Research Article

Phytochemical, antibacterial, antioxidant and wound healing properties of *Aloe inermis* latex extract obtained from Yemen

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Abstract

Objective: To determine the phytochemical content and to evaluate the antibacterial, antioxidant, and wound healing activities of the methanol extracts of *A. inermis.* **Material and Methods:** The methanol extracts of the latex, gel and green skin of *A. inermis* were screened for their phytochemical content. The antibacterial activity of the extracts was investigated using disc diffusion and broth microdilution assays and the antioxidant activity was evaluated using the DPPH free radical scavenging assay. The wound healing activity of the latex extract was evaluated via histological analyses and area measurements of healing wounds in rats. **Results:** The methanol extracts of *A. inermis* parts contained carbohydrates, steroids, phenolic compounds, tannins, anthraglycosides, anthrons and bitter compounds. The latex extract of *A. inermis* exerted a strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition zones of 18 and 19 mm, respectively, with MIC values of 1.25 mg/ml and MBCs of 2.50 mg/ml against both microorganisms. The latex and green skin extracts exhibited the highest antioxidant activity on DPPH assay with IC_{50} values of 55.9 and 67.4µg/ml, respectively. Topical application of the latex extract significantly accelerated the rate of wound healing and was associated with less scarring. The newly-formed granulation tissue of healing wounds contained less inflammatory cells and more fibroblasts compared to wounds treated with the vehicle. **Conclusion:** The latex extract of *A. inermis* is a potential source of bioactive compounds that may be exploited in the treatment of bacterial infections and to promote wound healing.

***Keyword*:** *Aloe inermis,* antibacterial, antioxidant, latex extract, phytochemical, wound healing

Introduction

Aloe inermis Forssk. (family; Aloeaceae) is a species native to many parts of the world including western Yemen where it mostly grows on dry rocky mountains as high as 1-2 kilometers (Wood, 1997). *A. inermis is* used traditionally, similar to other *Aloe species*, in the management of constipation, wounds, conjunctivitis, and as a disinfectant. Compared to *A. inermis*, *Aloe vera* is better established in folk medicine for treating constipation, skin disease, and healing wounds in various cultures (Gao et al., 2019). Aloe vera has also been shown to possess anticancer, hypoglycemic, hepatoprotective, gastrointestinal modulating, antibacterial and antiviral activities (El-Shemy et al., 2010; Nejatzadeh-Barandozi, 2013; Sharma et al., 2013; Zakieh et al., 2014; Zandi et al., 2007). The active components involved include anthraquinones (e.g. barbaloin), phytosterols, carbohydrates, chromes, enzymes, vitamins, amino acids, and proteins (Hamman, 2008).

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Wound repair remains a big concern for many clinicians as traumatic injuries constitute one of the leading causes of mortality, even in the developed countries of Europe and North America (Eming et al., 2014). Numerous orchestrated histological and physiological mechanisms take place *in situ* to ensure proper wound healing including hemostatic coagulation, inflammation, granulation tissue and extracellular matrix formation, connective tissue remodeling, collagenization and new tissue strength acquisition (Reddy et al., 2002). On the other hand, factors that may slow these mechanisms and thus slow down the healing process include bacterial contamination and oxidative stress (Mayes et al., 2015; Schäfer and Werner, 2008).

The idea of exploiting the surrounding natural reservoirs as a source of well-tolerated medications to treat wounds is rooted in the history of humanity since ancient times. *A. vera* is among the promising resources as it has been reported to accelerate the healing of burnt skin in rats via reducing inflammation and creating a more mature granulation tissue that is more conducive to skin recovery (Hamid and Soliman, 2015; Takzare et al., 2009). Thus, in this work, we sought to assess whether *A. inermis* possess a comparable therapeutic profile via investigating its phytochemical content and assessing its antibacterial, anti-oxidant and wound healing properties.

To the best of our knowledge, this work is the first to report the phytochemical, antibacterial, antioxidant and wound healing of *Aloe inermis* and this work is the first investigation on this species.

Materials and Methods

Chemicals, reagents and drugs

Solvents used in plant extraction and chemical tests include 99.8% methanol (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), tween 80 (Uni-Chem, Beograd), dimethylsulfoxide (DMSO) (Scharlae, Spain), formalin (BDH Chemicals, UK). The reagent used for the antioxidant assay was 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, USA). Ceftriaxone 1 g (Cipla, India), Gentamicin (80 mg/2 mL; Alexandria Co., Egypt) and Vitamin C (Sigma, USA) were used as positive control agents. For the antibacterial analyses, media used included human blood agar (HuBA) (Rapid laps, UK), Mueller-Hinton agar (MHA) (Scharlau, Spain) and Mueller-Hinton broth (MHB) (Oxoid, UK). Antibiotic discs containing 30 µg ceftriaxone, 30 µg nitrofurantoin and 120 µg gentamicin (Oxide, England) served as positive control antibiotics. Other agents used included 2% fusidic acid ointment (Fucidin®, LEO Pharma, Ireland), 40 mg/kg thiopental (Rotexmedica, Germany) and Ketoprofen (Ketofan®, Amriya Pharm Ind, Egypt).

Plant materials

The leaves of *A. inermis* were collected in Taiz city, Yemen in January 2016 and were 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, Sana'a University, with a voucher number of (Ain1 2016).

Preparation of the methanol extracts

A. inermis leaves were thoroughly cleaned before latex collection via cutting the fresh leaves and draining them into clean glass dishes for an hour before drying for 2 days. The gel of the plant was also obtained from the cut leaves and was collected in clean glass containers. The green skin of the leaves from which the gel has been removed was air-dried for a week and then ground using a mortar. The obtained latex, dried gel, and green skin were then extracted separately using methanol (99.8%) before filtration. The filtrates were dried via a rotary evaporator (Buchi rotavapour R-215; Switzerland) in a water bath (Buchi water bath B-491; Switzerland) at a temperature not exceeding 45 ˚C. The percentages of yield for extracts (68.67% for the latex, 1.31% for the gel, and 12.32% for the green skin) were calculated based on initial dry weights. The final dried extracts were stored in desiccators for subsequent phytochemical screening and biological activity tests.

Experimental Animals

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 maintained under standard conditions (12-hour light-dark cycle; 25 ± 3°C; 35–60% humidity) with strict hygienic conditions, and were fed food and water 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 in 9th September 2016 (02/FPhSana'a/2016).

Phytochemical screening

Chemical tests and thin layer chromatography (TLC)

Chemical tests were performed according to Banu and Cathrine (2015) to identify alkaloids, carbohydrates, fixed oils fats, steroids, anthraquinones, phenol, tannins, proteins, saponins, gums, and mucilage compounds. Chemical constituents and their retention factor (Rf) values for each extract of *A. inermis* were identified using thin layer chromatography (TLC) via conventional one-

dimensional ascending method using silica gel 60 F254, 20 x 20 cm (Merck, Germany). Thin layer chromatography was performed as previously described by Wagner and Bladt (1996) and by Moharram et al. (2018).

Antibacterial activity

Test microorganisms

The methanol extracts for the latex, gel and green skin of *A. inermis* were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* using disc diffusion and broth microdilution methods. All bacteria under investigation were sub-cultured on HuBA medium. All bacteria used were obtained from the Microbiology Department of YemenLab Laboratory, Sana'a, Yemen.

Antibacterial assays

Disc diffusion and broth microdilution assays were performed to determine the antibacterial activity of the extracts. Bacterial suspensions were prepared following standard methods recommended by the National Committee for Clinical Laboratory Standard (Coyle, 2005) with minor modifications (Moharram et al., 2018).

The disc diffusion method was performed as described by Moharram et al. (2018). Broth microdilution assay was performed according to National Committee for Clinical Laboratory Standard (Coyle, 2005) with few modification (Moharram et al., 2018). The assay was performed only on the extracts that have had inhibition zones of ≥15 mm.

Five controls were used: negative control [DMSO: methanol (1:1)], growth control (100 µl of bacterial suspension in 100 µl MHB), positive control (ceftriaxone or gentamicin), sterile control of plant extracts (100 µl of plant extract in 100 µl MHB) and 200 µl sterile plain MHB control (to confirm the sterility of the broth). The microtiter plates were incubated for 24 h at 35°C. The antibacterial activity was categorized as strong if the MIC was smaller than 1 mg/ml, moderate if the MIC was between 1 and 4.9 mg/ml, and weak if MIC was greater than 5 mg/ml (Jantan et al., 2008). Sterile conditions were maintained throughout the experiments. All experiments were performed in triplicates.

Minimum bactericidal concentration (MBC) determination

Twenty microliters of broth was taken from the microtiter plate wells (that had been used for determining MICs and which did not show visible bacterial growth) and was inoculated into MHA. The inoculated agar was then incubated for 24 h at 35 ºC. The MBC value was calculated as the lowest concentration of test materials that did not show any bacterial growth on the plates after incubation (Warnock, 1989).

Anti-oxidant activity

The antioxidant activity of *A. inermis* extracts was carried out according to the method described by Moharram et al. (2018).

Wound healing activity

The wound healing activity was assessed according to Amin et al. (2015). Rats were weighed prior to the surgical procedure and anesthetized using 40 mg/kg intraperitoneal thiopental. For analgesia, subcutaneous 5 mg/kg ketoprofen was injected into all animals right before the operation and then every 24 h afterwards for 2 consecutive days. The animal skin was shaved by electrical shaver and swabbed with 70% alcohol. An area of uniform 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. (2012). The wounds involved the lower subcutaneous tissue.

Rats were randomly divided into 5 groups of 6 rats each. Group 1 rats were untreated (negative control). Group 2 rats were topically dressed at the wound site with 0.2 ml of gum acacia (vehicle) at twice a day for 14 days. Groups 3 and 4 were topically dressed with 0.2 ml of 100 (for group 3) or 200 mg/ml (for group 4) of the *A. inermis* extract while group 5 were dressed with 0.2 ml fusidic acid ointment 2% and served as a positive control.

Each injured rat was housed in an individual cage and the contraction of the wound area was measured manually in square centimeters. The wound closure area was assessed by tracing the wound area at days 0, 7 and 14 after wounding. The wound closure rate was expressed as the ratio of wound area at a specific day to the wound area at day 0, implementing a transparent paper and a permanent marker as prescribed by Tsala et al. (2013).

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

Wound closure (%) = (1- Ad/A0) x 100

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

Histopathological studies

Biopsies 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, embedded in paraffin, and blocked out. Subsequently, 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. (2012). 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-epithelization, granulation tissue formation, and collagen

deposition and organization.

Statistical analysis

The data were analyzed using *Statistically Package for Social Sciences* (SPSS) version 11.5. Antibacterial and antioxidant experiments were performed in triplicate and the results were presented as means ± standard deviations (SD). Paired T-test was used to test the significance of the differences between every two groups in the wound healing activity. Significance level was set at 0.05.

Results

Phytochemical screening

Thin layer chromatography (TLC) and the chemical tests performed on the latex, gel and green skin of *A. inermis* indicated the presence of various bioactive components in all extracts including carbohydrates, steroids, phenols, tannins, and anthrones. Components, solvent system and Rf values for the three methanol extracts are listed in Table 1.

Table 1. TLC analyses showing the active constituents for the methanol extracts of *A. inermis.*

Chemical Constituents	Solvent System	Part of *A. inermis*	NO. spots	Rf Values
Anthraglycosides, anthrones	EtOAc: MeOH: Water (100:13.5:10)	Latex	3	0.19 0.30 0.59
		Gel	2	0.19 0.61
		G. skin	2	0.19 0.59
Bitter compounds	EtOAc: MeOH: Water (100:13.5:10)	Latex	-	0.38
		Gel	-	-
		G. skin	-	0.38
Coumarins	Diethyl ether: Toluene (1:1)	Latex	-	-
		Gel	-	-
		G. skin	1	0.13

EtOAc= ethyl acetate, MeOH= methanol, a.= acid, - = not detected

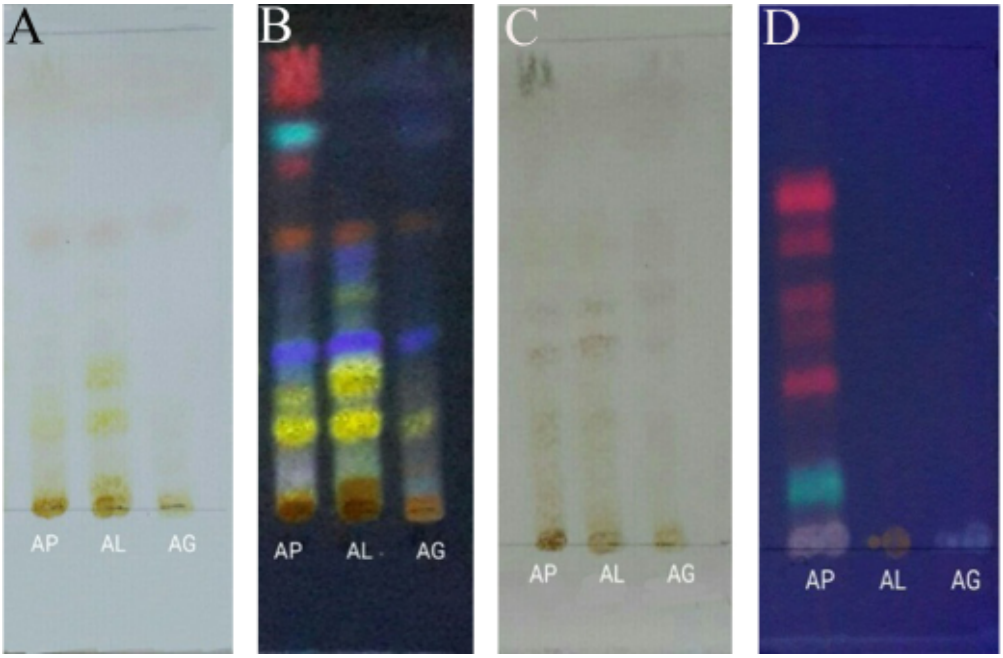

Figure 1. TLC analyses for *A. inermis* extracts (AP = green skin, AL = latex, AG = gel of *A. inermis),* (A) Detection of anthraglycoside in the TLC plate in visible light after spraying. (B) Detection of anthraglycoside in UV light (365 nm) after spraying. (C) Detection of bitter compounds in visible light after spraying. (D) Detection of coumarins in UV light (365 nm).

Our findings indicate that all extracts of *A. inermis* were negative for alkaloids, flavonoids and saponins. However, all extracts of *A. inermis* contained anthrones as evidenced by the yellow spots formed in visible (Photo 1-A) and in UV-365 nm (Photo 1-B) after spraying the TLC plates with 10% alcoholic KOH. In addition, formation of red spots in visible (Photo 1-A) and in UV-365 (Photo 1-B) in all TLC plates sprayed with 10% alcoholic KOH in the TLC of all extracts indicated the presence of anthraglycosides. It is noteworthy that the chemical composition for the three extracts was not identical. For instance, bitter compounds were found only in the latex and green skin extracts of *A. inermis* (Table 1, Photo 1-C) while coumarins were found only in the green skin as evidenced by the appearance of bright blue spots following the application of 10% alcoholic KOH (Table 1, Figure 1-D).

Antibacterial activity

Based on the disc diffusion assay, *A. inermis* latex extract showed strong antibacterial activity against *S. aureus* and *P. aeruginosa* with inhibition zones (IZ) ranging between 19 and 18 mm, respectively (Table 2). However, *E. coli* was weakly sensitive to the latex extract (IZ= 9 mm). Subsequently, MICs were determined based on the microdilution method (only when the IZ exceeded 15 mm). The latex extract had MIC values of 1.25 mg/ml against *S. aureus* and *P. aeruginosa*, and MBC values of 2.50 mg/ml (Table 2). Interestingly, these MIC values were comparable to those of ceftriaxone against *S. aureus* (MIC for ceftriaxone = 1 mg/ml) and gentamicin against *P. aeruginosa* (MIC for gentamicin = 5 mg/ml).

Compared to the latex extract, the green skin extract showed only moderate antibacterial activity against both *S. aureus* (IZ, 14 mm) and *P. aeruginosa* (IZ, 11 mm) while the gel extract was not active against any of the tested bacteria (Table 2).

To the best of our knowledge, this work is the first to report an antibacterial activity of *A. inermis* against *S. aureus*, *P. aeruginosa* and *E. coli*.

Anti-oxidant activity

The DPPH antioxidant assay showed that all 3 extracts of *A. inermis* exhibited a dose-dependent antioxidant activity *in vitro*. However, the latex extract exhibited the highest activity (IC_{50} 55.9 µg/ml) followed by the green skin extract (Figure 2).

Wound healing activity

A minor surgical procedure was carried out on rats to create a wound with an area of 4 cm^2 (at day 0) for all rats. Areas of wounds in all tested animal groups and the percentage of wound closure (WC %) on day 7 and 14 post-intervention were measured (listed in Table 3).

Our findings showed that wounds treated with the latex extract of *A. inermis* exhibited considerable signs of dermal healing and recovered faster than those of the vehicle group

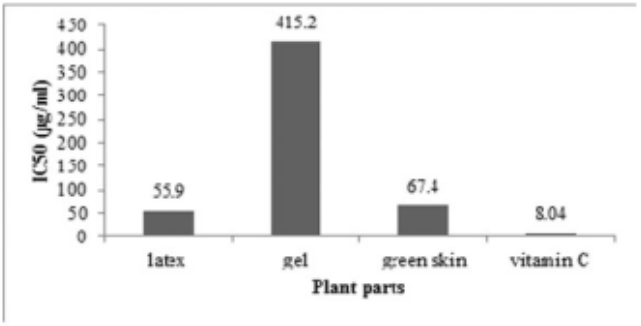

Figure 2. IC_{50} values (µg/ml) for the methanol extracts of the latex, gel, and green skin of *A. inermis* compared to vitamin C.

Table 2. Antibacterial activity of the methanol extracts of *A. inermis* as determined by disc diffusion (inhibition zone) and broth microdilution [minimum inhibitory concentration (MIC), minimum bactericidal concentrations (MBC)] assays

Samples	Part	Inhibition zone (mm)			MIC in mg/ml (MBC in mg/ml)	
		S. aureus	*E. Coli*	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*
A. inermis	Gel	-	-	-	N.D.	N.D.
	Latex	19.0 ± 0.0	9.0 ± 0.0	18.0 ± 0.0	1.25 ± 0.0 (2.5 ± 0.0)	1.25 ± 0.0 (2.5 ± 0.0)
	Green Skin	14.0 ± 0.5	-	11.0 ± 0.8	N.D	N.D.
CRO 30 µg	+ve	20.0 ± 0.0	N.D.	N.D.	N.D.	N.D.
Nit 30 µg	+ve	N.D.	35.0 ± 0.0	N.D.	N.D.	N.D.
CN 120 µg	+ve	N.D.	N.D.	27.0 ± 0.0	N.D.	N.D.
CRO					1.0 ± 0.0	N.D.
GN					N.D.	5.0 ± 0.0

Each value is the mean of three triplicate ± SD. CRO = Ceftriaxone, AMP = Ampicillin, Nit = Nitrofurantoin, CN and GN = Gentamicin, - = no activity, +ve = positive control, N.D. = not determined.

or of the untreated group (Figure 3). The percentages of wound closure (WC%) achieved by the 100 mg/ml *A. inermis* latex extract were 69.3% and 99.7% on days 7 and 14, respectively, and were 74.8% and 99.7% on days 7 and 14, respectively, by the 200 mg/ml extract. In contrast, the application of the vehicle control resulted only in 45.2% and 92.4% improvement on days 7 and 14, respectively (Figure 3 and Table 3).

Interestingly, on both day 7 and day 14 post-treatment, the 200 mg/ ml latex extract of *A. inermis* showed a more pronounced reduction in wound size compared to that induced by the well-known fusidic acid ointment, which was utilized as a reference control (74.8% and 99.7% for the latex vs. 58.1% and 89.0% for fusidic acid).

Table 3. Effect of the latex extract of *A. inermis* on the percentage (%) of wound healing/closure in experimental rats

Groups	Concentration	Day 0	Day 7		Day 14	
		Wound area in cm ^{2}	Wound area in cm ^{2} (WC%)	*P* values	Wound area in cm ^{2} (WC %)	*P* values
Untreated		4.0	3.12 ± 0.6 (22.1)	-	0.82 ± 0.2 (79.5)	-
Gum acacia	0.2 ml	4.0	2.19 ± 0.2 (45.2)	0.007 *	0.31 ± 0.11 (92.4)	0.001 *
A. inermis	100 (mg/ml)	4.0	1.23 ± 0.6 (69.3)	0.004 * 0.011 † 0.2 ‡	0.01 ± 0.02 (99.7)	2.3x10^{-5} * 0.002 † 0.0002 ‡
A. inermis	200 (mg/ml)	4.0	1.01 ± 0.3 (74.8)	0.0004 * 9.9x10^{-5} † 0.05 ^{c}	0.01 ± 0.02 (99.7)	2.8x10^{-5} * 0.001 † 0.0002 ‡
Fusidic acid	0.2 ml	4.0	1.68 ± 0.5 (58.1)	0.01 * 0.04 †	0.44 ± 0.1 (89.0)	0.004 * 0.12 †

WC% = percentage of wound closure. Data represent mean values ± SD, n = 6, *= significance compared to the untreated groups. † = significance compared to the gum acacia group, ‡, significance compared to the fusidic acid group. Statistical analysis was carried out using paired T-test to compare between each two groups (SPSS software).

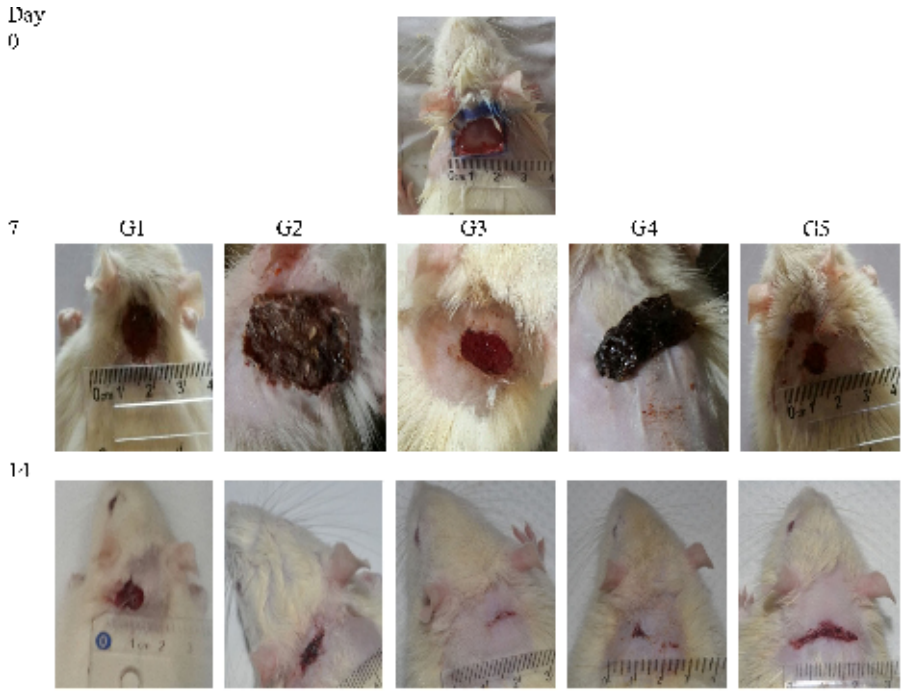

Figure 3. Macroscopic appearance of healing wounds at days 0, 7 and 14 after surgery. G1: represent the untreated rats; G2: represent rats treated with 0.2 ml gum acacia and show a wide wound closure area at day 7 and 14; G3: represent rats treated with 100 mg/ml of *Aloe inermis* and show almost complete wound closure; G4: represent rats treated with 200 mg/ml of *Aloe inermis* and show almost complete wound closure area; G5: represent rats treated with fucidic acid ointment and show better wound closure compared to G1.

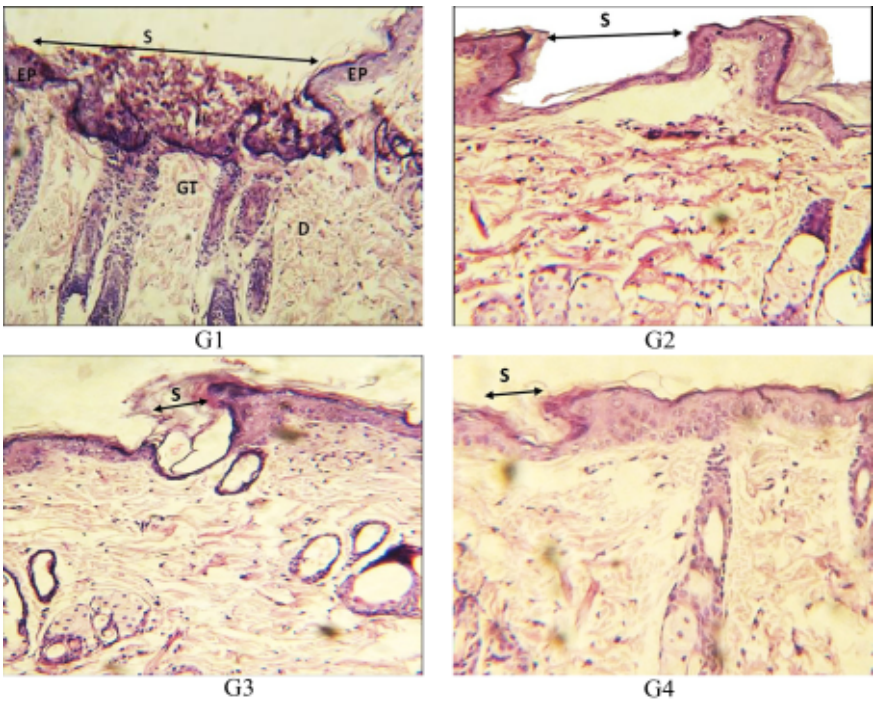

Figure 4. Histological sections for the 14-day-old granulation tissue of healed wounds G1: animals treated with the vehicle control had a wide scar area (arrow) that is not fully epithelialized. G2: animals treated with 100 mg/ml of *A. inermis* latex extract show a moderate scar (arrow) at the wound closure area. G3: animals treated with 200 mg/ml latex extract show a narrow scar area (arrow). G4: animals treated with fucidic acid show a small scar area (arrow) and absence of inflammatory cells. S = scab, EP = epidermis, D= dermis and GT = granulation tissue (H&E stain, 400x).

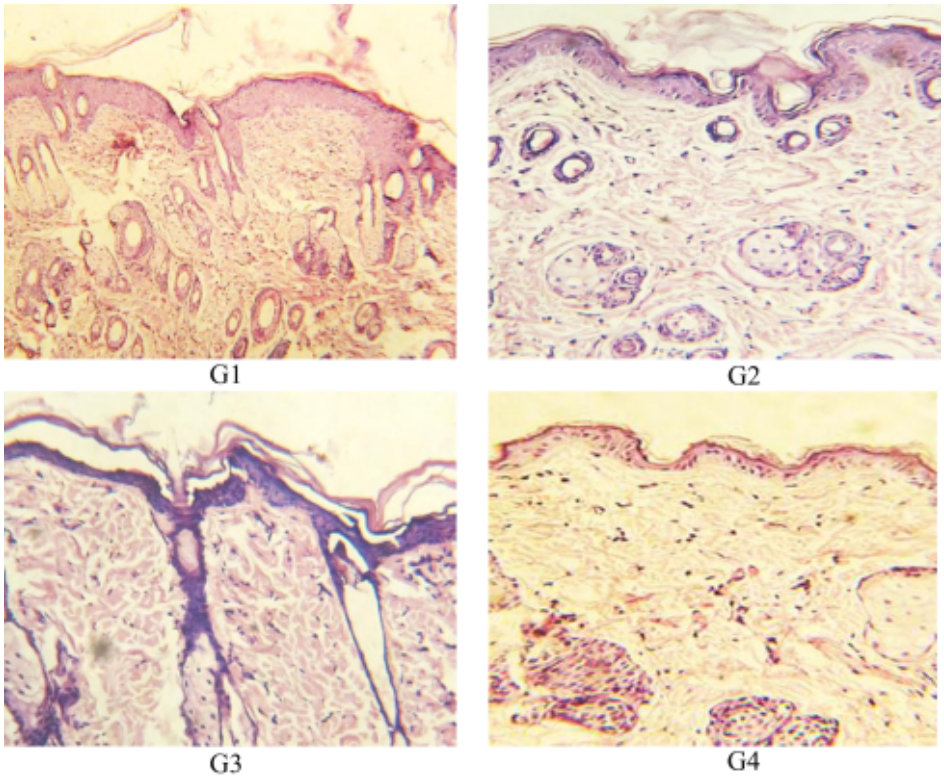

Figure 5. Histological sections of the healed tissues at day 14 post treatment. G1: Granulation tissue of the gum acacia treated group animal showing less collagen deposition and more macrophages. G2 and G3: Granulation tissue of latex extract treated (100 and 200 mg/ml, respectively) animals showing well-organized collagen bundles and well-developed blood vessels. Cutaneous appendages can also be visualized. G4: Granulation tissue of the fusidic acid-treated group animal showing moderate collagen deposition (H&E stain, 200x).

To corroborate the findings, histological analyses of the healed wounds were performed on day 14 post-therapy. Indeed, wounds dressed with the *A. inermis* latex extract showed significantly smaller scar width at the wound closure area in both the treated and the positive control groups (fusidic acid ointment) compared to that seen in the vehicle group (treated with gum acacia). No difference in scar width was observed between the latex-treated groups and the positive control group (Figure 4). In addition, in the latex-treated group, healthy epithelium covered a big portion of the wound area and the extracellular matrix was extensively rich in newly-formed collagen, indicating progressive physiological wound healing. Other signs of enhanced wound healing induced by the latex include that the nascent granulation tissue in the wound area contained higher density of small capillaries (indicating enhanced angiogenesis) and collagen fibers (Figure 5). The data also showed increased cellular infiltration and re-epithelialization as well as scarcity of macrophages or edema in the treated group compared to the control group (Figure 5). Also, histological evaluation revealed increased cellular infiltration, and re-epithelialization particularly in the treated and the positive control groups.

Discussion

Chemical screening revealed that the latex, gel and green skin of *A. inermis* extracts contained carbohydrates, steroids, phenols, tannins, anthrones and bitter compounds. In accordance with these results, previous studies have shown that Aloe species mostly contain carbohydrates (e.g. glucomannans), phenols (e.g. chromone, anthra-quinone and anthrone derivatives), enzymes (e.g. bradykininase), and other organic compounds (e.g. magnesium lactate and salicylic acid) (Mukherjee et al., 2013; Nejatzadeh-Barandozi, 2013). These components have been reported to exhibit significant anti-inflammatory, wound-healing, immunostimulant, antibacterial, antioxidant, anti-diabetic, antiulcer and antitumor effects (El-Shemy et al., 2010; Mukherjee et al., 2013; Nejatzadeh-Barandozi, 2013; Okyar et al., 2001).

In the current study, among the parts (the latex, gel and green skin) investigated of *A. inermis*, the latex extract exerted the highest antibacterial activity against both *S. aureus* and *P. aeruginosa*. In fact, the results showed a bactericidal effect of the latex against both microorganisms that was comparable to powerful conventional antibiotics such as ceftriaxone and gentamicin. In accordance with the shown antibacterial activity of *A. inermis*, a previous study has reported that *A. vera* juice (latex and gel), exhibited a significant antibacterial effect on *Klebsiella pneumoniae,* but had negligible effects on *E. coli* and *P. aeruginosa* (Cock, 2008; Thiruppathi et al., 2010). The pool of anthraquinone content of *A. vera was* demonstrated to be responsible for the exhibited antimicrobial effects against *Mycobacterium tuberculosis* and *Bacillus subtilis*. Aloe juice has also been found to exert a bacteriostatic effect against gram-positive *S. aureus*, *Streptococcus pyogenes* and gram-negative *Salmonella paratyphi* (Arunkumar and Muthuselvam, 2009; Thiruppathi et al., 2010).

In addition to the antibacterial effect of *A. inermis* latex extract, the data obtained in this study revealed a significant antioxidant activity for the latex extract, as evidenced by the DPPH free radical scavenging assay. In line with our findings, a previous investigation on *A. vera* juice, has reported that both methanol and acetone extracts of *A. vera* possessed a strong DPPH free radical and superoxide radical scavenging activities (Saritha, 2010). However, it is noteworthy that various Aloe species may exhibit variations in their phytochemical content, biological activities, and toxicity profiles (Nejatzadeh-Barandozi, 2013).

Another interesting aspect of the present investigation is the significant wound-healing activity that *A. inermis* exhibited *in vivo.* Wounds are associated with significant cellular damage and death, and are classified based on location, size, depth or cause (e.g. surgical, traumatic, ischemic). Wound healing is a physiological process that repairs the damaged tissues and is considered efficient when the wound heals in the shortest time possible, with minimal pain, discomfort, and scarring (Alam et al., 2011; Singh et al., 2011). It is a complex dynamic process of restoring cellular structures and tissue layers in the damaged tissue, as closely as possible, to their normal state (Abdulla et al., 2010). The overall healing process is comprised of three major phases; namely: inflammatory, proliferative, and maturational, and involves multiple overlapping processes such as wound contraction, granulation, and epithelization as well as connective tissue collagenation and repair (Singh et al., 2011).

The potential of *A. inermis* to promote wound healing *in vivo* was shown in this work by the ability of the topical application of *A. inermis* latex extract to accelerate the healing of induced wounds. The newly-formed tissues at the wound site exhibited normal collagenation and contained healthy-looking fibroblasts, hair follicles and blood capillaries in the granulation tissue. In addition, no inflammatory cells were observed by the end of the two week experiment. These wound healing effects could be ascribed to induced upregulation of collagen I (Bonte et al., 1994), to increased tensile strength of wounds (Suguna et al., 1996) or to enhanced angiogenesis (Shukla et al., 1999; Trabucchi et al., 1986). Sufficient angiogenesis in the newly-formed granulation tissues improves blood delivery to the wound site, thus providing oxygen and nutrients essential for the healing process (Szabo et al., 1995).

The thorough histopathological analyses of *A. inermis* latex-healed wounds revealed less inflammation, increased

collagen deposition and neovascularization in the latex extract-treated groups as compared to the vehicle control. In addition, with the passage of time, these latex extract-treated wounds undergo an efficient proliferative phase as indicated by the abundance of fibroblasts and collagen fibers in the healing tissues. This means that the treated wound had quickly passed the inflammatory phase and moved into the subsequent healing-conducive stages of proliferation and maturation. This short inflammatory phase suggests an anti-inflammatory effect exerted by the latex extract. Alleviating inflammation is indeed a key factor in promoting the healing process (Rezaie et al., 2013). Like other plant extracts, it is also likely that *A. inermis* latex extract may have initially activated macrophages, increased their phagocytic activity and increased secretion of macrophage-derived cytokines. These cytokines are thought to accelerate fibroblast proliferation, resulting in enhanced collagen synthesis and connective tissue repair. Cytokines also promote fibroblast differentiation and angiogenesis (Luettig et al., 1989; Rezaie et al., 2013; Stimpel et al., 1984).

Several mechanisms may explain the wound healing activity of *A. inermis*. For instance, a study has reported that *A. vera* accelerated wound healing by reducing inflammation and providing a more mature granulation tissue (Hamid and Soliman, 2015). In addition, significant antibacterial activity shown by the latex extract of *A. inermis* may likely contribute to the beneficial effects of the extract. Indeed, a previous study has reported that the wound healing mechanism of *A. vera* leaf gel is due to their antimicrobial properties, which appear to be responsible for wound contraction and increased rate of epithelialization (Tsuchiya et al., 1996). These therapeutic properties may be attributed to the presence of bioactive compounds present in *Aloe sp.* such as carbohydrates (e.g. glucomannan, acemannan), enzymes, glycoproteins, growth factors, vitamins and minerals (Davis et al., 1994; Rodriguez et al., 2010; Subramanian et al., 2006).

Conclusion

The results of the current work show strong antibacterial, antioxidant and wound healing potential for *A. inermis* latex. The *in vivo* wound healing activity of the latex extract was evidenced by improved epithelization, enhanced collagenation and neovascularization and by the presence of new healthy hair follicles at the wound site, that were comparable to those induced by fusidic acid. These encouraging findings provide an insight into the traditional usage of *A. inermis* in treating skin wounds and warrants further investigations to assess the possibility of utilizing the latex by mainstream physicians in the management of skin injuries especially those associated with bacterial infection.

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Conflict of interests

The authors declare that they have no conflicts of interests.

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