Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
www.plantsjournal.com
JMPS 2021; 9(2): 19-27

Received: 10-01-2021
Accepted: 15-03-2021

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Pharmacological justification for the ethnomedicinal use of stem bark extract of *Stereospermum kunthianum* Cham. (Family: Bignoniaceae) for wound healing effects

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Abstract
The stem bark extract of *Stereospermum kunthianum* Cham (Family; Bignoniaceae) is used traditionally for the treatment of wounds, ulcers, gastritis, bronchitis and other inflammatory and pain-related health conditions. The aim of the study was to evaluate the plant extract for wound healing effects. The stem bark was successively macerated in 80% v/v methanol to obtain the extract used for the evaluation. The aqueous-methanol extract of *S. Kunthianum* was analysed for phytochemical constituents. Its acute toxicity profile was determined in rats. Its effects on wound healing were tested using excision and incision wound models. The results revealed that saponins, terpenes, tannins and steroids were present in the extract. The estimated oral Median Lethal Dose (LD_{50}) of the extract in rats was ≥ 5,000 mg/kg. *S. Kunthianum* extract (200 - 400 mg/kg p.o) caused significant reduction of wound area. The excision wound contraction (%) in rats showed that *S. Kunthianum* extract (200 mg/kg p.o) was most effective when compared with the negative control and all other treatment groups. *S. Kunthianum* extract (200 mg/kg p.o) had the shortest epithelialization period of 14 days. The extract (400 mg/kg p.o) and Vitamin C (50 mg/rat/day p.o) had epithelialization period of 16 days while *S. Kunthianum* (100 mg/kg p.o) and the negative control had epithelialization period of 19 days. The extract (200 mg/kg p.o) had the shortest Median Wound Closure Time (WC_{50}) of 4.2 days while the negative control and the extract (100 mg/kg p.o) had the longest Median Wound Closure Time (WC_{50}) of 6.1 days and 7.1 days respectively. Incision wound studies also revealed that *S. Kunthianum* (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 decreases in skin-breaking strength with values of 560.0 g, 540.0 g and 470.0 g respectively. The results corroborated the ethno-medicinal claims and have given the scientific justification for the use of *S. kunthianum* stem bark extract for wound healing. *S. kunthianum* stem bark extract therefore has the potential to be developed as wound healing agent.

Keywords: *Stereospermum kunthianum,* wound, excision, incision, phytoconstituents

Introduction
Wound is an inescapable event of life that occurs due to a disruption of cellular, anatomical and functional continuity of living tissue like skin mucous and tissue surfaces among others produced by physical, chemical, electrical, thermal, microbial or immunological insults to the tissue [1-4] Wound can occur unexpectedly but also during medical interventions such as surgery. Wounds occur under acute conditions and are healed through a natural process that may be prolonged without treatment [5]. Wounds are expected to heal in an orderly set of stages and in a predictable amount of time ranging between 4 weeks and 3 months [6]. However, there are some physiological and environmental impairments that slow or prevent wound healing. These lead to chronic non-healing wounds which are wounds that have failed to progress through a timely sequence of repair, or one that proceeds the wound healing process without restoring anatomic and functional results [7].

Wound healing is an important biological and dynamic process involving regeneration and repair of broken tissue [4]. It is the interaction of a complex cascade of cellular and biochemical actions initiated in response to an injury leading to the restoration of structural and functional integrity with regain of strength of injured tissues and it proceeds in three overlapping phases *viz*. Inflammation (0-3 days), cellular proliferation (3-12 days), and remodeling (3-6 months) [8].

The phases of wound healing normally progress in a predictable, timely manner, and if they do not, healing may progress inappropriately to either a chronic wound such as a venous ulcer or pathological scarring such as a keloid scar [8, 9]. Chronic wounds represent a major health burden and drains on resources of patients [10, 11]. Such patients with chronic wound experience chronic pain, loss of function and mobility, increased social stress and isolation, depression and anxiety, prolonged hospitalization, increased financial burden, increased morbidity and mortality [12]. Chronic wounds and pressure ulcers are characterized by a chronic inflammatory response which impedes healing [13]. These wounds are characterized by endogenous inflammatory chemicals that lower the threshold of peripheral nociceptor stimulation. This lowering of the nociceptor is called facilitated or primary hyperalgesia. The consequence is that when the firing threshold of the nociceptors is lowered, their responsiveness to stimulus is increased [14].

According to [15], the skin is the biggest organ of the body and any breach in its continuity in form of wound leads to compromised health and immunity. Hence, an utmost necessity to treat wound on urgent basis for restoration of the wounded or inflamed to normal condition.

In addition, management of chronic wounds is another major problem due to the high cost of therapy and the presence of unwanted side effects [16, 17]. Udupa *et al* [18] also stated that tremendous advances have been made in the pharmaceutical drug industry, yet, the availability of drugs that stimulate wound repair is still limited. There is, therefore, the need for further investigations for new pharmaceutical agents with wound healing effects that would be readily available and affordable.

Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effects. The medicinal value of these plants lies in their bioactive phytochemical constituents which can be and have been utilized in the treatment and cure of human and other animal diseases [19, 20].

Stereospermum kunthianum Cham (Family; Bignoniaceae) is a plant that has its different parts used in traditional medicine for the treatment of different ailments and conditions such as wounds, ulcers, gastritis, bronchitis and other pain and inflammatory related health conditions. It is a deciduous shrub or small tree widely spread across Africa with some species distribution in Asia. The species is well spread all over the Sahel region [21]. It is commonly called 'Pink Jacaranda' in English. In Nigeria, it is known as dan Sarkin-itatuwa, sansami (Hausa), weknavunihi (Gwari), buldumhi golombi (Fula-fulfude), golombi (Kanuri) umana tumba (TIV), ajade, ayada, afe (Yoruba).

The present study aimed at evaluating the stem bark extract of *S. kunthianum* to authenticate its ethnomedicinal usage for wound healing and for future drug development.

Materials and Methods

Plant Collection and Identification

Fresh plant materials of *S. kunthianum* stem bark was collected in the month of December from Suleja, Niger State, which is situated at 10º00'N 6º00'E, Nigeria. The plant was identified by Mr. I. Muazzam, a Plant Taxonomist with the Herbarium Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The specimen was deposited in the NIPRD Herbarium with voucher specimen number: NIPRD. H. 7072.

Preparation of the Plant Extract

The stem bark of *S. kunthianum* was air-dried and pulverized in a mortar. About 1.6 kg (1600 g) of the pulverized sample was macerated successively in 5 litres of 80% v/v methanol under a temperature of 40 $^{\circ}C$ on a shaker (GFL D 3006 mgH, Germany) for agitation to ensure maximum extraction. Double maceration was done over a period of 24 h each and the extract was then filtered with Whatman no. 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:

% Yield = (W1 X 100)/W2

Where: W1 = Weight of dry extract; W2 = Weight of dry plant

The extract was then kept in a refrigerator (4 $^{\circ}C$) for subsequent studies.

Animals

Wistar rats (150 – 260 g) of both sexes were used for the studies. They were obtained from the Animal Facility Centre, Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The experimental animals were separated for at least two weeks in the experimental room for acclimatization. The animals 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.

Chemical and Drugs

Methanol (Fluka Chemie, Switzerland), Xylazine 2% (VMD nu/sa Hoge Mauw, Arendonk, Belgium), Ketamine (Nirma Limited, India), Vitamin C (Ascorbic acid) were used for the studies.

Ethical Approval

Approval of research protocol was obtained from University of Abuja Ethics Committee on Animal Use (UAECAU) with reference number: UAECAU/2018/006. The research was conducted according to the internationally accepted principles for laboratory animal use and care of the NIH publication no. 85–23.

Phytochemical Analyses

The phytochemical screening of the crude extract was done using the standard method of Trease and Evans [22]. The extract was screened for the presence or absence of various chemical constituents such as saponins using the froth test, terpenes using Liebermann-Burchard test, tannins using ferric chloride test, steroids using Salkowski's test, flavonoids using ferric chloride test, anthraquinones using Borntrager's test, carbohydrates using Molisch test and alkaloids using general tests.

Acute Toxicity Studies

The modified method of Lorke [23] was adopted for the study. The method estimates the dose of the extract that will kill 50% of the animal population (LD_{50}) to which it would be administered to. The study was carried out in both Wistar rats and Swiss albino mice and the test routes were oral and intra-peritoneal.

The method involved administration of the extract in a biphasic manner. In the first phase, widely differing doses of

the extract (10, 100 and 1000 mg/kg) were administered intraperitoneally in the rats and mice to determine the range within which toxicity would occur. The same doses of the extract (10, 100 and 1000 mg/kg) were also administered to rats orally in the first phase. The second phase was dependent on the observations made in the first phase and involved administration of higher doses of the extract (2000, 3000 and 5000 mg/kg) intraperitoneally to new set of experimental rats and mice. The same doses of the extract (2000, 3000 and 5000 mg/kg) were also administered orally to new set of rats in the second phase.
The treated animals were observed for 72 h for behavioural and\or toxic effects such as nervousness, ataxia, excitement, alertness, dullness and death.

Wound Healing Activity Studies
Excision and incision wound models were used to evaluate the wound healing activity of the stem bark extract of *Stereospermum Kunthianum*.

Excision Wound Studies
Wistar rats were inflicted with excision wounds using modified method of Rupesh *et al* [8] and this model was used to monitor wound contraction and wound closure time. The pre-determined area 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 2% (6 mg\kg i.p) followed by Ketamine (60 mg/kg i.p.) prior to the creation of the wounds. The anticipated area of the wound to be created was outlined on the back of each of the rats using a marker.
The excision was inflicted on the dorsal region 1.5 cm away from the vertebral column and 5 cm away from the ear. Sterilized cerclage wire modeled to these specifications was used on all the rats to ensure accuracy of dimensions for the excision wounds. A full thickness of excision wound of circular area of 314.3 mm^2 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* [24]. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline [25, 26].
The rats were then grouped into five (n=5). Group 1 rats received distilled water (10 ml\kg p.o) to serve as negative control. Groups 2, 3 and 4 rats received *S. kunthianum* stem bark extract at 100, 200 and 400 mg/kg p.o. respectively while Group 5 rats were given Vitamin C (50 mg/rat/day po).
The respective treatments were administered orally to the rats of the respective groups from the day of wound creation and continued until the wound was healed (i.e. until complete epithelialization).
Percentage Wound Contraction, Period of Epithelialization Parameters and the Median Wound Closure Time (WC_{50}) were studied.

Wound contraction which contributes to wound closure was expressed as a reduction in percentage of the original wound size from the day of wound creation until the day of complete epithelialization. It was evaluated to calculate the degree of wound healing.
The progressive reduction in the wound area was monitored by tracing the raw wound boundaries on transparent paper every alternate day until the wounds were completely covered with epithelium. These wound tracings were retraced on a millimeter scale graph paper to determine the wound area.
Percentage wound contraction was calculated using the relation:

$$\text{Wound Contraction (\%)} = \frac{W_{D0} - W_{DE}}{W_{D0}} \times 100$$

Where:
W_{D0} = wound area on Day 1; W_{DE} = wound area post excision days on or before complete epithelialization

Period of Epithelialization was expressed as the number of days required for falling of the eschar (dead tissue remnants) without any residual raw wound. It is considered as the end point of complete epithelialization [27].

Median Wound Closure Time (WC_{50}) was the time taken for 50% of wound closure and was read off on a plot of wound closure (%) against time (days).

Incision Wound Studies
Wistar rats were used for the incision wound studies. The dorsal hair of each rat was shaved with shaving sticks and razor blades and the area cleaned with 70% alcohol. Each of the rats was anaesthetized with Xylazine 2% (6 mg\kg i.p) followed by Ketamine (60 mg/kg i.p.).prior to the creation of the wounds.
Two (2) 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 6 cm in length. Sterilized cerclage wire modeled to these specifications was used on all the rats to ensure accuracy of dimensions for the incision wounds and after complete haemostasis, the parted skin was stitched with interrupted sutures at intervals of about 0.5 – 0.6 cm using black braided silk surgical thread (no.0) and a curved needle (3/8 semicircle-curved cutting 35 mm)). The threads on both wound edges were tightened for good closure of the wound. The wounds were left undressed and mopped with a cotton swab [28, 29].
The rats were then grouped into five (n=5) and treatment was given from the day of the wound creation until the 8th day as follows:
Group 1 (negative control) received distilled water (10 ml/kg p.o.), Groups 2, 3 and 4 received *S. kunthianum* extract (100, 200 and 400 mg/kg p.o.) respectively while Group 5 received Vitamin C (50 mg/rat/day p.o).
On the 8th day of the wound creation and of treatments, the sutures were removed while the treatments continued. The skin-breaking strength of the healed wound was measured on the 10th day according to the method described by Garg *et al* [30] and Nayak *et al* [31].
The anaesthetized rats were secured to the table and a line drawn on either side of the wound 3 mm away from the suture line. 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 weights added to the bag was determined and noted as a measure of breaking strength in grams.
The procedure was repeated on the contra lateral wound. The mean reading for the group was taken as an individual value

of breaking strength. The mean value gave the skin breaking strength for a given group [30, 31].

Data Analyses

IBM SPSS Statistics 23 was used for the statistical analyses. The results of the studies were expressed as mean ± SEM. The differences among the treatment groups were analyzed using one-way Analysis of Variance (ANOVA). 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, diverse charts, plates and tracings as appropriate.

Results

Plant Extract

The weight of the pulverized *S. kunthianum* stem bark was 1600.0 g (1.6 kg) while the weight of *S. kunthianum* extract was 279.0 g giving a percentage yield of 17.44%. The extract appeared dark brown and was oily and slurry in consistency.

Phytochemical Analyses

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.

Chemical Constituents	Test	Inference
Saponinins	Froth test	+
Terpenes	Lieberman-Burchard test	+
Tannins	Ferric chloride test	+
Steroids	Salkowski's test	+
Flavonoids	Ferric chloride test	-
Anthraquinones	Borntrager's test	-
Carbohydrates	Molish test	-

Key: + = Present; - = Absent

Acute Toxicity Studies

No overt toxicity signs or death was observed in rats 72 h post oral treatment with *S. kunthianum* extract (10 – 5,000 mg/kg). The estimated oral median lethal dose (LD_{50}) of the extract in rats was therefore ≥ 5,000 mg/kg.

No death was observed in rats and mice 72 h post intraperitoneal treatment with *S. kunthianum* extract (10 – 5,000 mg/kg). No overt toxicity sign was observed in rats while mice were calm within the first 20 min. post administration with the extract (3,000 – 5,000 mg/kg i.p). The estimated intraperitoneal median lethal dose (LD_{50}) of the extract in rats and mice was therefore ≥ 5,000 mg/kg

Wound Healing Activity Studies

Excision Wound Studies

Wound Contraction

S. kunthianum extract (100 mg/kg p.o) did not cause reduction in wound area when compared to the negative control. However, *S. kunthianum* extract (200 – 400 mg/kg p.o) caused significant reduction in wound area (especially 200 mg/kg dose). The wound area reduction effect caused by the extract (200 – 400 mg/kg p.o) was comparable to that caused by Vitamin C (50 mg/rat/day; Table 2). The excision Wound Contraction (%) in rats showed that *S. kunthianum* extract (200 mg/kg p.o) was most effective when compared with the negative control and all the other treatment groups (Figure 1). Different healing stages are depicted in Plates I.

Table 2: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o) on excision wound area (mm^2) in rats

Treatment	Wound Area ($mm^{2)}$							
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	Day 16	Day 19
Negative Control (D/water, 10 ml/kg p.o) *S. kunthianum*	303.2 ± 26.6	205.6 ± 26.9	128.8 ± 21.1	68.8 ± 16.0	15.2 ± 3.7	4.0 ± 1.3	0.8 ± 0.8	0.0 ± 0.0
(100 mg/kg p.o)	306.0 ± 12.4	228.0 ± 18.1	170.4 ± 27.2	72.0 ± 15.2	47.2 ± 13.4*	24.0 ± 14.0	20.0 ± 10.0*	1.6 ±1.6
200 mg/kg p.o	261.6 ± 15.9	135.2 ± 14.2**	49.6 ± 9.3**	13.6 ± 2.4	8.0 ± 0.0**	0.0±0.0	0.0±0.0**	0.0 ± 0.0
400 mg/kg p.o	279.2 ± 17.2	196.0 ± 17.5	136.0 ± 21.5	48.0 ± 12.5	8.0 ±0.0**	3.2 ± 1.5	0.0±0.0**	0.0 ± 0.0
Vitamin C (50 mg/rat/day p.o)	274.4 ± 17.5	192.0 ± 4.6	133.6 ± 31.0	65.6 ± 29.9	11.2 ± 0.8**	1.6 ± 1.6	0.0±0.0**	0.0 ± 0.0

Values are expressed as mean ± SEM (n = 5); *P < 0.05, significantly different from the control; **P < 0.05 significantly different from *S. kunthianum* (100 mg/kg); One-way ANOVA; Tukey post hoc. NB: Wound area on Day 1 = 314.3 mm^2

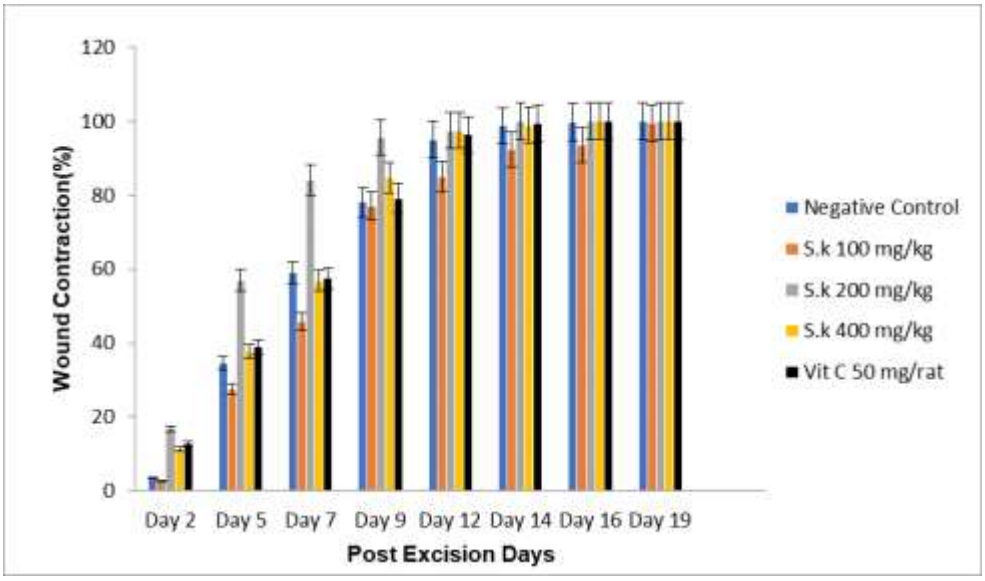

Fig 1: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o) on excision wound contraction (%) in rats.

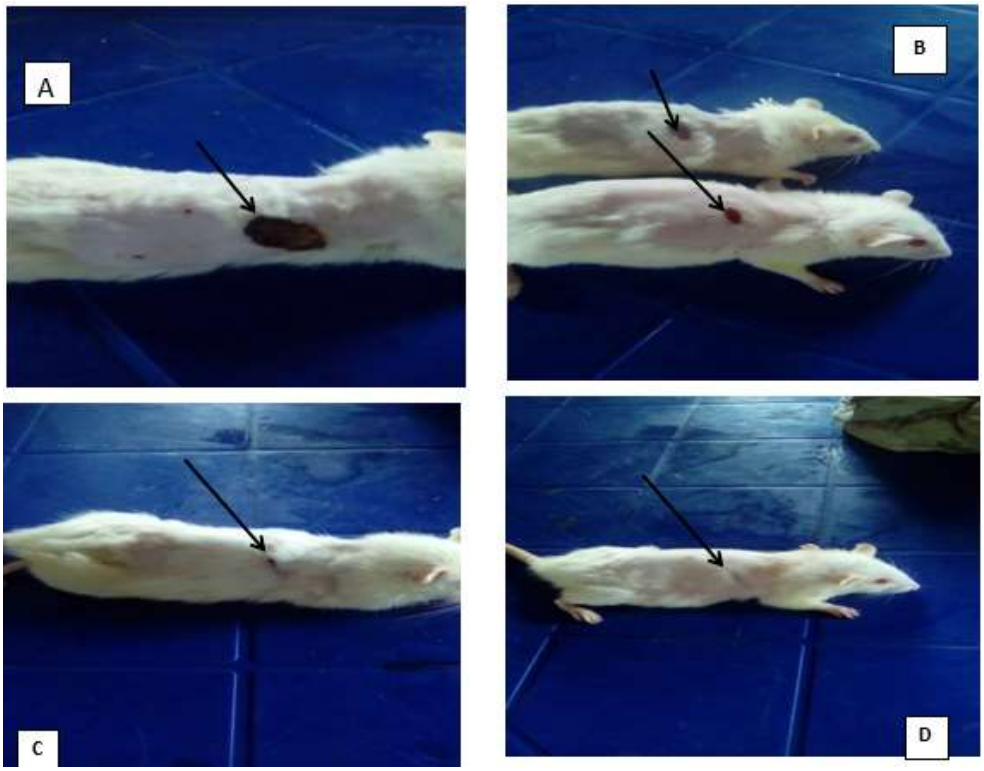

Plate I: Different stages of the excision wound contraction in rats treated with aqueous-methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o).

Key: A = Wound area on Day 1 = 314.3 mm^2; B, C, D = Pattern of wound contractility (Measurement for the different degrees of wound contractility in all the groups is shown in Table 2)

Period of Epithelialization

S. kunthianum at 200 mg/kg p.o had the shortest epithelialization period of 14 days while *S. kunthianum* at 400 mg/kg p.o and Vitamin C at 50 mg/rat/day p.o. had epithelialization period of 16 days. *S. kunthianum* at 100 mg/kg p.o and the negative control had epithelialization period of 19 days (Table 3).

Table 3: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o) on epithelialization period of excision wounds in rats

Treatment	Degree (%) and Period (Day) of Epithelialization		
	Day 14	Day 16	Day 19
Negative Control (D/water, 10 ml/kg p.o) *S. kunthianum*	20.0	60.0	100.0
100 mg/kg p.o	0.0	40.0	100.0
200 mg/kg p.o	100.0	100.0	100.0
400 mg/kg p.o	40.0	100.0	100.00
Vitamin C 50 mg/rat/day p.o	80.0	100.0	100.0

Values are expressed as percent of number of rats with complete epithelialization of excision wound within a period (number of rats = 5)

Median Wound Closure Time (WC_{50})

S. kunthianum at 200 mg/kg p.o had the shortest Median Wound Closure Time (WC_{50}) of 4.2 days while the negative control and *S. kunthianum* at 100 mg/kg p.o had the longest Median Wound Closure Time of 6.1 days and 7.1 days respectively (Table 4)

Table 4: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o) on Median Wound Closure Time (WC_{50}) of excision wounds in rats

Treatment	Median Wound Closure Time (Day)
Negative Control (D/water, 10 ml/kg p.o) *S. kunthianum*	6.1
100 mg/kg p.o	7.1
200 mg/kg p.o	4.2
400 mg/kg p.o	5.1
Vitamin C 50 mg/rat/day p.o	6.0

Values were read off on a plot of wound closure (%) against time (days)

- **Incision Wound Studies**

Rats treated with aqueous-methanol stem bark extract of *S. kunthianum* at 100, 200, 400 mg/kg p.o showed a progressive rate of wound repair with increasing doses of the extract. This was comparable with Vitamin C (50 mg/rat/day)-treated rats. The wounds healed relatively fast and, in a dose-dependent manner. The incision lines were hardly visible and neatly healed by Day 10. On the other hand, the negative control rats showed a lot of scar tissue formation, gapping and dry skin edges (skin edges did not appose very well). The different stages of the incision wound studies from the day of wound creation to the day of skin-breaking strength measurement are shown in Plate II.

The aqueous-methanol extract of *S. kunthianum* at 100, 200, 400 mg/kg p.o showed skin-breaking strength of 560.0 g, 540.0 g and 470.0 g respectively. Vitamin C (50 mg/rat/day) showed skin-breaking strength of 670.0 g while the negative Control group showed skin-breaking strength of 497.0 g (Figure 2).

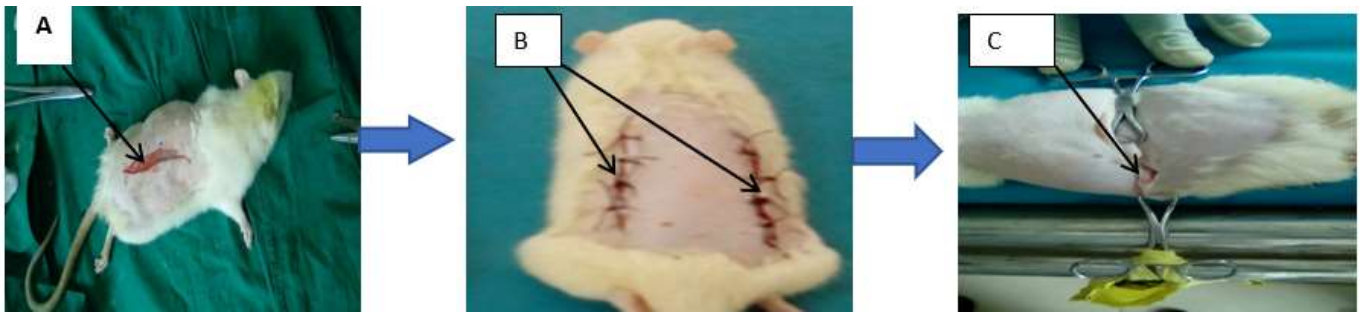

Plate II: Different stages of the incision wound studies on rats treated with aqueous-methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o).

Key: Day of wound creation (A); Stitching of the incision wound using interrupted suture pattern (B); Day of skin-breaking strength measurement of the healed wound (C).

Wound gaping in (C) marked the end point for incision wound breaking strength (The values for all the groups are depicted in Figure 2)

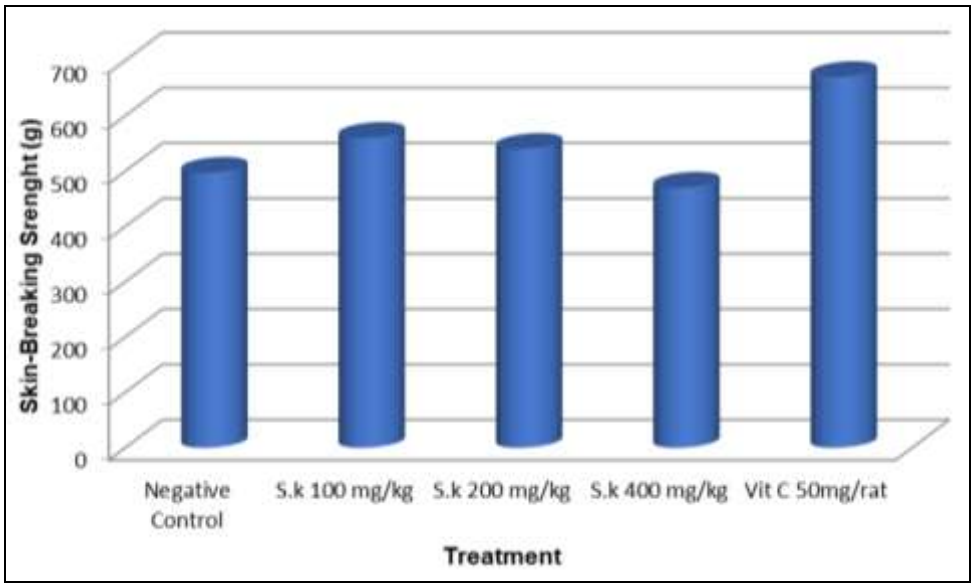

Fig 2: Effect of aqueous- methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o) on skin-breaking strength of incision wound in rats; S.k = *Stereospermum kunthianum*

Discussion

Acute toxicity study was used to establish the Median Lethal Dose (LD_{50}) of stem bark extract of *S. kunthianum* in mice and rats treated orally and intraperitoneally. The present investigation has revealed the toxicity profile of the extract in order to determine the extent of its safety if in use as drug. This is because as important as it is for drugs to be efficacious, cheap and available, there is extreme need for these drugs to be safe for short and long term uses. The fact that different substances have different toxicity levels is shown in the classification of substances into very toxic, toxic, less toxic or only slightly toxic [23]. This further indicates that evaluation of safety profile of a drug is paramount in the development of drugs and in their subsequent clinical uses.

The present study showed that the stem bark extract of *S. kunthianum* caused no overt toxicity sign or death in rats 72 h post oral treatment. The oral median lethal dose (LD_{50}) of the extract was estimated to be $\geq$ 5,000 mg/kg in rats. The Organization for Economic Cooperation and Development (OECD)[32] recommended chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values as: very toxic, $\leq$ 5 mg/kg; toxic, > 5 $\leq$ 50 mg/kg; harmful, > 50 $\leq$ 500 mg/kg and no label, >500 $\leq$ 2,000 mg/kg [33, 34]. Based on this classification, the oral LD_{50} of 5,000 mg/kg established for rats in this study indicates relative oral safety. Lack of overt toxicity signs in these experimental animals also points to that fact.

Furthermore, no death was observed in rats and mice 72 h after intraperitoneal treatment with *S. kunthianum* extract (10 – 5,000 mg/kg). No overt toxicity sign was observed in rats while mice were calm within the first 20 minutes of administration with the stem bark extract (3,000 – 5,000 mg/kg i.p). The estimated intraperitoneal median lethal dose (LD_{50}) of the extract in rats and mice was therefore $\geq$ 5,000 mg/kg. This also means that intraperitoneal administration of the extract is also relatively safe. Lorke [23] considered LD_{50} values greater than 1g (1000 mg/kg) for a test substance or chemical as only slightly toxic (relatively safe). However, attention should be given to the calmness observed (though at high doses) few minutes post treatment in mice.

The oral acute toxicity value $\geq$ of 5,000 mg/kg obtained in this study for stem bark extract of *S. kunthianum* corroborated with the oral LD50 value of $\geq$ 8,000 mg/kg obtained by Ching *et al* [35]. These results suggest that the stem bark extract of *S. kunthianum* is relatively safe.

In the present study, excision wound model in rat revealed that *S. kunthianum* extract caused significant ($p < 0.05$) reduction of wound area (mm^2) only at doses of 200 and 400 mg/kg p.o. The excision Wound Contraction (%) showed that *S. kunthianum* extract (at 200 mg/kg p.o.) was most effective if compared with the negative control and all the other treatment groups. However, the wound contractile effect of the extract at the dose of 100 mg/kg p.o was lower than that of the negative control. This suggests that the wound contractile effect of the stem bark extract of *S. kunthianum* occurred at doses equal to or greater than 200 mg/kg p.o. It also suggests that the stem bark extract of *S. kunthianuma* at the doses of 200 and 400 mg/kg p.o had better wound contractile effect than vitamin C at the tested dose of 50 mg/rat/day p.o

The model further revealed that *S. kunthianum* stem bark extract (at 200 mg/kg p.o.) had the shortest epithelialization period of 14 days and that *S. kunthianum* stem bark extract (400 mg/kg p.o.) and Vitamin C (at 50 mg/kg p.o) had epithelialization period of 16 days while *S. kunthianum* stem bark extract (at 100 mg/kg p.o.) and the negative control had epithelialization period of 19 days. This suggests that the stem bark extract of *S. kunthianuma* at the dose of 200 mg/kg p.o had the shortest number of days (14 days) for falling off of the eschar (dead tissue remnant) without any residual raw wound. This was followed by the number of days (16 days) taken by both *S. kunthianum* stem bark extract (at 400 mg/kg p.o.) and Vitamin C (at 50 mg/rat/day p.o) while *S. kunthianum* extract (at 100 mg/kg p.o.) and the negative control had the longest epithelialization period of 19 days. This suggests that *S. kunthianum* stem bark extract (at 200 and 400 mg/kg p.o.) facilitated epithelialization by shortening the period of epithelialization. However, the dose of 200 mg/kg p.o produced a faster epithelialization effect and may be clinically preferable.

The stem bark extract of *S. kunthianum* (at 200 mg/kg p.o.) also had the shortest Median Wound Closure Time (WC_{50}) of 4.2 days while the negative control and *S. kunthianum* extract (at 100 mg/kg p.o.) groups had the longest Median Wound Closure Time (WC_{50}) of 6.1 days and 7.1 days respectively. This suggests that the stem bark extract of *S. kunthianuma* at the dose of 200 mg/kg p.o had the shortest number of days (4.2 days) for wound closure for 50% of the treated rats. This was followed by those of *S. kunthianum* stem bark extract at 400 mg/kg p.o. (5.1 days), Vitamin C at 50 mg/rat/day p.o (6.0) while the negative control and *S. kunthianum* stem bark extract (100 mg/kg p.o.) had the longest number of days (6.1 and 7.1 days respectively.) for wound closure for 50% of the treated rats. This shows that *S. kunthianum* stem bark extract (200 and 400 mg/kg p.o.) has the potential to cause fast wound healing in an average population of treated wound patients and may be preferable to the tested dose of Vitamin C (50 mg/rat/day p.o). It could also be used as an adjuvant to other drugs used in wound healing such as Vitamin C.

The results for the wound contraction, period of epithelialization and Median Wound Closure Time (WC_{50}) consistently suggest that *S. kunthianum* stem bark extract has the potential to accelerate wound healing process especially at the doses equal to or greater than 200 mg/kg p.o. These corroborate the ethno-medicinal use of *S. kunthianum* for wound healing [36].

The incision wound model showed that aqueous-methanol stem bark extract of *S. kunthianum* (100, 200, 400 mg/kg p.o)-treated rats had a progressive rate of incision wound repair with increasing doses of the extract. This was comparable with Vitamin C (50 mg/rat/day)-treated rats. The wounds healed relatively fast and also in a dose-dependent manner. The incision lines of extract-treated rats were hardly visible and neatly healed by Day 10, suggesting early remodeling. On the other hand, the negative control rats showed a lot of scar tissue formation, gapping and dry skin edges (skin edges did not appose very well) suggesting poor healing for the untreated rats. This probably shows that *S. kunthianum* stem bark extract has the potential to be developed into an oral wound healing agent.

Furthermore, *S. kunthianum* extract (100, 200, 400 mg/kg p.o) showed skin-breaking strength of 560.0 g, 540.0 g and 470.0 g respectively. Also, Vitamin C (50 mg/rat/day) showed skin-breaking strength of 670.0 g while the negative Control group showed skin-breaking strength of 497.0 g. This shows that the stem bark extract of *S. kunthianum* at 200 and 400 mg/kg p.o doses took shorter time for the cutaneous healing to occur than did *S. kunthianum* stem bark extract at 100 mg/kg p.o. but the extract (100 mg/kg p.o) exhibited higher skin-breaking strength. than those of the extract at 200 and 400 mg/kg p.o doses. It should also be noted that the previous report on

excision wound also suggested that *S. kunthianum* (100 mg/kg p.o) may not have facilitated wound healing because the cutaneous layer did not heal as fast as other treated groups. The stem bark extract at 100 mg/kg p.o showed poor and slow cutaneous response to healing process in comparison with the negative control group but the incision wound model for testing skin-breaking strength suggested sufficient deposition of collagen to account for the substantial breaking strength recorded for *S. kunthianum* stem bark extract at 100 mg/kg p.o. It is worth noting that the skin-breaking strength shown by the extract (100, 200 and 400 mg/kg p.o) decreased in a dose-dependent manner 560.0 g, 540.0 g and 470.0 g respectively). The reason for the inverse effect could not immediately be deduced. However, the doses of *S. kunthianum* stem bark extract equal to or greater than 200 mg/kg p.o. are preferable for clinical work because they caused faster and better cutaneous wound closure which reduces vulnerability of wound to infection and the healed wounds still exhibited appreciable skin-breaking strength.

The general results from the excision and incision wound models suggest that *S. kunthianum* stem bark extract might have facilitated the growth of the dermal and epidermal layers resulting in fast wound healing through one or more of the mechanisms documented by some authors. For instance, Nayak *et al* [25] and Vipin and Sarvesh [37] stated that there are three stages to the process of wound healing: inflammation, proliferation and remodeling. According to their report, inflammatory response occurs following injury and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. The proliferative phase is characterized by angiogenesis (involving new blood vessel growth from endothelial cells), collagen deposition, granulation tissue formation, epithelialization and wound contraction. In fibroplasia and granulation tissue formation, fibroblasts excrete collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently, epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells [25]. The present study suggests that the stem bark extract of *S. kunthianum* probably facilitated the proliferative phase because of the increased epithelialization and wound contraction rates. The skin breaking strength model also suggests an improved collagen deposition. Collagen provides strength, integrity and structure in normal tissue and when tissues are disrupted following injury, collagen is needed to repair the defects and restore anatomic structure and function.

The wound healing potential of *S. kunthianum* may be attributable to the presence of a mixture of phytochemical constituents present in the plant. The accelerated wound healing process could be a function of either the individual phytochemical constituents or the additive or interactive effects of the phytochemical constituents. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms [37]. The role of phytochemical constituents in wound healing is supported by different studies. For instance, saponins are compounds extensively found in most plants and research has revealed that saponins can accelerate numerous biological activities including anti-bacterial, anti-viral and antioxidative functions [38, 39, 40, 41]. In addition, saponins reportedly have anti-inflammatory activity which can reduce oedema and skin inflammation [42]. A saponin extracted from ginseng, known as ginsenoside has been shown to accelerate neovascularization in burn wounds of the skin in mice and increase vascular endothelial growth factor and interleukin (IL)-1β which is one of the inflammatory cytokines known to induce accumulation of macrophages at skin wound sites and accelerate wound healing [43]. Tannins also inhibit bacterial growth and are shown to be active detoxifying agents [44]. Terpenoids promote wound healing process mainly due to their astringent and antimicrobial property [45]. Therefore, the presence of phytochemical constituents such as the saponins, tannins and terpenes in the crude stem bark extract of *S. kunthianum* may have contributed to the wound healing effect by functioning independently or synergistically through additive or interactive reactions.

Conclusion

These results corroborate the ethnomedicinal use of *S. kunthianum* stem bark extract for the treatment of ulcers, gastritis, bronchitis and other wound-related health conditions [36]. The results have also shown that *Stereospermum kunthianum* stem bark has the potential to be developed into a wound healing therapeutic agent.

Conflict of interests

No conflict of interest was declared by the authors.

Funding

The study was self-sponsored

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