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Healing effects of aqueous extract of leaves of *Petrosolinum crispum* (Apiaceae) on induce wounds in rats

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ABSTRACT: *Petrosolinum crispum* (Apiaceae) is a plant commonly used in traditional African medicine for improving the health of populations including wound care; the aim of this study was to evaluate the healing properties of *Petrosolinum crispum* (Apiaceae) leaves on second degree incision wounds in Wistar rats. This study was carried out on 28 rats divided into 7 batches of 4 rats, one of which was an untreated control and the other 6 received daily applications of test substances. The results showed that the wounds treated with 500 mg/ml of the aqueous extract of leaves of *Petrosolinum crispum* (EAPc) healed first. At this concentration, the healing time is the same as that of rats treated with Flammazine, 16 days against. For the batches treated with Betadine and EAPc at 1000 mg/ml, the wounds had completely healed in 26 days. However, those of the untreated rats had healed 95% by the 28th day. This effect of the extract is due to the bioactive substances present within the plant. These results therefore suggest that this extract has healing activity.

KEYWORDS: *Petrosolinum crispum,* incision wounds, cicatrization, rats.

1 INTRODUCTION

The skin is a complex organ covering the entire body and ensuring several functions necessary for the survival of the organism such as protection against external physical, chemical and biological attacks [1], [2]. These attacks cause wounds. Skin wound healing is a coordinated dynamic process that involves cell multiplication, active cell migration and extracellular matrix production [3], [4]. Research undertaken for the therapeutic management of wounds has greatly improved their prognosis [5]. However, the use of many "conventional" products is often limited because of their variable effectiveness, their possible adverse effects, their unavailability. The solution to these many disadvantages is the use of natural products. These products from traditional medicine have proven their effectiveness for the treatment of wounds and have advantages from the point of view of availability, harmlessness and low cost For the treatment of wounds, several medicinal plants are used [6]. Among these plants is parsley (*Petrosolinum crispum*).

Petrosolinum crispum (Apiaceae) is a herbaceous plant, commonly used in cooking. Traditionally, it is used for menstrual disorders, galactagogue, diabetes, edema and hematoma [7], [8]. *Petrosolinum crispum* is also used in the treatment of intestinal cramps, diarrhea, against lice [9] and for its anticancer, analgesic, spamolytic activity, beneficial effects on diueresis and for wounds [10], [11]. For the treatment of wounds, many medicinal plants are used, however very few scientific studies have been carried out [6]. It is in this context that the present work takes place, the general objective of which was to evaluate the healing properties of an aqueous extract of leaves of *Petrosolinum Crispum* (Apiaceae) on wounds induced in Wistar rats.

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2 MATERIAL AND METHODS

2.1 MATERIAL

2.1.1 VEGETAL MATERIAL

Petroselinum crispum leaves were used. They were collected in the commune of Adjamé (Abidjan Ivory Coast). This plant was identified at the Center National de Floristique (CNF) of the Félix Houphouët-Boigny University of Cocody (Abidjan, Ivory Coast) in comparison with the herbarium UCJ 0190.

2.1.2 ANIMAL MATERIAL

Mice (male and female) of the species Mus Musculus (Muridae) of Swiss strain, with a weight of 17 to 28 g, were used for the toxicity test. Rats of the species Ratus Norvegecus (Muridae) of the Wistar albino strain with a body weight of between 190 and 220 g were used in the healing study. These animals came from the Vivarium animal facility, located at the ENS (Ecole Normale Supérieure). They had free access to food and water. They were fed standard pellets manufactured by IVOGRAIN. The room had received lighting and darkness on a cycle of (12/12h) with a temperature of 28° ± 3°C. The experimental protocols were followed in accordance with the protocols for the protection of experimental animals of the European Council on Legislation 2012/707.

2.2 METHODS

2.2.1 PREPARATION OF THE AQUEOUS EXTRACT OF *PETROSELINUM CRISPUM* LEAVES

One hundred (100) grams (g) of fresh leaves of *Petroselinum crispum* were ground in a blender and then boiled for 15 min in 1.5 L of distilled water. The decoction obtained was filtered on absorbent cotton then on Whatman No. 2 filter paper. The filtrate collected was dried in an oven (Thermo SCIENTIFIC VT 6060 M, Finland) at 50° C. for 48 hours. After drying, the aqueous extract of *Petroselinum crispum* leaves was in powder form. It has been used for toxicity testing and pharmacological wound healing studies in rats.

2.2.2 PHYTOCHEMICAL SCREENING OF THE AQUEOUS EXTRACT OF *PETROSELINUM CRISPUM* LEAVES

The identification of secondary metabolites (alkaloids, phenolic compounds, terpenoids, sterols, coumarins, flavonoids, tannins and saponosides) consisted of characterization tests of the major groups of chemical compounds contained in the aqueous extract. The detection of these compounds based on the principle that they induce chemical reactions in the presence of appropriate reagents [12].

These tests were carried out using the analytical techniques described in the work of [13] and those of [14].

2.2.3 STUDY OF THE CURATIVE EFFECTS OF THE AQUEOUS EXTRACT OF *PETROSELINUM CRISPUM* LEAVES ON INDUCED WOUNDS IN WISTAR RATS INDUCTION OF WOUNDS

This study was carried out using a skin healing model following the protocol described by [15]. A circular incision 2 cm in diameter was made in the dorso-scapula region of each rat anesthetized with ether. It was important to ensure that the rats were unconscious during wound induction to minimize animal suffering during the experiment [16]. This was achieved by ensuring that the ocular and withdrawal reflexes had disappeared and breathing had weakened and become predominantly abdominal. After making the wounds, these were treated according to the batch to which each rat belonged. The wounds were not protected by a dressing.

2.2.3.1 ANIMAL TREATMENT

Twenty-eight (28) rats were used. These were divided into 7 groups of 4 rats including an untreated control group and the 6 others receiving daily applications of substances for 28 days.

- Group 1: rats with wounds without treatment (NT),
- Groups 2 and 3: rats with wounds treated with reference substances Betadine (TRB) and flammazine (FMZ),
- Groups 4, 5, 6 et 7: wounds treated respectively with the concentrations 100, 250; 500 and 1000 mg/ml of the paste of the aqueous extract of *Petrosolinum crispum* (EAPc)

2.2.3.2 HEALING ASSESSMENT

Measurements of the diameter of the wounds were made every two days using a graduated ruler and then by digital planimetry. The images obtained were processed with the Java ImageJ software [17]. All animals were monitored regularly until complete wound healing. They had access to food and water ad libitum. The percentage (%) of wound retraction was then determined using the following formula [18]:

$$\%\text{Rétraction} = \frac{(\text{initial wound size - Jn wound size}) \text{ X } 100}{\text{initial wound size}}$$

3 STATISTICAL ANALYSIS

The statistical analysis of the values and the representation of the data were carried out using the Graph PadPrism 8 software (San Diego, Carlifonia, USA). The statistical difference between the results was carried out using the Analysis of Variances (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance level $P < 0.05$. All values were presented as the mean ± SEM (Standard Error of the Mean).

4 RESULTS

4.1 PHYTOCHEMICAL STUDY OF THE AQUEOUS EXTRACT OF *PETROSOLINUM CRISPUM* LEAVES

Phytochemical screening carried out using the aqueous extract of *Petrosolinum crispum* leaves (EAPc) revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, saponosides, quinone compounds, alkaloids and catechin tannins. But the absence of gallic tannins was noted (Table 1).

Table 1. . Phytochemical composition of aqueous extract of Petroselinum crispum leaves.

Composés recherchés		*Test ou réactifs*	Résultat
Stérols et polyterpènes		*Liebermann*	+
Polyphénols		*Chlorure ferrique*	+
Flavonoïdes		*Cyanidine*	+
Saponosides		*Agitation vigoureuse*	+
Composés quinoniques		*Borntraeger*	+
Alcaloïdes		*Dragendorff*	+
		Bouchardat	+
Tanins	Catechiques	*Stiasny*	+
	Galliques	*Acide chlorhydrique*	-

(+): Presence of the compound (–): Absence of the compound

4.2 EFFECTS OF AQUEOUS EXTRACT OF *PETROSOLINUM CRISPUM* LEAVES ON WOUND HEALING IN WISTAR RATS

4.2.1 EFFECTS OF AQUEOUS EXTRACT OF *PETROSOLINUM CRISPUM* LEAVES ON HEMOSTASIS IN WISTAR RATS

Bleeding from the wounds of animals treated with the extract and with Flammazine ceased after 1 min 45 s for the longest time. While untreated wounds and those treated with Betadine saw their hemorrhage time reach 3 min for the maximum time, i.e. double that of animals treated with the extract.

4.2.2 MACROSCOPIC APPEARANCE

The appearance of crusts was observed in the batches treated with the extract after the 1st day, on the other hand in the untreated and Betadine reference batches, the observation was made from the 3rd day. Signs of inflammation (redness and oedema) were observed on all wounds 24 hours after induction. These signs had regressed and disappeared after 2 days in the rats treated separately with the plant extract and Flammazine (reference substance) against 3 days with Betadine (reference substance) and 5 days for the animals not having received treatment. The buds had appeared earlier and more numerous on the wounds treated with EAPc than those treated with the reference substances (Betadine and Flammazine). These buds had appeared after 4 days in the animals of the batches treated with the extract versus 6 days in those treated with the reference substances (Fig 1).

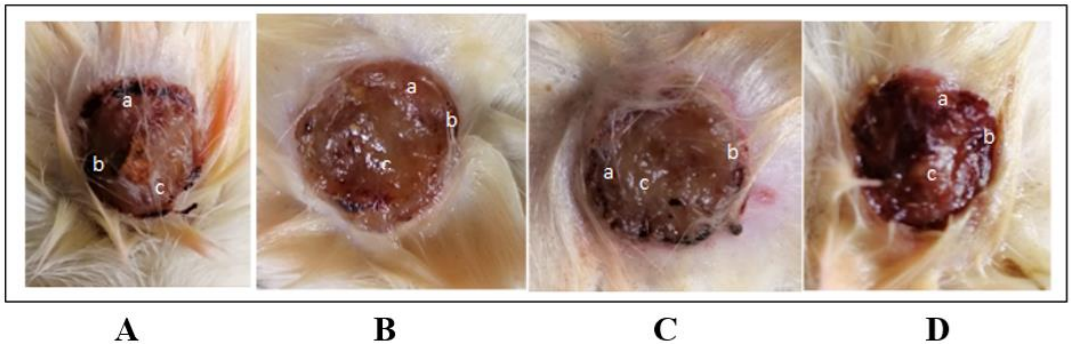

Fig. 1. General appearance of incisions in rats after 4 days of treatment.

a: edema b: dermis c: exudate
A: Photograph of incision of untreated rats
B: Photograph of incision of rats treated with Betadine
C: Photograph of incision of rats treated with Flamazine
D: Photograph of incision of rats treated with aqueous extract of Petrosolinum crispum leaves

4.3 EFFECTS OF AQUEOUS EXTRACT OF *PETROSOLINUM CRISPUM* LEAVES (EAPC) ON THE SURFACE OF INCISIONS IN WISTAR RATS.

A reduction in the mean surface areas of the wounds was observed in the rats of the different batches, but unevenly (Fig 2). The wounds treated with EAPc as well as those treated with Flammazine closed faster than those of animals treated with Betadine and those of untreated animals. Indeed, the initial surface of the wounds for the untreated controls was equal to 2.45 ± 0.05 cm2 against 2.38 ± 0.03; 2.51 ± 0.29; 2.50 ± 0.13 and 2.43 ± 0.03 cm2 in rats treated with EAPc at 100, respectively; 250: 500 and 1000 mg/ml. During the first 3 days, the surface of the wounds of the rats of the different batches did not vary. In contrast, a significant decrease at $P < 0.05$ in wound area was observed on day 14 for wounds treated with EAPc. It had gone to 0.53 ± 0.13 respectively; 0.38 ± 0.03; 0.15 ± 0.15 and 0.95 ± 0.25 cm2 in rats treated with EAPc at 100; 250: 500 and 1000 mg/ml.

In addition, wounds treated with the 500 mg/ml concentration of EAPc healed first. With the application of EAPc at 500 mg/ml, the mean surface area of the wounds which was 1.08 ± 0.53 cm2 corresponding to a contraction percentage of 56.80% on the 6th day of treatment decreased to 0, 15 ± 0.15 cm2 or a contraction of 94% on the 14th day. In untreated rats, the surface of the wounds decreased from the 10th day with an average which went from 2.45 ± 0.05 cm2 to 1.15 ± 0.08 cm2 giving 53.19% contraction of the surface of wounds.

The healing time of the rats treated with aqueous extract of *Petrosolinum crispum* leaves (EAPc) at 500 mg/ml just like that of the rats treated with Flammazine was 16 days against 18 and 20 days for the rats treated with EAPc at 100 and 250 mg/ml. The wounds of the Betadine-treated rats and those treated with EAPc at 1000 mg/ml had completely healed in 26 days. However those of the untreated rats had healed to 95% on the 28th day (Fig 3 and 4).

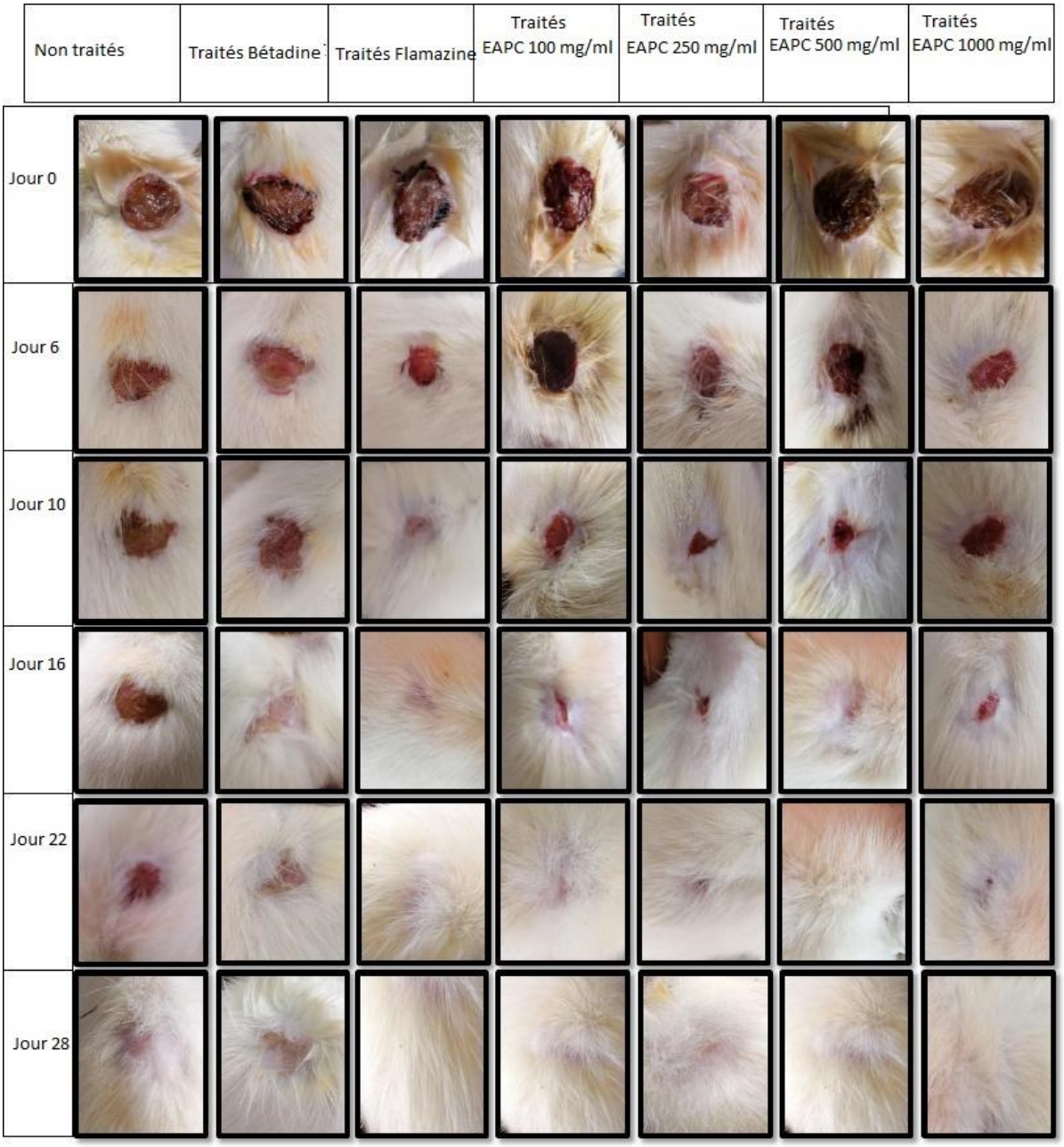

Fig. 2. Evolution of wound surface in rats over 28 days.

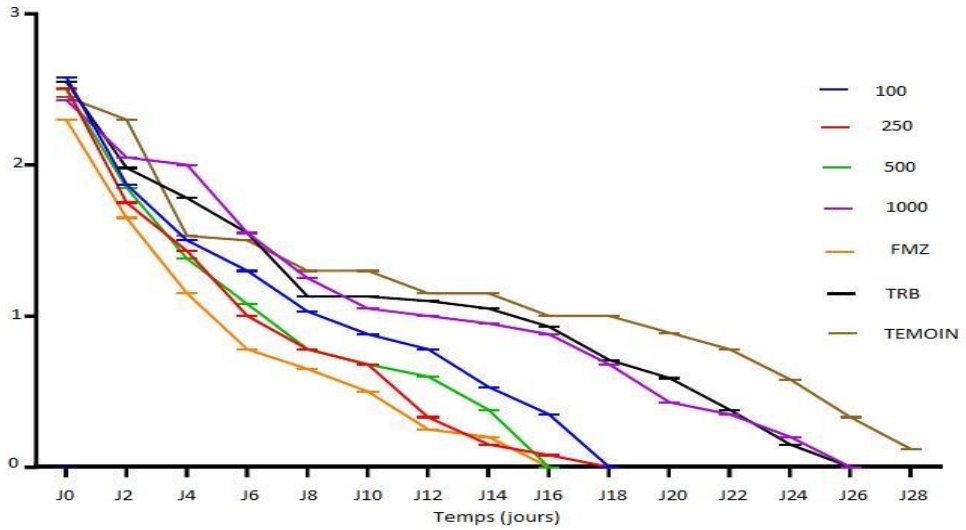

Fig. 3. Evolution of wound surface in rats over 28 days.

***CONTROL**: Untreated **TRB**: treated with Betadine **FMZ**: treated with Flamazine*
***100, 250, 500 and 1000**: treated with the aqueous extract of Petrosolinum crispum leaves (EAPc) at different concentrations (100, 250, 500 and 1000 mg/ml).*

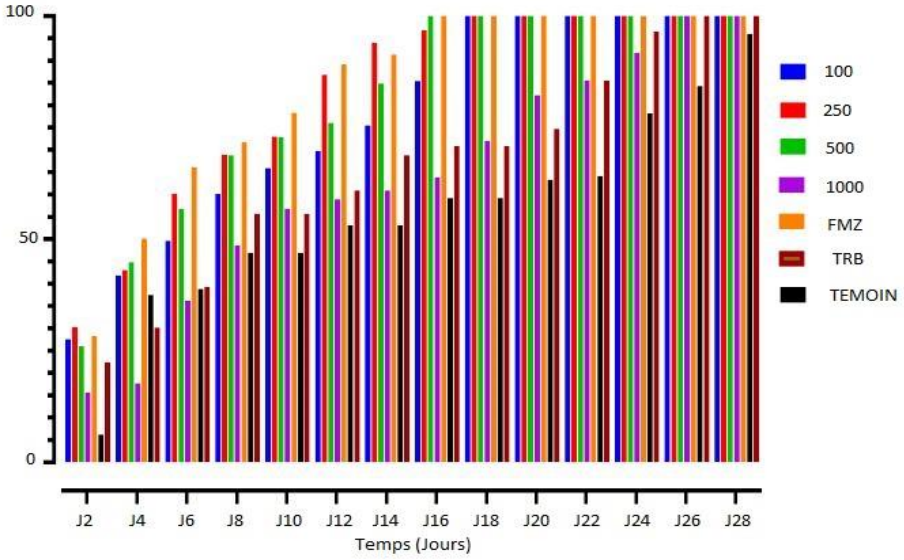

Fig. 4. Percentage of wound contraction in rats as a function of day.

***CONTROL**: Untreated **TRB**: treated with Betadine **FMZ**: treated with Flamazine*
***100, 250, 500 and 1000**: treated with the aqueous extract of Petrosolinum crispum leaves (EAPc) at different concentrations (100, 250, 500 and 1000 mg/ml).*

5 DISCUSSION

The pharmacological study of the aqueous extract of leaves of *Petrosolinum crispum* (EAPc) on the healing of incisional wounds in rats showed that EAPc has healing properties. After incision, the immediate application of the extract to the wounds reduced the duration of bleeding and accelerated the appearance of scabs. This would suggest an action of aqueous extract of leaves of *Petrosolinum crispum* on hemostasis. This action on haemostasis would be attributable to secondary metabolites

revealed by phytochemical screening. [19] reported that tannins are responsible for the haemostatic activity of plant extracts. Tannins, by precipitating plasma proteins, promote the formation of a plug at the level of the vascular breach. This mechanism involving tannins could be borrowed by the aqueous extract of *Petrosolinum crispum* leaves.

According to [20], flavonoids and triterpenoids possess antimicrobial property. Studies carried out by [21] on the aqueous extract of Stevia rebaudiana (Asteraceae) in mice also show that the flavonoids contained in this extract are responsible for its anti-inflammatory effect and that the latter improve vascularization, thus preventing necrosis. wound cells. According to [22], who worked on an extract of Argania Spinosa (Sapotaceae), flavonoids have an anti-inflammatory effect, they activate macrophages which phagocytose foreign bodies. The aqueous extract of Termina Petrosolinum crispum leaves (EAPc) containing flavonoids and triterpenoids could participate in wound cleansing, which reduces the duration of the inflammation phase of wounds treated with this extract.

The rapid appearance of the granules and the number of buds at the level of the surface of the wounds treated with EATs were higher than those of the animals not treated or treated with the reference substances (Betadine and Flammazine). These buds are made up of neo-vascularizations which irrigate the injured area and the fibroblasts. This would improve the supply of nutrients and oxygen necessary for angiogenesis, the proliferation of fibroblasts and keratinocytes in the wound [23]. Studies carried out on the extract of *Verbascum speciosum* (Scrophulariaceae) show that the flavonoids it contains stimulate the proliferation of fibroblasts and the release of growth factors [24]. In this sense, EATs could stimulate the proliferation of fibroblasts, as well as neogenesis thanks to the flavonoids it contains. The appearance of epithelial tissue was faster in wounds treated with EAPc. Epithelialization is due to the migration of keratinocytes from the edges of the wound to reconstitute the damaged epidermis [25].

According to studies carried out on an alcoholic extract of *Kaempferia galanga* (Zingiberaceae), flavonoids stimulate fibroblasts and keratinocytes, thus accelerating wound contraction and the appearance of epithelial tissue [25]. The acceleration of wound closure observed in rats treated with EAts could be due to the flavonoids in this plant extract. From these results obtained, the healing activity of EATs could be due to the combined action of the triterpenoids, flavonoids and tannins it contains.

6 CONCLUSION

The aqueous extract of petrosolinum crispum leaves (EAPc) has been shown to be non-toxic by the oral route. The present study showed that cEAP has healing effects. These effects would be linked to the combined action of the chemical compounds contained in the leaves of petrosolinum crispum. It appears from this study that the plant has a healing activity in accordance with the effectiveness recognized in traditional medicine. Additional research is needed to isolate and identify the molecules responsible for these effects, in order to clarify their mechanism of action and to carry out more in-depth studies, in particular histological studies.

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