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ORIGINAL ARTICLE

Evaluation of antibacterial and wound-healing activities of alcoholic extract of *Boswellia carterii*, in vitro and in vivo study

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Abstract

Background: Today, despite the existence of various chemical and physical treatments for wound healing, the use of traditional medicine including herbal medicine is still widely used in most developed and developing countries.

Objectives: To investigate the antimicrobial and wound-healing activities of alcoholic extract of *Boswellia carterii* (BC) plant.

Methods: The BC extract was prepared using alcohol 70%. The chemical groups and extract compounds were determined using Fourier transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC) analysis, respectively. The antimicrobial and wound-healing activities of different concentrations of BC extract and its combination with penicillin–streptomycin were assessed by agar well diffusion and infected wound model in albino rabbits, respectively.

Results: FTIR revealed the presence of hydroxyl, amide, carboxyl, alkyl C–H stretches, aromatic C=C bends, and aromatic C–H bends in the BC extract. The HPLC revealed 14 different compounds including thujene (48.0%) as the most abundant ingredient. All BC concentrations showed antibacterial and wound-healing activities. The 10% concentration of BC extract had the strongest antibacterial effect. Also, the combination of penicillin–streptomycin with BC extract showed synergistic antibacterial effect. The 5% concentration of BC was the best wound-healing compound which healed the wound in 6 days and decreased the wound size 10 mm each day.

Conclusions: This study demonstrated the potential abilities of BC as an antibacterial and wound-healing medicinal plant. Further studies are required to justify the in vivo use of this plant.

KEYWORDS
antibacterial effects, *Boswellia carterii*, FTIR, HPLC, in vivo

1 | INTRODUCTION

Wounds are defined as the cellular and anatomical destruction of tissues that can be caused by chemical, physical, microbiological, thermal, or immunological damage, leading to skin rupture.[1] Wound healing is one of the most complex biological processes, with four overlapping phases: hemostasis, inflammation, proliferative, and maturation. These phases lead to the regeneration of new tissue. In burn injuries, blood coagulation occurs due to damaged capillaries, followed by inflammation.[2,3]

The global cost of wound care averaged 2.8 billion dollars in 2014 and is expected to reach 3.5 billion dollars by 2021.[4] A 2018 analysis

of Medicare beneficiaries found that 8.2 million people had infectious or noninfectious wounds that cost them between 28.1 and 96.8 billion dollars in wound treatment. Moreover, they are more likely to experience severe morbidity and mortality due to their wounds.[4–6] Therefore, wound care and management are important issues in the medical field and medical consultants pay much attention to these areas.

The most common microorganisms in healthy skin and wounds are bacteria, which can delay or prevent wound healing.[7] Today, the prevalence of multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* is increasing in infections associated with a variety of skin lesions, including chronic wounds.[8,9] This may lead to increased morbidity, mortality, and medical costs. Hence, the development of new drugs from medicinal herbs has long been considered as an approach to combat antibiotic resistance in bacteria.[10] In this context, there is much evidence of synergistic effects of combining herbal compounds with chemical drugs in the treatment of various disorders including wound scars and bacterial infections. This may reduce the dosage and side effects of antibiotics, increase their antibacterial efficacy, and slow down the spread of antibiotic resistance.[11,12]

Despite the existence of various treatments for wound healing including plastic surgery, skin grafting, cell transplantation, and plasma therapy, traditional medicine, including herbal medicine, is still widely used in most developed and developing countries.[5,11,13] The potential of plants for the treatment of wounds is enormous. Several plants are used as traditional medicines for the treatment of a variety of wound injuries and skin disorders.[7,9] Herbal treatment that has been shown to be safe and effective in this situation is recommended and promoted by the World Health Organization (WHO).[14]

Frankincense (Kondor) or olibanum is an oleogum resin obtained from *Boswellia* species including *Boswellia carterii* (BC). These species belong to the Burseraceae family of medicinal plants. Burseraceae is a family with 17 genera and 500–600 species distributed in the tropical and subtropical regions.[15–17] BC has beneficial treatment effects on various diseases including bronchitis, diarrhea, rheumatoid arthritis, and asthma.[18] Also, the different *Boswellia* species have antidiabetic, antineoplastic, antioxidant, and anti-inflammatory properties.[19] Previous studies have shown that BC is able to relieve pain and inflammation.[16,20] However, to the best of our knowledge, there are little data on the wound-healing properties of BC worldwide. Also, the antibacterial effects of this plant have rarely been investigated. Infections can cause the orderly process of wound healing to be delayed and disrupted.[16] Medicinal plants promote blood clotting, treat infections, and accelerate wound healing. They demonstrate wound-healing effects through various mechanisms such as modulation of wound healing, increase in fibroblasts and fibrocytes, reduction of bacterial count, improvement of collagen deposition, production of glycosaminoglycans, and formation of granulation tissue.[11,21] Therefore, finding an herbal remedy that has the ability to simultaneously heal wounds and control infections can greatly help the medical community in the treatment of burn wounds, diabetic wounds, and other wounds that respond late to routine treatments. Hence, the aim of this study was to investigate the antimicrobial and wound-healing activities of the alcoholic extract (5%, 10%, 20%, and 30%) of BC plant and its possible synergistic property in combination with standard antibiotics penicillin–streptomycin.

2 | MATERIALS AND METHODS

2.1 | Plant materials and alcoholic extract preparation

The BC oleogum resins used in this study were purchased from the local markets of Al-Hilla (Ibn Witwit store for medicinal plants) in January 2020. The resins were washed and air dried after the identity of the plant was confirmed and authenticated by a botanist. On a heated magnetic stirrer (VELP Scientifica, Italia), 100 g of BC was dissolved in 150 ml of 70% alcohol (Merck, Germany) for 24 h. After filtration with Whatman filter paper (No. 1) (Maidstone, UK), the extract was kept in a plate in an incubator at 40°C for 3 days to dry before being collected in a clean container and stored in the refrigerator for ointment preparation (Figure 1).

2.2 | Formulation of the BC extract ointment

Because the ointment can remain on the wound surfaces for a more extended duration than liquid formulations and can be easily rubbed on the wounds, the initial formulation was prepared as ointment. To prepare a therapeutic ointment containing 4 different concentrations of BC (5%, 10%, 20%, and 30%), the plant extract was first powdered. The extract powder (5, 10, 20, and 30 g) was then mixed with a certain amount of vaseline (up to 100 g) (w/w) by levigation on the surface of the ointment plate to produce an ointment with uniform consistency and smooth texture. Finally, the prepared ointment was transferred into a clean container to be used as a topical agent during the experiment. The selection of different concentrations of 5%, 10%, 20%, and 30% was performed randomly and as primary investigation.

2.3 | Preparation of penicillin–streptomycin (P-S) ointment

To prepare 5% P-S ointment, 95 g of vaseline and 5 g of penicillin–streptomycin (w/w) (Sigma-Aldrich, USA) were mixed by levigation on the surface of the ointment plate to produce an ointment with uniform consistency and smooth texture.

2.4 | Preparation of P-S-BC mixture

Penicillin–streptomycin and 5% BC extract were combined; then, vaseline was added to a final concentration of 100 g. They were well mixed together, and the resulting mixture was placed in a clean container. Before use, the combination was kept for one week to observe any particular reactions or color changes.

FIGURE 1 Steps of extraction: A = *Boswellia carterii* (BC), B = BC with 70% alcohol on hot plate with magnetic stirrer, C = BC extraction, D = Final extraction after dry and filtration

2.5 | Fourier transform infrared spectroscopy (FTIR) analysis of the alcoholic extract of BC

The dried powder of the plant solvent extract was used for FTIR analysis. To prepare transparent sample discs, 10 mg of the dried extract powder was encapsulated in 100 mg KBr pellets. The powdered sample was loaded in FTIR spectroscopy (Perkin Elmer, Spectrum GX, USA), with a scan range of 4000 to 400 cm^{-1} with a resolution of 0.15 cm^{-1} to evaluate the functional groups involved in the particle preparation process.[22]

2.6 | High-performance liquid chromatography (HPLC) analysis of the alcoholic extract of BC

2.6.1 | Reagents

All chemicals and reagents used in this research were of the highest quality. HPLC grade acetonitrile, methanol, and Milli-Q HPLC grade water were purchased from Merck Co, Germany. Merck analytical grade acetic acid was used for the mobile phase. Also, HPLC grade methanol was used for sample preparation. The ultrasonic bath used was from Elma Sonic (Elma S 100 H) Singen, Germany. The HPLC system included an Agilent 1100 Series HPLC instrument (Agilent Technologies, Santa Clara, CA, USA) with a binary pump, an auto-sampler, an automatic electronic degasser, an automatic thermostatic column oven, a diode array detector, and a computer with Chemstation software (version 06.03 [509]) for data analysis. HPLC separations were optimized using RP-18, a C18 reversed-phase chromatography column (46 × 250 mm, 5 μm) with an isocratic mixture of acetonitrile (40:60 ratio). The mobile phase was dispensed at a flow rate of 1.0 ml/min. The temperature of the column was maintained at 30°C to ensure the sharpness of the eluting peaks. UV chromatograms were recorded at 205 and 250 nm. Exact weights of the dried extract sample from the BC gum resin were dissolved in known amounts of HPLC grade methanol. After filtration of the plant extract using Millipore (0.45 m) filters, a total volume of 10 μl of the sample was injected into the HPLC system.

2.6.2 | Preparation of bacterial sample and suspension

In this study, a clinical strain of *Staphylococcus epidermidis* isolated from wound cultures was used to investigate the antibacterial effects of BC extract. A sample swab was taken from the wound after it had been cleansed (with sterile saline and gauze) and debrided (removal of necrotic tissue, foreign material, calluses, and undermined wound edges). No antimicrobial agent (e.g., alcohol or iodine) or antiseptic was put into the wound prior to collection of the specimen. The wound was swabbed using the Levine method, in which a swab was rotated over a 1 cm^2 area of the wound for 5 s while

applying enough pressure to remove fluid from the inner section of the wound. Within 20 min, the specimen was placed in sterile transport containers and sent to the microbiology laboratory for aerobic culture. The specimens were inoculated onto nutrient agar (Merck, Germany), mannitol salt agar (Merck, Germany), and blood agar (Merck, Germany) and incubated at 37°C for 24 h. Grown isolates were identified using the Vitek2 system (bioMérieux, USA).[23]

2.6.3 | Antibacterial activity assay

The antibacterial activity of BC extract ointment, P-S ointment, and P-S-BC combination was determined using the agar well diffusion technique. Six wells of 6 mm diameter were prepared on a Muller-Hinton agar (Merck, Germany) plate previously inoculated with 1.5×10^8 CFU/ml of *S. epidermidis* isolates (equivalent to 0.5 McFarland) using a sterile cork borer. Each well was filled with 50 μl of different BC ointment concentrations (5%, 10%, 20%, and 30%), P-S ointment, and P-S-BC combination. All plates were incubated at 37°C for 24 h. Finally, the inhibition zones formed were measured to the closest millimeter. The experiment was repeated three times, and the average values were used to calculate the diameter of the inhibition zone.

2.7 | Experimental animals

A total of 24 healthy adult male albino rabbits (1.5–2 kg, 1–2 years old) were obtained from Al-Magmoaa Veterinary Clinic (Iraq). Animals were housed in cages in a typical animal shelter with a natural 12/12 h light/dark cycle at room temperature and were given food and drink. All rabbits were given one week to adapt to the home environment before the experiment began. Throughout the research, animal handling and care were carried out in accordance with international laboratory animal use and care norms.

2.8 | Wound-healing activities assay

2.8.1 | Grouping and dosing of animals, infected wound model

To evaluate the wound-healing effects of BC extract, animals were randomly divided into eight groups (A-H) (each containing 3 rabbits) as shown in Table 1. The 8 groups of rabbits were divided according to their treatment with BC ointment at different concentrations, P-S ointment, P-S-BC mixture, negative control, and positive control groups. Group A received 2% Tween 80 (Merck, Germany) as negative control. Group B received indomethacin (10 mg/kg) (Ritual Drugs Pvt. Ltd, India) as standard triterpenoid control. Groups C-H were treated with BC ointment at different concentrations (5%, 10%, 20%, and 30%), P-S ointment, and P-S-BC mixture, respectively.

TABLE 1 Grouping and dosing of studied albino rabbits based on their treatment

Group	Treatment	Number of tested animals
A	Negative control	3
B	Positive control	3
C	5% *Boswellia carterii* extract ointment	3
D	10% *Boswellia carterii* extract ointment	3
E	20% *Boswellia carterii* extract ointment	3
F	30% *Boswellia carterii* extract ointment	3
G	Penicillin–streptomycin (P-S) ointment	3
H	Combination (P-S and 5% BC extract)	3

The effect of BC extract was evaluated using the infected wound model in albino rabbits. On wounding day, animals were anesthetized using I/M injection of ketamine (1 ml/kg) and xylazine (1 ml/kg) (Sigma-Aldrich, USA) in femoral muscle. The fur of each rabbit was then shaved, and a 6 cm linear incision was made through the skin and subcutaneous tissue. The wounds were infected with *S. epidermidis* (1.5×10^8 CFU/ml) and left undressed to the open environment (Figure 2). The day of wounding was considered as Day 0. After 48 h of wound creation (on Day 2), animals were treated with topical formulation of ointments daily as described in the grouping and dosing section until the wound was completely healed. A transparent paper and a 1 mm^2 graph sheet were used to measure the wound area in the morning of each day until the wound was completely closed.

2.8.2 | Assessment of wound contraction

Contractions contribute to wound closure during the first two weeks. A percentage of the initial wound size was used to calculate wound contraction.[20] Wounds were monitored and wound area was measured every day before and after treatment with BC extract ointment, P-S ointment, and their combination until complete healing. The wound-healing effect was calculated (considering the initial size of the wound as 100%) using the following formula:

$$\%\,\text{wound contaction} = \frac{\text{wound area on day 0} - \text{wound area on day n}}{\text{wound area on day 0}} \times 100$$

n = number of days at which the measurement was taken.

2.9 | Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 22 (Armonk, NY, USA) was used to enter, code, and analyze the data. The data of wound contraction were analyzed as the mean ± Standard Error of the Mean (SEM). The p-value <0.05 was considered statistically significant using one-way analysis of variance (ANOVA) and post hoc Tukey's test.

FIGURE 2 Infected wound model to evaluate the *Boswellia carterii* extract wound-healing activity

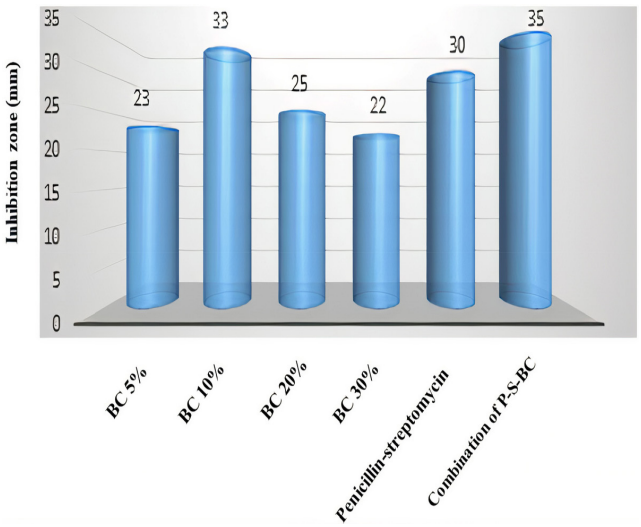

FIGURE 3 Inhibition zone of different BC extract concentrations, penicillin–streptomycin, and combination of penicillin–streptomycin–*Boswellia carterii* (P-S-BC) extract against *Staphylococcus epidermidis*

TABLE 2 High-performance liquid chromatography (HPLC) analysis of *Boswellia carterii* alcoholic extract

Peak	Analyte	Retention time (min)	Percentage
1	*E*-Nerolidol	5.76	0.3
2	α-Thujene	14.78	48.0
3	Cembrene A	15.08	2.1
4	α-Pinene	15.08	8.5
5	Cembrene C	15.52	0.5
6	Camphene	15.52	1.1
7	Verticillate-4(20),7,11-triene	15.65	0.4
8	Sabinene	15.65	0.7
9	Incensole	16.95	1.3
10	Incensole acetate	17.14	2.3
11	Myrcene	17.14	1.3
12	Incensole oxide	18.07	1.7
13	Hexyl acetate	18.07	0.5
14	Incensole oxide acetate	18.51	2.1

3 | RESULTS

3.1 | FTIR analysis

Based on the peak value in the infrared radiation area, FTIR analysis revealed the presence of different active chemical groups in the BC extract. The absorption peaks at 3437 and 1634 cm^{-1} are related to OH and COOH, respectively. The presence of the peaks at 3437.14, 2075.91, and 2924.3 cm^{-1} in FTIR spectrum confirmed the presence of amide, carboxyl, and O–H functional groups, respectively. The frequency area between 2950–2850 cm^{-1}, 1700–1400 cm^{-1}, and 860–680 cm^{-1} showed the accurate existence of alkyl C–H stretch, aromatic C=C bending, and aromatic C–H bending, respectively. Moreover, FTIR analysis revealed the existence of the major functional groups including C–O bond at 1710 cm^{-1}, C=C bending at 1450 cm^{-1}, and aromatic C–H bending at 733–739 cm^{-1}.

3.2 | HPLC analysis

The HPLC analysis revealed 14 different compounds in BC extract as shown in Table 2. The most abundant ingredients were as follows: α-thujene (48.0%), α-pinene (8.5%), and incensole acetate (2.3%).

3.3 | Antibacterial activity assay (in vitro)

This study showed the potent antibacterial effect of BC extracts in different concentrations against *S. epidermidis* isolate by the agar well diffusion method (Figure 3). The 10% concentration of BC extract had the stronger antibacterial effect compared to other BC extract concentrations and penicillin–streptomycin. The inhibition zone of the BC 10% extract (33 mm) was significantly larger ($p = 0.008$) than those of other BC concentrations 5%, 20%, and 30% with the inhibition zones of 23, 25, and 22 mm, respectively. Also, the inhibition zone of 10% BC extract was larger than inhibition zone of penicillin–streptomycin (30 mm). However, this difference was not significant ($p = 0.08$). Moreover, the combination of penicillin–streptomycin and 5% BC extract affected

the growth of *S. epidermidis* with the most significant inhibition area (35 mm) compared to penicillin–streptomycin alone or BC extract alone.

3.4 | Wound-healing activity and wound contraction

Forty-eight hours after contamination with the *S. epidermidis*, signs of inflammation (redness, heat, swelling, and pain) and purulence appeared in the infected area. After inflammation signs appear, BC extract ointment, P-S ointment, and P-S-BC mixture were applied to the infected wound. Planimetric analysis was used throughout the 23-day trial period to assess the rate of wound healing of each compound and comparison with negative and positive controls. According to Figures 4 and 5, the best concentration of wound-healing BC ointment was 5% which healed the wound in 6 days and decreased the wound size 10 mm each day (Figure 6). The wound contraction related to BC 5% in Day 6 (mean ± SEM = 0.00 ± 0.00) was significantly better than the control group (mean ± SEM = 2.04 ± 0.01) (p = 0.001). The wound-healing effects of BC 5% were significantly (p-value = 0.013) better than those of the other BC concentrations. The promising healing activity of the extract on excision wound rabbits model in comparison with control group confirm the release of the phytochemicals. BC 10% ointment healed the wound entirely in 8 days and contracted the wound 0.75 mm every day. Other concentrations (20% and 30%) of BC extract showed less effects than 5% and 10% concentrations. The P-S ointment healed the wound in 8 days, and P-S-BC mixture was the most effective compound than antibiotics alone and different concentrations of BC extract where the wound healing occurred in 5 days. The wound-healing effect of P-S-BC was significantly better than those of BC concentrations 10%, 20%, and 30% (p = 0.001). Also, the wound contraction related to P-S-BC in Day 5 (mean ± SEM = 0.00 ± 0.00) was significantly better than the control group (mean ± SEM = 2.70 ± 0.05) (p = 0.001). The fastest contraction of wound was observed in animals treated with the P-S-BC combination ointment on the 1th, 2th, 3th, 4th, and 5th days, which were 20%, 40%, 60%, 80%, and 100%, respectively. All these results were better than the positive control group in which the speed of the wound-healing process reached up to 23 days and wound contracted every day 0.26 mm. During the experiment period, wound post-infection was not observed, all animals were in healthy status, and no animal was dead.

3.5 | Dermal toxicity observation

Application of different concentrations of BC extracts on the created wounds in tested animals was found to be safe. No signs of erythema, edema, or toxicity were observed during the time of the experiment.

4 | DISCUSSION

In this study, we focused on the less studied BC extract to shed light on its antibacterial and wound-healing properties. Herbal medicines are usually produced with less quality control than commercially produced pharmacological drugs and may contain

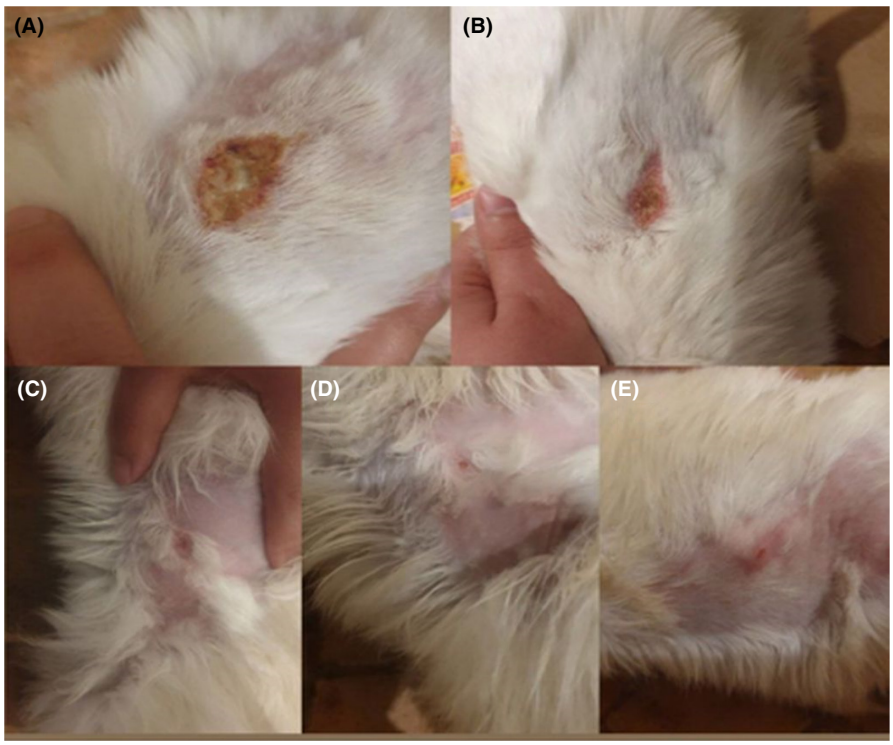

FIGURE 4 Various phases of wound healing during 6 days with 5% BC extract ointment. A: first day. B: second day, C: fourth day, D: fifth day, and E: sixth day

a number of bioactive components.[24] In this study, the result of HPLC analysis revealed that α-thujene (48.0%) and α-pinene (8.5%) were the main constituents of BC extract. So far, more than 300 volatile chemicals have been identified in *Boswellia* species with a wide variety of pharmacological activities including antibiotic resistance modification, antibacterial, anti-inflammatory, and analgesic properties, of which α-pinene and α-thujene are the most abundant.[25–28] Incensole acetate was another constituent present in trace amounts (2.3%) in BC extract in this study. Incensole acetate (diterpenoid), which is abundant in the *Boswellia* plant, has been shown to have anti-inflammatory properties.[29] In contrast to the previous investigation by Woolley et al.,[30] myrcene was found in trace quantities (1.3%) in this study. It is noteworthy that excessive levels of myrcene may not be safe, as the International Agency for Research on Cancer (IARC) categorized it as a probable carcinogen in 2017.[28] Differences in the results of various studies may be due to influencing factors such as the geographical location of the plant, seasonal variations, and the extraction technique used, which affect the quality and quantity of chemical constituents of extracts of different *Boswellia* species.[31,32] In this study, the functional groups of BC extract compounds were analyzed using FTIR to determine the main active groups. FTIR is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds.[33] FTIR spectroscopy revealed the presence of alcohol, phenols, alkanes, alkyl, aldehydes, carboxylic acids, aromatics, nitro and amides in the alcoholic extract of BC in the different spectra ranging from 4000 to 400 cm^{-1}. These results were consistent with previous studies by Fatimah et al.,[33] Sharma and Jana,[34] and Adebayo et al.[35]

It has been previously shown that BC has antibacterial properties against Gram-positive and Gram-negative bacteria.[36,37] In a research conducted by Kaániová et al.,[37] BC showed the best activity against *S. epidermidis* strains among different *Staphylococcus* species. The present investigation also revealed significant antimicrobial effects of BC extracts at different concentrations against *S. epidermidis* isolate obtained from wound infection using agar well diffusion assay. HPLC and FTIR analyses showed that the plant extract contained phenolic compounds, fatty acids, and other antibacterial substances. Also, Sultan et al.[38] found that fatty acids and phenols of *B. serrate* had higher antibacterial potential against *Bacillus subtilis*, *Streptococcus pneumonia*, and *Proteus vulgaris* than ciprofloxacin. Recently, the synthesized BC-AgNPs showed strong

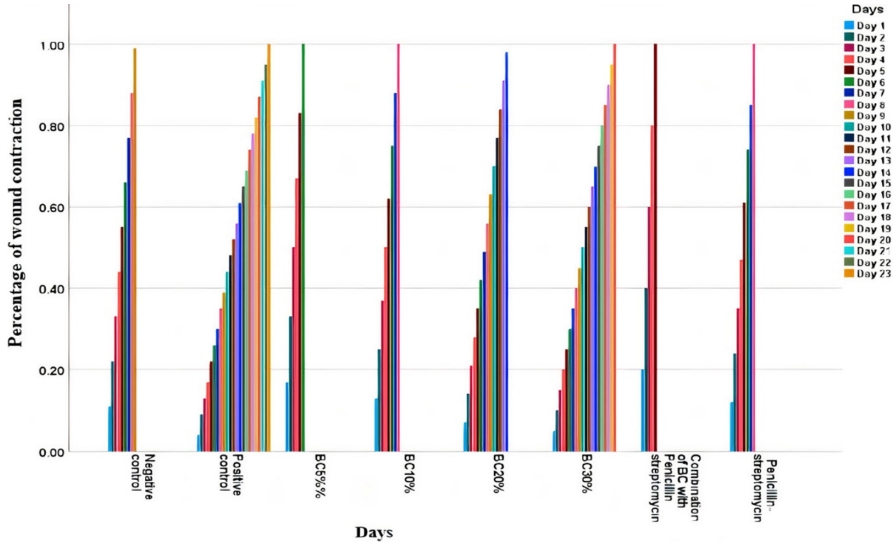

FIGURE 5 Percentage of wound contraction as a result of the different concentrations of *Boswellia carterii* (BC), penicillin–streptomycin, and combination of penicillin–streptomycin with 5% *Boswellia carterii*

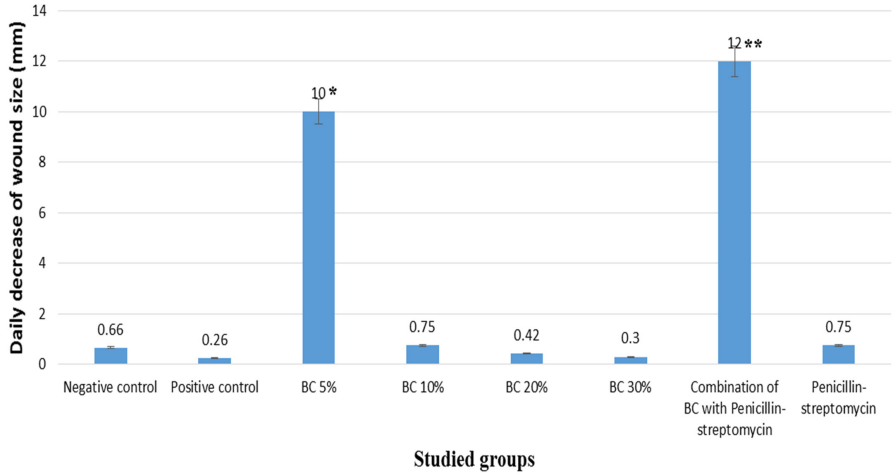

FIGURE 6 Daily decrease of wound size (mm) as a result of the different concentrations of *Boswellia carterii* (BC), penicillin–streptomycin, and combination of penicillin–streptomycin with 5% *Boswellia carterii*, * and ** The significant daily decrease of wound size (mm) of BC 5% (p-value = 0.013) and P-S-BC (p = 0.001) compared to other studied groups

antibacterial activity against the oral pathogen *S. mutans*, indicating that they may have a wide range of biological uses as an alternative therapy.[39] In another study, the antibacterial activity of aqueous, ethanolic, and chloroform BC extracts was ranged from 12.5 to 50 mg/ml against *S. aureus*, *Escherichia coli*, *P. aeruginosa*, *Bacillus subtilis*, and *Klebsiella pneumoniae*.[40] According to a study published in 2021 by Klūga et al.,[41] BC had a significantly higher antibacterial effect on *Aerococcus* spp., and *Enterococcus* spp. than *Escherichia* and *Pseudomonas* species. The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria may be related to the different morphological nature of these microorganisms.[41] Also, when the antibacterial properties of frankincense essential oil were investigated by Beyzaei et al.[42] and Pirnia et al.,[43] the best inhibitory activities were found against Gram-positive bacteria. The antibacterial effects of BC extract may be due to the presence of several antimicrobial components including phenolic constituents, fatty acids, and monoterpenoids such as α-pinene which inhibit the growth of a wide variety of bacterial pathogens. A review of the literature revealed a number of publications on the wound-healing properties of *Boswellia* species from both animal and human research.[44–47] Also in this study, wound treatment with a combination of 5% extract and the standard antibiotics (penicillin–streptomycin) significantly increased the rate of wound contraction and epithelialization substantially. The rate at which the unhealed area decreases during the healing process is indicated by wound contraction. Wound contraction is important because it reduces the size of the wound, thus shortening the healing time. The wound-healing effects of BC showed significant improvement in wound-healing activity with both 5% (w/w) and 10% (w/w) extracts in infected wound model as compared to control group. The 5% BC extract showed more healing activity than the other concentrations. The wound-healing efficacy of the 10% extract was comparable to that of standard antibiotics. Wound contraction time decreased from 23 days (control) to 6 days with the 5% extract. Also, the combination of 5% BC with penicillin–streptomycin decreased the wound contraction time to 5 days compared to the 23 days of the control group. The strength of healed wound tissue in this study may be due to collagen remodeling and formation of stable intra- and intermolecular cross-links required for collagen maturation, as described elsewhere. In 2021, an in vivo study by Rouhi-Broujeni et al.[46] revealed that *Boswellia* cream 2.5% increased the rate of epithelial tissue and collagen fiber synthesis and wound healing in rats with diabetic wounds. A clinical trial was conducted to evaluate the benefits of frankincense gel on the healing of 75 hospitalized patients with second and third degree pressure ulcers. The results of the study showed that the ulcers healed after five weeks and that growth factors increased in the study groups. Furthermore, there were no adverse reactions to frankincense.[45] The effects could be attributed to the presence of secondary metabolites such as flavonoids, tanins, and saponins, which are responsible for promoting collagen maturation that ensures the strength and integrity of the wound matrix.[48] Thus, the wound-healing effects of BC extract can be related to the phytoconstituents present, which may be due to their individual or additive action. Several studies have found that BC has a potent anti-inflammatory effects.[49,50] Microbes and their endotoxins can lead to a sustained increase in pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor α (TNFα). This can result in a persistent inflammatory state that increases the production of matrix metalloproteases (MMPs) and inhibits wound healing.[51] Thus, the antibacterial effects of BC extract on these wounds may contribute to wound healing by eliminating the infection and facilitating the initiation of normal tissue repair processes.[52] In an in vivo study, frankincense was shown to promote healing by reducing scar formation,[53] and in an animal investigation, it was shown to enhance vascularization, suppress inflammation, and improve wound healing in diabetic mice.[54] The findings of the study by Faraji et al.[16] showed that BC can be used as a safe natural medicine in the healing of episiotomy wounds. Today, various studies have been performed to evaluate the different chemical and plant compounds as nanoemulsion in wound healing, which have yielded significant results.[55,56] The result of this study also provided a new horizon for the use of BC plant as nanoparticles for wound healing in future studies.

4.1 | Limitations

In this study, we could not evaluate the antibacterial effects of the plant on a wider spectrum of Gram-negative and Gram-positive pathogens due to the traffic restrictions caused by the COVID-19 coronavirus pandemic. Due to this critical situation, the remaining wound-healing models, such as excision wound model, were also not investigated. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of BC were not determined. The normality of this study was not determined using a standard reference bacterium. The biosafety of the BC extract was not investigated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Also, the histology evaluation was not performed to confirm the tissue regeneration and absence of inflammatory cells. Finally, the active ingredients responsible for wound healing were not determined.

5 | CONCLUSIONS

In this study, wound healing and contraction, and epithelialization were improved by the alcoholic extract of BC compared to the control group. The extract was also found to have anti-inflammatory and antibacterial activities. The observed results justify the use of BC extract as an herbal supplement for wound healing. Although this study demonstrated the potential wound healing, anti-inflammatory, and antibacterial activities of BC extract, it does not indicate which metabolites are responsible for the observed benefits. It is therefore expected that future research on BC extract will focus on

fractionation, isolation, and characterization of the active constituents, as well as exploring the mechanisms of action. It is also recommended that some clinical trials be conducted to approve the use of BC extract in humans.

AUTHOR CONTRIBUTIONS

All authors have read and approved the final manuscript. Shaimaa Obaid Hasson, Adnan Mansour Jasim, Sumod Abdul Kadhem Salman, and Maryam Adil Hassan performed the research. Shaimaa Obaid Hasson and Adnan Mansour Jasim designed the research study. Sousan Akrami and Morteza Saki analyzed the data. Sousan Akrami and Morteza Saki wrote the paper.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

All methods in this study were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and its associated guidelines, and the EU Directive 2010/63/EU for animal experiments. All experimental protocols in this study were approved by the Research Ethics Committee of the Al-Qasim Green University, Al-Qasim, Iraq (code: 533FD2), and all methods were performed in accordance with the ARRIVE guidelines.

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