

# *Senna podocarpa* Emulgel: A Herbal Alternative for Chemical Burn Wound Treatment

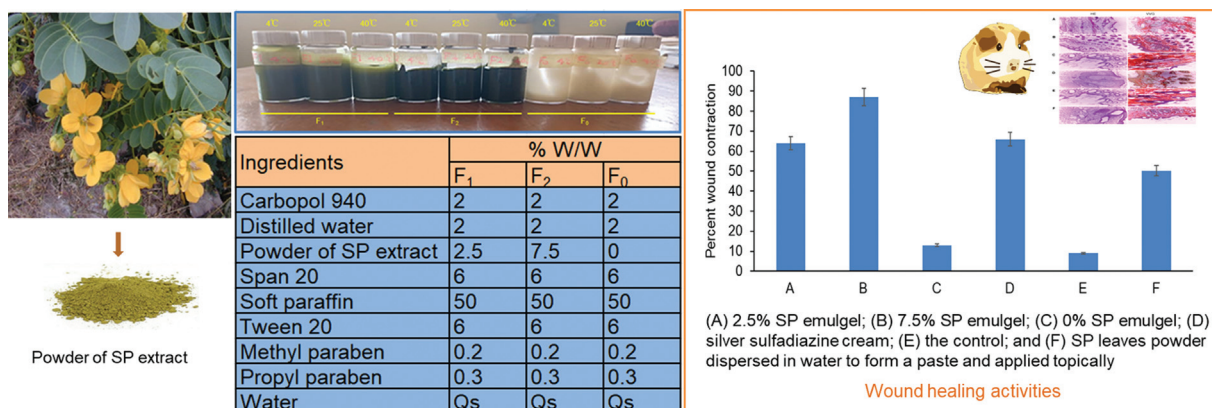
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## Abstract

*Senna podocarpa* (SP) leaves are used in folk medicines for treatment of burns and wounds as poultices on wound surface. However, to the best of our knowledge, the wound healing ability of this plant has not been scientifically evaluated. This work aimed to determine the wound healing potential of the crude extract of SP leaves, and to evaluate the benefit of its preparation as an emulgel. In this study, the formulations of 2.5% of SP emulgel (F<sub>1</sub>) and 7.5% of SP emulgel (F<sub>2</sub>) were prepared by mixing the emulsion phase with the gel phase in a ratio of 1:1, and then physical appearance, globule size, pH, viscosity, swelling, water activity, extrudability, occlusion, spreadability, stability, and wound healing ability were determined. Phytochemical screening showed the presence of alkaloids, saponins, tannins, cardiac glycosides, flavonoids, anthraquinones, and phenols within the hydro-ethanolic extract of SP leaves, and high flavonoid content is believed to be responsible for its healing attributes. Our formulations showed acceptable physical properties. Hematoxylin-eosin and Verhoeff–Van Gieson stain showed that F<sub>2</sub> could induce the accumulation of fibroblasts, fibrocytes, inflammatory cells, gland cells, epidermal cells, adipocytes, and collagen

## Keywords

- ▶ *Senna podocarpa*
- ▶ emulgel
- ▶ hematoxylin-eosin
- ▶ Verhoeff–Van Gieson
- ▶ burn
- ▶ wound healing

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in the process of wound healing in mice injured with hydrochloric acid. Encouragingly, the percent of wound contractions in mice treated with F<sub>1</sub>, F<sub>2</sub>, and SP leaf poultice were 64, 87, and 50, respectively, suggesting the superior healing properties exhibited by SP emulgel over SP leaf poultice, and this may be due to the occlusive property of emulgels. In conclusion, F<sub>2</sub> of crude extract of SP leaves has better pharmacological effects on burn and wound healing, and may represent a preferred choice to treat burn wounds in the future.

## Introduction

*Senna podocarpa* (SP), formerly known as *Cassia podocarpa* Guill. et Perr (Leguminosae - Caesalpinioideae), is a glabrous shrub that is widely distributed in West Africa and could be found in the Savannah Forest of the region.<sup>1</sup> The phytochemical screening of the leaves of Nigerian species of *Senna* including SP revealed some major groups, for example glycosides (anthraquinone, naphthopyrone, etc.), phenolic compounds, and flavonoids, with pharmacological importance such as anti-inflammatory, hepatoprotective, hypolipidemic, antigenotoxic, spasmogenic, antinociceptive, antiproliferative, hypotensive, purgative, antidiabetic, estrogenic and antiestrogenic, antiulcer, antioxidant, antifungal, antishigellosis, anthelmintic, antimutagenic, antibacterial, and antiplasmodial activity.<sup>2</sup> The extract of SP leaves shows inhibitory effects on the growth of several strains of bacteria and fungi, and its killing ability for microorganisms is mainly attributed to leaking potassium and sodium ions from their cell cytoplasm causing plasmolysis, leading to death of the organisms.<sup>3,4</sup> Amazingly, SP pods did not show any lethal effect on mice and rats; as well as no adverse effect on their brain, kidney, liver, and testis.<sup>5</sup> SP is a folk medicine used to treat burns and wounds by pulverizing the dried leaves and applying it as poultices on the wound surface.<sup>2</sup> To the best of our knowledge, the study of the wound healing of this plant remained largely unknown.

Burns is a skin injury caused by heat, radiation, radioactivity, electricity, friction, or chemicals. It has been a public health issue accounting for an estimated 180,000 deaths per year, and a leading cause of disability-adjusted life-years lost in low- and middle-income countries, probably due to poor hygienic condition.<sup>6</sup> Easy access to chemicals used for assault (such as in acid violence attacks) has been identified as one of the risk factors for burns.<sup>6</sup>

Infection accompanying burn is the major cause of mortality in burn patients, hence it is recommended that burn should be managed within a short possible time.<sup>6</sup> The wound healing process involves coagulation, inflammation, tissue granulation, formation of matrix, remodeling of connective tissue, collagenization, and the final wound strength acquisition.<sup>6</sup> Commonly, silver sulfadiazine and mafenide are used to relieve symptoms and promote burn healing, yet, due to the need of the activation of multiple biological pathways, the healing process is usually prolonged with these agents.<sup>7</sup>

Emulgels are semisolid preparations formed when emulsion is combined with the gel. Emulgel has the advantages

of thixotropic properties, greaseless, spreadable, washable, emollient, nonstaining, stable, longer half-life, bio-friendly, beautiful appearance in comparison to other topical preparations, and represents as an ideal choice for delivering hydrophobic drugs.<sup>8</sup> In this work, we first determined the wound healing potential of the crude extract of SP leaves, and further evaluated the benefit of formulating it into an emulgel. This will offer a cost-effective alternative to commercially available preparations for the treatment of chemical burn.

## Materials and Methods

Carbopol 940, ethanol, sulfuric acid, ferric chloride, and ammonia solution were obtained from Sigma-Aldrich. Methyl paraben and propyl paraben were obtained from Zeb Pharma, India. Paraffin wax was purchased from Unicorn Pet, India. Span 20 and Tween 20 were purchased from LobaChemie, India. Dragendorff's reagent was obtained from Ganesh Chem Tech, India. All reagents were of analytical grade.

### Plant Collection and Extraction

The fresh leaves of *S. podocarpa* Linn. were collected from the National Institute for Pharmaceutical Research and Development (NIPRD, Botanical Garden, Abuja, Nigeria). Identification and authentication of the plant were done by Mr. Akeem Lateef of the Herbarium Unit of the Institute, and the voucher number of the plant was NIPRD/H/7264. The leaves were rinsed with plenty of water, spread for 24 hours to drain water, dried in an oven at 40°C for 6 hours, and then blended using an electrical blender. The powder (300 g) was added into a solution of ethanol-water (v/v = 1:1, 600 mL), covered with an aluminum foil, placed on a mechanical shaker (GFL 3017, Germany), and continued shaking for 48 hours. The extract was filtered and concentrated to dryness at 100°C in a water bath, pulverized, and stored in an airtight container in a dark cupboard until used.<sup>9</sup>

### Phytochemical Screening of Plant Extract

The presence of alkaloids, saponins, tannins, steroids, cardiac-active glycoside, flavonoids, anthraquinones, and phenols in the extract was assessed respectively as the following according to the reported studies<sup>10,11</sup>:

- The presence of alkaloids was detected by observing the precipitate by shaking a portion of the extract with 1%

HCl and adding Dragendorff's reagent dropwise to the filtrate.

- The presence of saponins was detected by observing the persistent frothing when the extract was shaken with distilled water in a test tube.
- The presence of tannins was detected by observing a blue-green coloration when ferric chloride was added to the filtrate of the extract with distilled water.
- The presence of steroids was detected by observing a reddish-brown coloration when a portion of the extract was dissolved in chloroform, and then concentrated sulfuric acid was added in a dropwise manner.
- The presence of cardiac-active glycoside was detected by dissolving a portion of the extract in glacial acetic acid containing a drop of ferric chloride solution, followed by the addition of sulfuric acid (1 mL) to determine the presence of a brown ring at the interface.
- The presence of flavonoids was detected by adding a small amount of magnesium powder and few drops of HCl to the extract to determine a red coloration.
- The presence of anthraquinones was detected by adding 10% ammonia solution to the filtrate of the extract and benzene (being used to separate the organic solvent layer) to determine a reddish coloration.
- The presence of phenols was detected by observing green precipitate when the extract was dissolved in ferric chloric solution.

### Preparation of Emulgel Formulation

The formulations of 2.5% of SP emulgel (F<sub>1</sub>) and 7.5% of SP emulgel (F<sub>2</sub>) were prepared according to the formula shown in ►Table 1. The choice of ingredients was referred to a report by Samala and Sridevi.<sup>12</sup> The choices of % w/w for different ingredients were referred to a method by Ali Khan et al with slight modifications.<sup>13</sup> Carbomer 940 was dispersed in distilled water separately and stirred at a constant speed to prepare the gel base. The oil phase of the emulsion was prepared by dissolving Span 20 in paraffin, and the

aqueous phase was prepared by dissolving Tween 20 in purified water. Methyl and propyl parabens were dispersed in the oil phase. The oily and aqueous phases were separately heated to 70 and 80°C, then the oily phase was added to the aqueous phase with continuous stirring until a stable emulsion was formed. The emulsion was mixed with the gel in a ratio of 1:1 and gently stirred to obtain the emulgel.<sup>12</sup> The formulation without the extract was prepared as a control (F<sub>0</sub>).

### Evaluation of Emulgel

#### Organoleptic Properties and PH Determination

The color, odor, consistency, and homogeneity of the prepared emulgel containing the SP extract were physically evaluated. The pH values of the formulations were determined using a digital pH meter (S220 Mettler-Toledo, Switzerland).

#### Spreadability, Occlusion, Extrudability, Swelling, and Viscosity Assays

The spreadability, occlusion, extrudability, and viscosity assays of F<sub>1</sub> and F<sub>2</sub> were performed according to a reported study.<sup>14,15</sup> In spreadability assay, the apparatus consisted of two glass slides (7.5 × 2.5 cm). The downward slide is fixed on a wooden board, and the upper slide is movable, tied to a thread which passed over a pulley. The formulation (1 g) was placed between the two glass slides. A weight (100 g) was placed on the upper slide to expel the entrapped air between the slides and to provide a uniform film of the formulation. Then the weight was removed, and pulled the top slide by attaching a 30 g weight to the pulley. The time (seconds) required for the moving slide to travel a predetermined distance (6.5 cm) was recorded and expressed as spreadability.

Occlusion assay was performed to determine the degree of water loss from the skin when covered with the emulgel according to a reported study.<sup>15</sup> Briefly, a 50-mL beaker was filled with 25 mL of water, covered with 0.45 µm filter paper, and sealed. Samples were spread on the filter papers and stored at room temperature at 75% relative humidity (RH) for 144 hours. A beaker covered with filter paper without samples was considered as control. The occlusion factor (F) was calculated as Eq. (1):

$$F = (A - B)/A \times 100\% \quad (1)$$

where *A* is the water loss of control, and *B* is water loss of the sample.

In extrudability assay, the syringe was weighted, and then filled with the herbal gel (2 g). The filled syringe was extruded. The weight of the extruded gel was determined and expressed as percentage extrudability.<sup>15</sup> The extrudability was calculated by using Eq. (2)

$$\text{Extrudability} = \frac{\text{Weight applied to extrude emulgel from tube (g)}}{\text{Area (cm}^2\text{)}} \quad (2)$$

The swelling index was determined by taking 1 g of emulgel in an aluminum foil, neatly wrapped, and mixed

**Table 1** Emulgel formula

Ingredients	% w/w		
	F <sub>1</sub>	F <sub>2</sub>	F <sub>0</sub>
Carbopol 940	2.0	2.0	2.0
Distilled water	2.0	2.0	2.0
Powder of SP extract	2.5	7.5	0
Span 20	6.0	6.0	6.0
Soft paraffin	50	50	50
Tween 20	6.0	6.0	6.0
Methyl paraben	0.2	0.2	0.2
Propyl paraben	0.3	0.3	0.3
Water	Qs	Qs	Qs

Note: F<sub>1</sub> was the formulation of 2.5% of SP emulgel; F<sub>2</sub> was the formulation of 7.5% of SP emulgel; and F<sub>0</sub> was the formulation of 0% of SP emulgel.

with acetate buffer solution (pH = 6.8, 10 mL) in Petri dishes. One hour later, the foil and its content were reweighed. The swelling index was assessed according to Eq. (3).<sup>16</sup>

$$\text{Swelling index \%} = (W_t - W_0)/W_0 \times 100\% \quad (3)$$

where  $W_t$  is the weight of swollen emulgel after 1 hour,  $W_0$  is the weight of emulgel at time zero.

Viscosity of the emulgel was determined using Biobase viscometer (NDJ-85). About 50 g of the emulgel was taken into a beaker and the spindle dipped in it. The viscosities were recorded at different rotational speeds of 10, 20, 50, and 100 rpm.<sup>16</sup>

### Moisture Sorption

Three glass chambers labeled A, B, and C were used. Chamber A contained distilled water and believed to have 100% RH. Chamber B contained a supersaturated solution of sodium chloride with 75% RH. The chamber C contained a supersaturated solution of potassium hydroxide with 8% RH. The glass chambers containing the supersaturated solutions were covered appropriately and allowed to equilibrate. The emulsion samples were weighed at 30-minute intervals for 3 hours to check the changes of their weight.<sup>17</sup>

### Globule-Size Analysis

The mean size of the globules of the emulgel was measured using an optical microscope (Olympus light microscope) with software MC 1000 (Motic China Group Co. Ltd.) for image analysis. The samples spread on a specimen slide were stained with an aqueous dye and covered with a slip. One hundred globules were measured from each preparation, and their average value was recorded.<sup>16</sup>

### Stability Studies

Using the ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines, formulations were kept at different temperatures (4, 25, and 40°C) at 75% RH for 3 months. Parameters such as pH, viscosity, color, odor, consistency, and homogeneity were assessed.<sup>18</sup>

### Selection and Conditioning of Animals

Wistar albino mice weighing between 32 and 36 g were maintained at the Animal Facility Center of NIPRD. They were housed in standard polypropylene cages with saw dust as beddings at  $25 \pm 2^\circ\text{C}$ . The animals were fed on standard rodent feed and had free access to clean drinking water *ad libitum*. The animals were handled according to the Institutional Animal Guidelines for Care and Use of Animals as recorded in the Standard Operating Procedure of the Department of Pharmacology and Toxicology, NIPRD (SOP No. 05:03:02), and the internationally accepted principles for laboratory animal use and care (National Institutes of Health publication No. 85–23, revised 2010).

### Skin Irritancy Test

The hair of mice was shaved using a clipper and razor blade. The prepared emulgels were applied to the shaved dorsal

skin of six mice (three males and three females) and their potential skin sensitization was assessed. The area of application was occluded with a gauze and covered with a non-sensitizing microporous tape. The presence of erythema and edema was observed for 3 consecutive days.<sup>18</sup>

### Establishment of Wound in Mice and Treatment

Thirty-six mice (18 males and 18 females) were anaesthetized with intraperitoneal ketamine (10 mg/kg). A clipper and a razor blade were used to shave the dorsal skin of the mice 3 cm from the base of the neck. The shaved area was cleaned with 70% alcohol. A piece of paper with a circular hole of 2 cm in diameter was prepared for burned areas. Then hydrochloric acid (0.2 mL of 37%) was dropped in the burned areas of the skin for 15 seconds to establish wounds in the mice. The mice were anesthetized in the whole process.

The wound mice were randomly divided into six groups ( $n = 6$  [3 males and 3 females]): group A (mice treated with  $F_1$ ); group B (mice treated with  $F_2$ ); group C (mice treated with 0% SP emulgel); group D (mice treated with a wound-healing agent [silver sulfadiazine cream]); group E (a control group without any treatment); group F (SP leaf powder dissolved in water to form a poultice and applied topically to the wound of the mice, and during this process the powder should be reconstituted daily throughout the study). The wounds were treated daily for 14 consecutive days, and on the 15th day, the mice were sacrificed by chloroform inhalation.

Wound-healing ability of the SP emulgel was assessed by histological analysis and wound contraction study. In histological analysis, the burned area was harvested. The skins were removed, fixed, dehydrated, embedded, and cut for microscopy section. Three slides were made for each wound and stained with hematoxylin-eosin (HE; basic stain), Verhoeff–Van Gieson (VVG; for collagen and elastic fiber staining).<sup>13</sup> Sections were evaluated by an independent observer. In the wound contraction study, the wound contraction rate was expressed as the percent change in the original area following the formula: wound contraction rate = (original wound area – wound area after day 14)/original wound area  $\times$  100%. The wound area was traced on a transparent paper after treatment for 14 days. The animals were restrained to a position during the tracing process. The tracings were then transferred to a 1 mm<sup>2</sup> graph sheet and the wound area was measured.<sup>9</sup>

## Results and Discussion

### Phytochemical Test

Phytochemical screening for secondary metabolites in the hydro-ethanolic extract of SP leaves was performed using standard phytochemical methods reported in the literature.<sup>10,11</sup> Results suggested that alkaloid, saponin, tannin, cardiac glycoside, flavonoid, anthraquinone, and phenol were present in the SP extract, while steroids were absent. Tannins, flavonoids, and phenols are potent antioxidants, and antioxidants have been implicated in wound healings by improving vascularization and reducing lipid peroxidation

which in turn prevents necrosis of cells and promotes cell proliferation.<sup>6</sup> Also, the ability of antioxidants to limit free radicals in the epidermal layer of the skin will assure of SP safety on application topically. These however provide a scientific basis for SP as a natural remedy in wound therapy.

### Evaluation of SP Emulgel

In the preparation process, it is very important to choose an ideal surfactant to favorably emulsify oil with water.<sup>7</sup> Evidence suggested that the most stable emulsion systems are usually consist of lipophilic and hydrophilic blends, which are connected to each other through hydrogen bonding, conferring more rigidity and strength to the film surrounding oil droplets.<sup>7</sup> Values between 10 and 18 are best suited for a stable O/W emulsion formulation.<sup>19</sup> In our work, a blend of Tween 20 with hydrophilic-lipophilic balance (HLB) = 16.7 and Span 20 (HLB = 8.6) was used to give a required HLB value of approximately 10.

### Organoleptic Properties of SP Emulgel and pH Value

The organoleptic parameters and the pH values of the formulations are presented in ►Table 2. The prepared emulgel was green in color, had herbal-characterized odor, and a consistent and homogenous appearance with no sign of instability related to phase separation. The color intensity and the odor of the formulation may be concentration-dependent.

Due to the sensitive nature of the skin, the pH value of a topical formulation is important. Too acidic or too basic pH of an emulgel could cause skin irritation.<sup>20</sup> Our data showed that SP emulgels have a pH value of between 4.12 and 4.15. Increased SP content in the formulation induced a slight increase in the pH value, which provides an important direction for the preparation of SP emulgel closer to that of human skin. More recent findings have shown that the natural skin surface has an average pH of less than 5, and this pH has been proven to be responsible for provision of a

**Table 2** Physical properties of different formulations of SP emulgel

	F <sub>1</sub>	F <sub>2</sub>	F <sub>0</sub>
Odor	++	+++	+
Color	Army green	Dark green	White
Consistency	+++	+++	+++
Homogeneity	+++	+++	+++
Viscosity (cp)	3013 ± 0.04	3290 ± 0.08	2597 ± 0.71
pH	4.12 ± 0.03	4.15 ± 0.01	3.64 ± 0.03

Abbreviation: SP, *Senna podocarpa*.

Note: F<sub>1</sub> was the formulation of 2.5% of SP emulgel; F<sub>2</sub> was the formulation of 7.5% of SP emulgel; and F<sub>0</sub> was the formulation of 0% of SP emulgel. + indicates slight level, ++ indicates moderate level, and +++ indicates high level.

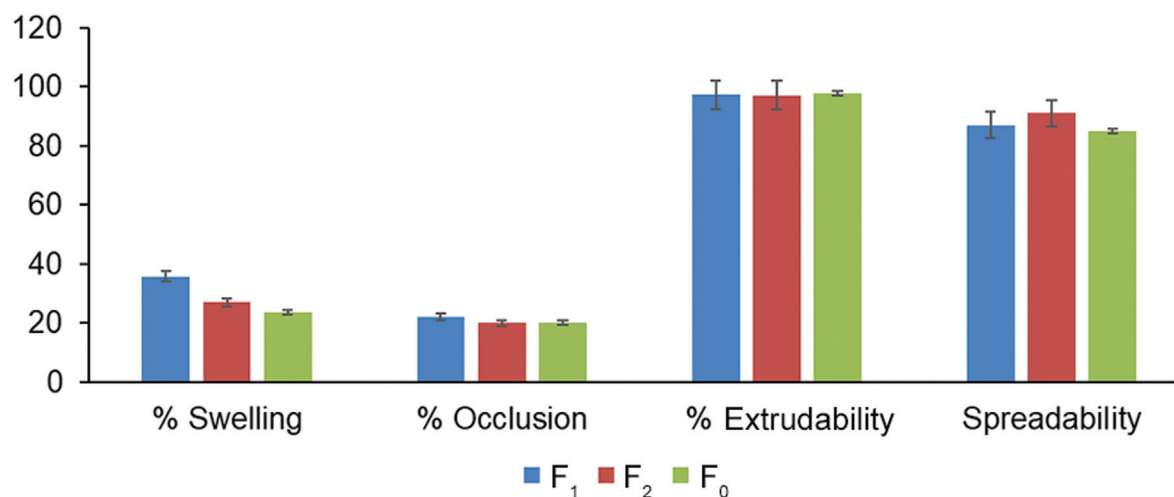
conductive environment for resident skin microflora and also support important physiological processes like the formation of an optimal structure of the lipid barrier and stratum corneum homeostasis.<sup>21</sup>

### Results of Spreadability, Occlusion, Extrudability, and Swelling Assays

Results of spreadability, occlusion, extrudability, and swelling assays are shown in ►Fig. 1. Spreadability is a term expressed to denote the extent of area on which the gel is easily spread on the skin during application. The correct delivery of a drug depends on its ability to be spread evenly with ease.<sup>20</sup> Our data demonstrated that SP emulgels enhanced the spreading coefficient in comparison to the control emulgel with the maximum being seen in F<sub>2</sub>.

The extrudability indexes of 2.5%, 7.5%, and the control SP emulgel were similar (~97%), suggesting that the prepared emulgel can be easily dispensed from the container upon use.

Besides, there was no remarkable difference in the occlusion of the emulgel. The type and amount of oil affect the



**Fig. 1** Extrudability, occlusion, spreadability, and swelling results for different formulations of SP emulgel. F<sub>1</sub> was the formulation of 2.5% of SP emulgel; F<sub>2</sub> was the formulation of 7.5% of SP emulgel; and F<sub>0</sub> was the formulation of 0% of SP emulgel. Each bar denotes mean ± standard deviation (n = 6). SP, *Senna podocarpa*.

occlusion property of an emulgel.<sup>22</sup> After 144 hours, all the emulgels showed excellent occlusion property, suggesting that the emulgel could form a barrier on the skin, which may affect skin hydration by restoring skin lipids, and also maintain skin elasticity.

The equilibrium swelling for F<sub>1</sub>, F<sub>2</sub>, and F<sub>0</sub> were 35.7, 27.0, and 23.5%, respectively. One hour later, the formulations swelled. This may probably be due to the porous nature of the emulgel, which has a large surface area that allows rapid uptake of the solvent. However, the swelling was inversely associated with the concentration of the SP extract. SP induced a significant enhancement in the swelling, suggesting that the binding force in the formula was weak.

### Viscosity Measurement of SP Emulgels

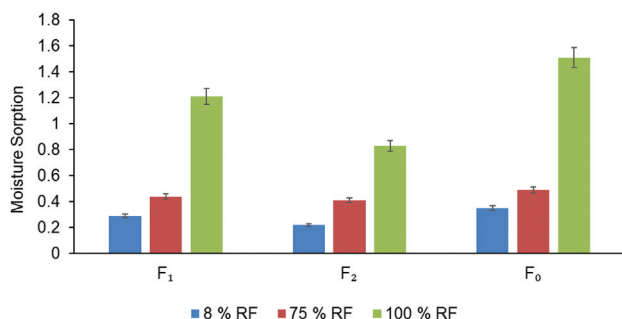
The viscosity characteristic of the emulgels is presented in ►Table 2. Viscosity is a tool to assess the rheology of a formulation. The SP extract appears to increase the viscosity of the emulgel in a dose-dependent manner, which could be as a result of entities like secondary metabolites present in the extract that can increase the gelling power of the gelling agent.<sup>20</sup> Viscosity values of our emulgels were good when compared with similar reports,<sup>6,16,17</sup> and the reason may be that the surfactants (Span 20 and Tween 20) used in the preparation were able to obtain energy from the interfacial film between the dispersed phase and the dispersion medium, and also the strong colloidal interactions between emulsion droplets.<sup>22</sup>

### Water Sorption Activity of SP Emulgel

Different samples of the emulgels showed relatively similar moisture interactions at different RH values (►Fig. 2). Water sorption activity for all formulations ranged from 0.2 to 1.6 at 8, 75, and 100% RH. Studies showed that a product with water activity greater than 0.8 is prone to grow mold,<sup>22</sup> hence, the formulation may not be suitable for storage in an environment of approximately 100% RH.

### Photomicrograph of SP Emulgel

Our emulgels have small droplet sizes ranging from 1.268 to 5.212 μm, (►Fig. 3) evenly distributed, and the specific



**Fig. 2** Moisture sorption results at 8, 75, and 100% RH for different formulations of SP emulgel. F<sub>1</sub> was the formulation of 2.5% of SP emulgel; F<sub>2</sub> was the formulation of 7.5% of SP emulgel; and F<sub>0</sub> was the formulation of 0% of SP emulgel. Each bar denotes mean ± standard deviation ( $n=6$ ). RH, relative humidity; SP, *Senna podocarpa*.

surface area per unit volume is high.<sup>22</sup> This also implies that our formulations was stable.

### Stability Study of SP Emulgel

Samples were transferred into transparent bottles, and easy visual inspection was performed at different temperatures (4, 25, and 40°C) after 3 months. Short-term accelerated stability studies after 3 months showed our emulgels retained their original color, homogeneity, and consistency (►Fig. 4). The pH was in the range of 3.96 to 4.10. Hence our formulations were stable under accelerated conditions, and there was no evidence of syneresis, which is a common drawback of gels.<sup>22</sup>

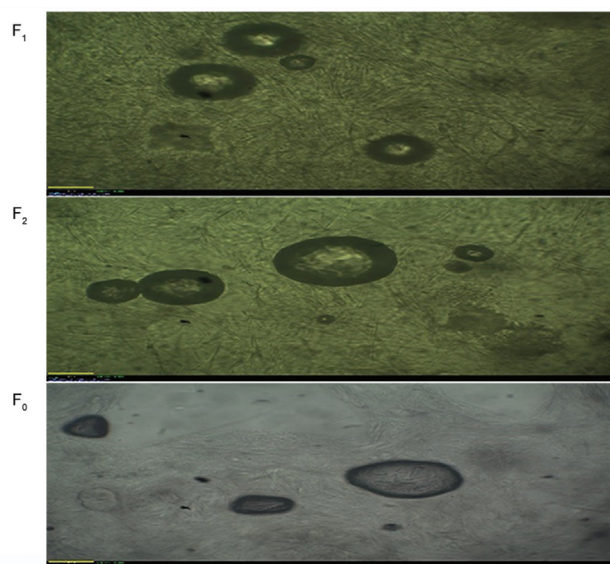
### Animal Studies

#### Skin Irritancy Test

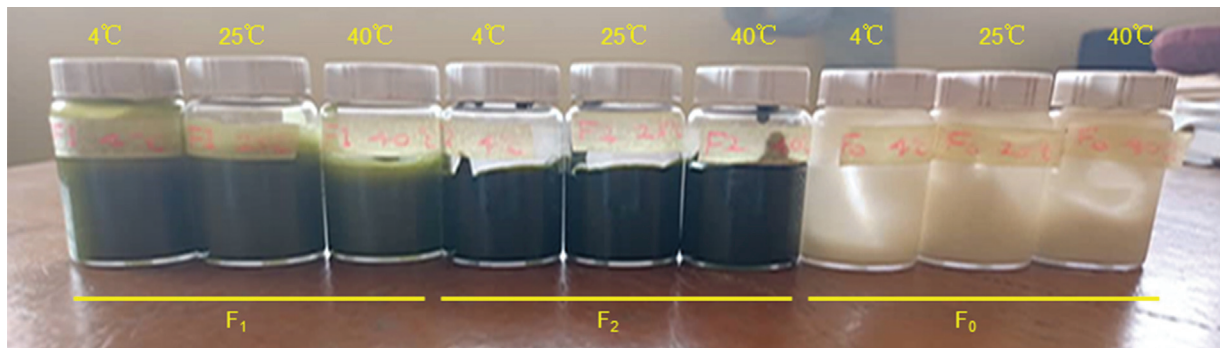
A skin irritancy test was performed to determine the potential risk of a formulation to harm the skin. Some topical preparations have been shown to pass through the epidermis into the dermis causing toxic effects in the form of redness, itching, or pain. This reaction has been tracked to an immediate immune response.<sup>23</sup> Our results showed that there were no visible signs of edema, erythema, or irritation in the wound mice treated with SP emulgel, suggesting the safety of our product when applied topically. This also revealed the biocompatibility of our extract with all the excipients used.<sup>23</sup>

#### Histological Study

The purpose of an intrinsic healing process is to stop further damage, prevent the wound against infection, and then restore strength and tissue function.<sup>23</sup> Ideally, wound healing itself can be accomplished through homeostasis and the



**Fig. 3** Photomicrograph globule size image of different formulations of SP emulgel (original magnification × 100, the scale bar is 10 μm). F<sub>1</sub> was the formulation of 2.5% of SP emulgel; F<sub>2</sub> was the formulation of 7.5% of SP emulgel; and F<sub>0</sub> was the formulation of 0% of SP emulgel. The experiment was repeated at least three times. SP, *Senna podocarpa*.



**Fig. 4** Image of different formulations of SP emulgel at 4, 25, and 40°C after 3 months. F<sub>1</sub> was the formulation of 2.5% of SP emulgel; F<sub>2</sub> was the formulation of 7.5% of SP emulgel; and F<sub>0</sub> was the formulation of 0% of SP emulgel. SP, *Senna podocarpa*.

activation of body immune systems. The process of wound healing begins with homeostasis, and stops bleeding by activating platelets to form a fibrin clot. This is followed by (1) the inflammatory phase to activate an elaborate immune response to destroy pathogens entering the wound, and then prepare the tissue for the restoration of anatomical integrity; then (2) the proliferative or angiogenesis stage to form new blood vessels by granulation of tissue, neovascularization, and re-epithelialization; and (3) the final remodeling phase where granulated tissue is replaced with a scar and the epidermis is freed from immune cells, which either dies by apoptosis or relocate to the dermis.<sup>24</sup>

Fibrocytes and fibroblast exhibit tissue remodeling properties by acting as antigen-presenting cells capable of stimulating T-cell immunity, promote wound closure through  $\alpha$ -smooth muscle actin-mediated contraction, and enhance angiogenesis by transformation into another mesenchymal cell type.<sup>25</sup> Light microscopy of wounds with HE staining revealed more deposition of fibrocytes and fibroblasts for groups A, B, and D after 14 days of treatment (**Table 3** and **Fig. 5**) when compared with other groups.

The inflammatory phase in wound healing is shaped by the presence of neutrophils and macrophages at the injury site.<sup>26</sup> Inflammatory cells inhibit bacteria invasion as a result of high efficiency in pathogen clearance by releasing reactive oxygen species and cytotoxic granules and forming neutrophil extracellular traps with subsequent phagocytosis of pathogens.<sup>27</sup> Aggregation of inflammatory cells was high with the mice in group B (treatment with 7.5% SP emulgel), moderate with mice in group A (treatment with 2.5% SP emulgel), and slight with mice in group D (treatment with silver sulfadiazine).

Evidence suggests that keratinocytes immigrate into the wound and expand during the regeneration of the burn-damaged tissue, and thus play an essential role for good wound healing.<sup>24</sup> Keratinocytes contribute to the initiation of the excessive inflammation in the first 2 days, and also express toll-like receptor 3 (TLR-3), TLR-4, and TLR-9, which are upregulated in acute wounds.<sup>24</sup> Our data confirmed that the group treated with 7.5% SP emulgel was associated with moderate deposition of keratin.

Epidermal layers were more for mice in group B, moderate with those in group A, and slight with those in group F.

Dermal adipocytes are known to take part in wound healing by recruiting macrophages; and trans-differentiate into myofibroblasts. Studies have shown that when dermal adipocytes are depleted prior to skin injury, macrophages' recruitment to the wound site are reduced while revascularization and re-epithelialization of the wound bed are delayed.<sup>27</sup> Our data showed that adipocyte's deposition was more in group B, moderate in groups D and F, and slight in group A.

The regeneration of a fully functional skin involves restoration of skin components such as the sweat glands.<sup>28</sup> The hyperplasia of glands in mice in group B was more than in other groups, while mice in groups A, D, and F showed moderate gland hyperplasia.

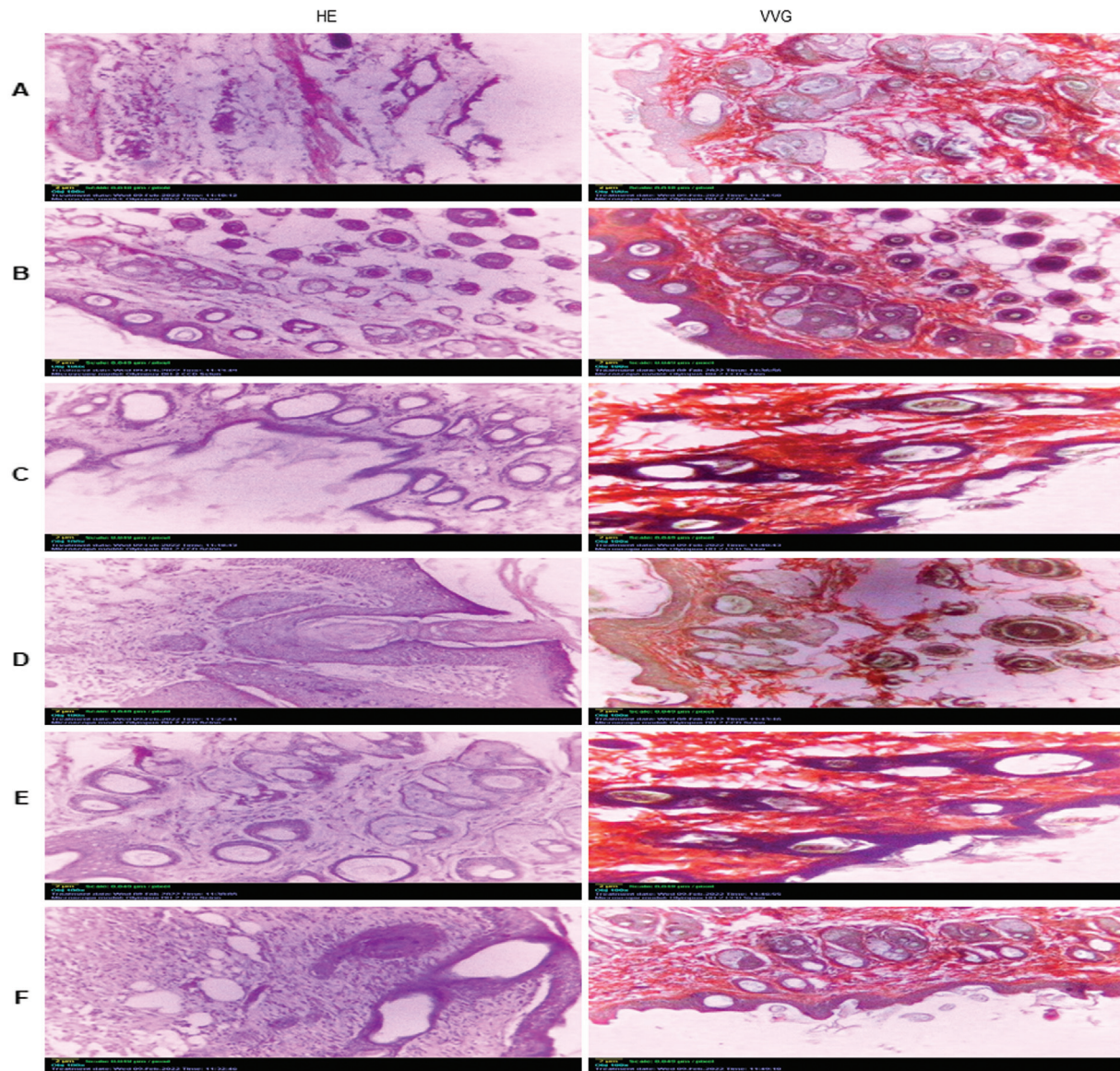
Collagen serves as a scaffold in connective tissue.<sup>27</sup> A collagen inflammatory response to injury leads to cellular differentiation, angiogenesis, and mitosis.<sup>26</sup> In this study, the collagen and elastic fiber were further stained by VVG. We noticed more deposition of collagen in mice in groups A and B, while mice in groups D and F had moderate collagen deposition.

Elastic fibers are known to liberate fiber-forming protein leading to fibroblast proliferation thereby enhancing collagen type I while increasing tropoelastin.<sup>29</sup> Our data showed that elastic fiber in mice in F group was more, and moderate in mice in A, B, C, and D groups.

Above all, histological results first prove that the SP emulgel's healing process is associated with stimulation of fibroblast, fibrocytes, inflammatory cells, glands, adipocytes, collagen and elastic fiber synthesis, and re-epithelialization, together with modulation of both inflammatory response and oxidative stress during tissue repair, which is consistent with the wounding healing process in a reported study.<sup>30</sup>

### Wound Contraction

The percent of wound contraction is shown in **Fig. 6**. Our data showed that the percent of wound contraction in group A (mice treated with 2.5% SP emulgel) was 64%, which is similar to that treated with silver sulfadiazine (Group D, 66%), while Group B (mice treated with 7.5% SP emulgel) showed a superior percent contraction of 87%, suggesting that the wound contractions of SP extract may be dose-dependent. Additionally, the SP poultice induced a 50% of



**Fig. 5** Histology evaluation of the wounds using HE and VVG staining (original magnification  $\times 100$ , the scale bar is  $2\ \mu\text{m}$ ). Mice with wounds were treated with (A) 2.5% SP emulgel; (B) 7.5% SP emulgel; (C) 0% SP emulgel; (D) silver sulfadiazine cream; (E) the control; and (F) SP leaf powder dispersed in water to form a paste and applied topically. After 14 days, HE and VVG staining was performed in the wounds. HE, hematoxylin-eosin; SP, *Senna podocarpa*; VVG, Verhoeff–Van Gieson.

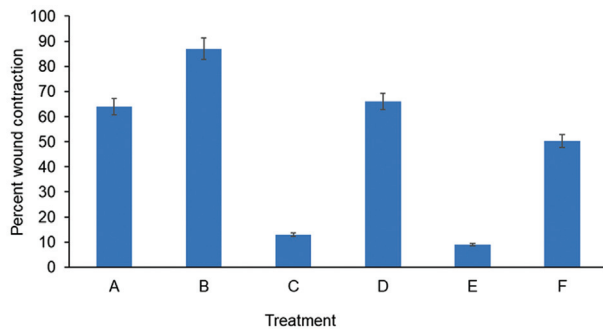
**Table 3** The percentage of different types of cells in the wounds after treatment

	Fibroblast	Fibrocyte	Inflammatory cell	Keratin	Epidermal cell	Gland cell	Adipocyte	Collagen	Elastic fiber
A	+++	+++	++		++	++	+	+++	++
B	+++	+++	+++	++	+++	+++	+++	+++	++
C	+	+	+						++
D	+++	+++	+			++	++	++	++
E	+	+	+						
F	+	+	+		+	++	++	++	+++

Abbreviations: HE, hematoxylin-eosin; SP, *Senna podocarpa*; VVG, Verhoeff–Van Gieson.

Note: Mice with wounds was treated with (A) 2.5% SP emulgel; (B) 7.5% SP emulgel; (C) 0% SP emulgel; (D) silver sulfadiazine cream; (E) the control; and (F) SP leaf powder dispersed in water to form a paste and applied topically. 14 days later, HE and VVG staining was performed in the wounds, and the percentages of different types of cells in the wounds were graded as: (+) slight level,  $<30\%$  staining; (++) moderate level,  $30\text{--}70\%$  staining; and (+++) high level,  $>70\%$  staining.





**Fig. 6** Percent wound contraction. Mice with wounds were treated with (A) 2.5% SP emulgel; (B) 7.5% SP emulgel; (C) 0% SP emulgel; (D) silver sulfadiazine cream; (E) the control; and (F) SP leaf powder dispersed in water to form a paste and applied topically. After 14 days, wound contraction was calculated. Each bar denotes mean  $\pm$  standard deviation ( $n = 6$ ). SP, *Senna podocarpa*.

wound contraction, which was much lower than that of SP emulgel, suggesting that the formulation exhibits the remarkably increasing effect in wound therapy. We proposed that the antiwound effect of SP extract may be attributed to its high content of flavonoid, which is responsible for healing attributes, as well as enhancing vascularization, and decreasing lipid peroxidation that eventually prevents cellular necrosis and promotes cell proliferation.<sup>31</sup> The occlusive potential of emulgel could be responsible for the better healing properties exhibited by SP emulgel over silver sulfadiazine cream and SP poultice.<sup>32</sup>

## Conclusion

SP emulgel prepared with Tween 20/Span 20 mixture showed acceptable physical properties. In this work, the promotion effect of SP extract and its formulation in the process of wound healing was first reported through a histology evaluation. Phytochemical analysis suggested several antioxidants in the extract, which may be responsible for the wound healing activities by controlling wound oxidative stress, and thereby accelerating the wound healing. SP emulgel has superior pharmacological activity over SP poultice. The formulation of 7.5% of SP emulgel has been proved to be the formula of choice with a maximum effect on inhibiting edema and burn while promoting wound healing. SP emulgel could be a promising natural dressing for effective treatment of burn wounds.

### Conflict of Interest

The authors declared no conflict of interest.

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