



## *In vivo* Evaluation of the Cicatrizing Activity of the Essential Oil of *Tetraclinis articulata*

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### ABSTRACT

*In the present work, we have evaluated the cicatrizing activity of a galenic formulation (dermal dough) based on essential oil, extracted from the leaves of Tetraclinis articulata on deep second-degree experimental burns caused on the skin of Wistar rats. Four groups of rats were formed and each group consisted of ten rats. The first is processed by the dough with the essential oil of T. articulata, the second group processed by the dough without essential oil (placebo), the third group will receive an application of Biafine® will represent the positive control, and the fourth will not receive any treatment and will serve as a negative control. The evolution of scar process is followed by a study planimetry of wounds and complement by histological examination during the entire duration of healing (28 days). According to the results of research the dermal dough to basis of (0.3g) essential oil of Tetraclinis articulata was more effective than the dough witness, their cicatrizing activity effect is intensified in the fourth week by a percentage reduction in the burned area of 97% and the healing phenomenon is accelerated.*

**Keywords:** Tetraclinis articulata, essential oil, skin burns, galenic formulation, cicatrizing activity

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### INTRODUCTION

Natural substances from plants have multiple interests exploited [18] in industry: food, cosmetology and dermopharmacy [5]. Among these compounds we find to a large extent the secondary metabolites which are especially illustrated in therapeutics [2].

A lesion of the skin can thus have harmful consequences for the health, even to present a danger of mortal, in the extreme cases. Indications in traditional medicine and data from previous studies served as a support for our study, whose objective is to produce a galenic formulation based on essential oil of leaves of *T. articulata*, to highlight and measure their activity cicatrizing of thermal burns, caused on the skin of the rat Wistar.

### MATERIAL AND METHODS

#### Plant material and essential oils extraction

A portion (100 g) of the leaves of plants (*Tetraclinis articulata*) has been hydrodistilled for at least three hours, using a device of type Clevenger. To remove all traces of water, the extracted oil was treated with anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>), Filtered and then stored in the dark at 4°C. The yield of essential oils was expressed in ml / 100g of dry matter [14].

According to the AFNOR standard [1], the yield of essential oil (YHE), is defined as the ratio between the mass of the essential oil obtained after extraction (M') and the mass of the plant material used (M) [16].

It is given by the following formula:  $YHE \% = (M'/M) \cdot 100$

#### Galenic formulation of dermal dough

##### Preparation Method

The constituents in powder form are finely pulverized (Bentonite 30g, zinc oxide 25g, sodium tetraborate 5g, salicylic acid 3g, copper sulfate 0.5g, H<sub>2</sub>O 6g, glycerol 14g). They are then mixed in a porcelain mortar in which a solution of formaldehyde is added. Triturate with glycerine and add water in small amounts, then add drop by drop the essential oils of *Tetraclinis articulata* with mixing until a homogeneous mixture is obtained, for having a better consistency of the dermal dough [17].

### Organoleptic and microbiological controls

This control based on the appreciation of odor,color, hydrogen potential (pH) and texture of the dough (consistency or spreadability)[19, 10].

Various microbiological controls and research are carried out on cosmetic products in order to assess their level of hygiene and to predict their behavior with regard to possible contamination according to the Pharmacopoeia had 8.0 (January 2014) [20], the search of microorganisms specified (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and mesophilic totals) and the search for indicators of faecal contamination to detect a failure of hygiene during the process of manufacture (*Escherichia coli*).

A quantity of prepared dermal dough (1 g) diluted in 1 ml of physiological saline and then seeded on petri dishes contains the culture medium specific for each pathogen sought.

The absence of microorganisms shows a good microbiological quality of the galenic formulation.

### Toxicological controls (PI = index of primary irritation of the skin by the dough)

The irritation is an important element in dermopharmacy. In a first time, the flanks of 6 Wistar rats albinos were shorn and then 3 parallel scarring of the epidermis have been practiced at the level of the right flank of each animal. A quantity of product equivalent to 1 g of the dermal dough with essential oil has been applied on the flanks the rights of animals while the left flank, intact, will serve as witnesses. The dermal irritation has been appreciated in the sites of application (scarified and non-scarified) using a numerical evaluation scale after 24h and 72h [7].

The index (PI) will be calculated by the following formula [9]:

$$PI = (\text{Edema} + \text{Erythema})_{\text{flank treaty}} + (\text{Edema} + \text{Erythema})_{\text{control flank}} / 24.$$

By adding, figures obtained for Erythema and Edema at 24 and 72 hours

**With: 24** = numbers of rats (06) x number of areas tested (treaty and control = 2) x number of type of irritation

Depending on the severity and duration of the observed effects, the dermal dough will be classified in:

- Action non-irritating, PI less than 0.5:  $PI < 0.5$
- Action slightly irritating, PI between 0.5 to 2:  $0.5 < PI < 2$
- Action moderately irritating, PI between 2 and 5:  $2 < PI < 5$
- Severely irritating Action, PI between 5 and 8:  $5 < PI < 8$ . [7].

### Wound healing activity:

#### Animals experience:

Experimental animals consist of male adult rats of Wistar strains of body weight between 300 and 320 grams from the petshop of University of Mascara. They received daily a standard composition diet and tap water at will [13].

The animals are distributed in individual cages equipped with a manger. The cages are stored in a standardized environment, with an ambient temperature of 22-32°C and a natural light-dark cycle. The litter used is sawdust, renewed twice a week.

#### Burn induction

The process is carried out according to the technique described by Hamdi Pacha [12].

- The back of rats was shaved with a razor blade, 24h before the beginning of the test.
- At day zero, all the rats were anaesthetized to chloroform by an intramuscular injection (1ml/10kg).
- The area to be burned is anesthetized locally by infiltration with xylocaine and at a dose of 1ml per burn.
- A weight of 200g and 2.3 cm in diameter is maintained for 3 minutes in boiling water (100 ° C). It is immediately dried and deposited on the skin of the rat during 15 seconds without the exercise of force.
- After cooling of the piece of metal, the other burns are performed in the same way.

The standardization of the size of induced lesions makes it possible to compare the process of:

#### Treatment of burns

Immediately after the achievement of burns, the tested products are applied topically, treat them daily, during 04 weeks by an application of the galenic formulation as indicated below:

**Batch n°1:** Treaty by the dough has basis of (03g) of essential oil of *T. Articulata*.

**Batch n°2:** Treaty by a cream of reference (**Biafine®**) (positive control).

**Batch n°3:** Treaty by the dough without essential oil (placebo)

**Batch n°4:** will not receive any treatment (negative control)

Each product is applied in the dorsolumbar region in rats. All products are administered once a day until complete epithelialization (28 days) is observed.

#### Cicatrizing activity:

The evaluation of cicatrizing process is done by a macroscopic study, photography Burns, body weights of rats, planimetry of wounds and histological study of the burned skin during four weeks among the four experimental group.

### Macroscopic study

The macroscopic appearance (appearance, color, odor) and external evolution of the wound are examined and noted daily after the application of healing products throughout the treatment. The depth of the wound, the appearance or not of edema, the presence or absence of bud and the thickness of the crust [4].

### Body weight evolution

The body weights of animals is followed regularly throughout the experience (D01, D07, D14, D21 and D28) using a precision balance (0.01 g).

### Planimetric study

The margins of wounds are plotted on a transparent paper, then the diameter vertical and horizontal each wound is measured each week using a vernier caliper.

The average surface area of wounds is calculated according to the formula:  $R^2 \times \pi$ , where R is the radius (the average of the two diameters of each surface).

The percentage of the contraction of the wound is calculated each week. The study has adopted of morphological parameters, including the surface of the wound, it has measured the percentage of contraction of the wound on (D01, D07, D15, D21 and D28) by using the following formula [21, 22] :

Percentage of the Contraction (%) =  $[\text{Initial wound area} - \text{Healed wound area (D)}] \times 100 / \text{Initial wound area}$ .

The initial surface is 4.15 cm<sup>2</sup>, with a diameter of 2.3 cm.

The photograph of the wounds is taken every week, for the creation of a real picture describing the chronological evolution of the burns at the same time as the progress of the cicatrization.

### Histological study:

The epithelialization period is a histological approach.

Every seven days of healing, one rat is sacrificed for each group. The collected pieces are washed and placed in labeled pill containers to make histological sections at the burned area [6]. The histological examination is carried out according to the standard technique at the laboratory of pathological and cytological anatomy of hospital establishment of Mascara-Algeria. Histological sections of different wounds are read and interpreted. The parameters retained are the presence of inflammatory cells, the epithelialization of the epidermis, the importance of fibrosis, the presence of granulation tissue and the detection of abnormalities. Photomicrographs were performed with an optical microscope equipped with a digital camera [6].

## RESULTS AND DISCUSSION

### Physicochemical characterization of essential oils

The essential oil of *Tetraclinis articulata* obtained by hydrodistillation of pale yellow to strong odor characteristic of Araar close to the original aromas of fresh plants. The physicochemical indices of the essential oils of *Tetraclinis articulata* are reported in Table 01.

**Table 01.** The constants physico-chemical essential oils obtained

Yield of essential oil (%)	Densityat (20°C)	PH	Index of ester	Index of acid
1.09	0.867	6.4	10.6	1.17

The average yield in essential oil, extracted from the dry leaves of *Tetraclinis articulata* (harvested during the period of flowering), is 1.09 %. This yield is higher than that cited by Toumi.F [23].

An index of acid less than two (1.17), is a proof of good conservation of the oil. In effect, a fresh oil contains only very few free acids. It is during the storage period that the oil may suffer degradation such as the hydrolysis of esters [15].

The formulation implementation in the course of this work has allowed the obtaining of a dough dermal beige white color, very homogenous (we see no lumps) and soft. Their odor is strong characteristic of *Tetraclinis articulata* it was t easy to spread. The pH of the dough dermal is equal 6.4 (Figure 01).



**Figure01.** Galenic formulation based on essential oil of *Tetraclinis articulata*.

All the microbiological results obtained during this study allowed us to show a total absence of germs in the Petri dishes (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, total mesophiles and *E. coli*) demonstrates a good microbiological quality of the dermal dough prepare.

### The index of primary irritation dermal (PI)

















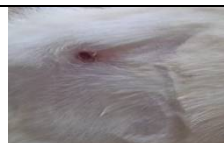



**Table 02.** Results of the index of irritation.

	Scarified skin		Skin not scarified	
	24 h	72h	24h	72h
<b>Erythema</b>	3	1	1	0
<b>Edema</b>	0	0	0	0
<b>Total</b>	4		1	
<b>Total general</b>	5			
<b>PI</b>	<b>0.208</b>			

According to the Table 02, the observed phenomena are only Erythema after 24 hours of the application of the dough on the skin of the Wistar rats (**PI = 0.208**) allows us to conclude that our galenic preparation is not irritating to the skin.

### Cicatrizing activity:

#### Macroscopic Study

Rats	group n°1 Treaty by dough of essential oil	group n°2 : Treaty by the dough without he (Placebo)	group n°3 : Treaty by <i>Biafine</i> ®	group n°4: untreated (Negative control)
<b>Photo of burns</b>	<b>01 day</b>			
				
	<b>07 days</b>			
				
	<b>14 days</b>			
				
	<b>21 days</b>			
				
<b>28 days</b>				
				

**Figure 02.** Visual observation of burns for four weeks

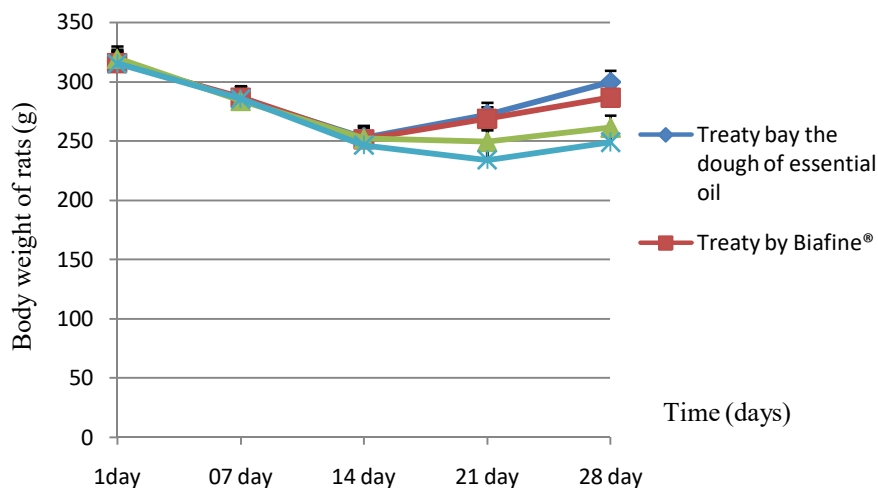
The study of the evolution of the parameters of the cicatrization made it possible to note a reduction of the surface and depth of the wound, vanishes at the 14th day, thus an appearance of the crusts thick brownish and blackish covered all the wound, easy a excised and buds in batches treated with dough

based on essential oil of *Tetraclinis articulata* and **Biafine**® with the exception of placebo and control groups (untreated).

Visual and daily observation of burns has shown the presence of signs of infection at the level of the wounds of the burns of most of the Rats untreated (control group) compared to the other groups Treaty. The absence of infection and the presence of hairs at the level of the burns Treaty by the dough has basis of essential oil interpreted by the presence of a antimicrobial power of the essential oil of *Tetraclinis articulata* as well that the antiseptic effect of the tetraborate of sodium this probably explains and/or justifies the slight advantage of the dough prepared without HE (used as placebo) during the period of natural cicatrizing.

Various studies mention the presence of an antimicrobial effect of the essential oils of *Tetraclinis articulata* [8].

#### Body weight evolution during the treatment of burns:



**Figure 03.** Evolution of the body weight (g) during the cicatrizing period according to the different types of treatment.

According to the (Figure 03) at the beginning of the experience there is no significant difference between the weights of the different groups with a initial average weight virtually identical of approximately 300 and 320 grams.

After 7 days of application of burns, the values obtained indicate a slight and significant decrease ( $p < 0.05$ ) in body weight in burned rats, with a decrease of approximately (17%) regardless of the nature of the animals treated or not treaty.

On the fourteenth day (day 14), severe body weight loss was observed in the different groups of animals up to (25%) compared to the initial body weight.

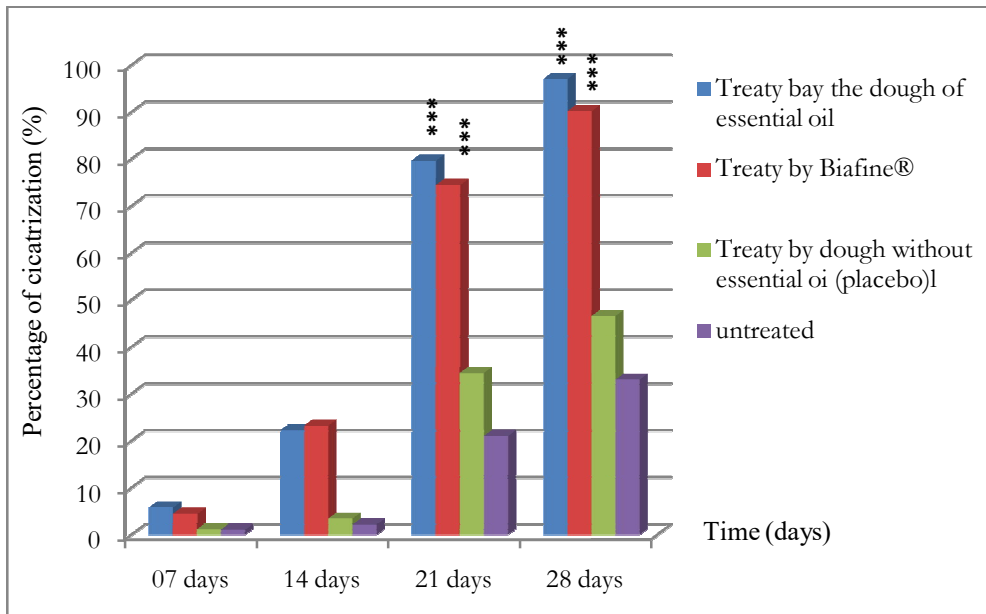
Several factors could be involved in this alteration of the weight growth of animals of experience. Given the importance of the defense process of the body trauma of the burn on which requires the intervention of several systems, organic and biological functions such as the protein synthesis (ex: collagen) and the division and growth of cell strain differences participating and / or involved in the pathophysiological process post-traumatic. The decrease in food consumption noted during the first period of the experiment may be indirectly involved in the slowing of weight growth in the burned animals.

The values obtained after twenty one days of the experiment clearly show a recovery of weight in the different groups with an average increase of over 25g except for the control and placebo groups did not significantly increase compared to the initial body weight.

#### Planimetry of Burns:

The results observed at the 14th day of the treatment (Figure 04) show a significant ( $p < 0.01$ ) potentiation of the contraction of the wounds of the rats treated with the dough based on essential oils ( $\approx 22\%$ ) compared to negative and placebo control groups ( $\approx 1,09\%$ ,  $\approx 1,15\%$ ) respectively.

This action inducing healing is much better in animals treated with dough of essential oil with a lesser degree for rats receiving dough without essential oil. On the other hand, the inductive effect of the cicatricial process is not clearly established in the group untreated



**Figure 04.** Evolution of the cicatrization process according to the percentage of healing. \*\*\* Statistically different to the control group and placebo (the difference is significant).

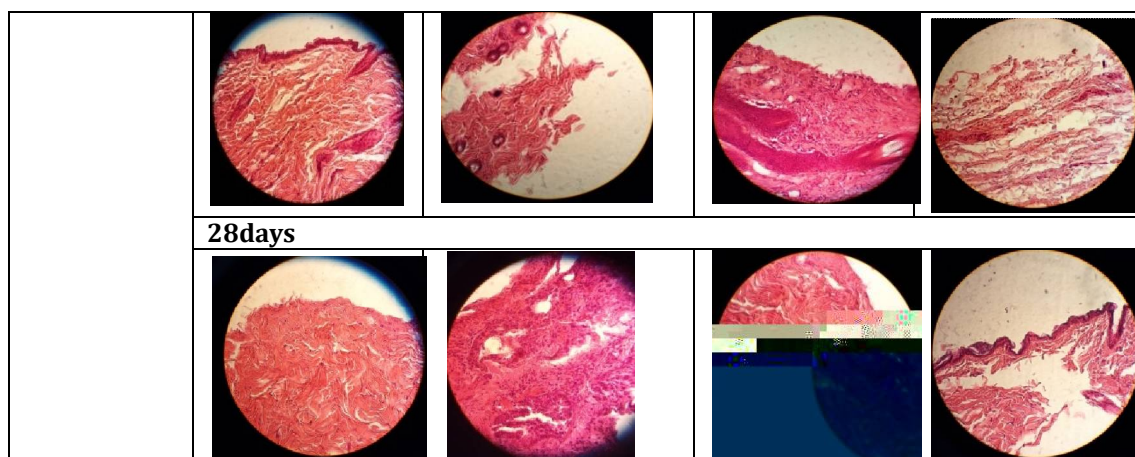
During the last period of (21 days of the experiment), the evolution of the contraction as well as the time of closure of the wounds become statistically identical ( $\approx 10\%$ ) in the groups treated with the dough of essential oil and *Biafine®* (figure 04).

The results suggest that the plant has a cicatrizing activity and competes with *Biafine®* at the first, second and third cicatrizing stages, until the 28th day. This result is well justified by a percentage reduction in the burned area of 97%, 90%, 46% and 33% respectively for the group treated with *T.articulata*, *Biafine®*, placebo and untreated control at the 28th day, this result is comparable to that reported by Ansari M. et al., 2009.

This cicatrizing phase may be affected by certain phytochemicals of the essential oil of *Tetraclinis articulata*.

**Histological study:**

Rats	group n°1 Treaty by dough of essential oil	group n°2 : Treaty by the dough without essential oil (Placebo)	group n°3 : Treaty by <i>Biafine®</i>	group n°4: untreated (Negative control)
<b>Histological sections in the skin burn</b>	<b>07 days</b>			
	<b>14 days</b>			
	<b>21 days</b>			



**Figure 05.** Microscopic observation of histological sections in the skin burn.

Microscopic observation of histological sections performed after 7th days of treatment, shows the appearance of mononuclear inflammatory elements at the level of the hypodermis and a granulation tissue rich in capillary vessels (endothelial cells) in rats treated with dough of *T. articulata*. On the other hand, coagulation necrosis with leukocyte inflammatory infiltrate was observed in the untreated group. The histology of the skin burned after 14th days is characterized by the presence of granulation tissue rich in fibroblasts which accelerates healing (promotes collagen formation) in the group treated with dough of essential oil of *T. articulata* and Biafine® compared to the control group. The potentiating effect of the dough of essential oil-based is specifically observed during the 21st day of application of burns. This period corresponds to the proliferative phase of the cicatricial process; it is characterized by the formation of the granulation tissue and the phenomenon of epithelialization. During the 28th day: healing phenomenon is accelerated, end of maturation and beginning of remodeling and formation of collagenized scar tissue in the group treated with dough of *T. articulata*.

## CONCLUSION

Many comparative studies have been conducted to evaluate the efficacy of *T. articulata* essential oil in the treatment of deep second degree burns in Wistar rats. The *in vivo* evaluation of the cicatrizing activity is done by a preparation of adermal dough based on essential oil extracted by the method of hydrodistillation of the leaves of *Tetraclinis articulata*. The microbiological and toxicological control of the dough gives a good quality of this galenic formulation for a dermal application. The statistical analysis of the planimetric results and the histological study of the burned skin during the cicatrizing period allow us to suggest the following conclusions. The **Biafine®** is a drug healing and it has a beneficial effect on the burns that at the final stage (maturation and remodeling). The essential oils of *Tetraclinis articulata* have a cicatrizing activity equivalent to that of the reference. *T. articulata* could therefore be an effective alternative and without harmful effect in the treatment of burns. This study showed that the plant has a cicatrizing activity evident completely in agreement with the effectiveness with which he is recognized in the traditional medicine.

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