragilis*), 0.9% [18], and *L. arborescens*, 0.07% [17], growing in the Algerian Sahara. A yield of 0.035% was obtained from *L. nudicaulis* grown in Oman [20].

In comparison with other species of the same genus, the composition of *L. lanifera* is completely different. This is corroborated by the reported high percentage of esters (60.61%) and the relatively low content (8.95%) of monoterpenes in the oil of *L. resedifolia* (*L. fragilis*) [19]. Alkenes and ketones were also found to be the major constituents (58.24%) of *L. arborescens* oil [17]. On the other hand, constituents reported for the essential oil of *L. nudicaulis* were mostly long chain hydrocarbons [38]. *L. lanifera* also contains hydrocarbons up to C18, mainly *n*-pentadecane, *n*-hexadecane, *n*-heptadecane and *n*-octadecane, but to a lesser extent (11.4%).

The absence of a characteristic scent of the *L. lanifera* oil was attributed to the absence of important aromatic compounds [39]. The plant material used in the current investigation contains a large amount of flowers. Irregular volatile terpenoids can often be encountered in the flavor of some flowers [40], including β-ionone and dehydro-β-ionone [41]. However, owing to their low odor threshold, these compounds contribute significantly to the overall fragrance even if they are present in very low concentration in the scent [42]. This may also explain the absence of a characteristic odor of *L. lanifera* essential oil.

Like several Asteraceae species, *L. lanifera* is a yellow flowered herb [7], where carotenoids are mostly responsible for yellow floral pigmentation [43]. A vast number of carotenoid breakdown products are apocarotenoids [44,45]. This is also the case of the essential oil of *L. lanifera*, analysed in the present study, where a high percentage of apocarotenoids that range from C11 (dihydroactinidiolide) to C18 (hexahydrofarnesyl acetone) has been observed. Apocarotenoids in the range of C9–C19 are volatile constituents and their much higher solubility permits their inclusion in essential oil [46,47].

Regarding the antibacterial activity, important bacterial activity has been reported for *Launaea residifolia* essential oil,

which is of the same genus as *L. lanifera*, against some bacterial strains, including those used in the current analysis (*S. aureus*, *P. aeruginosa* and *E. coli*) [18]. This result may be due to the complete different chemical composition of the two oils. Antagonistic effect of some compounds present in the essential oil may also influence the biological activity of some active constituent [48]. The components found in *L. lanifera* essential oil, especially those in higher amounts, are not included in the composition of essential oils usually reported to possess *in vitro* antibacterial properties [48,49].

In conclusion, it is of highly scientific interest to investigate the essential oils composition of various plants grown in Algeria. The present study provides, for the first time, important data about the chemical composition of *L. lanifera*. The studied essential oil does not possess significant activity against the tested microorganisms. However, further investigations should be carried out on other biological activities, including other bacterial species, as well as for its antifungal and antioxidant properties. Furthermore, antagonistic effects of known and/or unknown compounds present in the volatile mixture should also be verified. Regarding the low yield obtained in the current analysis, it would be better to try other extraction processes to improve the result. A further study on the essential oils obtained from different plant organs and in different seasons of the year is also recommended to evidence a possible variability in the yield and the composition of the essential oils.

Conflict of interest statement

We declare that we have no conflict of interest.

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