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Chemical profiling and total thickness-excised wound-healing activity of *Pistacia lentiscus* L. fruits growing in Algeria

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Abstract: The aim of this study was to determine the chemical characterization and to assess full-thickness-excised wound-healing activity of the oil of *Pistacia lentiscus* L. fruits grown in Algeria. The fatty acid composition of *P. lentiscus* L. extracts were analyzed by GC–MS and their total protein amounts were determined by elemental analysis. Total thickness-excised wound-healing activity of hexane extract was carried out on rabbits; comparing oil and wax effects. The results showed that *P. lentiscus* L. fruits contain considerable levels of oil and protein. Additionally, oleic (46.91 ± 1.03, 48.34 ± 0.07) in hexane and cold pressure extracts, respectively, palmitic (25.75 ± 0.82, 25.44 ± 0.65) and linoleic (20.59 ± 0.03, 20.27 ± 0.81) acids were the major fatty acids. Wound-healing activity was significantly higher in *P. lentiscus* L. fatty oil compared to the wax, therefore, fats refining is wildly recommended.

Subjects: Agriculture; Analysis & Pharmaceutical Quality; Clinical Trials - Pharmaceutical Science; Dermatopathology; Food Chemistry; Laboratory Animal Science; Natural History; Natural Products; Nutrition; Pharmaceutical Laboratory

Keywords: *Pistacia lentiscus* L.; GC–MS; fatty acids; total protein; excised wound healing

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PUBLIC INTEREST STATEMENT

Pistacia lentiscus L. (Anacardiaceae), known locally as “ed’drew”, is an evergreen shrubby tree 1–3 m high with edible fruit containing a fixed oil, used in traditional medicine, specially at eastern part of North Africa (Algeria, Tunisia), for its wound-healing effect and as remedy against respiratory allergy ailments. Separation of wax from this oil enhances wound-healing activity. Cutaneous and oral toxicities evaluations of *P. lentiscus* oil in animal (mouse, rabbit) concluded with the absence of short time toxicities at used concentrations.

In this study, *P. lentiscus* oil has shown promising healing properties, by reducing the inflammatory phase, stimulation of wound contraction, and reducing the epithelization period.

The study concludes that *P. lentiscus* fatty oil is effective for healing wounds. It is tolerable in the short term, but may cause skin sensitization after prolonged use.

1. Introduction

Medicinal plants have played an important role in health care for centuries. More than 80% of the world's population rely on traditional medicines to treat various skin disorders, including wound infections (Priya, Gnanamani, Radhakrishnan, & Babu, 2002; World Health Organization, 2002). Wound healing is a physiologic process involved to repair damaged tissues, including hemostasis, inflammation, proliferation, and maturation (Enoch & Leaper, 2005; Nguyen, Orgill, & Murphy, 2009; Rieger, Zhao, Martin, Abe, & Lisse, 2014; Stadelmann, Digenis, & Tobin, 1998; Toporcer et al., 2006). Diabetes, infection, and metabolic deficiencies are the main factors that contribute to nonhealing wounds (Lazarus et al., 1994). The care of the wounds prevents infection and enhances wound healing via disinfection, treatment, and protection from reinjury (Alizadeh, Ahmadi, Mohammadzadeh, Paknejad, & Mohagheghi, 2009). Several drugs for wound healing take their origin from plants (Habbu, Joshi, & Patil, 2007). Researches of new plant products that may promote wound healing have recently taken advantages and can serve as new lead compounds for wound healing (Wang et al., 2013). *Pistacia lentiscus* L. (Anacardiaceae) is an evergreen shrub 1–5 m tall with globulous edible fruits first red then black at maturity, cultivated for the extraction of oil and the resin (Iserin, 2001; Sharma, 1993). It is native to the Canary Islands, throughout the Mediterranean region, from Morocco and Iberian Peninsula in the west throughout Southern France and Turkey to Iraq and Iran in the east (Simpson, & Ogorzaly, 2013; Tingshuang, Jun, Avi, & Dan, 2008). Previous pharmacological studies indicated that the fatty oil extracted from *P. lentiscus* L. fruits is used to care respiratory diseases, diarrhea, and pharyngitis, and for treatment of wounds and burns (Djerrou et al., 2010; Trabelsi et al., 2012). The objectives of the current study were to investigate the healing effect of *P. lentiscus* L. fruits hexane extract on the total thickness-excised wounds, and the composition on fatty acids and total protein.

2. Results and discussion

2.1. Total fat and protein contents

The protein contents of *P. lentiscus* L. fruits have been determined on the basis of total nitrogen contents according to the elemental analysis method. The protein contents were calculated as total nitrogen amount multiplied by 5.68 ± 0.3 (Sosulski & Imafidon, 1990). Nitrogen content of the sample was found to be 4.2% ± 0.006; consequently, the value for total protein contents ranged from 23.86% (Table 1). It is said that *P. lentiscus* L. mature black fruits are rich in terms of protein contents as well as fat contents (Table 1).

2.2. Fatty acids composition

The results of total oil from *P. lentiscus* L. fruits showed that hexane extract has higher rate of total oil (25.67%) than cold pressure extract (20.84%) as shown in Table 1. The results of GC–MS study indicated three dominant fatty acids: oleic, palmitic, and linoleic acids, and two moderate fatty acids: palmitoleic and stearic acids for hexane and cold pressure extracts, with similar percentages in both types of extraction (Table 2). Benhammou, Atik Bekkara, and Panovska (2008) reported good nutritive quality of this oil due to its contents in unsaturated fatty acids (Oleic + linoleic = 73%) and saturated fatty acids (Palmitic + stearic = 25.8%). The cold pressure extract had more oleic acid comparing to hexane extract with similar amounts for the remaining fatty acids. In addition, oleic (18:1) acid has been found as the major fatty acid in both hexane and cold pressure extracts with maximum values of 46.91 and 48.34%, respectively, followed by palmitic (16:0) and linoleic (18:2) fatty acids. From the literature reviews, several studies gave the same three dominant fatty acids with different amounts (Charef, Yousfi, Saidi, & Stocker, 2008; Mezni et al., 2012; Trabelsi et al., 2012); they may vary not only due to the locations and the harvest times, but also to the extraction methods. In addition, some of the presented fatty acids have been described as bioactive molecules that

Table 1. Oil and protein contents of *P. lentiscus* L. fruits

Species	Protein (%)	Oil (%) hexane extract	Oil (%) cold pressure extract
P. lentiscus L.	23.856 ± 1.260	25.671 ± 0.852	20.84 ± 1.318

Note: Values presented are the means of triplicate experiments for each extract.

Table 2. Fatty acid analysis results of hexane and cold pressure extracts of *P. lentiscus* L. by GC–MS

Rt (min)[a]	Constituents[b]		Hexane extract[c] (%)	Cold pressure[c] (%)
25.78	Stearic acid, methyl ester	(C18:0)	1.38 ± 0.34	1.29 ± 0.07
18.60	Myristic acid, methyl ester	(C14:0)	0.05 ± 0.02	0.04 ± 0.02
19.83	Pentadecanoic acid, methyl ester	(C15:0)	0.01 ± 0.01	0.01 ± 0.01
21.94	Palmitic acid, methyl ester	(C16:0)	25.75 ± 0.82	25.44 ± 0.65
23.86	Heptadecanoic acid, dimethyl ester	(C17:0)	0.08 ± 0.02	0.06 ± 0.02
29.22	Arachidic acid, methyl ester	(C20:0)	0.21 ± 0.02	0.15 ± 0.02
	∑ SFA		27.48 ± 0.21[a]	26.99 ± 0.13[a]
22.57	Palmitoleic acid, methyl ester	(C16:1)	4.24 ± 0.12	3.74 ± 0.02
24.43	10-Heptadecanoic acid, methyl ester	(C17:1)	0.08 ± 0.06	0.09 ± 0.01
26.38	Oleic acid, methyl ester	(C18:1)	46.91 ± 1.03	48.34 ± 0.07[a]
29.67	11-Eicosenoic acid, methyl ester	(C20:1)	0.15 ± 0.01	0.19 ± 0.01
	∑ MUFA		51.38 ± 0.31[a]	52.36 ± 0.03[a]
27.26	Linoleic acid, methyl ester	(C18:2)	20.59 ± 0.03	20.27 ± 0.81
28.46	Linolenic acid, methyl ester	(C18:3)	0.55 ± 0.04	0.39 ± 0.01
	∑ PUFA		21.14 ± 0.03[a]	20.66 ± 0.41[a]
	SFA/UFA		0.38	0.37
	PUFA/SFA		0.77	0.76
	Cox value		2.71	2.65

Note: Values presented are the means of triplicate experiments for each extract.

[a]Retention time (in minutes).

[b]Compounds listed of elution from a HP-5 MS column.

[c]Percentage of relative weight.

can solve human health disorders, such as antihyperlipidemic, lowering cholesterol level, and blood pressure (Djerrou, 2014; Kris-Etherton, 1999). It was obvious that the prominent class of fatty acid was represented by MUFA at 51.38%, followed by SFA at 27.48%, then PUFA at 21.14%. The SFA/UFA ratio was accounting for 0.4; it indicates that *P. lentiscus* L. fatty oil has much more unsaturated fatty acids than saturated ones, which attributes dietetic and nutritive properties to this oil.

2.3. Excised wound healing

This study investigated the efficiency of *P. lentiscus* L. hexane extract (PLHE) for full-thickness-excised wounds healing. The skin of different rabbits has been inspected and the percentage of wound contraction has been recorded every three days (see Table 3). The results showed that both PLHE and Madecassol® significantly ($p \leq 0.05$*) promote wound healing compared to wounds dressed with PLW and the untreated wounds, during all observation times with absence of wound infection in PLHE treatment. However, as there is very high significant difference ($p \leq 0.001$***) in the last healing phase (day 15) between MAD and PLW, it can be concluded that PLW reduces cell proliferation and maturation and seemed to delay cicatrization effect. Also high significant difference ($p \leq 0.01$**) in the second healing process (day 6) between PLHE and CONT- groups showed that PLHE promoted wound healing during inflammation phase without infections. Consequently, the period of total healing in CONT (-) and PLW treatment groups were at least 18 days, while MAD and PLHE groups have been completed within 15 and 16 days, respectively. PLHE showed better contraction percentage than MAD during the first and the second measurement days (day 3 and day 6) which correspond to the cell hemostasis and cell inflammation phases of wound-healing process, while MAD produced the highest healing percentage during the last six days of treatment, that were in accordance with the proliferation and maturation phases (Enoch & Leaper, 2005; Nguyen et al., 2009;

Table 3. Evolution of the healing process (contraction %) of total thickness-excised wounds on rabbits

Treatment	Wound healing (%)				
	Day 3	Day 6	Day 9	Day 12	Day 15
CONT (-)	24.62 ± 1.41	36.80 ± 4.10	60.80 ± 1.64	81.73 ± 2.99	92.68 ± 1.46
MAD	38.47 ± 2.08	50.73 ± 1.30	72.68 ± 1.33	93.05 ± 3.70	98.50 ± 0.76
PLHE	41.11 ± 1.42	54.64 ± 1.28	70.85 ± 1.53	92.93 ± 4.18	95.29 ±1.39
PLW	18.04 ± 3.26	41.08 ± 2.03	60.30 ± 1.25	79.93 ±1.23	85.38 ± 0.55
Statistical data					
PLHE–CONT-	Signif-$p \le 0.05$*	Signif-$p \le 0.01$**	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Nonsignificant
PLHE–PLW	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Signif-$p \le 0.01$**	Signif-$p \le 0.05$*
PLHE–MAD	Non-significant	Non-significant	Non-significant	Non-significant	Non-significant
MAD–CONT-	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*
PLW–CONT-	Non-significant	Non-significant	Non-significant	Non-significant	Non-significant
MAD–PLW	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Signif-$p \le 0.05$*	Signif-$p \le 0.01$**	Signif-$p \le 0.001$***

Note: The results represent the mean ± SD ($N = 4$).

Rieger et al., 2014; Stadelmann et al., 1998; Toporcer et al., 2006). In the 15th day, PLHE healing activity decreased during maturation phase and gave no significant difference in contraction rate with CONT (-) treatment group. It was obvious that PLW reduces proliferated cells to be mature; therefore, it might be necessary to remove the wax from *P. lentiscus* L. extracted oil in order to increase its healing effects. We have demonstrated previously the effect of this oil on burn healing in rabbits' skin (Djerrou et al., 2010).

3. Conclusions

PLHE promotes equally wound healing on the skin of rabbits, after three weeks of daily application comparing to reference drug, in hemostasis and inflammatory phases of cicatrization, without infection effect. Therefore, further studies of antimicrobial and antifungal activities of its fatty oil are strongly recommended. Additionally, effective wound healing properties of *P. lentiscus* L. may be attributed to the synergistic effect of its fatty acids, especially oleic, palmitic and linoleic acids, proteins, and other compounds. Therefore, the studied species which is widely used in traditional therapies needs to be screened well. Moreover, *P. lentiscus* L. fruits acquire considerable levels of total oil and protein content. The results of our study can also be useful for nutritional research.

Supplementary material
Supplementary material for this article can be accessed here http://dx.doi.org/10.1080/23312025.2016.1182611.

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Competing Interests
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