International Journal of Biological Macromolecules 88 (2016) 443–450

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Phoenix dactylifera L. sap enhances wound healing in Wistar rats: Phytochemical and histological assessment

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ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 24 March 2016
Accepted 6 April 2016
Available online 8 April 2016

Keywords:
Date palm sap
Minerals
Wound healing
LC–MS analysis
Antioxydant
Bioactive phenolic compounds

ABSTRACT

The sap of the date palm "Lagmi" is a clear liquid, rich in sugars and minerals, with a pleasant flavour. Folk remedies based on the use of "Lagmi" for wound healing are still practiced. However, no studies investigated the relevance of "Lagmi" for wound healing. Therefore, the aim of this study was to identify the *in vivo* healing properties of "lagmi" on mechanically wounded wistar rats. Injured rats were divided into three groups: a first group treated by "lagmi", a second reference group processed by CICAFLORA® and a third untreated control group. On the 12th day of the experiment, total healing in the first group was reached, while healing was incomplete in the other groups. The sap seems to accelerate cell proliferation and contribute to faster healing with a gain of more than 30% as compared to CICAFLORA®. Chemical Analysis of "Lagmi" showed important radical scavenging activity and high total antioxidant capacity. Features reported to help healing process and/or provides a favourable environment for tissue healing in wound sites. Extensive characterization of "Lagmi" phenolic and flavonoid compounds by High Resolution LC–MS (LC-HRESIMS) analysis indicates "Lagmi" is an important source of known anti-inflammatory compounds as well as promising wound healing candidates.

1. Introduction

Date palm (*Phoenix dactylifera* L.), a tree widely distributed throughout the southern regions of Tunisia, is cultivated for its edible sweet fruit. The tree represents a source of raw materials. Virtually every part of the tree is utilized to make functional items for construction, consumption and/or other daily life functions [1,2]. The sap of the date palm is a clear liquid, rich in sugars and minerals, with a pleasant flavour reminiscent of coconut milk. It is a very fermentable product widely used in the region [1,2]. Most parts of date palm are also popular in folk medicine: fresh pulp, fruit, pollen and the date palm sap "Lagmi". The use of products and by-products of the date palm in traditional medicine is an ancient practice [3,4]. For thousands of years in Egypt and the Middle East, the tree has been used for Pharmacopoeia [5]. The Date palm sap aids in the treatment of anaemia and dehydration, stimulating lactation in women, improving vision and regulating blood pressure [6]. Mixed with various ingredients, it heals sore stomach, fever, and respiratory diseases. The sap is also used as a beauty product [6]. Other medicinal properties of the tree include antioxidant activity [7,8], memory and learning stimulation [9] and gastrointestinal transit activity [10,11].

Wound healing is a dynamic process where, after wound, the skin or other body tissue repairs itself. Three phases have been identified during active wound healing processes:

Abbreviations: ICP-MS, inductively coupled plasma mass spectrometry; LC–MS, liquid chromatography mass spectrometry; LC-HRESIMS, high resolution hyphenated LC–MS; CRMs, the certified reference materials; GAE, gallic acid equivalent; QE, quercetin equivalent; DPPH, 1,1-diphenyl-2-picryl-hydrazyl.

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http://dx.doi.org/10.1016/j.ijbiomac.2016.04.015
0141-8130/

i) Inflammatory phase: characterized by contraction of blood vessels and the clot formation. Once haemostasis achieved nutrients, enzymes, antibodies as well as specialized white blood cells are recruited to achieve host response.
ii) Proliferation phase: new granulation tissue composed of collagen and extracellular matrix is rebuilt and vascularised (angiogenesis). Sufficient levels of oxygen and nutrients are critical for healthy granulation. The tissue in the wound site is pink/red in colour and does not bleed. Then the epithelialisation take place allowing epithelial cells to resurface the wound.
iii) Maturation phase: during this phase, that occurs when the wound is closed, collagen is remodelled from type III to type I.

Recently there was a new trend in characterizing active molecules from folk medicine recipes and remedies [12]. Folk remedies based on the use of "Lagmi" for wound healing are still practiced in the south of Tunisia and are of surprisingly high curative value [6]. To our knowledge, there were no reports regarding the wound healing effect of Date palm sap. Hence, we decided to evaluate its wound healing potential in rats. Additionally, in order to provide the basis of the putative wound healing activity, we investigated minerals, flavonoids and polyphenols content of "Lagmi". Finally, extensive characterisation of "Lagmi" using LC-HRESIMS was carried out in order to identify polyphenols and flavonoids, frequently associated with anti-inflammatory, antimicrobial and wound healing activities.

2. Materials and methods

2.1. Products used

The sap extracted from the Beser date palm variety was used as treatment for wounds healing. "CICAFLORA®", a restorative emulsion which promotes repair of altered epidermis: open or closed wounds (cuts, burns...), was used as reference standard medicine to conduct the comparative study with the date palm sap. "CICAFLORA®" contains extract rich in tannins, trace elements and bioflavonoid derived from the bark powder of a Mexican tree, *Mimosa Tenuiflora*. "CICAFLORA®ẅas shown to have an action promoting cell stimulation and repair of weakened skin and a bacteriostatic power. The cleaning of the wounds was performed using physiological serum which was the only treatment for the control rats. The animals were obtained from the animal housing facility of the Faculty of Medicine (University of Sfax, Tunisia). Lignocaine HCl (2%) was applied to the rat's muscle layer for anaesthesia prior to wound making. For the determination of trace elements by ICP-MS water (18.2 MΩ cm) provided from a MilliQ Millipore water purification system (Millipore, UK) was used. Nitric acid (≥69.0%, TraceSELECT®) was purchased from Fluka (Buchs, Switzerland). Calibration standards were prepared from a 10 mg/L multi element standard AccuTrace® (AccuStandard®, New Haven, USA), and 1000 mg/L B, Sb and Mo single element standards (High-Purity Standards, USA). The certified reference materials (CRMs), Rice 1568a and Whole Egg Powder 8415 both from the National Institute of Standards and Technology (NIST, Gaithersburg, USA), IAEA-140 from the International Atomic Energy Agency (Vienna, Austria) and DOLT4 from National Research Council Canada were used for quality control.

Table 1
Average weight (g) of rats before and after treatment.

	Group 1	Group 2	Group 3
Initial body weight	237.2 ± 7.56	235.6 ± 10.32	234 ± 4.89
Final body weight	237.8 ± 7.42	232 ± 7.54	234.6 ± 5.02

2.2. Date palm saps collection

Date Palm Sap samples were collected from date palm *P. dactylifera* L. trees of the Beser variety from a palm grove in Tozeur; in the south of Tunisia. The local traditional sap collection method was used. It consisted in cutting off the growing point of the palm tree. The juice was then collected from a shallow depression scooped out at the top [2,13]. The sample (fresh sap) was collected in sterile plastic containers and immediately stored in an ice box (+4 °C) to avoid fermentation during transportation to the laboratory.

2.3. Experimentally induced wounds

Wistar adult male rats were randomly divided into 3 groups of 5 rats each. Each rat that weighed 235.6 ± 1.6 g was housed separately (one rat per cage). The animals were maintained on standard pellet diet and tap water. The animals were anesthetized by diethyl ether and the skin shaved using an electrical shaver, disinfected with 70% alcohol and injected with 1 mL of Lignocaine HCl (2%, 100 mg/5 mL). An area of uniform wound (1.5 cm × 1 cm) was excised from the nape of the dorsal neck of all rats with the aid of round seal as described by Suguna et al. [13]. Incision of the muscle layer and tension of skin were constantly avoided during the procedure.

2.4. Topical application of vehicles

Wounds of Group 3 rats were treated twice daily with sterile physiological serum as a negative control. Group 2 wounds were treated with a thin layer of "CICAFLORA ®" twice daily as a positive control. Group 1 animals were treated topically with a date palm sap twice daily. The wounds were observed on a daily basis until complete wound-healing enclosure occurred.

2.5. Evaluation of healing effect

The evaluation of the healing effect was based on macroscopic and microscopic criteria.

2.6. Qualitative assessment of wound healing

The wounds were photographed daily. Based on the colour of the wounds, we assigned a chromatic code to the wound of each rat (bright red = blood covering the wound, dark red = coagulation of dermal elements of skin (crust), red = granulation tissue and pink = the phase of epithelialisation).

2.7. Quantitative evaluation of the healing

2.7.1. Evaluation of the wound area

A measurement of the wound area was performed daily by drawing a borderline on the edge of the wound with a marker in order to determine the evolution of wound surfaces. The calculation of the wound surface was obtained by applying the following formula:

$$\text{The area}(\text{cm}^2) = \frac{\text{mass of paper sheet corresponding to the shape of the wound}}{\text{the mass of a 1 cm}^2 \text{ paper sheet}}.$$