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Wound healing potential of the crude leaf extract of *Stachytarpheta Jamaicensis* Linn. *Vahl* (Kandikandilaan) on inducedwounds in rats

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Abstract
The wound healing activity of the ethanolic crude leaf extract of *Stachytarpheta jamaicensis* was evaluated in albino rats by excision wound model for a period of 20 days. In this study, the rats were divided into four groups with 9 replicates; T_1, the control group where no treatment was given to the animals, T_2 for animals treated with commercial 10% povidone- iodine, T_3 for animals treated with 95% ethyl alcohol and T_4 for animals treated with the crude extract of *S. jamaicensis*. Wound healing was monitored on day 4, 8, 12 and 16. Scabs collected on day 4 and 8 were subjected to Biuret Test and protein content was indicated by the intensity of blue color. External application of the crude leaf extract of *S. jamaicensis* significantly accelerates wound healing in albino rats. Phytochemical investigations on the crude leaf extract of *S. jamaicensis* revealed the presence of phytoconstituents such as tannin, flavonoids, saponin, terpenoids, glycosides, phenols which exhibit wound healing potential.

Keywords: Complete scab formation, Excised wound, Extract, Extraction, Initial scab formation, Wound contraction.

1. Introduction
The skin is the largest organ of the body which acts as a barrier against external agents. The loss of skin tissue integrity like in the development of wound can cause lesions or illnesses which could be fatal. Wounds are inevitable occurrences of life, which arise due to physical injuries that typically involve laceration of skin. The healing of wound varies, some may take a longer period to recover and some may even fail to heal. Although there are different methods and degrees of injury, the basic phases of healing are essentially the same for most wounds. They are the inflammatory, epithelialization, proliferative and remodeling (Sussman and Bates-Jensen, 2007)[1].

Wounds heal naturally yet they can easily be infected, and even the simplest of infections can lead to the serious disease complications. While the body is currently recovering the destroyed tissues, it should at the same time be protected from various kinds of infections and this is usually done by applying substances that act against organism that might cause further damage through infection process.

For decades, medicinal plants have been utilized as a natural source containing bioactive compounds that offer therapeutic benefits and affordable treatments against wide spectrum of diseases. The usage of medicinal plants as an alternative to chemically synthesized drugs in the treatment of diseases has been accepted on a global scale (Liew and Yong, 2015)[2].

Several herbal plants that have medicinal benefits can be obtained in the locality. Extracts of these plants have immense potential for the management and treatment of wounds as some of them were used traditionally.

One of the plants traditionally applied to wounds for healing purposes is *Stachytarpheta jamaicensis* Linn. Vahl, locally known as kandikandilaan. The plant is a common weed distributed in open and waste places at low medium altitude in settled areas throughout the Philippines. The medicinal use of *S. jamaicensis* would considerably be enhanced through the effective integration of chemical composition of extracts on understanding its wound healing property.

2. Materials and Methods

Collection and Preparation of Plant Sample

The plant samples were collected from the vacant lot situated in front of Pangal Norte Elementary School, Pangal Norte, Echague, Isabela. The plants were uprooted and the leaves were isolated and were washed with tap water to remove soil and other adhering particles. The clean leaf samples were left to air dry for 1 week. The air dried leaves were cut into pieces and ground resulting to powdered form.

Extraction of Bioactive Components

Five hundred grams of the powdered leaves were soaked in 2450 ml of 95% ethanol for seventy two hours using conical flasks at room temperature. The resultant mixture was filtered using Whatman No. 1 filter paper and the filtrate was concentrated in vacuo (Stuart) at 50 °C to remove excess solvent. The resulting crude extract was transferred in beaker wrapped with thick white paper, covered tightly, stored and refrigerated for succeeding steps while the plant residue was discarded.

Preparation of the Experimental Animals

Authorization to use animals in the experimental research was secured prior to the conduct of the experiment from the Bureau of Animal Industry Visayas Avenue, Diliman, Quezon City, the agency responsible for animal welfare. Thirty six albino rats of either sex weighing 200- 250g were purchased and used as experimental animals. The rats were housed individually in clean cages made of steel wire screen measuring 12 x 10 x 10 inches in length, height and width, respectively. Commercially pelleted rat feeds and clean water *ad libitum* were given to the experimental animals. Animals were acclimatized to laboratory conditions for 72 hours before conducting the experiments. Care was observed in handling the animals.

Assay for Wound Healing Activity

Wound induction

Excision wound model was adapted in inducing wound on the experimental animals. The wound site was prepared by a veterinarian by shaving the hairs on the skin of dorsal surface of the animals. The animals were anaesthetized by open mask method using ether before inducing wound. A round seal of 15 mm in diameter was impressed on the dorsal region of the rat. A full skin thickness was excised from the mark area to get a wound measuring 15mm (circular area = 176.625 mm^2) in diameter and 2 mm depth using toothed forceps, surgical blade and pointed scissors. To achieve complete blood clotting the wound was blotted with 1 ml warm saline. The animals were placed singly in individual properly labeled cages.

Evaluation of wound healing activity

Treatment started immediately after wound induction on the experimental rats. The experimental animals were treated with 0.5 ml of the treatments by applying directly the solutions on the induced wounds. The treatments were given once daily in the morning from 1st day of wounding until the day when the wound closure was observed which for T_4 is 12 days, for T_1 and T_2 are 20 days, and for T_3 is 24 days.

The wound induced to laboratory rats were monitored daily and observed for wound size, wound color, development of fluid and signs of infections. Number of days to formation of brown color on the induced wound, an indication of initial scab formation was also noted. Intense brown color and hardening of the scab were observed and taken as complete scab development.

The observation of wound contraction was made after every 4 days and was determined by placing a transparency sheet over the wound and measured in square millimeter using graphing paper. The circular area was computed (Garg, 2011) [3]. using the formula:

$$A=\pi r^2$$

Where:

A = circular area of the wound

π = 3.1416

r = radius derived from the half of the diameter of the induce wound

A reduction on wound size was also monitored as indicated by the percentage decrease on the size of the wound. One hundred (100%) percent indicates total wound closure. The percentage of wound contraction was computed using the formula of Mageswari (2015) [4].

$$\% \text{ wound contraction} = \frac{\text{Initial Area of wound} - n^{th} \text{ day area of wound}}{\text{Initial Area of wound}} \times 100$$

Qualitative Estimation of Protein on Scabs

a. Hydrolysis

Initial wet scabs formed were collected and dried. To each tube containing 40 mg of dried granulation tissue, 1 ml of 6N HCl was added. The scabs were then kept on boiling water bath for 24 hours (12 hours each day for 2 days) for hydrolysis. The hydrolysates were then cooled and excess acid was neutralized by 10N NaOH using phenolphthalein as indicator. The volume of neutral hydrolysate was diluted to a concentration of 20 mg/ml distilled water. The final hydrolysate was used for the estimation of protein using Biuret test.

b. Biuret Test

To 0.3 ml of hydrolysate, 25N NaOH and 0.01M $CuSO_4$ were added. Tubes were shaken vigorously and placed immediately in water bath at 80 °C. After 15 minutes, tubes were removed and cooled for 5 minutes in cold water. Intensity of the blue color solution containing scabs from different treatments was noted and these were compared against control using distilled water and Biuret reagent.

Phytochemical Screening

Qualitative tests were performed to identify the bioactive constituents present in the leaf crude extract of *S. jamaicensis* using the standard method (Guevarra, 2005; Sasidharan *et al*, 2011; Singh *et al*, 2012) [5, 6, 7].

a. Test for alkaloid

Mayer's Test: A 2 ml of the extract was treated with few drops of dilute hydrochloric acid and filtered. To the filtrate, 3 drops of Mayer's reagent was added. There was no change observed upon the addition of Mayer's reagent.

b. Test for flavonoids

NaOH Test: To 2-3 ml of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow color that became colorless on addition of few drops of dilute HCl indicated the presence of flavonoids.

c. Test for phenols
Phenol Test: When 0.5 ml of $FeCl_3$ (w/v) solution was added to 2 ml of test solution, formation of deep blue color indicated the presence of phenols.

d. Test for phytosterols
Salkowski Test: To 2 ml of extract, 2 ml of chloroform and 2 ml concentrated H_2SO_4 were added then shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols.

e. Test for terpenoids
A 1 ml of the crude extract was added to 2 ml acetic anhydride and concentrated H_2SO_4, resulting to formation of blue/green rings indicative of the presence of terpenoids.

f. Test for tannins
Ferric Chloride Test: Small quantity of extract was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for blue black coloration which indicated the presence of tannins.

g. Test for carbohydrates
Molish's Test: To 1 ml of extract, 2 drops of Molisch's reagent was added in a test tube and 2 ml of concentrated H_2SO_4 was added carefully along the sides of slightly slanted test tube. Formation of violet ring at the junction indicated the presence of glycoside.

h. Test for coumarins
A 3 ml of 10% NaOH was added to 1 ml of the crude extract; formation of yellow color indicated the presence of coumarins.

i. Test for saponin
Foam Test: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. Formation of a 1 cm layer of foam indicated the presence of saponin.

j. Test for amino acid
Ninhydrin Test: To 5 ml of extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. Deep blue color was observed in the solution

k. Test for Anthraquinones
A 0.5 g of the extract was boiled with 10 ml sulfuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia (NH_3) was added. The resulting solution formed a slight yellow color.

Statistical Analysis
The data gathered were tabulated and subjected to descriptive statistics. The number of days to scab formation and wound healing parameters were tabulated following the format of completely randomized design (CRD). Since there were treatments having zero values, the square root transformation was resorted to statistically analyze the data collected. The The formula used was $\sqrt{(x+0.5)}$, where x is the variate and 0.5, a constant value (Gomez and Gomez, 1984) [8]. These data were analyzed using the Analysis of Variance (ANOVA) F-test. The Tukey's Honestly Significant Difference (HSD) method was resorted to, in identifying which among the treatment means is different for significant results in the F – test.

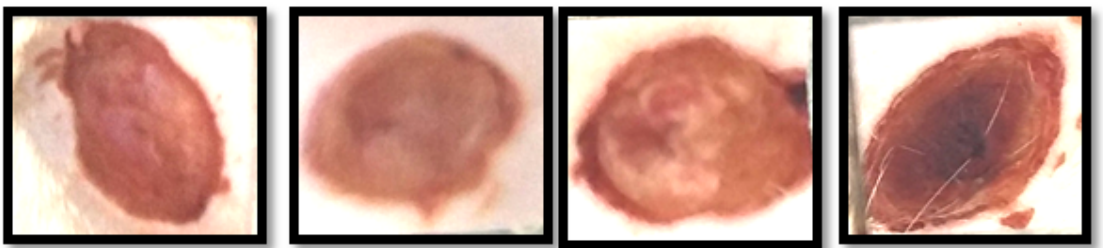
III. Observation and Discussion of Results

The plant extract
Exhaustive extraction and in vacuo concentration, resulted to a sticky and dark green colored extract weighing 140 grams. The crude extract was kept in a beaker wrapped with clean thick white paper to prevent exposure to light, labeled as *S. jamaicensis* crude extract and stored in refrigerator. The crude extract was subjected to subsequent bioassay and phytochemical analysis.

Behavioral Response of the Albino Rats
The experimental animals exhibited varying behavioral response when the different treatments were applied. When the *Stachytarpheta jamaicensis* crude extract (T_4) was applied on the wounds of the albino rats, it was observed that slight sign of restlessness was exhibited by the animals such as trying to reach and lick the induced wound as hard as they could. However, the location of the wounds prevented the animals from reaching the wound. The experimental animals treated with commercial solution (T_2) did not show any sign of restlessness and kept still as the treatment was applied. The experimental animals treated with 95% ethyl alcohol (T_3) were moving around their cage and were agitated. The animals which were not treated (T_1) with any solution displayed normal behavior since no solution was applied to their wounds.
The observed reactions of the albino rats toward the treatments given to them were the same up to the 4th day. On the 8th day, it was noted that animals in T_2 and T_4 did not manifest aggressive behavior toward the respective treatments applied on the wounds except on those animals in T_3 which displayed a slight sign of irritation on the wounds as shown by animals trying to scratch the induced wound. Typical behavior of animals in T_1 as no treatment was given to them.

Health Status of the Experimental Animals during the Duration of the Study
The albino rats during the first day of the experiment were observed to be in good condition, and appeared to be adjusting with their new environment as to temperature and space. It was also noted that the hair of the animals was dull, with teary eyes and unstable perhaps due to transportation trauma. After 72 hours of acclimatization and conditioning of the rats, it was observed that the animals became active and aggressive. They responded well to the external environment especially when given water and food.
The weights of the experimental animals were noted to have increased gradually throughout the duration of the study. Before the experiment, the mean weight of the albino rats in T_1 is 211.67g; T2 is 196.67; T3 is 195g and T_4 is 211.11g. After the experiment, the mean weights of the animals increased and recorded accordingly; T_1 is 222 g; T_2 is 210 g; T_3 is 202.78 g; and T_4 is 223.88 g. No mortality rate was recorded on the experimental animals.

Changes on the Wound Surface
Healing of wounds is initiated by development of scab observed as brown-like coloration on the surface of the wound (Nayeem *et al*, 2008) [9]. Similar changes were noted on the wounds induced on the experimental rats. The appearance of color on the surface of induced wounds was noted on the third day of treatment. This was indicated by the brown coloration on the wounds of all the experimental animals. However, the degree of brown coloration varies with the treatment received by the animals.



T_1 T_2 T_3 T_4

Changes in the color of the induced wounds.

Animals treated with *S. jamaicensis* extracts (T_4) showed a darker brown coloration, the wounds were dry and the development of blood clot on the surface of the wound was observed. For the group of animals treated with commercial solution (T_2), the wounds were noted to be light brown in color, while the wounds of animals of the control group (T_1) displayed a lighter brown color and dry surface. The experimental animals receiving the 95% ethanol treatment (T_3) had a lighter brown color and showed a small amount of fluid like substance on the surface of the wound, resulting to a fresh wound appearance.

Scab Formation on the Induced Wound

Initial scab formation was noted to have occurred earlier on the wounds of animals receiving T_4 solution. Dark brown coloration on the surface of the wound was developed after three to five days of treatment with scabs appearing to be thicker. Table 1 shows the number of days to initial scab formation.

Table 1: Number of Days to Initial Scab Formation

Treatments	Mean number of days
T_1	4
T_2	4
T_3	5
T_4	3

Wounds of animals in T_4 treated albino rats were observed to develop darker colored thicker scabs formed, an indication of initial scab formation after a mean number of three days. Lighter brown color of wound surface was noted on T_3 animals with thin scabs that were initially formed after 5 days of treatment. The observations made on T_1 and T_2 treated albino rats showed similar results. They had pale brown color with the formation of thin scabs on the wound surface on the 4^{th} day of post treatment.

Scabs that developed on the wounds were noted to be complete between 4 to 8 days after treatment. Treatments 1, 2 and 3 showed the same number of days for scabs to completely form and that was from 6.43 to 7.57 mean numbers of days. Rats receiving 20% *S. jamaicensis* (T_4) were noted to have caused similar activity on the induced wounds. The surfaces of the wounds were totally covered with dark brown colored materials though at a shorter period of 4.63 mean days.

Table 2 shows the analysis of variance revealing a significant result on the days to complete scab formation.

Table 2: Number of Days to Complete Scab Formation

Treatments	Mean number of days
T_1	6.43^{ab}
T_2	6.43^{ab}
T_3	7.57^{a}
T_4	4.63^{b}

Result = *

HSD = 2.50

Treatment means with common letters are not significantly different at 5% using HSD.

Complete scab formation is the development of scab indicated by a dark brown color of the wound and hardening of granulation tissue covering the induced wound. Complete scab formation was first observed on wounds treated with 20% S. *jamaicensis* extracts (T_4) on the 4th day of treatment.

The result revealed that experimental animals treated with 20% *S. jamaicensis* extract showed the shortest period of complete scab formation indicated by early onset of brown coloration on the surface of the induced wounds of T_4 treated albino rats. The report of Udegbunam (2014) [10] on the acceleration of wound healing by the use of 20% *Pupalia lappacea* ointment having the highest rate of epithelialization at 4, 7 and 14 days post treatment supports the result obtained by the study. Period of epithelialization was indicated by complete scab formation in which the granulation tissue and collagen fibrils dominate the space as shown on the result of the study conducted by Pandian *et al* (2013) [11] on the evaluation of wound healing activity of hydroalcoholic extract of leaves of *S. jamaicensis* in streptozotocin induced diabetic rats.

Wound Area

Another indicator of wound healing is wound area contraction. Wound area of the experimental animals was monitored by tracing the wound and the value obtained was used in computing the wound area shown in Table 3.

Table 3: Records of wound area from 0 days until complete wounds in albino rats. Numbers in parenthesis are data transformed using √(x+0.5)

Treatments	Mean wound area, ($mm^{2)\,1}$				
	0	4^{th}	8^{th}	12^{th}	16^{th}
T_1 (Control group)	176.625 (13.31)	166.37^{a} (12.92)	68.73^{ab} (8.32)	16.46^{a} (4.11)	3.58 (2.00)
T_2 (20% Povidone-iodine)	176.625 (13.31)	159.11^{ab} (12.63)	64.11^{a} (8.01)	15.34^{ab} (3.88)	2.17 (1.34)
T_3 (95% ethyl alcohol)	176.625 (13.31)	166.45^{a} (12.92)	96.91^{b} (9.86)	26.25^{a} (5.08)	12.59 (3.24)

T_4 (20% SJE))	176.625 (13.31)	144.44[b] (12.04)	49.20[a] (6.99)	2.79[b] (1.46)	0 (0.707)
Grand Total	2,119.50 (13.31)	1,915.06 (151.51)	836.84 (99.54)	182.51 (43.58)	56.82 (21.86)
Grand Mean	176.625 (0.707)	150.09 (12.63)	69.74 (8.30)	15.21 (3.63)	4.74 (1.82)
F-values(computed)	-	7.115	7.825	6.407	2.781
F- values (tabular)	4.066	4.066	7.59	4.066	4.066
Result	-	*	**	*	Ns
CV	-	0.193	1.541	2.741	1.876
HSD	-	0.627	1.712	2.455	-
Level of Significance	-	5%	1%	5%	-

[1]Means with common letters are not significantly different.

As shown in Table 3, the initial wound area of the experimental albino rats are the same, an indication that the area of the wound induced on the animals is equal.

On the 4th day of post wound treatment, the wound areas computed were significantly influenced by the treatments received. Animals treated with 20% *SJE* (T_4) resulted in the lowest wound area of 144.44 mm^2, followed by 159.11 mm^2, 166.37 mm^2 and 166.45 mm^2 for T_2, T_1 and T_3 respectively. The application of T_2 and T_4 were found to be the same statistically in reducing the area of the induced wound.

Data on Table 3 further show the computed area of the wound applied with the treatments, on the 8th day of post wound treatment. Statistical analysis detected a significant ($p > 0.01$) difference among the treatments. Application of 20% *SJE* (T_4) had the lowest wound area of 49.20 mm^2, which was followed by 64.11 mm^2, and 68.73 mm^2 for T_2 and T_1 respectively. The largest wound area was computed for T_3 with an area of 96.91 mm^2 which was found to be statistically the same with the control (T_1).

Further evaluation of the wound area on the 12th day of post wound treatment resulted to a significant reduction of the wound area of 2.79 mm^2. Of the 12 albino rats allocated for T_4, total wound closure was noted on seven rats, indicated by the total closure of the induced wounds. The reduction of the wound area on T_2 treated rats was statistically the same as that of T_4 with a mean area of 15.34 mm^2. Rats treated with T_1 and T_3 resulted to an area of 16.46 mm^2 and 26.25 mm^2 respectively.

All wounds on T_4 treated rats were found to have closed completely, giving a zero (0) value for the wound area on the 16th day of post treatment. Analysis of the data showed that the wound area for all treatments is not significantly different. It was noted that the lowest mean wound area was noted on T_2 which is 2.17 mm^2; T_1 is 3.58 mm^2 and T_3 is 12.59 mm^2.

Wound contraction was observed to have started on the 4th day of treatment as indicated by the decrease of wound size on the induced wound of experimental animals. The mechanism of wound contraction plays an important role in wound healing. Wound contraction implies the reduction in wound size and is believed to take place as a result of movement of wound edges towards the center (Paarakh, *et al*, 2009) [12].

The results of the present study conforms to the findings of Nayak (2008) [13] when avocado extracts were topically and orally administered on rats using excision and dead space wound models. Healing was assessed by the rate of wound contraction and period of epithelialization.

Total closure of wounds indicated by zero value for the wound area on the 12th day of post wounding treatment conforms to Paarakh (2009) [14] observation on wound healing activity of *Annona muricata* using open wound model.

Days to Wound Closure

One of the parameters in wound healing that was observed and monitored was wound closure. Wound closure was indicated by 0 mm^2 wound area. Table 4 summarizes the number of days to wound closure.

Table 4: Days to wound closure in albino rats

Treatments	Mean days to wound closure
T_1	17.30[a]
T_2	16.42[a]
T_3	18.20[a]
T_4	12.43[b]

Result = *

HSD= 3.765

Treatment means with common letters are not significantly different at 5% using HSD

It was noted that wound closure took place from day 12 to day 19 in the experimental rats. As shown in table 5, the *S. jamaicensis* extract treated animals (T_4) had the shortest period to wound closure in all the groups. In comparison however, the T_1 animals had a wound closure between 17 to 18 days, 16 to 17 for T_2, 18 to 19 for T_3 and 12 to 13 for T_4.

The analysis of variance of the data gathered showed a significant result at 5% level of significance. The mean number of days for animals in T_1, T_2, T_3 and T_4 were as follows: 17.30, 16.42, 18.20 and 12.43.

Wounds of *S. jamaicensis* extract treated rats healed faster as indicated by significant increase in complete scab formation, wound contraction and wound closure. This is in line with the results of the study reported by Nayak (2008) [12]; Udegbunam (2014) [10]; Karodi (2009) [14] and Mageswari (2015) [4] showing that the plant extracts have better wound healing property, as evidenced by the rate of wound contraction and reduction in the period of epithelialization.

Qualitative Protein Estimation

Protein plays a central role in wound healing through the production of collagen (Egozi, 2003) [15]. Collagen is the predominant extracellular protein in the granulation tissue of a healing of wound and there is a rapid increase in the synthesis of this protein in the wound area soon after an injury. In addition to providing strength and integrity to a tissue matrix, collagen also plays an important role in homeostasis (Pandian *et al*, 2013) [11]. Hence, increase in protein content confirms the healing of wound (Udegbunam, 2014) [10].

The scabs collected on the 4th and 8th day of post wound induction from the different treatments were subjected to protein content analysis using Biuret test.

The analysis on the scabs collected on the 4th day resulted to different color intensity. Protein content in experimental animals treated with T_4 indicated to be high as revealed by the

tube with the bluest colored solution when treated with Biuret reagent. This result is supported by the findings of Pandian (2013) [11] on the evaluation of wound healing activity of hydroalcoholic extract of leaves of *S. jamaicensis* in streptozotocin induced diabetic rats. The present investigation also conforms to the findings of Mageswari (2015) [4] that increase in collagen concentration facilitates wound healing.

The scabs collected on the 8th day were stiff and desiccated. However, bigger amount of scabs were collected from T_3 since the area of the wound is bigger compared to the other treatments.

The blue color intensity of the tube after adding Biuret reagent indicated the protein content of T_1, T_2, T_3, and T_4. The amount of scabs which were collected from T_4 was lesser, hence greater wound contraction was observed on the induced wound. T_4 scab had lesser protein content as indicated by the lesser intensity of the blue colored solution compared to T_3. This may be due to the fact that the wound is almost healed as shown by a greater wound contraction. T_2 and T_4 were almost similar in terms of the intensity of its blue colored solution.

The estimated protein content of the scabs treated with the different treatments was determined using Biuret test. It could be summarized that T_4 scab had the bluest colored solution on the 4th day indicating that the induced wound was at its peak of wound healing. The study conforms to the result obtained by Ikobi, *et al* (2012) [16] that wound healing is observed on the 1st to 4th day of post treatment with methanolic extract of dried fresh *Gossypium barbadense* indicated by high collagen content on the granulation tissue collected from the experimental animals.

Phytochemical Screening

Qualitative tests were performed on the leaf crude leaf extract of *S. jamaicensis* to identify the observable phytoconstituents responsible for the observed wound healing property of the extract. The result of qualitative phytochemical screening is shown in Table 5.

Table 5: Results of Phytochemical Screening

Secondary constituents	Observation	Result
Alkaloid	No change was observed	-
Flavonoids	Formation of intense yellow color that became colorless on addition of few drops of dilute HCl.	+
Phenols	Formation of deep blue color	+
Phytosterols	The chloroform layer appeared red and the acid layer showed greenish yellow fluorescence	+
Terpenoids	Formation of blue/green rings	+
Tannins	A blue black coloration was observed	+
Carbohydrates	Formation of violet ring at the junction	+
Coumarins	Formation of yellow color	+
Saponins	1 cm layer of foam was observed	+
Amino acids	Formation deep blue color	+
Anthraquinones	Change in the color from colorless to slight yellow solution was observed	+

Legend: + indicates the presence of secondary metabolites on *S. jamaicensis* extract
- indicates the absence of secondary metabolites on *S. jamaicensis* extract

The result obtained from phytochemical analysis has shown the presence of flavonoids, phenol, phytosterol, terpenoid, tannin, coumarin, saponin, anthraquinone, carbohydrates and amino acid. Alkaloid was not found present in the crude leaf extract of *S. jamaicensis.*

Phytochemical constituents present in the *S. jamaicensis* extract may be responsible for wound-healing potential. The exhibited antibacterial properties of *S. jamaicensis* could be attributed to the presence of saponins and phenolics in the plant. (Ruma & Zipagang, 2015) [17]. Previous studies with plant extracts have shown that constituent like flavonoids possess potent antioxidant and free radical –scavenging effect, enhancing the level of antioxidant enzymes in granuloma tissue (Mughrabi *et al*, 2014) [18].

Saponins are responsible for wound contraction and elevated rate of epithelialization known to promote the wound-healing process mainly due to their antibacterial and antimicrobial properties.

Tannins are free radical scavengers and promote wound healing due to their astringent and antimicrobial property (Soni *et al*, 2012) [19]. Tannins are also known to possess antioxidant activity and are able to improve wound healing and protect tissues against oxidative damage (Akanji and Sonibare, 2015) [20].

Flavonoids have been recognized as agents that can be used to antagonize lipid peroxidation that usually occurs in case of wound injury. Similar to antioxidant like vitamin C and vitamin E, any drug that antagonizes lipid peroxidation helps in increased circulation therefore increased collagen viability, thus increasing DNA synthesis and reducing cell damage (Pandian *et al*, 2013) [11]. Phytochemical screening of *S. jamaicensis* which showed presence of flavonoids attributed to its wound healing property.

With respect to wound healing mechanism, the antibacterial, antioxidant and antimicrobial properties of *S. jamaicensis* extracts could be effective on the proliferation and remodeling phase of wound healing. The wound healing potentials of S. *jamaicensis* extract may be associated to the phytoconstituents present in the crude extract and the faster process of wound healing could be a function of either the individual or the additive effects of the secondary metabolites.

Conclusion

The result of the present investigation suggests that *S. jamaicensis* leaf extract has a beneficial effect and plays major roles in wound healing. External application of the crude leaf extract of *S. jamaicensis* accelerates wound healing in albino rats. Phytoconstituents such as tannins, flavonoids, saponin, terpenoids, glycosides, phenols could have been responsible for wound healing activity due to antibacterial, antioxidant and antimicrobial property. The result revealed that the leaf crude extract of *S jamaicensis* could be effective for the treatment of wounds as shown by scab formation, better wound contraction and promote faster wound closure as the parameters to wound healing.

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