Phytochemicals Screening and evaluation of phoenix dactylifera L. seeds oil functional properties on wound healing in rats

Sura Louy Al-Ghabban1, Manar Serhan Ahmed2, Ali Taha Metaib1, Israa Ghassan Hussein4
1,2Department of pharmaceutical chemistry, college of pharmacy, Al Farahidi University., Baghdad, Iraq
4Department of pharmacology, college of pharmacy, Al Farahidi University., Baghdad, Iraq
Email: Surahalghaban@yahoo.com

Abstract

The seeds of phoenix dactylifera L. plants have many traditional uses of various kinds for disease, one folklore use of the oil of the plant seeds is the treatment of wound healing. this study undertaken the composition and content of certain bioactive constituents of the oil obtained from phoenix dactylifera L. seeds and the effect of the phoenix dactylifera L. seeds on wound healing in the rats. Uniform wounds were induced on the dorsum of 18 rats, randomly divided into three groups. The wounds were photographed, and topically treated with nothing (control group), 0.13 mg/mm2 of a reference drug (“Mebocream®”), and 0.52 μl/mm2 of phoenix dactylifera oil daily for 12 days and biopsies were histologically assessed. morphometric assessment and histological findings revealed healed biopsies from the plant oil-treated group of rats, unlike untreated group, and a full re-epithelialization with the reappearance of skin appendages and well organized collagen fibers without inflammatory cells.

Keywords: phoenix dactylifera L. seed oil, Fatty acids, Phytosterols, Wound healing.

INTRODUCTION

P. dactylifera L. family Arecaleae commonly known as date palm is a flowering plant species in the palm, cultivated for its edible sweet fruit. Its place of origin is the lands of Iraq. The species is widely cultivated and is naturalized in many tropical and subtropical regions worldwide(1,2) It belong to the monocotyledon plant from family Arecaleae the fruit has five growth stages were name khababauk, kimri, khalal, rutaband tamer .It is used as a source of food for humans and animals the first domestication of date palm is believed to have taken placed at least 6000 years ago in Mesopotamia the land between the tigris and Euphrates rivers in Iraq phoenix dactylifera L. date plam seed is used traditionally to wounds, lesions, inflammation demulcent laxative and relieve toothache and ague also it has many medicine activity as Anticancer activity, Anti-diarrheal Activity, Hepato-protective activity, Antioxidant activity, Anti-inflammatory activity antiviral activity, Nephroprotective activity.

Date palm seeds oil is considered a rich source of fatty acid and phytoerol compounds which have anti-inflammatory activity. The main target of the present study is to investigate the phytochemical compounds present in oil of powder seed extract in the phoenixa dactylifera L. which is cultivated in Iraq by chemical tests and determine the presence of some phytosterol compound by GC /MASS, HPLC and evaluation oil plant extract seeds functional properties on wound healing in rats.

Address for correspondence: Sura Louy Al-Ghabban, college of pharmacy, Al Farahidi University., Baghdad, Iraq
Email: Surahalghaban@yahoo.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: pnjournal@gmail.com

How to cite this article: Sura Louy Al-Ghabban, Manar Serhan Ahmed, Ali Taha Metaib, Israa Ghassan Hussein, Phytochemicals Screening and evaluation of phoenix dactylifera L. seeds oil functional properties on wound healing in rats, J PHARM NEGATIVE RESULTS 2022;13:382-388.
**Material and Methods:**

Plant material: Plant collected from a garden in Diyala during the month of July – October in the year 2020. It was in khalal stage. The variety of plant is Barhee. The plant was identified by Date palm Zaaafaranya station/Ministry of Agriculture.

Extraction: Amount of 100g of dried powder seeds of *P. dactylifera L.* was maceration with 500ml n-hexane for 24 hours at room temperature, and filtrated. The extracted plant material dried at room temperature then maceration in water for 24 hours, then filtered and dried at room temperature, the residual plant material was refluxed with 500ml of 2N HCl solution for 2 hours after filtration, then 5% ammonia solution was added to the extracted plant part, and washed by distilled water several time to neutralization. The extracted plant part dried and extracted by using soxhlet apparatus with 500ml petroleum ether at 60-90°C for 10 hours. The petroleum ether filtrate evaporated to get the crude extract.(5)

Analytical methods:

1. **Libermann Burchard test:** The presence of sterol compound in the crude extract of *P. dactylifera L.* seed was detected by adding a few drops of acetic anhydride to 1ml of the crude extract indicated the result, then a few drops of sulfuric acid was added, this result indicated the presence of sterol. (6)

2. **Qualitative Identification of sterol:** Qualitative identification of sterol (betasitosterol) compound in the crude extract of *P. dactylifera L.* seed in Iraq was detected by using two techniques which are (TLC),(HPLC) and GC-MS. The first technique (TLC) is done by using readymade Silica gel aluminum plates GF 254, detection by 5% H2SO4 solution was added to the extracted plant part, and washed with distilled water several time to neutralization. The second technique (HPLC) is done by using Shimadzu LC C18 high-performance liquid chromatography at which identical chromatographic conditions, and identification were made by comparison the retention time plant extract and authenticated sterol standard. (10)

The second technique (HPLC) is done by using Shimadzu LC C18 high-performance liquid chromatography at which identical chromatographic conditions, and identification were made by comparison the retention time plant extract and authenticated sterol standard. (10)

GC-MS screening was carried out on a Shimadzu GCMSQP2010Ultra system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite- fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), helium (99.999%) was used as carrier gas at a Flow Control Mode Pressure:100.0 kPa, Column Flow: 1.6 ml/min, Linear Velocity: 47 cm/sec, Purge Flow: 3.0 mL/min, Split Ratio:5.0, injector temperature 280.0°C; ion-source temperature 50°C. The oven temperature was programmed from 50°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 300°C, ending by increasing The temperature 10 degrees per minute until complete elution. Mass spectra were taken at 70 eV, then the time required for sample chromatography was 32 minutes. (11)

c. **Determination of betasitosterol in the extract:** is done by using High-Performance Thin-layer chromatography of the crude extract confirms the presence and determine the concentration of betasitosterol which done by using development solvent system S1, that gives same RF value which is reading at UV 196nm and For doing a calibration curve determine concentration.

Evaluation of *P. dactylifera L.* crude extract pharmacological properties on wound healing: the evaluation occurred the vivo experiment and was done by the following:

a. **Experiment Animals:**

Eighteen adult female albino rats with (180-200 gram) body weight were used. Aged between (2 to 3) months. They were obtained from the animals house of Baghdad university /college pharmacy and placed in sterilized cages subjected to constant environmental conditions.

These animals were kept under the conditions of controlled temperature at 21-25°C and a photoperiod of 12 hours daily. The food was given as pellets of freshly prepared ration.

b. **Induction of wounds:**

On the first day, each rat was anesthetized by using diethyl ether then the rats were shaved and cleaned. (1.5 cm×1 cm) full thickness open excision wound was made by removing a patch of skin.

c. **Wound model:**

A total of 18 animals were divided into three groups of six animals each:

- **Group 1:** rats were the negative group not treated with drug (control group).
- **Group 2:** rats were treated with 0.13 mg/mm2 of a reference drug “Mebo cream®” (standard group).
- **Group 3:** wounds were treated with 0.52 μl/mm2 of crude extract of *P. dactylifera L.* (tested group).

d. **Measuring bleeding time in rats:**

Hemostasis is a physiologic defense mechanism that guards the integrity of the vascular system. It involves platelet aggregation and coagulation. Bleeding time is a basic test of primary hemostasis to assess platelet function and the body’s ability to form a clot. Female adult Wistar rats were divided into three groups. The tail of each rat was cut with a scalpel.
blade, and a drop of the test substance was applied to the cut simultaneously with the start of the stopwatch. The control group was not treated and determined the bleeding time while the standard group chopped tails were dipped in distilled water or normal saline. The tested groups’ chopped tails were dipped in crude extracted sterols. All the chopped tails were then positioned vertically on top of the filter paper and bleeding time was taken as the time for the first drop of blood to show to the time when the filter paper stopped showing blood stains.

e. Measuring body weight:
Body weight was measured every 4 days.

f. Measurement of wound area:
The wound area was measured by tracing manually the wound every 4 days. The shapes of the wounds and the wound surface areas were measured. The wound contraction was expressed as a reduction of the original wound size percentage. The percentage of wound contraction was calculated by using the following equation:

Percentage of wound contraction =
(Initial wound size – specific day wound size) x 100/Initial wound size.

g. Histological examination:
The rats were sacrificed and the tissues from the wound site of the individual rat were collected for histopathological examination purposes. All tissue samples were fixed in 10% neutral buffered for formalin solution for 4 weeks as fixation time, embedded in paraffin wax, cut into 5 μm-thick sections and stained with hematoxylin-eosin.

h. Statistical analysis:
All the data were expressed as mean values±standard deviation (S.D.). Statistical comparisons between groups were carried out using SPSS. In the case of multiple comparisons, repeated measurements of Analysis of Variance (ANOVA) were performed to compare the percentage of wound healing mean differences between and within groups and body weight while a oneway analysis of variance was used to compare bleeding time.

Result

Libermann Burchard test: In this test, 1 ml of crude extract was added to it drop by drop acetic anhydride that gave pink color which then changed to green color when added few drops of sulfuric acid this indicated the presence of sterols.

Qualitative Identification of sterol by TLC technique: The Table (1) lists the Rf value of spot reference betasitosterol standard and the spot result from the extract method of sterols in three different mobile phase (S1,S2, and S3) against beta-sitosterol reference standard. The spots of extract appear the same color and same Rf value as that in beta-sitosterol reference standard on TLC plates which were detected by vision after using 5% H2SO4 spray and heating in an oven for 5 minutes at 100°C.

Table (1): Rf of Betasitoterol reference standard and Betasitosterol compound in the crude extract of seed using different developing solvent systems in TLC

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf value beta-sitosterol standard</td>
<td>0.36</td>
<td>0.76</td>
<td>0.28</td>
</tr>
<tr>
<td>Rf value of beta-sitosterol in extract</td>
<td>0.36</td>
<td>0.75</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Qualitative Identification of sterol by HPLC technique: As shown in table (2) and figures 1,2 the retention time of reference standard and compound in the sterol extract.

Table (2)

<table>
<thead>
<tr>
<th>Compound</th>
<th>The retention time of standards</th>
<th>Retention time in extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betasitosterol</td>
<td>7.766</td>
<td>7.646</td>
</tr>
</tbody>
</table>

Figure (1): HPLC Peak of the beta-sitosterol reference standard

Figure (2): HPLC Peaks of the extracted standard

The result from the sterols method extraction the petroleum ether of sterols cruds extract was subjected to GC/MS analytic. The GC/MS chromatogram, as shown in figure (3) in which the higher peak detected was oleic acid and the most important fragments results from it, were shown in figures Fragment of (4) oleic acid and (5) fragment from result extract:
Evaluation of pharmacological properties on wound healing:

a. Body weight:

The investigation of body weight changes is monitored through the rat’s weight. Those findings showed an increase in rat weight in the standard group and test groups. These variations did not have a significant difference in the mean body weight between the studied groups of rats at the end of the experimental period which proves that the growth of the rats is affected due to complications that occur in the wound.

b. Bleeding time in rats:

Bleeding time is the primary hemostasis test, the crude extract of *P. dactylifera* L. seeds seems to help blood clotting as it has shortened the bleeding time. The hemostatic effect of this tested oil reported in this work could explain its healing effect. As shown as in table (4).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bleeding time (mean /min:sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>3:39±17.6</td>
</tr>
<tr>
<td>Normal saline</td>
<td>2:15±4.9</td>
</tr>
<tr>
<td>Crude extract of <em>P. dactylifera</em> L.</td>
<td>1:21±8.1</td>
</tr>
</tbody>
</table>

Values are given as mean±SD (n=6/group). Data with different letters for each group represent a significant difference at p<0.05.

c. Wound area:

The healing process was checked for 11 days through the experimental period to examine the wound healing potential of the three tested groups by following the size of the wound area and the percentage of contraction rates. The rate examination of the wound percentage of healing of all groups is shown in Table (5). In the tested group (group 3) that was treated with the crude extract of *P. dactylifera* L., as well as the standard group (group 2), significant healing effects on contraction were observed from day 3 to day 11 of the experiment period. Delayed wound healing processes were observed in the control group compared to all the other groups; After 11 days, in contrast to the crude extract of *P. dactylifera* L. seeds(tested group), in which a good recovery of the wounds was achieved, the untreated animals still show an open wound (23.74%) at the end of the experiment while the standard group show open wound(14.9%) and the open wound of crude extract of *P. dactylifera* L. seeds only(9.23%).

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 8</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>0</td>
<td>9.8%±9.70</td>
<td>42.10%±14.40</td>
<td>76.36%±11.83</td>
</tr>
<tr>
<td>Group2</td>
<td>0</td>
<td>21.79%±15.30</td>
<td>63.65%±8.76</td>
<td>85.95%±5.36</td>
</tr>
<tr>
<td>Group3</td>
<td>0</td>
<td>38.52%±12.00</td>
<td>71.17%±6.124</td>
<td>91.77%±7.71</td>
</tr>
</tbody>
</table>
Values are given as mean±SD (n=6/group). Data with different letters for each group represent a significant difference at p<0.0001. Group 1: that not give any thing (control group); Group 2: rats were treated with reference drug “Mebo cream®”; Group 3: rats were treated with the crude extract of P. dactylifera L..

d. Result of histological examination:

The biopsies from wounds were studied on day 12 post excision the result was that the control group (group1) is concerned the epithelial regeneration was incomplete and other are absence of epithelial with inflame moderate presence of inflammatory cell around the wounds as show in figure (4).

The standard group (group2) shows thin epithelial regeneration was complete and other incomplete the epithelial with the moderate presence of inflammatory cell in all rats group as shown in figure (5).

In the tested group (group3) the epithelial regeneration was complete in all biopsies of rats with a mild presence of inflammatory cell as show in figure (6). In all sections found granulation tissue formation and collagen deposition. the result from histological data found wound healing in rats treated with sterol crude extract was better than in control or reference groups due to healing of wounds with less complication.

Figure (6): Sections of the skin biopsies of rats of the control group after 11 days of the induced wound. Showing complete re-epithelization associated with granulation tissue formation and moderate chronic inflammation. Reading in power20X10.
Figure (7): Sections of the skin biopsies of rats of the standard group after 11 days of the induced wound. Showing complete re-epithelization associated with granulation tissue formation and moderate chronic inflammation Reading in power20X10.

Figure (8): Sections of the skin biopsies of rat of the tested group after 11 days of the induced wound. Showing complete re-epithelization associated with granulation tissue formation and mild chronic inflammation Reading in power20X10.
Discussion:

The result has revealed that crude extract of P. dactylifera L seeds is an important source of many healthy components such as antioxidant and antimicrobial agents, the presence of sterols, polyunsaturated fatty acids, and some essential oil in extract make it an excellent drug in pharmaceutics and cosmetics which would provide potential protection against skin problem. Our findings revealed also that cutaneous wound healing in rats treated with crude extract of P. dactylifera L seeds extract was better than control or reference groups by the means of macroscopic, morphometric and histological data.

References

7. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30