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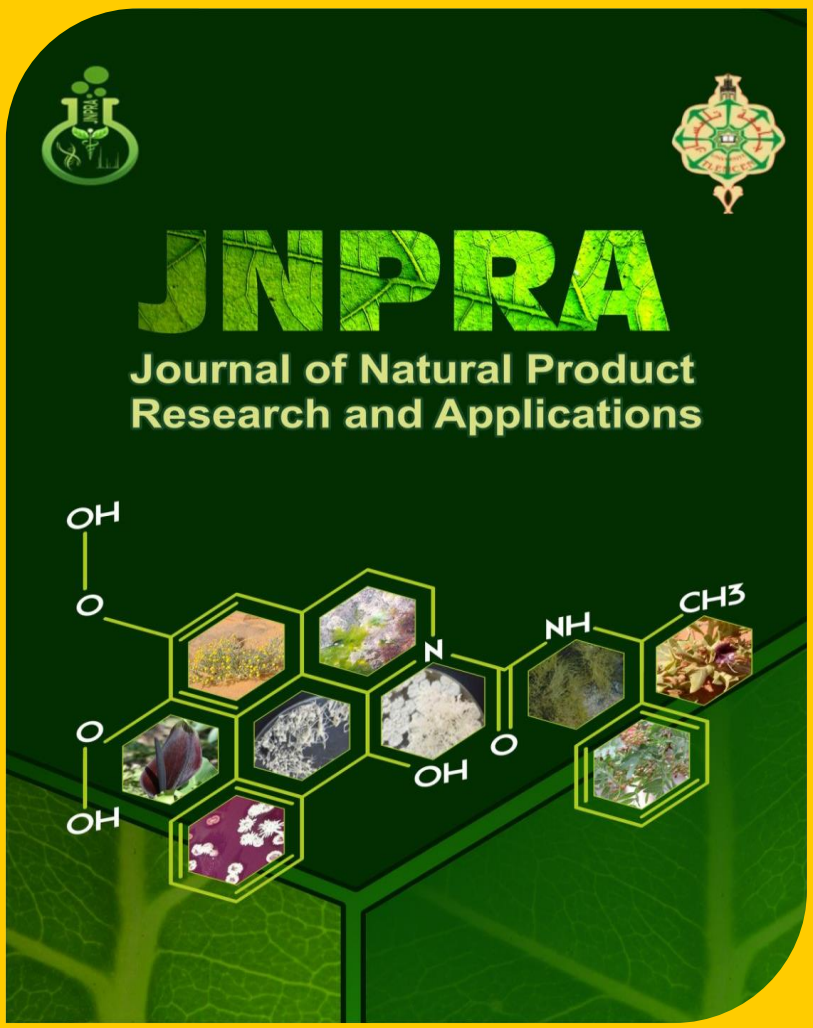
Journal of Natural Product Research and Applications
Volume 1, Issue 2



Journal of Natural Product Research and Applications (JNPRA)

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Research Article

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Effect of *Ammodaucus leucotrichus* Coss. & Dur. Essential Oil on the Viability of Erythrocytes and its Antiradical Activity Assessment

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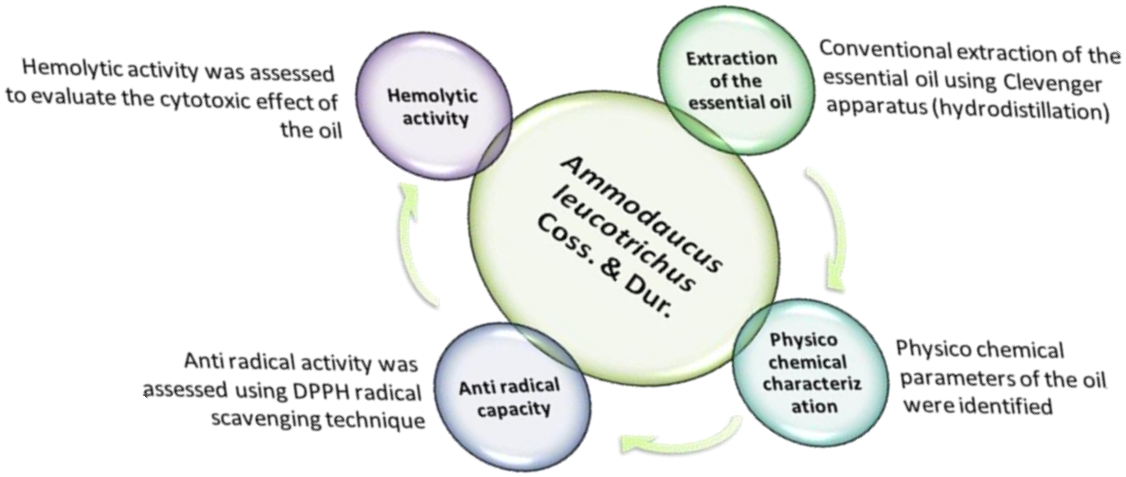
Highlights

- *A. leucotrichus* oil was extracted by hydrodistillation and characterized.
- The oil presented a weak hemolytic activity.
- The results obtained demonstrated the safety of use of *A. leucotrichus* oil.

Graphical Abstract



Abstract
Plants for medicinal purposes are considered as the main source of health care for the majority of the world population. In order to promote medicinal plants in Algeria, the present work aimed to assess the physico-chemical characterization, hemolytic, and antioxidant activities of *Ammodaucus leucotrichus* essential oil of an endemic plant from south-west of Algeria. The plant was harvested from the region of Bechar (south-west of Algeria). The oil was obtained by hydrodistillation method using Clevenger apparatus. The antioxidant activity of the oil was carried out using DPPH scavenging assay, and its effect on erythrocyte cells was evaluated by measuring the hemolytic degree. The obtained results showed that this oil presented a low antioxidant activity compared to positive control (ascorbic acid). The hemolytic activity was very low since the oil diluted to 1/10, while it was low in the pure state. This fact proves the safety of *A. leucotrichus* oil, which can explain its use without risk by the indigenous population.

Keywords: *Ammodaucus leucotrichus*; hydrodistillation; essential oil; medicinal plant; antioxidant activity; hemolytic activity.

1. Introduction

Renewed attention in recent decades has been paid to alternative medicines and natural therapies. This fact has stimulated a new wave of research interest in traditional practices (Dalar et al., 2012). Indeed, the plant kingdom has become a target for the search and the study of new drugs and bioactive compounds (Bouaziz et al., 2009; Hossain et al., 2012).

Composed of a variety of volatile, lipophilic secondary metabolites, essential oils are characterized by a strong and generally pleasant flavor (Turek and Stintzing, 2012). Essential oils have been widely used for their pharmacological activities such as: antibacterial (Burt, 2004; Adorjan and Buchbauer, 2010; Idm'hand et al., 2020; Barao Paixao and de Carvalho, 2021), antifungal (Tampieri et al., 2005; Salem et al., 2016), antiviral (Hussein et al., 2000; Gomes et al., 2013), antiparasitic (Pillai et al., 2012; Gainza et al., 2015), insecticidal, antispasmodic and many other applications (Bakkali et al., 2008; Essid et al., 2015; Sebaaly et al., 2016). Today, essential oils are being used in pharmaceutical, sanitary, cosmetic, agricultural, and food industries (Pavlović et al., 2012; Dawidowicz et al., 2016; Fillatre et al., 2016).

Ammodaucus leucotrichus Coss. & Dur. is a small annual plant, 10-12 cm high, glabrous with erect, finely striated stems. The leaves are finely dissected and slightly fleshy. The flowers are grouped in umbels of 2 to 4 branches with 5 free petals. The fruit is a diachene, 6-10 mm, long and is covered with dense silky white hairs. The plant has a strong smell of anise. It usually flowers in early spring (February to April). The plant is common in the Algerian Sahara; its presence is also mentioned in the Canary Islands (Quezel and Santa, 1963; Benhouhou, 2005).

A. leucotrichus is used for the treatment of many diseases in folk medicine (table 1).

In our previous work carried out on *A. leucotrichus* essential oil from the fruit part, perillaldehyde and limonene were identified as main compounds (El Haci et al., 2014). This oil has demonstrated a very interesting antimicrobial effect against several strains of bacteria, and fungi. The most sensitive bacterial strains were: *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Bacillus cerius* (El Haci et al., 2014). In addition, an interesting antifungal activity was observed against *Candida albicans*. A higher level of activity was

demonstrated against filamentous fungi; *Fusarium oxysporum*, *Aspergillus flavus*, and *Aspergillus fumigatus*.
However, physico-chemical and several other biological proprieties of the oil have not been assessed. Hence, the present study aims to conduct some physico-chemical characterizations and to assess antioxdant power and hemolytic activity of *A. leucotrichus* oil.

Table 1. Uses of *A. leucotrichus* (Coss. & Dur.) in traditional medicine (Benhouhou, 2005; Hammiche and Maiza, 2006).

	Arabic denomination	**Part used**	**Traditional uses**
Ammodaucus leucotrichus Coss. & Dur. (Apiaceée)	Arabic: kammun es-sofi, el messoufa	Leaves	Are used for chest complaints. In the Tassili (South of Algeria), it is mainly used as a powder or an infusion to treat the symptoms aforementioned. In the Djanet area (In Tassili), leaves are used to aromatise tea, and its powder is a much appreciated spice for food.
		Fruits	Are used either as a powder or in a decoction to treat gastric-intestinal pain and indigestion. It is also frequently used, as an infusion, for diverse infantile diseases of the digestive apparatus: dysentery, nausea, regurgitation, vomiting
		Seeds	Are used to treat diseases related to the digestive apparatus and to ease stomach and liver pain. It can be used by mixing them with milk or millet

2. Materials and Methods

2.1. Plant material and essential oil isolation

The whole plant was harvested in the province of Beni-Abes (west-Southern of Algeria-Region of Bechar). A voucher specimen was deposited in the herbarium of the Laboratory of Natural Products (University of Tlemcen) under the reference A-1918. Dried fruits of *A. leucotrichus* (150 g) were hydrodistilled for 180 min using Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate, and stored in amber vials at +4°C. The extractions were performed in triplicate.

2.2. Physico-chemical characterization

Some physico-chemical parameters (density, refractive index, optical rotation, solubility in ethanol, acid value, and ester value) were identified by conventional methods (AFNOR, 1992).

2.3. Antioxidant activity

DPPH scavenging activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging effect was evaluated according to the method used by Bouaziz et al. (2009). Briefly, different concentrations of *A. leucotrichus* oil in ethanol (50 µL) were added to 1.95 mL of a 6×10^{-5} M of DPPH. After 30 min of incubation at room temperature, the absorbance was read against a blank at 515 nm.
The absorbance (A) of the control and samples was measured, and the DPPH scavenging activity (SA), in percentage, was determined as follow:
$SA\% = [(A_{control} - A_{sample})/ A_{control}] \times 100$

Oil concentration providing 50% inhibition (IC_{50}, expressed in µg/mL) was calculated from the graph plotting inhibition percentage against oil concentration. Ascorbic acid was used as positive control and all tests were carried out in triplicate.

2.4. Hemolytic activity

Hemolytic activity of *A. leucotrichus* oil on human erythrocytes was measured by a modified method according to Lee et al. (2002). Freshly collected human blood samples were immediately mixed with an anticoagulant to prevent blood coagulation.

To obtain a pure suspension of erythrocytes, the blood sample was washed three times in 9 volumes of sterile 0.9% NaCl solution. After each washing, cells were pelleted by centrifugation at 72×g for 15 min at +4°C.

The supernatant was then removed by gentle aspiration. Erythrocytes were, finally, resuspended in phosphate buffer saline (PBS) (pH 7.4, 100 mM).

The erythrocyte suspension was incubated at 37 °C with two concentrations of the oil (pure, and diluted to 1/10) under continuous agitation for 90 min. Samples of 500 µL from the reaction solution were taken at regular intervals (15 min), to which, 2 mL of cold washing solution (150 mM NaCl, 2 mM $MgCl_2$) was added. After centrifugation at 4000 rpm for 5 min, absorbance was measured at 548 nm. Control samples of 0% lysis (in PBS) and 100% lysis (in triton-X100) were employed in all experiments.

The hemolysis percentage was calculated by the equation:

$$\text{Hemolysis (\%)} = [(A - A_0)/(A_{100} - A_0)] \times 100$$

Where: A, A_0, and A_{100} were sample absorbance, control with PBS and control with triton X-100, respectively.

3. Results and discussion

3.1. Physico-chemical characterization

The extraction yield of the oil obtained from *A. leucotrichus* fruit parts was of the order of 1.5 ± 0.02%, which is a fairly important yield. Previous studies reported higher extraction yield of this oil; 2.15 %, 2.58 %, 2.76 % and 3 % reported by Khaldi et al. (2017), Halla et al. (2018), Velasco-Negueruela et al. (2006) and Abu Zarga et al. (2013), respectively. These differences can be related to several factors: geographical origin, climate, nature of the soil, and edaphic conditions.

Table 2 reports the results obtained for physico-chemical parameters of the oil.

Table 2. Physico-chemical parameters of *A. leucotrichus* essential oil.

Physico-chemical parameters	Essential oil
Color	Blue
Density	0.957
Optical rotation at 20°	-120
Refractive index	1.502
Solubility in ethanol	2v:1v
Acid value	2.244
Ester value	56.1

The physico-chemical characterization was performed on a fresh sample. In the study of Khaldi et al. (2017), different values of physico-chemical parameters of *A. leucotrichus* oil

were reported which were similar to those founded in our study, and this in respect to density, refractive index, and miscibility in ethanol parameters. For chemical indices (acid and ester), important difference was found. This fact can be explained by the difference of the origin of the species, as well as by the method of extraction, and the conservation of the oil.

3.2. Antioxidant activity

Figure 1 reports the antioxidant activity of *A. leucotrichus* oil.

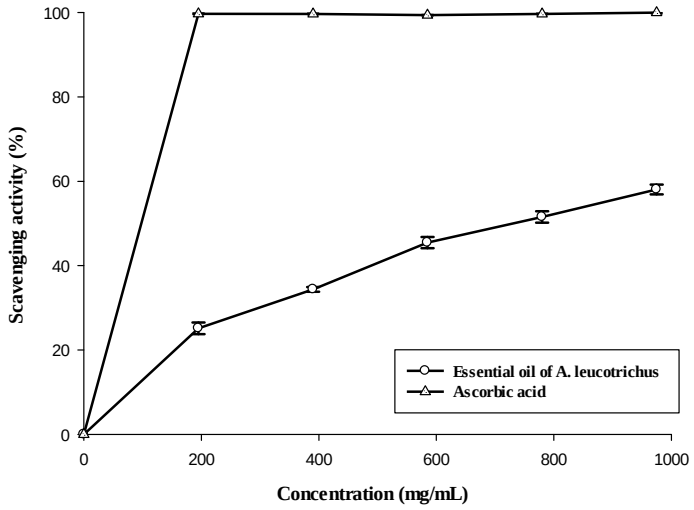


Figure 1. The antioxidant activity of *A. leucotrichus* essential oil.

A. leucotrichus oil was tested for its scavenging effects on DPPH radical and its activity was compared to ascorbic acid used as positive control. From these results, it is demonstrated that the oil tested showed a low antioxidant activity. This fact was confirmed by some studies carried out on this oil (Idm'hand et al., 2020; Manssouri et al., 2020).

The model for scavenging stable DPPH free radicals can be used to evaluate the antioxidant activity in a relatively short time. The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through a donation of hydrogen to form the stable DPPH-H molecule (Conforti et al., 2009). The effect of antioxidants on DPPH radical-scavenging was thought to be due to their hydrogen donating ability.

This result can be explained by the absence of substances-donors of electrons or protons in the oil. The study of the chemical composition of this oil revealed the presence of perillaldehyde as a major compound (87 %) followed by limonene (8.2 %) (El Haci et al., 2014; Sadaoui et al., 2018). α-pinene, β-pinene, myrcene, p-cymene, cuminaldehyde, perillyl alcohol, methyl perillate, γ–decalactone, and δ-3-carene, were also identified. According to this composition, no substances with antioxidant activity are present, such as: terpenoids (thymol, carvacrol…), and phenylpropanoids (eugenol, guaiacol …) (Saleh et al., 2010; Amorati et al., 2013).

3.3. Hemolytic activity

Hemolytic activity was analyzed against human erythrocytes with triton-X100 as positive control. Hemolytic activity after 90 min of treatment of blood with *A. leucotrichus* essential oil was shown in Figure 2.

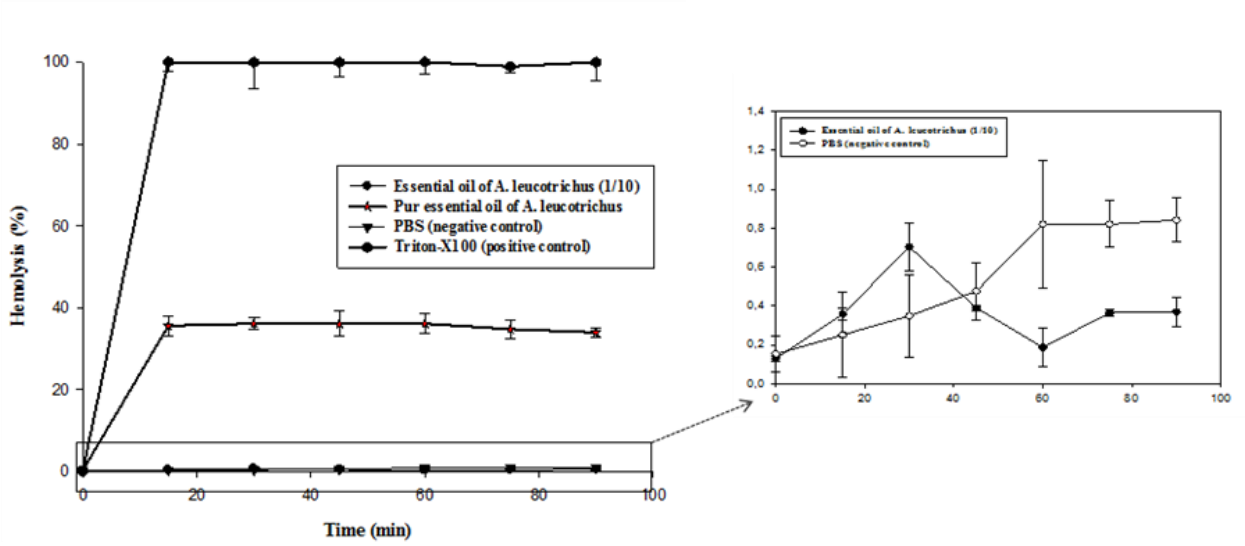


Figure 2. Kinetic of hemolysis at 90 min caused by *A. leucotrichus* essential oil.

A. leucotrichus essential oil showed hemolytic effect (39 %), but very less than triton-X100. The last one expressed a very high activity (100 %) since the first contact with erythrocytes.
On the other hand, when oil diluted at 1/10, no hemolytic effect was registered, and this fact during the 90 minutes of the kinetic.
The stability of the membrane of human red blood cells is an indicator to evaluate, *in vitro*, the cytotoxic effect of various compounds (Sharma and Sharma, 2001). Cells exposure to a cytotoxic agent can cause various health problems. The cells can loss the integrity of the membrane then they undergo cell death (Riaz et al., 2012). Erythrocytes represent an appropriate model for a first evaluation of the cytotoxicity of an extract or a substance. The release of hemoglobin following the hemolysis of red blood cells can be measured spectrophotometrically, which constitutes an important advantage of an experimental point of view.

4. Conclusion

The present study on the oil of *A. leucotrichus* fruits is considered as a continuity of precedent works on this oil. Indeed, the produced promising antimicrobial activity pushed us to investigate other biological activities such as the antioxidant activity. The last one has not been remarkable given the constitution of the oil. The traditional uses of *A. leucotrichus* made us thought to investigate also its cytotoxic activity. A very low cytotoxic effect has been exercised by this oil when this last is diluted. This suggests that the use of this plant does not present a danger to human health.

Acknowledgment

The authors are grateful for the support of the laboratory of research on Natural Products LAPRONA- University of Tlemcen-Algeria, and the General Directorate of Scientific Research and Technological Development DGRSDT- Higher Education and Scientific Research Ministry- Algeria.

Author Contribution Statement

All authors contributed equally in this article (idea, experimentation, writing, editing, and formatting the final manuscript).

Conflict of interest
The authors declare that they have no competing interests.

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