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Antimicrobial and Wound Healing Activity of *Gardenia aqualla* Stem Bark Methanol Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at examining the antimicrobial and wound healing activities of *Gardenia aqualla* stem bark methanol extract (*GASBME*) in order to provide scientific basis for its antimicrobial and wound healing properties.
Methodology: Preliminary phytochemical screening was done and antimicrobial activity determined using some pathogenic micro-organisms were evaluated following its wound healing effectiveness in Wistar rats using the model of superficial skin excision wound. Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnet's t-test.
Results: The results of preliminary phytochemical screening revealed the presence of carbohydrate, cardiac glycosides, saponins, flavonoids, triterpenes, tannins, alkaloids with anthraquinones being absent. The extract showed antimicrobial activity on *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia and Salmonella typhi* and significant ($p< 0.05$, $p< 0.001$) decrease in wound measurement by the 11th day.
Conclusion: This study demonstrated that GASBME possesses antimicrobial activity with wound healing properties that justifies the ethno-medicinal use of the plant in wound/ulcer healings.

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1. INTRODUCTION

Medicinal plants have been identified and known throughout human history as plants whose roots, stems, leaves and seeds possess therapeutic, tonic, and other pharmacological potentials. They are used as drug, food additives and for their nutritive values. Medicinal values of some plants lie in the chemical substances that produce definite physiological actions in their body. Among the chemical substances present in plants are phytochemicals which are bioactive natural compounds [1]. Many plants that are used in traditional medicine to alleviate symptoms of illnesses have been found to possess phytochemicals. Chemical compounds present in these plants mediate their effects on human body through processes identical to those already well understood from chemical compounds in conventional drugs. Thus herbal medications do not differ greatly from conventional drugs in terms of their effects on the body .This enables herbal medicines to be effective as well as having the potentials to cause harmful side effects [2,3].The use of herbs and search for drugs and dietary supplements derived from plants have accelerated in recent years because medicinal plants are known to contain some chemical substances which can be used for treatment purposes or to produce drugs [4]. Medicinal plants play vital roles in the health of individuals, in fact most modern drugs are derived from them [5]. Medicinal- plant -based drugs have the advantage of being simple, effective and have broad spectrum activity .An ethno-botanical and ubiquitous plant serves as rich resources of natural drugs for research development [6].

Traditional medicine has become a potential source of therapeutic agents for various communities around the world as primary health care to address their healthcare needs and concerns [5,6] and this encompasses all kinds of folk medicine, unconventional medicine and indeed any kind of therapeutically available method that had been taken over from the fore fathers [7,8]. High performance liquid chromatography (HPLC) and preliminary phytochemical screening of the methanol extract of *Gardenia aqualla* stem bark have revealed the presence of saponins, flavonoids and tannins [9]. Previous studies have also revealed the analgesic and anti- inflammatory effects of the methanol extract of *Gardenia aqualla* stem bark in different assays including acetic-acid induced writhing, formalin-induced nociception, and albumin- induced oedema in mice [10]. *Gardenia aqualla* haven been shown to possess some bioactive constituents like tannins, saponins and flavonoids with anti-inflammatory potential and relatively safe from its previous toxicological studies. The antioxidant and hepatoprotective potential of this plant have also been demonstrated suggesting that GASBME can be utilized as a natural source to protect the liver in hepatotoxin liver injury [11,12].

2. MATERIALS AND METHODS

2.1 Animals

Fifty one (51) male Wistar rats weighing 140-200 g were obtained from and housed in the Animal house, Department of Pharmacology and Therapeutics, A.B.U, Zaria under room temperature. They were all kept in plastic cages. The animals were fed on Vital Grower feeds (Vital feeds, Jos-Plateau State) and water *ad libitum*. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

2.2 Drugs and Chemicals

DPPH (Sigma-Aldrich, Germany), Chloroform (Sigma Chemical Co. USA), 1% Silver sulphadiazine (Salutas Pharma GmbH, Germany), L-Ascorbic acid- (Sigma-Aldrich Co. LLC), Methanol (Synthetic colour chemical Industry, India), Molisch's reagent, Concentrated Sulphuric acid (H_2SO_4) (BDH Ltd Poole, England), Concentrated hydrochloric acid (BDH Ltd Poole, England), Ferric Chloride (BDH Ltd Poole, England), Silymarin tablets 140 mg film coated tablet (Micro Labs Ltd, India) and Lidocaine Injection 2% (Erica Life Science Ltd. UK).

2.3 Preparation of Plant Extract

The *Gardenia aqualla* stem bark was freed from sand particles by carefully scrapping with a spatula. It was chopped to pieces, air dried and milled into coarse powder using pestle and mortar. Extraction was carried out by cold maceration of 500 g of the coarse powder with 2.5 L of 70% V/V aqueous methanol for 72 h, with constant shaking using the Gesellschaft für Labortechnik (GFL shaker No. 3017 mbH, Germany). The resultant mixture was filtered

using Whatman filter paper (No.1) and the filtrate was evaporated to dryness in vacuum at 40°C using rotary evaporator to give a yield of 25% w/w of the extract. Aliquot portions of the extract were weighed and dissolved in distilled water for use in the study.

2.4 Phytochemical Screening

The preliminary phytochemical screening of the plant was carried out using standard method for the presence of phytochemical constituents [13]. Molisch's test was used to test for carbohydrate. Keller-Killiani and Kedde's test were used for cardiac glycosides. Frothing's test for saponins, Liebermann-Burchard's for steroids and triterpenes. Shinoda and sodium hydroxide tests were used for flavonoids. Lead sub-acetate and ferric chloride tests were used for tannins and Mayer's, Dragendorff's and Wagner's test were used for alkaloids [13].

2.5 Acute Toxicity Studies

LD_{50} determination was conducted using Lorke's method [14] for both intra-peritoneal and oral routes in rats. This method was carried out in two phases. In the first phase, 3 rats per group of different weights were treated with the methanol extract of *Gardenia aqualla* stem bark at a dose of 10 mg/kg, 100mg/kg and 1000 mg/kg body weight orally and were observed for signs of toxicity and death for 24 hours. Based on the results of the first stage, another set of animals (n=1) were treated with four (4) more specific doses of the extract at 1000 mg/kg, 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg body weight respectively and observed for signs of toxicity and mortality for 24 h. The procedure was also repeated for intra-peritoneal route (i.p.). The LD_{50} value was determined by calculating the geometric mean of the highest non-lethal dose (0/1) and lowest lethal dose (1/1) as shown in the formula

$LD_{50} = \sqrt{}$ (Highest non-lethal dose) x (lowest lethal dose)

2.6 Formulation of Creams

The formulation components were sorbitan monostearate and stearic acid which were melted in the liquid paraffin and cooled at 54°C.The initial quantity of the extract used was 30 g and the weight of the beaker used when empty was 25.65 g. The dissolution of all the quantities used were in 10 ml. The *Gardenia aqualla* stem bark methanol extract (*GASBME*) in different concentrations of 1%, 5% and 10% were determined. For 1%, it was calculated as 1/30 x 100 and this gave 3.3 g which was weighed and mixed with propylene glycol 4.02% W/W, glycerin 4.13% W/W and 10 ml of distilled water, all heated in one beaker till dissolution and this were allowed to cool to a room temperature. This procedure was also repeated for 5% and 10% respectively. Others like acetyl alcohol 3.56% W/W, stearic acid 4.80% W/W, olive oil 5.78%, span 60 1.78% W/W and tween 80 0.75% W/W were also heated in different beakers and all finally dissolved, stirred together until it cooled. The creams were then separated in different transparent container for onward use for the wound healing activities.

2.7 Antimicrobial Assessment

The antimicrobial activity of *Gardenia aqualla* stem bark methanol extract (*GASBME*) was determined using the following pathogenic microorganisms (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Proteus mirabilis, Pseudomonas florescence, Klebsiella pneumonia and Salmonella typhi*). GASBME was weighed and dissolved in 10 ml of distilled water so as to obtain a concentration of 60 mg/ml. This was the initial concentration used to check the antimicrobial activities of the plant. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to manufacturer's instruction sterilized at 121°C for 15 min. The medium was poured into sterile Petri-dish, the plates were then allowed to cool and solidify. Agar well diffusion method was the method employed for the screening of the extract. The sterilized medium was then seeded with 0.1 ml of the standard inoculums of the test microbes. The inoculum was spread evenly over the surface of the medium with a sterile swab. By the use of a standard cork borer of 6 mm diameter, a well was cut at the center of each inoculated medium. The extract solution (0.1ml) in concentration of 60 mg/ml was then introduced into each well on the inoculated medium. The inoculated plates were incubated at 37°C for 24 h, after which each plate was observed for zone of inhibition of growth. The zone was measured with a transparent ruler and the results recorded in millimetres [15].

2.8 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of GASBME was carried using broth dilution

method [16]. Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and was sterilized at 121^{o}C for 15 min, and the broth was then allowed to cool. McFarland's turbidity standard scale number 0-5 was prepared to give turbid solution. Normal saline was prepared, 10 ml was dispersed into sterile test tube and the test microbe was inoculated and incubated at 37^{o}C for 6 hours. Dilution of the test microbe in the normal saline was done until the turbidity marched that of the McFarland's standard by visual comparison; at this point the test microbe has a concentration of about 1.5 x 108 cfu/ml (colony-forming unit/ml). Two- fold serial dilution of the GASBME in the sterilized broth was made to obtain the concentration of 60 mg/ml, 30 mg/ml, 15 mg/ml, 7.5 mg/ml and 3.75 mg/ml. The initial concentration was observed by dissolving 0.6 g of the GASBME in 10 ml of the sterile broth. On obtaining the different concentrations of the GASBME in the broth, 0.1 ml of the test microbe in the normal saline was then inoculated into the different concentrations. Incubation was made at 37^{o}C for 24 h after which the test tube was observed for turbidity (growth), the lowest concentration of the GASBME in the broth which shows no turbidity was recorded as the MIC.

2.9 Minimum Bactericidal Concentration (MBC)

The MBC was carried out following the method of Celiktas et al. [16] to check whether the test microbes were killed or only the growth was inhibited. The Mueller Hinton agar was prepared, sterilized and was poured into sterile petri-dishes and the plate were allowed to cool and solidify. The content of the MBC in the serial dilutions were then sub-cultured onto the prepared medium, incubation was made at 37^{o}C for 24 h, after which each plate was observed for colony growth. The MBC was the plate with the lowest concentration of the extract without colony growth.

2.10 Wound Healing Studies

An excision wound healing model was used as described by Nayak et al. [17]. Wistar rats were weight- matched and placed into six (6) groups of 5 rats each, but the rats in the same group were also separated in different cages after excision to prevent cannibalism and subsequent application of cream prepared for wound healing at the dorsal region where excision was made. The dorsal fur of the animal was shaved with a razor blade and the area of the wound to be created was outlined on the back of the animals using permanent marker. All rats used for the experiment were anaesthetized with lidocaine at the region of the excision and a full thickness excision wound of 1cm in width was created along the markings using toothed forceps, a surgical blade and pointed scissors.

Group I rats served as control and was treated with base cream only, while groups II, III, IV were treated with cream extract of 1%, 5% and 10% w/w of the animal in each group measured. Group V served as synergism group and was treated with the cream 1% silver sulfadiazine + 10% extract w/w. Group VI served as standard and was treated with 1% silver sulfadiazine only. All the treatment lasted for eleven (11) days and wound area measurements were taken using caliper, thread and ruler on days 3, 5, 7, 9, and 11. All the animals were left for observation for two (2) weeks post complete wound closure and healing for any observable signs of toxicities on the wound area. The percentage wound healing was calculated using the formula below:

$$\%\ \text{Wound healing} = \frac{\text{Initial wound area} - \text{Unhealed wound area}}{\text{Initial wound area}}\ \text{X}\ 100$$

2.11 Statistics

All experiments were performed in replicates (n=3) for validity of statistical analysis. Results were expressed as mean ± standard error of mean (S.E.M.). One way analysis of variance (ANOVA) and student t- tests were performed on data set using SPSS analytical tool. Values of $p< 0.05$ were considered significant.

3. RESULTS

3.1 Phytochemical Screening

The results of preliminary phytochemical screening show the presence of carbohydrate, cardiac glycoside, saponin, flavonoids, triterpenes, tannins, alkaloids but anthraquinones was absent (Table 1).

Effects of GASBME on the studied microbes. The results of antimicrobial susceptibility effects of GASBME and 1% silver sulfadiazine against the tested organisms (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli, Proteus mirabilis, Pseudomonas flourescense, Klebsiella pneumonia and Salmonella typhi*) showed that the microbes *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli,*

Klebsiella pneumonia and *Salmonella typhi* are sensitive to GASBME but the microbes *Streptococcus pyogenes*, *Proteus mirabilis* and *Pseudomonas flourescense* were resistance to GASBME. Meanwhile, the microbes *Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia* and *Salmonella typhi* were sensitive to 1% silver sulfadiazine but *Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis* and *Pseudomonas flourescense* were resistant to 1% silver sulfadiazine (Table 2).

Effects of GASBME and 1% silver sulfadiazine on zone of inhibition on test microbes. The results of zone of inhibitions of the GASBME against the tested microbes (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli, Proteus mirabilis, Pseudomonas flourescense, Klebsiella pneumoniae* and *Salmonella typhi*) showed respectively as 22, 0, 24, 20, 0, 0, 27 and 20 all in millimeters (mm). Also for 1% silver sulfadiazine against the microbes (*Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Escherichia coli, Proteus mirabilis, Pseudomonas flourescense, Klebsiella pneumonia* and *Salmonella typhi*) are respectively 0, 0, 32, 29, 0, 0, 35 and 27 (Table 3).

3.2 Effects of Minimum Inhibitory Concentration (MIC) and Antimicrobial Susceptibility Using GASBME

For MIC, the results showed that *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli* and *Salmonella typhi* were inhibited by the GASBME at 15 mg/ml turbidity and of slight growth at 7.5 mg/ml with moderate turbidity at 3.75 mg/ml respectively. *Streptococcus pyogenes, Proteus mirabilis* and *Pseudomonas flourescense* were not inhibited by the extract. Lastly, *Klebsiella pneumoniae* has the minimum inhibition concentration of 7.5 mg/ml when treated with the extract and turbidity of 3.5mg/ml was also observed (Table 4)

3.3 Effects of Minimum Bactericidal Concentration (MBC) on Test Microbes Using GASBME

The MBC results showed that the methanol extract of GASBME can kill the microbes *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi* at a minimum bactericidal concentration of 30 mg/ml, 30 mg/ml, 60 mg/ml, 15 mg/ml and 60 mg/ml respectively. This means that the extract will kill these microbes completely at this concentration stated in their respective regions. The extract has no effects on *Streptococcus pyogenes, Proteus mirabilis, Pseudomonas fluorescence* and *Klebsiella pneumonia* (Table 5)

3.4 Effects of Topical Application of GASBME Cream Extract on Excision Wound in Rats Treated for 11 Days

The topical application of 1% GASBME cream showed that there was no statistical ($p > 0.05$) difference at day 3 (0.80±0.03) compared to the base formulation (0.86±0.02), but there was statistical ($p < 0.001$) decrease at day 3 (0.56±0.05, 0.48±0.04) of the cream extract 5% and 10% respectively in relation to the wound area when compared to the base formulation (0.86±0.02) with the 5% and 10% showing 100% effectiveness in the healing of the wound. The standard drug (1% silver sulfadiazine) offered effectiveness of 100% (0.56±0.05) also at day 3. At day 5, extract 1% preparation had statistical ($p < 0.05$) decrease (0.52±0.04) while that of 5%, 10% cream extract had statistical ($p < 0.001$) difference (0.38±0.04, 0.24±0.03). This showed that at day 5, 1% cream extract had 95% wound healing effectiveness while 5% and 10% had 100% wound healing effectiveness (Table 6). The standard silver sulfadiazine had also 100% wound healing effectiveness. At both day 7 and 9, there was statistical difference ($p < 0.001$) showing 100% wound healing effectiveness of all the percentage cream extracts. At day 11, there was statistical ($p < 0.05$) difference in all the cream percentage extract used for the excision sites on the Wistar rats (Table 6).

4. DISCUSSION

Some medicinal plants were scientifically proven to be used for the healing of wounds which are mentioned in the African folk medicine [18,19]. Plants have the ability to synthesize aromatic substances, most of which are phenolic compounds and their oxygen-substituted derivatives [20]. In many cases, these substances serve as plant defense mechanisms against predation by micro- organisms, insects, and herbivores while some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment [21]. The mode of action for plant-derived agents

targets biochemical features of the invading pathogens that are not prossessed by the normal host cell. Sensitivity of the infecting micro-organism to a particular agent is some of the factors important for anti- microbial treatment [22].

Table 1. Phytochemical constituents of *Gardenia aqualla* stem bark methanol extract

Phytochemical	Inference
Carbohydrate	+
Saponins	+
Tannins	+
Flavanoids	+
Triterpenes	+
Alkaloids	+
Anthraquinones	–

Key + = Present – = Not detected

Table 2. Antimicrobial susceptibility effect of GASBME and 1%silver sulfadiazine

Test Organism	GASBME	Silver sulfadiazine cream
Staphylococcus aureus	Sensitive	Resistant
Streptococcus pyogenes	Resistant	Resistant
Corynebacterium ulcerans	Sensitive	Sensitive
Escherichia coli	Sensitive	Sensitive
Proteus mirabilis	Resistant	Resistant
Pseudomonas flourescense	Resistant	Resistant
Klebsiella pneumoniae	Sensitive	Sensitive
Salmonella typhi	Sensitive	Sensitive

Table 3. Effects of GASBME and 1% silver sulfadiazine on zoneof inhibition on test microbes

Test Organism	Zone of inhibition (mm)	
	GASBME	Silver sulfadiazine cream
Staphylococcus aureus	22	0
Streptococcus pyogenes	0	0
Corynebacterium ulcerans	24	32
Escherichia coli	20	29
Proteus mirabilis	0	0
Pseudomonas flourescense	0	0
Klebsiella pneumoniae	27	35
Salmonella typhi	20	27

Table 4. Minimum inhibitory concentration (MIC) (mg/ml) and antimicrobial susceptibility effect of GASBMEagainst test microbes

Test organism	60	30	15	7.5	3.75
Staphylococcus aureus	-	-	0*	+	++
Streptococcus pyogenes	-	-	0*	+	++
Corynebacterium ulcerans	-	-	0*	+	++
Escherichia coli	-	-	0*	+	++
Proteus mirabilis	-	-	-	-	-
Pseudomonas flourescense	-	-	-	-	-
Klebsiella pneumonia	-	-	-	0*	+
Salmonella typhi	-	-	0*	+	++

- = No turbidity (no growth), 0 = Minimum Inhibitory Concentration, + = Turbidity (high growth), ++ = Moderate Turbidity, MIC = Minimum Inhibitory Concentration*

Table 5. Minimum bactericidal concentration (MBC) of GASBME on test microbes

Test organism	60	30	15	7.5	3.75
Staphylococcus aureus	-	0*	+	++	+++
Streptococcus pyogenes	-	-	-	-	-
Corynebacterium ulcerans	-	0*	+	++	+++
Escherichia coli	0*	+	++	+++	++++
Proteus mirabilis	-	-	-	-	-
Pseudomonas flourescense	-	-	-	-	-
Klebsiella pneumoniae	-	-	0*	+	++
Salmonella typhi	0*	+	++	+++	++++

- =No turbidity i.e. no growth, 0 = Minimum bactericidal concentration, + = Scanty colonies growth, ++ =Moderate colonies growth, +++ = Heavy colonies growth*

Table 6. Effects of topical application of *Gardenia aqualla* cream extract on excision wound in rats treated for 11 days

Cream	Reduction in diameter of wound (mm)				
(% $^{w}/_{v}$)	Day 3	Day 5	Day 7	Day 9	Day 11
Base	0.86 ± 0.02	0.68 ± 0.04	0.56 ± 0.04	0.42 ± 0.02	0.30 ± 0.03
Ext. 1 %	0.80 ± 0.03^{ns}	0.52 ± 0.04^{a}	0.36 ± 0.02^{c}	0.12 ± 0.01^{c}	0.06 ± 0.02^{a}
Ext. 5%	0.56 ± 0.05^{c}	0.38 ± 0.04^{c}	0.22 ± 0.02^{c}	0.13 ± 0.03^{c}	0.06 ± 0.02^{a}
Ext. 10%	0.48 ± 0.04^{c}	0.24 ± 0.03^{c}	0.16 ± 0.02^{c}	0.10 ± 0.03^{c}	0.12 ± 0.10^{a}
1% Dermazin	0.56 + 0.05^{c}	0.38 + 0.04^{c}	0.22 + 0.02^{c}	0.13 + 0.01^{c}	0.06 + 0.02^{a}

Values are expressed in Mean ± SEM, n=5, ns=not significant, df = degree of freedom, f=frequency, α= degree of significant, $p < 0.05^{a}$, $p < 0.001^{b \text{ and } c}$ One Way ANOVA followed by Dunnet's t-test

Phytochemical constituents present in plants are said to be active secondary metabolites responsible for the important pharmacological activities and they play a major role in wound healing. For example, tannins have been shown as an active detoxifying agent and also inhibit bacterial growth [23]. Also the astringent and antimicrobial property of terpenoids have been said to promote the wound healing process [24] and flavonoids are said to be potent antioxidants, free radical scavengers [13,25,26]. Polyphenols and flavonoids which prevent the synthesis of prostaglandins are said to possess anti-inflammatory properties and have antimicrobial activities [27]. Glycosides isolated from plants are also said to possess antioxidant, antimicrobial, analgesic, antitumor, immunomodulatory, and anti- inflammatory effects [28,29]. Therefore, the presence of phytochemicals in the crude extract of our plant such as terpenoids, flavonoids, glycosides, saponins, tannins, and phenolic compounds may have contributed to the wound healing activities serving as detoxifying agent with the inhibition of the bacterial growth [23], fast promotion of the wound healing [24], acting as an anti-inflammatory agent [29] as shown in the previous work using the plant (*Gardenia aqualla*), antioxidant and antimicrobial [13,24,29] on the Wistar rats.

The presence of pathogenic organisms such as *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia and Salmonella typhi* in wounds have been shown to result in infection of wounds which may lead to formation of chronic wounds [30]. The study showed that the extract exhibited strong broad spectrum antimicrobial activity against these pathogens (Table 2). The extract has been shown to be sensitive to *Staphylococcus aureus, Corynebacterium ulcerans, Escherichia coli, Klebsiella pneumonia and Salmonella typhi* hence preventing the wound from getting infected with microbes [30,31]. The topical application of antimicrobial agents/extract is an efficient method of microbial populations' destruction because of the availability of the active agents at the wound site which led to enhanced wound healing activity [31]. The GASBME had significant influence on the rate of wound closure based on different days of treatment of the wounds with the extract compared with the base cream treated group and the standard. The 5% cream extract had the same strength compared to the standard 1% silver sulphadiazine at day 3. At day 5 there were significant wound contraction (p< 0.001) which showed the same in day 7 and day 9 and wound closure of 81.56%, 80.85% and 88.9% for 1% cream extract respectively. Also there was different percentage wound closure for

5% and 10% cream extract as shown in the result. The wound of the Wistar rats treated with base cream showed no statistical (p> 0.05) difference compared to the cream extracts (1%, 5% and 10% cream preparations) and standard, 1% silver sulphadiazine treated wounds. This may also indicate that the components of the base cream do not interfere with the activities of the extract hence the enhanced effectiveness of the GASBME which may be primarily due to the bioactive principles present in the extract [12]. The above findings may support the claims that wound healing process may be due to their direct action on the wound repair process, phytochemical bioactive present and antimicrobial effects or the combination of the above effects [17].

5. CONCLUSION

This study has shown that *GASBME* has phytochemical constituents which have the ability to enhance wound healing in Wistar rats. The effectiveness of the extract in wound healing may also be attributed to its earlier study showing its anti-inflammatory and antioxidant effects with good potent antimicrobial ability as shown in this our present studies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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