Original Article:

Antibacterial and Wound Healing Activities of Topical Gel of *Jatropha variegata* Vahl Extract in Rats

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ABSTRACT

Background: *Jatropha variegata* Vahl is a well-known Yemeni medicinal shrub, used traditionally to treat wounds.

Objectives: To investigate the wound-healing and antibacterial properties of the *J. variegata* extract as a new gel formula.

Methods: The leaves and stems of *J.variegata* were collected from Ibb Province, Yemen, and extracted by maceration in methanol. To ensure the quality and effectivity of the extract, physicochemical and biological investigations were conducted. Finally, the extract was prepared as a gel dosage form for bacterial and experimental animal studies. Twelve female Albino rats were divided into three groups: each rat was inflicted by three wounds. The first wound was treated thrice a day using the gel containing the *J. variegata* extract, the second was treated by a gel without the extract, and the third was control. The first, second, and third groups were treated for 5, 10, and 15 days, respectively, with follow-up of the wounds' diameters. After treatment, the rats were anesthetized before analyzing the anatomy of the rats and the histological examination.

Results: The antibacterial activity of the extract was competent to the positive control against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* with a Minimum Inhibitory Concentration (MIC) of 0.05%. Similarly, 100% of the wounds healed within 15 days when treated using the gel containing the *J. variegata* extract. While just 33% of the control group healed.

Conclusion: *J. variegata* exhibited high antibacterial activity against the targeted strains and remarkable wound healing activity when applied topically on the skin of rats in a gel dosage form. This finding proves an opening for further pharmacological, toxicological, and clinical studies.

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Introduction

The interest in nature as a source of potential chemotherapeutic agents continues, with natural products and or their derivatives representing more than 50% of all drugs in medical use [1]. At least 80% of people around the world use herbal medicine as a part of their primary health care [2].

Jatropha variegata Vahl is a member of the Euphorbiaceae family according to Plantlist.org, World Checklist of Selected Plant Families. Its common name is Blood Epki or Epki. *Jatropha variegata* is a small evergreen tree with lanced and reticulate-veined leaves and pink flowers. The fruits when immature are small and green and when mature become reddish-green. *Jatropha* grows up to 1 m in height, with several slender trunks, and the roots descend to 1 m. However, in cultivation in the mountains of the Yemeni provinces of Taiz and Ibb, it is usually smaller. *Jatropha* contains approximately 170 species of succulent plants, shrubs, and trees. Most of these are native in America, with 66 species found in the old world [3].

There are not enough medical and toxicological studies on this species. However, there are several studies on other *Jatropha* species' pharmacological uses around the world. *J. gossypifolia* has been reported to be of use in connection with cancer, diarrhea, dysentery, skin diseases (leprosy), arthritis, ulcers, gum infections, and wound healing [4]. The young stem of *J. gossypifolia* is used to stop bleeding and itching of cuts, and as a toothbrush, as well as to clean the tongue [5], and the leaf is used for sores, sprains, and rashes [6]. The latex of *Jatropha* is used as a hemostatic and for livestock infection and has anti-cancerous properties [7]. *J. tanjorensis* also exhibits pharmacological activities, including hematological, antimalarial, antimicrobial, hypoglycemic, hypolipidemic, and antihypertensive properties [8]. Furthermore, the roots of *J. glandulifera* are used for diabetes, after preparation of boiling [9].

Wound healing can be defined as a process of restoring cellular structures and tissue layers. The wound healing process in human adults can be divided into three stages: the inflammatory phase, the proliferative phase, and the remodeling phase. Within these phases, there are complex and coordinated events that include chemotaxis, phagocytosis, neocollagenesis, collagen degradation, and collagen remodeling [10]. Besides, angiogenesis, epithelization, and the production of new glycosaminoglycans and proteoglycans are vital to the wound healing process.

The accumulation of these biological processes results in the replacement of normal skin structures with fibroblastic mediated scar tissue. The objective of wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient [11]. The use of herbal medicines in dermatological disorders and skin ulcers has been increasing. Herbal medicines not only heal the wound, as well as internal and external ulcers due to its antiseptic and anti-inflammatory properties but also improves blood flow in the affected area [12, 13].

The topical gel was selected as an appropriate dosage form. This topical dosage form is not greasy and spreads quickly on the skin. The weak oily effect of the topical gel makes it more compatible than any other topical preparations for the patient [14]. Thus, this study was designed to investigate the activity of extract of *J. variegata* Vahl (Appendix 1) against some strains of bacteria and for wound healing after topical application of the extract in gel dosage form using Albino rats, and to evaluate the stability of the prepared gel formula under various conditions.

Materials and Methods

Study area

This study was carried out in the pharmaceutical laboratories at Al-Nasser University, while the evaporation process was carried out in the Yemeni Organization for Standards and Quality Control. Animal trials were carried out in the Department of Biology, Faculty of Applied Sciences, Sana'a University, and the histological study for skin specimens in the Central Laboratories, Sana'a, Yemen.

Ethical considerations

Ethical clearance and approval of the study protocols were obtained from the Ethics Research Committee of Sana'a University on March 3, 2018 (code: 321/2018), and the study followed common ethical principles adopted in phytochemical and experimental pharmacology research. The study animals were investigated and housed according to the guidelines for the Housing of Rats in Scientific Institutions.

Plant collection and extraction

J. variegata Vahl leaves and stems were collected from Ibb Province, Yemen, in November 2017, identified by the taxonomist Dr. Hassan Ibrahim, and stored under

voucher number NU092018 in the Al-Nasser University Pharmacognosy Lab. The sample was sterilized by spraying with methanol and dried in the shade at room temperature for three days until complete drying.

The fresh powder of *J. variegata* Vahl leaves and stems (20 g) was macerated in 400 mL methanol for three days with intermittent shaking before being filtered. Then, a rotary evaporator (Buchi Rotavapor R-200, Germany) was used to concentrate the extract, which was then dried in an oven at 60°C and subsequently stored at 4°C in dark bottles.

Physical and Phytochemical screening of the crude extract

Organoleptic properties, pH, and solubility of the crude extract were assessed. Phytochemical screening for the phytochemical constituents was accomplished by chemical tests [15] and thin layer chromatography under UV lamp, wavelength 365 nm (Bibby Scientific Ltd, UK), using the mobile phases and spray chemical reagents specified in British Pharmacopeia [16].

Verification of the analytical method

About 4 g of *J. variegata* Vahl extract was dissolved in 20 mL of distilled water before being filtrated into a 50 mL volumetric flask. Series dilutions were prepared (200, 100, 50, and 25 mg/mL) to detect the absorbance by UV spectrophotometry at a wavelength of 280 nm, and the mean was calculated to construct the calibration curve.

Antibacterial screening

Three bacterial strains (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922) included in this study were obtained from the National Center Health of Public Health Laboratories, Sana'a, Yemen.

The antibacterial screening was carried out by disk diffusion assay. The turbidity of bacterial cell suspension was matched with the McFarland Standard, i.e., a mean of 3.33*106 CFU/mL, which matches with the standard turbidity of 1% barium sulfate solution. About 200 mL of each bacterial cell suspension was inoculated in 20 mL of sterile nutrient agar and poured into sterile Petri dishes. Discs of 6 mm were prepared from sterile filter paper and immersed in the extract with serial concentrations, using sterile water for dilution of 0.025%, 0.05%, 0.1%, 0.5%, 1%, and 2% (1% and 2% for gel) then allowed to evaporate. The disks saturated with extract were placed on agar plates and kept in a refrigerator for 2 h before being incubated at 37° C for 48 h. The inhibition of bacterial growth of the concentrations was measured and compared with the positive control (ofloxacin), and the vehicle of extract and gel base as the negative control [17, 18].

Formulation of *J. variegata* Vahl gel

The formulations F1 and F2 were prepared by the agitation of the gelling agent with water for 20 minutes, 0.8 g carbomer 974 in F1 and 2.5 g xanthan gum in F2, before adding the preservatives and a sufficient quantity of distilled water. Next, the buffer solution was added to neutralize the gel, 0.1 g of citric acid monohydrate, and 3.5 g of tri-sodium citrate dehydrate in water. Finally, 2 g of *J. variegata* Vahl extract was dispersed in the prepared base, and 3 mL of 10% NaOH was added, with continuous stirring until a homogenous gel formed, then this was filled and packed in airtight glass jars.

Quality control of gel formulas

The prepared formulas were stored under stress conditions 40°C and relative humidity 75±5% for 3 months then the following tests were performed.

Physicochemical evaluation and microbial test

Physical examination of the formulations was conducted for color, odor, general appearance, and washing ability using the visual methods. The viscosity and homogeneity were tested by pressing a small amount of the gel between the thumb and the index finger. A small quantity of each sample was rubbed on the back skin of the hand to determine the homogeneity and spreading ability for 3 months under different conditions as established in Table 1. From each formula, 10 g was dispersed in 100 mL of distilled water to detect the pH value by immersing the electrode of a digital pH meter in the sample. A small amount of each formula was inoculated on a nutrient agar plate and incubated for 48 h at 37°C to check the microbial contamination.

Quantitative analysis

One gram of the formulation was dissolved and filtered to 50 mL of distilled water to quantify the extract in the formula using UV spectrophotometry at 280 nm.

Experimental study on rats

Animals and housing

Twelve Albino Rats (female), aged two months with a median weight of 250 g, were obtained from the biology

laboratory, Faculty of Applied Sciences-Sana'a University. The rats were housed individually in stainless steel cages at a well-ventilated animal house according to Guidelines for the Housing of Rats in Scientific Institutions [19]. The rats were fed on the diet formula consisting of white corn, soybean, wheat bran, melt bran, yellow corn, and dried fishes as a source of animal protein and vegetable oil. The ingredients were mixed and supplemented with multivitamins and minerals (Vitamin-M, Aman Veterinary Manufacturing Company, Sana'a-Yemen) then rolled in cylindrical pellets and dried. Each rat received 100 g/d of the dried pellets.

Experimental study design

A total of 12 female rats were divided into three groups: the first group [1] to be examined histologically on the 5th-day post-wounding, the second group [2] on the 10th-day post-wounding, and the third [3] on the 15th-day post-wounding. After shaving the dorsal part of each rat and cleaning with 70% ethanol, suitable areas are detected and performed a full-thickness circular excisional wound, including all layers of skin with a diameter of 1.5 cm [20]. Three wounds were made in each rat; the first wound, A, was treated by 0.5 g gel with the extract thrice

Table 1. Comparison of physicochemical properties of gel formulas

Parameter		Gel Formulas			
		F1		F2	
		Stress Conditions (40°C, 75% RH)	Room Conditions (25°C, 65% RH)	Stress Conditions (40°C, 75% RH)	Room Conditions (25°C, 65% RH)
First week	Color	Dark green	Dark green	Brownish green	Brownish green
	Odor	Cocoa	Cocoa	Cocoa	Cocoa
	pH	8.14	8.14	5.2	5.21
	Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
	Consistency	Suitable	Suitable	Suitable	Suitable
First month	Color	Dark green	Dark green	Brownish green	Brownish green
	Odor	Cocoa	Cocoa	Cocoa	Cocoa
	pH	8.16	8.14	4.82	5.13
	Homogeneity	Homogenous	Homogenous	Turbid	Turbid
	Consistency	very good	very good	Liquefied	Suitable
Second month	Colour	Dark green	Dark green	Brown	Greenish brown
	Odour	Cocoa	Cocoa	Cocoa with acrid smell	Cocoa
	pH	8.16	8.14	4.65	5.06
	Homogeneity	Homogenous	Homogenous	Turbid	Turbid
	Consistency	Good	Good	Liquefied	Good
Third month	Colour	Dark green	Dark green	Brown	Greenish brown
	Odour	Cocoa	Cocoa	Acrid smell	Cocoa
	pH	8.17	8.15	4.53	5.01
	Homogeneity	Homogenous	Homogenous	Turbid	Turbid
	Consistency	Good	Good	Liquefied	liquefied

Table 2. Bacterial inhibition produced by *J. variegata* extract and gel

Bacteria	Diameter of Inhibition Zone (MM)				
	Concentration of Extract		*J. variegata* Gel		Positive Control
	0.05% (MIC)	0.1%	1%	2%	Ofloxacin
*E. coli**	11	15	18	21	20
*P. aeruginosa**	10	14	15	17	18
*S. aureus**	11	13	16	19	19

*P<0.01.

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a day, the second wound, B, by 0.5 g gel base (vehicle) without the extract, and the third wound, C, was control.

At the end of the first 5 days, the diameter of the wounds in the first group was recorded and the rats were anesthetized by chloroform then sacrificed immediately. Then, their three wounds were dissected out and preserved in 10% formalin (pH 7.2) before the histological examination. The same procedure was performed on the second group after 10 days and on the third group after 15 days [21].

Statistical analysis

The IBM SPSS V. 22.0 was used in this study. The study variables were described as a percentage and the mean.

Results

Evaluation of the extract

The *J. variegata* Vahl extract was a dark green solid powder with a cocoa odor and slightly bitter taste, soluble in highly polar solvents (water and alcoholic solvents) with a pH of 5.11 (weakly acidic). Thin-layer chromatography and chemical test results confirmed that *J. variegata* Vahl extract has numerous phytochemical constituents (confirmed by the colors), including saponins, tannins, phenols, glycosides, steroids, flavonoids, and alkaloids. The results are similar to a previous study reported by Pramod [22] for *Jatropha curcas* leaves.

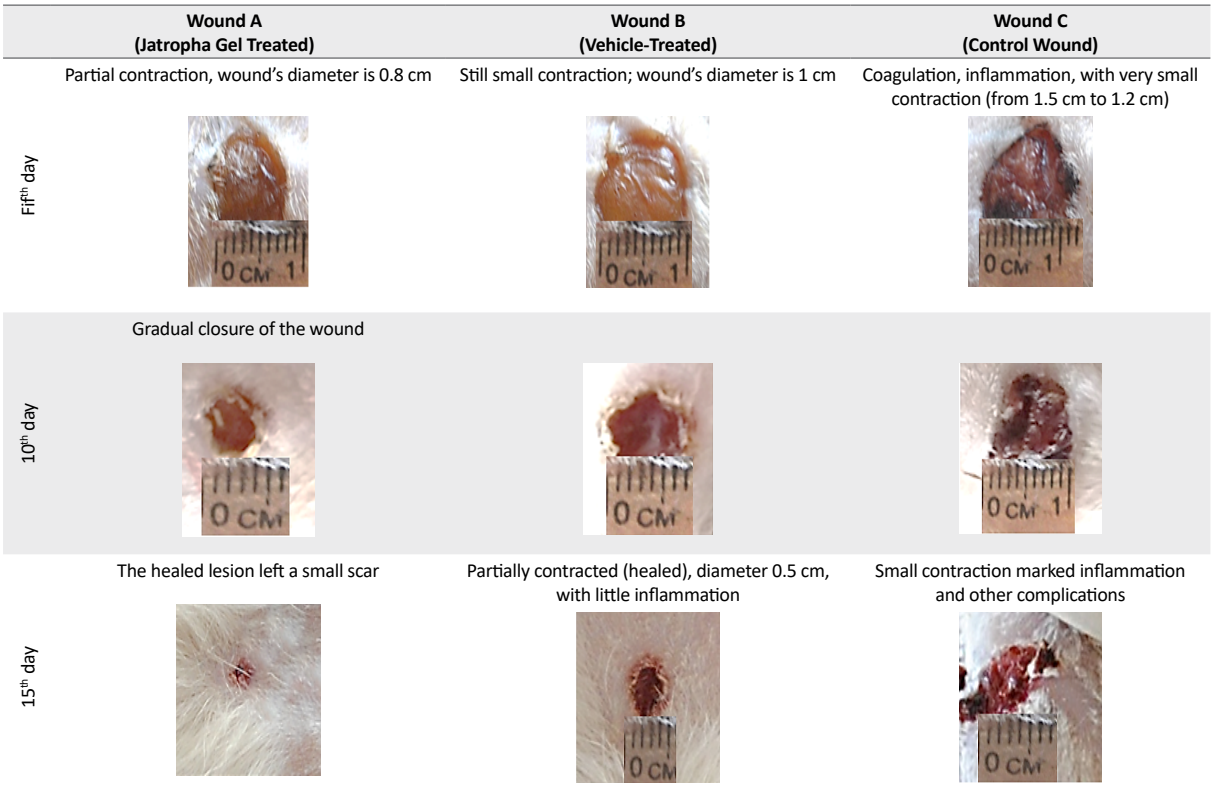

Figure 1. Changes in wounds' diameter during the treatment period

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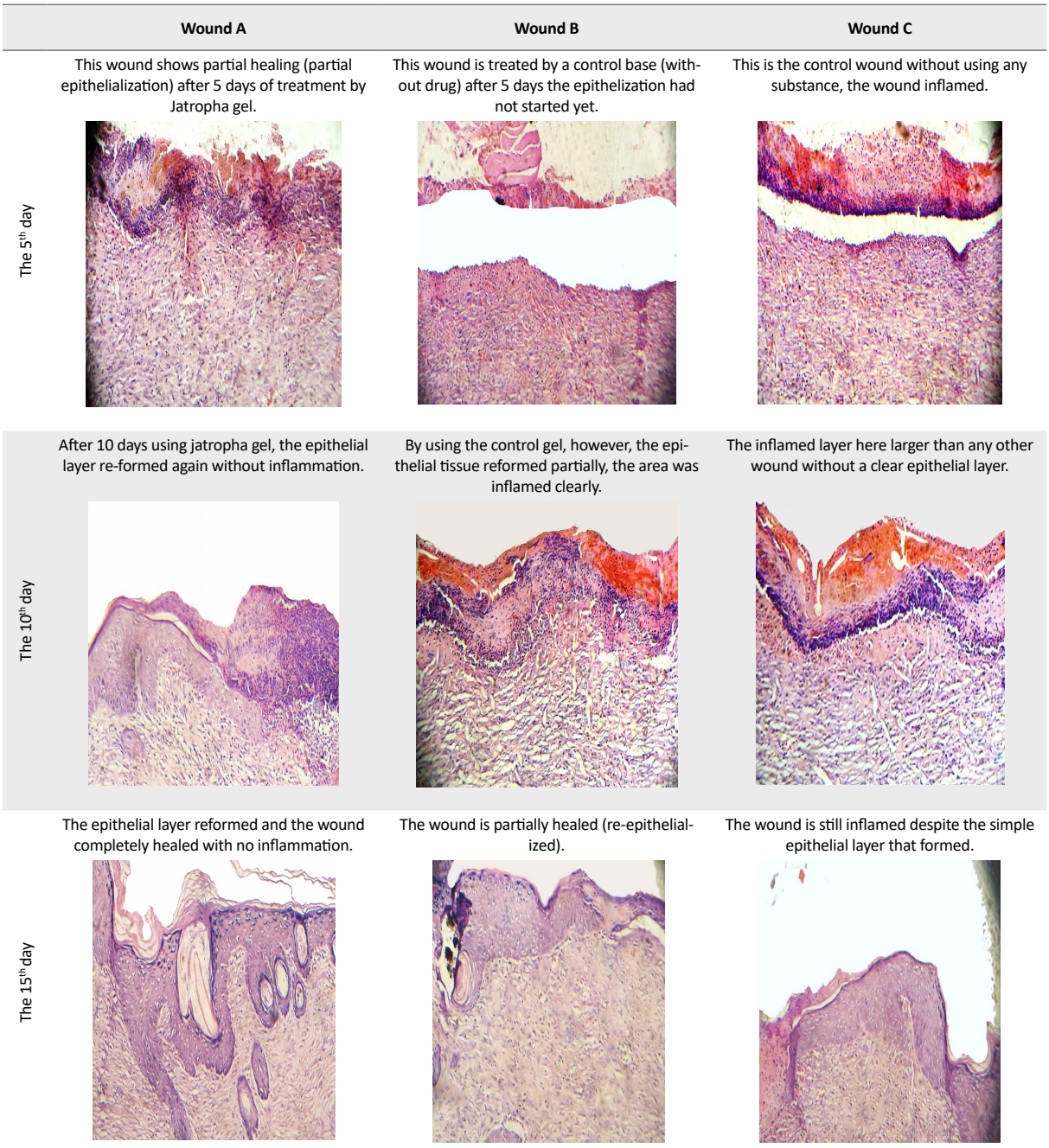

Figure 2. Re-epithelization process of wounds in 15 days

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Antibacterial screening of *J. variegata* Vahl extract and gel

As revealed in Table 2, the minimum inhibitory concentration (MIC) of the extract is 0.05% and the zone of inhibition increased dramatically with the higher concentrations for E. coli, which showed more sensitivity to the extract, followed by S. aureus and *P. aeruginosa*, respectively (P<0.01). The extract at 2% is competent to the positive control (ofloxacin). A similar study was carried out on *J. variegata* Vahl, which showed considerable activity against *Staphylococcus aureus* (ATCC 6538) and *Micrococcus flavus* (SBUG 16) [17].

Antibacterial effect of the extract in a gel formula with 1% showed high antibacterial effect 18, 15, and 16 mm against E. coli, S. aureus, and *P. aeruginosa*, respectively and with increasing the extract concentration in the formula to 2%, the result was similar to the crude extract 21, 17, 19 mm against *E. coli, S. aureus,* and *P. aeruginosa*, respectively and slightly more than the zone of the posi-

tive control (ofloxacin). The vehicle of extract and gel base (negative control) showed no antibacterial activity.

Similar studies were carried out on other species of *Jatropha*, *J. elliptica*, and *J. gossypifolia*, which exhibited an antibacterial effect against Gram-positive bacteria [23]. Another study indicated that methanolic extracts of roots and leaves of *Jatropha gaumeri* have high antimicrobial and antioxidant activities [24].

Stability of Jatropha gel

F1 is more stable and physically acceptable than F2 during the stability study period as shown in Table 2, since, the pH is relatively constant and the consistency of F1 has remained unchanged for 3 months. The microbial growth in both formulas was negative.

The calibration curve illustrated the linearity of the working standard solution of *Jatropha variegata* extract using UV spectrophotometer and R2=0.9959 indicates the accuracy and linearity of the analytical method. Assay of gel extract formula was calculated using the following formula:

Assay %=[(absorbance of sample (gel) / absorbance of standard)×(concentration of standard/concentration of sample)]×100=(0.2958/0.312×50/50)×100=94.4%

This means that the active ingredient in the 2% of the extract is 94.4% (approximately equivalent to 1.94 g in the 2 g of the extract included in the gel), SD 0.0043.

Wound healing measurement

Macroscopic wound contraction

Figure 1 showed the photomicrograph of the contraction percentage of the wounds on rats' skin. In the fifth-day post-wounding, the average contraction of wounds A was 53% in the test gel (F1) treated wounds while it was 33% in the wounds B treated with vehicle, and 20% in the control wounds C, without treatment. On the 10th day of post-wounding, the percentage of wounds healing in all wounds increased by an average of 15%. At the end of the second week, the contraction rate of wounds A was completely healed; however, the wound B was healed by 66.6%, and still half-healed in the control wounds C.

In summary, the contraction rate is greater in treated wounds by the *Jatropha* gel than vehicle and control, and complete wound closure was achieved on the 15th-day post-wounding in only the *Jatropha* gel treated wounds.

Histological study

Evaluation of the histopathological re-epithelization of extract-treated and control wounds is clarified in Figure 2. Throughout the experiment, the progression of the new epithelium to cover the defected area was greater in extract-treated than control gel treated and control wounds, and the re-epithelization process was completed on the 15th day in the extract-treated wounds. These observations were consistent with macroscopic results proving that the applying of *J. variegata* Vahl extract has noticeably accelerated the healing process.

Figure 2 indicated that on the fifth day, the extract-treated wounds have shown gradual closure of the wound, whereas the other wounds (B and C) were not re-epithelized. On the 10th day of study, the extract-treated wounds (A) showed a moderate re-epithelization, though in B and C wounds a mild re-epithelization has happened. At the end of the period (day 15), wounds A had re-epithelized completely, but, in gel-base treated wounds (B) and control wounds (C), the re-epithelization was partial.

Discussion

The previous studies on *J. variegata* extract's wound healing activity are insufficient; there is one study on *J. variegata* leaves and fruits reported that it is used as antiseptic for wounds and hemostatic [25]. However, several studies reported on other *Jatropha* species for wound healing such as *Jatropha curcas* L. extract ointment, which demonstrated wound healing potential in both excision and incision models [26]. Another study had shown that application of methanol leaf extract of *J. curcas* incorporated into ointment base on the excision wound in rats caused a significant ($P<0.05$) higher rate of wound healing and reduced the epithelialization period in a dose-dependent manner [27]. Moreover, another two studies on crude bark extract and seed oil of *J. curcas* using Wistar albino rats confirmed its activity in accelerating the healing process by increasing the skin breaking strength, granulation of tissue, wound contraction, dry granulation tissue weight, and hydroxyproline levels [28, 29].

The risk of wound infection increases as local conditions favor bacterial growth rather than host defense. Consequently, the primary objective in wound management is to redress the host-bacterial balance, and this is most effectively achieved by ensuring that the wound is cleared of devitalized tissue and foreign bodies, the bacterial load and inflammation are controlled, and that adequate tissue perfusion is maintained [30].

All of these confirmed the local use of this plant for the treatment of some ailments traditionally and verified the ability of *J. variegata* Vahl as an antimicrobial with potential application to wound healing may be through enhancing fibroblast proliferation, angiogenesis, keratinization, and epithelialization and reduce the inflammation as compared with the vehicle-treated group or the control group.

This study shed light on the contribution of Yemeni flora in the treatment of different diseases. It presents new preliminary information about the pharmacological efficacy of a local Yemeni plant. However, future phytochemical, toxicological, and clinical investigations are necessary to ensure the safe therapeutic use of such traditional medicinal plants.

Ethical Considerations

Compliance with ethical guidelines

Ethical clearance and approval of the study protocols were obtained from the Ethics Research Committee of Sana'a University on March 3, 2018 (Code: 321/2018), and the study followed common ethical principles adopted in phytochemical and experimental pharmacology research. The study animals were investigated and housed according to the guidelines for the Housing of Rats in Scientific Institutions.

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Authors' contributions

Conceptualization, supervision: Abdulkarim K Alzomor; Writing the original draft, review, and editing: Nahlah Mansour Sallam; Investigation, recources, writing the original article, methodology: All authors.

Conflict of interest

The authors declared no conflict of interest.

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Appendix 1. Yemeni *J. variegata* Vahl