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In Vivo Diabetic Wound Healing and in Vitro Anti-Staphylococcus Aureus Activities of Some Medicinal Plants from Sudan

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ABSTRACT: Diabetic wound infections are among the most severe complications of diabetes, often resulting in delayed healing and poor response to conventional therapies. The emergence of multidrug-resistant pathogens, particularly Staphylococcus aureus, has increased the need for effective alternative treatments. In Sudanese traditional medicine, Quercus infectoria nut galls, Guiera senegalensis, and Aloe vera are widely used for wound management, although their therapeutic efficacy remains insufficiently validated. This study evaluated the in vivo wound-healing and in vitro antibacterial activities of these plants cultivated in Sudan. The ethanolic extracts (80%) were formulated into 10% topical gels (8 mg/mL) and applied to streptozotocin-induced diabetic rats using an excision wound model. Solcoseryl® gel (10%) served as the reference treatment. Wound healing was assessed macroscopically and histologically, while antibacterial activity against S. aureus was determined using the disc diffusion method. Rats treated with the ethanolic extract gels of Q. infectoria, G. senegalensis, and A. vera exhibited complete wound closure within nine days, significantly faster than the Solcoseryl®-treated group, which required twenty days, whereas untreated rats showed no significant healing. Histopathological examination confirmed restoration of normal skin architecture in the treated groups. Additionally, the extracts demonstrated strong antibacterial activity against S. aureus at concentrations ranging from 6.25–100 mg/mL, producing larger inhibition zones than gentamicin (30 mg/mL). These findings support the traditional use of these plants and highlight their potential as affordable natural therapies for diabetic wound management.

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1. INTRODUCTION

Guiera senegalensis J.F. Gmel. (Combretaceae), Quercus infectoria Olivier (Fagaceae), and Aloe vera L. Burm.f. (Asphodelaceae) are commonly known in Sudan as Ghubaysh, Affsa (or Oak), and Sabbar, respectively. These species naturally thrive in the arid zones of the Ghubaysh region in western Sudan and are widely distributed across the savannah areas of western and central Africa [1].

The phytochemical composition of these plants is well documented: G. senegalensis primarily contains flavonoids, carbohydrates, saponins, and triterpenes [2]; Q. infectoria is particularly rich in tannins, flavonoids, and carbohydrates [3]; while A. vera includes phenolic acids, carbohydrates, coumarins, steroids, and triterpenes [4]. These plants are recognized both locally and regionally as valuable traditional remedies, especially for their topical application in treating wounds, cuts, eczema, psoriasis, and other dermatological disorders. Moreover, they have been traditionally employed for the management of fever, cough, respiratory congestion, and malaria [5].

Diabetic wound infections constitute one of the most prevalent and severe complications among diabetic patients and are a significant global health issue [6]. The increasing incidence of multidrug-resistant (MDR) pathogens- particularly Staphylococcus aureus - has made the treatment of diabetic wounds increasingly difficult, leading to extended hospitalization, higher treatment expenses, and elevated morbidity and mortality rates in severe cases [7,8]. This growing challenge underscores the urgent need for novel and effective therapeutic alternatives for managing diabetic wounds.

In Sudan, medicinal plants such as G. senegalensis, Q. infectoria nut galls, and A. vera leaves have been traditionally applied for wound care with notable empirical success. However, there remains a lack of scientific validation regarding their wound-healing efficacy, particularly in the context of diabetic wound infections. Furthermore, comprehensive pharmacological investigations on these Sudanese plant species are still limited. Therefore, the present study was designed to scientifically evaluate the wound-healing efficacy of these plants in experimentally induced diabetic wounds, along with assessing their antibacterial potential against Staphylococcus aureus, a principal pathogen in skin and diabetic wound infections.

2. MATERIAL AND METHODS

Plant materials

Leaves of Guiera senegalensis and Aloe vera stem, along with nut galls of Quercus infectoria, were collected from the Ghubaysh region in western Sudan. The plant specimens were taxonomically identified and authenticated by a specialist at the Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Khartoum, Sudan.

Extraction of plant materials and gel formulation

The collected plant materials were first rinsed with sterile distilled water to eliminate dust and other contaminants. Q. infectoria and G. senegalensis samples were shade-dried and coarsely powdered. Approximately, 500 gm of each powdered plant material was macerated in 1,500 mL of 80% ethanol for 72 hours with periodic agitation. The extracts were filtered using a vacuum filtration system and concentrated under reduced pressure.

Gel formulations were prepared using methyl cellulose as a water-soluble base, serving both as an emulsifier and thickening agent. Following a previously described laboratory procedure with slight modifications [9], 3 gm of methyl cellulose was dispersed in 50 mL of hot distilled water (70–90 °C) and stirred until completely dissolved. The ethanolic extracts were added individually to the gel base and mixed until homogeneous. Subsequently, 50 mL of cold distilled water (or ice chips) was added with gentle stirring to ensure complete gel formation. The final formulations contained 8 mg/mL of plant extract. A commercial Solcoseryl® gel (10%) served as the reference standard. All gels were refrigerated until use.

Experimental animals

Healthy young male and female Swiss Wistar albino rats (100–300 gm) were obtained from the National Experimental Animal House (NEAH), Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Khartoum, Sudan. Ethical approval for the animal experiments was granted by the same institution. The rats were housed in ventilated cages under

controlled temperature conditions (25–30 °C) and a 12-hour light/dark cycle, with access to standard NEAH-prepared rat chow and tap water ad libitum [10].

Induction of diabetes

Intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dose of 50 mg/kg body weight dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) [11]. Blood glucose levels were determined on days 3, 4, 5, 6, and 7 post-STZ administration as described by Tay et al. in 2005 [12]. Rats exhibiting fasting blood glucose levels exceeding 250 mg/dL were considered diabetic and selected for the study. Thirty rats were randomly distributed into five groups (n = 6 per group).

Creation of diabetic infected excision wound model

An excision wound model was established following the method of Morton and Malone (1972) [13]. Rats were lightly anesthetized with diethyl ether, and the fur on the lower dorsal region and right flank was shaved. The skin was gently stretched, and a circular area (1 cm diameter) was outlined using a marker. A full-thickness wound was created within the marked area using a sterile scalpel and forceps, extending down to the loose subcutaneous tissue. Artificial infection was introduced by evenly applying 0.1 mL of Staphylococcus aureus suspension over each wound using a micropipette.

Grouping of animals

After wound creation and infection, the rats were kept individually to prevent cross-contamination and divided into five groups (n = 6):

- Group 1: Untreated control (negative control).
- Group 2: Treated with Q. infectoria ethanolic extract gel.
- Group 3: Treated with G. senegalensis ethanolic extract gel.
- Group 4: Treated with A. vera ethanolic extract gel.
- Group 5: Treated with Solcoseryl® gel (10%) as positive control (Protein-free hemodialysate of calf blood).

All topical treatments were applied every 12 hours.

Wound healing evaluation

Healing progress was monitored through wound area measurements, taken every 24 hours, using a Vernier caliper and millimeter ruler. Wound contraction was calculated as the percentage reduction in wound area relative to the initial wound size. Each reading was repeated three times, and the mean value was recorded. The wound area on day 0 was considered 100%, and subsequent measurements were compared using the Walker and Mason formula:

Percentage of wound closure/contraction = 1- [Ad/ Ao] × 100%

Where (Ao) is the wound area on day zero, and (Ad) is the wound area on the respective day [14].

Histological evaluation

Tissue samples from both treated and untreated groups were collected on day 20 post-treatment to assess microscopic healing and tissue remodeling. Cross-sectional wound scars (approximately 6 mm thick) were excised and processed following a slightly modified method of Santos et al. in 2021 [15]. Samples were fixed in 10% buffered formalin for 24 hours, dehydrated using graded ethanol and xylene, embedded in paraffin (40–60 °C), and sectioned at 5–6 μm thickness. Sections were deparaffinized, stained with hematoxylin and eosin (H&E), and examined under a light microscope at (100×) magnification.

In vitro antibacterial assay

The in vitro antibacterial activity of the ethanolic extracts was evaluated against Staphylococcus aureus, a Gram-positive bacterium frequently implicated in skin and diabetic wound infections [8]. The disc diffusion technique on Mueller–Hinton agar (MHA) was

employed, following the guidelines of the National Committee for Clinical Laboratory Standards (1999) [16]. The bacterial suspension was adjusted to 10^8 CFU/mL (0.5 McFarland standard). A 100 µL aliquot of the suspension was evenly spread on the MHA surface and allowed to dry for 5 minutes. Sterile Whatman No. 1 filter paper discs (6 mm) were impregnated with 20 µL of each extract and placed on the inoculated agar plates. After incubation at 37 °C for 24 hours in an inverted position, the diameters of inhibition zones (including disc diameter) were measured in millimeters. Gentamicin (30 µg/disc) served as the positive control.

Statistical analysis

Data related to wound contraction and antibacterial activity were analyzed using the Statistical Package for the Social Sciences (SPSS), version 16. Results are expressed as mean ± standard deviation (SD). Differences among groups were determined using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for wound contraction comparisons at $\alpha = 0.05$. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

Wound healing is a dynamic biological process involving the restoration of injured tissue to its normal physiological condition. Wound contraction, a major component of this process, refers to the progressive reduction in wound area. The rate and quality of wound healing depend primarily on the intrinsic regenerative capacity of the tissue, the nature and severity of the injury, and the general health of the organism [18]. In this study, an excision wound model was employed to evaluate the wound-healing potential of ethanolic extracts prepared from selected plant species. The results demonstrated that extract-treated rats exhibited a significantly accelerated healing rate, comparable to the standard treatment ($p < 0.05$) and similar to other medicinal plants such as Anogeissus leiocarpus (Combretaceae), which achieved complete healing within 15 days [19]. Likewise, Amoolya et al. in 2015, reported that Michelia champaca flower extract promoted complete wound contraction in diabetic rats within 21 days [20]. In the present investigation, gel-based formulations were selected due to their favorable properties- such as non-irritancy, clarity, spreadability, and viscosity- which make them effective and stable topical dosage forms. Both the dosage and formulation were aligned with the reference drug (Solcoseryl® gel) to enable valid comparison.

In the excision model, there were no statistically significant differences among the groups treated with 10% ethanolic extracts gels (8 mg/mL) of Q. infectoria, G. senegalensis, and A. vera ($p = 0.937$, 0.784, and 0.852, respectively). These treated tissues appeared drier, exhibited shrinkage, and showed improved tensile strength (Table 1). However, the differences between all extract-treated groups and the untreated control were statistically significant (Table 2). Interestingly, the wounds of rats treated with Solcoseryl® gel (positive control) remained moist and tended to reopen during the healing period. The extract-treated groups achieved complete wound closure within 7 to 9 days, whereas the Solcoseryl® group required approximately 20 days for complete healing. In contrast, the untreated group displayed no notable improvement during the observation period (Tables 1–2). Histopathological analysis further supported these findings. Skin sections from rats treated with plant extract gels displayed normal epidermal and dermal architecture, including hair follicles and sebaceous glands (Figure 1). In comparison, the Solcoseryl®-treated group showed a broad area of denuded epidermis accompanied by fibrous tissue proliferation, indicative of ongoing repair (Figure 2). Meanwhile, untreated control sections revealed loss of epidermal continuity, disrupted connective tissue with fibroblast and capillary proliferation, and surface neutrophil infiltration (Figures 3–4). These observations suggest that the ethanolic extracts promoted cell proliferation and facilitated the restoration of normal skin structures, as evidenced by the histological results.

The wound-healing properties of these medicinal plants can be attributed to their bioactive phytochemical constituents. Several studies have highlighted that antioxidant-rich plant extracts enhance tissue regeneration by mitigating oxidative stress and preventing damage to dermal components [21]. Phenolic compounds, flavonoids and tannins, commonly found in these plants, play a major role in free radical scavenging and tissue repair [22]. The wound healing and antibacterial effects observed here likely arise from concentrated phenolic and flavonoid constituents, as described in previous reports, where such compounds were associated with antioxidant and antibacterial activities [23]. Therefore, the high phenolic content of the studied extracts likely contributed to their wound-contraction activity through mechanisms involving redox balance and enhanced collagen synthesis.

Chronic diabetic wounds are frequently complicated by infections that prolong inflammation and delay tissue regeneration. Gram-

positive bacteria, especially Staphylococcus aureus, are among the most prevalent pathogens associated with such infections. The ethanolic extracts of these plant species revealed high sensitivity against S. aureus, there was no significant different variation in means of zones of inhibition between the ethanolic extracts ($p = 0.085$), while there was significant different variations between the extracts and standard $p = 0.012$, as reported in (Tables 3-4), and (Figure 5). The ethanolic extracts produced substantial inhibition zones against S. aureus, and this finding supported the effectiveness of ethanolic extracts with in vitro antibacterial activity of many plant extracts, such as Ficus carica and Olea europaea [24]. The current study revealed that ethanolic extracts exhibited pronounced antibacterial activity against S. aureus, comparable to gentamicin (30 mg/mL), across a concentration range of 6.25–100 mg/mL (Table 3). Moreover, no pus cells were detected in treated wounds, confirming the extracts' antimicrobial efficacy which may probably due to the presence of phenolic compounds (Table 4; Figure 5). Therefore, the dual action of the tested plant extracts- both in reducing oxidative stress, which facilitates tissue preparation, and in exhibiting antibacterial activity- contributed to the shortened duration of complete wound healing observed with these ethanolic extracts (only 9 days).

Overall, this study provides experimental validation supporting the traditional use of G. senegalensis, Q. infectoria, and A. vera in Sudan for the management of diabetic wounds. The ethanolic extracts, formulated as 10% gels containing 8 mg/mL of active components, demonstrated both wound-healing and antibacterial properties. Our gel formulations and rapid wound closure are consistent with previous reports on topical plant extract creams, which documented both phytochemical contributions, such as flavonoids, and antibacterial efficacy in cream formulations [25]. These findings suggest that such formulations hold promise for the development of affordable, plant-based therapeutic alternatives that could serve as adjunct or complementary treatments for diabetic wound care.

4. CONCLUSION

The present study investigated that the ethanolic extracts of Quercus infectoria, Guiera senegalensis, and Aloe vera, cultivated in Sudan, possess significant diabetic wound-healing and anti-Staphylococcus aureus infection activities. Topical application of the formulated gels from these alcoholic extracts, significantly accelerated wound contraction and restored normal skin architecture in streptozotocin-induced diabetic rats ($p < 0.05$), and healing outcomes superior to the standard Solcoseryl® gel treatment. In addition, the alcoholic extracts exhibited pronounced antibacterial activity against Staphylococcus aureus, producing inhibition zones significantly greater than those of Gentamicin ($p < 0.05$). The observed pharmacological effects are likely attributed to the rich phytochemical composition of these plants, particularly phenolic compounds, tannins, and flavonoids, which may enhance tissue regeneration and suppress bacterial proliferation. These findings scientifically validate the traditional use of these medicinal plants grown in Sudan, and highlight their potential as safe, affordable, and effective natural alternatives for the management of diabetic wound infections, although further toxicological, phytochemical, and clinical investigations are still required.

5. ACKNOWELEDGEMENT

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6. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this research work.

7. AUTHORS CONTRIBUTION

Conceptualization: Areeg Ahmed Satti, and Yahia M.A. Elimam. Data curation: Areeg Ahmed Satti, and Asmaa E. Sherif. Formal analysis: Areeg Ahmed Satti, and Mohamed Mirghani. Methodology and Experimental: Areeg Ahmed Satti, Ahmed Ashour, and Yahia M.A. Elimam. Project management: Wadah Osman. Resources: Wadah Osman, and Asmaa E. Sherif. Supervision: Yahia M.A. Elimam. Validation: Hisham Ali Waggiallah. Drafting - original draft: Areeg Ahmed Satti, and Yahia M.A. Elimam. Writing - proofreading and editing: Mohamed Mirghani, Wadah Osman, Ahmed Ashour, Asmaa E. Sherif, and Hisham Ali Waggiallah

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Table 1. Effect of topical application of ethanolic extracts from plant species on excision wound healing, expressed as percentage wound closure.

Days / Groups	Day 1	Day 3	Day 5	Day 7	Day 9
G. senegalensis	0.00 ± 0.28 (0.00%)	0.13 ± 0.05 (49.8%)	0.09 ± 0.03 (86.5%)	0.02 ± 0.14 (96.8%)	0.00 ± 0.00 (100%)#
Q. infectoria	0.69 ± 0.19 (0.00%)	0.37 ± 0.25 (47.8%)	0.09 ± 0.06 (89%)	0.00 ± 0.00 (100%)#	-
A. vera	0.748 ± 0.53 (0.00%)	0.31 ± 0.13 (58.2%)	0.15 ± 0.06 (79.4%)	0.07 ± 0.04 (89.8%)	0.00 ± 0.00 (100%)#
Standard (Positive control)	2.43 ± 4.10 (0.00%)	2.36 ± 4.03 (2.9%)	0.52 ± 0.36 (68.3%)	0.56 ± 0.43 (76%)	0.44 ± 0.38 (81%)
Negative control	0.68 ± 0.49 (0.00%)	0.68 ± 0.49 (0.00%)	0.68 ± 0.49 (0.00%)	0.68 ± 0.49 (0.00%)	0.68 ± 0.49 (0.00%)

*Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 16.0.

**Values are presented as mean ± SD (standard deviation) of wound contraction; numbers in parentheses indicate percentage

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wound closure.

***(#): Indicates complete wound closure.

****All values for all groups, including the positive standard group, showed statistically significant differences compared with the negative control group ($p < 0.05$).

Table 2. Multiple comparison of mean wound contraction between ethanolic plant's species extracts and standard-treated groups, using one-way ANOVA.

ANOVA			
	Sum of Squares	**Mean of Squares**	**Sig**
Between Groups	25.888	6.472	*.000
Within Groups	242.622	.825	
Total	268.509		

*At $p < 0.05$, one-way ANOVA showed a statistically significant difference between groups ($p = 0.000$).

Table 3. Antibacterial sensitivity of the plant's species ethanolic extracts and Gentamicin (Positive control) against *S. aureus*.

Concentrations	**Zone of inhibition (mm)***			
	Gentamicin	**G. senegalensis**	**Q. infectoria**	**A. vera**
30 mg/mL	15mm.	-	-	-
100 mg/mL	-	19 mm.	23 mm.	19 mm.
50 mg/mL	-	18 mm.	20 mm.	18 mm.
25 mg/mL	-	15 mm.	18 mm.	15 mm.
12.5 mg/mL	-	14 mm.	18 mm.	14 mm.
6.25 mg/mL	-	14 mm.	16 mm.	14 mm.

* Interpretation of sensitivity results: > 18 mm = Sensitive (susceptible); 14-18 mm = Intermediate; < 14 = Resistant[17].

Table 4. Multiple comparison of mean zones of inhibition of ethanolic plant's species extracts and standard-treated groups using one-way ANOVA.

Zone of inhibition	**ANOVA**		
	Sum of Squares	**Mean Square**	**Sig.**
Between Groups	147.733	73.867	*.034
Within Groups	196.000	16.333	
Total	343.733		

Statistical analysis was performed using SPSS 16.0. *The difference between groups was considered significant at ($p < 0.05$).

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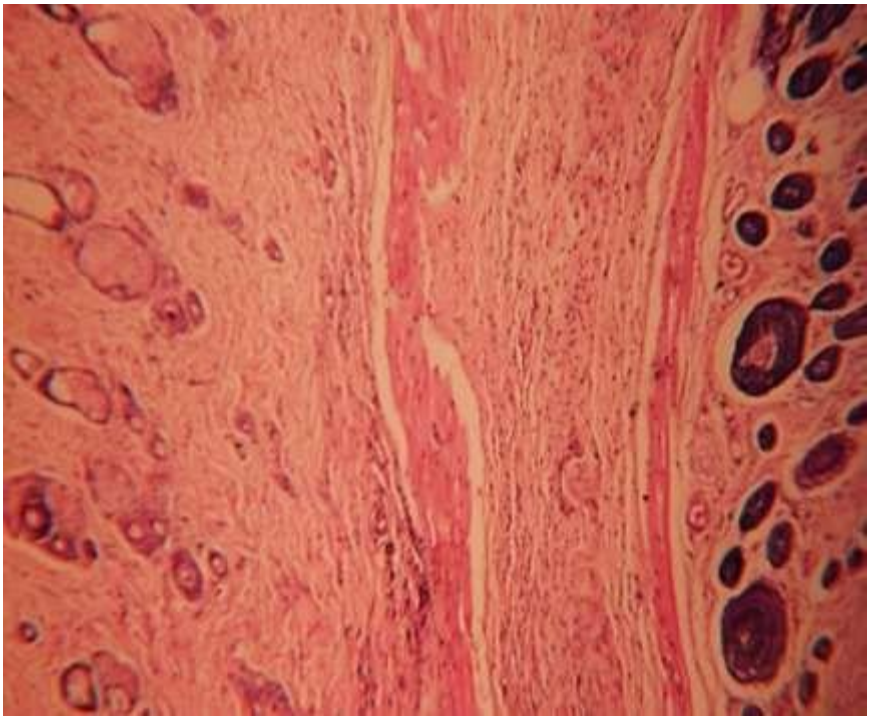


Figure 1. Section showing normal skin structure with hair follicles and sebaceous glands, stained with haematoxylin and eosin (100×) (*Q. infectoria* group).

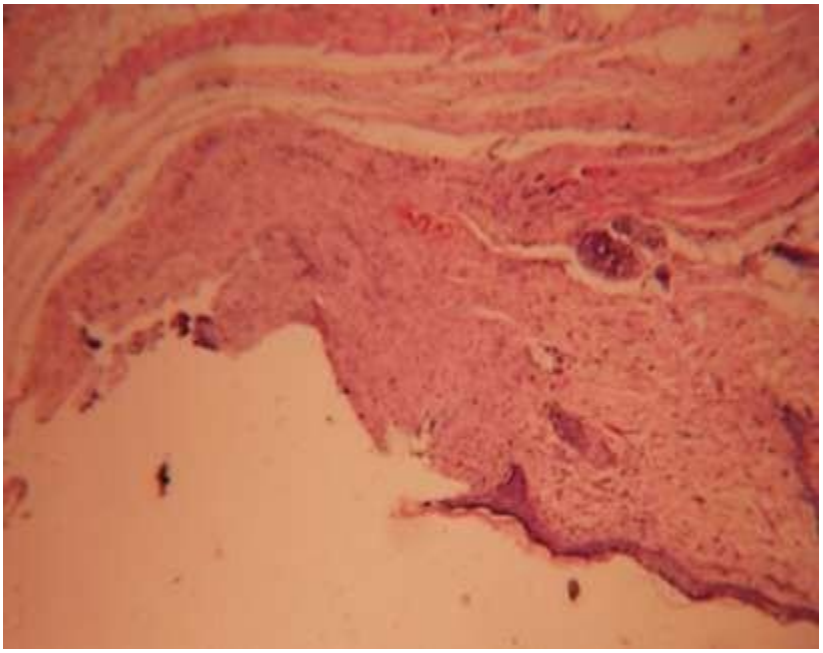


Figure 2. Section showing damaged skin with eroded epidermis and dermal fibroplasia, indicating an ongoing healing process. Stained with haematoxylin and eosin (100×) (Positive control group).

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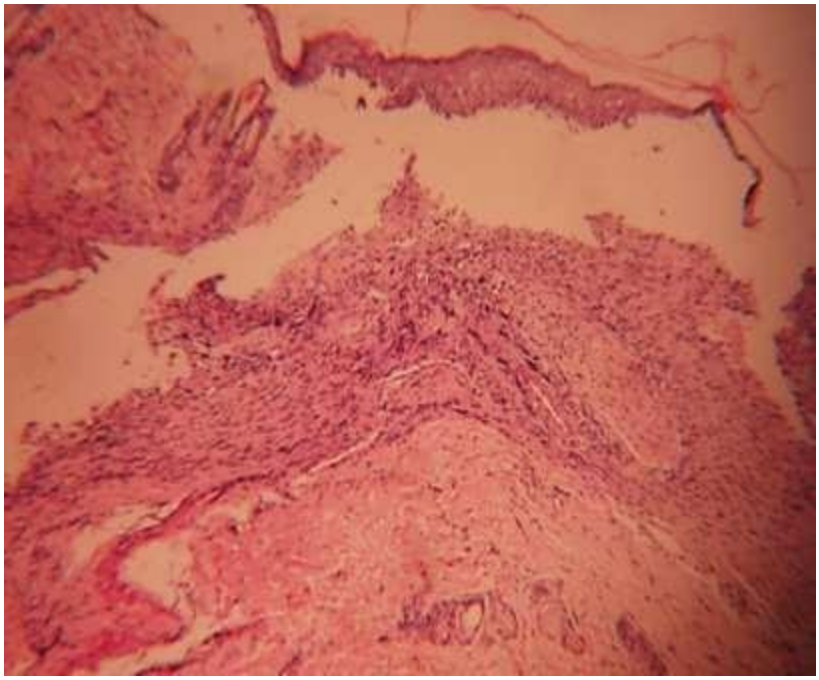


Figure 3. Section showing disrupted epidermis with dermal proliferation of connective tissue, neutrophils, and fibroplasia. Stained with haematoxylin and eosin (100×).

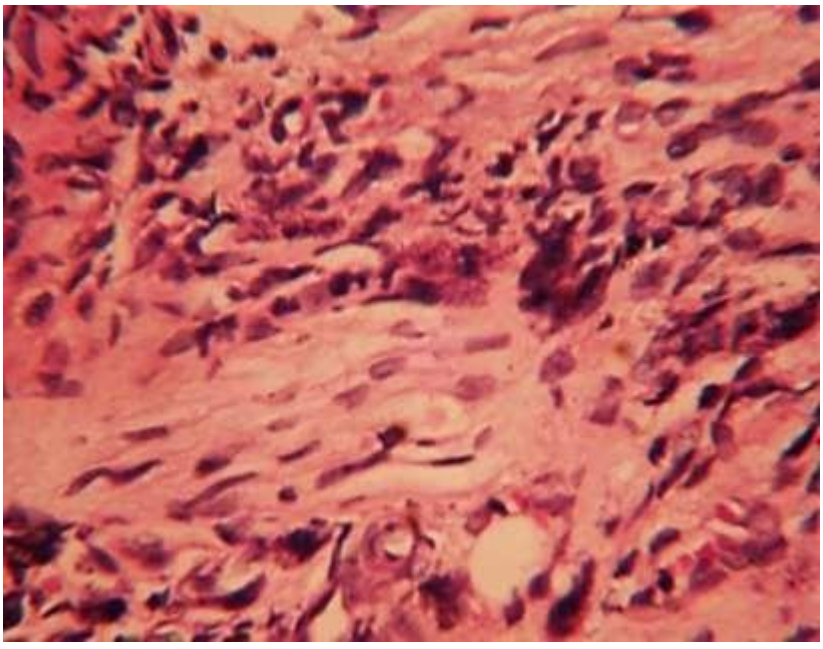
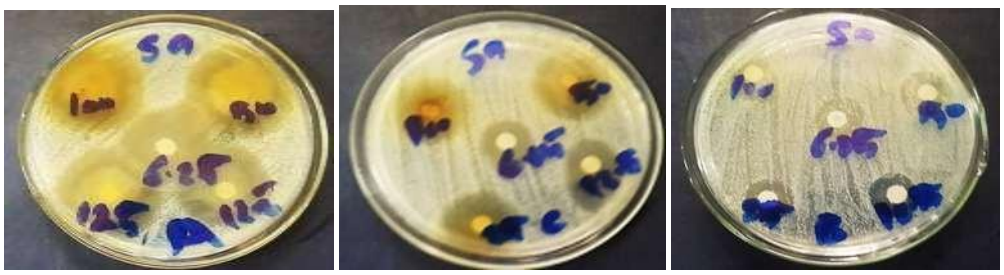


Figure 4. Higher magnification (100x) showing fibroplasia and infiltration of neutrophils (Negative control group).

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(a) (b) (c)

Figure 5. Antibacterial sensitivity of the ethanolic extracts of (a): *Q. infectoria*; (b): *G. senegalensis*; (c): *A. vera* against *S. aureus* using the disc diffusion method.