Gas chromatography coupled to mass spectrometry characterization, anti-inflammatory effect, wound-healing potential, and hair growth-promoting activity of Algerian *Carthamus caeruleus* L (Asteraceae)

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**Abstract:**

**OBJECTIVES:** The roots of *Carthamus caeruleus* have been used by the population of Northern Algeria to treat several pathological conditions, including wound healing and hair growth. The present study was conducted to evaluate the anti-inflammatory activity, wound-healing potential, and hair growth-promoting activity attributed to *C. caeruleus* root.

**MATERIALS AND METHODS:** In this study, we have investigated the anti-inflammatory effect using carrageenan-induced paw edema test, evaluated the wound-healing potential by linear incision wound model, and evaluated hair growth activity using *in vivo* hair growth-promoting test attributed to *C. caeruleus* root. Preliminary phytochemical screening and gas chromatography coupled to mass spectrometry (GC/MS) characterization were also performed.

**RESULTS:** It was found that the methanolic extract of *C. caeruleus* was characterized by the presence of tannins, flavonoids, anthocyanins, leucoanthocyanins, sennosides, free quinones, saponins, glycosides, mucilage, and coumarins. The GC/MS analysis could identify 22 compounds and showed that the major chemical constituents were palmitic acid (12.88%), mono(2-ethylhexyl) phthalate (12.75%), and 5-(hydroxymethyl)-2-furancarboxaldehyde, (9.19%). The phytoextract strongly inhibited (*P* < 0.001) paw edema formation in mice. The roots of *C. caeruleus* also showed a significant (*P* < 0.05) wound-healing and hair growth-promoting effects.

**CONCLUSION:** The results indicate the richness of the roots of the Algerian *C. caeruleus* in biomolecules. These molecules exhibit an excellent reducing inflammation activity, a wound-healing property, and an interesting hair-promoting growth activity. All in all, the findings promote the usage of the Algerian *C. caeruleus* as an effective and a safe potential skincare alternative remedy.

**Keywords:** Biomolecules, *Carthamus caeruleus*, gas chromatography coupled to mass spectrometry, skincare

**Introduction**

Fighting diseases has been an important aspect of human beings interactions with natural environment. In this fight, the scientific interest has been diverted toward the natural compounds as they are biocompatible, safe, and cost-effective as well. Plants have been forever a major ally in the healing process. Treatment with medicinal plants is considered very safe as
Inflammation is a complex biological response of vascular tissues to invasion by an infectious agent, antigen challenge, physical, chemical, or traumatic damage. A striking feature of allopathic medications is the debilitating and lethal consequences of adverse drug reactions (ADRs), which rank as one of the top 10 causes of death and illness in the developed world, claiming 100,000–218,000 lives in the United States annually. Currently used anti-inflammatory drugs are associated with some severe side effects. Common adverse effects such as gastric lesions caused by nonsteroidal anti-inflammatory drugs (NSAIDs) and tolerance and dependence induced by opiates have been registered; as a result, the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases. Thus, the development and the investigation of potent anti-inflammatory and analgesic drugs with fewer or lacking side effects are necessary; these could substitute NSAIDs and opiates. Several studies have reported an anti-inflammatory effect and an antitumor activity of Carthamus sp.

Wound is a rupture in the epithelial integrity of the skin which resulted from violence or trauma and may be followed by disruption of the structure and function of underlying normal tissue. Each year, millions of people experience burns, suffer from chronic wounds, or have acute wounds that become complicated by infection, dehiscence, or problematic scarring. Therefore, more efforts toward the development of new lead compounds for effective and evidence-based wound-healing strategies are needed. Recent investigations have shown that plant-derived secondary compounds can serve as new lead compounds for the improvement of wound healing. Polyphenols are used nonspecifically against wound infection and stimulate keratinocyte proliferation, and oligo-polysaccharides induce proliferation of keratinocytes.

The term androgenetic alopecia frequently used to express the patterned loss of scalp hair in genetically vulnerable men and women. To address this problem, the markets of cosmetics and pharmaceuticals for the hair regrowth and protection from hair loss have been markedly growing for a few decades. In dermatology, Carthamus tinctorius has many beneficial effects against skin problems such as erythematous, psoriasis, and other dermatitis.

The Carthamus caeruleus species growing in the north of Algeria is commonly used in traditional medicine by healers and local population, because of their therapeutic benefits to treat skin problems and burns. The aims of this study were to evaluate the anti-inflammatory, wound-healing, and hair growth-promoting activities of the roots of C. caeruleus variety.

Materials and Methods

Harvesting and preparing the plant powder
The harvesting of the plant was conducted in March and April 2014, under hot and dry weather after sunrise, to avoid wetting of the plant. Samples of roots, free of insects and molluscs, were retained. Location of sampling region was in Draâ El Mian (a municipality in the Wilaya of Tizi Ouzou in Algeria, located 42 km southwest of Tizi Ouzou and 110 km southeast of Algiers).

Preparation of extract
The roots of C. caeruleus are dried in open air and at obscure for a week at room temperature. The plant material is crushed into a clean mortar and then finely ground using an electric grinder. The obtained plant powder (20 g) was macerated in 100 ml of methanol (95%) for 3 days and then proceeded to a purification of the extract from residues. After filtration, the alcoholic layer was evaporated under reduced pressure by a rotary evaporator at 70°C. The obtained filtrate was stored at 4°C until use.

Phytochemical screening
A set of colorimetric methods were carried out on either the plant powder or the infusion, and this allows the detection of secondary metabolites. We searched mainly anthocyanins, leucoanthocyanins, total tannins, iridoids, gallic tannins, catechin tannins, alkaloids, flavonoids, saponosids, senosids, quinones, coumarins, and mucilages. Methods in this study were conducted as described by Yadav and Agarwala.

Gas chromatography coupled to mass spectrometry
The analysis of the methanolic extract was performed on a Hewlett-Packard gas chromatography coupled to mass spectrometry (GC/MS) system (GC 6890, 5973 MSD). A capillary column HP-5 MS (30 m, 0.25 mm, and film thickness 0.25 lm) is directly coupled to the mass spectrometer. The carrier gas used was helium (1 ml/min). The temperature program used was 3 min isothermal at 60°C, then 270°C at a rate of 5°C/min, and then held for 10 min isothermal. The injector temperature was 250°C. The ionization of sample components was performed by electron ionization mode (70 eV).
The compounds are detected in order of elution and identified by their mass spectrum and retention time. Reading the chromatographic profiles is provided by a computer system managing a mass spectra library: NIST002 (National Institute of Standards and Technology).

**Animals**

Male NMRI mice (20–25 g) and Wistar rats (130–160 g) provided by Pasteur Institute of Algeria were left for 3 days for adaptation period into laboratory conditions and maintained on standard pellet diet and water. For the evaluation of pharmacological activities, the food was withdrawn on the day before the experiment, but free access to water was allowed. Six animals were used in each group. All experiments complied with the Algerian Legislation (Law Number 12-235/2012) inherent to the protection of animals designed to experimental and other scientific purposes as well as with the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA) and were specifically approved by the latter (AASEA authorization number 45/DGLPAG/DVA/SDA/14).

**Anti-inflammatory activity**

In this test, the used mice were divided into three groups (six mice per group). The different groups were treated intraperitoneal with methanolic extract of *C. caeruleus* (2.5 ml/kg), diclofenac (2.5 ml/kg), and vehicle control (0.9% NaCl). The administration of extract and drugs was 1 h before injection of 0.025 ml of 1% freshly prepared suspension of carrageenan (in 0.9% NaCl) in the right hind paw subplantar of each mouse. The paw volume was measured 4 h later, the animals were killed by cervical dislocation, and the right and left paw of each animal were removed. The left paw was considered as a control. Circular sections were taken with a cork borer (diameter of 7 mm) and weighed. The edematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as a percentage of the edema reduction in treated mice compared to the control group.[11]

The anti-inflammatory effect of methanolic extract of *C. caeruleus* was calculated by the following equation:

\[
\text{Anti-inflammatory activity (\%)} = (1 - \frac{D}{C}) \times 100,
\]

where *D* represents the percentage difference in paw volume after the administration of drugs to the mouse and *C* represents the percentage difference of volume in the control groups.[12]

**Wound-healing activity**

**Linear incision wound model**

Animals were anesthetized with 0.05 mL xylazine (2% Alfazine®) and 0.15 mL ketamine (10% Ketasol®) and the back hair of the rats were shaved and cleaned with 70% alcohol. Two 1-cm length linear-paravertebral incisions were created with a sterile blade at a distance of 0.5 cm from the dorsal midline on each side. The test ointments, the reference drug (Madecassol®), and the ointment base were topically applied on the dorsal wounds in each group of animals once daily throughout 9 days.[13]

**Percent wound contraction**

The wound contraction of individual animal from control and treatment groups was measured in each and healing rate was expressed as percentage contraction:[14]

\[
\text{Percent wound contraction} = \frac{\text{healed area}}{\text{total area}} \times 100
\]

**In vivo hair growth-promoting test**

The animals were divided into two randomized groups (*n* = 6) to study the hair growth-promoting activity of the *C. caeruleus* root. All animals were shaved by clipping to observe the anagen phase hair follicle development. Daily treatment was applied for 9 days.[15]

**Statistical analysis**

Pharmacological data were presented as mean ± standard error (SD). Student’s *t*-test was used to assess the differences between groups using *P* < 0.05 as the level of statistical difference.

**Results**

**Phytochemical screening**

The results show that *C. caeruleus* is rich in tannins, flavonoids, anthocyanins, leucoanthocyanins, senosides, free quinones, saponins, glycosides, mucilage, and coumarins [Table 1].

**Table 1: Phytochemical screening of Carthamus caeruleus roots**

| Tests                | Abondance |
|----------------------|-----------|
| Total tannins        | +++       |
| Catechin tannins     | -         |
| Gallic tannins       | ++        |
| Flavonoids           | +++       |
| Anthocyanins         | +++       |
| Leucoanthocyanins    | +++       |
| Senosids             | +++       |
| Amidon               | +         |
| Quinones fibers      | +++       |
| Saponosides          | +++       |
| Alcaloïdes           | -         |
| Glucosides           | +++       |
| Mucilages            | +++       |
| Iridoids             | -         |
| Coumarins            | +++       |

*=Absence, +=Presence in low concentration, ++=Presence, +++=Presence in high concentration*
Chromatographic analysis of *Carthamus caeruleus* roots extract
Figure 1 represents the GC-MS chromatogram of *C. caeruleus* roots extract, which showed a set of peaks indicating the presence of the phytochemical compounds.

The active principles of the roots with their retention time and peak area in percentage are presented in Table 2.

Anti-inflammatory activity
Percentage increase in paw volume was significantly reduced in treated mice after 4 h pretreatment with *C. caeruleus* extract compared to negative controls. As expected for Diclofenac® sodium with its anti-inflammatory effect, an important diminution in percentage increase in paw volume induced by carrageenan was observed. The anti-inflammatory activity of methanolic extract of *C. caeruleus* was very important following postinjection showing a greater anti-inflammatory activity similar to the positive controls (Diclofenac®) [Figure 2].

**Wound contraction**
Wound contraction was tested to determine the wound rate reduction during healing. The fast rate of wound closer indicates the better efficacy. The progressive

Table 2: Gas chromatography coupled to mass spectroscopy results of the bioactive compounds identified in the methanolic extract of *Carthamus caeruleus*

| Peak | Retention time | Area (%) | Name of compounds                                    |
|------|----------------|----------|------------------------------------------------------|
| 1    | 4.9            | 0.61     | Furfural                                             |
| 2    | 12.86          | 2.24     | 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one   |
| 3    | 13.63          | 0.56     | 7-octenoic acid                                     |
| 4    | 15.07          | 9.16     | 5-HMF                                               |
| 5    | 20.62          | 0.42     | 1-pentadecene                                       |
| 6    | 22.8           | 2.54     | Caryophylen oxide                                   |
| 7    | 23.76          | 1.64     | Caryophylla-3(15),7(14)-dien-6-ol                   |
| 8    | 24.03          | 1.12     | 13-tetradec-11-yn-1-ol                              |
| 9    | 24.13          | 8.94     | 8-methylene-3-oxatriocyclo[5.2.0.0(2,4)]nonane      |
| 10   | 24.17          | 0.97     | E,Z-2,13-octadecadien-1-ol                          |
| 11   | 24.58          | 1.01     | 1-octadecene                                        |
| 12   | 25.93          | 0.44     | Tetradecanoic acid                                  |
| 13   | 28.7           | 1.41     | Methyl isohexadecanoate                             |
| 14   | 29.35          | 12.88    | n-hexadecanoic acid (palmitic acid)                 |
| 15   | 31.41          | 0.9      | 9,12-octadecadienoic acid, methylester, (E, E)-     |
| 16   | 32.08          | 2        | 9,12-octadecadienoic acid (Z, Z)-                   |
| 17   | 32.17          | 1.38     | Octadec-9-enio acid                                 |
| 18   | 32.51          | 1.65     | 2-ethylhexyl trans-4-methoxycinamate                |
| 19   | 37.44          | 4.24     | Hexadecanoicacid, 2-hydroxy-1-(hydroxymethyl) ethyl ester |
| 20   | 37.66          | 12.75    | 1,2-Benzenedicarboxylic acid, mono (2 ethylhexyl) ester or MEHP |
| 21   | 44.02          | 2.18     | Gamma-sitosterol                                     |

MEHP=Mono (2-ethylhexyl) phthalate, 5-HMF=5-hydroxymethyl, 2-furancarboxaldehyde
reduction in wound area of different groups of treated animals over 9 days for the negative control, the ointment of C. caeruleus, and Madicasol® is presented in Figure 3. The fastest and complete (98.33% ± 4%) wound contraction healing was observed in animals treated with the ointment of C. caeruleus, compared to Madicasol® (86.66% ± 15.05%) and the negative control (81.66% ± 15.05%).

**Effect of Carthamus caeruleus ointment on anagen induction**

Wistar rats were used to investigate the effect of C. caeruleus ointment on anagen phase induction. The shaved skin of telogen phase rats is pink and whiteness with the initiation of anagen. The day after the animals were shaved using a clipper, the dorsal skin of these rats was treated with C. caeruleus ointment or negative control daily for 9 days. Visual scoring of the hair growth-promoting effect of Carthamus caeruleus ointments by using the scoring guideline shown in Table 3. C. caeruleus ointment-treated groups exhibited a significant hair growth (4.83 ± 0.98) compared to negative control (0.67 ± 0.81) [Figure 4].

**Discussion**

The phytochemical screening of C. caeruleus showed an important wealth in tannins, flavonoids, anthocyanins, leucoanthocyanins, sennosides, saponins, glycosides, mucilage, and coumarins. The existing literature reports that these chemical components present several pharmacological activities.\[16\]

GC/MS analysis plays a key role in the analysis of components of plant origin. Generally, the plant materials are highly complexes, which make GC/MS well suited for their analysis because of its high sensitivity and selectivity. The main compounds are n-hexadecanoic acid (palmitic acid), mono (2-ethylhexyl) phthalate (MEHP), 5-(hydroxymethyl)-2-furancarboxaldehyde (5-HMF), and 8-methylene-3-oxatricyclo[5.2.0.0(2,4)]nonane. To the best of our knowledge, this is the first time the roots in this species were analyzed by GC/MS. The GC/MS analysis of C. caeruleus showed the presence of MEHP the biomolecule with multiple biological activities such as antifungal, antiretroviral, antitumor, antidiabetic, antioxidant, anti-inflammatory, and antimicrobial.\[2\] A number of other studies have found additional therapeutic properties including anti-inflammatory.\[17\]

Methanolic extracts of C. caeruleus roots produced a significant inhibition of carrageenan-induced paw edema. The inhibition was more important than that of the standard drug Diclofenac® sodium. There are several mediators or multiprocesses underlining the pathogenesis of inflammatory.\[18\] Inhibition of any of these mediators may bring down the inflammatory process.\[3\] In a preliminary phytochemical screening of our methanolic extract of C. caeruleus roots, we have found several compounds among flavonoids which are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception.\[19\]

Another major biomolecule of C. caeruleus root, palmitic acid, might function as an anti-inflammatory agent as it had shown a significant inhibitory activity in the enzyme kinetic study of phospholipase A.\[20\] Pharmacological studies on the components showed that 5-HMF had good biological activities, such as anti-inflammatory activity and bacteriostatic action.\[21\] Moreover, the caryophyllene oxide shows analgesic and anti-inflammatory activities.\[22\]

Wound healing is an interactive process involving mediators, cells, and extracellular matrix components and has three successive stages; inflammation, tissue formation, and tissue remodeling that overlap in time.
Traditional wound-healing remedies are expected to contribute the wound-healing process by acting in one or more of these stages.[1] Medicinal plant-based ointments used in folk medicine are reported to have beneficial effects in wound care and healing. The wound-healing activity of *C. caeruleus* ointments from the underground part of this medicinal plant was evaluated comparatively.

According to the experimental results, *C. caeruleus* possessed better wound-healing activity compared to Madecasol® and the control groups. Several factors could be involved in the enhancement of the wound-healing process. The presence of the antioxidant, anti-inflammatory, and antimicrobials properties may be among the factors that contributed to the potential wound-healing effect of *C. caeruleus* root. Moreover, interesting *in vitro* pharmacological activities of *C. caeruleus* have been reported such as antimicrobial and antioxidant activities.[23]

Pharmacological tests showed that the lipid concentration from the medicinal plant had a healing activity.[24] Furthermore, the main compound of the roots of *C. caeruleus* is palmitic acid; this molecule induces the wound healing by reduction of oxidation and inflammation.

In recent years, many herbal topical formulations have been marketed worldwide to prevent hair loss or promote hair growth. Hair loss is not a serious disorder but often causes mental stress and affects both men and women, especially at the age of 50 years and up.[9] The results of *in vivo* hair growth tests strongly suggest that *C. caeruleus* promoted induction of the anagen phase in the dorsal skin of rats. The results of the chromatographic analysis show that *C. caeruleus* contains MEHP. This biomolecule significantly induced mRNA expression of prostaglandin-endoperoxide synthase 2, an enzyme important for synthesis of prostaglandins with PGE2 mediated vasodilation and increased microvascular permeability.[25] The *C. caeruleus* ointments probably improve hair growth activity by increasing the distribution of the sebaceous glands, capillaries around the hair follicles, and vasodilation to assist in forming the necessary environment for hair growth, thereby promoting hair growth by stimulating hair follicles. These findings may lead to the development of new treatments for hair loss patients. The results of the present investigation also complement the ethnobotanical usage of the studied plant which possesses several phytoconstituents with multiple biological activities.

Based on the present investigation, it is concluded that *C. caeruleus* L. is a potential source of bioactive compounds with great pharmaceutical value. These results confirm that *C. caeruleus* is a rich medicinal plant with many active molecules responsible for the anti-inflammatory, wound-healing, and hair growth properties observed by the local population of the north of Algeria. The study can be extended for the purification and isolation, *in vitro* and *in vivo* evaluation of bioactive compounds of novel drug discovery programs.

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**Conflicts of interest**

There are no conflicts of interest.

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