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Evaluation of Aqueous Methanol Stem Bark Extract of Stereospermum kunthianum for Wound Healing Effects in Alloxan-induced Diabetic Rats

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Abstract

Background and Purpose: The stem bark extract of *Stereospermum kunthianum* (Family: *Bignoniaceae*) is used in traditional medicine to treat wounds, bronchitis, pneumonia, coughs, gastritis, rheumatic arthritis, ulcers, dysentery, leprosy, venereal diseases, and other inflammatory and pain-related health conditions in humans. The aim of this study was to evaluate the wound healing, analgesic and anti-inflammatory properties of the stem bark extract of S. kunthianum in alloxan-induced diabetic rats for development into a drug development. **Experimental Approach:** The aqueous-methanol extract of S. kunthianum was analyzed for phytochemical constituents. The aqueous-methanol stem bark extract of the plant was evaluated for 72 h for its acute toxicity in the adult Wistar rats. Similarly, the extract of *Stereospermum kunthianum* was evaluated for wound healing using excision and incision wound healing models in diabetic-induced rats.

Results: The results revealed that saponins, terpenes, tannins and steroids were present in the extract. Its acute toxicity profile was determined in the diabetic rats. In the acute toxicity studies, there was no observed toxicity sign up to the dose up to 5000 mg/kg p.o. and i.p. The estimated oral and intraperitoneal median lethal dose (LD_{50}) of the extract was ≥ 5,000 mg/kg. The *S. kunthianum* stem bark extract (100 mg/kg p.o, 200 mg/kg p.o, 400 mg/kg p.o) caused significant reduction of wound area. The excision wound contraction (%) in rats treated with *S. kunthianum* stem bark extract (100 mg/kg p.o, 200 mg/kg p.o, 400 mg/kg p.o.) was most effective and

had 100% wound contraction as when compared to the controls groups (distilled water; 10 ml/kg and Vitamin C; 100 mg/kg, p.o.) which had 92.3% and 88.0% respectively. *S. kunthianum* stem bark extract (100, 200, 400 mg/kg, p.o.) caused a complete (100%) epithelialization after 25 days of treatment, while Vitamin C (100 mg/kg p.o.) and the negative control (distilled water; 10 ml/kg) had epithelialization of 85% and 86% within the said period. The extract (400 mg/kg, p.o.) produced the shortest mean wound closure time (WC_{50}) of 5 days, the extract (100, 200 mg/kg, p.o.) had WC_{50} of 6 days and 7 days, respectively, while Vitamin C (100 mg/kg, p.o) and distilled water (10 ml/kg, p.o.) had the mean wound closure time (WC50) of 8 days each. In the Incision wound studies, the *S. kunthianum* stem bark extract (100, 200,400 mg/kg, p.o) showed a progressive rate of wound repair with increasing doses of the extract. However, there was a dose-dependent increase in the tensile strength with values of 983.5 g, 984.4 g and 1066.0 g when the extract was tested at 100, 200 and 400 mg/kg, respectively.

Conclusion: The results obtained from the research corroborated the ethno-medicinal claims and have given the scientific justification for the use of *S. kunthianum* stem bark extract for wound healing.

Implication: The *S. kunthianum* stem bark extract therefore has the potential to be developed as a wound healing agent.

Key Words: Wound healing, Diabetes mellitus, *Stereospermum kunthianum* stem bark extract, surgical wounds (excision and incision), tensile strength, phytoconstituents, wound contraction, mean wound closure time, wound epithelialization.

Introduction

There is urgent need for discovery of targeted drugs that will enhance wound healing in diabetic patients. The skin is the body's largest organ which covers the entire external surface of the body [1]. The skin as the external envelope of the body serves as a physical barrier, mainly by the structure of the *Stratum corneum* which constitutes the primary defense against environmental physical aggressions and external pathogen invasion [2]. Wounds are injuries that break or cause disruption of the cellular and anatomical continuity of the skin or other body tissues [3]. Causes of injury may be mechanical, chemical, microbial, immunological, electrical, thermal, or nuclear sources [4]. Following skin injury, the damaged tissue is repaired through the coordinated biological actions that constitute cutaneous healing. In mammals, repaired skin is not identical to intact uninjured skin; however, this disparity may be caused by differences in the mechanisms that regulate post-natal cutaneous wound repair compared to embryonic skin development [5]. Wounds represent a major global health challenge, which puts much economic, financial, and social stress on health institutions, caregivers, patients, and their families causing disability and loss productivity [6]. A Chronic Non-Healing Wound (CNHW) is typically correlated with comorbidities such as diabetes [7, 8]. Diabetes is associated with impaired wound healing, making patients susceptible to chronic non-healing wounds [9]. Diabetes mellitus is a chronic, non-communicable metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads to serious damage of the heart, blood vessels, eyes, kidneys and nerves [10]. This disease is also associated with symptoms such as polyuria, fatigue, weight loss, delayed wound healing, blurred vision, increases in urine glucose levels, etc. [11]. Impaired healing in diabetes is the result of a complex pathophysiology involving vascular, neuropathic, immune, and biochemical components [12]. One of the main

consequences of diabetes is the impairment of self-repairing abilities [13]. Diabetes mellitus is one of the leading and major contributors to chronic wound healing problems. Diabetic patients with ulcers are at high risk for major complications which include infection and amputation [14, 15]. The risk for lower extremity amputation is 15 to 40 times higher in people with diabetes than people without diabetes [16]. The main reason for skin wound healing is the restoration of the barrier function in order to prevent further damage or infection. This process of wound healing requires the distinct interplay and crosstalk of a multitude of cells and mediators from the onset [17]. Wound healing is a dynamic, interactive, complex process that involves soluble mediators, blood cells, extracellular matrix, parenchymal cells, and tissue layers of replacing devitalized and missing cellular structures, and tissue layers [18, 19]. The intricate skin repair process has been organized in four sequential and overlapping steps: the hemostasis phase, the inflammatory phase, the proliferative phase, and the remodeling phase [20]. Due to simplicity in the measurement of wound healing responses, the excision and incision skin-wound healing models in rodents are by far the most convenient and reliable methods of study for potential new therapeutic agents [21]. Medicinal plants are the richest biosource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [22]. In recent years, several studies have been carried out on herbal drugs to explicate their potential in wound management and these natural remedies proved their effectiveness as an alternative treatment to available synthetic drugs for the treatment of wounds [23]. *Stereospermum kunthianum* belonging to the family Bignoniaceae, also referred to as pink jacaranda in English, is an African deciduous shrub found widespread across Africa to the Red Sea. There are over thirty species within Central Africa and Asian distribution [24, 25]. The *Stereospermum kunthianum*, Cham, Sandrine Petit (Family: *Bignoniaceae*) is used in traditional medicine to treat bronchitis, pneumonia and cough, gastritis, wounds, rheumatic arthritis, ulcers, dysentery, leprosy and venereal diseases in humans [26]. The plant is mainly used by the local people of Mayo–Danay Cameron as a wound healing agent [21].The present study aimed at evaluating the stem bark extract of *S. kunthianum* to authenticate its ethnomedicinal usage for wound healing in alloxan-diabetic rats and for future drug development.

Methods

Study area

The study was carried out at the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja. The period of research was from May, 2021 - May, 2022.

Plant collection and identification

Fresh stem bark of *Stereospermum kunthianum* was collected from Suleja, Niger State, which is situated at 10000’N 6000’E, Nigeria. The plant sample was identified by a plant Taxonomist with Herbarium Unit, Department of Medicinal Plants Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja.

Preparation of plant extract

Stem bark of *Stereospermum kunthianum* was washed with clean water after collection and then cut into small pieces with a knife. Then, the stem bark of *S. kunthianum* was air-dried and pulverized using a mortar and

pestle. One thousand six hundred grams (1.60 kg) of the pulverized sample was macerated successively in 5 liters of 80% v/v methanol under a temperature of 40°C on a shaker (GFLD 3006 mgH, Germany) with agitation to ensure maximum extraction. Double maceration was done over a period of 24 h each and the extract was then filtered with Whatman size 1 filter paper. The filtrate was concentrated using rotary evaporator (KNF RC 900, Neuberger, USA). The concentrate was then placed over a water bath to ensure proper dryness of the extract. The percentage yield of the extract was calculated as follows:

$$\% \text{ Yield} = \frac{W_1}{W_2} \times 100$$

Where,

W1=weight of dry extract after extraction;

W2=weight of the stem bark before extraction.

The extract was stored in a closed container, kept in a refrigerator at 4°C for subsequent studies.

Phytochemical analyses

The phytochemical screening of the crude extract was done using the standard method of Trease and Evans [27]. The extract was screened for the presence or absence of various chemical constituents like saponins using the froth test, terpenes using Libermann-Buchard test, tannins using ferric chloride test, steroids using Salkowski test, flavonoids using ferric chloride test, anthraquinones using Borntrager's test, carbohydrates using Molisch test and alkaloids using Dragendorff's test.

Chemicals and drugs

Methanol (Fluka Chemie, Switzerland), Alloxan Monohydrate (Sigma Aldrich, USA), Accu-Chek active test strips (mg/dl: Indiana USA), Xylazine (20 mg/ml: Bioverta Czech Republic), Ketamine Hydrochloride injection (Jawa Ketamine, India), Vitamin C (Ascorbic acid; Emzor Pharmaceutical Industries Ltd., Lagos Nigeria), were used for the studies.

Animals

Adult Wistar rats of both sexes used for the study were procured from the Animal Facility Centre, Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRID), Abuja. The experimental rats were separated for at least two weeks in the experimental room for acclimatization. The rats were maintained under normal environmental temperature, approximately normal 12 h day and night illumination cycle. They were allowed free access to standard feed and water *ad libitum* throughout the experimental period except when starvation was needed during the experiment.

Ethical approval

The study was approved by the Ethical Committee on Animal Use (UAECAU), Faculty of Veterinary Medicine, University of Abuja, Nigeria, with reference number: UAECAU/2020/0002. The animal handling was in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council [28].

Acute toxicity study

The modified method of Lorke was adopted for the study [29]. The method was used to estimate the dose of the extract that will kill 50% of the treated rat population (median lethal dose: LD). The study was carried out in adult Wistar rats using both oral and intraperitoneal routes (two phases). Nine rats were randomly allocated into three groups of three rats each. Groups 1, 2, and 3 were administered with the stem bark extract of *Stereospermum kunthianum* intraperitoneally at doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg, respectively. Similarly, another set of animals were grouped as described above and given the stem bark extract of *Stereospermum kunthianum* by oral route. In the second phase of the study, three rats were placed in three groups of one animal each. Groups 1, 2, and 3 received the extract at doses of 2000 mg/kg, 3000 mg/kg and 5000 mg/kg, respectively; using the intraperitoneal route. The second of oral administration was carried out as described for the intraperitoneal route. The treated rats were then observed for 72 h for behavioral and/or toxic effects such as nervousness, ataxia, excitement, alertness, dullness, and death. The LD_{50} of the extract was calculated using the formula [30]:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where D_0 = Highest dose that gave no mortality, and D_{100} = Lowest dose that produced mortality. All rats that survived were further monitored for two weeks for toxic effects.

Induction of diabetes by alloxan monohydrate

Alloxan monohydrate (Sigma Aldrich Inc. USA) was used to induce diabetes mellitus. The drug was prepared for injection by dissolving in distilled water (20 mg/ml) A total of fifty (50) adult Wistar rats (150 g-200 g) were fasted for 24 h (depriving them access to feed and water). The rats were then individually weighed with a weighing balance. Blood was drawn from the tail of each rat, spotted on glucose test strips and the baseline blood glucose levels of the rats were obtained using Accu-chek glucometer and glucose test strips. Alloxan (115 mg/kg) was administered by the route intraperitoneally to each rat. The rats resumed normal feeding after the induction. The blood glucose level in mmol/L of each rat was measured after 48 hours of induction using a glucometer by using the tail tipping blood sample technique [14]. Rats with blood glucose of ≥ 11 mmol/L were considered diabetic. Diabetes in rats is frequently defined as fasting blood glucose above 7 mmol/L (126 mg/dL) [31, 32]. The diabetic rats were subsequently used for further studies.

Wound healing studies in diabetic rats

Excision and incision wound models were used to evaluate the wound healing activity of the stem bark extract of *Stereospermum kunthianum* in diabetic-induced rats.

Excision wound study in diabetic rats

Twenty-five diabetes-induced adult Wistar rats (150 g-200 g) of both sexes were used for the study. The rats were inflicted with excision wounds using the modified methods of Rupesh *et al* and Nwinyi *et al* [33, 34]. The predetermined areas of wound infliction at the back of the rats were prepared for surgery by shaving their

dorsal hair with shaving sticks and razor blades followed by cleaning with 70% alcohol. Each of the rats was anaesthetized using xylazine (10 mg/kg, i.p.) followed by ketamine (80 mg/kg, i.p.) prior to the creation of the wounds. The excision wound site was outlined on the back of each of the rats using a marker and ruler. The excision was inflicted on the dorsal region 1.5 cm away from the vertebral column and 5 cm away from the ear. A measuring ruler was used to measure the distance marked for wound creation. A full thickness of excision wound area of 4 cm^2 (2 cm × 2 cm) was created along the outlined area of the shaved back using a surgical blade, toothed forceps, and pointed scissors. The entire wound was left open according to Diwan *et al* [35]. Haemostasis was achieved by dabbing the wound with a cotton wool swabs soaked in normal saline [34, 36, and 37]. Following recovery from anaesthesia after surgery (creation of wounds), the rats were housed individually and allotted into five groups (A-E), of five rats each. Distilled water (10 ml/kg, p.o.) was administered to the rats in group A and served as a negative control. Three doses (100 mg/kg, 200 mg/kg, 400 mg/kg) of the stem bark extract of *Stereospermum kunthianum* were administered orally to the second, third, and fourth groups of rats (groups B, C and D), respectively. Vitamin C (100 mg/kg p.o.) was given to the fifth group (group E) and serves as the positive control group. Treatment commenced on the day of wound creation and continued until the wound healed (i.e., until complete epithelialization). Changes in the size of the excision wound in each experimental rat on days 5, 10, 15, 20 and 25 were recorded accordingly [38]. Gross observation of the wounds was made; wound contraction and period of epithelialization were also determined. The diabetic level of each rat was monitored throughout the period of the research.

Wound contraction

The size of the wound on each of the diabetic-induced rats was traced on transparent paper and placed on millimetre scale graph paper to estimate the wound area every five days until the wound was completely healed. Wound contraction contributes to wound closure and was expressed as a reduction in the percentage of the original wound size from day of wound creation until the day of complete epithelialization. It was used to calculate the degree of wound healing and represented as a percentage of healing wound area [39]. Percentage wound contraction was calculated using the formula:

$$\text{Wound Contraction (\%)} = \frac{W_{DO} - W_{DE}}{W_{DE}} \times 100$$

Where: W_{D0}=wound area on Day 0;

W_{DE} =wound area post excision day on or before complete epithelialization.

Period of epithelialization

This was expressed as the number of days required for falling of the eschar (dead tissue remnants) without any residual raw wound. Wounds were considered closed (completely healed) when moist granulation tissue was no longer apparent and the wound was covered with new epithelium [40, 41]. Epithelialization is useful for assessing the progress of wound healing in patients. In each wound, the percentage of wound epithelialization was calculated by the following formula [42].

Percentage epithelialization = (open wound area/total wound area) × 100

Mean wound closure time (WC_{50})

This is the time taken for 50% of wound closure and was read off on a plot of wound closure (%) against time (days).

Incision wound study in diabetic rats

Twenty-five diabetic-induced adult Wistar rats (150 g-200 g) of both sexes were used for the study. The dorsal hair of each rat was clipped and disinfected with 70% alcohol. Each of the rats was sedated with xylazine (10 mg/kg, i.p.) followed by ketamine (80 mg/kg, i.p.) to achieve anaesthesia prior to the creation of the wounds. Two paravertebral incisions were made through the skin and cutaneous muscles at a distance of 1.5 cm from the midline on either shaved side of the vertebral column with a sterile sharp blade. Each incision was 4 cm in length. A measuring ruler was used to measure the distance marked for wound creation and was used on all rats to ensure the accuracy of dimensions for the incision wounds [43]. After complete haemostasis, which was achieved by dabbing the wound with cotton wool soaked in normal saline, the parted skin was stitched with interrupted sutures at intervals of 0.5 cm-0.6 cm using a black braided silk surgical thread (size no.0) and a curved needle (3/8 semicircle-curved cutting: 35 mm). The wounds were left undressed and mopped with cotton wool [44, 45]. The alloxan-induced diabetic rats were grouped into five groups (A-E), of five rats each. Distilled water (10 ml/kg, p.o.) was administered to the first group of rats and served as a negative control. Three doses (100, 200, 400 mg/kg) of the stem bark extract of *S. kunthianum* were administered orally to the second, third, and fourth groups of rats, respectively. Vitamin C (100 mg/kg, p.o.) was given to the fifth group (group E) and to serve as the positive control group. On the 7^{th} day of the wound creation and of treatment, the sutures were removed while the treatment continued. The skin-breaking strength of the healed wound was measured on the 10^{th} day according to the method described by Garg *et al*, Krishnaveni *et al*, and Nwinyi *et al*. The anaesthetized rats were secured on the table and a line was drawn on either side of the wound 3 mm away from the suture line [46]. These lines were gripped using two forceps applied firmly on to the line facing each other. One of the forceps was supported firmly; whereas the other was connected to a freely suspended light-weight bag. Weight was added gradually to the bag. A gradual increase in weight got transmitted to the wound site pulling apart the wound edges. As soon as wound gaping appeared, the addition of weight was stopped and the weight added to the bag was determined by weighing it on a measuring scale. The procedure was repeated on the contra lateral wound of each of the rats. The two values obtained from each rat were summed together and the mean weight for each rat was obtained to represent the tensile strength of the wound of each rat. The mean reading for the group was then given as the tensile strength for a given group which was measured in grammes. Gross observations of the incision wounds were made and determination of tensile strength of the incision wounds in respect of the varying group treatments at different time intervals was performed accordingly. The diabetic level of each rat was monitored throughout the period of the research.

Data analysis

IBM SPSS Statistics version 23 was used for the statistical analyses. The results of the study were expressed as mean $\pm$ SEM. The data generated from the studies were analysed using One-way analysis of Variance (ANOVA) where appropriate. Tukey Post Hoc Test was used to determine the differences between treatment groups. P-values <0.05 were taken to be statistically significant. Results were presented as tables, figures, and plates as appropriate.

Results

Plant extract

The weight of the pulverized *S. kunthianum* stem bark was 1600.0 g, while the weight of *S. kunthianum* extract was 279.0 g. The percentage yield was 17.44%. The extract was dark brown in colour, oily, and slurry in consistency.

Phytochemical analysis

The phytochemical analyses carried out on the crude stem bark extract showed the presence of saponins, terpenes, tannins, and steroids **(Table 1).**

Table 1: Phytochemical constituents of aqueous methanol extract of *S. kunthianum* stem bark extract.

Chemical Constituents	**Inference**
Anthraquinones	Absent
Carbohydrates	Absent
Flavonoids	Absent
Saponinins	Present
Steroids	Present
Tannins	Present
Terpenes	Present

Acute toxicity studies

The results of the acute toxicity tests with *S. kunthianum* stem bark extract are shown in **Table 2**. No toxicity sign or death was observed in the rats 72 h after oral treatment with *S. kunthianum* stem bark extract (10 mg/kg–5,000 mg/kg). The estimated oral median lethal dose (LD50) of the extract in rats was therefore >5,000 mg/kg. The experimental rats did not exhibit acute signs of toxicity or death within 72 h even at the maximal dose of 5000 mg/kg following intraperitoneal administration of *S. kunthianum* stem bark extract. The intraperitoneal median lethal dose (LD_{50}) of the extract in rats was also estimated therefore to be greater than 5,000 mg/kg.

Table 2: Acute toxicity study of *Steroespermum kunthianum* stem bark extract.

Treatment, *S. kunthianum* (p.o.; i.p.)	**Number of dead rats**	**Number of rats alive**
Phase I		
10 mg/kg	0/3	0/3

100 mg/kg	0/3	0/3
1000 mg/kg	0/3	0/3
Phase II		
2000 mg/kg	0/3	0/3
3000 mg/kg	0/3	0/3
5000 mg/kg	0/3	0/3

Key: 0/3 =Number of animals which died/number of animals used; 3/3 = Number of animals alive/number of animals used.

Excision wound studies in diabetic rats

Gross Observation of the Excision wounds

Gross observation of the excision wounds on the diabetic rats showed that rats in the negative control group (distilled water: 10 ml/kg, p.o.) healed more slowly with scar tissues which were very visible when compared with rats treated with *S. kunthianum* at 100 mg/kg, 200 mg/kg, 400 mg/kg; p.o. from day 5 to day 25 after excision. The wounds in the positive control group (Vitamin C; 100 m/kg, p.o.) were fresh and healed at a much slower pace, while all wounds in rats treated with Stereospermum kunthianum stem bark extract at 100 mg/kg, 200, mg/kg 400 mg/kg; p.o. completely healed with no scar formation by the 25th day post treatment (Plates 1-5).

On Day 0 of creating excision wounds, the mean wound size of all the groups was 4.00 cm^2.

Wound contraction

The effect of *S. kunthianum* stem bark extract on the wound closure of diabetic rats can be seen in **Table 3**. The percentage wound contraction post treatment in each experimental group was determined on days 5, 10, 15, 20 and 25.

At day 5, administration of distilled water (10 ml/kg, p.o.) caused the wound to decrease in size from 4.00 cm^2 ± 0.00 cm^2 to 3.58 cm^2 ± 0.4 cm^2. Thus, a 10.5% contraction was produced. This contraction was significantly ($p<0.05$) different from the contraction in the treatment groups. Vitamin C produced 40.5% contraction while *S. kunthianum* extract had 41.0%, 42.5%, and 46.0% contraction at 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively **(Table 1, 3 and 4, Plate 1).**

At day 10, The mean excision wound size of non-treated control group (Group A; distilled water; 10 ml/kg, p.o.) was 1.57 cm^2 ± 0.3 cm^2 relative to 3.58 ± 0.4 cm at Day 5; the percentage wound contraction was 60.8%. The wound size in 100 mg/kg extract treated rats was 1.11 cm^2 ± 0.1 cm compared to 2.36 cm^2 ± 0.1 cm2 on day 5; the percentage wound contraction produced was 72.3%. Similarly, the extract at 200 and 400 mg/kg significantly ($p<0.05$) reduced the excision wound sizes of 2.30 cm ± 0.0 cm and 2.16 ± 0.1 on day 5 to 0.93 cm ± 0.1 cm and 0.64 cm^2 ± 0.1 cm which are equivalent to 76.8% and 84.0% wound contraction, respectively. The contraction produced by the extract was significantly ($p<0.05$) different from the negative control at all tested doses (100 mg/kg, 200 mg/kg, 400 mg/kg, p.o.). Vitamin C, however, produced the lowest wound contraction of 57.0% compared to the other groups; the mean wound size was reduced from 2.38 cm ± 0.2 cm to 1.72 ± 0.2 cm **(Table 1, 3 and 4, Plate 2).**

At day 15, administration of distilled water (10 ml/kg, p.o.) was able to reduce the excision wound size from 3.58 cm ± 0.4 cm to 0.90 cm ± 0.1 cm, (77.5% wound contraction) within the period. *S. kunthianum* stem bark extract at 100 mg/kg and 200 mg/kg, p.o. achieved a reduction in wound sizes between 2.36 cm ± 0.1 cm to 0.58 cm ± 0.1 cm (85.5%) and 2.30 cm ± 0.0 to 0.62 ± 0.0 cm (84.5%), respectively. The extract at 400 mg/kg, p.o. was however, able to significantly ($p<0.05$) produced a reduction in the size of excision wound from 2.16 cm ± 0.1 cm to 0.42 cm ± 0.1 cm (89.5% wound contraction). In the positive control treated with Vitamin C (100 mg/kg, p.o.), the wound size was reduced from 2.38 cm ± 0.2 cm to 1.33 cm ± 0.2 cm, (66.8% wound contraction). Vitamin C was observed to have exerted the lowest percentage wound contraction in all test groups (**Table 3, 4, Figure 1, and Plate 3**).

At day 20, administration of distilled water (10 ml/kg, p.o) was able to reduce the excision wound size in rats from 3.58 cm ± 0.4 cm to 0.64 cm ± 0.1 cm, (84.0% wound contraction) within the period. *S. kunthianum* stem bark extract at 100 mg/kg, 200 mg/kg, 400 mg/kg, p.o. achieved significant ($p<0.05$) reductions in the wound sizes in rats from 2.36 cm ± 0.1 cm to 0.25 cm ± 0.0 cm (93.8%) 2.30 cm ± 0.0 cm to 0.27 cm ± 0.0 cm (93.3%) and 2.16 cm ± 0.1 cm to 0.18 cm ± 0.0 cm respectively. In the positive control treated with Vitamin C (100 mg/kg, p.o.), the wound size got reduced from 2.38 cm ± 0.2 cm to 0.74 cm ± 0.1 cm, (81.5% wound contraction (**Table 1, 3 and 4, Plate 4**).

At day 25; administration of distilled water (10 mg/kg, p.o) reduced the excision wound size of 3.58 cm ± 0.4 cm to 0.31 cm ± 0.1 cm, (92.3% wound contraction). *S. kunthianum* stem bark extract at 100, 200, 400 mg/kg, p.o. significantly ($p<0.05$) achieved a complete reduction in the wound size of 100% in all treated groups. In the positive control (Vitamin C; 100 mg/kg, p.o.) group, the wound size reduced from 2.38 cm ± 0.2 cm to 0.48 cm ± 0.1 cm (88.0% wound contraction) (**Table 1, 3 and 4, Plate 5**).

The wound area contraction effect caused by the *S. kunthianum* stem bark extract (100 mg/kg, 200 mg/kg, and 400 mg/kg, p.o) was significant compared to that caused by Vitamin C (100 mg/kg, p.o.) on the days post treatment **(Table 4 and Figure 1).** The S. kunthianum stem bark extract (100 mg/kg, 200 mg/kg, 400 mg/kg p.o) caused significant ($p<0.05$) contraction of wound area on days 5, 10, 15, 20 and 25 when compared to the negative control (distilled water; 10 ml/kg p.o.; **Table 3**). However, *S. kunthianum* stem bark extract (400 mg/kg, p.o.) was the most effective dose **(Plate 5).**

Table 3: Effect of aqueous methanol stem bark extract of *Stereospermum kunthianum* administered orally on excision wound contraction in diabetic rats.

Treatment	**Wound Contraction (cm^2)**				
	Day 5	**Day 10**	**Day 15**	**Day 20**	**Day 25**
Distilled water (10 ml/kg)	3.58 ± 0.4	1.57 ± 0.3	0.90 ± 0.1	0.64± 0.1	0.31 ± 0.1
***S. kunthianum* (100 mg/kg p.o)**	2.36 ± 0.1*	1.11 ± 0.1*	0.58 ± 0.1*	0.25± 0.0*	0.00 ± 0.0*
***S. kunthianum* (200 mg/kg p.o)**	2.30 ± 0.0*	0.93 ± 0.1*	0.62 ± 0.0*	0.27± 0.0*	0.00 ± 0.0*

***S. Kunthianum* (400 mg/kg p.o)**	2.16 ± 0.1*	0.64 ± 0.1*	0.42± 0.1*	0.18±0.0*	0.00 ± 0.0*
Vitamin C (100 mg/kg p.o)	2.38 ± 0.2*	1.72 ± 0.2	1.33 ± 0.2	0.74 ±0.1	0.48 ± 0.1
S.k = *Stereospermum kunthianum*; n=5					

Values are expressed as mean ± SEM (n=5); *P<0.05, significantly different from the control; One-way ANOVA; Tukey post hoc; *the mean difference is significant at the level of 0.05.

Table 4: Effect of aqueous methanol extract of *Stereospermum kunthianum* stem bark on excision wound contraction (%) in diabetic rats.

Treatment (mg/kg)	**Wound contraction (%) Days**				
	Day 5	Day 10	Day 15	Day 20	Day 25
D.w (10 ml/kg)	10.50%	60.80%	72.50%	84.00%	92.30%
S.k (100)	41%	72.30%	85.50%	93%	100%
S.k (200)	42.50%s	76.80%	84.50%	93%	100%
S.k (400)	46%	84%	89.50%	95%	100%
Vitamin C (100)	40.50%	57%	66.80%	81%	88%
S.k: *Stereospermum kunthianum*; D.w: Distilled water					

Values are expressed as percent wound contraction (n=5)

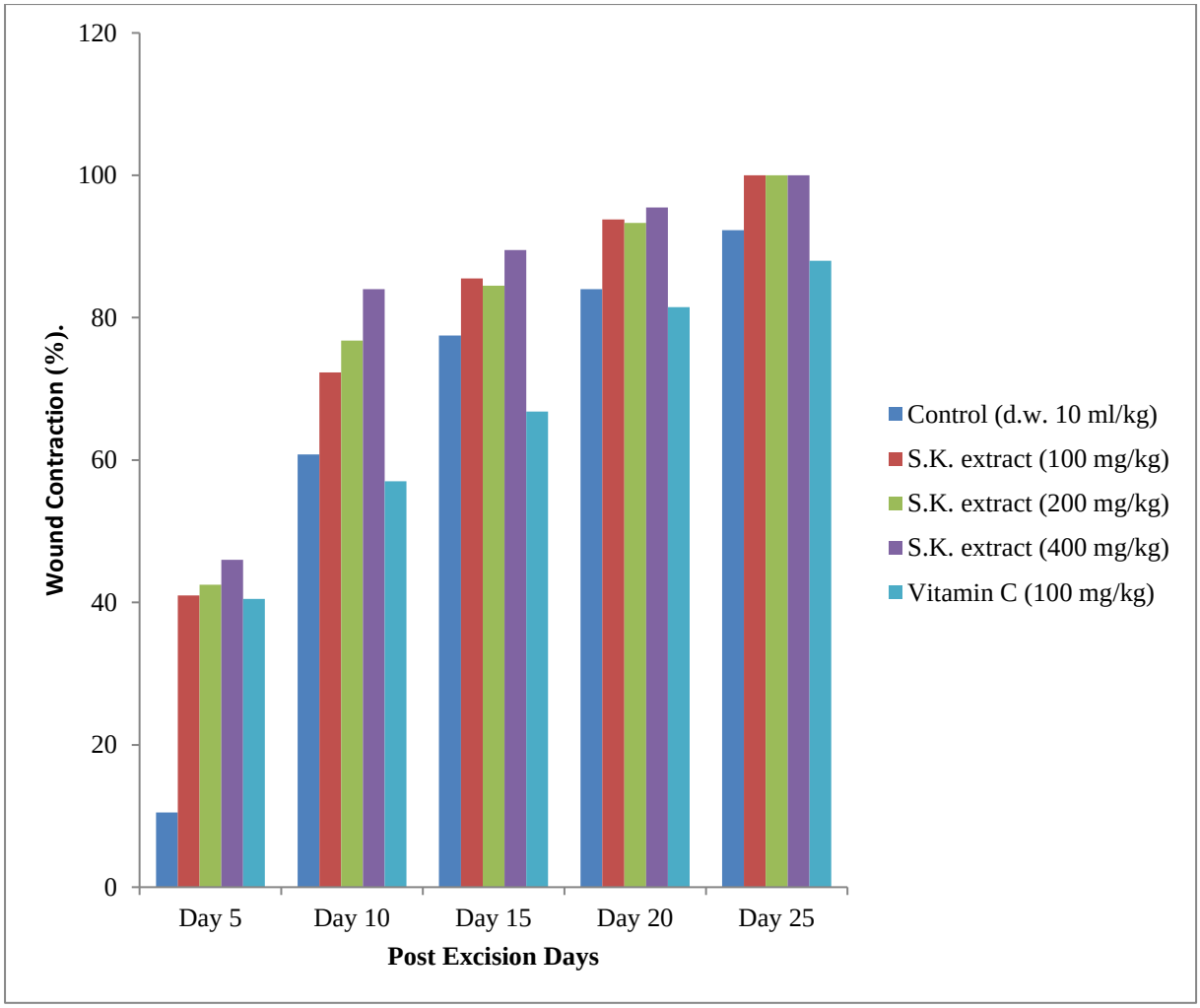

Figure 1: Effect of aqueous- methanol extract of *S. kunthianum* stem bark on excision wound contraction percentage (%) in diabetic rats.

Significantly ($p<0.05$) different compared to control; d.w. = distilled water; S.K. = *Stereospermum kunthianum* stem bark extract**.**

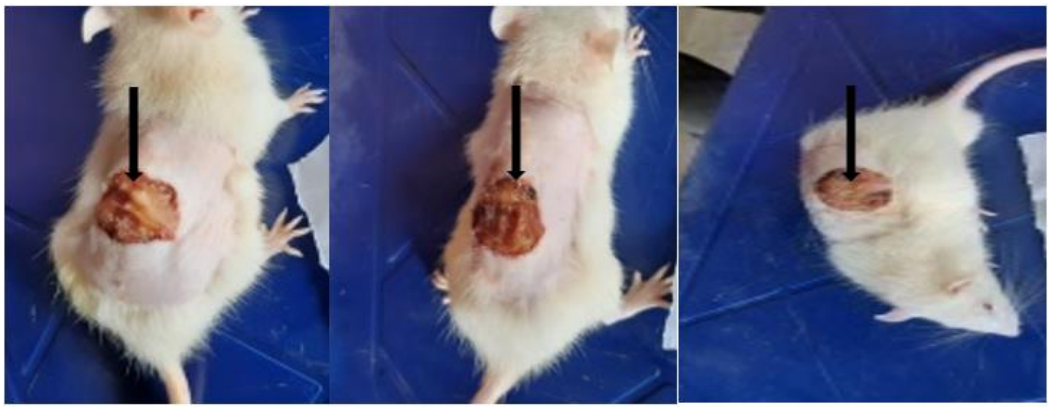

Plate 1: The wound healing effect of *Stereospermum kunthianum* stem bark extract on excision wound in diabetic rats on day 5 post excision.

A: Negative control (distilled water: 10 ml/kg p.o); B: *S.kunthianum* (100 mg/kg p.o.);

C: *S.kunthianum* (200 mg/kg p.o.); D: *S.kunthianum* (400 mg/kg p.o.); E: Vitamin C (100 mg/kg p.o.)

Plate 2: The wound healing effect of *Stereospermum kunthianum* stem bark extract on excision wound in diabetic rats on day 10 post excision.

A: Negative control (distilled water: 10 ml/kg p.o.); B: *S.kunthianum* (100 mg/kg p.o.);
C: *S. kunthianum* (200 mg/kg p.o.); D: *S.kunthianum* (400 mg/kg p.o.); E: Vitamin C (100 mg/kg p.o.)

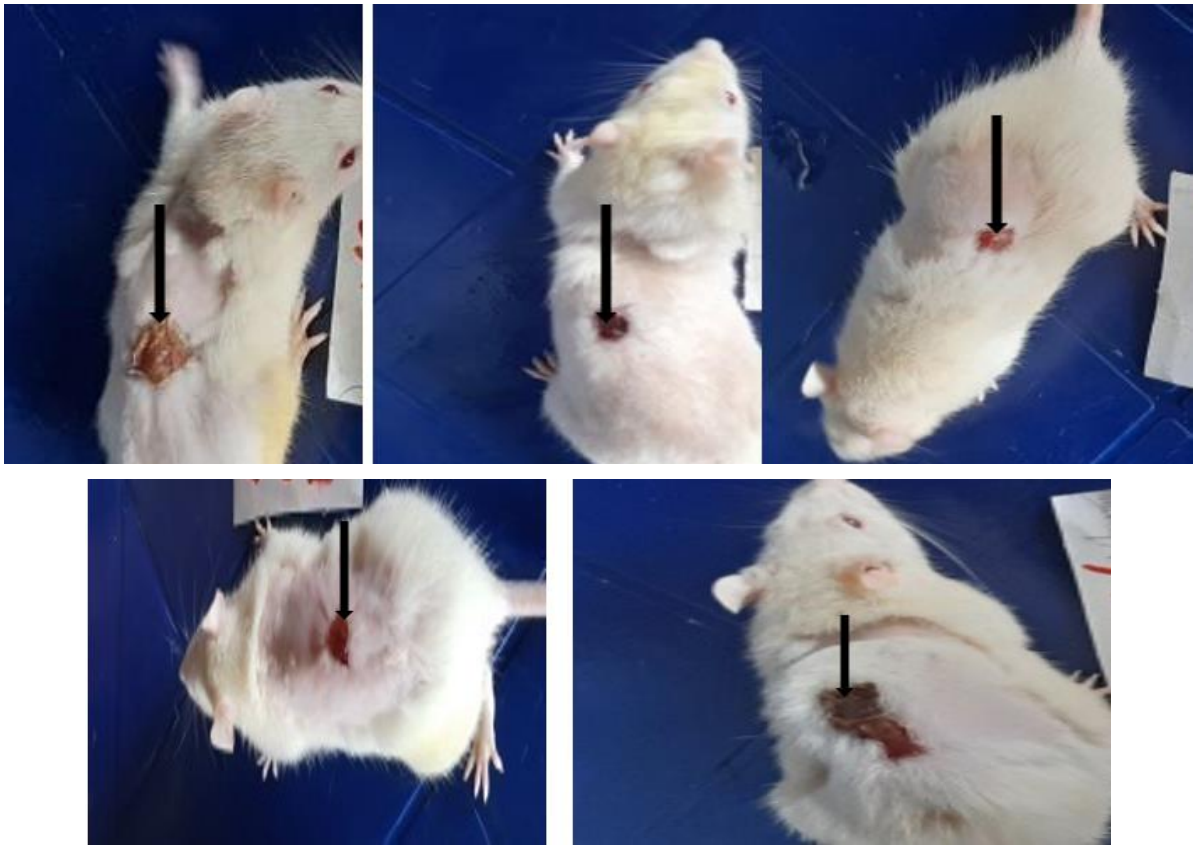

Plate 3: The wound healing effect of *Stereospermum kunthianum* stem bark extract on excision wound in diabetic rats on day 15 post excision.

A: Negative control (distilled water: 10 ml/kg p.o.); B: *S. kunthianum* (100 mg/kg p.o);
C: *S. kunthianum* (200 mg/kg p.o.); D: *S. kunthianum* (400 mg/kg p.o.); E= Vitamin C (100 mg/kg p.o.)

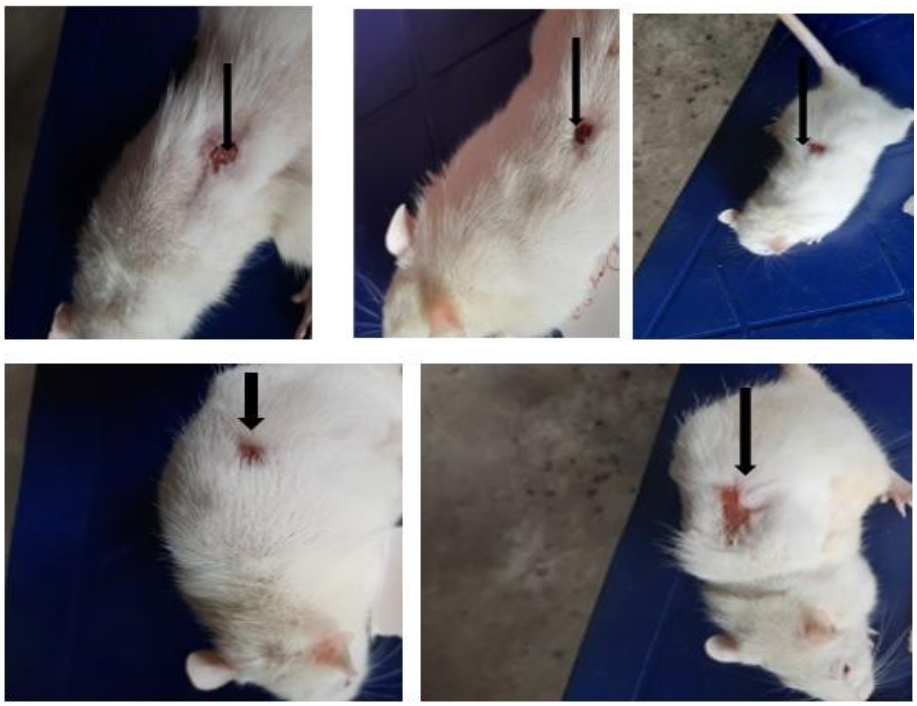

Plate 4: The wound healing effect of *Stereospermum kunthianum* stem bark extract on excision wound in diabetic rats on day 20 post excision.

A: Negative control (distilled water: 10 ml/kg p.o.); B: *S. kunthianum* (100 mg/kg p.o.); C: *S. kunthianum* (200 mg/kg p.o.); D: *S. kunthianum* (400 mg/kg p.o.); E: Vitamin C (100 mg/kg p.o.).

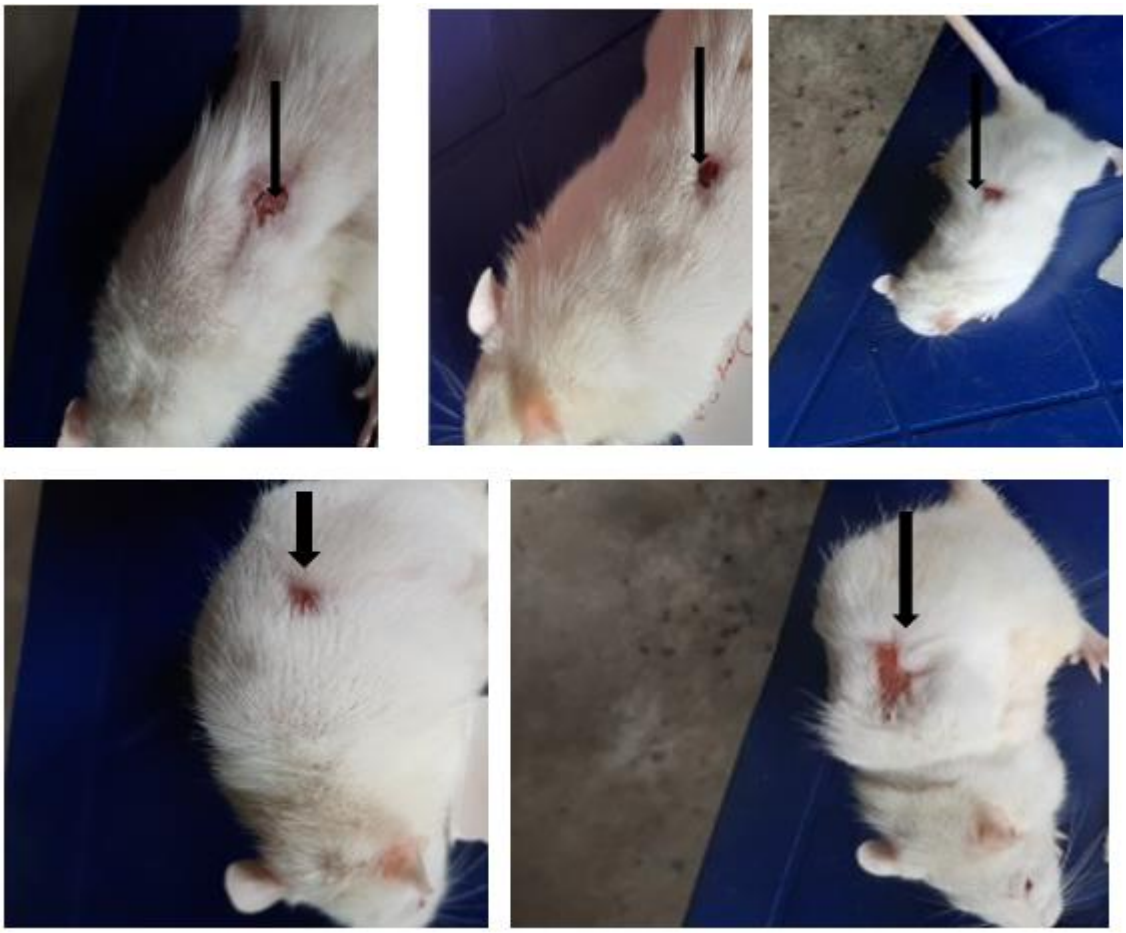

Plate 5: The wound healing effect of *Stereospermum kunthianum* stem bark extract on excision wound in diabetic rats on day 25 post excision.

A: Negative control (distilled water: 10 ml/kg p.o.); B: *S.kunthianum* (100 mg/kg p.o.); C: *S. kunthianum* (200 mg/kg p.o.); D: *S. kunthianum* (400 mg/kg p.o.); E: Vitamin C (100 mg/kg p.o.)

Period of epithelialization

In all test rats, there was no epithelialization of the excision wounds at days 1 and 5 **(Table 5).** Epithelialization commenced, however, on day 10 in the negative control (distilled water, 10 mg/kg, p.o.) rats had 65% compared to 62.3%, 63%, 64% with the respective oral doses of 100 mg/kg, 200 mg/kg, 400 mg/kg of *S. kunithanum* extract. Vitamin C (100 mg/kg p.o.) however, induced 62% epithelialization. Moreover at day 15, distilled water produced 69% epithelialization compared to 71%, 72%, 80%, and 74% and for Vitamin C (100 mg/kg), 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract respectively. In the same manner, distilled water induced 72%, Vitamin C 77%, but 100 mg/kg, 200 mg/kg, and 400 mg/kg of the extract had 81%, 90%, and 88% accordingly. All doses (100 mg/kg, 200 mg/kg and 400 mg/kg) of the stem bark extract of *S.*

kunthianum produced complete (100%) epithelialization at day 25, while the negative control (distilled water; 10 ml/kg p.o.) had 85% and Vitamin C (100 mg/kg p.o.) had 86% epithelialization at the end of the study period. Stem bark extract of *S. kunthianum* at 100 mg/kg p.o, 200 mg/kg p.o and 400 mg/kg p.o. produced the shortest epithelialization period of 25 days each while, animals in the negative control (distilled water; 10 ml/kg, p.o) and positive control (Vitamin C; 100 mg/kg, p.o.) did not achieve full epithelialization period at 25 days post excision **(Table 5).** All doses (100 mg/kg, 200 mg/kg, 400 mg/kg) of the extract enhanced excision wound healing with greater percentage wound contraction and complete epithelialization with no scar tissue formation compared to either distilled water or Vitamin C, the reference drug from day 10 to the end of the study. Again, at day 25, the various doses of the extract displayed maximal wound healing potency with 100% wound contraction relative to Vitamin C (100 mg/kg, p.o.) which produced 88.0% within the same period. The extract doses (100 mg/kg, 200 mg/kg, 400 mg/kg, and p.o.) demonstrated greater wound healing efficacy compared to Vitamin C (100 mg/kg, p.o).

Table 5. Effect of aqueous-methanol stem bark extract of *S. kunthianum* given orally to diabetic rats on epithelialization period of excision wounds.

Treatment (mg/kg)	**Degree (%) and Day of Epithelialization**			
	Day10	**Day 15**	**Day 20**	**Day 25**
Distilled water (10 ml/kg p.o.)	65	69	72	85
S.kunthianum (100 mg/kg p.o.)	62	72	81	100
S.kunthianum (200 mg/kg p.o.)	63	80	90	100
S.kunthianum (400 mg/kg p.o.)	64	74	88	100
Vitamin C (100 mg/kg p.o.)	62	71	77	86
S.K.= Stereospermum kunthianum; n=5				

Values are expressed as percent of number of the rats with complete epithelialization of the excision wound within a period.

Mean Wound Closure Time (WC_{50}) in Diabetic Rats

Diabetic rats treated with the stem bark extract of *S.kunthianum* at 100 mg/kg, 200 mg/kg, and 400 mg/kg p.o. had a mean wound closure times of 6, 7, and 5 days, respectively when compared to the control groups (distilled water 10 ml/kg p.o. and Vitamin C 100 mg/kg p.o.) with median wound closure time of 8 days (**Table 6).**

Table 6: Effect of aqueous methanol stem bark extract of *S. kunthianum* given orally to diabetic rats on the Mean Wound Closure Time (WC_{50}).

Treatment (mg/kg)	**Median wound closure Time (Days)**
Distilled water 10 ml/kg	8
S.*kunthianum* 100 mg/ kg	6

S.*kunthianum* 200 mg/ kg	7
S. *kunthianum* 400 mg/ kg	5
Vitamin C 100 mg/kg	8
S.K. = Stereospermum kunthianum; n=5	

Incision wound study in diabetic rats

Gross Observation of the Incision wounds

Rats treated with the stem bark extract of *S. kunthianum* at 100 mg/kg, 200 mg/kg, and 400 mg/kg had complete epithelialization at day 10. Healing was observed to occur in the negative control group with scar tissue formation, but a thin line depicted the area of wound healing in each of the treated rats. It was observed that wounds in rats treated with *S. kunthianum* extract at 100 mg/kg, 200 mg/kg, 400 mg/kg, p.o. healed completely by the 10^{th} day with complete epithelialization. The wounds in the control group healed with scar tissue formation. Incision wounds on the diabetic rats in the treatment groups (100 mg/kg, 200 mg/kg, 400 mg/kg, and Vitamin C) healed completely leaving thin lines of scars.

Tensile strength of incision wounds on diabetic rats treated with *S. kunthianum* stem bark extract

Diabetic rats treated with aqueous methanol stem bark extract of *S. kunthianum* at 100 mg/kg, 200 mg/kg, 400 mg/kg, p.o. showed a progressively significant ($p<0.05$) increase in the tensile strength of the healed wound muscle fibres in the diabetic rats compared to the control groups **(Plate 3)**. The *S. kunthianum* extract at 100, 200, and 400 mg/kg, p.o. produced a dose-dependent increase in the tensile strength of incision wounds in diabetic rats with values of 983 g, 984.4 g and 1066.0 g respectively compared to 446.3 g (distilled water) and 542.0 g (Vitamin C) of the control groups. The test doses of the extract (100 mg/kg, 200 mg/kg, 400 mg/kg) produced a significantly ($p<0.05$) higher tensile strength compared to the control groups (distilled water and Vitamin C) **(Figure 2).**

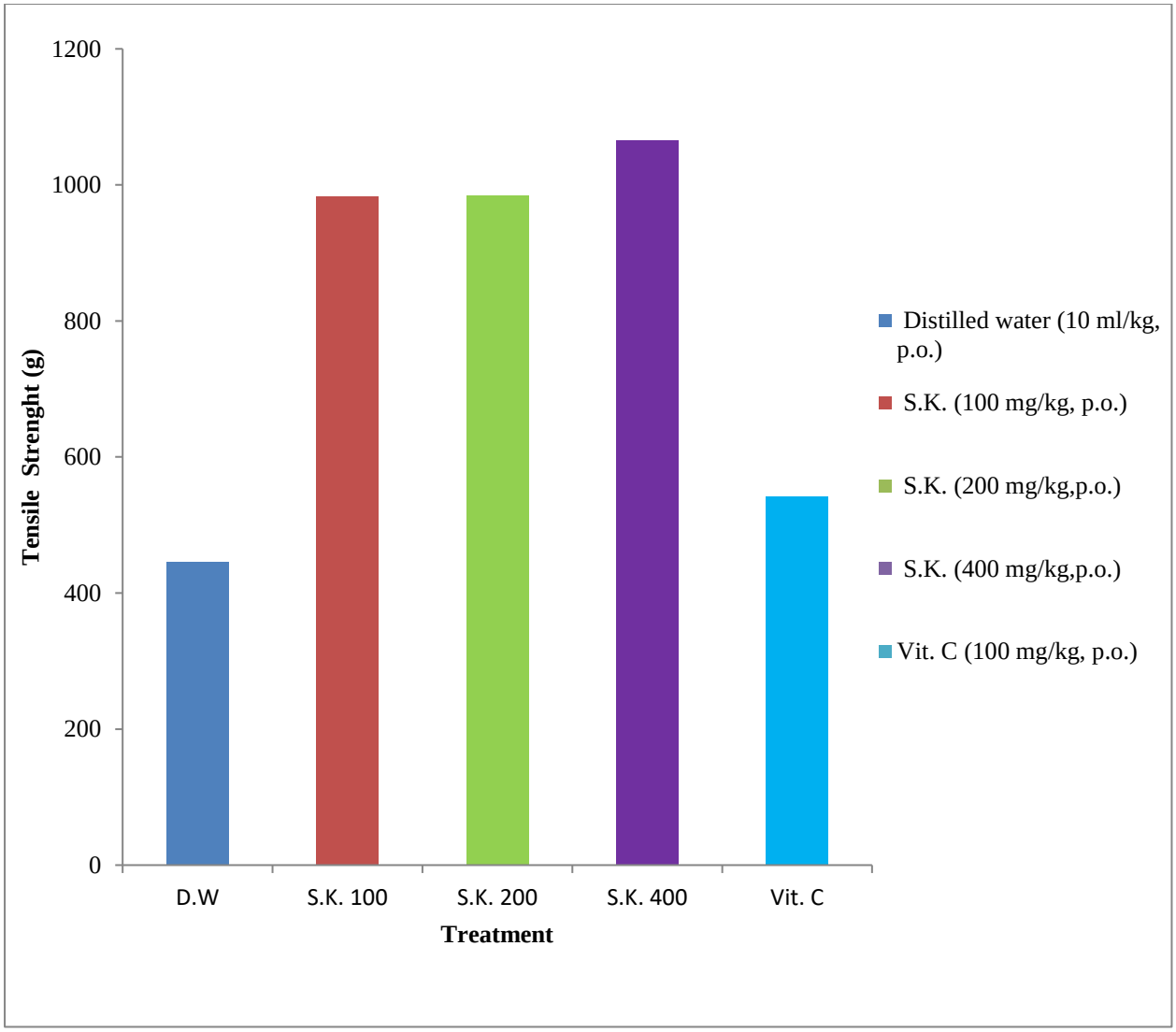

Figure 2: Effect of aqueous-methanol stem bark extract of *Stereospermum kunthianum* on the tensile strength of healed incision wounds in diabetic rats.

Significant difference at $p<0.05$; S.K. = *Stereospermum kunthianum* stem bark; Vitamin C= Vitamin C; n=5.

Discussion

The phytochemical analyses of the stem bark extract of *S. kunthianum* revealed the presence of saponins, terpenes, tannins, and steroids. These plant constituents are responsible for the biological activity of the plant including antidiabetic and wound healing properties. This finding agrees with Bhattacharjee *et al* which show that these phytochemicals have bioactive properties [39-48]. The aqueous methanol stem bark extract of *Stereospermum kunthianum* was evaluated for its acute toxicity (72 h) in rats using the oral and intraperitoneal routes. The extract did not produce any toxic effect up to a dose of 5000 mg/kg in the adult Wistar rats. The extract induced no acute toxic manifestation or death of the Wistar rats at 5000 mg/kg. According to the guidelines set by OECD, this level is considered practically non-toxic. Similarly, Lorke showed that LD value greater than 1g (1000 mg/kg) for a test substance or chemical is considered as only slightly toxic (relatively safe). In diabetes mellitus, glucose cannot be converted into energy due to lack of insulin or resistance of body cells to insulin or both [29, 49, and 50]. The acute complications of diabetes mellitus are hyperglycaemia,

ketoacidosis, and hyperosmolar non-ketonic coma, while the delayed systemic complications include diabetic nephropathy, which may lead to amputation of joints, microangiopathy, diabetic neuropathy retinopathy, artherosclerosis, and infections [51]. Wound healing is generally delayed in diabetic patients due to factors considered localized to the wound and these include desiccation, infection or abnormal bacterial presence, maceration, necrosis, pressure, trauma and edema. Alloxan monohydrate is a toxic glucose analogue used to induce diabetes in laboratory animals; it selectively destroys insulin-producing cells in the pancreas [52, 53. Excision and incision wound models were then used to evaluate the wound healing effect of *Stereospermum kunthianum* since the plant is used by traditional herbalists in northern regions of Cameroon for treating wounds. In this study that effect was evaluated to further justify the use of the plant in wound treatment [21]. The extract at all doses tested had a significant effect on wound healing. The stem bark extract doses demonstrated greater wound healing ability when compared with the standard control drug (Vitamin C). The underlying reason behind the reduced efficacy of Vitamin C observed in this study is not very clear. However, increased doses of Vitamin C may be useful in further studies. Vitamin C reportedly plays critical roles in tissue regeneration [54-56]. Tissue repair and regeneration are influenced by vitamin C through the synthesis of connective tissue, particularly collagen; it provides tensile strength to newly formed collagen to stretch without tearing, acts as an antioxidant which removes and neutralizes oxidants in the body and increases the proliferation of dermal fibroblast [57]. The extract probably facilitated excision wound healing by primary intention, hence scar tissue formation was not observed, while the treated and untreated control groups healed by secondary intention with visible scars [58]. The extract of *S. kunthianum* stem bark may also have caused wound healing effects due to its antimicrobial properties whereby bacterial activities due to wound contamination were greatly suppressed, allowing the wound to heal, devoid of scar formation. This corroborates the findings of [59-61]. The extract of *S. kunthianum* did not only induce the healing of the incision wounds in the diabetic rats but also increased the tensile strength of the healed wounds. This effect could be attributed to increased collagen concentration and stabilization of the fibres [62, 63]. The stem bark extract of *S. kunthianum* produced a dose-dependent increased in tensile strength of the wound. This finding corroborated the report by Nwinyi et al, that *S. kunthianum* stem bark extract enhanced wound healing in a dose-dependent manner in normal rats [37]. Thus, *S. kunthianum* extract aided wound healing in diabetic rats by inducing greater percentage wound contraction and increase in the tensile strength of the muscle fibres involved in the healing process. The wound healing potential of *S. kunthianum* may be attributable to the presence of a mixture of phytochemical constituents (saponins, terpenes, tannins, and steroids) present in the plant. The role of phytochemical constituents in wound healing is supported by different studies. Saponins extracted from ginseng, known as ginsenoside, have been shown to accelerate neovascularization in burn wounds of the skin in mice and increase vascular endothelial growth actor and Interleukin (IL)-1β which is one of the inflammatory cytokines known to induce the accumulation of macrophages at skin wound sites and accelerate wound healing [64].Tannins also inhibit bacterial growth and are shown to be active detoxifying agents, which has antioxidant and anti-inflammatory properties which helps to stabilize collagen needed for wound healing [65, 66]. Terpenoids promote the wound healing process mainly due to their astringent and antimicrobial properties [67]. Therefore, this study has revealed the possible scientific justification for the use of *S. kunthianum* for the treatment and management of wounds in traditional medicine.

Conclusion

In conclusion, the stem bark of *S. kunthianum* extract was relatively safe in adult Wistar rats; the oral and intraperitoneal LD of the extract was found to be ≥ 5000 mg/kg. Phytoconstituents detected in the extract were saponins, terpenes, tannins, and steroids. At all tested doses of the extract tested (100 mg/kg, 200 mg/kg, 400 mg/kg, p.o), the extract enhanced wound healing with greater percentage contraction, epithelialization, and tensile strength of the muscle fibres which may be due to the phytochemical constituents of the *S. kunthianum* stem bark extract suggesting a remarkable wound healing effect of the extract in diabetic rats. The results validated the ethno-medicinal use of *S. kunthianum* stem bark extracts in the treatment of wounds. The stem bark extract of *S. kunthianum* therefore has the potential to be developed as a wound healing agent in diabetic patients.

Recommendations

I recommend an Isolation and identification of the active compound (s) from the plant. The stem bark extract of *S. kunthianum* should be evaluated for its effects on mediators of inflammation such as prostaglandins, leukotrienes, histamine, bradykinin, platelet activating factor and interleukin-1. Sub-acute and chronic toxicity studies are required for determining the effects of long terms, low dose exposure to the stem bark extract of *S. kunthianum.* The mechanism of action of the extract on the measured parameters should be evaluated.

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