Evaluation of Arabic gum antibacterial and wound healing activities against *Staphylococcus aureus* isolates obtained from skin infections

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ABSTRACT

*Staphylococcus aureus* is a Gram-positive bacterium that has reflected a noteworthy problem for public health. Nearly all *Staphylococcus* strains secrete lipase enzymes, which may cause damage to host tissues and deActivate host immune systems. This pathogen may colonize the skin and soft tissues. This study aimed to investigate the effectiveness of Arabic gum in inhibiting the lipolytic activity of pathogenic *S. aureus* commonly found in skin infections, through both lab-based and animal experiments. We obtained 300 specimens from individuals who met the clinical requirements for various skin diseases from four Egyptian hospitals: Al-Minshawi general hospital, Tanta University hospital, Kafr El-Zayat general hospital, and Mahalla general hospital, respectively. We determined the lipase activity for the *S. aureus* isolates and examined the effect of Arabic gum on mice against the lipase enzyme activity. Arabic gum treatment reduced the lipase activity at 1/2 MIC, 1/4 MIC, 1/8 MIC, and 1/16 MIC by 84.1%, 68.7%, 81.6%, and 68.6%, respectively. In male Sprague-Dawley mice, Arabic gum revealed a significant improvement in burn healing. All of the mouse groups used in the experiment showed better neovascularization and re-epithelialization in the histological tests on Arabic gum. Our results suggest that Arabic gum may help burn wounds heal faster by affecting tissue regeneration. We concluded that Arabic gum has promising antibacterial and wound-healing properties against *S. aureus* skin infections.

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Introduction

Staphylococcal skin infections involve a wide range of condition. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive coccus often found on the skin and mucous membranes of healthy individuals. It is estimated that 15-40% of people are carriers of *S. aureus*, meaning they have the bacteria on their skin without any active infection. Staphylococcal skin infections can occur when the bacteria enter the body through a break in the skin (Bisola et al. 2024). Preventing the spread of Staphylococcal infections is crucial and can be achieved through proper hand hygiene and avoiding contact with infected individuals or contaminated surfaces. Some minor Staphylococcal infections may heal without treatment. In severe cases, treatment for Staphylococcal skin infections typically involves antibiotics (Traoré et al. 2024). *S. aureus* infections were treated with antibiotics...
from the β-lactam class, such as cephalosporin and penicillin, but the development of resistance to these antimicrobial agents has complicated therapy. Methicillin-resistant *S. aureus* (MRSA) strains, which are resistant to nearly all β-lactam antimicrobials, have emerged and are now a significant public health concern (Bavaro et al. 2024).

Natural products are considered a valuable source of antimicrobial compounds that can serve as alternatives to antibiotics in combating bacterial infections. The increasing prevalence of antibiotic resistance has led to a renewed focus on these plant-based substances, which have the potential to provide effective and sustainable solutions to this growing crisis in medicinal industry, and in agricultural field (Hashem et al. 2023a; Khattab et al. 2022; Hashem et al. 2023b). Additionally, ongoing efforts explore the use of natural products combined with conventional antibiotics to enhance their efficacy and combat antibiotic resistance (Sadeer and Mahomoodally 2021; MacNair et al. 2024).

Arabic gum is a natural branched-chain multifunctional hydrocolloid with a highly neutral or slightly acidic, arabino-Glactan-protein complex containing calcium, magnesium, and potassium. Arabic gum is dried exudate obtained from the stem and branches of *Acacia* trees manly *Acacia senegal* (*Senegalia senegal*) and *Acacia seyal* (*Vachellia seyal*).

Arabic gum was used by the ancient Egyptians as an adhesive when wrapping mummies and in mineral paints when making hieroglyphs since the second millennium BC. In modern times, Arabic gum is used in foods, pharmaceutical, and many other industries (Musa et al. 2018). The main medicinal uses of Arabic gum include respiratory disorders, gastrointestinal issues, liver and kidney health, and skin and inflammatory disorders. Additionally, Arabic gum extract has been used to treat chronic hepatitis and have therapeutic benefits against various viruses.

Studies have found that Arabic gum extract exhibits potent antibacterial activity against both standard *S. aureus* and MRSA strains by reducing lipase activity and inhibiting virulence gene expression (Lakshmi et al. 2020; Ng'uni et al. 2017). Arabic gum has potential benefits in accelerating burn wound healing through the following properties: anti-inflammatory effects, antimicrobial properties, antioxidant activity, wound contraction and shrinkage, reduced pain and discomfort, faster epithelialization, and enhanced angiogenesis (Andreu et al. 2015; Sharma et al. 2020).

The objective of this study was to investigate the antibacterial activity of Arabic gum against *S. aureus* a significant public health problem due to its ability to colonize skin and soft tissue and deactivate host immune systems. Additionally, the study aims to assess the histopathological effects of Arabic gum on burn wounds.

**Materials and Methods**

**Source of Arabic gum**

Arabic gum was bought as crystals from a medical plants market (Shana health shop) at Menoufia governorate, Egypt.

**Samples collection and isolation media**

Skin swabs were collected from different infected sites of the skin of Egyptian patients in several medical centers. About 100 samples were collected from Al-Minshawi General Hospital, 80 samples from Tanta University Hospital, 60 samples from Kafr El-Zayat General Hospital, and 60 samples from Mahalla General Hospital. The swabs were streaked aseptically on blood and MacConkey agars (Oxoid, UK), incubated for 24 h at 37 °C. After incubation, the obtained *S. aureus* isolates were characterized morphologically and biochemically using several assays, including Gram-stain, coagulase tests, and catalase (Paleczny et al. 2021). The isolates were persevered in glycerol medium brain heart infusion broth with 15 % glycerol at -20 °C. Subcultures were prepared by activate the preserved isolates on nutrient broth medium (Sigma Aldrich).

**In vitro sensitivity assay of Staphylococcus isolates**

All isolates were confirmed as *S. aureus* by VITEK® 2. As a reference control, a reference strain of *S. aureus* ATCC 25923 was used. Multidrug-resistant (MDR) bacteria were defined as bacterial isolates that were resistant to three or more types of antibiotics (CDC). Moreover, antibiotic susceptibility was performed using VITEK® 2 AST GP67 cards to determine the resistance profile. The obtained results were automatically analyzed using VITEK 2 system and interpreted as sensitive, intermediate, or resistant according to the minimal inhibitory concentration (MIC) of each tested drug (Rai et al. 2023).

**In vitro detection of lipolytic activity of S. aureus isolates**

Qualitative assay

All isolates that were confirmed to be *S. aureus* were assessed qualitatively for lipase production on tributyrin agar plates. Petri plates containing tributyrin (1 %, w/v), is an ester composed of glycerol and butyric acid (India) and agar (Oxoid, UK) (2 %, w/v) were streaked with 24 h old bacterial cultures and incubated for 48 h at 37°C (Bala et al. 2020). After incubation, the diameter (mm) of the developing clear zones surrounding the colonies was measured using a calibrated ruler, demonstrating the production of lipase by the tested isolate. Overnight tryptic soy broth (TSB) (Oxoid, UK) cultures of the bacterial
isolates were diluted with sterile distilled water 1:100, then inoculated into 6 mm wells of tributyrin agar plates made using a cork borer, and incubated at 37 °C for 24 h. After incubation, the diameter of the developing clear zone was measured. After recording the enzyme production measurements, they were divided into weak, medium, and strong lipase producing isolates. Two ml of glycerol cultures were inoculated in 100 ml nutrient broth medium, then shaken at 150 rpm, and kept at 37°C for 12 h. If the clear zone about 0 to 12 mm the isolate classified as weak while the medium is about 12 to 20 mm and the strong is more than 20 mm (Arora et al. 2013).

**Quantitative lipolytic activity by spectrophotometric detection assay**

The spectrophotometric quantitative method suggested by Pera et al. (2006), was used to determine the lipolytic (Lipase) activity in the samples. In which, p-Nitrophenyl palmitate (pNPP), BIO SYNTH USA, was used as a substrate for rapid measurement of lipase activity but with slight modifications. Substrate solution was prepared as follows: 1.0 mL of solution A (40 mg of pNPP in 12 mL of isopropanol) was added drop wise with stirring to 19 mL of solution B (0.1 g of Arabic gum and 0.4 mL of Triton X-100 in 90 mL of distilled water) and the obtained emulsion remained stable up to 2 h. The enzyme assay mixture, composed of 0.5 mL of Tris-HCl Biotech Ltd. buffer (pH=7.6, 0.1 M), 1.0 mL of the substrate solution, 0.1 mL of suitably diluted enzyme and 1.4 mL of distilled water, was incubated at 40 °C for 15 min. The reaction was terminated by the addition of isopropanol (0.2 mL) and the released p-nitrophenol was measured at 410 nm in Cary-100 UV-Vis spectrophotometer (Agilent Technologies, Frankfurt, Germany). One unit of enzyme activity is defined as the amount of lipase that releases one µmol of p-nitrophenol per min under the standard conditions. Enzyme activity was expressed as U/mL for each sample.

**Antibacterial potential of Arabic gum and determination of its MIC**

The MICs of Arabic gum samples were determined using sterile 96-well plates. Each well was filled aseptically with 0.1 ml of Mueller Hinton (MH) broth (Oxoid, UK), with 50 µg of isolate combined with serially diluted Arabic gum samples, resulting in a concentration of 1000 to 0.98 µg/ml. All plates were incubated at 37 °C for 72 h. The ensuing turbidity was measured using a micro-plate reader (CLX800BioTek, USA) to measure optical density (OD) readings at 600 nm. The standard blank of the test was turbidity equivalent to a 0.5 Mc-Farland (Nicodemo et al. 2004). For every test, three replicates were used. The MIC was established as the minimal concentration of Arabic gum at which no growth was observed (Wiegand et al. 2008).

**In-vitro anti lipase activity of the Arabic gum**

*S. aureus* were injected with nutritional broth and kept at 37°C for 24 hours. Using a Spectrophotometer (10000 CFU), the turbidity of the broth was adjusted to 0.5 (optical density). Using sterile cotton swabs, the bacterial cultures were injected onto Mueller–Hinton Agar (MHA) plates. These plates all had wells with gel borer cut out of them. The Arabic gum was added to the agar wells to test the lipase production of the test pathogens quantitatively, a 500 ml Erlenmeyer flask holding 100 ml of liquid media was inoculated with 2 ml of the submerged microbial cultures. The flasks were then incubated at 30°C after being incubated at 37°C for 4-5 hours. The shaker rotated at 150 rpm. After a 24-hour incubation period, the culture was centrifuged for 20 minutes at 10,000 rpm at 4°C, and the fluid from the cell-free culture supernatant was used to extract the extracellular enzyme and measured as mentioned previously (Patel et al. and Shah et al. 2020).

**In vivo wound healing investigation**

The Institutional Animal Care and Use Committee (IACUC) of the Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, approved the study, and it was carried out entirely in accordance with its rules of the National Institute of Health Guide for the Care and Use of Laboratory Animals. The ethical approval number is (Azhar-Pharmacy-2024-012). Twelve -weeks-old adult male Sprague Dawley rats, weighing approximately (200±10 g) were purchased from the animal house at the El-Nile Pharmaceutical Company, Cairo. Rats were allowed to roam freely on a regular diet while being housed in polyethylene cages with regulated living conditions (room temperature 25±2°C, humidity 50–70%), and 12/12 h light–dark cycles. Ad libitum access to food and water was provided for rats. A total of 36 male Wistar rats were randomly allocated into four treatment groups/6 rats per each as following: Group 1: Control group: rats were left untreated. Group 2: Positive non-treated group: rats underwent burn induction and left untreated. Group 3: Rats underwent burn induction followed by local treated with marked preparation (Mupirocin 2% w/w) specifically in the right burn area. Group 4: Rats underwent burn induction followed by local treated with Arabic gum specifically in the right burn area.

**Induction of mice burn**

A rat skin burn model was designed on the backs of these rats; the dorsal fur was totally removed one day prior to the introduction of burns. Sharp scissors were used to first trim the fur, and then skin depilatory lotion was used.
Next, two 20 mm-diameter circular burns were created on the right and left dorsal sides of each rat using an aluminum punch. In this experiment, the aluminum stamp was boiled for 30 seconds at 100°C in water. Subsequently, the stamp was positioned on both sides for ten seconds without any force. Every rat in the research had two circular burns that were 20 mm in diameter and penetrated the whole skin thickness. After that, the strongest lipase bacterial isolate in the models was injected subcutaneously with a 20 mg 0.5 Mc-Farland insulin syringe. After induction of burn animals were housed in separate cages for each group (Mendes et al. 2012). Cages were cleaned daily and kept free of infectious agents. Throughout the investigation, the wounds were kept dry. The rats in the control group were treated in the same way as the other rats, but they were immersed in water that was 37°C for 10S and rats of groups III to VI were treated with specific protocol as mentioned above.

Assessment of wound closure rate
A successful course of therapy culminates in the full and permanent healing of the wound. In the present study, wound healing was compared with the control group and assessed by digital photographs of the wounds every week. The photo was shot using a 12-MP digital camera with a 3-inch LCD at a distance of 10 cm, with the lens parallel to the burn injury. The EOS 4000D DSLR camera used was manufactured by Canon Inc. in Tokyo, Japan, histopathological analysis (vascularization, granulation tissue, total polymorph nuclear inflammatory cells and collagen fibers) were investigated. The most accurate method of measuring the healing process is to use the rate of change in wound surface area, which is also referred to as wound healing rate, were performed by measuring the length and width of wound closure were done by the aid of digital Vernier caliper and image analysis algorithms (Ali 2012). Photography is an invaluable instrument in the field of dermatology, as it can yield information that is frequently interchangeable with diagnosis. Additionally, photography is non-invasive, aids in case documentation, and can provide significant insights into morphological changes, color variations, and other related aspects of wound healing in an experimental setting. Digital photographs were taken with high resolution camera (Olympus SZ-20, Indonesia).

Histopathological examination
Rats in various groups had autopsy samples collected from their skin, which were then preserved for 24 h. in 10% formal saline. The individuals were dehydrated using methyl, ethyl, and 100% ethyl alcohol dilutions in order after being cleaned with tap water. Specimens were immersed in paraffin for a whole day at 56 °C in a hot air oven after being washed with xylene. Tissue blocks constructed of bee paraffin wax were sectioned to a thickness of 4 microns using a sliding microtome. The obtained tissue slices were placed on glass slides, deparaffinized, and stained with hematoxylin and eosin stain for routine examination. After that, the examination was done through the light electric microscope. Images were captured and processed using Adobe Photoshop version 8.0 (Mehrvarz et al. 2017).

Statistical analysis
The information was displayed as mean± standard deviation (S.D.). One-way analysis of variance (ANOVA) was used for multiple comparisons, and where necessary, Tukey multiple comparison was used as a post-hoc test. The significance threshold was set at the 0.05 level of probability. Version 5 of GraphPad Prism (ISI® software, USA) was used for data analysis and graph display.

Results
Sample collection and characterization
In this study, swabs were collected from patients of the mentioned medical centers. About 300 samples (61.82% from males and 38.18% from females) 22.73% of patients aged from 20 to 39 years, 37.27% of patients aged 40-59 years and 40% of patients were ≥ 60 years. Samples have been taken from different skin sites, 43.33%, 38%, 10.67%, 5% and 3% were taken from wounds, abscesses, diabetic foot, burn, and conjunctiva, respectively. The standard biochemical characterization was used to identify the Staphylococcus isolates and the results revealed that 74.54% was Staphylococcus aureus.

Antimicrobial susceptibility of S. aureus
The susceptibility pattern of Staphylococcus isolates. Multidrug-resistant (MDR) bacteria were defined as bacterial isolates that were resistant to three or more types of antibiotics (CDC). Moreover, antibiotic susceptibility was performed using VITEK® 2 AST GP67 cards to determine the resistance profile as shown in the (table 1).

Lipolytic activity of S. aureus
The result revealed that among the S. aureus, 20% were exhibit strong production of lipase enzyme while 34.55% were exhibit moderate production of lipase enzyme and 45.45% classified as weak production of lipase enzyme according to the qualitative tributyrin agar plates result. After testing S. aureus bacteria with Arabic gum, the isolates were measured on the production of the lipase enzyme. The following was found to be the strongest as code (A50- A98-B75-C11-C36) (table2).
MIC of Arabic gum against most different isolates of *S. aureus*

The MICs for Arabic gum were determined using plates with a peak of 96 μg/ml, where the Arabic gum had the MIC concentration, which was 3.9 μg/ml for the organism *S. aureus* C11 (table3).

In vitro anti-lipase activity of the Arabic gum against *S. aureus* isolates

Table 4 shows that the anti-lipase activity of Arabic gum extracts against *Staphylococcus* isolates, the 1/2 MIC, 1/4 MIC, and 1/8 MIC of Arabic gum were tested against the most potent isolates and the results revealed the lipase ratio in *Staphylococcus* isolates C36 that reached 48.93% at a concentration of 3.9 µg/ml where the ratio decreased by lowering the concentration since it was reached 37.33% and 13.62% at concentration 1.95 µg/ml respectively without affecting the bacterial growth.

**Histopathological results**

Figure 1 (A-C) shows that the rat skin in the control group was characterized by intact epidermis with distinct layers, an average dermis, and an outermost layer with varied thickness that showed densely packed collagen bundles, average subcutis, muscles, pilosebaceous units, and hair follicles. In addition, the skin’s arrector pili muscles and dermal base were home to nerves and arteries.

The burn, which had been treated with bacteria, was sacrificed after one day. The skin displayed an ulcerated and markedly necrotic epidermis, with the upper dermis displaying marked necrosis and few bacterial colonies, and the deep dermis displaying marked necrosis that extended to the subcutis, with many bacterial colonies and a marked inflammatory infiltrate (Fig. 1-C).

After 14 days, the skin of burns treated with bacteria and sacrificed showed markedly ulcerated epidermis covered by an infected crust; the upper dermis showed excess granulation tissue with proliferating blood vessels; and the deep dermis showed excess granulation tissue with proliferating blood vessels extending to underlying muscles.

**Table 1: Resistance pattern of the isolates with strong lipolytic activity to different antimicrobial agents by VITEK 2**

<table>
<thead>
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<th>Code of <em>S. aureus</em> isolates</th>
<th>Antibiotic MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cefoxitin</td>
</tr>
<tr>
<td>1 (A50)</td>
<td>+</td>
</tr>
<tr>
<td>2 (A98)</td>
<td>-</td>
</tr>
<tr>
<td>3 (B75)</td>
<td>+</td>
</tr>
<tr>
<td>4 (C11)</td>
<td>-</td>
</tr>
<tr>
<td>5 (C36)</td>
<td>+</td>
</tr>
</tbody>
</table>
The burn infected with bacteria and treated with Arabic gum; skin showed intact thin epidermis, upper dermis showing large scar with proliferating blood vessels, and deep dermis showing excess collagen and average blood vessels (Fig. 1 G and J). The burn infected with bacteria and treated with Mupirocin: skin showed intact thin epidermis covered by crust with marked sub-epidermal edema, upper dermis showing large scar with proliferating blood vessels, and deep dermis showing large scar with thick collagen and markedly dilated congested blood vessels (Fig1 D and F).

Table 2: Determination of Lipolytic activity *S. aureus* isolates quantitative by spectrophotometer

<table>
<thead>
<tr>
<th>Code</th>
<th>Lipase activity (U/mL)</th>
<th>Code</th>
<th>Lipase activity (U/mL)</th>
<th>Code</th>
<th>Lipase activity (U/mL)</th>
<th>Code</th>
<th>Lipase activity (U/mL)</th>
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<td>16.3</td>
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<td>C32</td>
<td>13.77</td>
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Table 3: MIC of Arabic gum against most different isolates of *S. aureus*

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<th>Arabic gum</th>
<th>Vancomycin</th>
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Table 4: Anti-lipase activity of Arabic gum against *S. aureus* isolates

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Where, LPR (Lipase Production Ratio) and LRR (Lipase Reduction Ratio).

Fig 1. Excision of wound healing activity of standard drug and natural products

Fig 2. Histopathological results where, A- The burn not infected with bacteria skin showing intact epidermis (black arrow), and average dermis (red arrow), (H& X 100), B- The burn not infected with bacteria, skin showing ulcerated and markedly necrotic epidermis (black arrow) with underlying marked inflammatory infiltrate (red arrow) (H& X 100) C- The burn infected with bacteria and treated with AG; skin showing intact thin epidermis (black arrow), and upper dermis showing large scar (red arrow) extending to deep dermis (blue arrow) (H& X 100) and D- The burn infected with bacteria and treated with Mupirocin: skin showing intact thin epidermis covered by crust (black arrow), and upper dermis showing large scar (red arrow) extending to muscles (blue arrow) (H& X 100).

Discussion

*Staphylococcus aureus* is a significant public health concern due to its ability to colonize skin and soft tissue, beside to its lipase enzymes can damaging host tissues and overturn the immune system (Mohammed & Ibraheim et al. 2023; Fayemi et al. 2024). *S. aureus* can cause a variety of infections, from minor skin infections to serious medical conditions like pneumonia, endocarditis, and bacteremia.
(Tong et al. 2015). Since S. aureus has become resistant to several medications, treating infections becomes more challenging. Drug-resistant bacteria have emerged as a result of overuse of antibiotics (Guo et al. 2020).

This study aimed to evaluate the activity of Arabic gum extract against the lipolytic activity of S. aureus clinical isolates from skin infections and in-vivo on the induced burn in male Sprague Dawley rats. Additionally, the histopathological assessment of the effects of Arabic gum on burn healing is investigated.

The antimicrobial susceptibility patterns of S. aureus vary across different settings and regions. MRSA isolates generally show high resistance to multiple antibiotics (Baddour et al. 2006). The lipolytic activity of S. aureus is an important characteristic of their biology and has implications for various applications. The lipolytic activity of S. aureus isolates can be classified into three categories: strong, moderate, and weak. Our results showed that, 20% were exhibit strong production of lipase enzyme while 34.55% were exhibit moderate, and 45.45% weak.

The strong antibacterial qualities of Arabic gum against a variety of bacteria, including Gram-positive and Gram-negative species, have been shown in numerous investigations (Sabri et al. 2019). The low concentrations of Arabic gum extract against certain organisms suggest that they may be effective alternatives or adjuncts to vancomycin in treating infections (Simoes et al. 2011).

Arabic gum antibacterial properties are ascribed to its capacity to rupture bacterial cell membranes, impede enzyme function, and obstruct bacteria absorption of nutrients. These antibacterial qualities of Arabic gum are thought to be attributed to its polysaccharide and phenolic constituents (Al-Beihadiy et al. 2020). The lipase ratio in S. aureus C36 was found to be 48.93% at a concentration of 3.9 µg/ml, which decreased to 37.33% at 1.95 µg/ml and 13.62% at 0.975 µg/ml. The results demonstrate the antibacterial activity of Arabic gum extract against S. aureus, which could be beneficial in the treatment of lipase-related infections.

Histological analysis is critical for assessing burn wound depth and healing progression. Burn wounds are characterized by epidermal loss, dermal necrosis, collagen denaturation, and vascular pathologies. After 14 days, a severely burned and infected wound shows a markedly ulcerated epidermis with an infected crust, excess granulation tissue with proliferating blood vessels in the upper and deep dermis extending to underlying muscles (Shupp et al. 2010). On the other hand, in a burn infected with S. aureus but treated with Arabic gum, the epidermis is thin but intact, the upper dermis shows a large scar with proliferating blood vessels, and the deep dermis has excess collagen and average blood vessels after 14 days (Ogawa et al. 2017). This significant effect on wound healing can be attributed to the presence of squalene in the Arabic gum (Kim et al. & Karadeniz et al. 2012). Arabic gum exhibits antioxidant and anti-inflammatory characteristics that may assist in the wound healing process. Research has demonstrated that it can regulate the expression of genes participating in cell growth, potentially mitigating inflammation and facilitating tissue repair (Aloqbi et al. 2020).

Conclusion
The most predominant bacteria were found in specimens was Staphylococcus aureus with high lipolytic activity. Using Arabic gum extract inhibited the development and synthesis of toxins by S. aureus, making it an excellent natural preservative. Arabic gum has been found to effectively inhibit the lipolytic activity of S. aureus and to accelerate burn healing in rats. The results suggest that Arabic gum may be a promising remedy for wound healing and antimicrobial applications against S. aureus.

Conflict of interest
The authors declare that they have no conflict of interest.

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