Trop J Nat Prod Res, December 2025; 9(12): 6099 - 6109
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Wound Healing Potential of Hydrogel Extract of *Saccharum spontaneum* Leaves Using Incision Wound Healing Model in Rats

Eko S. Wahyuningsih[1], Asman Sadino[2], Farhamzah Farhamzah[3], Neni S. Gunarti[1], Putri Agustina[1], Sudrajat Sugiharta[3], Yogi R. Nugraha[4], Andi N. Amal[5], Maulana Y. Alkandahri[5]*, Siska R. Dewi[6], Siti S. Dhaniaty[6], Tati Kholisoh[6], Balebat A. Sinangling[6], Audia N. Z. Adam[6]

[1]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Buana Perjuangan Karawang, Karawang, West Java 41361, Indonesia.*
[2]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Garut, Garut, West Java 44151, Indonesia.*
[3]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Universitas Buana Perjuangan Karawang, Karawang, West Java 41361, Indonesia.*
[4]*Diploma Program of Pharmacy, Karsa Husada Garut College of Health Sciences, Garut, West Java 44151, Indonesia.*
[5]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Buana Perjuangan Karawang, Karawang, West Java 41361, Indonesia.*
[6]*Faculty of Pharmacy, Universitas Buana Perjuangan Karawang, Karawang, West Java 41361, Indonesia.*

ARTICLE INFO

Article history:
Received 08 September 2025
Revised 24 November 2025
Accepted 25 November 2025
Published online 01 January 2026

ABSTRACT

Saccharum spontaneum is a medicinal plant known for treating various diseases, such as wounds. Despite the potential use of this plant, there are no studies confirming its wound-healing activity. This study assessed *S. spontaneum* extract hydrogel formulation (HESs) and examined its effects on wound healing, antioxidant properties, and antibacterial activity. The disk diffusion method was used to evaluate the antibacterial activity, while DPPH and FRAP were used to measure the antioxidant activity. The rats were divided into 5 groups of 4 rats each to assess wound healing using the incision wound model. Group 1 served as the negative control, while group 2 was given 0.05% octenidine. *S. spontaneum* extract hydrogel formulation (HESs) was administered to groups 3, 4, and 5 at 5%, 10%, and 15% (%w/w) concentrations, respectively. The results showed that *S. spontaneum* extract has antioxidant activity with IC_{50} values of 80.16 (in DPPH) and 82.70 µg/mL (in FRAP), while exhibiting an inhibitory effect on the growth of bacteria in a concentration-dependent manner. The wound healing activity test results indicated that 10% and 15% HESs significantly closed the wound area on days 4, 8, and 16. The *S. spontaneum* leaf extract exhibited wound healing potential.

Keywords: *Saccharum spontaneum*, Wound healing, Antioxidant, Antibacterial, Hydrogel.

Introduction

The skin is the outermost organ that covers the entire body surface of humans. This organ prevents the entry of pathogens, minimises fluid loss, blocks ultraviolet radiation, and regulates body temperature.[1,2] The skin is also susceptible to injury due to several factors, including physical, chemical, thermal, microbial, and immunological. In living tissues, these elements may disrupt cellular and anatomical or functional continuity.[3] After an injury, an inflammatory response is triggered, prompting the cells under the dermis to produce more collagen, and then the epithelial tissue begins to grow.[4] Wound healing is a repair mechanism that takes place after the skin or other soft tissues are injured, to restore both the structural and functional integrity, along with replacing any damaged cellular structures and tissue layers.[5] This process is complex and dynamic, including various cell types, leukocytes, erythrocytes, platelets, fibroblasts, and epithelial cells, as well as growth factors, inflammatory mediators, and cytokines.[6]

*Corresponding author. E mail: alkandahri@gmail.com
Tel: +6282167757738

Citation: Wahyuningsih ES, Sadino A, Farhamzah F, Gunarti NS, Agustina P, Sugiharta S, Nugraha YR, Amal AN, Alkandahri MY, Dewi SR, Dhaniaty SS, Kholisoh T, Sinangling BA, Adam ANZ. Wound Healing Potential of Hydrogel Extract of *Saccharum spontaneum* Leaves Using Incision Wound Healing Model in Rats. Trop J Nat Prod Res. 2025; 9(12): 6099 – 6109 https://doi.org/10.26538/tjnpr/v9i12.26

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In general, the wound healing process takes place through four different phases. This process begins with hemostasis, which lasts for ±24 hours, followed by the inflammatory phase for 2–5 days. The proliferation phase includes angiogenesis, ECM (extracellular matrix) deposition, granulation tissue development, epithelialisation, and wound contraction, lasting for 5 days to 2 weeks. Lastly, the connective tissue deposition occurs during the remodelling/tissue maturation phase, which lasts for 3 weeks to 24 months.[7] Multiple components, such as the size of the wound, the blood supply to the wound area, and the presence of infection, determine the wound healing process.[8] Bacterial infections, specifically *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, can delay the process by prolonging the inflammatory phase.[9] This prolongation causes excessive formation of ROS that fight pathogens, such as macrophages and neutrophils. Meanwhile, excessive ROS production reduces the natural antioxidant activity of the body, reducing free radical scavenging and causing oxidative stress. This causes damage to biomacromolecules, including proteins, DNA, lipids, and carbohydrates, along with cell membranes and the ECM. The damage triggers an increase in the inflammatory mediators as well as proteolytic enzyme production, causing a harmful cycle of inflammatory amplification that worsens tissue injury.[10,11] This process can halt wound healing during the inflammatory phase,[12,13] thereby leading to the formation of scar tissue.[14]

Most wounds can heal naturally, but it can take quite a long time in some conditions. This extension of time causes a chronic wound with more severe conditions. Consequently, treatments that incorporate bioactive healing agents and/or wound dressings are essential to facilitate the healing process.[15] Many drugs are used to treat wounds, but some are expensive and can also cause several issues, including digestive problems, allergies, and drug resistance.[16] Therefore, drugs

derived from medicinal plants have become the subject of the latest pharmaceutical formulations to treat various diseases, including wound healing, due to their affordable and safe nature.[17] At present, more than 75% of people on the planet use medicinal herbs to cure wounds. Research has identified 62 families and 109 genera that exhibit wound-healing properties, which include *Saccharum spontaneum*, but its use is still empirical.[18]

S. spontaneum is a plant belonging to the Poaceae family. This perennial grass can grow up to four meters tall with deep roots and rhizomes. It can withstand various biotic and abiotic stressors, has the ability to root, higher morphological variability, and has the ability of its seeds to grow vigorously under both optimal and suboptimal conditions. The plant is widely distributed in tropical regions of the world, such as Australia, Asia, Africa, and the Americas. *S. spontaneum* is known to contain alkaloids, coumarins, saponins, quinones, steroids, terpenes, phenolics, carbohydrates, and proteins.[19] Prior investigation showed that it has anti-inflammatory, antidiarrheal, antifungal, and analgesic activity.[19,20] However, there has been no study reporting the *S. spontaneum*'s wound healing activity. The leaf extract cannot be used instantly because it does not remain on the injured experimental animals' skin for a sufficient duration. Therefore, its extract formulation into a topical preparation is necessary to achieve sustained drug delivery at the wound site, which can be carried out using a hydrogel preparation. The formulation is a clear, flexible film that exhibits strong adhesive properties, releasing and spreading the agent on the skin well.[21] Hydrogel offers soothing moisture to the wound spot as well as a cool sensation that reduces swelling and accelerates the healing process. According to a previous study, this material reduces pain around the wound and increases comfort for patients.[22] Therefore, this study aimed to determine the wound healing potential of *S. spontaneum* leaf extract hydrogel (HESs) using an incision wound model in rats, as well as determine its antioxidant and antibacterial activity.

Materials and Methods

Drugs, chemicals, and instruments

Octenidine dihydrochloride gel (PT. Ikapharmindo Putramas Tbk., Indonesia), ketamine HCl (PT. Bernofarm Pharmaceutical Company, Indonesia), folin-ciocalteu reagent, ascorbic acid, 1,1, diphenyl-2-picrylhydrazyl (DPPH), pulvis gummi arabicum, sodium bicarbonate, aluminum chloride, sodium formate, acetic acid anhydride, potassium bromide, carbopol 940, hydroxypropyl methylcellulose (HPMC), glyserin, propylparaben, methylparaben, 96% ethanol, vanillin, mercury (II) chloride, sulfuric acid, sodium hydroxide, magnesium, potassium dihydrogen phosphate, hydrogen chloride, aquadestilata, oxalic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, zinc, gelatin, potassium iodide, bismuth nitrate, tartaric acid, iodine, 70% methanol (EMSURE® ACS Merck, Darmstadt, Germany). Bacterial cultures were purchased from Remel Inc., a part of Thermo Fisher Scientific Inc.: *Staphylococcus aureus* ATCC BAA-976 PK/5, *Streptococcus pyogenes* ATCC 19615 PK/5, and *Pseudomonas aeruginosa* ATCC 15442. The instruments used in the present study were a UV-Vis spectrophotometer (Shimadzu UV 1601), Shimadzu IRPrestige-21 FT-IR spectrophotometer (Shimadzu Corporation), rotary evaporator (Eyela OSB-2100), magnetic heater stirrer (Bioevopeak Co., Ltd), pH meter (NeoMet, Istek Inc.), and viscometer (Lamy Rheology).

Sample collection, determination, and extraction

Fresh leaves of *S. spontaneum* were collected from Tegalwaru Sub-district (Google coordinates: 6°16'15" S and 107°34'27" E) in Karawang Regency, West Java, Indonesia, in February 2024. The leaves were identified at the Jatinangoriense Herbarium, Biosystematics and Molecular Laboratory, Universitas Padjadjaran, Indonesia. The sample (2.0 kg) of powdered *S. spontaneum* leaves was macerated in 96% ethanol (20 L) for three consecutive days at the Pharmacognosy Laboratory at Universitas Buana Perjuangan Karawang. The macerate was filtered in Whatman No. 1 filter paper and concentrated using a rotary evaporator set at 50°C.[23]

Phytochemical screening

Simple phytochemical screening was conducted to identify the presence of secondary plant metabolites, including polyphenols, flavonoids, quinones, saponins, monoterpenoids, tannins, alkaloids, sesquiterpenes, triterpenoids, and steroids contained in the plant extract using a standard procedure.[24]

Determination of total phenolic content (TPC)

A total of 10 mL (70%) of methanol was used to extract 100 mg of powdered *S. spontaneum* leaves for 15 minutes. 2.5 mL of Folin-Ciocalteu reagent with 0.4 mL of methanol was mixed with 0.1 mL of extract, and the mixture was then allowed to sit at 25°C for 3-5 minutes. The mixture was then mixed with 0.8 mL of $NaHCO_3$ (75 g/L) and left at the same temperature for one hour. The absorbance was measured using a UV-Vis spectrophotometer set to 765 nm, and the results were reported in GAE.[25]

Determination of total flavonoid content (TFC)

A total of 100 mg of powdered *S. spontaneum* leaves was extracted using 10 mL of 70% methanol for 15 minutes. A 0.1 mL sample of the extract was then liquefied in 2.4 mL of methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M NaCOOH, and 2.3 mL of water. The mixture was maintained at 25 °C for 30 minutes. The absorbance was measured at a 432 nm wavelength, and the test results were reported as QE.[26]

Fourier-transform infrared spectroscopic (FT-IR) analysis

S. spontaneum (2 mg) extract was thoroughly combined with 100 mg of dry potassium bromide (KBr) powder. The combination was then compacted in a mould using a hydraulic pump to form pellets or thin sheets. The results were evaluated using a Shimadzu IRPrestige-21 FTIR spectrophotometer at a wavelength in the mid-infrared region (500–4500 cm^{-1}) along with the 4 cm^{-1} resolution.[27]

Hydrogel preparation

The gel was prepared by mixing hydroxypropyl methyl cellulose (HPMC) in distilled water at 70°C and stirring with a magnetic stirrer at 300 rpm until homogeneous. Then, carbopol 940 was added and solubilised in distilled water, and stirred until homogeneous. The *S. spontaneum* extract was made into several concentrations by solubilising the substance in distilled water. At room temperature, the extract was mixed with the gel phase and agitated until the mixture was homogeneous (mass I). Glycerin was combined with methylparaben and propylparaben until the mixture was homogenous (mass II). Lastly, I and II were mixed and blended slowly until uniformity was obtained.[28] The composition of the hydrogel formulation utilised in this investigation is shown in Table 1.

Evaluation of S. spontaneum extract hydrogel (HESs) preparations

Physical examination

The evaluation commenced with a physical examination, which included visual observations of colour, homogeneity, and consistency.[28]

Measurement of pH

The pH of the different hydrogel formulations was determined using a pH meter (NeoMet, istek inc, Seoul). The formulation for pH determination was placed in a container, the electrode was then inserted, and the results were recorded. As a standard for topical preparations, the pH value should approximate the pH of the skin, which ranges from 4.5 to 6.5. This is because a topical preparation with an overly acidic pH value can lead to skin irritation. The topical preparation can also cause scaly skin when it is too alkaline.[29]

Spreading coefficient

Additionally, to assess the hydrogel formulation's spreadability and softness for smooth application on the skin, a spreading coefficient experiment was carried out. One gram of each HESs formulation was placed on a sheet of transparent glass covered with graph paper. To determine the area that each HESs formulation supplied, a glass plate was set over the transparent glass and loaded with 5–30 g of the gel for 60 seconds. A good topical preparation requires a spread diameter between 5–7 g.cm/sec.[30]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: HESs formulation.

Ingredient	Concentration (%w/w)				
	NC	PC	F1	F2	F3
S. spontaneum extract	-	Octenidine dihydrochloride gel	5.00	10.00	15.00
Propylparaben	0.02		0.02	0.02	0.02
Methylparaben	0.18		0.18	0.18	0.18
HPMC	0.25		0.25	0.25	0.25
Carbopol 940	0.25		0.25	0.25	0.25
Glyserin	5.00		5.00	5.00	5.00
Aquadestilata	ad 100		ad 100	ad 100	ad 100

NC: negative control; PC: positive control; F1: formula 1; F2: formula 2; F3: formula 3.

Rheological (viscosity) studies
A cone and a plate viscometer were used to measure the viscosity of each HESs formulation, and spindle 7 was used to measure the duration of HESs formulation in contact with the skin. The assembly was associated with a thermostatically regulated water bath at a temperature of 25°C. Furthermore, viscosity was tested by placing all formulations in a glass covered with a thermostatic jacket. The spindle was free to move around inside the HESs for 10 minutes at 100 rpm.[31] Good viscosity in a hydrogel preparation ranges from 2000 to 4000 cPs, and this value gives the hydrogel a longer contact time with the skin.[32]

Stability test
Hydrogel samples of *S. spontaneum* extract were stored at cold (4±2°C), room (27±2°C), and hot temperatures (40±2°C) for ninety days, and the physical appearance, pH, spreadability, and viscosity tests on all formulations and all temperatures were observed. This is an accelerated test aimed at obtaining the optimum HESs formula for proper storage due to changes that usually occur in normal conditions [33]

Antioxidant activity
DPPH assay
Different test concentrations of *S. spontaneum* extract (25, 50, 75, 100, and 125 µg/mL) were solubilised in 25 mL of methanol and subjected to DPPH antioxidant screening. 2.5 mg of DPPH was solubilised in 50 mL of methanol to create a DPPH stock solution with a concentration of 50 µg/mL. Additionally, 2 mL of each *S. spontaneum* extract solution was mixed with 2 mL of the DPPH stock solution, allowed to mix thoroughly, and then incubated at 30°C for half an hour. The blank solution was prepared by combining 2 mL of DPPH with 2 mL of methanol, which was then measured at the same wavelength as the extract. Ascorbic acid in concentrations of 2, 4, 6, 8, and 10 µg/mL was used as a standard drug in this experiment. A UV-Vis spectrophotometer was used to measure the antioxidant activity in 4 replicates at 515.50 nm wavelength.[34] Antioxidant activity was expressed in % inhibition with the equation:

$$\text{Inhibition } (\%) = \frac{\text{Cb}-\text{Cs}}{\text{Cb}}\ x\ 100 \qquad (1)$$

Where Cb denotes blank absorption, while Cs indicates sample absorption.

Ferric reducing antioxidant power (FRAP) assay
S. spontaneum extract (100 mg) was dissolved in 100 mL of 96% ethanol and diluted to obtain various test concentrations, including 25, 50, 75, 100, and 125 µg/mL. This was followed by adding 1 mL of each *S. spontaneum* extract solution to 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$, and stirring until a homogeneous mixture was formed. The mixture was incubated at 50°C for 20 minutes. 1 mL of trichloroacetic acid was added and then centrifuged at 3000 rpm for 10 minutes. Furthermore, 1 mL of supernatant (top layer) was taken into a test tube and mixed with 1 mL of water and 0.5 mL of 0.1% $FeCl_3$. The solution was left for 10 minutes, and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 720 nm in 4 replicates. A 1% oxalate solution mixture was used as a blank.[35] The same steps were carried out on standard ascorbic acid solutions with concentrations of 2, 4, 6, 8, and 10 µg/mL. Antioxidant activity was represented as a percentage of reducing power using the following formula:

$$\text{Reducing power } (\%) = \frac{\text{Cb}-\text{Cs}}{\text{Cb}}\ x\ 100 \qquad (2)$$

Where Cb represents blank absorption, and Cs represents sample absorption.

Antibacterial activity
This study utilises the disc diffusion technique to determine antibacterial activity. A total of 300 µL (1.0×10^8 cells) of bacterial suspension (*Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*) was pipetted from a sterile petri dish and then poured into 15 mL of MHA media until homogeneous and solid. Subsequently, discs containing 15 µL of HESs with concentrations of 6.25, 12.5, 25, 50, and 100% were placed on the media. This experiment used the hydrogel base and chloramphenicol as a negative and positive control, respectively. The clear zone was measured using a calliper after the plate was incubated for 18–24 hours at 37°C. The MIC was simultaneously determined using the serial dilution approach. After the MIC determination of the HESs, aliquots of 50 µL from all the tubes that showed no visible bacterial growth were seeded on MHA plates and incubated for 24 hours at 37°C. The MBC endpoint is the lowest concentration of an antimicrobial agent at which 99.99% of the bacterial population is killed. This was done by observing pre and post-incubated agar plates for the presence or absence of bacteria.[36]

Wound healing screening
Experimental animals
This experiment used 20 healthy male Wistar rats weighing 150–250 g and 8–12 weeks old. The rats were obtained from Animal House, CV. Mitra Putra Animal, Indonesia, and kept in plastic cages with softwood shavings at the Pharmacology and Toxicology Laboratory, Universitas Buana Perjuangan Karawang. A 12-hour light and 12-hour dark cycle was ensured, and free access to standard pellets and water *ad libitum* was provided.

Randomisation procedure and blinding
Before randomisation, each rat was assigned an identification number. The randomisation was performed using online software (https://www.graphpad.com/quickcalcs/randomize1/) that generated random numbers and assigned the rats to study groups. Meanwhile, during the experiment, alphanumeric codes were utilised to label vials and syringes, and each rat was assigned a number to maintain blinding. Subsequently, each sample code was enclosed in a sealed envelope and disclosed after the experiment.

Incision wound model
Before the wound was made, all rats were given a dose of 120 mg/kg of ketamine HCl intramuscularly to induce anaesthesia.[37] The intended region for the incision was marked, and the rats' back fur was shaved. Using a punch biopsy and a scalpel, a 30 mm incision was created on the back, 50 mm from the ear, cutting through the muscle and running parallel to the vertebral column.[38]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Wound healing activity of HESs

In this experiment, the rats were divided into 5 groups, consisting of four rats each. Group 1 was given a hydrogel base as the negative control, while group 2 received a gel containing octenidine dihydrochloride at a concentration of 0.05% (%w/w). Meanwhile, groups 3, 4, and 5 were treated with HESs at 5% (%w/w), 10% (%w/w), as well as 15% (%w/w) concentrations, respectively, for 15 days following the incision. On days 0, 2, 4, 8, and 16, the diameter of the wound was measured and recorded for every treatment group. A calliper was used to measure the average wound diameter in vertical, horizontal, and diagonal directions to determine the closure percentage.[39] Wound healing activity was expressed as a closure percentage using the following formula:

$$\text{Wound closure area (\%)} = \frac{\text{Wound area on 0 day} - \text{Wound area on } n \text{ day}}{\text{Wound area on 0 day}} \; x \; 100 \qquad (3)$$

Where *n* is the number of days (2nd, 4th, 8th, and 16th).

Ethical approvals

The Research Ethics Committee of Universitas Padjadjaran in Bandung, Indonesia, approved the research protocol under the number 553/UN6.KEP/EC/2024, in compliance with ARRIVE guidelines.

Statistical analysis

The experiment's data were presented as mean ± SEM, with $p < 0.05$ considered significantly different. The Tukey HSD post-hoc test, as well as one-way ANOVA, were performed for statistical analysis, and GraphPad Prism version 9 (Dotmatics) was used to obtain the IC_{50} values.

Results and Discussion

The phytochemical screening revealed that several secondary metabolites, including flavonoids, quinones, saponins, alkaloids, polyphenols, monoterpenoids, and sesquiterpenoids, were present in the *S. spontaneum* extract, as shown in Table 2. Furthermore, Table 3 shows that the TPC and TFC of *S. spontaneum* extract are 2.66 ± 0.22 mg GAE/gram and 4.54 ± 0.31 mg QE/gram, respectively. TPC and TFC are the standard tests for phytochemical studies to determine antioxidant activity in a plant extract. This is because phenolic compounds have been reported to scavenge ROS without causing other oxidative reactions, and 5,7,3',4'-hydroxy substituted flavonoid compounds have good free radical scavenging abilities.[40,41]

Table 2: The *S. spontaneum* extract's phytochemical constituents.

Phytochemical compounds	Results
Polyphenols	+
Flavonoids	+
Tannins	-
Quinones	+
Saponins	+
Alkaloids	+
Monoterpenoids and sesquiterpenoids	+
Triterpenoids and steroids	-

(+): contained; (-): not contained.

Table 3: *S. spontaneum* extract's TPC and TFC contents.

Samples	TPC (mg GAE/g)	TFC (mg QE/g)
S. spontaneum extract	2.66 ± 0.22	4.54 ± 0.31

Values are shown as mean ± SEM from four replicates in each group. TPC is denoted as mg of GAE per gram of sample, while TFC is shown as mg of QE per gram of sample.

Similarly, the FT-IR analysis result of *S. spontaneum* extract showed several different functional groups confirmed by the presence of 85 peaks in the chromatogram. Clear peaks were observed at 561.31-881.51 cm^{-1}, representing the stretching mode of alkyl halides (C–I stretch 587.35-561.31 cm^{-1}, C–Br stretch 683.79-607.60 cm^{-1}, C–Cl stretch 881.51-709.83 cm^{-1}), and peaks at 944.20-910.44 cm^{-1} showing the bending mode of alkenes (C=C). There was also a peak at 1026.17 cm^{-1} with strong intensity, representing the stretching mode of alkyl aryl ether (C–O). Furthermore, peaks were observed at 1716.72-1087.90 cm^{-1}, showing the bending mode of imine (C=N stretching), aldehyde (C–H stretching), as well as amide (C–N stretching). Several other functional groups were found in the peak range of 3748.81-2832.59 cm^{-1}, including alcohol (O-H stretching), carboxylic acid (C=O stretching), amine (N–H stretching), and alkane (C–H stretching). The FTIR analysis results of *S. spontaneum* extract are shown in Figure 1. In the HESs evaluation experiment, visual inspection was carried out for the formulations' colour, homogeneity, and consistency. The findings showed that all HESs formulations were brown and mixed homogeneously and had excellent viscosity, as shown in Figure 2. The pH value measurements also revealed that all HESs formulations, F0, F1, F2, and F3, had an average pH value of 4.98, 4.86, 4.80, and 4.62. Each formula, F0, F1, F2, and F3, had an average spreadability value of 6.60, 5.93, 5.60, and 5.56 g.cm/sec. Meanwhile, for all measurement results of formulations' viscosity, each formula F0, F1, F2, and F3 has an average viscosity value of 6227, 5414, 4629, and 3901 cPs. The study's findings indicate that all HESs formulations have good physical examination, pH value, spreadability, and viscosity. The HESs evaluation results are shown in Table 4. The stability test results showed that all formulations of HESs maintained consistent colour, homogeneity, and viscosity at different test temperatures over a period of ninety days. Additionally, each formulation exhibited pH values, spreadability, and viscosity that satisfy the standards for good hydrogel preparations. The antioxidant activity of the *S. spontaneum* extract was assessed using the DPPH and FRAP methods. The extract's stability as a free radical and its ability to evaluate free radical scavenging activity *in vitro* in biological systems served as the foundation for the DPPH method.[42] In this research, the antioxidant activity was assessed by evaluating the capability of *S. spontaneum* extract to neutralise the DPPH free radical cation, which has a peak absorption at 515.50 nm wavelength as measured by a UV-Vis spectrophotometer.[43] Based on the results, the *S. spontaneum* extract can scavenge DPPH radicals in a concentration-dependent manner with an IC_{50} value of 80.16 µg/mL. Additionally, the overall strength of the extract was directly tested using the FRAP method. This method relied on the extract's ability to decrease Fe^{3+} ions to Fe^{2+} ions, measured at 720 nm wavelength.[44] The results showed that *S. spontaneum* extract could inhibit free radicals in a concentration-dependent manner, with an IC_{50} value of 82.70 µg/mL. Meanwhile, it appears that the antioxidant activity of ascorbic acid was still higher than that of *S. spontaneum* extract, where the IC_{50} value for each test method was 6.26 µg/mL (DPPH method) and 8.40 µg/mL (FRAP method). Table 5 shows the extract antioxidant activity using the DPPH and FRAP methods.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

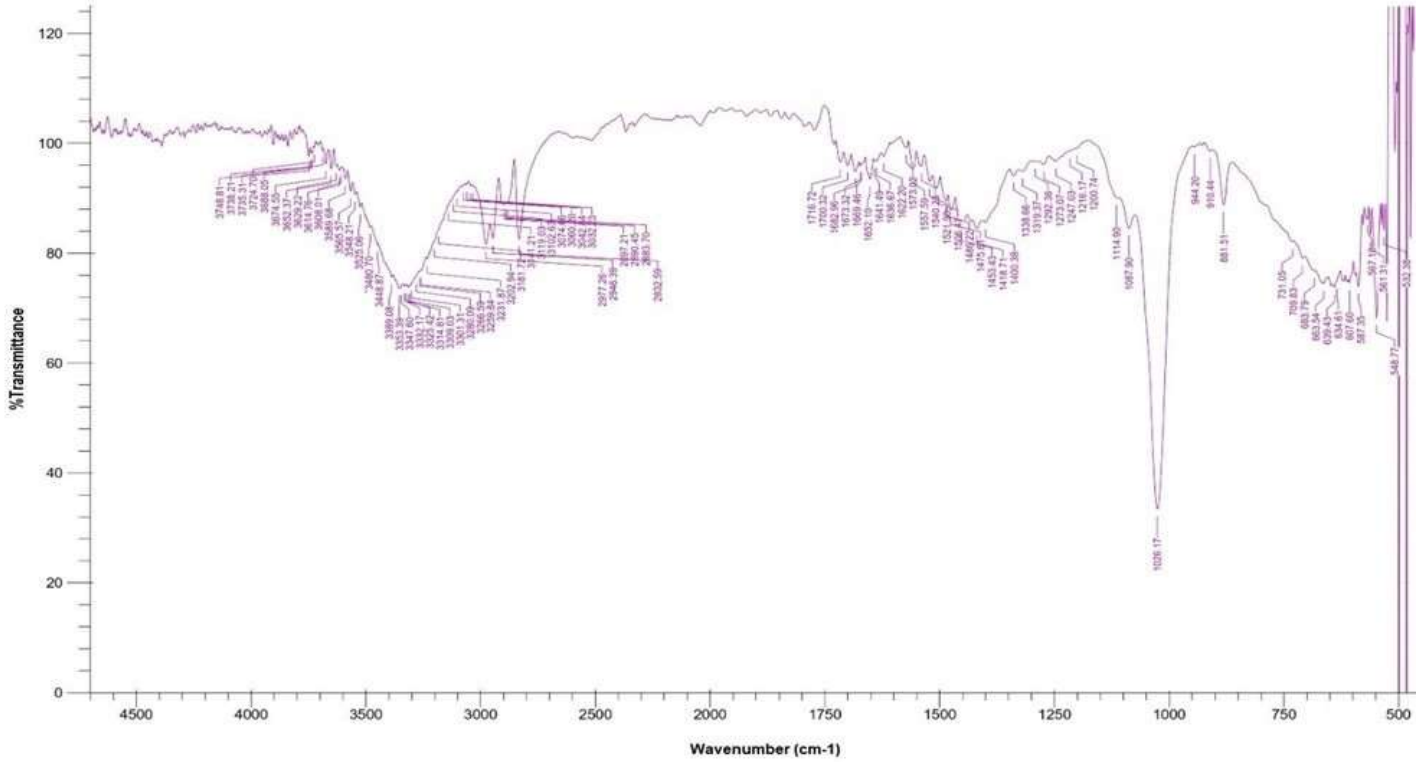

Figure 1: Fourier transform infrared spectroscopy analysis of *Saccharum spontaneum* extract.

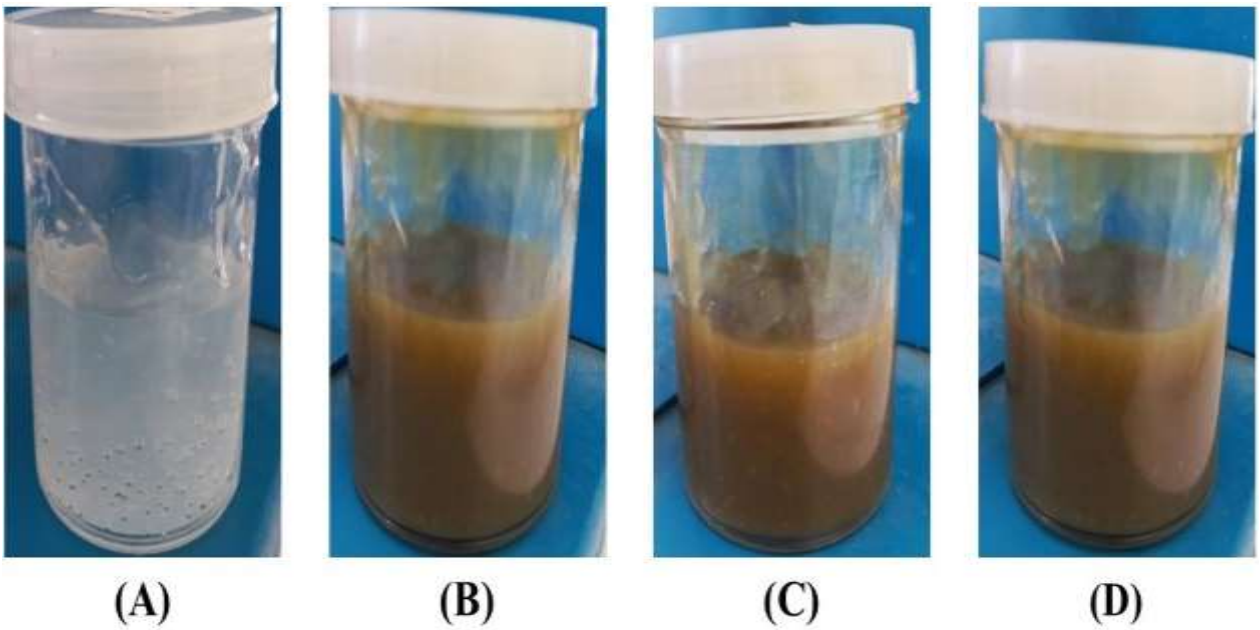

Figure 2: Hydrogel formulations of *S. spontaneum* extract. A: Formula 0; B: Formula 1; C: Formula 2; D: Formula 3.

Table 4: HESs' formulation evaluation physical chemical parameters.

Physical parameters Formulations	Color	Homogeneity	Consistency
F0	Clear	Homogeneous	Excellent
F1	Brown	Homogeneous	Excellent

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

F2	Brown	Homogeneous	Excellent
F3	Brown	Homogeneous	Excellent
pH value			
F0	4.98 ± 0.09		
F1	4.86 ± 0.10		
F2	4.80 ± 0.12		
F3	4.62 ± 0.18		
Spreading coefficient (g.cm/sec)			
F0	6.60 ± 0.10		
F1	5.93 ± 0.11		
F2	5.60 ± 0.10		
F3	5.56 ± 0.23		
Viscosity (cPs)			
F0	6227 ± 8.51		
F1	5414 ± 11.45		
F2	4629 ± 14.61		
F3	3901 ± 16.39		

The data are shown as the mean ± SEM of four replicates in each group

Table 5: Antioxidant activity of *S. spontaneum* extract and ascorbic acid using DPPH and FRAP methods.

Samples	DPPH method			FRAP method		
	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
	25.00	22.70 ± 1.63		25.00	31.45 ± 1.29	
	50.00	32.78 ± 1.91		50.00	39.17 ± 1.47	
S. spontaneum extract	75.00	42.82 ± 1.47	80.16 ± 1.75	75.00	45.25 ± 1.82	82.70 ± 1.86
	100.00	54.70 ± 1.35		100.00	59.80 ± 1.68	
	125.00	73.06 ± 1.82		125.00	72.78 ± 1.87	
	2.00	22.96 ± 1.15		2.00	7.05 ± 1.58	
	4.00	37.07 ± 1.68		4.00	13.55 ± 1.82	
Ascorbic acid	6.00	46.36 ± 1.96	6.26 ± 1.52	6.00	22.45 ± 2.08	8.40 ± 1.45
	8.00	57.67 ± 1.29		8.00	28.50 ± 1.73	
	10.00	64.11 ± 2.04		10.00	55.86 ± 1.78	

Data are shown as mean ± SEM from four replicates in each group. IC_{50} shows the half-maximal inhibitory concentration.

The results of this study showed that HESs exerted an inhibitory effect on the growth of *S. aureus*, *S. pyogenes*, and *P. aeruginosa* in a concentration-dependent manner. A higher concentration of HESs' leads to a larger inhibition zone for the development of *S. aureus*, *S. pyogenes*, and *P. aeruginosa*. These results also showed that the MIC and MBC value of HESs on the growth of *S. aureus*, *S. pyogenes*, and *P. aeruginosa* was 6.25%, 12.50%, 12.50%, 25.00%, 25.00%, and 50.00%, respectively. Meanwhile, chloramphenicol provided antibacterial activity based on the MIC and MBC against all three species of bacteria (*S. aureus*: MIC = 1.75×10^{-3}%; MBC = 1.25×10^{-2}%, *S. pyogenes*: MIC = 15×10^{-3}%; MBC = 3×10^{-2}%; and *P. aeruginosa*: MIC = 6.2×10^{-3}%; MBC = 5×10^{-2}%). Table 6 shows the inhibition zone and MIC and MBC of HESs and chloramphenicol on *S. aureus*, *S. pyogenes*, and *P. aeruginosa*.

In this study, the administration of topical HESs significantly affected the healing process of the incision wound in rats. Wound closure assessments were conducted on days 0, 2, 4, 8, and 16. According to the results, wounds in all treatment groups were nearly fully healed by day 16. The group with HESs 15% (%w/w) exhibited the most significant healing effect compared to the others. This shows that a greater concentration of HESs administered led to improved wound closure in the rats. Figure 3 illustrates the wound healing activity of HESs in the rat incision wound model. In comparison to negative controls, the results showed that HESs considerably aided wound contraction on days 4, 8, and 16. On days 4 ($p < 0.05$; $p < 0.01$), 8 ($p < 0.01$; $p < 0.001$), and 16 ($p < 0.01$; $p < 0.01$), the 10% and 15% (%w/w) HESs groups displayed significant wound closure. The group that received 5% (%w/w) HESs showed a higher wound area closure than the negative control, but there was no discernible difference in healing activity ($p > 0.05$). On days 4, 8, and 16, the group that received 0.05% octenidine gel demonstrated a considerable closure of the wound area ($p < 0.01$, $p < 0.001$, and $p < 0.01$, respectively). The study found that HESs administration promoted wound healing in a manner that depended on both time and concentration in the rat incision wound model. Figure 4 shows the HESs' effect on the percentage of wound closure area. Wound healing is the result of intricate interactions between dermal and epidermal cells, ECM, angiogenesis regulation, and proteins produced from plasma. Several cytokines and growth factors significantly influence this interaction. Hemostasis, inflammation, proliferation, and remodelling are the four overlapping, ongoing phases that make up the dynamic process.[45,46] The first phase begins with hemostasis immediately following an injury, followed by the formation of a fibrin

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

clot and vascular constriction. Platelet-derived alpha granules then release growth factors, like TGF-*β*, EGF, PDGF, and IGF, which activate and draw neutrophils, macrophages, endothelial cells, and fibroblasts to aid in the healing of wounds. The inflammatory phase features infiltration by neutrophils and monocytes, along with differentiation related to macrophage and lymphocyte presence. This phase marks the beginning of the granulation tissue creation as macrophages produce growth factors and pro-inflammatory cytokines variation, including IL-1 and IL-6. In the proliferation phase, fibroblast migration occurs with the newly synthesised ECM, angiogenesis deposition, and the formation of granulation tissue. In the final remodelling phase, type III collagen fibres are largely substituted by type I collagen, which can better endure substantial pressure. In this phase, there is also an increase in cross-linking between collagen fibre monomers that are consistent with the skin pressure line.[47–49]

Table 6: The inhibition zone along with MIC and MBC of HESs and chloramphenicol in *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*.

Samples	Concentration (%w/w)	*Staphylococcus aureus*			*Streptococcus pyogenes*			*Pseudomonas aeruginosa*		
		Zone of inhibition (mm)*	MIC (%w/w)	MBC (%w/w)	Zone of inhibition (mm)*	MIC (%w/w)	MBC (%w/w)	Zone of inhibition (mm)*	MIC (%w/w)	MBC (%w/w)
HESs	6.25	9.83 ± 3.40			6.98 ± 1.86			5.35 ± 2.15		
	12.50	14.60 ± 1.25			14.85 ± 1.65	12.50	25.00	6.18 ± 1.55		
	25.00	19.50 ± 1.32	6.25	12.50	22.68 ± 2.11			15.57 ± 2.84	25.00	50.00
	50.00	20.10 ± 1.75			26.45 ± 1.52			18.77 ± 1.65		
	100.00	28.00 ± 1.80			29.73 ± 1.28			21.85 ± 1.12		
Chloramphenicol	30.00	22.50 ± 2.17	1.75×10^{-3}	1.25×10^{-2}	24.10 ± 2.85	15×10^{-3}	3×10^{-2}	10.05 ± 0.01	6.2×10^{-3}	5×10^{-2}
Hydrogel base	-	UD	UD	UD	UD	UD	UD	UD	UD	UD

*Data are shown as mean ± SEM of four replicates in each group. HESs: hydrogel extract of *S. spontaneum*; UD: undetected.

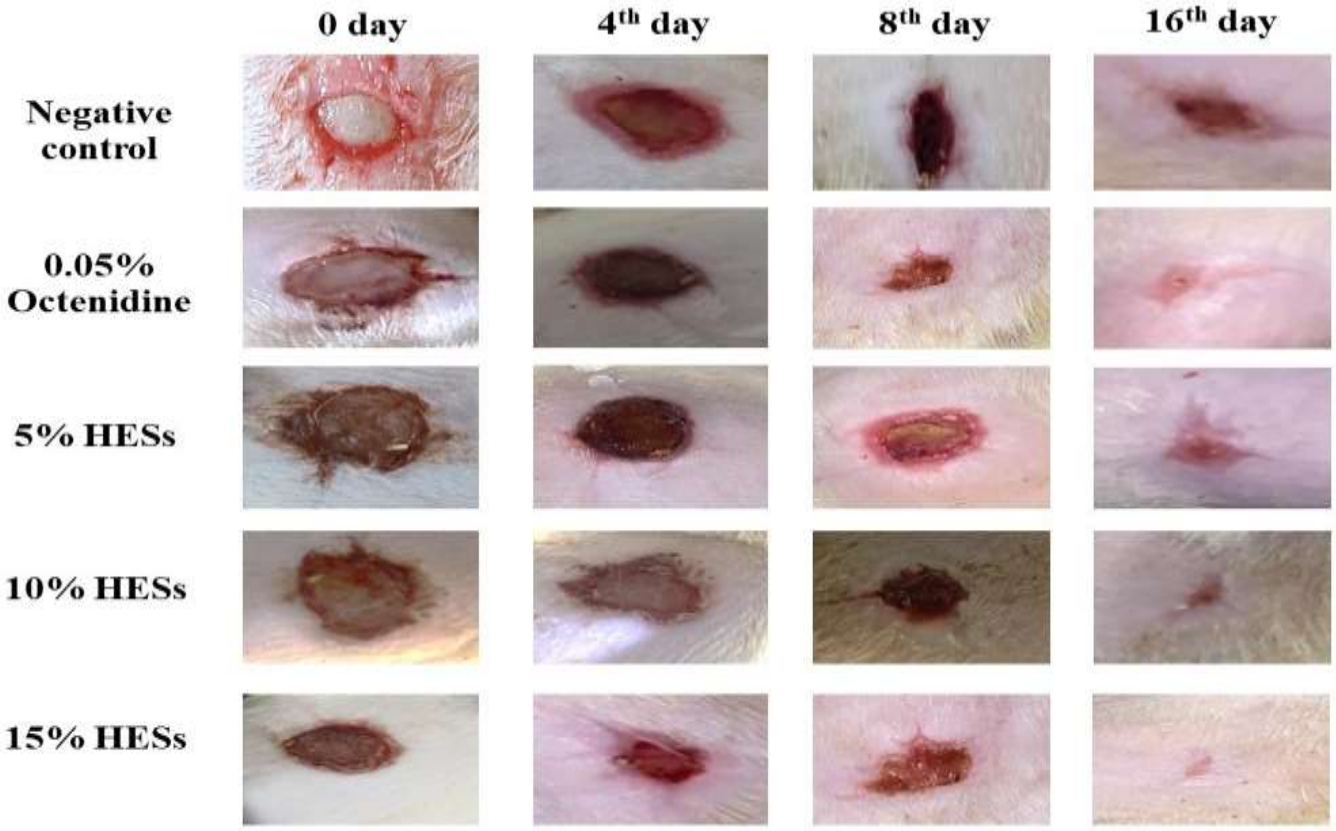

Figure 3: Wound healing effect of HESs on rats. 5% HESs: hydrogel extract of *S. spontaneum* concentration 5% (%w/w); 10% HESs: hydrogel extract of *S. spontaneum* concentration 10% (%w/w); 15% HESs: hydrogel extract of *S. spontaneum* concentration 15% (%w/w).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

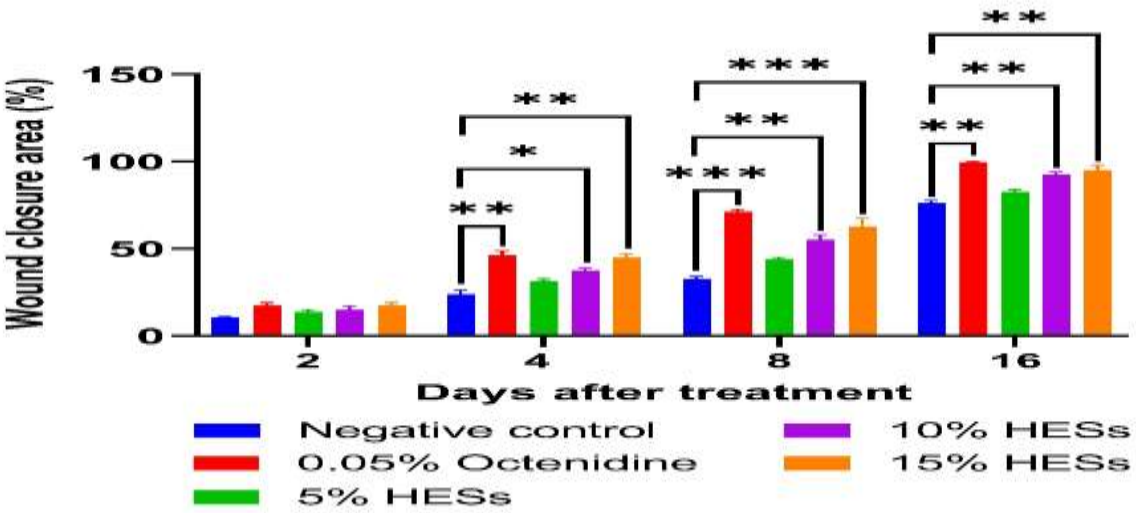

Figure 4: The effects of HESs on the wound closure area percentage in a rat incision wound model. Data are shown as mean ± SEM for four animals in each group. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus negative control. 5% HESs: hydrogel extract of *S. spontaneum* concentration 5% (%w/w); 10% HESs: hydrogel extract of *S. spontaneum* concentration 10% (%w/w); 15% HESs: hydrogel extract of *S. spontaneum* concentration 15% (%w/w).

In general, wound healing aims to restore the structure and function of tissue damaged by injury, enabling a return to normalcy. Therefore, effective wound management is needed to accelerate the repair of injured tissue and prevent complications.[50] Previous studies have conducted various investigations to determine the effective wound healing medications,[51] one of which is to explore medicinal plants that have the potential to treat wounds.[52] The use of these plants in traditional medicine offers numerous benefits, such as reduced side effects, affordability, easy access, and widespread cultural acceptance.[53] Reports show that medicinal plants hold significant promise as wound healing agents due to their capacity to speed up the healing process, alleviate pain and discomfort, minimise scarring, and prevent infections in wounds.[54] In this study, the wound healing activity of HESs was tested using an incision wound model in rats. The results showed that topical administration of HESs to rats with incision wounds enhanced the reduction rate in wound area, facilitated a superior healing process, and provided adequate wound edge hydration through tissue regeneration. This is inseparable from the ability of secondary metabolites contained in *S. spontaneum* extract to improve the wound healing process. According to the phytochemical screening result, *S. spontaneum* extract contains various secondary metabolites, like flavonoids, quinones, saponins, polyphenols, alkaloids, monoterpenoids, and sesquiterpenoids, as shown in Table 2. This can affect one or more phases and increase wound healing. Flavonoids are known to reduce inflammatory mediator levels, such as IFN-γ, PGE2, IL-6, IL-1β, TNF-α, and LTB-4, decrease NF-κB expression, inhibit COX activity, and increase IL-10 levels. In addition, this compound affects cell proliferation, migration, and differentiation, as well as angiogenesis, by increasing VEGF (the main molecule that regulates vascular growth) and the expression of MMP 2, 8, 9, and 13.[55–57] The compound also reduces lipid peroxidation by preventing or slowing down the onset of cell necrosis.[4,58] Meanwhile, quinone compounds were reported to increase wound closure rate by forming new blood vessels (angiogenesis), cell migration and proliferation, collagen synthesis, re-epithelialisation, and TGF-β1 regulation.[59] Saponin compounds were known to stimulate fibronectin synthesis through changes in TGF-β receptor expression in fibroblasts,[60] increasing VEGF and IL-1β production,[61] and reducing lesion size and suppressing scar tissue formation.[62] Alkaloids are another component of *S. spontaneum* extract that contributes to wound healing. These compounds promote wound healing and tissue granulation by enhancing fibroblast and collagen production while decreasing inflammatory cell counts.[63] Additionally, alkaloids influence the SRC/MEK/ERK signalling pathway, which plays a crucial role in the wound healing process.[64] Moreover, monoterpenoids and sesquiterpenoid compounds contained in *S. spontaneum* extract contribute to the wound healing process by decreasing the IL-6 and TNF-α production, stimulating fibroblast migration, which can help re-epithelialise the wound.[65,66]

The creation of free radicals, along with pathogens like *S. aureus*, *S. pyogenes*, and *P. aeruginosa* that frequently infect wounds, is known to hinder the healing process synergistically.[67,68] Consequently, wound healing agents that also have antioxidant and antibacterial activity are needed to accelerate the process.[69,70] Based on the results, *S. spontaneum* extract has antioxidant activity in the DPPH and FRAP models, as well as good antibacterial activity against *S. aureus*, *S. pyogenes*, and *P. aeruginosa*. This is also inseparable from the content of phytochemical compounds in *S. spontaneum* extract, such as polyphenols, flavonoids, and saponins, which have antioxidant activity through ROS scavenging, thereby preventing cell and tissue damage.[71,72] Additionally, these compounds also have antibacterial effects through inhibition of nucleic acid synthesis, energy metabolism, porins in cell membranes, cytoplasmic membrane function, attachment, and biofilm formation, as well as changes in bacterial cell membrane permeability.[73,74] According to the results of previous studies, quinone compounds, alkaloids, monoterpenoids, and sesquiterpenoids act as antioxidants by scavenging ROS and neutralising electrophiles, such as SOD, GPx, catalase, glutathione reductase, NQO1 and HO-1.[75–77] These compounds also have antibacterial effects by inhibiting the synthesis of bacterial nucleic acids and proteins, modifying the permeability of bacterial cell membranes, damaging cell membranes and cell walls, and inhibiting bacterial metabolism.[78–80] Therefore, the antioxidant and antibacterial effects of *S. spontaneum* extract contribute to increasing wound healing both independently and synergistically. Based on the evaluation results, all HESs formulations met the requirements for good hydrogel and are stable at various temperatures in stability testing for ninety days.

Conclusion

In conclusion, HESs formulations enhanced wound healing activity in a rat model with incision wounds, which was concentration and time-dependent. *S. spontaneum* extract also had good antioxidant and antibacterial effects. All HESs formulations also met the requirements of a good and stable hydrogel at various temperatures in ninety days stability testing. Therefore, extracts from *S. spontaneum* show promise for development as wound healing medications from medicinal plants, potentially formulated as hydrogel preparations. Future studies may examine cellular histopathology, LC-MS or HPLC characterisation of phytoconstituents of the plant.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

This research was supported by the Institute for Research and Community Services, Universitas Buana Perjuangan Karawang (grant no. 191/LPPM/III/2024).

References

1. Kalva SN, Augustine R, Al Mamun A, Dalvi YB, Vijay N, Hasan A. Active agents loaded extracellular matrix mimetic electrospun membranes for wound healing applications. J Drug Deliv Sci Technol. 2021; 63:1–16. doi: https://doi.org/10.1016/j.jddst.2021.102500
2. Muharni M, Annisa A, Fitrya F, Anas M. Wound healing activity of *Dillenia ochreata* leaves ethanol extract in Wistar rats. J Pharm Pharmacogn Res. 2022; 10(5):896–904. doi: https://doi.org/10.56499/jppres22.1444_10.5.896
3. Tessema Z, Molla Y. Evaluation of the wound healing activity of the crude extract of root bark of *Brucea antidysentrica*, the leaves of *Dodonaea angustifolia* and *Rhamnus prinoides* in mice. Heliyon. 2021; 7(1):1–8. doi: https://doi.org/10.1016/j.heliyon.2021.e05901
4. Arun M, Satish S, Anima P. Evaluation of wound healing, antioxidant and antimicrobial efficacy of *Jasminum auriculatum* Vahl. Avicenna J Phytomed. 2016; 6(3):295–304. doi: https://doi.org/10.22038/ajp.2016.5723
5. Yiblet TG, Tsegaw A, Ahmed N, Dagnew SB, Tadesse TY, Kifle ZD. Evaluation of wound healing activity of 80% methanol root crude extract and solvent fractions of *Stephania abyssinica* (Dill. & A. Rich.) Walp. (Menispermaceae) in mice. J Exp Pharmacol. 2022; 14:255–273. doi: https://doi.org/10.2147/JEP.S364282
6. Yates CC, Whaley D, Babu R, Zhang J, Krishna P, Beckman E, Pasculle AW, Wells A. The effect of multifunctional polymer-based gels on wound healing in full-thickness bacteria-contaminated mouse skin wound models. Biomaterials. 2007; 28(27):3977–3986. doi: https://doi.org/10.1016/j.biomaterials.2007.05.008
7. Sofrona E, Tziveleka LA, Harizani M, Koroli P, Sfiniadakis I, Roussis V, Rallis M, Ioannou E. In vivo evaluation of the wound healing activity of extracts and bioactive constituents of the marine isopod *Ceratothoa oestroides*. Mar Drugs. 2020; 18(4):1–15. doi: https://doi.org/10.3390/md18040219
8. Kumari MK, Vittalrao AM, Charitha C, Kumar PSE, Prabhath S. Evaluation of wound healing activity of an ethanolic extract of *Anacardium occidentale* leaves in Wistar rats. Biomed Pharmacol J. 2020; 13(4):2061–2068. doi: https://dx.doi.org/10.13005/bpj/2086
9. Uberoi A, McCready-Vangi A, Grice EA. The wound microbiota: microbial mechanisms of impaired wound healing and infection. Nat Rev Microbiol. 2024; 22(8):507–521. doi: https://doi.org/10.1038/s41579-024-01035-z
10. Li C, Li C, Ma Z, Chen H, Ruan H, Deng L, Wang J, Cui W. Regulated macrophage immune microenvironment in 3D printed scaffolds for bone tumour postoperative treatment. Bioact Mater. 2022; 19:474–485. doi: https://doi.org/10.1016/j.bioactmat.2022.04.028
11. Sun X, Gao Y, Li Z, He J, Wu Y. Magnetic responsive hydroxyapatite scaffold modulated macrophage polarisation through PPAR/JAK-STAT signalling and enhanced fatty acid metabolism. Biomaterials. 2023; 295:122051. doi: https://doi.org/10.1016/j.biomaterials.2023.122051
12. Deng A, Yang Y, Du S, Yang X, Pang S, Wang X, Yang S. Preparation of a recombinant collagen-peptide (RHC)-conjugated chitosan thermosensitive hydrogel for wound healing. Mater Sci Eng C. 2021; 119:1–13. doi: https://doi.org/10.1016/j.msec.2020.111555
13. Qu J, Zhao X, Liang Y, Xu Y, Ma PX, Guo B. Degradable conductive injectable hydrogels as novel antibacterial, antioxidant wound dressings for wound healing. Chem Eng J. 2019; 362:548–560. doi: https://doi.org/10.1016/j.cej.2019.01.028
14. Wu H, Li F, Wang S, Lu J, Li J, Du Y, Sun X, Chen X, Gao J, Ling D. Ceria nanocrystals decorated mesoporous silica nanoparticle-based ROS-scavenging tissue adhesive for highly efficient regenerative wound healing. Biomaterials. 2018; 151: 66–77. doi: https://doi.org/10.1016/j.biomaterials.2017.10.018
15. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol. 2005; 23(1):47–55. doi: https://doi.org/10.1038/nbt1055
16. Halim MBA, Eid HH, Deeb KSE, Metwally GF, Masoud MA, Ahmed-Farid OA, Messiry HME. The study of wound healing activity of *Thespesia populnea* L. bark, an approach for accelerating healing through nanoparticles and isolation of main active constituents. BMC Complement Med Ther. 2024; 24(1):1–16. doi: https://doi.org/10.1186/s12906-024-04343-2
17. Thakur R, Jain N, Pathak R, Sandhu SS. Practices in wound healing studies of plants. Evid Based Complement Alternat Med. 2011; 2011:1–17. doi: https://doi.org/10.1155/2011/438056
18. Cedillo-Cortezano M, Martinez-Cuevas LR, López JAM, Barrera López IL, Escutia-Perez S, Petricevich VL. Use of medicinal plants in the process of wound healing: A literature review. Pharmaceuticals. 2024; 17(3):1–27. doi: https://doi.org/10.3390/ph17030303
19. Hassan A, Malik K, Naqvi SAM, Sadia H, Khan K. A comprehensive review of *Saccharum spontaneum*, its traditional uses, phytochemistry and pharmacology. Ethnobot Res Appl. 2024; 29(5):1–13. doi: http://dx.doi.org/10.32859/era.29.5.1-13
20. Okokon JE, Udobang JA, Davies K, Edem UA, Bassey AI. Analgesic activity of ethanol leaf extract of *Saccharum officinarum*. Trop J Nat Prod Res. 2021; 5(6):1142–1145. doi: https://doi.org/10.26538/tjnpr/v5i6.27
21. Zagórska-Dziok M, Sobczak M. Hydrogel-based active substance release systems for cosmetology and dermatology application: A review. Pharmaceutics. 2020; 12(5):1–43. doi: https://doi.org/10.3390/pharmaceutics12050396
22. Alberts A, Moldoveanu ET, Niculescu AG, Grumezescu AM. Hydrogels for wound dressings: Applications in burn treatment and chronic wound care. J Compos Sci. 2025; 9(3):1–37. doi: https://doi.org/10.3390/jcs9030133
23. Alkandahri MY, Maulana YE, Subarnas A, Kwarteng A, Berbudi A. Antimalarial activity of extract and fractions of *Cayratia trifolia* (L.) Domin. Int J Pharm Res. 2020; 12(Suppl 1):1435–1441. doi: https://doi.org/10.31838/ijpr/2020.SP1.218
24. Gunarti NS, Alkandahri MY, Wahyuningsih ES, Agustina P, Mursal ILP, Hidayah H, Nurviana V. Evaluation of antipyretic and antioxidant activities of ten indigenous medicinal plants of Tirtajaya, Karawang Regency, West Java, Indonesia. Indian J Pharm Educ Res. 2025; 59(1):252–263. doi: https://doi.org/10.5530/ijper.20256381
25. Alkandahri MY, Kusumiyati K, Renggana H, Arfania M, Frianto D, Wahyuningsih ES, Maulana YE. Antihyperlipidemic activity of extract and fractions of *Castanopsis costata* leaves on rats fed with high cholesterol

diet. RASĀYAN J Chem. 2022; 15(4):2350–2358. doi: http://doi.org/10.31788/RJC.2022.1547015
26. Sytar O, Hemmerich I, Zivcak M, Rauh C, Brestic M. Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. Saudi J Biol Sci. 2018; 25(4):631–641. doi: https://doi.org/10.1016/j.sjbs.2016.01.036
27. Alkandahri MY, Sadino A, Abriyani E, Hermanto F, Oktoba Z, Sayoeti MFW, Sangging PRA, Wardani D, Hasan N, Sari SW, Safitri NA, Ikhtianingsih W, Safitri S. Evaluation of hepatoprotective and nephroprotective activities of *Castanopsis costata* extract in rats. Biomed Rep. 2025; 22(2):1–11. doi: https://doi.org/10.3892/br.2024.1902
28. Yuniarsih N, Hidayah H, Gunarti NS, Kusumawati AH, Farhamzah F, Sadino A, Alkandahri MY. Evaluation of wound-healing activity of hydrogel extract of *Sansevieria trifasciata* leaves (Asparagaceae). Adv Pharmacol Pharm Sci. 2023; 2023:1–10. doi: https://doi.org/10.1155/2023/7680518
29. Forestryana D, Hayati A, Putri AN. Formulation and evaluation of natural gel containing ethanolic extract of *Pandanus amaryllifolius* R. using various gelling agents. Borneo J Pharm. 2022; 5(4):345–356. doi: https://doi.org/10.33084/bjop.v5i4.1411
30. Nurman S, Yulia R, Irmayanti, Noor E, Sunarti TC. The optimisation of gel preparations using the active compounds of arabica coffee ground nanoparticles. Sci Pharm. 2019; 87(4):1–16. doi: https://doi.org/10.3390/scipharm87040032
31. Bonacucina G, Cespi M, Palmieri GF. Characterisation and stability of emulsion gels based on acrylamide/sodium acryloyldimethyl taurate copolymer. AAPS PharmSciTech. 2009; 10(2):368–375. doi: https://doi.org/10.1208/s12249-009-9218-1
32. Edityaningrum CA, Kintoko, Zulien F, Widiyastuti L. Optimisation of water fraction gel formula of binahong leaf (*Anredera cordifolia* (Ten.) Steen) with gelling agent of sodium alginate and carboxymethyl chitosan combination. Trad Med J. 2018; 23(3):97–105. doi: https://doi.org/10.22146/mot.36604
33. Farhamzah, Kusumawati AH, Alkandahri MY, Hidayah H, Sujana D, Gunarti NS, Yuniarsih N, Apriana SD, Agustina LS. Sun protection factor activity of black glutinous rice emulgel extract (*Oryza sativa* var glutinosa). Indian J Pharm Educ Res. 2022; 56(1):302–310. doi: https://doi.org/10.5530/ijper.56.1.36
34. Kusumawati AH, Farhamzah F, Alkandahri MY, Sadino A, Agustina LS, Apriana SD. Antioxidant activity and sun protection factor of Black Glutinous Rice (*Oryza sativa* var. glutinosa). Trop J Nat Prod Res. 2021; 5(11):1958–1961. doi: https://doi.org/10.26538/tjnpr/v5i11.11
35. Tamuly C, Hazarika M, Bora J, Gajurel PR. Antioxidant activities and phenolic content of *Piper wallichii* (Miq.) Hand.-Mazz. Int J Food Prop. 2014; 17(2):309–320. doi: https://doi.org/10.1080/10942912.2011.631250
36. Pertiwi D, Hartati R, Julianti E, Fidrianny I. Antibacterial and antioxidant activities in various parts of *Artocarpus lacucha* Buch. Ham. Ethanolic extract. Biomed Rep. 2024; 20(4):1–11. doi: https://doi.org/10.3892/br.2024.1755
37. Moglad EH, Hamad AM, Fatima F, Seshadri VD, Naz M. Antimicrobial and wound healing activities of certain Sudanese medicinal plants. Saudi J Biol Sci. 2020; 27(7):1766–1772. doi: https://doi.org/10.1016/j.sjbs.2020.05.017
38. Lambebo MK, Kifle ZD, Gurji TB, Yesuf JS. Evaluation of wound healing activity of methanolic crude extract and solvent fractions of the leaves of *Vernonia auriculifera* Hiern (Asteraceae) in mice. J Exp Pharmacol. 2021; 13:677–692. doi: https://doi.org/10.2147/JEP.S308303
39. Belachew TF, Asrade S, Geta M, Fentahun E. In vivo evaluation of wound healing and anti-inflammatory activity of 80% methanol crude flower extract of *Hagenia abyssinica* (Bruce) J.F. Gmel in mice. Evid Based Complement Alternat Med. 2020; 2020:1–12. doi: https://doi.org/10.1155/2020/9645792
40. Tsimogiannis DI, Oreopoulou V. The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3',4'-hydroxy substituted members. Innov Food Sci Emerg Technol. 2006; 7(1-2):140–146. doi: https://doi.org/10.1016/j.ifset.2005.09.001
41. Platzer M, Kiese S, Tybussek T, Herfellner T, Schneider F, Schweiggert-Weisz U, Eisner P. Radical scavenging mechanisms of phenolic compounds: A quantitative structure-property relationship (QSPR) study. Front Nutr. 2022; 9:1–12. doi: https://doi.org/10.3389/fnut.2022.882458
42. Gulcin I, Alwasel SH. DPPH radical scavenging assay. Processes. 2023; 11(8):1–20. doi: https://doi.org/10.3390/pr11082248
43. Alkandahri MY, Arfania M, Abriyani E, Ridwanuloh D, Farhamzah F, Fikayuniar L, Hasyim DM, Nurul, Wardani D. Evaluation of antioxidant and antipyretic effects of ethanolic extract of cep-cepan leaves (*Castanopsis costata* (Blume) A.D.C.). J Adv Pharm Educ Res. 2022; 12(3):107–112. doi: https://doi.org/10.51847/twcOIyzqTM
44. Spiegel M, Kapusta K, Kołodziejczyk W, Saloni J, Żbikowska B, Hill GA, Sroka Z. Antioxidant activity of selected phenolic acids-ferric reducing antioxidant power assay and QSAR analysis of the structural features. Molecules. 2020; 25(13):1–15. doi: https://doi.org/10.3390/molecules25133088
45. Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol. 2016; 91(5):614–620. doi: https://doi.org/10.1590/abd1806-4841.20164741
46. Dehkordi AN, Babaheydari FM, Chehelgerdi M, Dehkordi SR. Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. Stem Cell Res Ther. 2019; 10:1–20. doi: https://doi.org/10.1186/s13287-019-1212-2
47. Subramanian S, Duraipandian C, Alsayari A, Ramachawolran G, Wong LS, Sekar M, Gan SH, Subramaniyan V, Seethalakshmi S, Jeyabalan S, Dhanasekaran S, Chinni SV, Rani NNIM, Wahab S. Wound healing properties of a new formulated flavonoid-rich fraction from *Dodonaea viscosa* Jacq. Leaves extract. Front Pharmacol. 2023; 14:1–16. doi: https://doi.org/10.3389/fphar.2023.1096905
48. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies for skin wound care and regeneration. Pharmaceutics. 2020; 12(8):1–30. doi: https://doi.org/10.3390/pharmaceutics12080735
49. Choudhary V, Choudhary M, Bollag WB. Exploring skin wound healing models and the impact of natural lipids on the healing process. Int J Mol Sci. 2024; 25(7):1–29. doi: https://doi.org/10.3390/ijms25073790
50. Demilew W, Adinew GM, Asrade S. Evaluation of the wound healing activity of the crude extract of leaves of *Acanthus polystachyus* Delile (Acanthaceae). Evid Based Complement Alternat Med. 2018; 2018:1–9. doi: https://doi.org/10.1155/2018/2047896
51. Kanlayavattanakul M, Khongkow M, Lourith N. Wound healing and photoprotection properties of *Acanthus ebracteatus* Vahl. extracts standardised in verbascoside. Sci Rep. 2024; 14(1):1–9. doi: https://doi.org/10.1038/s41598-024-52511-8
52. Taufik AY, Yasin HM, Ahmad N, Arai M, Ja'afar F. An investigation into the phytochemical content and antioxidant, antidiabetic, and wound-healing activities of *Curculigo*

latifolia found in Brunei Darussalam. Sci World J. 2024; 2024:1–22. doi: https://doi.org/10.1155/2024/5656744
53. Alkandahri MY, Sujana D, Hasyim DM, Shafirany MZ, Sulastri L, Arfania M, Frianto D, Farhamzah, Kusumawati AH, Yuniarsih N. Antidiabetic activity of extract and fractions of *Castanopsis costata* leaves on alloxan-induced diabetic mice. Pharmacogn J. 2021; 13(6 Suppl):1589–1593. doi: https://doi.org/10.5530/pj.2021.13.204
54. Tekleyes B, Huluka SA, Wondu K, Wondmkun YT. Wound healing activity of 80% methanol leaf extract of *Zehneria scabra* (L.f) Sond (Cucurbitaceae) in mice. J Exp Pharmacol. 2021; 13:537–544. doi: https://doi.org/10.2147/JEP.S303808
55. Carvalho MTB, Araújo-Filho HG, Barreto AS, Quintans-Júnior LJ, Quintans JSS, Barreto RSS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phytomedicine. 2021; 90:153636. doi: https://doi.org/10.1016/j.phymed.2021.153636
56. Alkandahri MY, Pamungkas BT, Oktoba Z, Shafirany MZ, Sulastri L, Arfania M, Anggraeny EN, Pratiwi A, Astuti FD, Indriyani, Dewi SY, Hamidah SZ. Hepatoprotective effect of kaempferol: A review of the dietary sources, bioavailability, mechanisms of action, and safety. Adv Pharmacol Pharm Sci. 2023; 2023:1–16. doi: https://doi.org/10.1155/2023/1387665
57. Alkandahri MY, Sadino A, Pamungkas BT, Oktoba Z, Arfania M, Yuniarsih N, Wahyuningsih ES, Dewi Y, Winarti SA, Dinita ST. Potential nephroprotective effect of kaempferol: Biosynthesis, mechanisms of action, and clinical prospects. Adv Pharmacol Pharm Sci. 2024; 2024:1–17. doi: https://doi.org/10.1155/2024/8907717
58. Alkandahri MY, Berbudi A, Subarnas A. Active compounds and antimalaria properties of some medicinal plants in Indonesia – A review. Sys Rev Pharm. 2018; 9(1):64–69. doi: https://doi.org/10.5530/srp.2018.1.13
59. Kumari P, Singh V, Kant V, Ahuja M. Current status of 1,4-Naphthoquinones and their derivatives for wound healing. Eur J Med Chem Rep. 2024; 12:1–23. doi: https://doi.org/10.1016/j.ejmcr.2024.100194
60. Liu E, Gao H, Zhao Y, Pang Y, Yao Y, Yang Z, Zhang X, Wang Y, Yang S, Ma X, Zeng J, Guo J. The potential application of natural products in cutaneous wound healing: A review of preclinical evidence. Front Pharmacol. 2022; 13:1–18. doi: https://doi.org/10.3389/fphar.2022.900439
61. Khan MI, Karima G, Khan MZ, Shin JH, Kim JD. Therapeutic effects of saponins for the prevention and treatment of cancer by ameliorating inflammation and angiogenesis and inducing antioxidant and apoptotic effects in human cells. Int J Mol Sci. 2022; 23(18):1–15. doi: https://doi.org/10.3390/ijms231810665
62. Men SY, Huo QL, Shi L, Yan Y, Yang CC, Yu W, Liu BQ. Panax notoginseng saponins promote cutaneous wound healing and suppress scar formation in mice. J Cosmet Dermatol. 2020; 19(2):529–534. doi: https://doi.org/10.1111/jocd.13042
63. Budiawan A, Purwanto A, Puradewa L, Cahyani ED, Purwaningsih CE. Wound healing activity and flavonoid contents of purslane (*Portulaca grandiflora*) of various varieties. RSC Adv. 2023; 13(15):9871–9877. doi: https://doi.org/10.1039/d3ra00868a
64. Shi XQ, Chen G, Tan JQ, Li Z, Chen SM, He JH, Zhang L, Xu HX. Total alkaloid fraction of *Leonurus japonicus* Houtt. Promotes angiogenesis and wound healing through SRC/MEK/ERK signalling pathway. J Ethnopharmacol. 2022; 295:115396. doi: https://doi.org/10.1016/j.jep.2022.115396
65. Vyas KS, Vasconez HC. Wound healing: biologics, skin substitutes, biomembranes and scaffolds. Healthcare. 2014; 2(3):356–400. doi: https://doi.org/10.3390/healthcare2030356
66. Scherer MMC, Marques FM, Figueira MM, Peisino MCO, Schmitt EFP, Kondratyuk TP, Endringer DC, Scherer R, Fronza M. Wound healing activity of terpinolene and α-phellandrene by attenuating inflammation and oxidative stress in vitro. J Tissue Viability. 2019; 28(2):94–99. doi: https://doi.org/10.1016/j.jtv.2019.02.003
67. Wang G, Yang F, Zhou W, Xiao N, Luo M, Tang Z. The initiation of oxidative stress and therapeutic strategies in wound healing. Biomed Pharmacother. 2023; 157:1–12. doi: https://doi.org/10.1016/j.biopha.2022.114004
68. Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, Prezioso S, Muraro R, Giovanni, PD. Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-year retrospective study. Antibiotics. 2021; 10(10):1–14. doi: https://doi.org/10.3390/antibiotics10101162
69. Comino-Sanz IM, López-Franco MD, Castro B, Pancorbo-Hidalgo PL. The role of antioxidants on wound healing: A review of the current evidence. J Clin Med. 2021; 10(16):1–22. doi: https://doi.org/10.3390/jcm10163558
70. Deng X, Gould M, Ali MA. A review of current advancements for wound healing: Biomaterial applications and medical devices. J Biomed Mater Res B Appl Biomater. 2022; 110(11):2542–2573. doi: https://doi.org/10.1002/jbm.b.35086
71. Hassanpour SH, Doroudi A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed. 2023; 13(4):354–376. doi: https://doi.org/10.22038/AJP.2023.21774
72. Chen Q, Wang J, Gao Y, Wang Z, Wang D, Gao X, Yan P. Fermentation, identification, and antioxidant activity of saponins produced by a wild ginseng endophytic fungus *Umbelopsis dimorpha* Strain NSJG. Fermentation. 2024; 10(1):1–13; https://doi.org/10.3390/fermentation10010009
73. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr Med Chem. 2015; 22(1):132–149. doi: https://doi.org/10.2174/0929867321666140916113443
74. Li J, Monje-Galvan V. In vitro and in silico studies of antimicrobial saponins: A review. Processes. 2023; 11(10):1–17. doi: https://doi.org/10.3390/pr11102856
75. Cores Á, Carmona-Zafra N, Clerigué J, Villacampa M, Menéndez JC. Quinones as neuroprotective agents. Antioxidants. 2023; 12(7):1–37. doi: https://doi.org/10.3390/antiox12071464
76. Sirin S, Dolanbay SN, Aslim B. Role of plant-derived alkaloids as antioxidant agents for neurodegenerative diseases. Health Sci Rev. 2023; 6:1–11. doi: https://doi.org/10.1016/j.hsr.2022.100071
77. Kokilananthan S, Bulugahapitiya VP, Manawadu H, Gangabadage CS. Sesquiterpenes and monoterpenes from different varieties of guava leaf essential oils and their antioxidant potential. Heliyon. 2022; 8(12):1–9. doi: https://doi.org/10.1016/j.heliyon.2022.e12104
78. Junior MAD, Edzang RWN, Catto AL, Raimundo JM. Quinones as an efficient molecular scaffold in the antibacterial/antifungal or antitumoral arsenal. Int J Mol Sci. 2022; 23(22):1–16. doi: https://doi.org/10.3390/ijms232214108
79. Yan Y, Li X, Zhang C, Lv L, Gao B, Li M. Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. Antibiotics. 2021; 10(3):1–30. doi: https://doi.org/10.3390/antibiotics10030318
80. Huang W, Wang Y, Tian W, Cui X, Tu P, Li J, Shi S, Liu X. Biosynthesis investigations of terpenoid, alkaloid, and flavonoid antimicrobial agents derived from medicinal plants. Antibiotics. 2022; 11(10):1–32. doi: https://doi.org/10.3390/antibiotics11101380