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Chemical profile and bioactive properties of the essential oil isolated from *Ammodaucus leucotrichus* fruits growing in Sahara and its evaluation as a cosmeceutical ingredient

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ABSTRACT

Ammodaucus leucotrichus is a medicinal plant commonly used in Algeria by the indigenous populations, especially due to its therapeutic effects. In this context, the aim of the present study was to chemically characterize the essential oil of *A. leucotrichus* fruits (EOALF) growing in Algerian Sahara, and to evaluate its bioactive properties (antimicrobial, antioxidant and anti-inflammatory). Considering the interest of the cosmetic industry for natural ingredients, and taking into account the obtained biological properties, the essential oil was also evaluated by incorporation in a base cosmetic (cream). The essential oil was extracted with a yield of 2.58 ± 0.17%, being perilla aldehyde identified as the main component, accounting for 85.6% of the total composition. Concerning the tested bioactivities, EOALF presented antioxidant potential, a strong anti-inflammatory activity, and was effective against the tested microbial strains (*Staphylococcus aureus*, *Eschericia coli* and *Pseudomonas aeruginosa*), being *S. aureus* the most sensitive bacteria. After incorporation in a base cosmetic, the developed formulation was able to preserve the EOALF bioactivities along 28 days under storage. The obtained results, with relevance for the strong-anti-inflammatory activity, pointed out the interest to exploit this essential oil as a cosmeceutical ingredient in the cosmetic industry.

1. Introduction

The Sahara is not only the largest desert, but also the most expressive and typical due to its extreme aridity, presenting a discontinuous and very irregular vegetation mat (Le Houerou, 1990; Boukerker et al., 2016). The Saharan climate is characterized, in particular, by a weak and irregular precipitation, intense brightness, high potential evapotranspiration and high thermal amplitude. These climatic characteristics create drastic conditions that cannot be easily tolerated by the living ecosystems, resulting in a phytochemical adaptation and the appearance of new protective molecules against this extreme environment (Sitouh, 1983; Koull and Chehma, 2015).

The status of the spontaneous flora in Sahara Desert, and its relationship with local populations, deserve special attention. In addition to their ecological importance, they found many traditional uses in terms of pharmaceuticals, food and other domestic applications (e.g. as garden decoration). These plants have the ability to synthesize many compounds called secondary metabolites constituting an immeasurable source of important molecules, including polyphenols and essential oils of high chemical diversity, and possessing a wide range of biological activities (Jean and Jiri, 1983; Baamer et al., 2015). Thus, studies on the biological effects of medicinal plants have increased remarkably in

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the recent years due to their potential to be used as sources of several drugs (Haddouchi et al., 2016).

Among the huge plant diversity of the Sahara Desert, *Ammodaucus leucotrichus* Cosson & Durieu, which belongs to the *Apiaceae* family (Umbelliferae), inhabits the maritime sands in the Saharan and sub-Saharan countries of North Africa, Morocco, Algeria and Tunisia, extending to Egypt and tropical Africa (Velasco-Negueruela et al., 2006). *A. leucotrichus,* known in Algeria as "KammÛnes-sofi", is a medicinal plant with an extended culinary use by the indigenous populations against stomach pain, indigestion, diarrhea, vomiting, fever, spasms and colic, intestinal worms, constipation (Merzouki et al., 2000; Didi et al., 2003; Benhouhou, 2005; Chehma, 2006; Hammiche and Maiza, 2006; Fakchich and Elachouri, 2014), in the treatment of allergy symptoms (Didi et al., 2003; Hammiche and Maiza, 2006) and also against coughing, as emmenagogue and against anorexia (Hammiche and Maiza, 2006). In Tassili (Algeria), the fruits and the leaves are commonly consumed in infusions, being the fruits most consumed for their bioactive capacity mainly in the treatment of heart palpitations (Jouad et al., 2001), and the leaves for their flavoring properties in tea. The powder form is also an appreciated spice for foodstuff (Benhouhou, 2005; Chehma, 2006; El-Haci et al., 2014).

A. leucotrichus from different regions has been studied regarding their bioactive properties. Dahmane et al. (2017) studied the antioxidant and the antimicrobial properties of *A. leucotrichus* essential oils from Algeria. Alaoui et al. (2014) reported also the antibacterial and antifungal activity of the essential oils obtained from this species from Morocco. The same species from Morocco were also studied as antifungal agents against postharvest phytopathogenic fungi in apples (Manssouri et al., 2016). Considering the application in cosmetics, as far as we know, there are no reports on the use of the essential oil of *Ammodaucus leucotrichus*.

In the cosmetic industry, the preservation against microbial contamination and oxidation is a highly relevant topic. In this context, the important antimicrobial activity and powerful antioxidant properties of some essential oils have led many researchers to propose their use in cosmetics as natural preservatives. In fact, different reports from the literature have shown the preservative efficacy of a high array of natural products in cosmetic formulations (Popescu et al., 2014; Patrone et al., 2010; Yorgancioglu and Bayramoglu, 2013; Kunicka-Styczyńska et al., 2009, 2011; Kerdudo et al., 2016). In addition, essential oils can be incorporated in cosmetic products due to several other associated properties such as anti-inflammatory, emollient and humectant capacity, dye power, wound healing, anti-mutagen, anti-aging, protective effect against UV-B damage and skin discoloration (Dreger and Wielgus, 2013). In this context, the aim of the present work was to chemically characterize the essential oil obtained from *Ammodaucus leucotrichus* fruits (EOALF), growing in Algerian Sahara, and study its biological activity, including antimicrobial, antioxidant, and anti-inflammatory. Furthermore, the essential oil was tested as a cosmeceutical ingredient and their biological efficacy in a base cream evaluated along storage time (7, 14, 28 and 43 days).

2. Material and methods

2.1. Standards and reagents

Methanol was of analytical grade and supplied by Pronalab (Lisbon, Portugal). *n*-Hexane (purity ≥ 99.0%) was purchased from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Dulbecco's modified Eagle's minimum essential medium (DMEM), fetal bovine serum (FBS), Griess reagent system (Promega), DMSO, lipopolysaccharide (LPS), were obtained from Sigma-Aldrich Co. (Saint Louis, MO, USA). The bacterial strains (*Eschericia coli* ATCC 10536, *Staphylococcus aures* ATCC 19213 and *Pseudomonas aeruginosa* ATCC 9027) were purchased from Liofilchem Bacteriology Products (Italy). The culture media Muller Hinton broth (MHB) and Tryptic Soy Broth (TSB) were obtained from Biomerieux (Marcy l'Etoile, France). Blood agar with 7% sheep blood and MacConkey agar plates were purchased from Biomerieux Marcy l'Etoile, France). The dye *p*-iodonitrotetrazolium chloride (INT) was purchased from Sigma-Aldrich (Spruce Street; St. Louis, MO, USA) and was used as a microbial growth indicator. Helium, hydrogen, and synthetic air were acquired from Air Liquide (Portugal). The base cream was purchased from Fagron Iberica S.A.U. (Barcelona, Spain).

2.2. A. leucotrichus fruits samples and essential oil extraction

A. leucotrichus fruits were collected in March of 2015 in Tiouliline (wilaya of Adrar), south of Algeria (27°52′N and 0°17′W). The plant material was identified by Dr. Tayeb Si Tayeb (Laboratory of Biotoxicology, Pharmacognosy and Biological recovery of plants, University of Moulay-Tahar, Saida, Algeria). A voucher specimen was deposited at the Herbarium of the Laboratory under the code number LBPBP-TS03-12. The plant fruits were dried in the dark at room temperature and preserved until extraction.

The essential oil was extracted using a Clevenger-type apparatus. Briefly, 400 g of the dried fruits were subjected to hydro-distillation with 4 L of distilled water during 4 h. The obtained essential oil was dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 °C prior to analysis.

2.3. Chemical characterization of A. leucotrichus

2.3.1. Gas chromatography (GC) analysis

Quantitative analysis of the essential oil from *A. leucotrichus* fruits was performed using a Varian CP-3800 chromatograph equipped with a flame ionisation detector (FID) and a CP-Wax 52 CB bonded fused silica polar column (50 m × 0.25 mm, 0.2 µm film thickness) from Varian. The oven temperature was set at 50 °C for 5 min, increasing by 2 °C/min to 200 °C and finally held isothermal for 20 min. The injector and detector temperatures were 240 and 250 °C, respectively. Helium (He N60) was used as the carrier gas at a constant flow rate of 1 mL/min. Samples were diluted in *n*-hexane (1:1) and injected (0.1 µL) using a split ratio of 20:1. The percentage composition of the components was calculated by normalisation of the GC peak areas without response factors. Reproducibility was verified by analysing the sample three times.

2.3.2. Gas chromatography–mass spectrometry (GC–MS) analysis

The essential oil of *A. leucotrichus* was analyzed using a Varian CP-3800 coupled with a Varian Saturn 2000MS ion-trap mass spectrometer (MS), a CP-Wax 52 CB bonded fused silica polar column (50 m × 0.25 mm, 0.2 µm film thickness) from Varian and a Rxi®-5Sil MSf used silica low-polar column (30 m × 0.25 mm, 0.25 µm film thickness) from Restek, and a Varian MS Workstation 6.9 software. The injector was set at 240 °C and the samples (diluted in *n*-hexane (1:1)) were injected (0.1 µL) using a split ratio of 50:1, with helium (He N60) at a constant flow rate of 1 mL/min. The oven temperature program was initially set at 50 °C for 5 min, then raised up to 200 °C at a rate of 2 °C/min, and finally held isothermal for 20 min. All mass spectra were acquired in electron impact (EI) mode. The transfer line, manifold and trap temperatures were 171, 83 and 150 °C, respectively. The mass to charge ratio, *m/z*, ranged from 80 to 500, the emission current was 10 µA, and the maximum ionisation time was 0.025 s. Reproducibility was verified by analysing the sample three times. The components were identified according to their retention indices relative to C_8−C_{40} *n*-alkanes and mass spectra, which were compared with those of the NIST98 Spectral Library, the mass spectral database of Flavors and Fragrances of Natural and Synthetic Compounds 2 (FFNSC2) from Wiley, an in-house library (with > 200 pure reference chemicals) and literature data (Adams, 2001; Babushok et al., 2011; Abu Zarga et al., 2013).

2.4. Bioactive properties of A. leucotrichus

To assess the antioxidant activity of the *A. leucotrichus* essential oil, concentrations ranging from 0.781 to 100 mg/mL (methanol was used as the diluting medium) were prepared. The DPPH radical-scavenging and the reducing power (RP) assays were used to evaluate the antioxidant capacity following the methodology described elsewhere (Taofiq et al., 2017). The concentrations providing 50% of activity in the DPPH or 0.5 absorbance in the RP assays (EC_{50}) were obtained from the graph of the antioxidant percentages (DPPH assay) or the measured absorbance at 690 nm (RP assay) against concentration of the essential oil. Trolox was used as positive control.

For the antibacterial activity, the tested bacterial were ATCC strains: one Gram+ bacteria (*Staphylococcus aureus* ATCC 19213) and two Gram– bacteria (*Eschericia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 9027). Kanamycin was used as positive control (1 mg/mL in sterile physiological saline). Briefly, the microorganisms were cultivated and the minimal inhibitory concentration (MIC) was determined on a 96-well microplate by the colorimetric assay using *p*-iodonitrotetrazolium chloride (INT). The sample for analysis was prepared by dissolving the essential oil in 25% DMSO at a concentration of 300 mg/mL. Thereafter 100 µL of this solution were added to 350 µL of culture medium. Afterwards, 50 µL of bacteria medium (1.5×10^8 CFU/mL) were added, achieving a concentration range of 60–0.234 mg/mL. The microplates were further incubated at 37 °C during 24 h, in an oven (Jouan, Berlin, Germany). The colorant (INT) was then added at a concentration of 0.2 mg/mL, and after incubation at 37 °C for 30 min, the MIC were determined. Viable microorganisms reduced the yellow dye to a pink color. The MIC was defined as the essential oil concentration that prevented this change, thus showing complete inhibition of bacterial growth. The MBC value was defined as the essential oil concentration able to kill the bacteria strains.

The anti-inflammatory activity was evaluated according to Taofiq et al. (2017). The LPS-induced NO production by Murine macrophage (RAW 264.7) cell lines was determined as nitrite concentration in the culture medium. Dexamethasone (50 mM) was used as a positive control. The nitric oxide (NO) production was evaluated with the Griess Reagent System kit. The sample for analysis was prepared by dissolving the essential oil in 25% DMSO at a concentration of 400 µg/mL and further diluted to achieve the range of 400–1.56 µg/mL. Results were expressed as IC_{50} values (µg/mL), i.e. the sample concentration providing a 50% inhibition of NO production.

2.5. Incorporation of A. leucotrichus essential oil in a base cosmetic

Two samples were prepared, one without EOALF (negative control) and another with the incorporation of the EOALF. The used base cosmetic (a cream purchased from Fagron Iberica S.A.U. (Barcelona, Spain)), is certified by the US Food and Drug Administration (FDA), Regulation (EC) No 1907/2006dREACH and the National Health Surveillance Agency (ANVISA) of Brazil. It is a colorless product without adding of fragrances, parabens, mineral oils, sodium lauryl sulphate (SLS), propylene glycol, and ethoxylates. The incorporation was achieved by adding the essential oil to the base cream, at a concentration able to provide all the tested bioactivities, meaning that the incorporation was done based on the exhibited EC_{50}/GI_{50}/MIC values and calculated to be 2.5-fold higher than the EC_{50} value. Thus, 50 mg of the essential oil were added per gram of base cream. The cosmeceutical formulations were analyzed at different storage times (0, 7, 14, 28 and 43 days) according to the recommendations of European Pharmacopoeia (EP, 2011). The samples were stored at 4 °C for further analysis Antioxidant, antibacterial and anti-inflammatory activities were evaluated following the general procedure described in Section 2.4.

2.6. Statistical analysis

For the experimental assays, three samples (one per each assayed bioactivity) were analyzed in triplicate. The results are expressed as mean values ± standard deviation (SD). The observed differences between samples were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$, coupled with Welch's statistic. This treatment was carried out using SPSS v. 22.0 program.

3. Results and discussion

3.1. Extraction yield

A. leucotrichus was collected in Tiouliline in the wilaya of Adrar (south of Algeria). Adrar (27°52′ N, 0°17′ W), the second-largest department of the country covering about 427,368 km^2 (Azzi et al., 2012). The EOALF had blue color, with a characteristic and pungent odor. The obtained extraction yield was 2.58 ± 0.17% (w/w). This yield is in agreement with that obtained by Velasco-Negueruela et al. (2006) for the same species harvested in Dakhla, Western Saharan, North Africa (2.76%). However, other recent studies on this species harvested from different regions in Algeria, reported yields such as 1.5% (Bechar) (El-Haci et al., 2014), 2.1% (Djelfa), 2.0% (Illizi) (Dahmane et al., 2017) and 3.0% (Ghardaia) (Abu Zarga et al., 2013). The results obtained by Alaoui et al. (2014) for this species harvested in Morocco, refer a yield of 1.5%. The registered variations may be attributed to the geographical origin of the plant, as well as to the harvest period. In fact, from the analysis of the published data, it can be noticed that the harvest season can significantly affect the essential oil yield. Moreover, the obtained yield is described to be greater than 2% when the plant is harvested in the spring season (Velasco-Negueruela et al., 2006; Abu Zarga et al., 2013).

Fruits maturation occurs mostly in spring (Benhouhou, 2005). Studies on other plants belonging to the Apiaceae family, which have analyzed the organs responsible for the essential oils production, from the starting buds to the stage of green fruits, have shown that the percentage of essential oil increase until the ripening stage (Bernáth et al., 1999; Németh, 2003). In Apiaceae familly, the essential oil is preserved in specialized vessels (oil tubes), called “Vittae”, and these structures exist in the early stages of fruit development (Kurian and Sankar, 2007). The production of essential oils is reduced as a result of the accumulation of photosynthetic products in the endosperm. An anatomical modification of the tubes due to their drying during fruit ripening leads to a decrease in the essential oil yield (Bernáth et al., 2001; Moghaddam et al., 2015). Also, the rapid growth of non-fruit storage materials leads to a yield decrease in late spring (Németh, 2003).

3.2. Chemical analysis

The chemical profile of the EOALF is presented in Table 1 and Fig. 1. Ten constituents were identified representing 98.6% of the whole essential oil composition. Oxygen-containing monoterpenes (87.2%) were found to be the main group of components, followed by monoterpene hydrocarbons (11.1%) and oxygen-containing sesquiterpenes (0.35%). Perilla aldehyde was identified as the main component present in the essential oil accounting for 85.6% of the total composition. The obtained results are in agreement with previous analysis, which likewise highlights the predominance of oxygen-containing monoterpenes, with perilla aldehyde as the most abundant component by a percentage of 87.0% (El-Haci et al., 2014) and 84.43% (Abu Zarga et al., 2013). This result is different from the one obtained by Louail et al. (2016), Khaldi et al. (2017) and Dahmane et al. (2017), maybe due to the fact that the characterized essential oils were extracted from the seeds and not from the fruits. These authors report a percentage of Perilla aldehyde of

Table 1
Chemical composition (relative%) of the essential oil isolated from *A. leucotrichus*.

		CP-Wax 52 CB column		Rxi®-5Sil MS column		%[c]
	Components	LRI[a]	LRI[b]	LRI[a]	LRI[b]	
1	α-Pinene	1011	1008	928	939	2.2 ± 0.1
2	Camphene	1047	1050	943	954	0.20 ± 0.01
3	β-Pinene	1101	1113	971	979	0.37 ± 0.02
4	3-Carene	1144	1147	1005	1011	0.36 ± 0.02
5	Myrcene	1161	1160	986	989	0.11 ± 0.03
6	Limonene	1176	1198	1023	1029	7.8 ± 0.4
7	Perilla aldehyde	1738	1793	1289	1272	85.6 ± 0.4
8	Methyl perillate	1831	–	1488	1394	0.92 ± 0.02
9	Perilla alcohol	1966	2002	1297	1295	0.67 ± 0.01
10	Spathulenol	2025	2085	1591	1578	0.35 ± 0.03
	% Identified compounds					98.6
	Monoterpene hydrocarbons					11.1
	Oxygen-containing monoterpenes					87.2
	Sesquiterpene hydrocarbons					–
	Oxygen-containing sesquiterpenes					0.35

[a] ExperimentalLinear Retention Indices (LRI).
[b] Literature LRI (Adams, 2001; Babushok et al., 2011; Abu Zarga et al., 2013).
[c] Normalised peak area abundances without using correction factors.

59.12% (Bechar), 81.62% (Bechar), 37.5% (Djelfa) and 60.1% (Illlizi). Other studies carried out on the same species in Morocco revealed different rates of Perilla aldehyde, which are of the order of 68.88% (Alaoui et al., 2014), and 73.5% (Manssouri et al., 2016). Another study described by Velasco-Negueruela et al. (2006) recorded a percentage of Perilla aldehyde of about 63.3% for a plant harvested in Western Saharan, North Africa.

3.3. Bioactive properties

3.3.1. Antioxidant activity

The antioxidant power of the essential oils is presented in Table 2. From the obtained results, it is possible to observe that the EOALF showed antioxidant potential according to the two used assays, revealing an EC_{50} of 27.82 ± 1.81 mg/mL in the DPPH assay and an EC_{50} of 4.55 ± 0.08 mg/mL in the reducing power assay. The EC_{50} obtained for the DPPH assay is weaker than the ones obtained by Dahmane et al. (2017), which was in the order of 9.36 mg/mL (Djelfa) and 6.50 mg/mL (Illizi) for the same species. This disagreement may be due to the difference in the harvest season, causing modifications in the chemical composition, such as in the Perilla aldehyde content that was less than 60%. Tian et al. (2014) revealed an IC_{50} of 2.8764 ± 0.4851 mg/mL for the essential oils of *Perilla frutescens* harvested in Nanning (China) where the level of Perillaldehyde was about 53.41%.

3.3.2. Antibacterial activity

The obtained results for the antibacterial activity (MIC/MBC) of the EOALF are presented in Table 2. The EOALF revealed activity against the three tested strains (*Staphylococcus aureus*, *Eschericia coli* and *Pseudomonas aeruginosa*), being *S. aureus* the most susceptible bacteria with MIC/MBC values of 1.25/10 mg/mL, and *P. aeruginosa*, the most resistant one, presenting MIC/MBC values of 5/10 mg/mL. The MIC value obtained for E. *coli* (2.5 mg/mL) is similar to those obtained by other authors that recorded MIC values of 3.68 mg/mL for *A. leucotrichus* harvested at Bechar (Algeria) (Louail et al., 2016), and 1.5 mg/mL for the ones harvested at Djelfa and Illizi (Algeria) (Dahmane et al., 2017), but higher than the ones obtained by other authors that report a MIC value of 1 μg/mL for *A. leucotrichus* harvested at Bechar (Algeria) (El-Haci et al., 2014), and 1–2 μg/mL for that harvested in Morocco (Alaoui et al., 2014).

Comparing with the results obtained here, Dahmane et al. (2017) found that *S. aureus* was highly sensitive to EOALF by presenting MIC values of 0.046 mg/mL (Djelfa) and 0.375 mg/mL (Illizi), as well as El-Haci et al. (2014) and Alaoui et al. (2014), reporting MIC values of 1.25 mg/mL, and 1–2 μg/mL (El Galta), respectively.

Regarding the EOALF activity against *P. aeruginosa*, the present results are slightly lower (5 mg/mL) than the ones obtained by Dahmane et al. (2017) from Illizi (Algeria) against *P. aeruginosa* (3 mg/mL).

Concerning the antimicrobial effect, it has been described that monoterpenes act largely against microorganisms by compromising the integrity and function of the cell membrane, thus, the effect of the tested essential oil can be related with its lytic action on the plasma membrane (Greay and Hammer, 2015).

3.3.3. Anti-inflammatory activity

The anti-inflammatory effect of the EOALF was assessed upon stimulation of RAW 264.7 macrophages with lipopolysaccharide for the production of the inflammatory mediator (NO). The essential oil showed a strong anti-inflammatory activity compared to dexamethasone (positive control) with an IC_{50} = 11.70 μg/mL. The ability of the studied essential oil to inhibit the secretion of cytokines in RAW 264.7 cells may be due to the high content in Perilla Aldehyde and

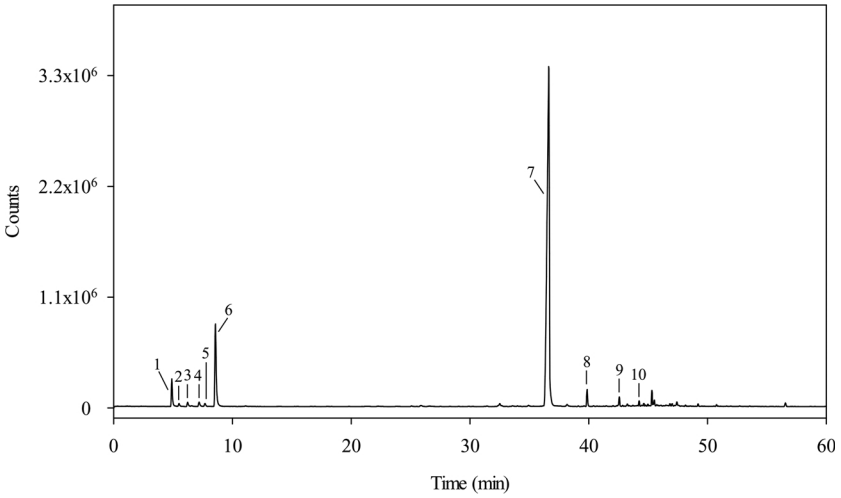

Fig. 1. GC–MS chromatogram of the essential oil isolated from *A. leucotrichus*.
1- α-Pinene, 2- Camphene, 3- β-Pinene, 4- 3-Carene, 5- Myrcene, 6- Limonene, 7- Perilla aldehyde, 8- Methyl perillate, 9- Perilla alcohol, 10- Spathulenol.

Table 2
Bioactivity of EOALF and of the developed cosmeceutical formulations in the different times.

Bioactivities.	*A. leucotrichus* essential oil	Cosmeceutical formulations									
		0 days		7 days		14 days		28 days		43 days	
		Cream	Cream with EOALF	Cream	Cream with EOALF	Cream	Cream with EOALF	Cream	Cream with EOALF	Cream	Cream with EOALF
Antioxidant activity (EC_{50} value, mg/mL)											
DPPH radical-scavenging activity	27.82 ± 1.81[d]	> 50	42.86 ± 1.74[c]	> 100	44.84 ± 1.27[c]	> 100	85,43 ± 1.15[b]	> 100	87.78 ± 1.31[a]	> 100	> 100
Reducing power	4.55 ± 0.08[e]	> 50	10.05 ± 0.77[d]	> 100	11.35 ± 0.19[c]	> 100	25.95 ± 0.13[b]	> 100	79.66 ± 1.31[a]	> 100	> 100
Antibacterial activity (MIC/MBC value, mg/mL)											
Staphylococcus aureus ATCC 19213 (Gram +)	1.25/10	> 200	25/200	> 200	50/200	> 200	50/200	> 200	100/200	> 200	> 200
Escherichia coli ATCC 10536 (Gram-)	2.5/10	> 200	50/200	> 200	100/200	> 200	100/200	> 200	200/ > 200	> 200	> 200
Pseudomonas aeruginosa ATCC 9027 (Gram-)	5/10	> 200	50/200	> 200	50/200	> 200	50/200	> 200	100/200	> 200	> 200
Anti-inflammatory activity (GI_{50} value, µg/mL)	11,70 ± 0,73[e]	> 400	140.4 ± 8.44[d]	> 400	198 ± 7.32[c]	> 400	292.5 ± 12.92[b]	> 400	325.6 ± 10.55[a]	> 400	> 400

Dexamethasone was used as positive control for anti-inflammatory activity (EC50 = 0.016 mg/mL). Trolox was used as positive control for antioxidant activity (EC_{50} = 0.041 mg/mL for reducing power and 0.042 mg/mL for DPPH scavenging activity. Kanamycin was used as positive control for the antibacterial activity (MIC/MBC = 0.0312/0.0625 mg/mL).

Limonene. The anti-inflammatory activity exhibited by plant matrices have been strongly correlated with the presence of Perilla Aldehyde and Limonene (Miguel, 2010). For instance, *Fortunella japonica* var. *margarita* which have high levels of limonene (61.58%) proved capacity to reduce the lipopolysaccharide (LPS)-induced secretion of nitric oxide (NO) in RAW 264.7 cells, demonstrating anti-inflammatory effects (Yang et al., 2010). Lin et al. (2016) have studied some species of *Perilla frutescens* that contain Perilla aldehyde with a varied rate of 54.35% to 93.19% and reported strong anti-inflammatory capacity for those plants, establishing a relationship between the content in Perilla aldehyde and the anti-inflammatory capacity.

3.4. Incorporation in a base-cosmetic

The results from the bioactivity of the prepared cosmetic formulations are presented in Table 2. The samples enriched with *A. leuctorichus* essential oil preserved the bioactive properties of the oil along 28 days under storage, even a decreasing pattern was registered. However, for all the tested assays (antioxidant, antibacterial and anti-inflamatory activities), it was possible to observe a decrease of activity right after incorporation of the EOALF (the active concentrations were higher than the ones obtained for the essential oil in its free form). The observed increase in the EC_{50}/GI_{50}/MIC values might be explained by the interference of the base cream that may limit the availability of the essential oil, and thus affect the ability to exert the bioactivity. After 43 days of storage the cosmetic formulation did not show any of the evaluated bioactivities, which can be associated with the degradation of the bioactive compounds present in the essential oil. Nevertheless, during 28 days the essential oil was able to exert bioactivity, meaning that it might be considered as an ingredient to act as a preservative in cosmetic formulations. To the authors best knowledge, there are no reports on the use of the essential oil of *A. leuctorichus* in cosmetic formulations.

4. Conclusion

A. leucotrichus plant was harvested in the Algerian Sahara Desert, region known for the characteristic climate. This plant showed a yield in essential oil higher than 2.5%, being Perilla aldehyde the major compound, accounting for 85% of its composition. EOALF revealed antioxidant, antibacterial and also anti-inflammatory activity, being these bioactivities related to the presence of Perilla aldehyde and limonene (the second major compound present in EOALF).

Considering the interest of the cosmetic industry for natural bioactive ingredients, tests concerning the evaluation of EOALF behavior when incorporated in a base cream have been performed. From the obtained results, it was possible to conclude that the bioactivity of the EOALF was evidenced during 28 days, even a decreasing pattern was observed with time. This was perceived right after the incorporation, pointing out possible interferences with the base cream matrix. In this context, the need to provide stabilization, and long-lasting effect was identified, which can be achieved through microencapsulation of the EOALF. Even so, the individual bioactive properties of EOALF pointed out the interest to exploit this essential oil as a natural cosmeceutical ingredient.

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