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Research Article

ALOE IRAFENSIS AN ENDEMIC PLANT OF YEMEN: PHYTOCHEMICAL SCREENING, ANTIBACTERIAL, ANTIOXIDANT, AND WOUND HEALING ACTIVITIES

BUSHRA ABDULKARIM MOHARRAM[1]*, HASSAN M. AL-MAHBASHI[2], TAREQ AL-MAQTARI[3,4], RIYADH SAIF-ALI[5], AMIN A. AL-DOAISS[6]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen. [2]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Sana`a University, Sana'a, Yemen. [3]Department of Pharmacology, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen. [4]Department of Pharmacology, Faculty of pharmacy, International University for Science and Technology, Daraa, Syria. [5]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Sana'a University, Sana'a, Yemen. [6]Department of Anatomy and Histology, Faculty of Medicine, Sana'a University, Sana'a, Yemen.

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ABSTRACT

Objectives: The objectives of the study were to determine the phytochemical constituents and to assess the antibacterial, antioxidant, and wound-healing properties of the methanol extracts of *Aloe irafensis.*

Methods: Methanol extracts of *A. irafensis's* latex, gel, and green skin were screened for their phytochemical constituents. All three extracts were investigated regarding their antibacterial potential using disc diffusion and microdilution assays, and their antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl free-radical scavenging assay. Histological analyses of wound healing areas were performed following the administration of the latex extract in male albino rats.

Results: The methanol extracts of *A. irafensis* revealed the presence of carbohydrates, steroids, phenols, tannins, and anthrones. The latex extract showed greater inhibition zones against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (24 and 17 mm, respectively) with minimum inhibitory concentration values of 1.25 and 2.50 mg/ml, respectively. The latex extract showed the highest antioxidant activity (IC_{50} of 65.54 µg/ml), followed by green skin extract (IC_{50} of 89.48 µg/ml). The latex extract significantly accelerated the rate of wound healing in rats ($p<0.01$), compared to the reference control fucidin ointment. Histological findings showed remarkably less scar width at wound closure site in the latex extract-treated wounds. Granulation tissue contained fewer inflammatory cells and more fibroblasts in wounds treated with the latex extract compared to those treated with the vehicle.

Conclusion: *A. irafensis* latex extract is a potential source of bioactive compounds that can be exploited for antioxidant, antibacterial, and wound healing purposes.

Keywords: *Aloe irafensis*, Phytochemical, Antioxidant, Antibacterial, Wound Healing.

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INTRODUCTION

Aloe (family Alliaceae) is a genus of about 500 species of flowering succulent plants [1]. They are commonly found in Southern Africa and on the eastern side of the continent. Many other species are found in the Arabian Peninsula and Madagascar [2]. There are about 33 *Aloe* species found in Yemen, 17 of which are considered endemic [3]. *Aloe irafensis* is one of the *Aloe* species endemic to Yemen, particularly in the city of Taiz where it is found in many parts of the city [3].

Several previous studies have shown that *Aloe vera*, a well-known *Aloe* species, possesses various biological activities including anticancer, hypoglycemic, hepatoprotective, gastroprotective, antibacterial, and antiviral activities [4-8]. The medicinal properties of *A. vera* are attributed to the presence of certain chemical components, such as anthraquinones, phytosterols, carbohydrates, chromes, enzymes, vitamins, amino acids, and proteins [9].

A. irafensis is traditionally used by locals in Yemen to speed healing wounds, to treat conjunctivitis, and as a laxative, among others. However, there is a significant shortage of data regarding *A. irafensis*, unlike its closely related species *A. vera.* Therefore, this study was undertaken to screen extracts of different parts of *A. irafensis*, regarding their phytochemical composition, as wells as their antibacterial, antioxidant, and wound-healing properties.

METHODS

Chemicals, reagents and drugs

Methanol (99.8%; Scharlae, Spain), ethyl acetate (HiMedia, India), formic acid (Fluka, Switzerland), glacial acetic acid (WinLab, UK), chloroform (Sigma, Germany), diethyl ether (Scharlau, Spain), toluene (Scharlau, Spain), methanol (High-performance liquid chromatography (HPLC) grade) (Sigma, MO, USA), acetonitrile (HPLC grade) (Scharlau, Spain), and phosphoric acid (Scharlau, Spain) were used in plant extraction and chemical testing. Tween 80 (Uni-Chem, Beograd) and dimethyl sulfoxide (DMSO; Scharlae, Spain) were used as suspending agents. Formalin was purchased from BDH Chemicals, UK. The reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, (USA). Ceftriaxone 1 g (Cipla, India), gentamicin (80 mg/2 mL; Alexandria CO, Egypt), and Vitamin C (Sigma, MO, USA) were used as standard drugs. Human blood agar (HuBA; Rapid labs, UK) and Mueller-Hinton agar (MHA; Scharlau, Spain) were used in the antimicrobial assay. Antibiotic discs, ceftriaxone (30 µg), aztreonam (30 µg), and gentamicin (120 µg) were obtained from Oxide, England. Fusidic ointment 2% (Fucidin®, LEO Pharma, Ireland), thiopental (40 mg/kg; Rotexmedica, Germany), and ketoprofen (Ketofan®, Egypt) were used in wound healing assays.

Plant materials

The leaves of *A. irafensis* were collected from Taiz, Yemen in January of 2016. The plant was identified and confirmed by Dr. Abdul Wali Al

Khulaidi, Public Authority for Research and Agricultural Extension, Dhamar city, Yemen. The voucher specimen of the plant was deposited in the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University with a voucher number of Air1 2016.

Preparation of the methanol extracts

Preparation of *A. irafensis* extracts were carried out as previously described [10]. Briefly, the latex was collected from leaves after cutting the fresh leaves and drying them at room temperature for 2 days. The gel was also obtained from the cut leaves while the green skin of leaves was air-dried for 1 week. The three obtained plant parts (gel, latex, and green skin) were extracted with 99.8% methanol, filtered, and dried using a rotary evaporator. The percentage of yield for each extract was calculated based on dry weights. Yields were 0.03% for the gel, 80.94% for the latex, and 15.9% for the green skin. The dried extracts were then stored in desiccators for subsequent phytochemical screening and biological activity assessment.

Experimental animals

Adult male albino rats (n=30), 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 maintained under standard conditions (12-h light-dark cycle cycle; 25±3°C; 35–60% humidity) with strict hygienic conditions. Animals were provided standard chow and water accessible *ad libitum*. The rats were acclimatized to the laboratory conditions for at least 48 h before experimentation. All animal experiments were approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana'a University, (02/FPhSana'a/2016).

Phytochemical screening and fingerprinting

Chemical tests were performed according to previous studies [10,11] to identify alkaloids, carbohydrates, fixed oils, fats, steroids, anthraquinones, phenolic compounds, tannins, proteins, saponins, gum, and mucilage.

Components and their retention factor (Rf) values for *A. irafensis* extracts were identified using thin-layer chromatography (TLC) as per the conventional one-dimensional ascending method using 60 F254, 20 cm × 20 cm silica gel sheets (Merck, Germany). TLC was performed as described previously [12,13].

HPLC analysis was used to look at the chemical profiling of *A. irafensis* latex extract. The method was performed as described elsewhere [14] with few modification. The HPLC/ultraviolet (UV)-visible system consisted of a Shimadzu HPLC system comprised of a pump (LC 10AD vp, Shimadzu, Japan), a manual injector (7725i, USA), a UV-visible detector (SPD-10A vp, Shimadzu, Japan) and a degasser (DGU-14A, Shimadzu, Japan). A shim-pack CLC-ODS (M) C18 reversed-phase column (5 µm, 4.6 × 250 mm, Shimadzu, Japan) was employed, at ambient temperature. Separation was done using acetonitrile (A) and 0.1% aqueous phosphoric acid (B) with a gradient elution of 24% A at 0–12 min, 24–50% A at 12–22 min, 50–24% A at 22–40 min, and 24% A at 40–50 min. The flow rate was 1.0 ml/min and the separation was monitored by measuring UV absorbance at 254 nm. The injection volume was 20 µl of 1 mg/ml methanol extract.

Antibacterial activity

Test microorganisms

The antibacterial activity of *A. irafensis's* extracts was assessed against three local bacterial isolates, namely: Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli*. The bacteria were sub-cultured on HuBA at 35°C for 24 h. The bacteria used were identified and obtained from the Microbiology by the Microbiology Department of Yemen Lab laboratory, Sana'a, Yemen.

Antibacterial assays

The antibacterial activity was performed using disc diffusion and broth microdilution assays. The disc diffusion method was performed as described previously [13].

Broth microdilution assay was performed according to the method of National Committee for Clinical Laboratory Standard [15] with few modifications [13]. The assay was done only for the extracts that exhibited an inhibition zone (IZ) greater than 15 mm. The antibacterial activity was considered "strong" if minimum inhibitory concentration (MIC) <1.00 mg/mL, "moderate" if 1.00≤ MIC ≤4.9 mg/mL, and "weak" if MIC ≥5.00 mg/mL [16].

Minimum bactericidal concentration (MBC) was determined by adding 20 µL of broth taken from microtiter plate wells (used for determining MICs) which did not show visible bacterial growth and the broth was inoculated in the MHA medium in triplicates and then incubated for 24 h at 35°C.

Antioxidant activity

The antioxidant activity of *A. irafensis* extracts was evaluated according to a previous work [13].

Wound-healing activity

The wound healing activity of the latex extract was assessed according to Moharram et al. [10]. Rats were weighed before the surgical procedure and anesthetized prior and during creation of wounds using 40 mg/kg intraperitoneal thiopental. Subcutaneous 5 mg/kg ketoprofen (an analgesic) was injected into all animals right before surgical operation and then every 24 h afterwards for 2 consecutive days. The dorsal fur of the animals was shaved by an electrical shaver and swabbed with 70% alcohol. An area of uniform wound of 4 cm^2 in length and 0.2 cm in depth was created at the nape of the dorsal neck of all rats with the aid of a square seal. The wounds made involved the lower subcutaneous tissue.

Rats were randomly divided into five groups of six rats each. Group 1 was untreated (negative control). Group 2 was topically dressed at the wound site with 0.2 ml gum acacia (vehicle) twice a day for 14 days. Groups 3 and 4 were topically dressed with 0.2 ml 100 or 200 mg/ml (for Group 3 and Group 4, respectively) of the *A. irafensis* latex extract while Group 5 was dressed with 0.2 ml 2% fusidic acid ointment and served as a positive control.

Each rat was then housed in an individual cage and the contraction of wound area was measured manually in square centimeters. The wound closure area was assessed by recording the wound area at days 0, 7, and 14 post-wounding using a transparent paper and a permanent marker as prescribed elsewhere [17]. The wound areas were measured using a graph paper.

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

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

A0 is the wound area at day 0 and Ad is the wound area on the corresponding day.

Histopathological studies

Tissue specimens were excised from healed cutaneous tissues obtained at day 14 post-operation from each rat. The excised tissue was preserved in neutral buffered formalin (10%) at 4°C. The tissue was then dehydrated in graded ethanol, cleared in xylene, embedded in paraffin, and blocked out. Then, 5 µm-thick sections of the tissues were mounted on glass slides. After dewaxing the sample, it was rehydrated in distilled water and stained with hematoxylin and eosin according to Suvarna *et al*. [18]. The prepared slides were examined under a compound microscope equipped with a digital camera to check the degree of re-epithelization, granulation tissue formation, and collagen deposition/organization.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS v. 11.5). Each sample was measured in triplicates, and the results

were presented as means plus or minus standard deviations. Wound healing was calculated as a percentage of initial wound areas. Paired t-test was used to test the significance of differences between every two groups. Significance level was set at <0.05.

RESULTS

Phytochemical screening

Chemical and TLC testing of *A. irafensis* latex, gel and green skin extracts showed the presence of various bioactive components including carbohydrates, steroids, phenols, tannins, and anthrones. The chemical components of each extract, Rf values and the solvent systems used are presented in Table 1. TLC results indicated the presence of anthrones as evidenced by appearance of yellow spots in the KOH-treated TLC plates containing latex (Rf. values 0.22, 0.34, and 0.45), gel (Rf. values 0.22, 0.32, and 0.45), and green skin (Rf. values 0.34 and 0.45) extracts under visible and UV light (UV wavelength = 365 nm) (Fig. 1a and b, respectively). However, coumarins were found only in the green skin extract as evidenced by the appearance of two light blue spots (Rf. values 0.13, 0.14) (Fig. 1c) under UV light (wavelength 365 nm) induced by spraying the plates with 10% alcoholic KOH. Furthermore, bitter components were found in the latex and green skin extracts as indicated by the appearance of one KOH-induced colored spot for each extract (Rf. = 0.38) under visible and UV-365 light.

HPLC analysis of *A. irafensis* latex extract showed the appearance of six major peaks and various minor others (Fig. 2). The major peaks appeared at retention times of 3.53, 9.18, 10.96, 13.52, 21.69, and 21.89 min.

Antibacterial activity

The latex extract of *A. irafensis* showed strong antibacterial activity against *S. aureus* and *P. aeruginosa* with inhibition zones (IZs) of 24 and 17 mm, respectively (Table 2). In addition, *E. coli* exhibited moderate sensitivity only to the latex extract (IZ = 10 mm). MICs were determined only for the latex extracts that showed an IZ of greater than 15 mm. The results indicated that the latex extract had MIC values of 1.25 and 2.5 mg/ml against *S. aureus* and *P. aeruginosa*, while MBCs values were 2.5 and 5.0 mg/ml, respectively (Table 2).

Antioxidant activity

The three extracts of *A. irafensis* showed significant antioxidant activity (Fig. 3). The latex extract exhibited the highest antioxidant activity (IC_{50} value of 65.54 µg/ml) followed by the green skin extract (IC_{50} value of 89.48 µg/ml).

Wound-healing activity

Wound closure area of wounds (in cm^2) for the different animal groups was measured on days 0, 7, and 14 after wounding (Table 3, Fig. 4). Both concentrations (100 and 200 mg/ml) of *A. irafensis* latex extract resulted in significant ($p<0.01$ and $p<0.001$) wound healing on day 7 (1.6 and 0.93 cm^2, respectively) and day 14 (0.03 and 0.01 cm^2, respectively) of inducing injury to rats' dorsal necks, compared to untreated and vehicle (gum acacia-treated) groups. Interestingly, on days 7 and 14 post-treatment, the 200 mg/ml latex extract of *A. irafensis* exhibited more reduction in wound area (0.93 and 0.01 cm^2, respectively) compared to the reduction induced by fusidic acid ointment, a reference drug (1.68 and 0.44 cm^2, respectively).

Histopathological examination of newly formed scars after 14 days after wounding is shown in Fig. 5. Histology of the wounds treated with the latex extract and fusidic acid ointment were associated with significantly less scar width compared to that induced by the vehicle. In addition, the wound of latex-treated group was partially to completely covered by newly formed epidermis and dermis layers. The extracellular matrix was also rich in newly-formed collagen fibers indicating rapid and progressive physiological wound healing in animals treated with the latex extract. Furthermore, the latex extract-treated group exhibited more proliferating blood capillaries (indicating enhanced angiogenesis) and dense collagen fibers in the granulation tissue of the wounded area with almost complete absence of macrophages or edema compared to the vehicle group (Fig. 6). Histological evaluation revealed increased

Table 1: TLC Investigation of methanol extracts of *A. irafensis*

Constituent	Solvent system	Part	NO. spots	Rf values
Anthraglycosid (anthrones)	Ethyl acetate: methanol: water (100:13.5:10)	Latex	3	0.22 0.32 0.45
		Gel	3	0.22 0.32 0.45
		G. skin	2	0.34 0.45
Bitter principles	Ethyl acetate: methanol: water (100:13.5:10)	latex	1	0.38
		Gel	-	-
		G. skin	1	0.38
Alkaloids	Ethyl acetate: methanol: water (100:13.5:10)	Latex	-	-
		Gel	-	-
		G. skin	-	-
Flavonoids	Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)	latex	-	-
		Gel	-	-
		Green skin	-	-
Saponins	Chloroform: glacial acetic acid: methanol: water (64:32:12:8)	latex	-	-
		Gel	-	-
		G. skin	-	-
Coumarins	Diethyl ether: toluene (1:1)	latex	-	-
		Gel	-	-
		G. skin	2	0.13 0.14

- = not detected, G. skin=green skin. A. *irafensis*: *Aloe irafensis*, Rf: Retention factor, TLC: Thin-layer chromatography

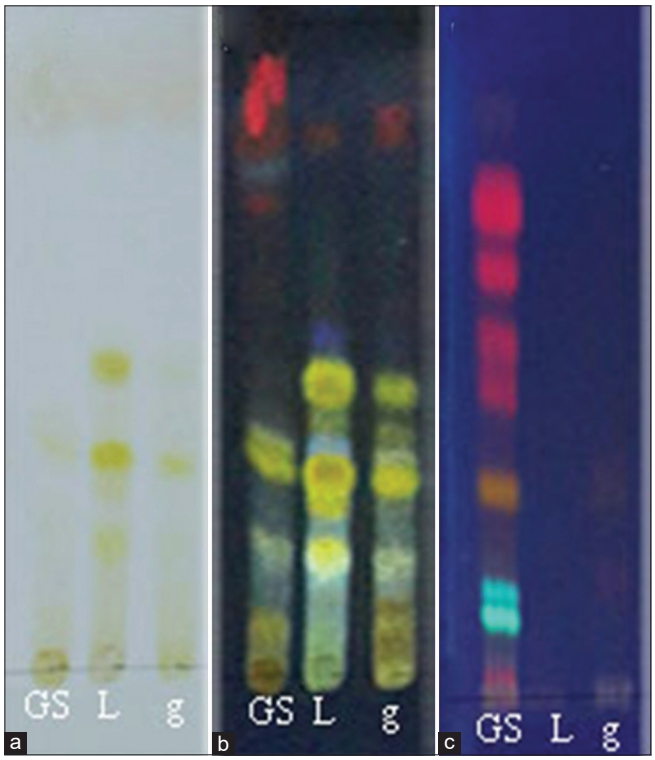

Fig. 1: Thin-layer chromatography plates of *Aloe irafensis* extracts (a-c). GS=green skin, L= latex, g= gel of *A. irafensis,* (a) TLC plate of anthraglycosides in visible light after spraying with 10% alcoholic KOH. (b) TLC plate of anthraglycosides in 365 nm after spraying with 10% alcoholic KOH. (c) TLC plate of coumarins in ultraviolet 365 nm light

Table 2: IZ, MIC, MBCs of *A. irafensis* methanol extracts

Samples	IZ (mm)			MIC (MBC) in mg/ml	
	S. aureus	*E. coli*	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*
Latex	24.5 ± 1.5	10.0 ± 0.0	17.0 ± 0.0	1.25 ± 0.0 (2.5 ± 0.0)	2.5 ± 0.0 (5.0 ± 0.0)
Gel extract	-	-	-	nd	nd
G. Skin	12.5 ± 0.5	-	11.5 ± 0.5	nd	nd
CRO 30 µg	20.0 ± 0.0	nd	nd	nd	nd
ATM 30 µg	nd	35.0 ± 0.0	nd	nd	nd
CN 120 µg	nd	Nd	27.0 ± 0.0	nd	nd
Ceftriaxone	nd	nd	nd	1.0 ± 0.0	Nd
Gentamicin	nd	nd	nd	nd	5 ± 0.0

Each value is the mean of three triplicates ± SD (standard deviation). CRO = Ceftriaxone, ATM = Aztreonam, CN = Gentamicin, - = no activity, (+ve) = positive control, nd = not determined. *A. irafensis: Aloe irafensis, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa,* MIC: Minimum inhibitory concentration, *E. coli: Escherichia coli,* MBC: Minimum bactericidal concentration, IZ: Inhibition zone

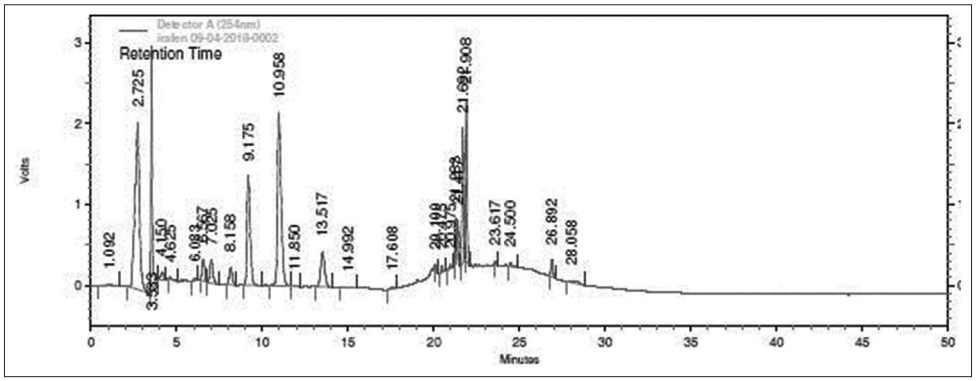

Fig. 2: High-performance liquid chromatography of *Aloe irafensis* latex extract at 254 nm absorbance using acetonitrile as solvent

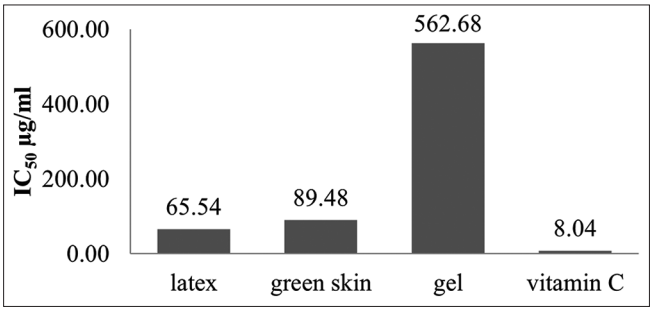

Fig. 3: IC_{50} values (µg/mL) of the methanol extracts of latex, green skin, and gel of *Aloe irafensis* and Vitamin C

cellular infiltration, collagen deposition, and re-epithelialization in the treated and the positive control groups but not in the vehicle-treated animals.

DISCUSSION

Several studies evaluated the biological activity, phytochemical screening, and clinical importance of various *Aloe* species. However, *A. irafensis* which is endemic to Yemen and traditionally used for antibacterial and wound healing, has not yet been well investigated. In this study, the chemical screening of *A. irafensis* indicated that the latex, gel, and green skin extracts contained carbohydrates, steroids, phenols, tannins, and anthrones which are commonly found in several *Aloe* species [6,19].

Due to the development of antibiotic resistance, the present study endeavored to investigate the antibacterial potential of *A. irafensis* against clinically isolated bacterial pathogens including *S. aureus* and *P. aeruginosa* and *E. coli*. Among the plant extracts, the latex extract exhibited the highest antibacterial results against *S. aureus* and *P. aeruginosa* (bactericidal effect). The activity was shown to be comparable to that of the conventional antibiotics ceftriaxone and gentamicin. These results are in accordance with a recent study which reported that *A. inermis* latex extract showed strong antibacterial activity against *S. aureus* and *P. aeruginosa* [10]. Compounds such as alkaloids, tannins, flavonoids, anthraquinones, tannins as well as saponins were responsible for the antimicrobial activity of *A. vera* [20-22]. The exhibited antibacteral activity may also be due to a synergistic action exerted by multiple bioactive compounds rather than by a single chemical substance.

In the present study, the latex extract of *A. irafensis* showed significant antioxidant activity as shown by the DPPH free radical scavenging assay. This result is in agreement with a previous study which reported that Aloe species such as *A. vera* and *A. inermis* possessed high DPPH free-radical and superoxide-radical scavenging activities [10,23].

In addition, the results of the present investigation clearly indicated the wound-healing properties of *A. irafensis.* A wound is the result of loss or damage of cellular, anatomical, or functional continuity of living tissues [24], whereas wound healing is the process of repairing injured tissues. Wound healing is considered effective if the wound is healed in a relatively short time with minimal pain, discomfort, and scarring [25,26]. The wound-healing process occurs in three phases: Inflammatory phase (hemostasis and inflammation), proliferative phase (granulation, contraction, and epithelialization), and remodeling phase, which organizes the newly-formed structures forming progressively increased tensile strength [25].

The findings of the present study confirmed the significant acceleration of dermal wound healing in rats following the topical twice-daily application of *A. irafensis* latex extract. The results of the present study are consistent with the previous studies that reported a significant wound healing activity of *A. vera* [27,28].

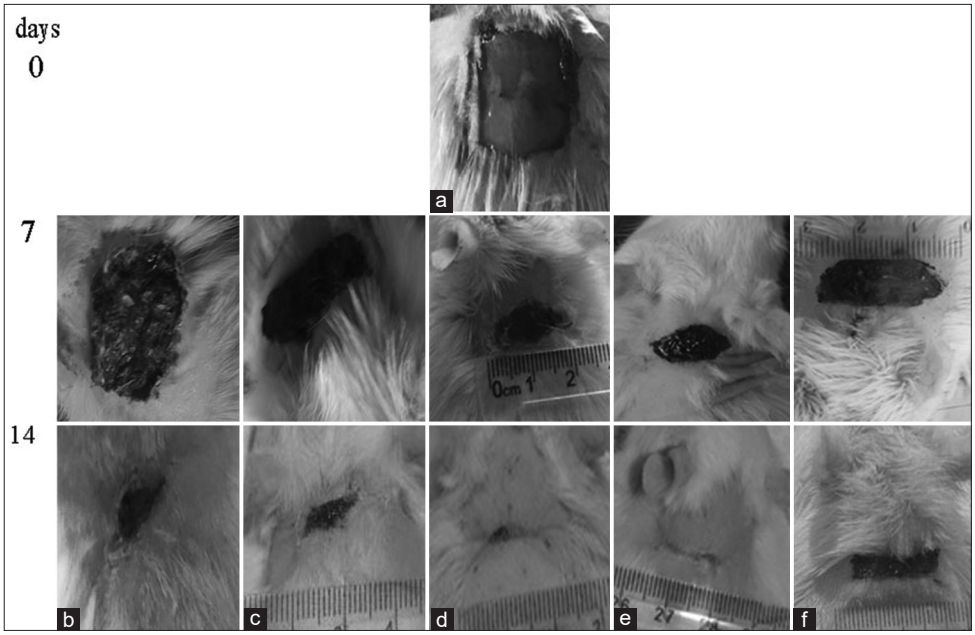

Fig. 4: Macroscopic appearance of wounds at days 0 (a) after surgery; (b): Untreated rats; (c) rats treated with 0.2 mL normal gum acacia (vehicle); (d) rats treated with 100 mg/ml of *Aloe irafensis;* (e) rats treated with 200 mg/mL of *A. irafensis;* (f) rats treated with fucidin ointment (positive control) at days 7 and 14

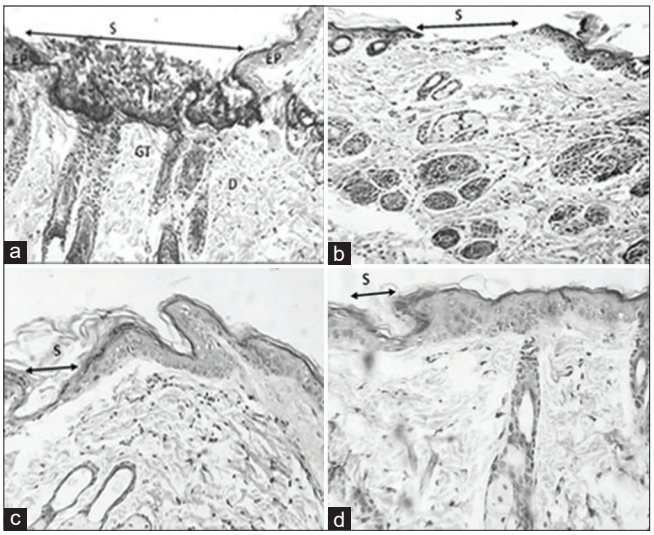

Fig. 5: Histological section of the 14-day-old granulation tissue of healed wound in: (a) Animals treated with vehicle, showing wide scar area (arrow) and not fully epithelialized, dense collagen fibers, fibroblasts with inflammatory cells showing incomplete healing, and showing focal ulceration of epidermis; (b) animals treated with *Aloe irafensis* 100 mg/ml showing a wide scar (arrow) area; (c) animals treated with *A. irafensis* 200 mg/ml showing a narrow scar (arrow) at the wound closure; (d) animals treated with fucidin showing a narrow scar area at the wound closure (arrow), S = scab, EP = epidermis, D= dermis and GT = granulation tissue (H and E 400×)

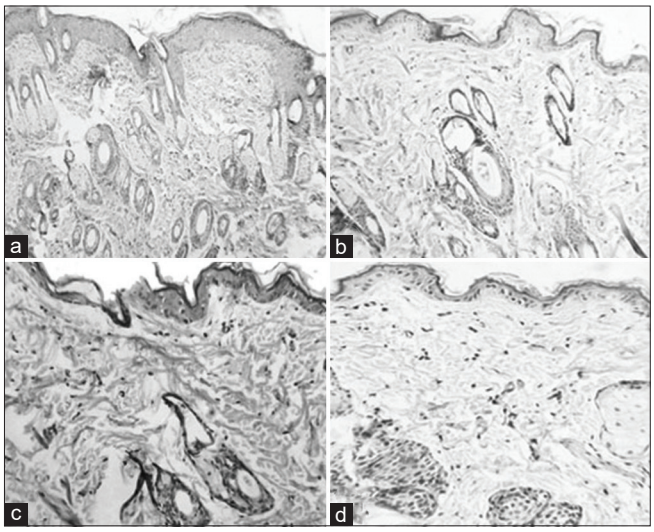

Fig. 6: Histological sections of the 14-day-old of healed tissue treatment with control, standard and test groups. (a) Granulation tissue of control group (vehicle) showing less collagen and abundent macrophages. (b,c) Granulation tissue of extract treated group animals showing well-organized collagen bundles with and well-developed blood vessels. There were dense collagen fibers and fibroblasts with neovascularization and few macrophages. (d) Granulation tissue of standard ointment (fucidin) treated group animal showing moderate deposition of collagen. Appearance of skin appendages can also be visualized on 14 day of the experiment (H and E stain, 200×)

In the current study, the histopathological findings showed presence of collagen, fibroblasts, hair follicles, and blood capillaries in the granulation tissue of wounds treated with the latex extract. The wounds treated with the latex showed a proliferative phase and were associated with no presence of inflammatory cells. The proliferation of fibroblasts and production of collagen fibers were much greater in the latex-treated wounds compared to the vehicle group, indicating that the treated wounds had already gone through the inflammatory phase and then entered the maturational phase. The anti-inflammatory activity of *A. irafensis* probably played a significant role in speeding the wound healing process by alleviating and shortening the early inflammatory phase post wounding [29]. In accordance, *A. vera* has also been shown to accelerate healing of burnt skin in rats by reducing inflammation and stimulating mature granulation tissue formation [27,28]. It is believed that rapid cellular proliferation, collagen deposition, epithelialization, myofibroblasts

Table 3: Effect of latex extract of *A. irafensis* on the wound closure area in experimental rats

Day	Wound area mm² (% wound healing)				
	Untreated	Gum acacia	*A. irafensis*		Fusidin
			100 mg/ml	200 mg/ml	
0	4.0	4.0±0.5	4.0±0.0	4.0±0.6	4.0±0.7
7	3.12±0.6 (22.1)	2.19±0.2^{a}** (45.2)	1.6±0.3^{ab}** (60.1)	0.93±0.2^{ab}***c* (76.7)	1.68±0.5^{ab}* (58.1)
14	0.82±0.2 (79.5)	0.31±0.11^{a}** (92.4)	0.03±0.02^{ab}***c* (99.3)	0.01±0.01^{ac}***b** (99.7)	0.44±0.1^{a}** (89.0)

WC% = percentage of wound closure. Data represent mean values±SD, n=6, ^{a}=significance compared to the untreated groups; ^{b}=significance compared to the gum acacia group; ^{c}=significance compared to the fusidic acid group, paired t-test; *p<0.05, **p<0.01, ***p<0.001 was considered significant. *A. irafensis: Aloe irafensis*

production, and angiogenesis in and around the wounded area play major roles in wound healing [30].

Factors such as presence of microbes and free radicals can delay wound healing. Therefore, agents with antioxidant and antimicrobial activities could be helpful in accelerating wound healing [31,32]. Interestingly, the present study confirmed that the latex of *A. irafensis* possessed both antibacterial and antioxidant activities. It has been reported that flavonoids, saponins, tannins, and phenolic bioactive compounds promoted wound healing due to their antimicrobial and astringent properties [33,34]. Other studies reported that tannins promoted wound healing through scavenging free radicals and oxygen reactive species, promoting contraction of the wound and stimulating angiogenesis [35,36]. The current study showed the presence of several bioactive phytochemicals in *A. irafensis* (carbohydrates, steroids, phenols, tannins, and anthrones) which may be responsible for the shown latex wound healing effect.

CONCLUSION

In this study, the phytochemical analysis revealed that *A. irafensis* extracts contained various bioactive compounds. The study also showed that *A. irafensis* latex extract exhibited strong antibacterial, antioxidant, and wound-healing activity. The wound-healing activity of the plant extract was apparently achieved through promoting epithelialization, collagenation, neovascularization and by exhibiting antibacterial, antioxidant effects. The findings provide insight into the potential utilization of *A. irafensis* in the treatment of wounds, particularly those associated with bacterial infections. For such future applications, pin-pointing and isolating the bioactive compounds responsible for the medicinal activities of *A. irafensis* are warranted.

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AUTHORS' CONTRIBUTIONS

Dr. Bushra Abdulkarim Moharram and Dr. Hassan M. AL-Mahbashi conceived, designed and performed the experiments. Dr. Bushra Abdulkarim Moharram, Dr. Riyadh Saifali and Tareq Al-Maqtari analyzed the data and wrote the paper. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

There were no conflicts of interest.

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