Research Article **IJTCM (2017), 2:1**

International Journal of Traditional and Complementary Medicine
(ISSN:2474-1361)

Antibacterial, antifungal and wound healing potentials of extract and fractions of *Nauclea diderrichii* root bark

Theophine Chinwuba Akunne[1*], Bonaventure C. Obi[1], Paul A. Akpa[2], Sunday O. Udegbunam[3], Adaobi J. Anaenugwu[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria; [2]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria; [3]Department of Veterinary Surgery, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

ABSTRACT

This study was aimed to evaluate antimicrobial and wound healing effects of root bark extract and fractions of *Nauclea diderrichii* Merrill (Rubiaceae). Dried root barks of N. *diderrichii* was extracted using methanol and dichloromethane (1:1) to obtain methanol-dichloromethane extract (MDE). The MDE was fractionated using chromatographic techniques to obtain n-hexane (HF), ethylacetate (EF) and methanol (MF) fractions. The antimicrobial effect was tested against selected organisms using agar diffusion and agar dilution technique to determine the inhibition zone diameters (IZD) and minimum inhibitory concentrations (MICs). The excision wound model was used to assess the wound healing properties of MDE (5, 10 and 20%) and compared it with that of bacitracin and neomycin powder (BNP, standard) in rats. Phytochemical screening and acute toxicity test were performed using standard procedures. Results showed that extract and fractions exhibited antimicrobial effect with IZD (mm, 40 mg/ml) against tested organisms. The EF exhibited the best antimicrobial activity with IZD of 30, 8, 15, 8 and 12 against *Staphylococcus aureus, Staphylococcus cohnii, Pseudomonas aeruginosa, Klebsiella aerogenes and Aspergillus flavus*, respectively. The EF also gave an MIC (mg/ml) of 0.63, 20.00, 1.25, 20.00 and 0.63 for *S. aureus, S. cohnii, P. aeruginosa, K. aerogenes, and A. flavus*, respectively. The MDE showed a comparable wound healing effect with BNP, although with better effect

***Correspondence to Author:**
Theophine Chinwuba Akunne, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, Nigeria. Email address: theophine.okoye @ unn.edu.ng ; Tel.: +23480 366 845 06

How to cite this article:
Theophine Chinwuba Akunne et al.,. Antibacterial, antifungal and wound healing potentials of extract and fractions of Nauclea diderrichii root bark.International Journal of Traditional and Complementary Medicine 2017, 2:1.

eSciencePublisher
eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

on day 20 with 100% wound contraction while BNP gave 93.79%, post wound excision. Therefore, *N. diderrichii* root bark possesses antibacterial, antifungal and wound healing properties.

Key words: *Nauclea diderrichii*, antibacterial and antifungal activity, phytochemical screening, wound healing.

1.0 Introduction

The high incidence of microbial resistance to current standard antimicrobial agents has spurred a renewed interest among researchers to explore medicinal plants and other natural products for possible isolation of potent phytoconstituents with antimicrobial properties[1]. Preparations of medicinal plants are used in folkloric medicine in diverse cultures of the world to treat infections caused by pathogens. Researchers in the area of natural products pharmacology have shown that extracts of several medicinal plants are endowed with antimicrobial activities[2, 3, 4]. In folkloric medicine, *Nauclea diderrichii* Merrill (Rubiaceae) is among the medicinal plants used in the treatment of infectious diseases. The plant, which is a native of West and Central Africa, has its root bark decoction being used in the treatment of gonorrhoea, anaemia, stomach ache and indigestion, while a decoction of its leaves is used as a wash for measles[5]. Additionally, antimalarial and antiplasmodial[6, 7], antileishmanial[8], and antitrypanosomal[9], activities of *Nauclea diderrichii* have been reported. In Nigerian folk medicine, the root bark preparation of *N. diderrichii* is used in treatment of bacterial infections such as gonorrhea, fungal infection, malaria, fever and cough. Therefore, the aim and objective of this research work is to ascertain the antimicrobial and wound healing effects of *N. diderrichii* in order to validate its folkloric use and also to pave way for the possible identification or isolation of the active phytoconstituent(s) responsible for the purported activity.

2.0 Materials and Methods

2.1 Microorganisms

Bacteria used are *Staphylococcus aureus*, *Staphylococcus cohnii*, *Enterococcus faecalis*, *Streptococcus pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes* and *Proteus mirabilis*, while fungi species are *Candida albicans* and A*spergillus flavus*. These are clinical isolates obtained from Adonai Medical Laboratory, Nsukka, Nigeria.

2.2 Animals

Adult Sprague-Dawley rats (150-200 g) and mice (18-30 g) of either sex were obtained from the Animal House facility of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. They were kept in standard cages under laboratory conditions and were fed with pellet feed (Guinea feed) and water *ad libitum*. All animal experiments were conducted in compliance with NIH Guidelines for Care and Use of Laboratory Animals (Pub. number 85-23, revised 1985) and in accordance with the University Ethics Committee on the use of laboratory animals.

2.3 Preparation of extract

Fresh root barks (2 kg) of *N. diderrichii* were collected from Calabar, Cross River State, Nigeria in July 2015 and taxonomically identified and authenticated by Mr. Alfred Ozioko of the International Centre for Ethno-medicine and Drug Development (InterCEDD), Aku Road, Nsukka, Enugu State, Nigeria. The number of voucher specimen is InterCEDD-0715. The root barks were washed, dried, pulverized into coarse powder and extracted with methanol and dichloromethane in the ratio of 1:1 by cold maceration for 48 h, filtered and concentrated. The yield was dichloromethane-methanol extract (MDE), which was stored in a refrigerator for further use.

2.4 Solvent guided fractionation

The MDE (50 g) was subjected to solvent-guided fractionation in a silica gel (70-220 mesh, Merck Germany) and successively eluted with n-hexane, ethyl acetate, and methanol in order of increasing polarity. The fractions were concentrated under reduced pressure in a rotary evaporator and yielded the various fractions; n-hexane fraction (HF), ethylacetate fraction (EF), and methanol fraction (MF) which were stored separately in amber-coloured containers in the refrigerator for

further studies.

2.5 Qualitative phytochemical screening

Qualitative phytochemical screening was performed on the MDE using standard procedures as described by Trease and Evans[10] and Harbone[11], to test for the presence or absence of phytochemicals or plant secondary metabolites.

2.6 Acute toxicity study

The estimated lethal dose (LD_{50}) of the plant extract was performed in accordance with the method described by Lorke[12]. Briefly, in the first phase, nine (9) mice were divided into 3 groups of 3 mice per group, and treated with the MDE per oral through a gavage, at the doses of 10, 100, and 1000 mg/kg respectively. In the second phase, 3 mice were treated with MDE doses of 1600, 2800 and 5000 mg/kg respectively, while the fourth mouse was used as control. The animals were observed for clinical signs and symptoms of toxicity like behavioural changes and mortality within 24 h and the LD_{50} determined.

2.7 Antimicrobial assay

The cup plate agar diffusion method was used according to International Society of the Learning Sciences (ICLS) in the determination of IZD. A 400 mg quantity each of the MDE, HF, EF and MF was dissolved in 10 ml DMSO (5% v/v). Then two-fold serial dilutions of this stock (40 mg/ml) were prepared up to five (5) concentrations. On the other hand, 1 mg each of the standard agents was dissolved in 10 ml of DMSO (5% v/v) and ten-fold serial dilutions made to obtain 10 µg/ml of the standard antibacterial, ciprofloxacin, and standard antifungal agent, fluconazole, which were used as positive control for the bacteria and fungi, respectively. The test organisms were prepared with a 0.5 McFarland standard and sub cultured at 37 ^{o}C and maintained on nutrient agar medium for bacteria, and Sabouraud agar medium for fungi. A 0.1 ml of the test organisms were transferred from the inoculums into the previously sterilized (dry heat 160 ^{o}C for I hour) Petri dishes. Then 20 ml of sterile molten agar (autoclaved at 121 ^{o}C for 1 hour) was poured and thoroughly mixed by swirling for proper homogenization. It was allowed to set. Indelible marker was used to divide the agar plate into sections corresponding to the different concentration of a particular sample. Using a sterile cork-borer of 4 mm in diameter five cups/wells was made, one cup to each section. A 1 ml of the prepared extract, fractions and standard agents were transferred into the various cups while 50 µL of DMSO (5% v/v) were used as a negative control. The seeded plates were allowed to stand at room temperature for about 15 minutes for pre-diffusion of the sample solution into the agar. After this, the plates were incubated at 37 ^{o}C and 25 ^{o}C for 24 hours for bacteria and fungi respectively. After 24 hours of incubation, zones of inhibition diameter (IZD) produced by these dilutions against each test organisms were measured and recorded.

2.8 Determination of the minimal inhibitory concentration (MIC)

The Agar dilution method based on ICLS and as previously described with modification[13], was used in the determination of the MIC. A 200 mg/ml of the extract, MDE, was prepared by dissolving 500 mg of the extract in 2.5 ml of DMSO. Two-fold serial dilution was prepared up to seven (7) different concentration of the extract. Twenty eighth (28) Petri dishes were sterilized using dry heat sterilization (160 ^{o}C for 1 hour). About a 20 ml volume of sterile molten agar was poured into each Petri dish and 50 µL of a particular concentration of a fraction was thoroughly mixed by swirling and allowed to set. The solidified agar was sectioned into 10 portions using indelible markers at the bottom back of the Petri dish. The microorganisms were streaked with the aid of an inoculums loop. It was incubated at 37 ^{o}C and 25 ^{o}C for the bacteria and fungi, respectively, for 24 hours. The MIC was then determined from the lowest concentration of each sample that showed no growth and results recorded. The same procedure was then repeated for the fractions; HF, EF and MF.

2.9 Preparation of Ointment

A 60 g quantity each of ointment was prepared from the root bark extract of *N. diderrichii* (MDE) in concentrations of 5%, 10% and 20% using white soft paraffin (WSP) as the ointment base. The preparations were stored in separate containers for further use.

2.10 Wound healing excision model

A total of thirty rats were divided into five groups

(n=5). The rats were pre-treated with xylazine HCl (10 mg/kg intramuscularly) while ketamine HCl at 50 mg/kg intramuscularly was used as the anaesthetic agent. Then the dorsum of the rats were shaved thoroughly and swabbed with cotton wool soaked in 5 % chlorhexidine HCl solution. A template of 20 mm in diameter was placed on the dorsum of the animal and an indelible marker used to outline the diameter of the wounds that were created. A full thickness skin excision wound was then created using size 24 scalpel blade and diameter of the wounds was measured with a meter rule as previously described[14, 15]. Groups A, B and C each was treated with 5%, 10% and 20% ointment of the MDE, respectively, while group D was treated with bacitracin 250 units and neomycin sulphate 3,300 units powder (Cicatrin®, GSK Pharma., Nigeria) (BNP) as positive control, while group E received white soft paraffin (WSP) as negative control. All treatments were done topically. The animals were housed individually in metal cages, the appropriate quantity of the MDE applied once a day on each animal from day zero till wound heals or up 21st post wounding day. The wound diameter was measured and recorded on alternate days by using a translucent paper and measuring the diameter therein, while the wound areas on subsequent days were compared with the wound area on day zero (surgical day) and percentage contraction was calculated as the difference between the initial wound area and the wound area of the each day divided by the initial wound area and multiplied by 100.

Percent wound contraction (%) = W_O - W_T/W_O x 100

Where; W_O is wound area on day 0; W_T is wound area on subsequent days

2.11 Statistical analysis

Data obtained was analyzed using one way analysis of variance (ANOVA) subjected to Dunnett's multiple comparison post hoc test. Differences between means were accepted to be significant at $P < 0.05$ and the results expressed as mean ± SEM.

3.0 Results

3.1 Qualitative phytochemical screening

The preliminary qualitative phytochemical screening of MDE of *N. diderrichii* revealed that alkaloids, saponins and carbohydrates were abundantly present while flavonoids, resins, and steroids were moderately present (Table 1).

3.2 Acute toxicity study (LD_{50})

The MDE gave an estimated LD_{50} greater than 5000 mg/kg as no death was observed in the groups after 48 h treatment. Also no visible sign of acute toxicity was observed. Therefore, MDE could be reasonably safe to animals and humans.

3.3 Antimicrobial assay

The result of the sensitivity test showed that the extract and fractions exhibited antimicrobial activity against the selected organisms. The EF manifested highest antibiotic activity with an IZD of 30.0 mm against *S. aureus* while HF gave 20.0 mm against same *S. aureus*. Based on the sensitivity assay the degree of order of antibiotic activity was EF > HF > MF > MDE (Table 2). On the other hand HF and EF exhibited antifungal activity with HF showing an IZD of 10.0 and 12.0 against *Candida albicans* and A*spergillus flavus*, respectively, while EF also showed an IZD of 12.0 against A*spergillus flavus* while the DMSO (5% v/v) showed no inhibition to the various organisms tested (Table 2).

3.4 Minimum inhibitory concentration (MIC) determination

The result of the MIC showed that the EF had the best MIC where; *S. aureus* (0.63 mg/ml), *P. aeruginosa* (1.25 mg/ml) and *A. flavus* (0.63 mg/ml). The MF showed the lowest MIC where; *S. cohnii* (20.00 mg/ml). The order of antibiotic activity with respect to the MIC is as follows; EF > HF > MDE > MF (Table 3). EF also exhibited MIC of 0.63 against *A. flavus* better than HF which showed an MIC of 5.0 (Table 3).

3.5 Wound healing excision model

The percentage wound contraction (PWC) of the MDE preparations showed comparable wound healing with the standard agent. The first two weeks (14 days) of treatment revealed that BNP exhibited better wound healing effect compared

Table 1: Qualitative phytochemical screening of extract and fractions

Phytoconstituent	Inference
Flavonoids	++
Alkaloids	+++
Saponins	+++
Tannins	-
Resins	++
Proteins	+
Carbohydrate	+++
Reducing sugars	+++
Hydrolysis test for glycosides	+
Fats and oils	-
Steriods	++
Terpenoids	+
Cardiac Glycosides	-

Key: + = present; ++ = moderately present; +++ = abundantly present; - = absent.

Table 2: Inhibition zone diameter of extract and fractions

S/N	Organisms	40 mg/ml (mm)				10 µg/ml (mm)	
		MDE	HF	EF	MF	Ciprofloxacin	Fluconazole
1	*Staphylococcus aureus*	6.0	20.0	30.0	+	20.0	NT
2	*Staphylococcus cohnii*	6.0	+	8.0	6.0	16.0	NT
3	*Enterococcus faecalis*	+	+	6.0	8.0	13.0	NT
4	*Streptococcus pneumonia*	+	+	+	+	18.0	NT
5	*Escherichia coli*	+	10.0	+	+	19.0	NT
6	*Pseudomonas aeruginosa*	10.0	15.0	15.0	10.0	22.0	NT
7	*Klebsiella aerogenes*	+	+	8.0	+	13.0	NT
8	*Proteus mirabilis*	+	+	+	+	11.0	NT
9	*Candida albicans*	2.0	10.0	+	+	NT	20.0
10	*Aspergillus flavus*	+	12.0	12.0	+	NT	16.0

Key: (+): no activity; (NT): not tested. 5% DMSO = no activity.

Table 3: Minimum inhibitory concentrations (MICs) in mg/ml

S/N	Organisms	MDE	HF	EF	MF
	Gram positive bacteria				
1	*Staphylococcus aureus*	0.63	5.00	0.63	+
2	*Staphylococcus cohnii*	20.00	+	20.00	20.00
3	*Enterococcus faecalis*	+	+	20.00	>20.00
4	*Streptococcus pneumonia*	+	+	+	+
	Gram negative bacteria				
5	*Escherichia coli*	+	5	+	+
6	*Pseudomonas aeruginosa*	20.00	10.00	1.25	>20.00
7	*Klebsiella aerogenes*	+	+	20.00	+
8	*Proteus mirabilis*	+	+	+	+
	Fungi				
9	*Candida albicans*	+	5.00	+	+
10	*Aspergillus flavus*	+	5.00	0.63	+

+ = No activity, > = Greater than

Table 4: Percentage rate of wound contraction post wound excision

Post wounding day	MDE ointment (w/w)			BNP	WSP
	5%	10%	20%		
0	0.00	0.00	0.00	0.00	0.00
2	4.74±3.88	7.71±3.59	21.67±2.69	7.72±1.40	8.00±0.63
4	10.01±2.44	17.39±4.81	22.04±4.90	23.40±2.32	19.20±0.40
6	25.68±4.48	43.53±6.88	33.00±11.20	44.69±6.02	31.60±0.86
8	45.09±4.39	59.49±6.56	48.34±10.54	62.07±6.11	37.60±0.66
10	47.92±4.75	64.17±5.93	54.72±8.66	72.44±5.48	46.80±0.27
12	62.91±4.13	76.58±5.09	73.84±5.60	77.99±7.05	61.32±0.27
14	60.02±5.43	75.00±6.79	73.84±5.60	82.65±7.13	72.00±0.41
16	93.56±4.39	93.47±4.00	88.49±4.02	88.30±10.03	83.32±0.29
18	96.52±2.17	97.20±1.96	95.14±3.05	86.41±8.41	86.68±0.87
20	100.00±.00	100.00±.00	100.00±.00	93.79±6.21	94.00±0.50
22	100.00±.00	100.00±.00	100.00±.00	100.00±.00	100.00±0.00
24	100.00±.00	100.00±.00	100.00±.00	100.00±.00	100.00±0.00

All data were expressed as mean ± SEM (n=5). Differences were considered significant at *P<0.05 when compared test groups versus control; BNP = bacitracin and neomycin powder; WSP=white soft paraffin.

to MDE, while afterwards MDE showed a better wound healing activity than BNP with 100% and 93.79% wound contraction, respectively on day 20 post wounding. However, MDE at tested doses showed non significant ($p < 0.05$) wound contraction effects compared to WSP, the negative control indicating less potent activity (Table 4).

4.0 Discussion

Research has shown that medicinal plants with antimicrobial effects often possess wound healing properties[16, 15, 17, 18].Wound normally occur when the continuity of the skin or mucous membrane is broken and can be healed through natural complex process involving series of coordinated events such as coagulation, acute inflammation, proliferation of connective tissues, synthesis of extra cellular matrix proteins, remodelling and collagen deposition[19]. Most cases of wound contamination often involves a variety of organisms such as *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Clostridium perfringes, Clostridium tetani, Coliform bacilli* and *enterococcus*[17]. The usual delay in the process of wound contraction, healing and epithelialisation could be due to the presence of these infectious bacteria. The extract and fractions of *Nauclea diderrichii* exhibited potent antibacterial and antifungal activities with wound healing properties. The antibacterial studies revealed activity against both gram positive and gram negative organisms indicating broad spectrum of activity for *Nauclea diderichii*. The extract and fractions of *N. diderichii* showed antibacterial activity against the following organisms; *Staphylococcus aureus*, *Staphylococcus cohnii*, *Enterococcus faecalis*, *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella aerogenes*. This potent antibacterial effect might therefore, be a possible major contributing factor to the observed wound healing and epithelialisation effects of the extracts. The EF showed the highest antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with an MIC value of 0.63 and 1.25 mg/ml respectively, which are often implicated in wound infection. The observed antifungal effect of the extract and fractions of *N. diderrichii* has also correlated with its ethnobotanical use. However, the EF based on the MIC showed the highest antifungal activity against *Aspergillus flavus*. This is an indication that *N. diderrichii* could be a good candidate for deep or systemic mycosis since *A. flavus* is one of the fungi often implicated.

The phytochemical studies revealed the possible phytoconstituents that could be responsible for the claimed activity of *N. diderrichii*. Alkaloids, flavonoids, terpenoids and saponnins which were observed to be abundantly present in the MDE have been reported to possess antimicrobial activity[20, 21]. Furthermore, with reference to the wound healing effect, flavonoids have been shown to increase the viability of collagen fibrils by causing an increase in the strength of collagen fibres while alkaloids in the plants may increase cell proliferation and thus increase the rate of wound healing[22, 23]. Hence any or combined effect of these phyto-constituents may be responsible for the antimicrobial or/and wound healing effect, although none could be ascribed to be specifically responsible and neither mechanism of action could be ascertained at this stage of the work.

4.1 Conclusion

The extract and fractions of *Nauclea diderrichi* showed potent antibacterial, antifungal and wound healing effects, and exhibited an estimated LD_{50} greater than 5000 mg/kg. Further studies are ongoing towards isolation of the active phytoconstituent(s).

4.2 Acknowledgement

The authors wish to thank Mr. A. Ozioko of Inter-CEDD for the plant collection.

4.3 Authors' contribution

TCA, BCO, SOU, PAA, AJA all contributed to the design, performing the experiment and writing up of the research work. All authors red and approved the work.

4.4 Conflict of interest

The authors declare no conflict of interest.

5.0 REFERENCES

1. Chah KF, Eze CA, Emuelosi CE, Esimone CO. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. J. Ethnopharmacol., 2006; 104:164-167.

2. Ramesh N, Viswanathan MB, Saraswathy A, Bal-

akrishna K, Brindha P, Lakshmanaperumalsamy P. Phytochemical and antimicrobial studies of Begonia malabarica. J. Ethnopharmacol, 2002; 79: 129-132.

3. Fleischer TC, Ameade EPK, Sawer IK. Antimicrobial activity of the leaves and lowering tops of Acathospermum hispidum. Fitoterapia, 2003; 74: 130-132.

4. Patel JD, Shrivastava KM, Kumar V. Evaluation of some medicinal plants used in traditional wound healing preparations for antibacterial property against some pathogenic bacteria. Journal of Clinical Immunology and Immunopathology Research, 2009; 1(1):007-012.

5. Orwac C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestree Database: a tree reference and selection guild version, 2009; 4.0. Available from http://www.worldagroforestry.org/af/treedbl

6. Lamidi M, Ollivier E, Gasquet M, Faure R, Nze-Ekekang L, Balansard G. Structural and antimalarial studies of saponins from Nauclea diderrichii bark. Adv Exp Med Biol. 1996;404:383.

7. Mustofa, Valentin A, Benoit-Vical F, Pélissier Y, Kone-Bamba D, Mallié M. Antiplasmodial activity of plant extracts used in West African traditional medicine. J Ethnopharmacol. 2000;73:145.

8. Di Giorgio C, Lamidi M, Delmas F, Balansard G, Ollivier E. Antileishmanial activity of quinovic acid glycosides and cadambine acid isolated from Nauclea diderrichii. Planta Med. 2006;72:1396.

9. Nwodo NJ, Agbo MO. Antitrypanosomal effects of methanolic extracts of Nuclea diderrichii (Merr.) and Spathodea campanulata stem bark. Journal of Pharmaceutical and Allied Sciences, 2010;7(5).

10. Trease GE, Evans WC, Test book of Pharmacognosy, 11th ed. Brailliare Tindall and Macmillian Publishers, London. 1989; Pp: 176- 180.

11. Harborne JB. Phytochemical Methods: a guide to modern techniques of plant analysis. 2nd edition. London, Chapman and Hall, 1988; pp:55–56.

12. Lorke D. A new approach to practical acute toxicity testing. Arch of toxicol, 1983; 53: 275- 287.

13. Akunne TC, Okafor SN, Okechukwu DC, Nwankwo SS, Emene JO, Okoro BN. Catfish (Clarias gariepinus) Slime Coat Possesses Antimicrobial and Wound Healing Activities. UK Journal of Pharmaceutical and Biosciences, 2016; 4(3):60-66.

14. Morton JJ, Malone MH. Evaluation of vulneary activity by an open wound procedure in rats. Arch. Int. Pharmacodyn, 1972; 196:117-126.

15. Okoli CO, Ezike AC, Akah PA, Udegbunam SO, Okoye TC, Mbanu TP, Ugwu E. Studies on wound healing and antiulcer activities of extract of aerial parts of Phyllanthus niruri L. (Euphorbiaceae). Am. J. Pharm. & Toxicol., 2009; 4(4):118-126.

16. Okoli CO, Akah PA, Okoli AS. Potentials of leaves of Aspillia Africana (composition) in wound care: an experiment evaluation. BMC Complement. Altern. Med., 2007; 7:24.

17. Nwonu P, Okoye TC, Akah PA, Ezike AC, Ejindu IA, & Eneh SE. Wound healing properties of stem bark extract of Tabebuia rosea. Journal of Pharmaceutical and allied sciences, 2010; 7(4):1115-1120.

18. Tohidi M, Khayami M, Nejati V. Meftahizade H. Evaluation of antimicrobial and wound healing of Pistacia atlantica and Pistacia khinjuk. Journal of Medical Plants Research, 2011; 5(17) pp. 4310-4314.

19. Velnar T, Bailey T, Smrkolj V. (2009). The wound healing process: an overview of the cellular and molecular mechanisms. The Journal of International Medical Research, 2009; 37 (5):1528-1542.

20. Özçelik B, Kartal M, Orhan I. (2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharmaceutical biology, 2011; 49(4), 396-402.

21. Okoye TC, Akah PA, Okoli CO, Ezike AC, Omeje EO, Odoh UE. Antimicrobial effects of a lipophilic fraction and kaurenoic acid isolated from the root bark extracts of Annona senegalensis. Evidence-Based Complementary and Alternative Medicine, 2012.

22. Barbakadze V, Mulkijanyan K, Gogilashvili L, Amiranashvil L, Merlani M, Novikova Z, Sulakvelidze M. Allantoin and pyrrolizidine alkaloids: free wound healing compositions from Symphytum asperum. Bull GA Nat. Acad. Sci., 2016; 3:159-164.

23. Panda P, Tripathy G. Wound healing activity of aqueous and methanolic bark extract of Vernonia arborea in Wistar rats. Nat Prod Radiance, 2009; 8:6–11.