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Research Article
Antioxidant, Antimicrobial and Wound Healing Potential of *Jatropha variegata* - An Interesting Plant Endemic to Yemen

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Abstract

Background and Objective: *Jatropha variegata* is traditionally used in Yemeni folk medicine for antiseptic and hemostatic purposes. In this study, the methanolic extract of the plant leaves was evaluated for its antioxidant, antibacterial and wound healing activity. **Materials and Methods:** The antioxidant activity was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was tested using disc diffusion and broth microdilution assays. *In vivo*, the ability of the extract to accelerate wound healing in rats was evaluated using both wound area measurements and histological analyses. **Results:** The leaves extract exhibited strong antioxidant activity with an IC_{50} value of 16.7 μg mL^{-1}. The extract exhibited moderate antibacterial activity against *S. aureus* with inhibition zones of 10.6 mm, and the Minimum Inhibitory Concentration (MIC) value was 5 mg mL^{-1}. The extract significantly accelerated the rate of wound healing closure compared to those treated with the vehicle. In addition, histopathological analyses of wound granulation tissues showed significantly better healing signs after 14 days in the extract-treated groups, with denser collagen deposition at the injury site. **Conclusion:** The leaves of *J. variegata* appear to contain bioactive compounds that may be utilized clinically in combating oxidative stress and in wound management.

Key words: *Jatropha variegata*, antibacterial, antioxidant, wound healing, phytochemicals, granulation tissue

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jatropha variegata (Forsk.) Vahl Euphorbiaceae family[1], has been used traditionally in Yemeni folk medicine for antiseptic and hemostatic purposes and in the management of wounds[2]. Previous studies have shown the therapeutic potentials of *J. variegata* both as an antibacterial and as an antioxidant probably due to the presence of bioactive steroids and flavonoids[3]. In accordance, five flavonoids were previously isolated from the stem of *J. variegata*, namely: kaempferol 3-O-α-L-rhamnopyranoside, 3-O-α-L-arabinopyranoside, 3-O-β-D-glucopyranoside, 3-O-β-D-galactopyranoside and kaempferol[1]. Both Kaempferol and its glycosides, have been shown to exhibit antioxidant, anti-inflammatory, anticancer, antimicrobial, antidiabetic, and cardiovascular protective activities[4], indicating that flavonoids may contribute, at least in part, to the therapeutic potential of the plant.

Wounds and burns can be major causes of the physical disability that induce a negative impact on human normal activity[5]. In fact, traumatic injury is considered one of the leading causes of mortality in the United States and Europe[6]. And although regeneration and restoration of damaged tissues are vital[7], the process of wound healing is gradual and multi-faceted; going through multiple consecutive stages of hemostasis, inflammation, proliferation, fibroplasia, collagen deposition, epithelialization, contraction, remodeling and finally maturation[8]. In addition, the rate of healing can be very slow, particularly with wide damaged areas, promoting the odds of microbial infection. Therefore, there is a need for medicinal agents that possess the ability to speed up the healing process and tissue recovery to prevent future complications[9].

Natural products and their phytochemical components have long been proven invaluable in wound management, displaying several potential repair mechanisms including combating Reactive Oxygen Species (ROS), activation of immune cells, cytokines and other inflammatory mediators, stimulating extracellular matrix (ECM), and enhancing the secretion of growth factors[10]. Therefore, the present study was performed to investigate the potential therapeutic wound-remedial profile of *J. variegata* by determining its phytochemical content and assessing its antibacterial, anti-oxidant and wound healing activity.

MATERIALS AND METHODS

Study area: This study was performed in the Faculty of Pharmacy, Alhikmah University, Sana'a, Yemen and Yemen Lab, Sana'a Yemen during August 2017-April 2018.

Chemicals and reagents: Methanol (99.8%) (Scharlae, Spain), ethyl acetate (HiMedia, India), formic acid (Fluka, Switzerland), glacial acetic acid (WinLab, UK), chloroform (Sigma, Germany), formalin (BDH Chemicals, UK) were used for plant extraction and phytochemical testing. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, USA) used for free radical scavenging. Ceftriaxone 1 g (Cipla, India), Ceftriaxone disc (Oxoid, England), 2% fusidic acid ointment (Fucidin®, LEO Pharma, Ireland) and Vitamin C (Sigma, USA) were used as positive control agents. Media included human blood agar (HuBA) (Rapid laps, UK), Mueller-Hinton agar (MHA) (Scharlau, Spain) and Mueller-Hinton broth (MHB) (Oxoid, UK) were used for the antibacterial analyses. 40 mg kg^{-1} Thiopental and Ketoprofen (Ketofan®) were purchased from Rotexmedica, (Germany) and Amriya Pharm Ind. (Egypt), respectively

Plant materials: *Jatropha variegata* was collected in Haifan, Taiz city, Yemen in July 2017 and was identified by Dr. Abdul Wali Al Khulaidi, Public Authority for Research and Agricultural Extension, Dhamar city, Yemen. The voucher specimen was prepared and deposited at the Pharmacognosy Department, Faculty of Pharmacy, Al-Hikma University, Sana'a. The voucher number is JV 2017.

Experimental animals: Thirty mature male albino rats, weighing 200-250 g, were obtained from the animal house of the Faculty of Science, Sana'a University. The animals were housed in polypropylene cages and kept 25±3 °C in 12 hrs light dark cycle, relative humidity 35-60% with strict hygienic conditions and were fed food and water ad libitum. The rats were acclimatized to the laboratory conditions for at least 48 hrs before experimentation. All animal experiments were approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana'a University on July 7th, 2017 (approval number: 01/FPhSana'a/2017).

Preparation of the methanol extracts: The leaves (1109.74 g) of *J. variegata* were cleaned, dried in the shade, grounded and then extracted with methanol before filtration. The filtrate was dried using a rotary evaporator (Buchi rotavapor R-215; Switzerland) in a water bath (Buchi water bath B-491; Switzerland) at a temperature not exceeding 45 °C. The yield percentage (10.58%) was calculated based on initial dry weights. The final dried extract was stored in a desiccator for subsequent phytochemical screening and biological activity tests.

Phytochemical screening

Chemical tests and thin-layer chromatography (TLC): Chemical testing for the *J. variegata* leaves extract was performed according to Banu *et al.*[11] to identify alkaloids, carbohydrates, fixed oils, fats, steroids, anthraquinones, phenol, tannins, proteins, saponins, gums, and mucilage compounds. Chemical components and their retention factor (Rf) values were identified by TLC techniques using silica gel 60 F254, 20×20 cm (Merck, Germany). TLC was performed as previously described elsewhere[12].

Total phenolic content (TPC): TPC was determined by the Foline Ciocalteu reagent assay. The total phenolic concentration in the extract was expressed as gallic acid equivalents (GAEs) and was measured according to the method described by Saboo *et al.*[13]. One milliliter aliquot of the methanolic extract (1 mg mL^{-1}) was mixed with 10 mL distilled water and 1.5 mL Folin-Ciocalteu reagent and left for 5 min. Then, 5 mL 20% sodium carbonate solution was added and then the mixture was brought to a final volume of 25 mL with distilled water. The reaction mixture was incubated for 30 min at room temperature after which the absorbance reading was taken at 765 nm against the blank (methanol: distilled water, 1:1). A standard concentration-absorbance calibration curve was drawn using gallic acid (40-280 µg mL^{-1}). The results were expressed as gallic acid equivalents (GAE) per mL of the test solution.

Anti-oxidant activity: The antioxidant activity of *J. variegata* extracts was assessed using DPPH radical scavenging method as previously described by Moharram *et al.*[12].

Antibacterial activity

Microorganisms: The antibacterial activity of *J. variegata* leaves extract was tested on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* using disc diffusion and broth microdilution methods. All bacteria were obtained from the Microbiology Department of YemenLab Laboratory, Sana'a, Yemen, and were sub-cultured on HuBA medium.

Antibacterial assays: The antibacterial activity of the extract was determined by disc diffusion and broth microdilution assays. Bacterial suspensions were prepared following Moharram *et al.*[12] The disc diffusion and broth microdilution methods were performed as previously described[12].

Five control solutions were prepared: The negative control (methanol), the growth control (100 µL bacterial suspension in 100 µL MHB), the positive control (ceftriaxone 30 µg), the sterile control of the plant extract (100 µL plant extract in 100 µL MHB) and the sterile medium control (200 µL sterile plain MHB) to confirm the sterility of the broth. The microtiter plates were incubated for 24 hrs at 35°C. The antibacterial activity was considered strong if the MIC was smaller than 1 mg mL^{-1}, moderate if the MIC was between 1 and 5 mg mL^{-1} and weak if MIC was greater than[14] 5 mg mL^{-1}. Sterile conditions were maintained during experimentation. All experiments were performed in triplicates.

Minimum bactericidal concentration (MBC) determination: About 20 µL of broth were taken from the microtiter plate wells (that did not show visible bacterial growth) to be inoculated into an MHA medium. The inoculated agar was then incubated for 24 hrs at 35°C. The MBC value was determined as the lowest concentration of test material that prevented any bacterial growth on the plates after incubation[15].

Wound healing activity: The wound healing activity was assessed as mentioned previously[16]. Rats were weighed prior to surgical intervention and anesthetized using 40 mg kg^{-1} intraperitoneal thiopental. Ketoprofen (5 mg kg^{-1}), an analgesic drug, was injected subcutaneously into all animals right before the operation and then every 24 hrs afterward for 2 consecutive days. The animal skin was shaved by an electrical shaver and swabbed with 70% alcohol. An area of wound (4 cm^2) was created at the nape of the dorsal neck of all rats with the aid of a square seal as described by Rawat *et al.*[17]. The wounds were made deep enough to involve the lower subcutaneous tissue.

Rats were randomly divided into 5 groups of six rats each. Group 1 was untreated and served as negative control. Group 2 was topically dressed at the wound site with 0.2 mL of normal saline (vehicle) twice daily for 14 days. Groups 3 and 4 were topically dressed with 0.2 mL of 100 mg mL^{-1} or 200 mg mL^{-1}, respectively, of *J. variegata* leaves extract. Group 5 was dressed with 0.2 mL fusidic acid ointment 2% and served as the positive control.

Each injured rat was housed in an individual cage with the wound area measured graphically (mm^2) on days 0, 3, 6, 9, 12 and 14 after injury. The wound closure rate at a specific day was expressed as the ratio of wound area at that specific day to the wound area at day 0, using a transparent paper and a permanent marker as described elsewhere[10].

The percentage of wound closure was calculated using the following equation:

$$\text{Wound closure at a specific } (\%) = 1 - \frac{Ad}{A0} \times 100$$

where, Ad is the wound area at a specific day, and A0 has wound area at day zero.

Histopathological studies: The skin specimens of healed cutaneous tissues obtained at day 14 post-operation from each rat were fixed in neutral buffered formalin (10%), dehydrated in graded ethanol, cleared in xylene and processed by paraffin tissue processing machine, 5 µm-thick sections of the tissues were then mounted on glass slides. After dewaxing the sample, it was rehydrated in distilled water and stained with hematoxylin and eosin as previously described[18]. All subsequent histopathological examinations were performed by an experienced pathologist blind to the prior treatments. The evaluation parameters were based on the degree of re-epithelialization, granulation tissue formation, and collagen deposition and organization.

Statistical analysis: The statistical analysis was carried out by one-way Analysis of Variance (ANOVA) using Statistically Package for Social Sciences (SPSS) version 11.5. Antibacterial and antioxidant experiments were performed in triplicates and the results were presented as Mean±standard deviation (SD). For wound healing results, the data were expressed as Mean±standard error of the mean (SEM). A comparison of mean values of different groups was tested by Tukey's multiple comparison test. A p-value of <0.05 was considered significant.

RESULTS

Phytochemical screening: The chemical and TLC tests of *J. variegata* leaves extract indicated the presence of various bioactive components including carbohydrates, proteins, alkaloids, phenols, tannins, phytosterols, bitters, gum and mucilage. The chemical components of the extract, retention factor (Rf) values and the solvent systems used are listed in Table 1. TLC results showed that the extract contained anthrones, as evidenced by two yellow spots with Rf values of 0.27 and 0.39 which formed (Fig. 1a) when spraying the TLC plates with 10% alcoholic KOH. Meanwhile, two red spots (Rf: 0.68, 0.87) formed under UV light (at wavelength 365 nm) indicated the presence of anthraquinones (Fig. 1b). Furthermore, bitter compounds were spotted as indicated by the appearance of multiple colored spots with different Rf values (red/ yellow, brown and dark green) under visible and UV-365 light as the TLC plates were sprayed with 10% KOH (Fig. 1c and d, respectively). Alkaloids were also detected in the extract as evidenced by the formation of the characteristic brown spot (Rf, 0.25) in visible light following the application of Dragendorff reagent (Fig. 1e).

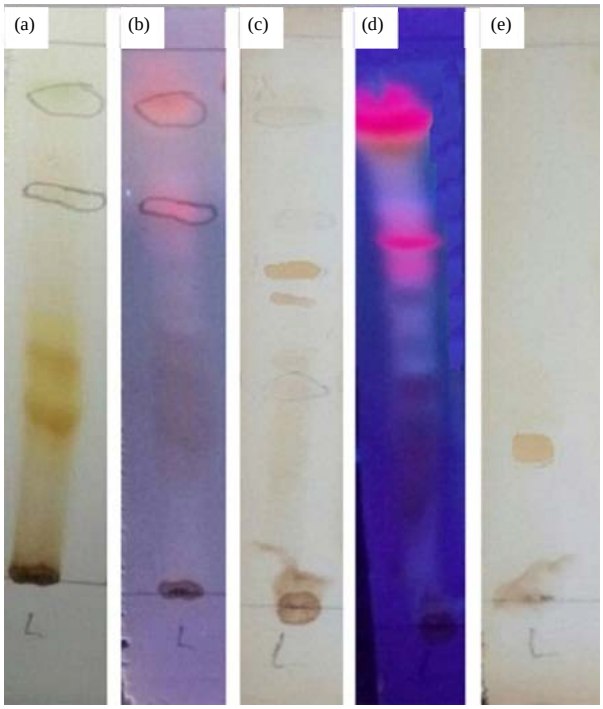

Fig. 1(a-e): TLC plates of *J. variegata* leaves extract (a-b) TLC plate of anthraglcosides in visible and UV 365 nm light, respectively, after spraying with 10% alcoholic KOH, (c-d): TLC plate of bitter compounds in visible light and UV 365 nm light, respectively after spraying with 10% alcoholic KOH, (e) TLC plate of alkaloids in visible after spraying with Dragendorff reagent

Phenolic content and antioxidant activity: Utilizing the Foline Ciocalteu reagent assay, the TPC of the methanol extract of *J. variegata* leaves was determined as 157.63 mg GAE g^{-1} extract (Table 2). In line with this finding, the leaves extract showed a strong free radical scavenging effect with IC_{50} value of 16.7 µg mL^{-1} (Table 2) as determined by the DPPH radical scavenging method.

Table 1: Thin layer chromatography investigation results for the methanolic *J. variegata* leave extract

	Solvent system					
Constituent	Ethyl acetate	Methanol	Water	Detection	No. spots	Rf values
Anthraglycosides	100	13.5	10	Visible	2	0.27
Anthrone						0.39
Anthraquinone				UV-365	2	0.68
						0.87
Bitter principles	100	13.5	10	UV-365	2	0.58
						0.85
				Visible	4	0.4
						0.45
						0.52
						0.6
Alkaloid	100	13.5	10	Visible	1	0.25

Table 2: Total phenolic content and antioxidant activity (IC_{50}) for *J. variegata* leaves extract

Parameters	Total phenolic content (mg GAE g^{-1} extract)	Antioxidant activity IC_{50} (µg mL^{-1})
Leaves extract	157.63±0.64	16.7
Ascorbic acid	-	5.7

Means±standard deviations (SD), the values are the means of three triplicates, -: Not detected

Table 3: Antibacterial activity of the methanol *J. variegata* extracts as determined by disc diffusion (inhibition zone), broth microdilution Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC)] assays

	Inhibition zone (mm)			MIC (mg mL^{-1}) (MBC mg mL^{-1})
Controls	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. aureus*
Extract	10.6±0.6	-	-	5±0.0 (5±0.0)
Ceftriaxone	15.0±0.0	25±0.0	20±0.0	<3.125±0.0 µg mL^{-1} (<3.125±0.0 µg mL^{-1})
MHB+2% MeOH (Negative)	nu	nu	nu	+
MHB+bacterial broth (Growth)	nu	nu	nu	+
MHB (Broth sterility)	nu	nu	nu	nil
Extract + MHB (Extract sterility)	nu	nu	nu	nil

Values of inhibition zone are expressed as Means±SDs, Each value is the mean of three triplicates, -: No activity, MHB: Mueller-Hinton Broth, MeOH: Methanol, Nil: No growth, +: Growth, nu: Not determined

Antibacterial activity: The antibacterial of *J. variegata* leaves extract against *S. aureus*, *P. aeruginosa* and *E. coli* were determined qualitatively and quantitatively using disc diffusion and broth micro-dilution methods, respectively (The results are summarized in Table 3). Only *S. aureus* showed moderate sensitivity to *J. variegata* leaves extract with Inhibition Zone (IZ) of 10.6 mm and identical MIC and MBC values of 5 mg mL^{-1}. However, gram-negative bacteria including *P. aeruginosa* and *E. coli* appeared resistant and were not inhibited by the extract.

Wound healing activity: A surgical procedure was performed on rats to create a wound with an area of 4 cm^2 (at day 0). Areas of wounds in all tested animal groups and the percentage of wound healing on days 3, 6, 9, 12 and 14 post-intervention were measured (Table 4). Figure 2a-k shows wound healing treated with J. variegata. The study showed that wounds treated with the leaves extract of *J. variegata* (100 and 200 mg mL^{-1}) exhibited significant wound healing (Fig. 2d and e, respectively) that was faster than the untreated wounds (Fig. 2b) or the wounds treated by the vehicle group (normal saline) (Fig. 2c). Compared to the vehicle-treated group, the extract-treated group showed significant (<0.01) wound area contraction starting at day 6 and onwards (Table 4). This effect was even more significant ($p<0.001$) in comparison with the untreated group starting on day 6. It is noteworthy that during the 14-day healing period following incision, the animals remained healthy and showed no clinical evidence of complications or infections.

The wound healing activity exhibited by *J. variegata* in rats was interesting as it exceeded the activity shown by fusidic acid. For instance, the wound closure rate in animals treated with 100 mg mL^{-1} extract on days 12 and 14 post-surgery reached 95.4 and 97.1%, respectively. Similarly, percentages of wound closure rate of 95.0 and 98.7% were seen in the animals treated with the 200 mg mL^{-1} extract. In

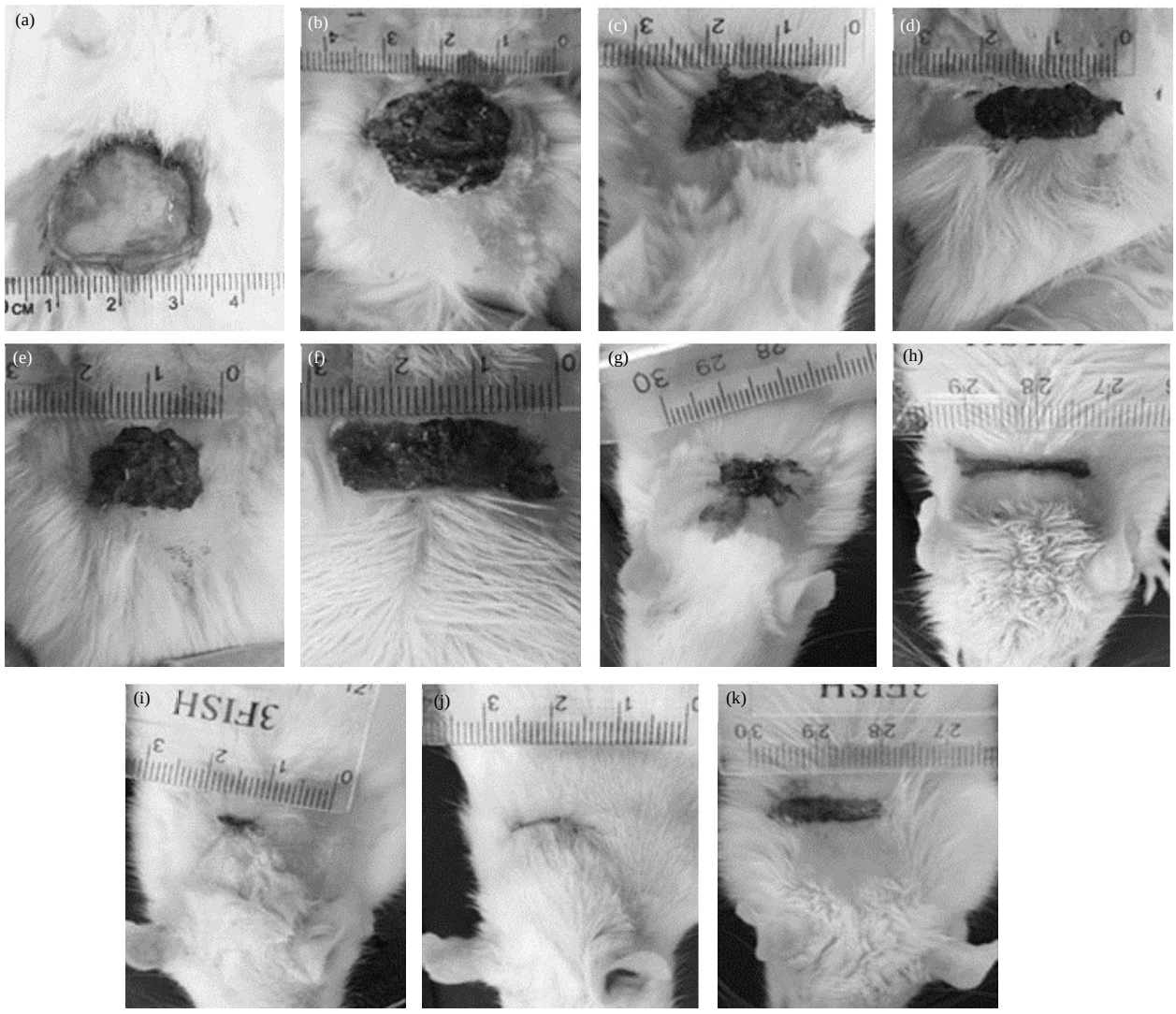

Fig. 2(a-k): Macroscopic appearance of wound healing at days (a) 0 after surgery: (b, g): Untreated rats, (c, h): Rats treated with 0.2 mL normal saline solution (d, i): Rats treated with 100 mg mL^{-1} of *Jatropha variegata* (e, j): Rats treated with 200 mg mL^{-1} of *J. variegata* (f, k): Rats treated with fucidin ointment (positive control) at day 7 (b-f) and 14 (g-k)

Table 4: Effect of *J. variegata* leaves extract on wound healing in experimental rats at different days interval

	Wound area mm^2 (Wound healing (%))				
			J. variegata (mg mL^{-1})		
Day	Untreated	Normal saline	100	200	Fucidin
0	400	400	400	400	400
3	373.2±13.3 (6.7)	323.3±21.2 (19.2)	351.8±12.6 (12.0)	301±11.5^{a*} ^{c**} (24.7)	382.6±10.0 (4.35)
6	357.0±8.7 (10.9)	250.5±10.6^{a***} (37.4)	236.2±7.6^{a***} (41.0)	197.2±7.7^{a***} ^{b**} ^{c**} (50.7)	236.0±7.5^{a***} (41.0)
9	238.8±4.0 (40.3)	133.7±17.9^{a**} (66.6)	71.5±10.3^{a***} ^{b**}^{c**} (82.1)	88.8±12.5^{a***} ^{c*} (77.8)	143±14.8^{a**} (64.3)
12	75.4±12.0 (81.0)	84.2±10.6 (79.0)	18.3±2.5^{a**} ^{b**} ^{c***} (95.4)	20.0±4.0^{a**} ^{b**}^{c***} (95.0)	82±5.7 (79.5)
14	42.0±2.8 (88.8)	34.2±5.0 (91.5)	11.5±0.4^{a***} ^{b**} ^{c**} (97.1)	5.5±0.7^{a***} ^{b**} ^{c**} (98.6)	41.2±4.7 (89.7)

Data represent mean values±SEM, n = 6, [a]Significance compared to the untreated groups, [b]Significance compared to the normal saline group, [c]Significance compared to the fusidic acid group one-way ANOVA; *p<0.05, **p<0.01, ***p<0.001 was considered significant

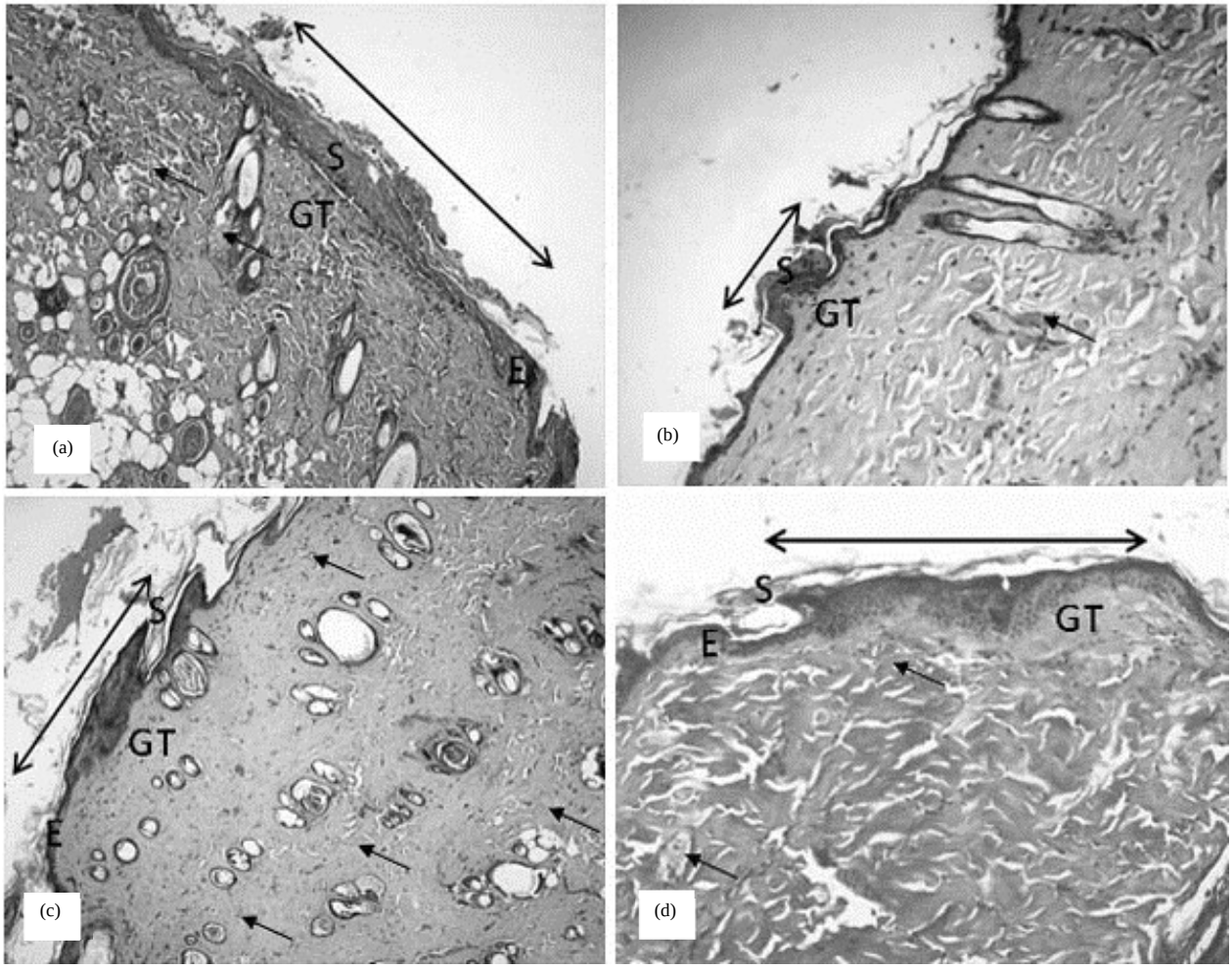

Fig. 3(a-d): Histology of healing wounds tissue at day 14 after wounding in rats: (a) 0.2 mL of normal saline (negative control) showed wide wound area (←→), (b) 100 mg mL^{-1} of *J. variegata*, (c) 200 mg mL^{-1} of *J. variegata*, showed smaller wound area (←→), angiogenesis (arrows) compared to negative control, (d) rats treated with fucidin ointment (positive control), showed moderate wound area (←→) and well epithelialization compared to negative control stained with hematoxylin and eosin (magnification 200x)
S: Scab, E: Epidermis and GT: Granulation tissue

comparison, the percentages of wound closure achieved by fusidic acid on day 12 and day 14 were only 79.5 and 89.7%, respectively.

To corroborate these findings, histological analyses of healed wounds were performed on day 14 post-treatment. The microscopic histological analyses in all groups confirmed marked improvement in tissues at the injury site. Histology of the healed wounds treated with extracts of *J. variegata* and fusidic ointment showed clear signs of rapid wound healing, growth of hair follicles, angiogenesis and less scarring at the wound enclosure site compared to the negative control group (Fig. 3a-d). Interestingly, no significant difference was observed between the fusidic acid group and the extract-treated groups (Fig. 3b-d) on day 14 post-treatment.

Looking at the extract-treated wounds, newly regenerated epithelium covered the wound area almost completely and the regeneration of the epidermis was also evident with barely seen signs of inflammation. Also compared with the negative control groups (Fig. 4a), there was extensive collagen deposition in both the extract-treated and the positive control groups (Fig. 4b-d). The granulation tissue showed the extensive formation of new blood

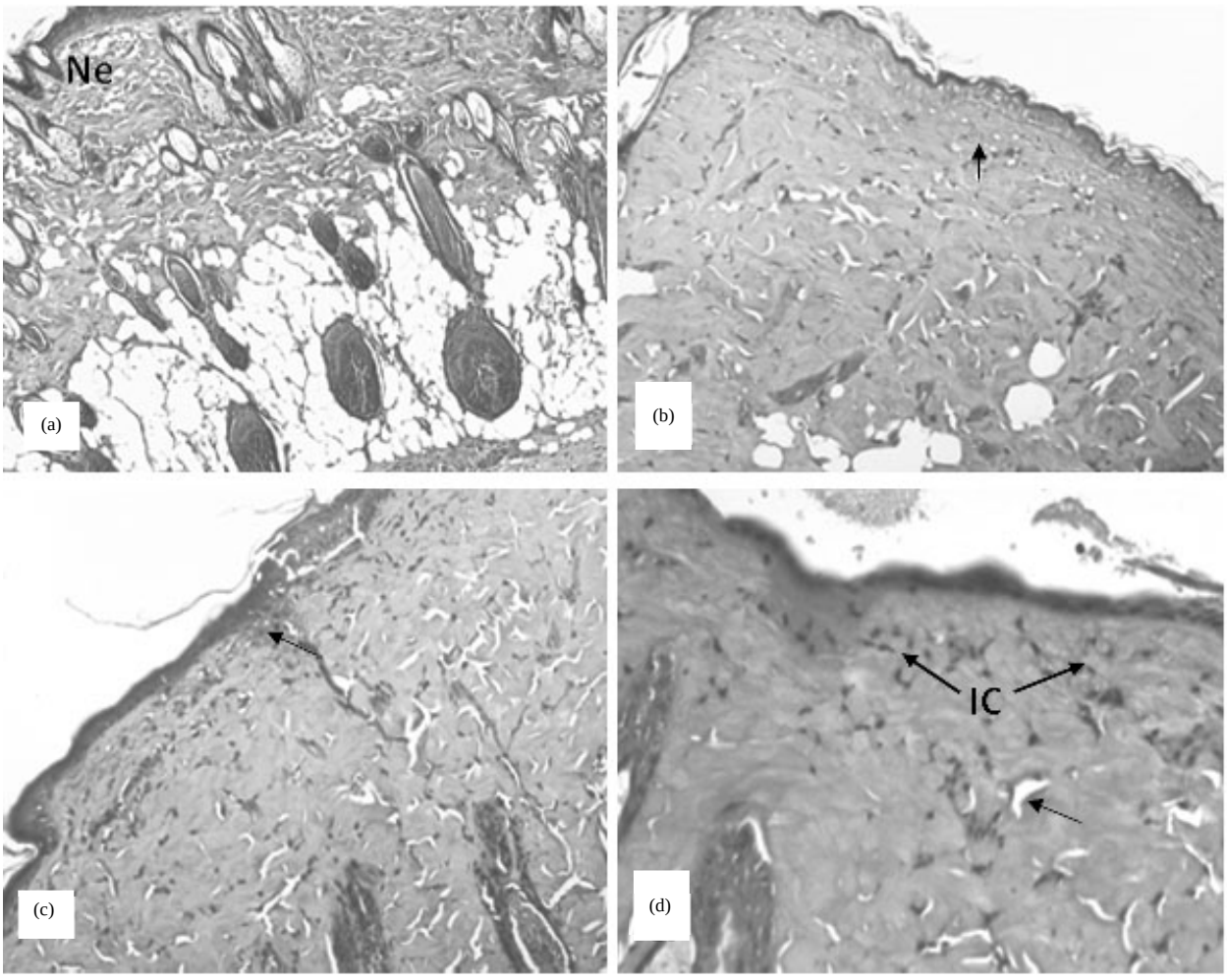

Fig. 4(a-d): High magnification histological analysis of wound granulation tissues with hematoxylin and eosin, at day 14 after wounding: (a) Group treated with 0.2 mL vehicle, normal saline, (b-c) Groups treated with 100 mg mL^{-1} or 200 mg mL^{-1}, respectively *Jatropha variegata* extract (d): Granulation tissue of the fucidin ointment-treated group (positive control), (magnification 400x).
Ne: Focal necrosis, IC: Inflammatory cells

capillaries (angiogenesis) and denser collagen fiber deposition compared to the negative control group. Moreover, the histological evaluation revealed increased cellular infiltration, and re-epithelialization only in the treated and positive control groups.

DISCUSSION

Plants are known to contain therapeutic bioactive compounds such as alkaloids, flavonoids, phenols, terpenoids, steroids and anthraglycosides that are utilized in the pharmaceutical, cosmetic and pesticide industries[19]. In the present study, a phytochemical screening for *J. variegata* leaves extract showed the presence of carbohydrates, proteins, alkaloids, saponins, phenols, tannins, phytosterols, bitters, gum and mucilage. It is noteworthy that a previous review listed similar phytochemical components for *Jatropha* species including diterpenes, triterpenes, lignans and coumarins, flavonoids, alkaloids and phytosterols[20].

Regarding the antioxidant potential of *J. variegata*, the leaves extract showed strong antioxidant activity which can be ascribed to the presence of various phenolic compounds as indicated by the high values of extract TPC. The positive correlation between the phenolic content and the free radical scavenging activity was consistently reported in several studies[21,22]. Moreover, the present study showed that the extract of *J. variegata* possesses moderate antibacterial activity. In accordance, a previous investigation showed that

the leaves extract exhibited strong antibacterial effect[3]. The modest differences between the findings of the present work and those of others may be due to differences in reagents and methodology. For instance, the efficiency of the extraction and thus the absolute quantities of active constituents per extract may vary considerably depending on several parameters, including extraction time, temperature, the volume and type of the solvents used. Environmental factors regarding the plant harvest and geographical location such as altitude, temperature, precipitation, humidity, and soil type may also play a role[23-25].

An ideal wound-healing agent should induce rapid appropriate wound healing in a short time without causing undesirable adverse effects[9]. Interestingly, the present study showed that *J. variegata* exhibited a significant wound-healing effect that was even faster than that exerted by the well-known antibiotic fusidic acid used widely by physicians in the management of skin infections. This result is in consistent with a previous study that reported that *Jatropha curcas* and curcain (a protein obtained from the seed oil of *J. curcas*) exhibited better wound-healing effect in rats and mice compared to standard drugs including povidone-iodine, nitrofurazone ointment and propamidine isethionate cream[26-28]. Other studies indicated that some *Jatropha* species including *J. curcas* and *J. multifida* were indeed capable of speeding up the rate of wound healing[28,29].

In the current study, the histological analysis of wounds at day 14 post-treatment indicated that the extract of *J. variegata* led to the generation of a new healthy epidermal layer, epithelial covering tissue, and intact dermal layer. The regeneration was associated with clear signs of fibroblast proliferation, collagen deposition, and new blood vessel formation (angiogenesis). In the same manner, a previous study reported that the crude bark extract of *J. curcas* possessed wound-healing properties evidenced by the observed accelerated epithelialization, increased granulation tissue breaking strength and skin breaking strength[28].

Several other natural plants such as *Strobilanthes crispus, Gymnema sylvestre, Acrostichum aureum green tea, Aloe vera, Aloe littoralis, Punica granatum, Aloe saponaria, Acrostichum speciosum* and *Datura metel* have also been proved effective in the treatment of wound injuries in animal models[30-33]. The ability of these plants to speed up wound repair were explained by the anti-inflammatory, anti-microbial and anti-oxidant properties of bioactive ingredients that may promote cutaneous healing and combat deleterious effects of free radicals and/or bacteria.

The results of this study indicate, for the first time, that *J. variegata* is a potential candidate for skin wound healing because of its effect on phases of the healing process and its antimicrobial and antioxidant properties. However, further depth and structured study would be useful to study its usefulness and mechanisms more exactly.

CONCLUSION

J. variegata is one of the medicinal plants that is often utilized in Yemeni traditional medicine for its wound healing properties. In the present study, *J. variegata* exhibited a potent and rapid healing effect on wound healing that was superior to fusidic acid. The phytochemical screening of the plant indicated the presence of carbohydrates, proteins, alkaloids, saponins, phenols, tannins, phytosterols, bitter, gum and mucilage compounds which appears to contribute to the wound-healing potential of the plant via exerting anti-oxidative, antibacterial and angiogenic properties. The study confirmed that *J. variegata* may be exploited to develop an effective natural wound healing remedy although additional studies are needed to confirm the findings clinically.

SIGNIFICANCE STATEMENT

This study discovers the antioxidant, antibacterial, and wound healing properties of *J. variegata* leaves extract that can be beneficial for skin infection and wound healing. The study can be helpful for upcoming researchers to use this plant for the formulation and evaluation of wound healing and other cosmetic applications. Thus *J. variegata* may be exploited to develop an effective natural wound healing remedy.

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