Society for Multidisciplinary & Advanced Research Techniques (SMART)
Trinity University, Lagos, Nigeria
SMART Scientific Projects & Research Consortium (SMART SPaRC)
Sekinah-Hope Foundation for Female STEM Education
Harmarth Global Educational Services
ICT University Foundations USA
IEEE Computer Society Nigeria Chapter

Proceedings of the 36th iSTEAMS Accra Bespoke Multidisciplinary Innovations Conference

Evaluation of the Wound Healing Activity Of *Picralima Nitida* Seeds

***Ibironke A. Ajayi, Bolaji S. Odejobi, Emmanuel N. Ifedi & Adewale A. Raji**
Industrial Unit, Chemistry Department
Faculty of Science
University of Ibadan
Ibadan, Nigeria
*Correspondent Author: frajayi@yahoo.com
Phone: +2348075041170

ABSTRACT

Picralima nitida seed extract was evaluated for wound healing activity. *P. nitida* seed methanolic extract was prepared by cold extraction method, labelled as PNME and evaluated for phytochemical constituents, antimicrobial activity and minimum inhibitory concentration using standard methods. 28 Wistar rats, distributed into four groups of seven rats each and fed with feed and water *ad-libitum* throughout the period of study, were incised with wound area of 550 mm^2. Rats from Groups 1 and 2 were treated with 15 % w/v and 20 % w/v of PNME prepared ointment respectively, Group 3 with 100 % ointment (positive control) while Group 4 were left untreated (negative control). The rate of wound healing of the animals was assessed by observing the wound contraction rate, wound area and epitheliazation time every four days until the end of the study. The effect of the incised wound on the rats' feed intake, change in body weight gain and behavioural pattern was also noted. Phytochemical analysis result showed that the extract contain different bioactive compounds such as alkaloid, reducing sugar, cardiac glycosides, flavonoids, terpenoids and sterols. Antimicrobial activity of 15 % w/v and 20 % w/v of PNME prepared ointments on *S. aureus*, *B. subtilis* and *P. aeruginosa* was significant. 20 % w/v PNME had the most potent wound healing capacity as evident from epitheliazation time of 19.86±2.34 days, followed by its 15 % w/v counterpart with 25.43±0.98 days; that of the positive control was 18.00±1.87 days. 20 % w/v *P. nitida* seed extract seemed to have potential of being used as wound healing ointment.

Keywords: *P. nitida* seeds; phytochemical; antimicrobial; wound healing; epitheliazation time

Proceedings Citation Format
Ibironke A. Ajayi, Bolaji S. Odejobi, Emmanuel N. Ifedi & Adewale A. Raji. (2023): Evaluation of the Wound Healing Activity Of Picralima Nitida Seeds. Proceedings of the 36th iSTEAMS Accra Bespoke Multidisciplinary Innovations Conference. University of Ghana/Academic City University College, Accra, Ghana. 31st May – 2nd June, 2023.
Pp 277-286. https://www.isteams.net/ghanabespoke2023. dx.doi.org/10.22624/AIMS/ACCRABESPOKE2023P25

1. INTRODUCTION

Akuammine is the most abundant active alkaloid found in the seeds from the tree *Picralima nitida*, commonly known as Akuamma. It is an opiate, but its action is principally on the kappa opioid receptor, as such it is not a plant that gives you direct spiritual experience in the way that opium or morphine does.

But it heals and is useful as a means of healing as such it is worthwhile including it because it indirectly helps spiritual experience in the suppression category by relieving pain and illness. The dried seeds from this plant are used in traditional medicine throughout West Africa, particularly in Ghana as well as in the Ivory Coast and Nigeria. The seeds are crushed or powdered and taken orally. *Picralima nitida* tree has been found to have several medicinal uses. A decoctin made from its stem bark is taken for the treatment of diarrhea, gonorrhoea and intestinal worms. The phytochemical screening reported by (Sumonu *et al.,* 2014) revealed the presence of some phytochemicals like alkaloids tannins, saponins, glycosides and steroids which probably suggests its usage for medicinal plant. This study evaluates the phytochemical constituent, and the wound healing acitivity of this seed extract on Wistar rats.

2. METHODOLOGY

2. 1 Extraction of *P. nitida*

The seeds were air dried and crushed to coarse powder with an electric blender before carrying out the extraction. 500 g of the powdered sample was put into an aspirator bottle and 1 L of methanol added. It was left at room temperature of about 32 ^{o}C for five days with continuous stirring after which it was filtered (Ajayi *et al.,* 2015). The filtrate was distilled to recover the solvent from the oil; the extract was stored in a universal bottle and refrigerated at 4 ^{o}C prior to use.

2. 2 Phytochemical screening

The extract and the ointments were subjected to phytochemical screening to detect the presence of secondary metabolites such as tannins, saponins, alkaloids, flavonoids, reducing sugar and glycoside using the methods described by Solomon *et al*. (2013). All determinations were done in triplicates.

2. 3 Pharmacological study of seed extracts

2. 3. 1 Antimicrobial activity assays

The antimicrobial activity of the seed extract was evaluated against a few pathogenic bacterial such as *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeuroginosa.* The pure bacterial strains were obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria. The bacterial strains were cultured overnight at 37 ^{o}C in nutrient agar.

2. 3. 2 Minimum inhibitory concentrations of seed extract

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the extracts that inhibit the visible growth on agar surface or turbidity in microwell broth. Minimum inhibitory concentrations both for bacterial and fungal strains were measured as reported in literature by Sarker *et al.* (2007).

2. 4. Experimental animals

Twenty-eight Wistar rats between the weights of 42-150 g were used for this research work. The rats, obtained from animal house of Veterinary Anatomy Department, University of Ibadan, were distributed into four groups and left to acclimatize in same venue for seven days before the commencement of the study. They were provided with water and feed *ad-libitum* throughout the study period. The weight of the rats was recorded on the first day of study and taken day zero; thereafter weekly weight was noted.

2. 4. 1 Wound excision model

The rats were injected with 0.2 mL ketamine hydrochloride anaesthesia based on the weight of each rat. After the numbness has been induced, the fur of the rats was scrapped off on the lateral right side of the animal using a scissors. A wound area of 2.65 cm diameter was impressed on the rats few meters away from the hind limb. The entire thickness of the skin was then excised using a scalpel, toothed forceps and a pointed scissors. Methylated spirit was used as antiseptic for the excised region. The wound were undressed, opened to the environment and returned to their cages.

2. 4. 2 Preparation of ointments

3.75 g of *P. nitida* extract was weighed with an analytical balance and poured into a 25 ml standard flask. This was then made up to the mark with paraffin oil to give 15 % (w/v) concentration of the extract. 5.0 g of same extract was also weighed and transferred into a 25 ml flask. This was also made up to the mark with paraffin oil to give 20 % (w/v) concentration of the oil extract.

2. 4. 3 Wound treatment

The grouped rats were treated with each of the extracts. The group 1 animals were treated by applying the prepared ointment using a cotton bud on the excised wound. This treatment was done every day for 24 days until the epithelisation stage of the animals was reached. The process was repeated for group 2 and group 3 animals. Group 3 animals were treated with 100 % paraffin oil while group 4 was left untreated for 24 days.

2. 4. 4 Wound healing activity

The diameter of the wound for each rat in each group was measured using a ruler. The percentage wound contraction was measured using the formula:

$$\frac{IA-FA}{IA} \times 100 \quad (1)$$

Where FA is the final area and IA is initial area.

The percentage wound contraction was measured on days 4, 8, 12, 16, 21 and 24 (Sharma *et al.,* 2010).

2. 4. 5 Growth parameters

2. 4. 5. 1 Biomass gain %

$$\text{Biomass gain} = \frac{\text{wt}-\text{wo}}{\text{wo}} \times 100 \quad (2)$$

Wo & wt are live weight at the time of starting and at the finishing the experiment respectively (Sharma *et al.,* 2010).

2. 4. 5. 2 Feed conversion ratio (FCR)

$$\text{FCR} = \frac{\text{feed ingested}}{\text{weight gain}} \quad (3)$$

2. 4. 5. 3 Specific growth rate (SGR)

The initial and final weights of the rats in each group were measured individually. Specific growth rate was determined according to the formulae below:

$$\text{SGR (\%)} = \frac{\text{In[final means biomass(g)]} - \text{In[initial means biomass(g)]}}{\text{time interval(days)}} \quad (4)$$

(Sharma *et al.,* 2010).

2. 4. 6. Epithelisation time

The epithelisation time for each of the rats was noted and recorded. Epithelisation time is the time it took the wound to heal completely.

2. 4. 7. Histological study

The skin tissues were collected on the 24th day ofthe experiment from all the six groups of animals and processed for histological study to determine the pattern of lay-down for collagen. The skin specimens from the treated animals were collected in 10 % buffered formalin and were subjected to sectioning and 6 m thickness sections were stained with hematoxylin and eosin. The stained slides were visualized for histological changes under a light microscope (Anderson, 1980).

2. 4. 8. Endogenous enzymes

The sample was homogenised in phosphate buffer (0.1 m pH 7.4) using homogenizer. The homogenates were centrifuged at 10.000 rpm at 4 °C for 10 mins using table top centrifuge and then the supernatants were stored in a deep freezer.Assay of superoxide dismutase (SOD), reduced glutathione and catalase.

2. 5. Statistical analysis

Statistical analysis was performed on data that were expressed as mean±SD and ANOVA test (IBM SPSS statistical 20.0 software) was used to compare the mean value of each treatment. Significant differences between the means of parameters were determined using the Duncan T test ($P< 0.05$).

3. RESULTS

Table 1. Phytochemical screening

Test	Group 1	Group 2	Group 3
Flavonoid	–	–	–
Reducing sugar	+	+	–
Alkanoid	+	+	–
Carbohydrate	+	+	–
Cardicglycosidic	+	+	–
Phenol	+	+	–
Saponins	+	+	–
Sterol	+	+	+
Tanins	+	+	–
Terpenoid	+	+	–

+ Presence of phytochemical constituents
- Absence of phytochemical constituents
Group 1- *P. nitida* seed at 15 % (w/v)
Group 2- *P. nitida* seed at 20 % (w/v)
Group 3- Control (ointment alone)

Table 2. Antimicrobial activities of *P. nitida* against flora microorganism

Test organisms	Zone of inhibition in diameter (mm)						
	Concentration of *P. nitida* seed extract (mg/mL)						
	Methanol	200	100	50	25	12.50	Gentamicin
S. aureus	-	18	14	12	10	-	38
B. subtilis	-	18	14	10	-	-	36
P. aueroginosa	-	20	18	14	12	10	34

Methanol is the negative control; gentamicin is the positive control; - indicates no antimicrobial activities

Table 3. Minimum inhibitory concentration of *P. nitida* (mg/mL)

Test organism	Concentration of the extract (mg/mL)			
	200	100	50	25
S. aureus	-	-	+	+
B. subtilis	-	+	+	+
P. aeroginosa	-	-	±	+

- indicates no growth of organism; + indicates the growth of organism; ± not determined

Table 4. Biomass gain of test and control rats

Groups	BMG (%)
Group 1	22.33 ± 10.41^{c}
Group 2	41.27 ± 14.22^{abc}
Group 3	47.88 ± 6.21^{ab}
Group 4	41.35 ± 21.51^{abc}

Group 1- *P. nitida* seed ointment at 15 % (w/v)
Group 2- *P. nitida* seed ointment at 20 % (w/v)
Group 3- Positive control (ointment only)
Group 4- Negative control (no treatment)
BMG- Biomass gain per group of the animal.
Values are expressed as mean±SD for groups of n=seven.
Data with different superscript letters along the same column are significantly different ($p<0.05$) using one ANOVA followed by Dunnett's test)

Table 5. Feed conversion ratio (FCR)

Groups	D4	D8	D12	D16	D20	D24
Group 1	-91.29	11.16	14.05	12.24	17.76	25.00
Group 2	6.16	4.89	17.21	482.15	5.91	18.75
Group 3	5.07	12.97	4.41	20.2	7.07	8.83
Group 4	-2.49	3.57	7.70	7.61	16.05	6.86

Group 1- *P. nitida* seed ointment at 15 % (w/v)
Group 2- *P. nitida* seed ointment at 20 % (w/v)
Group 4- Positive control (ointment only)
Group 5- Negative control (no treatment)

Table 6. Specific Growth Rate (SGR)

Groups	SGR
Group 1	0.95
Group 2	1.38
Group 3	1.63
Group 4	1.41

Group 1- *P. nitida* seed ointment at 15 % (w/v)
Group 2- *P. nitida* seed ointment at 20 % (w/v)
Group 3- Positive control (ointment only)
Group 4- Negative control (no treatment)

Table 7. Epithelialization time (Days)

Groups	E. Time
Group 1	25.43 ± 0.98^{a}
Group 2	19.86 ± 2.34^{b}
Group 3	18.00 ± 1.87^{b}
Group 4	24.00 ± 1.78^{a}

Group 1- *P. nitida* seed ointment at 15 % (w/v)
Group 2- *P. nitida* seed ointment at 20 % (w/v)
Group 3- Positive control (ointment only)
Group 4- Negative control (no treatment)

Table 8. Endogenous Enzymes

Groups	GSH	SOD	CAT
Group 1	40.76	87.56	57.99
Group 2	57.23	45.56	41.79
Group 3	44.90	97.57	90.22
Group 4	34.54	85.07	17.27

Group 1- *P. nitida* seed ointment at 15 % (w/v)
Group 2- *P. nitida* seed ointment at 20 % (w/v)
Group 3- Positive control (ointment only)
Group 4- Negative control (no treatment)
SOD- Superoxide Dimutase
GSH- Glutathione
CAT- Catalase

Table 9. Histology examination

Groups	Histological examination of the skin scar area
Group 1	The epidermis (thick arrow) is slightly hyperplastic and thickened. Dermis contains congested blood vessels (arrow) and numerous inflammatory cells (star).
Group 2	Epidermis (thick arrow) is discontinuous. There are numerous hair follicles (arrows).
Group 3	The epidermis (thick arrow) is intact and thick. The dermis (star) contains numerous hair follicles (thin arrows) enmeshed within dense fibrous connective tissue.
Group 4	The epidermis (thick arrow) is thin and intact. Dermis appears normal with hair follicles and sebaceous glands (arrows)

Histological examination of the skin scar area (H&E x100)

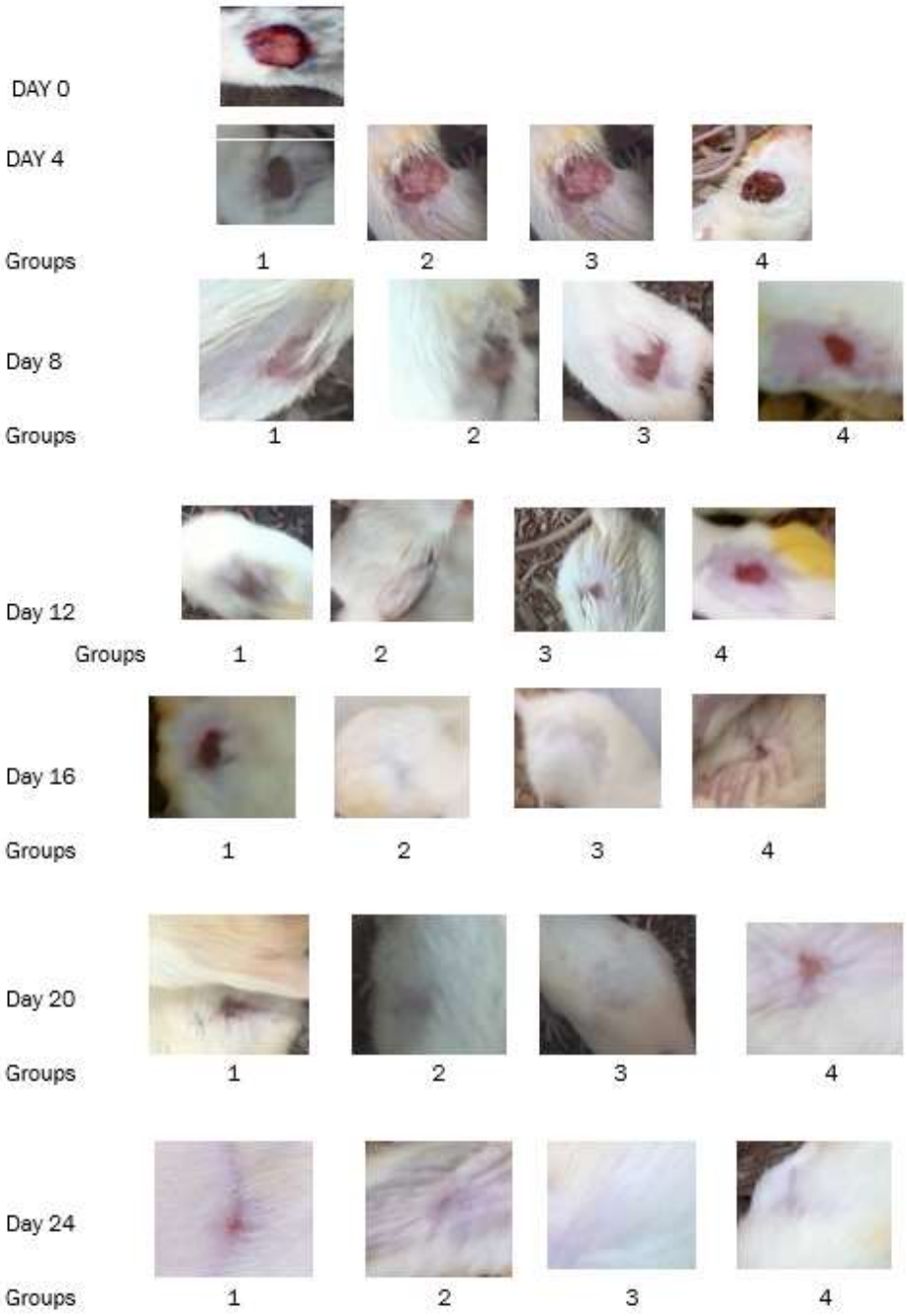

Fig 1: Pictures showing the wound area of each group at days 4- 2

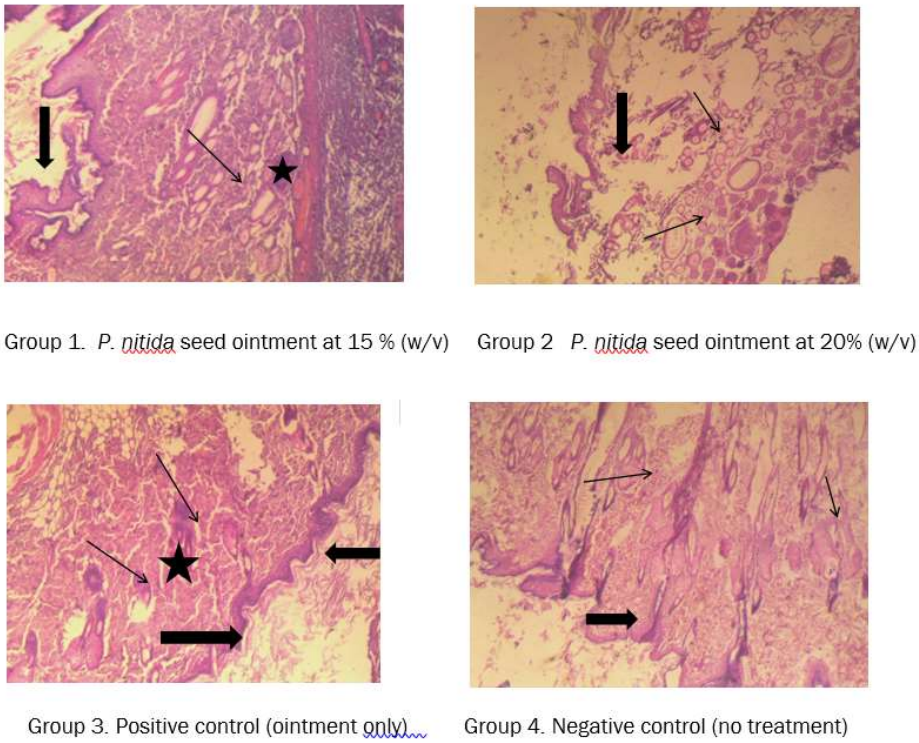

Fig. 2. Pictogram of the histology on the wound surface

4. DISCUSSIONS

The phytochemical result on table 1 revealed the presence of reducing sugar, alkanoids, carbohydrate, cardiac glycosidic, sterols, terpenoids and tanins in the extract. Tannins serve as chelating agents for metals ion, antioxidants in biological systems, and as protein precipitating agents (Warra *et al*., 2013).

The result of the antimicrobial activity of PNME is summarised on table 2. Methanol the negative control showed no antimicrobial activity against the three test microganisms; *S. aureus, B. subtilis* and *P. auerοginosa.* The highest zone of inhibition of PNME against *S. aureus* microorganism was 18.00 mm diameter at 200 mg/mL concentration. The analysis revealed 14 mm zone of inhibition at 100 mg/ mL of PNME, 12.00 mm zone of inhibition at 50 mg/mL and 10 mm zone of inhibition at 25 mg/mL. However, at 12.50 mg/mL, no antimicrobial activity was observed which indicates that at low concentrations, lower than 25 mg/mL of PNME, the ointment will not be effective in impeding the growth of *S. aureus* microorganism. This is an indication that at low concentrations, PNME will not be effective for wound treatment and healing.

PNME was also found to inhibit *B. subtilis* microorganism with the highest zone of inhibition 18.00 mm diameter at the concentration of 200 mg/mL. The analysis showed 14 mm zone of inhibition at 100 mg/mL of the extract, 10mm zone of inhibition at 50 mg/mL. However, no antimicrobial activity was observed at concentrations 25 mg/mL and 12.50 mg/mL. This implies that at concentrations lower than 50 mg/mL of PNME, it will not inhibit the growth of the microorganisms. Therefore, for maximum result and for good wound healing activity, the concentration of the extract should be at 50 mg/mL of the extract and above. PNME at 200 mg/mL concentration inhibited *P. auerοginosa* microorganism with zone of inhibition of 20 mm diameter.

This value is quite close to the value recorded for the positive control gentamicin with its zone of inhibition at 34 mm. Other zones of inhibition for PNME are 18 mm at 100 mg/mL, 14 mm zone at 50 mg/mL, 12 mm at 25 mg/mL and 10 mm at 12.50 mg/mL. PNME is found to be good at concentrations as low as 12.50 mg/mL for inhibiting the growth of *P. auerοginosa*; it is more susceptible to the effect of the ointment.

The minimum inhibitory concentration of *P. nitida* against flora microorganism as summarised in table 3. At 200mg/mL concentration of PNME ointment, no growth of the organism *S. aureus* was observed; also at concentration 100 mg/mL. It did not inhibit the growth of the organism at concentrations of 50 mg/mL and 25 mg/mL. At concentrations below 100mg/mL, PNME ointment did not inhibit the growth of the microorganism. This showed that it has a MIC of 100mg/mL against *S. aureus.* Consequently, for maximum wound healing ability, it should not be kept at concentrations below 100mg/mL. *B. subtilis* is inhibited at a concentration of 200 mg/mL but at concentration below 200 mg/mL, it showed the growth of the organism which signifies that *P. nitida* has a MIC of 200 mg/m L against *B. subtilis* and so will not inhibit the growth of the microorganism at concentrations below 200 mg/mL. PNME inhibited the growth of *P. aeroginosa* at concentrations of 200 mg/mL and 100 mg/mL but at concentrations below 100 mg/mL, the result was undetermined. This indicates that *P. nitida* has a MIC of 100 mg/mL against *P. auerοginosa* and so concentrations below 100 mg/m L should not be used for maximum wound healing ability.

The biomass gain of the model rats is summarized in table 4. The higher the biomass gain of the rats, the higher the growth rate of the rats. The lowest biomass gain was recorded for group 1. Group 2 and group 4 were not significantly different from each other with a biomass gain of 41.27 ± 14.22 % and 41.35 ± 21.51 % respectively. Epithelialization is the formation of granulation tissue unto an open wound. Epithelialization denotes the formation of epithelia cells over the open wound; the shorter the epithelialization time, the faster the wound healing process. The result of the epithelialization time for the rats is presented on table 7. Group 1 had the highest epithelialization time which indicates that the 15 % (w/v) concentration of *P. nitida* is not efficient in wound healing compared to Group 3 with epithelialization time at day 19.

5. CONCLUSION

20 % (w/v) concentration of *P. nitida* methanolic extract is more effective in wound healing compared to the 15 % (w/v) concentration. The presence of phytochemicals like taninns and alkaloids with anti-bacteria and anti-oxidant properties in higher concentration in *P. nitida* methanolic extract is probably responsible for its wound healing ability.

Acknowledgment

The authors appreciate the Department of Chemistry, Faculty of Science, University of Ibadan, Nigeria for making their facility available.

REFERENCES

Ajayi I.A., Raji, A.A., and Umeh, A.R. (2015).Investigation into the wound healing activity of *Monodora myristica* and *Monodora tenuifolia* seed extracts in albino rats. *American Chemical Science Journal* 4: 1-16.

Anderson K.R., Sutton M.G., Lie J. T., O'Brien P.C. and Frye R.I. (1980). Histopathological specification of hypertropic obstructive cardiomyopathy: myocardial fibre disarray and myocardial fibrosis. *British Heart Journal* 44: 433-443.

Sarkar S., Banerjee S., Erikson P.G. and Catuneanu O. (2005). Microbial mat control on siliciclastic precambrian sequence stratigraphy architecture; examples for India. *Sedimentory Geology* 176: 195-209.

Sharma B.B., Saha R.K.and Saha H. (2014). Effects of feeding detoxified rubber seed meal on growth performance and haematological indices of *Labeorohita* (Hamilton) fingerlings. *Animal Feed Science and Technology* 3: 8-17.

Solomon C.U., Arukwe U. and Onuoha I. (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Barker. *Asian Journal of Plant Science* 3: 10-13.

Sunmonu T.O., Oloyede O.B., Owolarafe T.A.,Yakubu M.T. and Dosumu O.O. (2014). Toxicopathological evaluation of *Picralima nitida* seed aqueous extract in wistar rats. *Turkish Journal of Biochemistry* 39: 119–125.

Warra A.A., Umar R.A., Sani I., Gafar M.K., Nasiru A. and Ado A. (2013). Preliminary phytochemical screening and physicochemical analysis of gingerbread plum (*Parinari macrophylla*) seed oil. *Journal of Pharmacognosy and Phytochemistry* 1:20-25.