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Ethnobotanical study, phytochemical characterization and healing effect of *Carthamus coeruleus* L. rhizomes

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Abstract: Objective of this work is ethnobotanical, phytochemical and healing effect of *Carthamus coeruleus* (Asteraceae) rhizomes a medicinal plant in the region of Baghlia (Algeria),.The result of ethnobotanical study shows that from 74, 98% of respondents were able to identify 66.66% women aged above 44 years who use this plant frequently for healing and therapy of different degrees' burns. We conducted a characterization study of rhizomes through a phytochemical screening reveals the presence of alkaloids, leucoanthocyanins, flavonoids, coumarins, saponins and absence of anthocyanins, tannins, and iridoids. To study the healing effect, we chose to formulate healing cream-based Total rhizomes of *Cartamus coeruleus* with participation of qualified person in this area. This cream was applied to wistar rats in which wounds and burns were caused by incision and burn of 2^{nd} degree, respectively. Healing and cell regeneration of the burnt skin and the wound closure caused, had resulted in the reduction of area. Monitor these areas over time has allowed us evaluation of decreasing diameter for 15 days with a percentage reduction of 85.66% in rats treated with our cream rhizomes *Carthamus coeruleus*, this being higher than that found in the rats treated with ointments and reference whose value is of 75.12%. The histopathological study has confirmed the effectiveness of the cream prepared by the regeneration of scar tissue.

Keywords: *Carthamus coeruleus*; ethnobotanical study; phytochemical characterization; healing effect; histopathological study.

Introduction

One of the most skin trauma response that represent a real public health problem still burns, which are the cause of 24,000 deaths per year in the European regions according to WHO (2006). Phenomenon is even more acute in socalled emerging countries such as Algeria, or burns are responsible for 37% of deaths among children. The long-term physical squeal, the most annoying of a burn are, without doubt, hypertrophic scars that cause major disfigurement, causing an imbalance psychological accordingly (Gal and Jeanguiot, 2010). Care of wounds is an art as old as the art of medicine, but still since antiquity as injuries especially burns can be treated with herbal saw the countenance of the latter as a bioactive component reacting in the heart mechanism of scar (Debuigne, 1984). From herbs having effect the healing process we note Lanneakerstingii roots and Aloe vera (Choi et al. 2001) and Carthamus caeruleus subject of this study.

This is an uncommon species that can be found in the land of lean Provence and Corsica. It prefers dry, sunny places in the Mediterranean. It is native to Southwest Asia (Milouane, 2004), is widespread in East and North Africa (Algeria, Morocco, Tunisia, Libya), Australia and the Americas, and Europe (Greece, Italy, France, Portugal, Spain) (Boullard, 2001). *Carthamus caeruleus* rhizomes are used in Algeria as a traditional cream, which contributes to the disappearance of scars generated by burning. Objective of our work is the botanical plant phytochemical characterization and the study of the healing effect of its rhizomes.

Materials and methods

Plant material

Our study focused on the rhizomes of the plant *Carthamus coeruleus* (Figure 1), harvested Baghlia (Boumerdes). The species identification was done at the Department of Botany of

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National School of Agronomy (ENSA) in Algiers.

According Quezel and Santa (1963), the species *Carthamus caeruleus* is classified as follows:

- Kingdom: Plantae
- Family: Asteraceae (Compositea)

- Sub-family: Carduoideae
- Tribe: Cardueae
- Sub-tribe: Centaureinae
- Gender: Carthamus
- Species: Carthamus caeruleus



Figure 1: *Carthamus coeruleus* rhizomes, a. tige feuillée; b. fleur non épanouie; c. fleur épanouie; d. rhizomes avec racines.

Ethnobotanical study

A survey was sent to 24 people living in Baghlia. It contains questions relating to *Carthamus caeruleus* as part of the plant used method of preparation and therapeutic uses. Les résultats obtenus portent sur les aspects suivants : Profil de personnes enquêtées (age, sexe, niveau d'étude) ; Plantes médicinales utilisées dans la région d'étude et les informations relatives à notre plante.(description générale de la plante, usage traditionnelle ,durée de traitement, sous quelle forme, les recettes de préparation, période de récolte, la partie utilisée)

Harvest of the plant

The harvest of crop plant was made when the plant is mature and during all seasons.

Phytochemical screening

These tests are performed either on the dried rhizome powder either infused or decocted following method (Konkon et al., 2006).

Preparation of Cartamus coeruleus traditional cream

Fresh rhizomes of *Carthamus coeruleus* are used to prepare traditional cream, rhizomes are cleaned, peeled and cut into small pieces and boiled in water for 12 hours. They are then allowed to cool and then filtered, to obtain cream beige ready for use (Figure 2).

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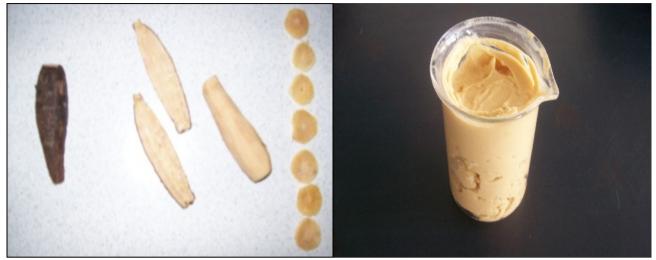


Figure 2: Traditional cream rhizomes *Carthamus coeruleus* (original).

Activity assessment healing cream traditional Carthamus caereulus

The evaluation criteria of the healing activity of the rhizomes of the plant Mersgousse a second degree burn and incisional wound is conducted by:

- A weight change of the animals used in experimentation;

- Comments macroscopic burns and sores;

- Criteria for assessment of incisional wound healing is done by:

- The calculation of wound area using AutoCAD

-The percentages of reduction in the area of wounds treated with the reference product Madécassol ® (CE1) and those treated with traditional cream Carthamus Caeureulus (CE2).

The percentage reduction is calculated as follows:

% of reduction (test product)

$$= \underline{\mu CE2 \ J_0} - \underline{\mu CE2 \ J_n} \\ \mu CE2 \ J_0$$

% of reduction (reference product)

$$= \underline{\mu CE1 \ J_0} - \underline{\mu CE1 \ J_n} \\ \mu CE1 \ J_0$$

 μ CE1: average area of the wounds treated with the reference product.

 μ CE2: average area of the wounds treated with the test product.

To give statistical significance to our results found through the different evaluation criteria, the data processing is performed by software XL ANOVA STAT version 6.0, which has been used to analysis confidence variance interval of 95%.

Evaluation of the healing activity of the traditional cream of Cartamus caeruleus

24 Wister rats are divided into 04 batches the day, before the experiment the animals were weighed marked and fasted. The next day, the animals were injected intra peritoneal anesthesia with Ketamine after depilation of the lumbar region (Figure 3).

Provocation injury

Case of a wound

Anesthetize rats by intra peritoneal injection of 120 mg of ketamine per kg. Then, two wounds are caused 3cm in diameter, one for testing and one for the witness. The wounds of test1 (E1) and 2 (E2) for 15 days receive an application Madecassol dermal cream and *Cartamus caeruleus* respectively, the wound remains parallel control without any treatment (figure 4).

Macroscopic observation is performed at days 3, 7, J11 and J15.



Figure 3: Animal's preparation. a. Animal's contention; b. Injection of Ketamine; c , d. Depilation of animal; e. Lumbar area bare; f. weight measurement.

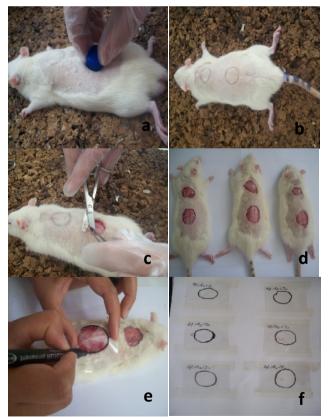


Figure 4: Wound's provocation, a. Areas to be cut; b. Two areas marked on animal's back; c. Skin incision; d. Rats after surgical incision; e.

trace surfaces; f. surfaces plotted on transparent paper.

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Case of a burn

Two burns (test 1 and control 2) are caused lumbar level with a weight in two places (Figure 5). The burns of test 1 and 2 are processed by Biafine cream and *Cartamus caeruleus* respectively (Figure 6). Monitoring the healing is done by macroscopic observation with a body weight gain of animal wholes two days (J0, J3, J5, J9, J11, J13, and J15) for 15 days. The 15th day of histological sections of skin regenerated is made.



Figure 5: Burn's provocation, a. The mass burn lot; b. Both burned areas.

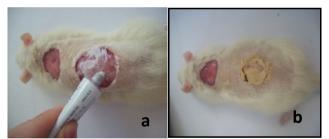


Figure 6: Applying products. a. Applying Madécassol®; b. Application of *Carthamus coeruleus*



Figure 7: Attaching and removing the regenerated skin.

Histological sections

The histological technique (Bensalem, Benjelloul, 1998) used is the inclusion in paraffin (Figure 7). After anesthetized rats,

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regenerated skin is removed (Figure 8), cut into pieces of 0.5 -1cm with a microtome (Figure 9) from 5 to 7 μ m thick and stained with haematoxylin and eosin and the fixed sections using the Ekkit. The observation of stained histological sections and taking pictures are made with a light microscope equipped with a digital camera (Figure 10).



Figure 8: Placing parts in the controller.



Figure 9: Making the microtome cuts.

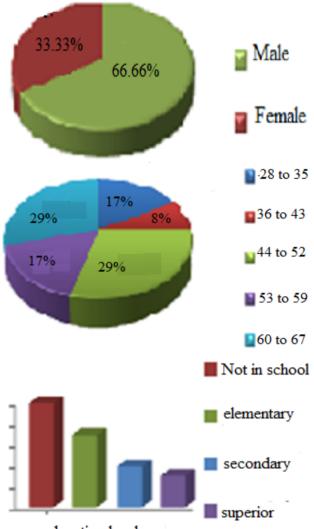


Figure 10: Microscope equipped with a Photonic digital camera.

Results and Discussion

The results of ethnobotanical study of *Cartamus caeruleus* are shown in Figure 11.

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education level Figure 11: Results of ethnobotanical study.

Phytochemical screening

Phytochemical screening performed on the crushed rhizome of *Cartamus caeruleus*, has highlighted the presence of saponins, coumarins, alkaloids, flavonoids and leucoanthocyane and the absence of tannins, iridoides and anthocyanins (Table 1).

Weight evolution In wound case

From Figure 12, we note that there is no distinct difference between the average weight of rats treated with Madecassol (μ PCE1) and those treated with *Cartamus caeruleus* (μ PCE2), weight gain increases with time after

a remarkable decrease of the initial weight at day J3. In addition, their daily food intake remained normal during the follow-up period.

 Table 1: Phytochemical screening results.

Substances	Observations	Results
Saponosids	Persistent foam	+++
Coumarins	yellow fluorescence	+++
Alcaloïds	Precipitated with flocation	+++
Iridoids	No black precipitate	-
Leucoanthocyan	Red color	+++
Anthocyan	No staining	-
Tanins	No staining	-
Flavonoïds	Red color	+++

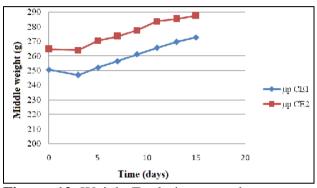
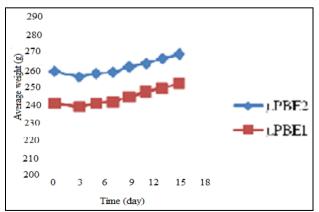
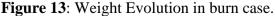


Figure 12: Weight Evolution wound case.

In burn case

From Figure 13, we see that the average body weight of rats treated with Biafine (μ PBE1) and those treated with *Cartamus caeruleus* cream (μ PBE1) decrease day after the provocation J3 burns performed at day 0, and then progressing normally from J5. Weight of the animals decreased J3days following the provocation of burns and wounds. This decrease is followed by a gradual increase incorporeal weight J5 during healing.





Macroscopic observations of the skin

a. Evolution of areas wounds treated / untreated by Madécassol: The results of the development of areas of test and control wounds1forover15 days are shown in Figure 14.

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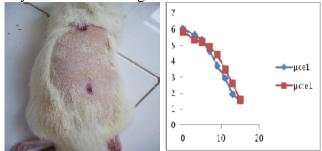


Figure 14: Evolution of in areas wounds treated/ untreated by Madécassol.

b. Evolution of wounds treated area / untreated by *Cartamus caeruleus:* The results of the development of areas of control wounds and wounds treated with *Cartamus caeruleus* cream are shown in Figure 15.

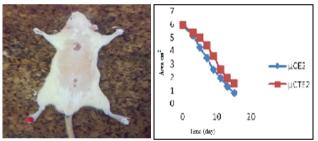


Figure 15: Evolution of areas wounds treated / untreated by *Cartamus coeruleus*.

c. Study of percentage reduction of wounds area: Percentage reduction of wounds surfaces treated with the reference Madecassol cream and *Cartamus coeruleus* are increased on Table 2.

	JO	J3	J5	J 7	J9	J11	J13	J15
% RS CE1	0	6,91	11,53	23,56	38,88	51,65	68,04	75,12
% RS CE2	0	13,32	27,99	41,65	56,49	66,78	77,9	85,66

The results concerning the percentage reduction in wound area to test E1and E2 test showed that rats treated with the cream of *Cartamus coeruleus* mark a best percentage reduction (85.66%) compared to those treated with reference cream (75.12%) (Figure 16).

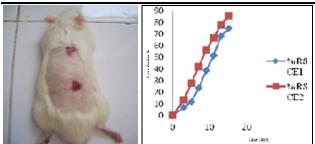


Figure 16: Percentage reduction of wounds area.

Microscopic examination of skin

Figure 17 (a, b, c, d) represents the histological scar tissue wounds burned and treated with cream *Cartamus caeruleus*. (a) corresponds to the general view, we see the different layers reconstitution: the epidermis, the dermis not completely reformed the hypodermis (b) corresponds to the highest Magnification; sebaceous follicle. (c and d) present a detail view of the dermis layer responsible for healing.

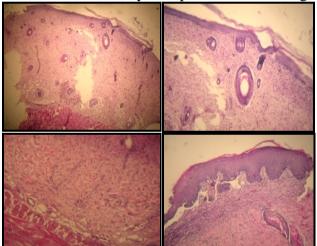


Figure 17: Histological scar tissue wounds burned and treated with cream *Cartamus coeruleus*.

In histological scar tissue wounds of burned rats treated with Biafine, there is a complete healing, the different layers are reconstructed: the epidermis with its layers, the dermis containing more nuclei than fibers and substances, activity of the hair follicle is apparent (Figure 18).

In histological scar tissue wounds burned untreated rats; there was a total absence of the layer of the epidermis and the dermis and the presence of the hypodermis. You can see the scar tissue and injured cells of the hypodermis (adipocyte) that appear round and transparent (Figure 19).

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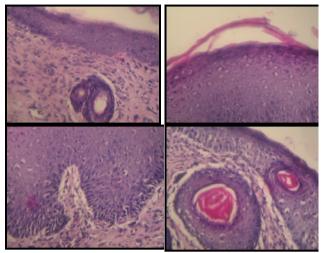


Figure 18: Histological scar tissue wounds of burned rats treated with Biafine.

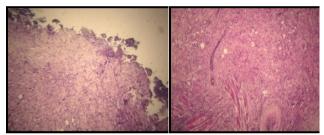


Figure 19: Histological scar tissue wounds burned untreated rats.

Conclusion

Although plant biomass is a promising therapeutic source, very few studies have focused on the chemical analysis of Carthamus caeruleus (Baghiani et al., 2010) and its healing effect has been no investigation. The ethno botany's studies of Cartamus caeruleus; showed that the majority of respondents (74, 98%) were age greater than 44 years and among them 66.66% are women. They use the roots of this plant for healing burns of varying degrees for the local population. The phytochemical study revealed the presence in the rhizomes Cartamus Coeruleus: flavonoids, alkaloids, saponins, coumarins and leucoanthocyanes and absence of anthocyanins, tannins and iridoides. Experiences healing the skin of animals burned, or having undergone an incision, by application of Cartamus Coeruleus cream, after macroscopic observation showed that there is an almost complete healing after 15 day; in the case of

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wounds, the percentage reduction in area is 85.66% which is significantly higher than that of animals treated with Madécassol (75.12%). This result inferred that the cream of this plant has a healing effect more powerful than pharmaceutical ointment. The results of healing by regeneration of epithelial tissue were confirmed by histological study. In view of continuing this work, it would be interesting to test the therapeutic formulations in order to make an application in the therapy of burning and regeneration.

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