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

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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.

Keywords: Date palm sap; minerals; Wound healing; LC-MS analysis; Antioxydant; bioactive phenolic compounds.

Abbreviations:

Inductively Coupled Plasma Mass Spectrometry: ICP-MS

Liquid Chromatography Mass Spectrometry: LC-MS

High Resolution hyphenated LC-MS (LC-HRESIMS)

The certified reference materials: CRMs

Gallic Acid Equivalent: GAE

Quercetin Equivalent: QE

1,1-Diphenyl-2-picryl-hydrazyl: DPPH

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:

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®" was 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.

2.2. Date palm saps collection

Date Palm Sap samples were collected from date palm *Phoenix 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

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:

The area (cm^2) = mass of paper sheet corresponding to the shape of the wound / the mass of a 1 cm^2 paper sheet.

Evaluation of wound contraction rate: This rate indicates the status of epithelialisation. It is calculated from the ratio between the healed area and the original area of the injury. The area (A) is calculated, after reproduction of the wound on a transparent sheet.

$$\text{Percentage of contraction} = \frac{\text{scarred area}}{\text{total wound area}} \times 100$$

$$= \frac{\text{initial surface(D1)} - \text{measured surface}}{\text{initial surface(D1)}} \times 100$$

2.8. Histological Evaluation of Healed Wounds

The skin specimens from wound healed areas were fixed in 10% buffered formalin and processed by a paraffin tissue processing machine. The healed skin was assessed by taking a 3-4 µm section followed by staining with hematoxylin and eosin.

2.9. Ethics

The experimental protocols were conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax, Tunisia, and approved by the Committee of Animal Ethics of the Faculty of Medicine of Sfax.

2.10. Sample preparation for trace element determination and ICP-MS analysis

The sap samples were diluted 1:100 by weight in 5 % nitric acid. The certified reference materials were digested using 2 mL conc. nitric acid in an Ethos Up microwave system with inserts (temperature program 0-15 min: RT to 200 ºC, 15 min holding at 200 ºC) and diluted with water. For the determination of the total element content an Agilent 8800 Triple Quadrupole ICP-MS (Agilent Technologies, Waldbronn, Germany) equipped with a Scott-type spray chamber and a MicroMist concentric glass nebulizer (Glass Expansion, West Melbourne, Australia) was used. The sample and skimmer cones were made of Ni. The ICP-QQQ was operated in no gas (MS-mode), helium (MS-mode), hydrogen (MS/MS-mode) and oxygen (MS/MS-mode) mode for different elements. Collision/ reaction cell (CRC) gas flow rates for helium, hydrogen and oxygen were 4.5 mL/min, 3.5 mL/min and 30% respectively. Germanium was used as an internal standard and was introduced externally via the peristaltic pump of the ICP-QQQ and a T-piece to the nebulizer. The ICP-QQQ was optimized for maximum sensitivity using robust plasma conditions. All samples were measured in triplicate with the results averaged. The recoveries for the CRMs were between 70 and 120 % of the certified values.

2.11. Extract preparation and LC-MS analysis

One hundred mg of the sap was dissolved in 100 mL of 10% of methanol, filtered and 1 mL was transferred to LC-MS vials. Reversed-phase column (Pursuit XRs ULTRA 2.8, C18, 100 × 2 mm, Agilent Technologies, UK) was used to carry out HPLC analyses. Twenty µL of the sample have been injected at a column temperature set at 30 °C. Mobile phases consisted of 0.1% formic acid in water (A) and 0.1 % formic acid in MeOH (B). A gradient program was used for separation at a flow rate of 1 mL/min. Mobile phases consisted of an initial composition of 100% solvent A, with a gradient to 100% solvent B over 20 minutes, hold on 100% solvent B for 5 min and to 100% solvent A for 25 min. Drying gas flow rate was 1 mL/min at 320 °C. MS was operated in the positive ion mode in a mass range of *m/z* 100-2000. High resolution mass spectral data were obtained on a Thermo Instruments ESI-MS system (LTQ XL/LTQ Orbitrap Discovery, UK) connected to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Accela Pump).

2.12. Statistical Analysis:

Mean weights and wound areas sizes are provided with their standard deviation. Comparing averages of the weight and sizes was carried out using analysis of variance (ANOVA). The difference was considered significant at the $P < 0.05$.

3. Results

3.1. General characteristics of rats: Follow up of Weight

The comparison of the average weight of the rats from the same group before and after treatment was not statistically significant ($P > 0.05$) for the five groups (Table 1). There was no death among all the rats during the experiment.

3.2. Qualitative evaluation of wound healing

Photographs of the wounds of a representative rat from each group were taken on day 1, 3, 6, 9 and 12, respectively, after wound induction, at the end of the inflammatory phase, during formation of granulation tissue, during re-epithelialisation phase and during the day of sacrifice (Figure 1). The wounds of the rats of all groups on the first day showed a bright red colour corresponding to the blood that covers the wound. After two successive daily applications of vehicles in the different groups (day 3), wound aspects differ across the different groups and vehicle used. In the third group (Control group treated with physiological serum) wounds showed perilesional inflammatory redness and thick beads. However, in the first group (sap treated group) and the second group (CICAFLORA treated group) a dark red colour with an apparent decrease in the area of wounds was observed. On the sixth day, the wounds in group 1 had a brown colour characteristic of the presence of the crust with a narrowing of the wound surface. In group 2, the wounds began to form their crusts and their surfaces began to decline but the third group had a greyish, yellowish white colour and a larger area. On, day 9 some scabs in Group 1 began to fall to let appear a pinkish colour of granulation tissue. In group 2 a brown coloration continued to appear. Wound surfaces were remarkably reduced compared to controls but never caught up with the first group. The last group (group 3) still had a red colour. On day 12, we noticed a complete healing in group 1. In addition, the fall of fragments of crusts let appear pinkish colour granulation tissues in the second group. However, a red colour was still noticed in group 3.

3.3. Quantitative assessment of wound healing

Wound healing activity was investigated in rats treated with date palm sap (group 1), CICAFLORA gel (group 2) and an untreated control group. The average of wounds in group 1 was significantly smaller than that in group 3 (Table 2 and Table 4). A total wound closure for group 1 (Table 2) was complete at the end of the 12^{th} day. However, according to previous

studies, the complete closure of a treated wound with "CICAFLORA®" in group 2 (Table 3) was completed on the 14th day. According to literature, the natural contraction of wounds was on the 21st day. Therefore, the date palm sap seems to accelerate cell proliferation and contribute to faster healing with a gain of more than 30% compared to the group treated with CICAFLORA®.

3.4. Histological study

To perform the histological evaluation of wound healing of the different groups, all rats were sacrificed on day 12 and biopsies were taken. Histological sections that were made after staining with hematoxylin-eosin are represented by the Figure 2. Complete tissue regeneration was observed in groups 1 and 2. However, total absence of tissue regeneration was noticed in group 3. Moreover, a total absence of skin regeneration annexes was noted (sebaceous glands and hair follicles) for all groups. Observation using optical microscopy of the various biopsies, showed that in the third group, healing was obviously delayed. The healing area consisted of a granulation tissue with many vessels and inflammatory cells showing chronic persistent inflammation. In Group 2, a very low epithelium inflammatory cell density was observed. However, granulation tissue rich with fibroblasts, blood capillaries and a huge content of collagen with the absence of inflammatory cells was observed in group 1 treated with Date Palm Sap.

3.5. Trace elements

The concentration of minor and major elements present in sap was determined by ICP-MS on a dry weight basis (see Table 5 for elements and concentrations). The sap proved rich in Ca (26.2±3.1 mg/kg), Cu (1.13±0.018 mg/kg) and Zn (3.65±0.026 mg/kg).

3.6. Sugar content

The sap of the Beser variety showed the presence of the 2-deoxy-scyllo-inososе as well as fructose, glucose, sucrose and difructose anhydride (Table 6).

3.7. Characterisation of phenolic compounds using LC-HRESIMS

LC-ESIMS analysis of DSP indicated the presence of at least 16 compounds belonging to different structural classes of phenolic compounds such as flavonoids and bi-flavonoids, phenolic glycosides, and gallic acid derivatives (Table 7): tubuloside A, tubuloside B, viscarticulide A, 5'-O-methyl-7'-ethyl ester of p-dehydrodigallic acid, 2-acetyl-1,3-di[(E)-feruloyl] glycerol, 2,4,5-tri-O-methylhiascic acid and (8R,7'*S*,8'*R*)-5,5'-dimethoxylariciresinol-9'-O-β-D-(6-O-E-4-hydroxy-3,5-dimethoxycinnamoyl) glucopyranoside, 7-O-(β-D-glucopyranosyl)diphysin, Ormocarpin, 5-O-[β-D-glucopyranosyl-(1->6)-β-D-glucopyranosyl]-8-hydroxybergaptol, ferunide, and 5-epipentenomycin I. The identification of these compounds was based on their MS^n characteristic fragmentation pattern after being suggested by the Dictionary of Natural products using the molecular structural formulae.

4. Discussion

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to their normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage where the area of the wound shrinks. It has 3 phases, inflammatory, proliferative and maturational depending on the type and extent of damage, the general state of the host's health and the ability of the tissue to repair. The inflammatory phase is characterized by haemostasis and inflammation, followed by epithelialisation, angiogenesis and collagen deposition in the proliferative phase [14]. In the maturational phase, the wound undergoes contracture resulting in a smaller amount of apparent scar tissue. The present study shows that date palm sap of the Beser variety could be used to significantly enhance the rate of wound healing. "Lagmi" possess a broad spectrum of biological activities. The production of antioxidants was shown to contribute to the stimulation of wound healing mechanisms [15], therefore, sap of the Beser

variety with a DPPH value of 63.09±1.63% and a Total antioxidant capacity of 136.28±0.31 mg vitamin/g provides a favourable environment for tissue healing in wound sites [2]. Wound healing was also linked to an up regulation of human collagen I expression [16] and an increase in tensile strength of the wounds [17]. Enhanced healing activity was attributed to increased collagen formation and angiogenesis [15,18]. Angiogenesis in granulation tissues improves circulation to the wound site thus providing oxygen and essential nutrients for the healing process with enhanced epithelial cell proliferation [19]. Analysis of the date palm sap "Lagmi" showed the presence of several minerals. Toxic elements like arsenic, lead, cadmium and mercury were near or below the detection limit. Sb concentration was near the detection limit. Date palm sap contained alkali and earth alkali elements at the expected ranges, with magnesium and calcium being the dominant elements. With the exception of Ni and Si which has not been observed in Beser sap, all the elements which have been reported as being important for wound healing processes Ca (26.2±3.1 mg/kg), Cu (1.13±0.018 mg/kg) and Zn (3.65±0.026 mg/kg) have been detected [20] (Lansdown, 1995). These minerals, may therefore, help the healing process by providing essential nutrients for the healing process [20]. Sugar content of the Beser variety have also been investigated and shows the presence of 2-deoxy-scyllo-inososе, a compound described for its anti-oxidative scavenging activity necessary for efficient wound healing [21]. We also recovered sucrose, glucose and fructose which have no beneficial effect on wound healing [22,23]. Flavonoids, biflavonoids and other polyphenols are known to promote the wound healing process due to their astringent and antimicrobial properties [24,25], which appear to be responsible for wound contraction and an increased rate of epithelialisation. Flavonoids and polyphenols contents of 0.69± 0.028 mg QE/g and 274.56±0.76 mg GAE/ g of the Beser variety may, therefore, be responsible for its wound healing effect [2].

In order to further investigate the polyphenols and flavonoid fractions of the sap we submitted the sap to LC-HRESIMS analysis (Table 7). This analysis shows that numerous polyphenols and flavonoids that have already been shown to have interesting wound healing activities with other coagulation-enhancing components are present. Tubuloside A and B have well documented anti-inflammatory activity [26,27,28]. Viscarticulide A and 2-acetyl-1,3-di [(E) -feruloyl] glycerol has also interesting anti-inflammatory activities that helps wound healing [29,30]. The presence of the bioflavonoids ormocarpin and 7-O-(β-D-glucopyranosyl)diphysin which exhibit strong antimicrobial activity against a diverse panel of Gram positive and Gram negative microbes as well as their antiprotozoal activities adds a great value in enhancing wound healing effect of the sap [31]. 5-O-[β-D-glucopyranosyl-(1->6)-β-D-glucopyranosyl]-8-hydroxybergaptol found during the course of this study have an anticoagulant activity, that could be useful for wound healing [32]. 5-epipentinomycin I described in Table 7 have an antibacterial activity [33]. Phenolic glycoside derivative (reported compound 1) also recovered have an anti-inflammatory and antioxidant activity [34]. Finally, Ferunide (Table 7) with its associated 5-lipoxygenase inhibitory effect is of benefit to wound healing [35,36,37]. The high costs associated with wound care, diabetic foot wounds in particular, make it important for clinicians and researchers to search for alternative therapies and to optimally incorporate them in the wound care protocols appropriately. Biswas et al. [38] examined the use of sugar as a treatment option in diabetic foot care and provided guidance for its appropriate use in healing foot ulcers. Mphande et al. [39] has compared honey and sugar for use as remedies for healing. We believe that more research is needed on Beser sap to efficiently provide a cost effective highly efficient mean for diabetic food wounds for example at least in the regions where the sap of the beser variety is consumed. Three compounds namely 2, 4, 5-tri-O-methylhiascic acid, 5’-O-methyl-7’-ethyl ester of p-dehydrodigallic acid and (8R, 7'S, 8'R) -5,5'-dimethoxylariciresinol-9'-O-β-D- (6-OE-4-hydroxy-3,5-dimethoxycinnamoyl)

glucopyranoside have been identified by LCMS analysis but no biological activity reported till now in literature. Ongoing research targeting putative activity of these compounds in undertaken in our laboratory. The current study indicated that the direct application of Beser date palm sap on wounds significantly enhanced the wound healing process in experimental rats. To our knowledge, this is the first study to show that date Palm sap enhances wound healing.

5. Conclusion

Our findings clearly demonstrate that Date Palm Sap has a significant stimulating effect on wound healing in rats. The minerals, flavonoids, and polyphenolic content as well as the antioxidant activity and the total antioxidant capacities seem to be the basis of the observed wound healing effect. Investigation of the polyphenol and the flavonoid fraction using LC-HRESIMS recovered compounds known for their anti-inflammatory and wound healing activities as well as promising candidates that have not yet been associated to a benefit wound healing activity. More importantly, our results contribute toward the validation of the traditional use of date palm sap for the treatment of many diseases and may provide an efficient remedy for diabetic food wounds for example.

Acknowledgment

Financial support of the Tunisian Ministry of Higher Education and Scientific Research is gratefully acknowledged.

Conflict of interest

The authors alone are responsible for the content of this paper and they declare no competing financial interests.

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450

451 **Figure captions**

452

453 **Figure 1:** Photographs of wounds in rats of L1, L2 and L3 groups at different days (Day 1 (D1)
454 to Day 12 (D12))

455 **Figure 2.** Histological sections of healed wounds. (A) Wound treated with CICAFLORA in a
456 Group 2 rat (G: 10 * 10). (B) Wound treated with Date Palm Sap in a Group 1 rat (G: 10 * 10).
457 (C) Wound treated with physiologic serum in a Group 3 rat (G: 10 * 10).

458 Der: Dermis; Ep: Epidermis; (↓) : Collagean; (▲) : Blood vessel ; (↖): Inflammatory nucleus
459

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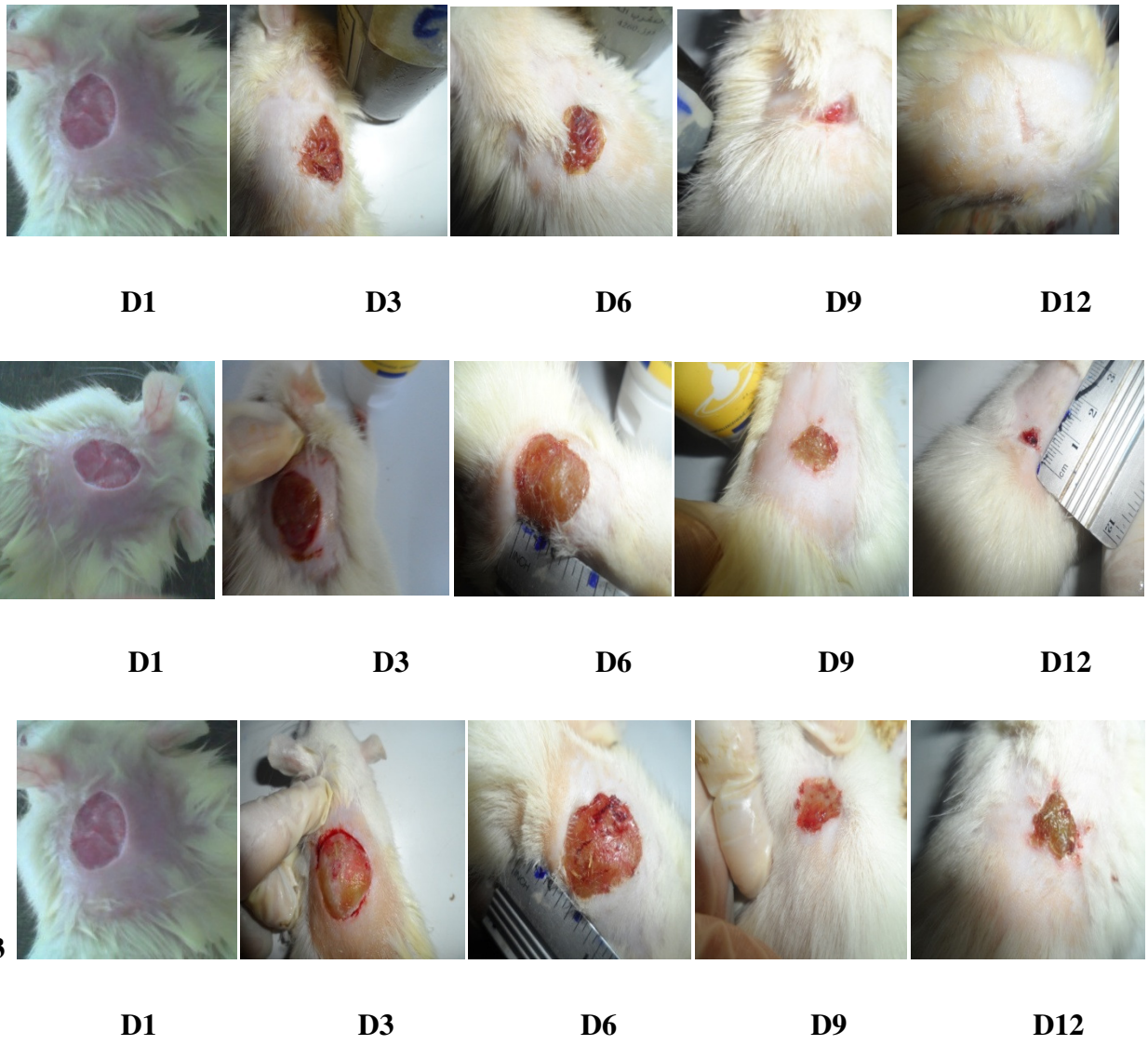

Figure 1. Photographs of wounds in rats of L1, L2 and L3 at different days (D1 until D12)

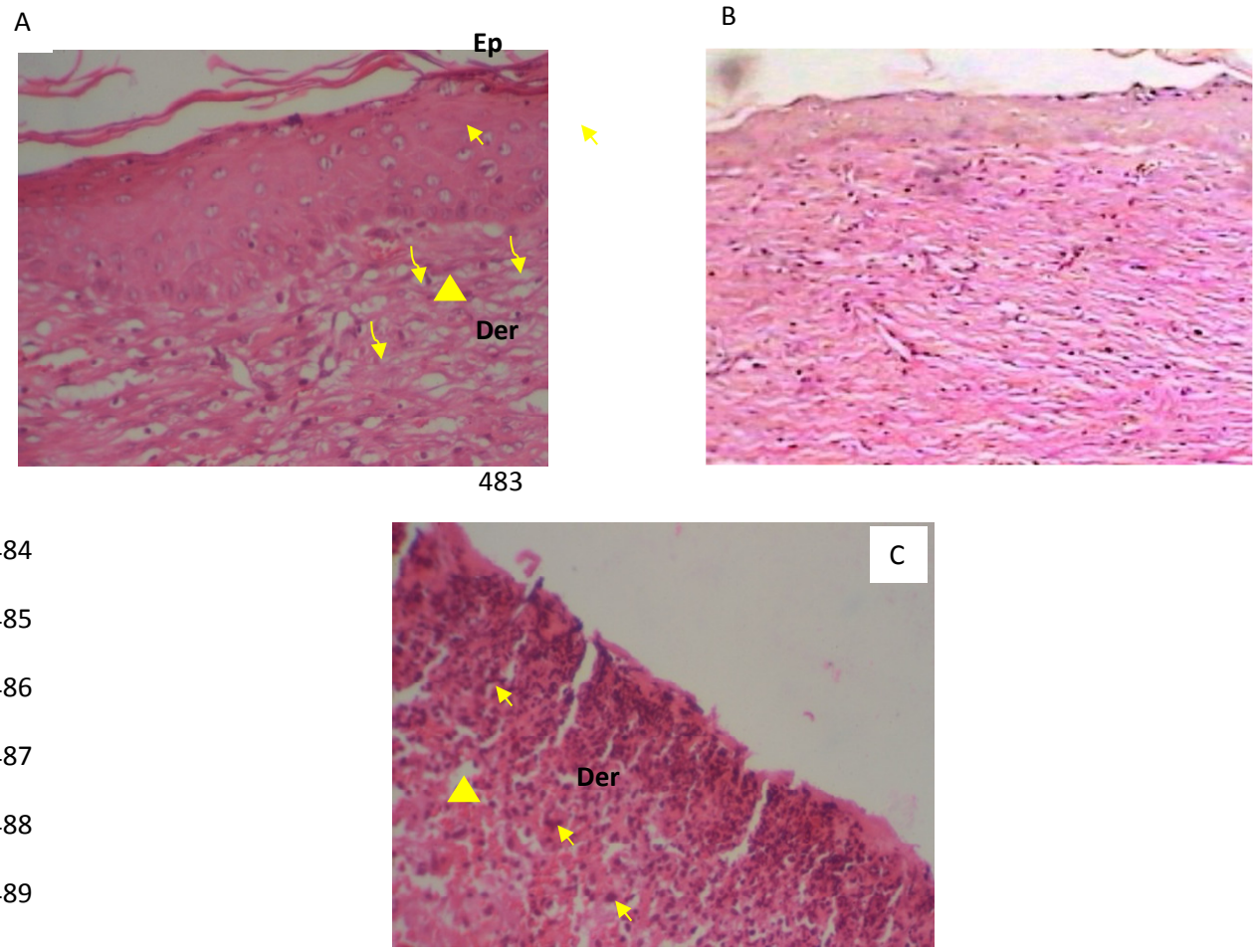

Figure 2. Histological sections of healed wounds. (A) Wound treated with CICAFLORA in a Group 2 rat (G: 10 * 10). (B) Wound treated with Date Palm Sap in a Group 1 rat (G: 10 * 10) .(C) Wound treated with physiologic serum in a Group 3 rat (G: 10 * 10).

Der : Dermis ; Ep : Epidermis ; ( ) : Collagean ; ( ) : Blood vessel ; ( ) : Inflammatory nucleus

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

Table 2: Group 1 (date palm sap) evolution of the wound surface

Area (cm^2)	D1	D3	D6	D9	D12
Rat 1	**1.18**	**0.609**	**0.565**	**0.235**	**0**
Rat 2	**1.178**	**0.942**	**0.504**	**0.282**	**0.15**
Rat 3	**1.179**	**0.918**	**0.705**	**0.439**	**0.19**
Rat 4	**1.178**	**0.758**	**0.545**	**0.274**	**0**
Rat 5	**1.179**	**0.863**	**0.436**	**0.192**	**0**
Average	**1.178 ± 0.0008**	**0.834 ± 0.102**	**0.551 ± 0.099**	**0.284 ± 0.093**	

Table 3: Group 2 (Cicaflora) evolution of the wound surface

Area (cm^2)	Day 1	Day 3	Day 6	Day 9	Day 12
Rat 1	**1.178**	**1.177**	**0.847**	**0.596**	**0.157**
Rat 2	**1.178**	**1.020**	**0.787**	**0.412**	**0.035**
Rat 3	**1.177**	**1.099**	**0.918**	**0.471**	**0.157**
Rat 4	**1.178**	**1.177**	**0.863**	**0.371**	**0.251**
Rat 5	**1.178**	**1.025**	**0.706**	**-**	**-**
Average	**1.178 ± 0.004**	**1.099 ± 0.077**	**0.824 ± 0.08**	**0.462 ± 0.097**	**0.150 ± 0.088**

Table 4: Group 3 (Control group) evolution of the wound area.

Area (cm^2)	Day 1	Day 3	Day 6	Day 9	Day 12
Rat 1	1.178	1.176	1.057	0.628	0.384
Rat 2	1.178	1.177	0.989	0.635	0.314
Rat 3	1.177	1.176	1.107	0.604	0.141
Rat 4	1.177	1.175	1.081	0.942	0.533
Rat 5	1.179	1.177	0.981	0.753	0.376
Average	1.178 ± 0.0004	1.176 ± 0.0009	1.043 ± 0.055	0.712 ± 0.014	0.349 ± 0.0141

Table 5: element concentrations determined in sap (n=3, mean ±sd) by ICP-MS on a dry weight basis

Element		Beser
Li	µg/kg	72.8±8.4
Rb	mg/kg	2.45±0.27
Cs	µg/kg	5.90±1.1
Mg	mg/kg	255±38
Ca	mg/kg	26.2±3.1
Sr	mg/kg	0.367±0.030
Ba	µg/kg	45.1±5.2
Mn	mg/kg	0.970±0.11
Fe	mg/kg	5.94±0.28
Cu	mg/kg	1.13±0.018
Zn	mg/kg	3.65±0.026
Se	mg/kg	0.276±0.020
V	µg/kg	25.1±2.5
Cr	µg/kg	39.1±6.1
Al	mg/kg	1.06±0.45
As	µg/kg	16.55±1.3
Sb	µg/kg	1.63±0.17
Cd	µg/kg	< 0.2
Hg	µg/kg	<40
Pb	µg/kg	<13

Table 6: Determination of saccharides using High Resolution Electrospray Ionization Mass Spectrometry (HRESIMS) and literature review of their biological properties.

HRESIMS [a]	Mol formula [a]	Suggested compound [b]	Biological properties	Reference
343.1234	$C_{12}H_{22}O_{11}$	Sucrose	No effect on wound healing	Kössi et al. (2000)
325.1130	$C_{12}H_{20}O_{10}$	difructose anhydride		
181.0710	$C_6H_{12}O_6$	fructose/glucose	No effect on wound healing	Kössi et al. (1999)
163.0599	$C_6H_{10}O_5$	2-deoxy-scyllo-inososе	Antioxidative scavenging activity	Ajisaka et al. (2009)

[a] High Resolution Electrospray Ionization Mass Spectrometry (HRESIMS) using Xcalibur 3.0 and allowing for M+H and M+Na adducts.

[b] The suggested compound according to Dictionary of Natural Products (DNP 23.1, 2015 on DVD) and characteristic fragmentation pattern.

Table 7: Determination of DSP phenolic compounds using High Resolution Electrospray Ionization Mass Spectrometry (HRESIMS) and literature review of their biological properties.

HRESIMS [a]	Mol formula [a]	Suggested compound [b]	Biological properties	Reference
851.26455	$C_{37}H_{48}O_{21}$	Tubuloside A	Has nitric oxide radical-scavenging activity, which possibly contributes to its anti-inflammatory effects.	Xiong et al. (2000)
689.21161	$C_{31}H_{38}O_{16}$	Tubuloside B	Prevents 1-methyl-4-phenylpyridinium ion (MPP +)-induced apoptosis and oxidative stress and may be applied as an antiparkinsonian agent Has the neuroprotective capacity to antagonize TNF alpha-induced apoptosis in SH-SY5Y cells and may be useful in treating some neurodegenerative diseases.	Sheng et al. (2002) Deng et al. (2004)
723.19603	$C_{34}H_{36}O_{16}$	Viscarticulide A	Improved survival of human endothelial-like immortalized cells after exposure to H_2O_2	Li et al. (2015)
487.16544	$C_{25}H_{26}O_{10}$	2-acetyl-1,3-di[(E)-feruloyl]glycerol	Improved survival of human endothelial-like immortalized cells after exposure to H_2O_2 Anti-inflammatory	Li et al. (2015) Shi et al., 2015.

867.2383	$C_{42}H_{42}O_{20}$	Ormocarpin (biflavonoid glycoside)	Antimicrobial activity	Dhooghea et al. (2010)
705.1852	$C_{36}H_{32}O_{15}$	7-O-(β-D-glucopyranosyl)diphysin (biflavonoid glycoside)	Antimicrobial activity	Dhooghea et al. (2010)
543.1318	$C_{23}H_{26}O_{15}$	5-O-[β-D-glucopyranosyl-(1->6)-β-D-glucopyranosyl]-8-hydroxybergaptol	anticoagulant	Weilie et al. (2005)
289.0920	$C_{12}H_{16}O_8$	ferunide	5-Lipoxygenase Inhibitory effect	Znati et al. (2014) Cottrell and O'Connor, (2009) Brogliato et al. (2014)
271.0814	$C_{12}H_{14}O_7$	Phenolic glycoside derivative (reported compound 1)	anti-inflammatory and anti-oxidant	Suo et al. (2012)
145.0492	$C_6H_8O_4$	5-epipentenomycin I	Antibacterial activity against Gram positive bacteria and weak against *Pseudomonas aeruginosa*	Baute et al. (1991)
527.15803	$C_{27}H_{26}O_{11}$	2,4,5-tri-O-methylhiascic acid	No biological activity reported.	-
381.07945	$C_{17}H_{16}O_{10}$	5'-O-methyl-7'-ethyl ester of p-dehydrodigallic acid	No biological activity reported.	-
811.27179	$C_{39}H_{48}O_{17}$	(8R,7'S,8'R)-5,5'-dimethoxylariciresinol 9'-O-β-D-(6-O-E-4-hydroxy-3,5-dimethoxycinnamoyl)glucopyranoside	No biological activity reported.	-
867.2383	$C_{42}H_{42}O_{20}$	Ormocarpin (biflavonoid)	Antimicrobial activity	Dhooghea et al. (2010)
705.1852	$C_{36}H_{32}O_{15}$	7-O-(β-D-glucopyranosyl)diphysin (biflavonoid)	Antimicrobial activity	Dhooghea et al. (2010)
271.0814	$C_{12}H_{14}O_7$	Phenolic glycoside derivative (reported compound 1)	anti-inflammatory and anti-oxidant	Suo et al. (2012)

[a] High Resolution Electrospray Ionization Mass Spectrometry (HRESIMS) using Xcalibur 3.0 and allowing for M+H and M+Na adducts.

[b] The suggested compound according to Dictionary of Natural Products (DNP 23.1, 2015 on DVD) and characteristic fragmentation pattern.

Graphical Abstract