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Wound Healing Activity of Total Alkaloidal Extract of the Root Bark of *Alstonia boonei* (Apocynacea)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JOK and KBM designed the study, wrote the protocol and managed the analysis of the study; author JPF performed the statistical analysis and wrote the first draft of the manuscript. Authors ED and JPF managed the literature searches and laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Screening total alkaloidal extract from *Alstonia boonei* root bark for antimicrobial and wound healing activity.
Methodology: The anti-microbial screening was performed using the agar well diffusion method and the excision wound model was employed for the wound healing experiment.
Results: Thin-layer chromatography (TLC) of the extract showed four (4) spots when glacial acetic acid, ethyl acetate and chloroform were used in the ratio 1:3:1 respectively. However, six (6) spots were observed when ethyl acetate, methanol and water were used in the ratio 15:3:2 respectively. The total alkaloid extract showed antimicrobial activity against *S. aureus, B. subtilis, P. aeruginosa and E. coli*. The extract also showed significant wound healing activity ($P<0.05$).
Conclusion: The TLC showed that the total alkaloid extract contains several individual chemical compounds. The alkaloid extract has good antimicrobial activity against the above listed microorganisms at concentrations of 1% (10mg/ml) and above. The total alkaloid extract of *Alstonia boonei* possesses significant wound healing activity which was evident by the increased rate of wound contraction and reduction in the period of epithelialization.

Keywords: Alstonia boonei; Anti-microbial; Excision wound; Phytochemical screening; Total alkaloids.

1. INTRODUCTION

Alstonia boonei belongs to the family *Apocynaceae*. Although all parts of the plant are very useful, the thick bark of the mature tree is the part that is mostly used for therapeutic purposes [1]. The bark of the tree is highly effective when it is used in its fresh state. Nonetheless, the dried one is equally used. Therapeutically, the bark has been found to have anti-rheumatic and anti-inflammatory [1], analgesic/pain-killing, anti-malarial, antipyretic, anti-diabetic (mild hypoglycaemic) and antimicrobial properties [2]. It is also used in folkloric medicine for the treatment of ulcers [3].

The alarming increase and spread of resistance among bacterial pathogens to most clinically useful antibiotics has necessitated the search for new anti-microbial agents (of which medicinal plants are a key source) directed toward new targets. Quite an extensive research has been done in relation to the antimicrobial activity of *Alstonia boonei.* For instance, Amole and Ilori reported that both the ethanolic and aqueous extracts of *Alstonia boonei* stem bark showed antimicrobial activity against *S. aureus, S. pyogenes, S. pneumoniae, E. coli, B. subtilis, Candida* sp and *P. aeruginosa.* According to the research, the ethanolic extract gave a minimum inhibitory concentration (MIC) of 500mg/ml against *P. aeruginosa* and 250mg/ml against all the other microorganisms. For the aqueous extract, the results obtained were twice as those obtained for the ethanolic extract in each case [4].However, a research conducted by Adomi and Umukoro showed that aqueous and ethanolic extracts of the root bark of *Alstonia boonei* showed no zones of inhibition against *S. aureus,S. typhi, K. pneumonia, B. subtilis, Flavobacterium* sp, P*. aeruginosa and E. Coli* [5].

Wounds are physical injuries that result in an opening or break of the skin. Wounds include cuts, scrapes, scratches and punctured skin [6]. Wound healing has three phases (inflammation, tissue formation, and tissue remodelling) that overlap in time [7]. The goals of wound care include reducing risk factors that inhibit wound healing, enhancing the healing

process and lowering the incidence of wound infections. Extensive research has been carried out in the area of wound healing management with medicinal plants. Recent studies with significant findings involving *Morinda citrifolia* (*Rubiaceae*), *Catharanthus roseus (Apocynaceae*), *Lycopodium serratum* (*Lycopodiaceae*) *and Euphorbia hirta (Euphorbiaceae)* among others have been emphasized in various articles. *Catharanthus roseus* is a key source of monoterpene indole alkaloids, vinblastine and vincristine which are found to be useful in the treatment of cancer [8]. In a study, ethanolic extract of the flowers of *Catharanthus roseus* in a dose of 100mg/Kg/day was demonstrated to possess good wound healing activity [9].*Lycopodium serratum* (*Lycopodiacece*) commonly known as club moss is reported to contain many alkaloids. An ethanolic extract of the leaves showed a significant decrease in period of epithelialization and an increase in wound contraction rate, tissue breaking strength and hydroxyproline content at the wound site [9].

Various alkaloids have been isolated from the bark of *Alstonia boonei,* some of which have been characterised. Some examples of these alkaloids include echitamine, echitamidine, voacangine and akuammidine among others [3].

To date, there is no available systematic work to test the antimicrobial and wound healing activity of total alkaloidal extract from the root bark of *Alstonia boonei*, hence the need for this research.

2. MATERIALS AND METHODS

2.1 Plant Collection

The plant material was collected from the KNUST campus and subsequently authenticated at Department of Herbal Medicine, KNUST by Dr George Henry Sam. Plant authentication voucher specimen number: KNUST/HM1/2013/R004

2.2 Extraction of Total Alkaloids

The plant material was air dried and milled with the aid of a hammer mill (Schutte Buffalo, New York, USA) into fine powder. 300.00g of the dried powdered root bark of *Alstonia boonei* was weighed and cold macerated in 1.2 L of petroleum ether for 24hrs. This extract was decanted. The plant material was then extracted with 96% methanol using hot maceration at a temperature of 50℃ and filtered to obtain the soluble extract which was subsequently concentrated in a rotary evaporator (Rotavapor R-210, Buchi, Switzerland) at 40℃.The concentrate was dissolved in 10% acetic acid and filtered to remove non-alkaloidal components. The filtrate obtained was basified with strong ammonia solution to a pH of 9 and transferred into a separating funnel and extracted with six 100ml quantities of chloroform. The aqueous layer was further extracted with six 60ml quantities of butanol. The chloroformic and butanolic extracts were concentrated separately with a rotary evaporator at 20°C.The extracts were combined and finally dried in a Gallenkamp hot air oven (Oven 300 plus series, England) at 40℃ to obtain the alkaloidal extract. The weight of total alkaloids obtained was 3.00g and the percentage yield was 1.00%.

2.3 Phytochemical Screening

Phytochemical screening for bioactive constituents such as steroids and terpenoids, alkaloids, tannins, saponins, flavonoids, anthraquinones and reducing sugars was carried out on portions of the plant material using standard phytochemical procedures [10-12].

2.4 Thin Layer Chromatography

Silica gel precoated aluminium plates 60 F254 (0.25 mm thick) were used for the TLC. A sufficient quantity of the alkaloidal extract was dissolved a suitable amount of methanol (96%) and applied as spots using capillary tubes on the TLC plates previously cut to an appropriate size so as to fit in a chromatographic chamber. The spots were applied on a line drawn about 2 cm from the edge and at equal distance from the margins. The spots were dried and the plates placed in a chromatographic chamber saturated with the mobile phase [glacial acetic acid: ethyl acetate: chloroform (1:3:1) in one instance and ethyl acetate: methanol: water (15: 3: 2) in another] for development by the one way ascending technique. The zones on TLC plates corresponding to separated compounds were detected under UV light 254 nm and also by spraying with anisaldehyde 0.5% w/v in glacial acetic acid: conc. sulphuric acid : methanol (10:5:85). The various retardation factor (RF) values of the spots were calculated as shown below.

$$\boldsymbol{RF} = \frac{distancetravelledbyspot}{distancetravelledbysolventfront} \quad (1)$$

2.5 Antimicrobial Screening

Pure cultures of two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis)* and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)* bacteria were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, KNUST. The experimental microorganisms were sub-cultured in sterile nutrient broth and incubated at 37℃ for 24 hours. The anti-microbial screening was done using the agar well diffusion method. Four concentrations of the total alkaloid extract (4.0%, 2.0%, 1.0% and 0.5%) were prepared as aqueous solutions. Four agar plates were used for each microorganism.

2.6 Animal and Husbandry

Healthy Sprague-Dawley rats of either sex (n=20, weighing 150 to 190g) were selected for wound healing study. They were housed under standard condition of temperature, humidity and light and fed with commercial pellet food (GAFCO, Tema, Ghana) and enough water, in a stainless steel cage with wood shavings as the bedding material and a wire screen top.

2.7 Wound Healing Experiment

Formulations were prepared from the total alkaloid extract, where 10% (500mg of extract in 5g of formulation), 3% (150mg of extract in 5g of formulation) and 1% (50mg of extract in 5g of formulation) of the extract was prepared using aqueous cream as the base. No preservative was added to the aqueous cream base.

A total of 20 animals were divided in 5 groups of four animals each. There were two control groups namely groups 1 (being the positive control) and 5 (being the negative control). The animals in group 1 were treated with 1% silver sulfadiazine cream (dermazin) whilst those in group 5 were treated with aqueous cream (without extract). Test group animals: groups 2, 3 and 4 received the 3%, 10% and 1% total alkaloid extract respectively.

Under light ether anaesthesia, an impression was made on the depilated dorsal thoracic central region of the rats, about 5cm away from the ears, by using a pair of surgical scissors.

Incisions in the tissues underneath the wound area were avoided and the skin tension was also kept constant during the experiment. The extracts were administered everyday up to a total of 21 days and the wound closure (contraction) was observed and recorded. The measurement of wound size was done with the aid of a transparent tracing paper on 1st, 3rd, 5th, 7th, 9th, 11th, 14th, 17th, 19th and 21st post wounding day. The transparent paper was subsequently placed on a 1 mm^2-grid graph sheet and the wound area was recorded. The percentage of wound contraction (wound healing) was calculated by using following formula:

$$\text{Percentage of Wound contraction} = \frac{woundsizeonday\ 0 - specificdaywoundsize}{woundsizeonday\ 0} \times 100\% \quad (2)$$

2.8 Data Analysis

All data analysis was done with the software Graph Pad Prism for Windows version 5 (GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1 Phytochemical Screening

Phytochemical screening of the powdered root bark showed the presence of alkaloids, tannins, saponins, reducing sugars, steroids and terpenoids whilst flavonoids and anthraquinones were absent.

3.2 Thin Layer Chromatography

Glacial acetic acid, ethyl acetate and chloroform in the ratio 1: 3: 1 gave four spots after TLC analysis with 0.75 and 0.29 being the highest and lowest retardation factor (RF)values respectively (Table 1). However, when ethyl acetate, methanol and water were used in the ratio 15: 2: 1, six separated spots were obtained with the highest and lowest RF values of 0.88 and 0.08 respectively (Table 2).

Table 1. Retardation factor (RF) for solvent system: glacial acetic acid: ethyl acetate: chloroform (1:3:1)

Spot	Retardation factor
A	0.29
B	0.48
C	0.65
D	0.75

Distance travelled by solvent front = 4.8cm

Table 2. Retardation factor (RF) for solvent system: ethyl acetate: methanol: water (15:2: 1)

Spot	Retardation factor (RF)
K	0.08
L	0.15
M	0.31
N	0.42
O	0.42
P	0.88

Distance travelled by solvent front = 4.8cm

$$RF = \frac{distancetravelledbyspot}{distancetravelledbysolventfront} \quad (1)$$

3.3 Antimicrobial Screening

The zones of growth inhibition measured during the antimicrobial screening by Agar well diffusion method is presented in Table 3 below. Zones of growth inhibition were observed for the microorganisms at all concentrations except for *P. aeruginosa* and *B. subtilis* which showed no zones of inhibition for the 0.5% (5mg/ml) extract. It could be observed that the total alkaloidal extract possessed a broad spectrum antimicrobial activity on both gram positive and gram negative bacteria at a concentration of 1.0% (10mg/ml) and above. But for the differences in concentration, these results are in agreement with the research conducted by Amole and Ilori [4], except that in addition to *P. aeruginosa, B. subtilis* also showed no inhibition with the least concentration of the extract.

Table 3. Average zones of growth inhibition for various microorganisms

	Mean zones of inhibition (mm)(Mean ± SEM)			
Conc. ($\%^w/_V$)	***E. coli***	***P. aeruginosa***	***B. subtilis***	***S. aureus***
4.0	7.50±0.29	3.50±0.29	6.25±0.25	7.75±0.48
2.0	5.00±0.00	3.25±0.25	3.50±0.29	4.00±0.00
1.0	3.75±0.48	3.00±0.00	2.75±0.48	3.00±0.00
0.5	3.50±0.29	-	-	2.50±0.29
Control	-	-	-	-

Results presented asMean ± SEM, **-** *= no inhibition.*

3.4 Wound Healing Studies

Healing wounds were compared between negative control group, rats treated with silver sulfadiazine as control group and total alkaloid extract-treated rats as experimental groups during the wound healing process. In contrast, the progress of wound healing in the control and negative control groups appeared to be impaired. In all groups, wounds were covered by a dehydrated wound crust or scab at day 1 after incision, and the scab was almost gone after day 14. By 21 days after the incision, the wounds fully healed in all total alkaloid extract-treated rats based on the macroscopic closure of the incision interface and restoration of an epithelial cover (Fig. 1). The wounds' diameters in the experimental group, which was treated with the total alkaloid extract, experienced a faster reduction than both the negative control and the control. A graph showing the percentage contraction of excision wounds treated with test and control formulations from days 1 to 21 is represented in Fig. 2 below.

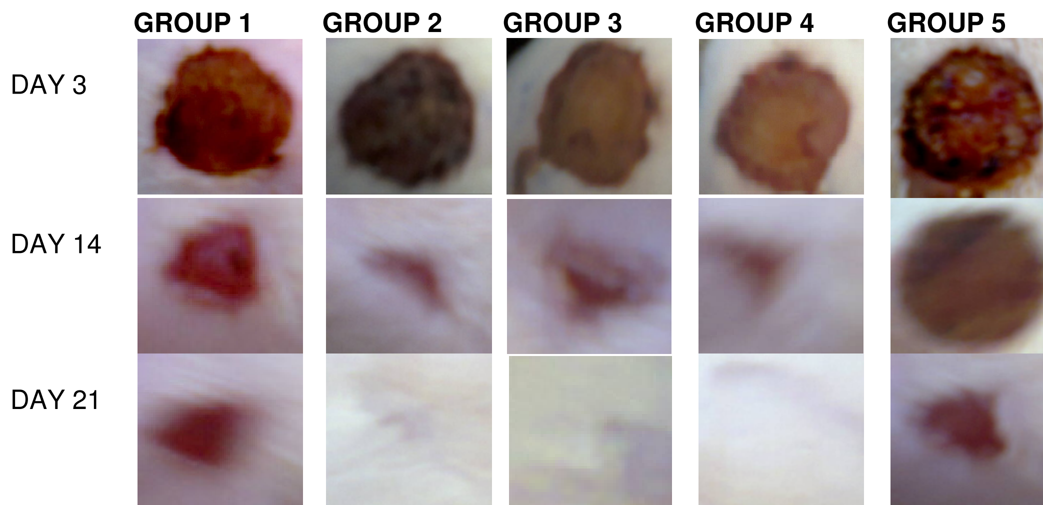

Fig. 1. Effect of both test and control formulations on the gross appearance of wound healing on day 3, 14, and 21. (Group 1) The sulfadiazine group, showing incomplete wound healing. (Group 2) The 3% extract group, showing complete wound healing. (Group 3) The 10% extract group, showing complete wound healing. (Group 4) The 1% extract group, showing complete wound healing. (Group 5) The aqueous cream group, showing incomplete wound healing

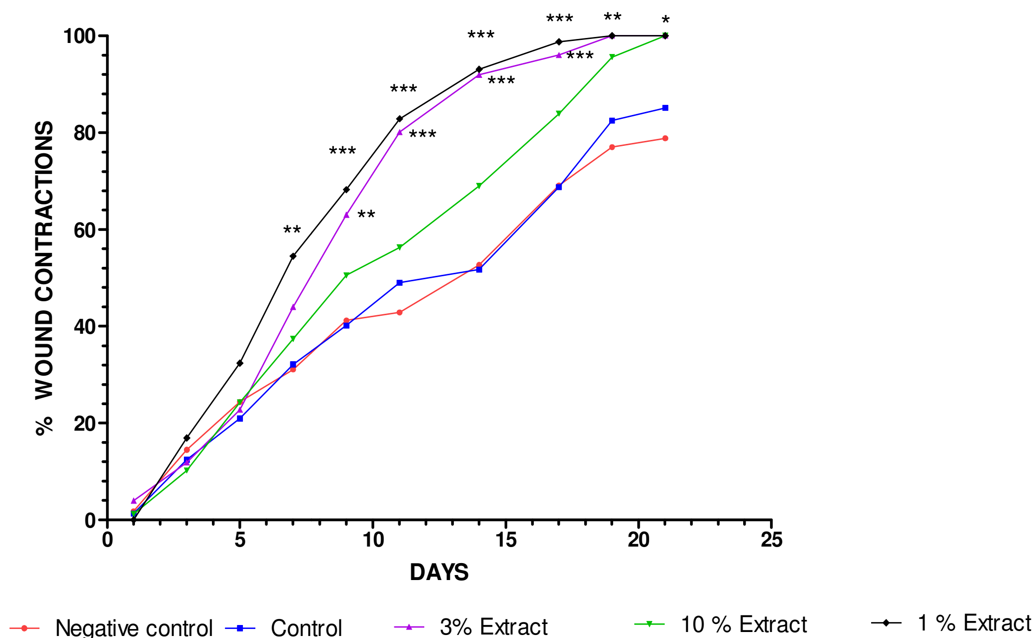

Fig. 2. Percentage contraction of excision wounds treated with test and control formulations. The results presented as mean ± SEM. Statistical analysis is by two-way ANOVA using Bonferroni post hoc test. * means P<0.05, ** means P<0.01, * means P <0.001**

$$\text{Percentage of Wound contraction} = \frac{wound size on day\ 0 - specific day wound size}{wound size on day\ 0} \times 100\% \qquad (2)$$

4. DISCUSSION

Phytochemical screening of the root bark of *Alstonia boonei* showed the presence of glycosides, tannins, alkaloids, saponins, steroids and terpenoids. The presence of several secondary metabolites in the plant could possibly account for the reason why the root bark has numerous therapeutic indications. Some tannins for instance, have inherent astringent activity and this is very important in the management of various ulcers. Various types of glycosides have different varieties of pharmacologic actions ranging from anti-diabetic, antihypertensive, to anti-rheumatic actions. Alkaloids in *Alstonia boonei* are generally known to have anti-inflammatory, antimicrobial and wound healing activity.

The percentage content of total alkaloids in the root bark obtained was 1%. This depicts the fact that secondary metabolites like alkaloids exist in the root bark in relatively low quantities. However, it could also be that the various alkaloids were of very diverse chemical properties and polarities such that the solvent systems used for the total alkaloid extraction was not effective in extracting all the alkaloids.

From the TLC results, the wide range of Rf values obtained for both solvent systems suggest that the alkaloids present in the root bark of *Alstonia boonei* are of varying polarities. For instance, an Rf value of 0.08 is generally suggestive of a compound with relatively high polarity and a 0.88 Rf value suggests that the spot may contain compounds that are less polar comparatively. Also, the fact that the solvent system that contained ethyl acetate, methanol and water produced more spots than the system containing acetic acid, ethyl acetate and chloroform suggests that the former will be a better solvent system to separate the extract for further analysis. The possible alkaloids that may be present in the various spots are echitamine, echitamidine, voacangine and akuammidine among others. Further analysis of the spots is however required to determine the exact alkaloids present in each spot.

The present study indicates that the total alkaloid extract may be active against both gram positive and gram negative bacteria. *P. aeruginosa* often infects extensive skin burns, which can be a source of sepsis. *P. aeruginosa* can be very resistant to antibiotics, making such infections difficult to treat. [13] This research finding therefore offers a major headway to the discovery of a potentially active anti-pseudomonal agent which also possesses a very good wound healing activity thus can be used to effectively manage burns and other wounds infected with P*. aeruginosa*. Considering the fact that *Staphylococcus aureus* and *Escherichia coli* are among the commonest strains of microorganisms associated with open wound infections, [13] this extract can be a promising remedy not only for the treatment of such wounds but also in managing infections in which these microorganisms are implicated. *Bacillus subtilis,* just like *P. aeruginosa* showed a relatively low susceptibility to the extract. The low susceptibility of *Bacillus subtilis* to the extract may be due to the ability of *Bacillus subtilis* to form endospores [14] which offers it some level of resistance against antimicrobial agents. Overexpression of active efflux systems with wide substrate profiles [15] among other factors, may account for the low susceptibility of *P. aeruginosa* to the extract.

The complex process of wound healing involves various phenomena like wound contraction, granuloma formation, collagen maturation etc. The contribution for healing of such processes depends on the type of wound. Wound contraction plays a significant role in healing of excision wound while granuloma formation plays a role in healing of sutured

incision and dead space wounds. From the results obtained, it was observed that the test formulation-treated animals showed a higher percentage closure of wound size and a rate of wound contraction compared to the values obtained for standard drug-treated and negative control group.

The results of the present study clearly demonstrate that the total alkaloid extract of *Alstonia boonei* possess a definite prohealing action in wound healing. The wound healing activity of the total alkaloid extract could be attributed to the fact that extract caused an increased rate of formation of epithelial cells thus speeding up the re-epithelialization process which is critical in wound healing. There is also the possibility that angiogenesis which is the formation of new blood vessels was accelerated. This will in turn increase blood supply to the newly formed epithelial cells and thus in effect cause an overall increase in the rate of wound contraction. Zahra et al. [16] showed that wounds treated with some plant extracts contain more collagen deposition and fewer inflammatory cells and angiogenesis. An increase in the rate of healing activity has been attributed to angiogenesis and collagen deposition in granulation tissue [17]. Acceleration of wound-healing potential of the total alkaloid extract may therefore be due to the deposition of more collagen fibres with angiogenesis and less inflammatory cells in granulation tissue of wound area. Alkaloids in the root bark of *Alstonia boonei* are known to have anti-inflammatory activity [1]. This could be achieved by the inhibition of the production of cytokines following a cutaneous injury. Inflammation results in trauma and in the presence of trauma wound healing is delayed [18]. On the other hand, the anti-inflammatory effect of the extract may give rise to a quickening of the wound healing process. Research conducted by Kulasekaran SP, et al. [19] also revealed that alcoholic extract of Celosia argentea (*Amaranthaceae*), which contains several alkaloids, has a good wound healing activity. The molecular and cellular mechanisms of wound healing were investigated and it was discovered that the extract promoted cell motility and proliferation of primary dermal fibroblasts at 0.1–1.0 µg/ml but did not alter these responses in primary keratinocytes. In an initial examination of molecular mechanisms, it was found that the extract did not alter fibroblast and keratinocyte responses to the wound repair-associated epidermal growth factor receptor ligands. Although these could be suggested as possible mechanisms of wound healing by the alkaloidal extract, a systematic research ought to be carried out to ascertain the actual mechanism of wound healing by the alkaloidal extract.

The differences in percentage wound contraction with respect to the various strengths of the total alkaloid extracts could be attributed to the fact that the rats treated with the 10% extract showed signs of irritation following the application of the extract. This led to the removal of the extract from the wound by the rats. As such, the formulation tends to have a relatively short contact time on the wound thus it is not able to elicit its full therapeutic effect before it is wiped off the wound. Also in wiping the formulation from the wound, the rats tend to scratch the wound sites against the walls and floor of the cage and this resulted in subsequent bruising of the healing wound. This observation was also made in the group treated with the 3% extract. However, it was to a lesser extent in this group. There is also the possibility that the higher concentrations of the alkaloid extract gave a toxic effect (typically a cytotoxic effect) rather than the expected therapeutic effect thus retarding the wound healing process.

5. CONCLUSION

The phytochemical investigations of the root bark of *Alstonia boonei* showed the presence of steroids and terpenoids, saponins, alkaloids, tannins and reducing sugars (from glycosides).

The TLC results showed that the total alkaloid extract contains several chemical constituents with varying physicochemical properties.

The present study revealed that the total alkaloidal extract obtained from the root bark of *Alstonia boonei* possesses significant wound healing activity which was evident by the increased rate of wound contraction and reduction in the period of epithelialization. The antibacterial activity of the total alkaloidal extract contributes to its medicinal properties.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. Allexperiments have been examined and approved by Committee on Animal Research, Publication and Ethics (CARPE) of the faculty of pharmacy and pharmaceutical sciences, KNUST, Kumasi, Ghana; Ethics Reference №: FPPS/PCOL/Et0052/2013. Laboratory study was carried out in a level 2 biosafety laboratory. All the technical team observed allinstitutional biosafety guidelines for protection of personnel and laboratory.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abbiw D.K. Useful Plants of Ghana: West African Uses of Wild and Cultivated Plants, Intermediate Technology Publications. The Royal Botanical Garden, Kew, London. 1990;(119,127,132,157,167-170).
2. Hadi S, Bremner JB, Initial studies on alkaloids from Lombok medicinal plants, Molecules. 2001;6(2):117–129.
3. Ayiku MNB. Ghana Herbal Pharmacopoeia. Science and Technology Policy Research Institute, 1st edition, Accra. 1992:1O-16.
4. Amole OO, Ilori OO. Antimicrobial activity of the aqueous and ethanolic extracts of the stem bark of *Alstonia boonei,* International Journal of Phytopharmacology. 2010;1(2):119-123.
5. Adomi PO, Umukoro GE. Antibacterial activity of aqueous and ethanol crude extracts of the root barks of *Alstonia boonei* and preliminary phytochemical test of *Morindalucida,* Journal of Medicinal Plants Research. 2010;4(8):644-648.
6. Brigham PA, McLoughlin E. Burn incidence and medical care use in the United States: estimate, trends, and data sources. J Burn Care Rehabil. 1996;17:95-107.
7. Clark RAF. The molecular and cellular biology of wound repair, 2nd edition, New York: Plenum Press. 1996;112-114.
8. Badri PN, Renu S, Role of Medicinal Plants in Wound Healing. Research Journal of Medicinal Plant. 2011;5(4):392-405.
9. Nayak B.S. Cecropiapeltata L (Cecropiaceae) has wound healing potential-a preclinical study in Sprague Dawley rat model. IntJ,LowExtrem Wounds. 2006;5(1):20–26.

10. Evans WC. Trease and Evans textbook of Pharmacognosy, 15th edition, Philadelphia: Elsevier limited. 2008;289-335,536.
11. Bruneton J. Pharmacognosy, Phytochemistry and Medicinal Plants, 2nd edition, Hampshire, Intercept Ltd. 1995;386.
12. Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S, et al. Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* inalloxan-induced diabetic rats. Pharmacognosy Res. 2010;2(4):258-63.
13. Kumar V, Abass AK, Fausto N, Aster J. Robbins and Cotran Pathological Basis of Diseases, 8th edition. 2010;357-365.
14. Nakano MM, Zuber P Anaerobic growth of a "strict aerobe" (*Bacillus subtilis*), Annu. Rev. Microbiol. 1998;52(1):165–190.
15. Strateva T, Yordanov D. Pseudomonas aeruginosa – a phenomenon ofbacterial resistance,Journal of Medical Microbiology. 2009;58(9):1133–1148.
16. Zahra AA, Kadir FA, Mahmood AA, Al hadi AA, Suzy SM, Sabrim SZ, et al. Acute toxicity study and wound healing potential of Gynuraprocumbens leaf extract in rats, Journal of Medicinal Plant Research. 2011;5(12):2551–2558.
17. Paladini RD, Takahashi K, Bravo NS, Coulombe PA. Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin. J Cell Biol. 1996;132(3):381-397.
18. Hess CT. Checklist for factors affecting wound healing, Lippincott Williams & Wilkins. 2011;24:192.
19. Kulasekaran S, Priya, Gnanamani Arumugam, Bhuvaneswari Rathinam, Alan Wells, Mary Babu. Celosia argentea Linn leaf extract improves wound healing in a rat burn wound model. Wound Repair and Regeneration. 2004;12(6):618-625.

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