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Chemical composition and burn wound healing effects of *Pistacia lentiscus* L. fruits and leaves from Algeria

Himour Sara[1,2*] Belattar Hakima[1,2] Bousmid Ahlem[1,2] Talhi Fahima[1,2] Yahia Abedelouhab[1,2]

1 Laboratory of Natural Sciences and Materials (LSNM), Abdelhafid Boussouf University Center. BP N°26 RP Mila 43000 Algeria.

2 Department of Biotechnology, Institute of Natural and Life Sciences, Abdelhafid Boussouf University Center, Mila, Algeria

***Auteur correspondant**: **s.himour@centre-univ-mila.dz.**

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Abstract

The aim of this study was to chemically characterize and evaluate the healing activity of the ointments prepared by leaves and fruits of *Pistacia lentiscus* L. grown in Algeria. The secondary metabolites in the extracts were determined through phytochemical screening, while the wound-healing activity of the ethanol extract was tested on rabbits. The phytochemical analysis revealed constituents in the ethanol extracts of both leaves and fruits that are documented to possess potential burn-healing properties. Deep third-degree burn wounds were inflicted on the feet of each animal with treatments applied daily until complete healing. Over a five day post-burns period, wound the contraction percentage and healing times were recorded. The results indicated that both ointments prepared from the leaves and fruits of *Pistacia lentiscus*, as well as and Biafine® significantly accelerated wound healing compared to Vaseline-dressed and untreated wounds. Notably, the ointment made from leaves demonstrated a significantly higher level of wound contraction and faster healing time.

Keywords: *Pistacia lentiscus*, burn, wound healing, rabbits, leaves, fruits.

Introduction

Plant extracts have been used for centuries as a popular remedy for various health disorders (Dellai et al .,2013) and are the primary source of phytochemicals present in conventional medicines. Phenolics are secondary plant metabolites found in the majority of herbs, vegetables and teas. They have been shown to exert diverse biological effects (Azaizeh et al .,2013).

Pistacia lentiscus belongs to the Anacardiaceae family, which consists of 70 genera and more than 600 species. The genus *Pistacia* includes deciduous resin-bearing shrubs and trees (Santarsiero et al .,2020). *Pistacia* are used in pharmacological activities (Sehaki, et al.,2023) including the treatment of eczema, paralysis, diarrhea, throat infections, renal stones, jaundice, asthma and stomach-ache, as well as possessing astringent, anti-inflammatory, antipyretic, antibacterial, antiviral, pectoral and and stimulant properties (Duru et al.,2003) .

Many studies have been conducted to uncover the chemical composition of *P. lentiscus* leaves and fruit extracts, identifying and quantifying numerous constituents (Longo et al .,2007). The observed metabolic profile explains the significant healing activity of *P. lentiscus* for burns. Wounds are defined as skin damage and can result from burns.

The wound healing process is characterized by three dynamic and complex phases: inflammatory, proliferative, and maturation (Velnar et *al* .,2009; Elloumi et al .,2022). The purpose of this study was to clinically assess the effect of leaves and fruits extracts on burn wounds healing in rabbit model.

1- Materials and Methods

1-1 Preparation of plant extract

The plant material used consisted of dried fruits and leaves of *Pistacia lentiscus* L. collected in December 2022 from El Milia wilaya Jijel , North Eastern Algeria. After harvest, the fruits and leaves were cleaned, washed with tap water and dried in hot air oven at 60° C, They were then ground into a fine powder using an electric grinder (Moulinex). The powdered material was exhaustively extracted with a 70/30 ethanol /water solution using the maceration process. The macerated mixture was filtered using Whatman N°1 filter paper and evaporated at room temperature to yield a solid extract. This extract was stored in refrigerator until analysis.

1-2 Animals

Twenty five healthy adult male rabbits, weighing between 1.33–1.85 kg, were used for the study. The Animals were kept in individual standard cages under standardized environmental conditions with an ambient temperature of 22 ± 2ºC and a 12 hour light-dark cycle. Food and water were provided ad libitum. All experimental procedures adhered to International guidelines for animal care.

1-3 Phytochemical compound determination (Phytochemical screening)

The phytochemical screening involved qualitative tests based on coloring or precipitation reactions specific to each class of secondary metabolites in the studied plants. Five grams of crushed *Pistacia* were added to a mixture of methanol and water (100 mL, 70: 30 (v / v)) to extract phenolic compounds . The mixture was allowed to stand at 4 C° in the dark for 5 days,. It was then filtered using Whatman filter paper (Al-Quraishy et al., 2017). Each extract was stored in a dark bottle until needed . The presence or absence of different classes of secondary metabolites containe *Pistacia* lentiscus L. extracts were qualitatively tested as follow

1-4 Burn wound model

The procedure used in this study was described by (Hamdi-pacha et al., 2002). On day zero, rabbits were anesthetized with 50 mg/kg ketamine hydrochloride, administered intramuscularly, along with 5 mg/kg diazepam. The hairs on the skin of the animals' backs was shaved using a sterilized razor blade. Four burns of identical size (3 cm in diameter) were created on the back of each animal, two cranially (left and right) and two caudally (left and right), using a metal cylinder weighing 200 g , which had been immersed in boiling water for 3 minutes and maintained on the animal's skin for 15 seconds. Each animal served as its own control.

1-5 Preparation of healing ointments

The extracts of the leaves and fruits of *Pistacia lentiscus* were homogenized with petroleum jelly to prepare a 6% concentration ointment, which was then kneaded in a mortar. The composition of the ointments is shown in (Table1):

Table 01: Composition of prepared ointments

Ingredient	**Placebo (vaseline)**	**Ointment (6%)**
P. lentiscus leaf and fruits extract (g)	/	**3**
Vaseline (g)	**49.955**	**46.955**
Sodium benzoate (g)	**0.075**	**0.075**
Total (g)	**50**	**50**

1-5 Treatment and assessment of healing process

Each wound was assigned to a test substance in such a way that each drug would be applied to different wound locations on each animal. preventing experimental bias from body movement (Bae et al., 2005). The treatments were as follows:

- Biafine® cream 1%
- 6% *Pistacia lentiscus* leaves and fruits 6% extract ointment (PL group)
- Vaseline gel (VAS group) at a dose of 0.5g

- No treatment (CRL group, control).

All treatments were applied topically using sterilized cotton swabs immediately after the burn procedure and repeated once daily until complete epithelialization. The wound size was traced on a transparent sheet every 5 days, and the wound surface area was measured. The percentage of wound contraction was calculated using the following equation, with the initial wound (706.5 mm^2) considered as 100 %:

Percentage of wound contraction= [(Initial wound size – specific day wound size) / Initial wound size] × 100 (Srivastava and Durgaprasad, 2008).

The healing process was evaluated using the following scale:

- 0: Complete healing
- 1: Tissue healing nearly complete
- 2: Remnants of the crust, lesion size decreasing
- 3: Removal of dead tissue, sores, and oozing
- 4: Partial removal of necrotic skin, ulceration, and oozing
- 5: Necrotic skin fully covering the burned area

1-6 Statistical analysis

The findings obtained were presented as mean ± SD. The data were subjected to one-way ANOVA to assess significant difference. Statistical analyses were performed using SPSS 24.0, with p-values < 0.05 considered statistically significant.

2 Results and Discussion

2-1 Phytochemical Screening

The results of the phytochemical screening on the ethanolic extracts from *Pistacia lentiscus* leaves and fruits are shown in (Table 02). These tests provided an overview of the chemical composition of the studied plants.

Table 02. Phytochemical screening results of *P. lentiscus* leaves and fruits ethanolic extracts.

Organs	*Phenolic compounds*	*Flavonoïds*	*Tannins*	*Saponines*	*Free quinones*	*Anthraquinones*	*Triterpenes*	*Coumarins*	*Anthocyans*	*Alcaloïds*	glycosides
Leaves	+	+	+	+	+	+	+	+	+	+	-
Fruits	+	-	-	+	+	+	+	+	-	-	+

The phytochemical analysis of *Pistacia lentiscus* ethanol extracts revealed compounds that may contribute to burn wound healing, such as polyphenols. According to (Preedy and Watson.,2020), certain phenolic compounds assist wound healing by preventing infection, reducing inflammation, increasing re-epithelization and cell proliferation, and controlling excessive healing.

The screening of phytochemicals, including tannins, saponins, , flavonoids, terpenoids, glycosides and alkaloids anthocyans coumarins anthraquinones free quinines, showed that both leaves and fruits contain phenolic compounds., Free quinones saponins ,flavonoids, anthraquinones and terpenoids. Bammou et al. (2015) reported that *P. lentiscus* L. leaves contains tannins, flavonoids, sterols, triterpenes and saponins, but lack alkaloids and glycosides.

Barbouchi et al. (2020) found that various parts of *P. lentiscus* (leaves and fruits) are rich in tannins, flavonoids, saponins, sterols, triterpenes, and reducing sugars, but anthraquinones are absent. While alkaloids were present in fruits, they were absent in leaves, Beraich et al . (2024) send that the investigated extracts of *P. lentiscus* might be considered valuable sources of bioactive compounds.

2-2 Healing Properties for Burn Wounds

During the experiment, no animals died. The efficacy of different ointments made from *Pistacia lentiscus* leaves and fruits were compared to Biafine, Vaseline, and untreated controls. The percentage of wound contraction was evaluated over time (Table 3, figure1). Wounds treated with healing ointments prepared from the leaves and fruits showed faster healing. Vaseline-treated wounds initially contracted less than untreated wounds, but after day 15, Vaseline-treated wounds contracted more rapidly.

The epithelization period was significantly shortened in the *Pistacia lentiscus* leaf group (15 ± 3.8 days) compared to the other groups. Biafine-treated wounds also healed faster than those treated with Vaseline (25.25 ± 3.8 vs. 32.66 ± 2.9 days).The Vaseline group, however, showed better healing time compared to the untreated wounds (35.16±3.24 days) (Table 3 ; Figure 1).

Table 3: **Percentage of wound contraction at different time intervals and epithelization period in treated and untreated wounds.**

Treatment (N=5)	**Wound contraction (%) Mean ±SD**				**Epithelization Period (days) Mean ±SD**
	5 th Day	**10th Day**	**15th Day**	**20th Day**	
CRL	**16,21±4.35**	**35,76±5.02**	**50.35±3.25**	**70.56±2.83**	**35.16±3.24**
VAS	**24,79±3.25**	**50,33±4.23**	**70.83± 2.28**	**85.98±2.25**	**32.66±2.9**
BIAF	**33,52±2.21**	**65,88±2.21**	**88.24±2.56**	**97.78±3.68**	**25.25±3.8**
PLVL	**57,79±4.56**	**78,40±2.58**	**100**	**100**	**15±3.8**
PLFR	**42,47±5.02**	**63,57±3.24**	**96,12±1.29**	**100**	**17.66±2.6**

CRL= Control (no treatment), VAS= Vaseline, BIAF= Biafine ,PLVL= *Pistacia lentiscus* leaves , PLFR= *Pistacia lentiscus* fruits, $P<0.05$.

The development of drugs for wound management has a long history in medical Science (Ramesh.1993). (Habbu et al. 2007) presented a detailed review of literature on natural pro-healers, phytoconstituants, polyherbal formulations and various nutraceuticals responsible for wound healing activity. They reported 81 plants studied by different authors.

Pistacia lentiscus is one of plante used in folk medicine with a strong reputation for its healing properties (Djerrou et al., 2010). This vegetable oil has demonstrated significant healing activity on experimental burns in a rabbit model model by decreasing the inflammatory phase, promoting wound contraction, and reducing the period of epithelialization (Djerrou et al. 2010).

Hemida et al.(2022) found that *Pistacia lentiscus* leaves exhibit wound healing effects, as they accelerate wound contraction and reduce the epithelialization period. Ointments prepared from ethanolic extracts of *Pistacia lentiscus* leaves showed effective wound healing action in Wistar rat skin excisions.

According to Nahida et al. (2012) Wound contraction i.e. healing was significantly ($P<0.05$) enhanced in the presence of *Pistacia lentiscus* L. .The topical application of the *P. lentiscus* L. fruit fatty oil and its unsaponifiable fraction has been found to be beneficial in the treatment of wounds.

The results of the present study confirm previous findings that have tested *Pistacia lentiscus* fruit oil (PLFO) or honey separately. While honey has been well studied for a long time, there are few reports available on PLFO. Our team has scientifically confirmed the healing properties of PLFO (Maameri et al., 2012).

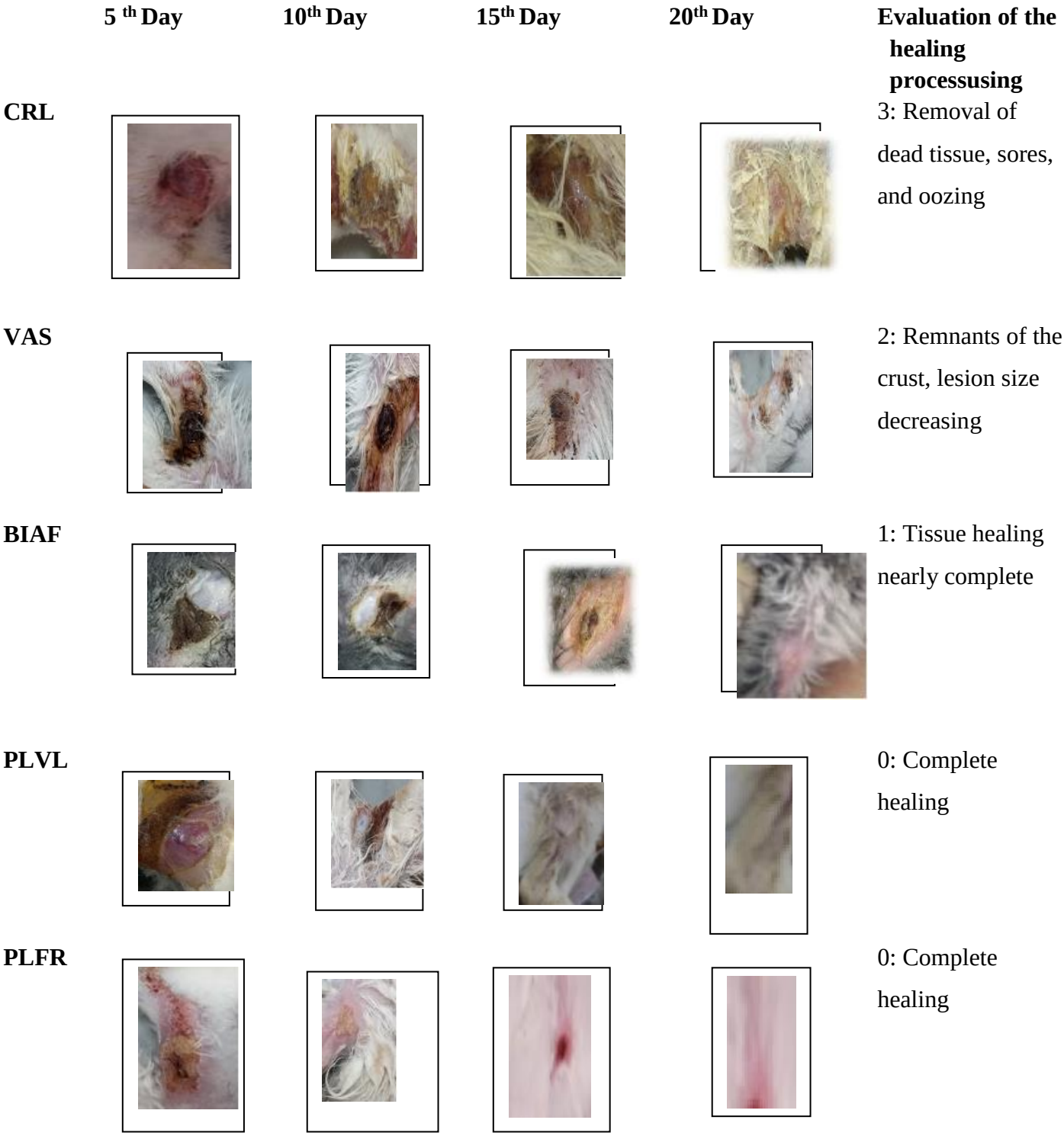

Figure 1: Burn wounds chronicity in the first rabbit- CRL= Control (no treatment), VAS= Vaseline, BIAF= Biafine ,PLVL *Pistacia lentiscus* leaves , PLFR= *Pistacia lentiscus* fruits.

Conclusion

The present study demonstrates that although it is not yet clear which constituents of *P. lentiscus* are responsible for improved wound healing, The analyses of *P. lentiscus* leaves and fruits revealed that the extracts

obtained through maceration leaf and fruit powders are rich in natural bioactive substances. Our research has shown that the regular topical application of *P. lentiscus* leaf and fruit ointment accelerates the wound healing process, confirming the traditional of the traditional use of this plant. This wound healing potential of P. *lentiscus* is likely due to the presence of these bioactive compounds. In conclusion, this study suggests that the extracts from *P. lentiscus* leaves and fruits possess significant healing properties.

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