JOURNAL OF VETERINARY AND APPLIED SCIENCES
2017 VOL. 7 (1): 21 – 28
Manuscript No. JVAS/2017/015; Received: 18/05/2017; Accepted: 10/10/2017
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

EVALUATION OF WOUND HEALING AND ANTIBACTERIAL ACTIVITIES OF *Palisota hirsuta* METHANOL LEAF EXTRACT

Chioma Unamba-Oparah*[1], Ihemdirim C. Unamba-Oparah[2], Chinedu A. Eze[3] and Aruh O. Anaga[4]

[1]Department of Veterinary Surgery and Theriogenology and [2]Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, Nigeria; [3]Department of Veterinary Surgery and Radiology and [4]Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria.

==========

ABSTRACT

This study evaluated the wound healing activity of methanol leaf extract of Palisota hirsuta (MLEPH) on excision wound and its antibacterial activity. Thirty albino rats, assigned into 5 groups (I – V) of 6 rats per group had excision wounds surgically created on their dorsa. The excision wounds in the various groups were treated daily by topical application of Petroleum Jelly (PJ, negative control, Group I), Cicatrin® (positive control, Group II), 1% w/w MLEPH (Group III), 2% w/w MLEPH (Group IV) and 4% w/w MLEPH (Group V) for 21 days. The groups were monitored for healing. No antibacterial activity was observed using the agar well diffusion assay. The results of the excision wound model showed that groups III and V treated with 1 % and 4 % w/w MLEPH (LD_{50} 260 mg/kg) had significantly ($p < 0.05$) increased wound contractions. Similarly, group V treated with 4 % w/w MLEPH had significantly ($p < 0.05$) decreased epithelialization period compared with groups I and II treated with PJ (negative control) and Cicatrin® powder (positive control) respectively. Wound scoring data also showed that group V treated with 4 % w/w MLEPH was the first to show complete healing. Consequently, topical application of 4 % w/w MLEPH had the best wound healing activity. This study therefore confirms the wound healing property of MLEPH and its potential as a wound healing agent.

Keywords: Excision wound, Epithelialization, Antibacterial activity, *Palisota hirsuta*, Rats

==========

INTRODUCTION

Wounds represent a significant burden on patients and health care professionals worldwide in that wounds do not only affect the physical and mental health of millions of people but also impose significant cost on them [1]. While animal records are not readily available, estimates show that nearly 6 million people worldwide suffer from chronic wounds [2] which have failed to progress through the normal stages of wound healing. In most cases, such chronic wounds result to a state of pathologic inflammation [3] that often lead to multiple organ failure or death of the patient [4].

The use of conventional medicine in wound healing has its challenges which include cost, availability, and microbial resistance. This has necessitated the search for cheaper, more effective, quicker and longer-acting alternatives to conventional medications [5]. The widespread availability of plant formulations with wound healing potentials, has led to the development and use of herbal remedies in wound healing, and thus its application in wound dressing.

Palisota hirsuta Reichb. Ex Endl (*Commelinaceae*) is a tropical West African plant and a member of the spiderwort family [6]. In Nigeria, *P. hirsuta* is known as *ikpere aturu* (Igbo), *akerejupon* (Yoruba) and *ighiguewe* (Edo) [7]. *P. hirsuta* is a robust perennial herb of about 3 m in height found in lowland rainforest areas. It has characteristic swollen nodes which may be responsible for the local names as *'swollen knee'* in Bassa, Liberia; *'sheep knee'* or *'knee cap'* in Igbo and Yoruba-speaking areas of Nigeria [7]. *Palisota hirsuta* is used in the treatment of diarrhea, dysentery, skin infection, hemorrhoid, kidney problems, nasopharyngeal infections, venereal diseases, swellings, edema and gout [7]. The Igbo people of Nigeria use it as ointment in the treatment of gun-shot wounds.

Anaga *et al*., [8] showed the phytochemical constituent of the methanol leaf extract of *P. hirsuta* to include tannins, flavonoid, glycosides and proteins. They also reported the lethal dose (LD_{50}) of 260.6 mg/kg and narrow spectrum antibacterial activity. Other reported biological activities of the plant include local anaesthetic [8], antiviral activity against herpes simplex, sindbis virus and poliovirus [9,10], anti-arthritic, anti-inflammatory and antipyretic [11], liver protective [12] and antinociceptive [13].

There is paucity of information on the wound healing activity of the plant, *Palisota hirsuta* in man and animals. The present study was designed to investigate the wound healing potentials of *Palisota hirsuta* using excision wounding model, in order to provide the scientific basis for its folkloric use as a traditional wound healing agent.

MATERIALS AND METHODS

Plant material

The leaves of the plant, *Palisota hirsuta* were collected in March, from Orba in Udenu Local Government Area of Enugu State, Nigeria. They were identified by Mr. A. O. Ozioko, a taxonomist in the Department of Botany, University of Nigeria, Nsukka and a voucher specimen duly annotated (UNN/BD.3912.04) was kept in the herbarium of the department.

Experimental animals

Thirty albino rats (15 males and 15 females) aged 3-4 months, with mean weight of 175.40 ± 4.42 g obtained from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. They were housed individually in stainless steel cages at room temperature and fed commercial feed (Vital® Growers feed, GCOML, Jos, Nigeria) and water *ad-libitum* throughout the course of the study. The rats were acclimatized for 2 weeks before the commencement of the experiment, and maintained in accordance with the recommendation of the *Guide for the care and use of laboratory animals* [14].

Plant preparation

The fresh leaves of *Palisota hirsuta* were air-dried under shade, and pulverized into coarse powder using a hammer mill. The pulverized leaves (500 g) were defatted with 100% hexane for 24 h with intermittent shaking. This was followed by extraction with 100% methanol for 48 h with intermittent agitation. The extract was concentrated *in vacuo* using rotary evaporator at 40°C and designated as methanol leaf extract of *Palisota hirsuta* (MLEPH). The extract was stored at 4°C before use.

Antibacterial assay

The agar diffusion method [15] was used in evaluating the antibacterial activity of MLEPH. The petri dishes used were sterilized by autoclaving at 121°C for 15 minutes. The media was prepared by strict adherence to the manufacturer's instructions. Three nutrient agar plates (labelled plate 1, 2 and 3) were used for this experiment and into the plates, *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* were inoculated as follows: Plate 1 was inoculated with *Staphylococcus aureus*, Plate 2 was inoculated with *Pseudomonas aeruginosa* while Plate 3 was inoculated with *Escherichia coli*. Five (5) wells were punched into each nutrient agar plate using a sterile cork borer and labelled 1-5. The extract at various concentrations was dispensed into the wells, using a micropipette as follows: Well 1 (50 µL 20 mg/ml MLEPH), Well 2 (50 µL 10 mg/ml MLEPH), Well 3 (50 µL 5 mg/ml MLEPH), Well 4 (50 µL 2.5 mg/ml MLEPH) and Well 5 (50 µL 100 % DMSO). The plates were incubated uninverted at 37°C for 24 h. After incubation, the plates were read (checked for zones of inhibition).

Excision wound model

A modified method of Morton and Malone [16] was adopted for this experiment. Thirty albino rats of either sex (15 males and 15 females) were randomly assigned into 5 groups of 6 rats each. The rats were sedated with xylazine at the dose of 5 mg/kg BW intramuscularly, followed by ketamine hydrochloride injection (35 mg/kg BW, intramuscularly) 5 min later to achieve dissociative anesthesia. The marked area on the dorsa of the rats, 1 cm from the vertebral column and 5 cm from the ear, were shaved liberally and scrubbed using 4% chlorhexidine hydrochloride solution. They were placed prone on the surgical table. A 3 cm diameter circular template was cut out from a tracing paper and placed on prepared dorsum of each rat. A marker was then used to outline the circumference of the template on the dorsum of each rat. With a sterile scalpel blade and a thumb forceps, excision wounds 3 cm diameter x 2 mm depth were aseptically created on the delineated areas. After recovery from anesthesia, the animals were placed in their cages. Wounding day was considered as day 0 (Fig.1).

The 1%, 2% and 4% w/w MLEPH were prepared by respectively suspending 1 g, 2 g and 4 g of MLEPH in 99 g, 98 g and 96 g of Petroleum Jelly (PJ). The rats were treated post-operatively as follows:

i. Group I – Rats in this group served as the negative control and the wounds on their dorsa were treated topically with 0.5 g PJ once daily.
ii. Group II – Rats in this group served as the positive control and the wounds on their dorsa was treated with Cicatrin® powder (Neomycin Sulphate BP-1650 units and Bacitracin Zinc BP-125 units) once daily.
iii. Group III – The wounds on the dorsa of these rats were treated with 1% w/w of MLEPH once daily.
iv. Group IV - The wound on the dorsa of these rats were treated with 2% w/w of MLEPH once daily.
v. Group V - The wound on the dorsa of these rats were treated with 4% w/w of MLEPH once daily

In all cases, the wounds were treated daily for 21 days post surgery (PSD). The wounds were monitored and the wound areas measured on PSD 3, 6, 9, 12, 15, 18, and 21, using tracing papers and pencil. The tracings were read off on a graph sheet and recorded. The graph readings were used in calculating the wound contractions which were expressed as a percentage of the original size [17] as follows:

$$\% \text{ wound contraction} = \frac{\text{wound area on day 0} - \text{wound area on day } n}{\text{wound area on day 0}} \times 100$$

Where n = number of days.

Epithelialization periods, which were the periods it took the scabs to fall off without raw wounds [18] were recorded for all the groups. As healing progressed, the gross appearances of the wounds were scored

as: wet (W) = 1, fairly wet (FW) = 2, dry (D) = 3, very dry and crusty (VDC) = 4, very dry with scar (VDS) = 5 and complete healing (CH) = 6.

Statistical analysis

The result of the wound scoring was analyzed using Mann-Whitney nonparametric test. The parametric data were presented as mean ± standard error of means (SEM) and analyzed using Univariate and Multivariate general linear model. The variant means were separated using least significant difference (LSD) *post hoc* test at $p < 0.05$.

RESULTS

Physical and antibacterial profile of MLEPH

The results of the plant extraction and antibacterial assay are presented in Table 1. The percentage yield of the plant extract was 5.98% w/w dry matter. The extract had a dark green colour, pungent smell and a pasty consistency. In the antibacterial assay, no inhibition zones were observed after incubating the inoculated plates.

Table 1. Summary of the physical and antibacterial properties of MLEPH

.

Physical properties	Antibacterial properties
Percentage yield = 5.98% w/w dry matter. Dark green colour with pasty consistency and a pungent smell.	No inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*

Excision wound model

The appearance of the excision wound on days 0 and 21 are shown in Figs. 1 and 2. The effect of MLEPH on percentage wound contraction in the excision wound model is presented in Fig. 3. On PSD 6, the percentage wound contraction at 1% w/w (Group III) and 4% w/w MLEPH (Group V) significantly ($p < 0.05$) increased when compared with Cicatrin® (group II). The increase in percentage wound contraction was sustained to the PSD 9 by the extract at both 1% w/w (group III) and 4% w/w (group V) concentration compared to PJ (group I) and Cicatrin® (Group II) treatments. There was no significant ($p > 0.05$) variation in percentage wound contraction on PSD 12 in all the groups. However, on PSD 15 and 18, 4% w/w MLEPH group V had significantly ($p<0.05$) increased percentage wound contraction when compared with the PJ-treated group I. Wound contraction was significantly ($p<0.05$) increased by 4% w/w MLEPH (group V) and 1% w/w MLEPH (group III) when compared with PJ (group I) on PSD 21, similar increase was also observed at 4% w/w(group V) when compared with Cicatrin® (group II).

The epithelialization period of group V rats treated with 4% w/w MLEPH (21.33 ± 0.21 days) was significantly ($p<0.05$) lower than those of the PJ-treated group I (29.83 ± 4.29 days) and Cicatrin®-treated group II (29.67 ± 2.56 days) (Fig. 4).

The gross appearance scores (Table 2) showed that on PSD 3, there was no significant ($p > 0.05$) difference across the groups. On PSD 6 and 9, Cicatrin® (group II), 1% MLEPH (group III), 2% MLEPH (group IV), and 4% MLEPH (group V) scored significantly ($p<0.05$) higher compared with PJ (group I). However, on PSD 12 and 15, there was no significant ($p > 0.05$) difference across the groups. But on PSD 18, 4% MLEPH (group V) showed significantly ($p<0.05$) higher scores when compared with the PJ (group I) and Cicatrin® (group II). On PSD 21, Groups III, IV and V scored significantly ($p > 0.05$) higher than groups I and II.

Table 2. Effect of MLEPH on Gross Appearance of the wound

	Days post treatment						
	3	**6**	**9**	**12**	**15**	**18**	**21**
GROUPS							
I (PJ)	1	2	3	4	4	4	4
II (CICATRIN)	1	3^{a}	4^{a}	4	4	4	4
III (1% MLEPH)	1	3^{a}	4^{a}	4	4	4	$5^{a,b}$
IV (2% MLEPH)	1	3^{a}	4^{a}	4	4	4	$5^{a,b}$
V (4 % MLEPH)	1	3^{a}	4^{a}	4	4	$5^{a,b}$	$6^{a,b}$

Values are expressed as median (n=6); ^{ab}Figures with different superscripts are significantly different ($p < 0.05$) with negative or positive controls.

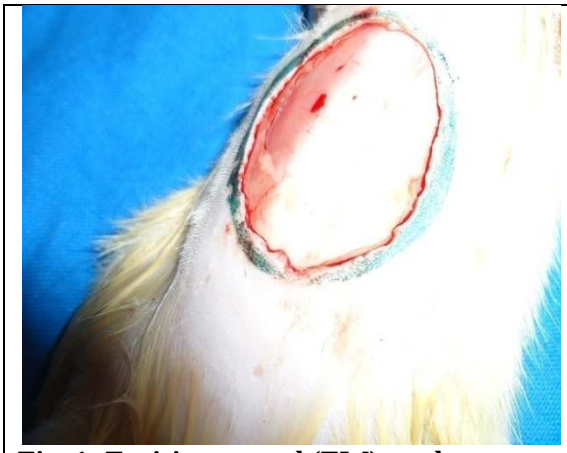

Fig. 1: Excision wound (EW) on the dorsum of the experimental rat (Day 0) x 100.

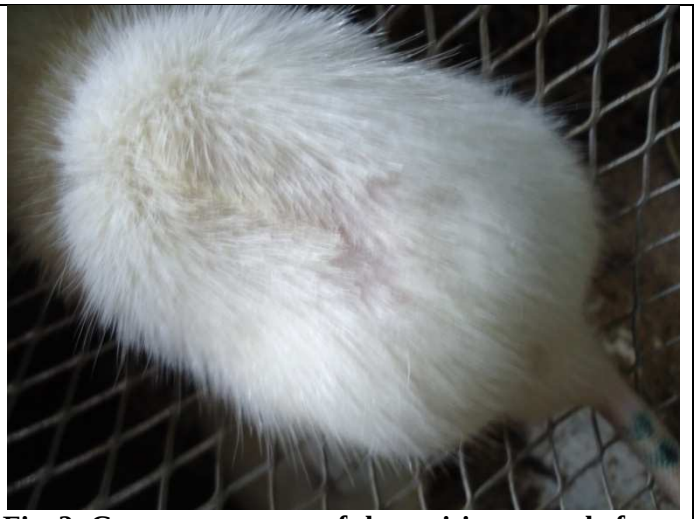

Fig. 2: Gross appearance of the excision wound of rats treated with 4 % MLEPH (group V) on PSD 21 showing complete healing (CH) x 100.

DISCUSSION

The excision wound model measured the wound contraction and epithelialization period. Wound contraction is the centripetal movement of wound margin to close up the open wound area. This movement is caused by myofibroblast activity [19,20]. Generally, MLEPH, Cicatrin® and the vehicle petroleum jelly (PJ) all exhibited different levels of wound contraction, but MLEPH at 1% w/w (group III) and 4% w/w (group V) enhanced wound contraction compared to the positive (group II) and negative (group I) controls. This demonstrated the efficacy of the methanol leaf extract of *Palisota hirsuta* (MLEPH) in wound management. The enhancement of wound contraction in groups III and V was either due to the ability of MLEPH to enhance the contractile property of myofibroblasts or increase myofibroblasts populations recruited into the wound site. The enhanced wound healing potential was also reflected in the gross appearance scores of the wounds, where MLEPH at 4% w/w (group V) scored highest indicating best healing activity, reaching the stage of complete healing fastest.

The period of epithelialization was also significantly reduced by MLEPH at 4% w/w concentration (group V) when compared with PJ- and Cicatrin®-treated groups I and II respectively. This means that the MLEPH increased the rate of epithelialization by enhancing the mobilization and proliferation of epithelial cells over the wound area. It is also possible that the wound healing activity of MLEPH is

derived from its flavonoid and tannins phytocomponent [8]. Some flavonoids and tannins have been reported to have wound healing activity due to their astringent, antimicrobial and antioxidant properties [21].

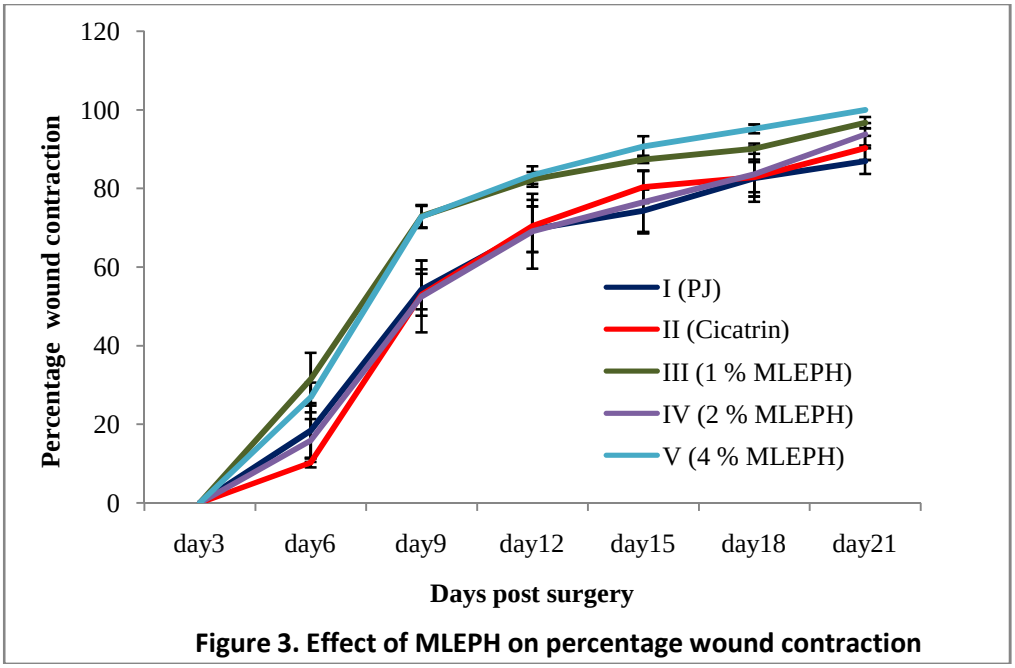

Figure 3. Effect of MLEPH on percentage wound contraction

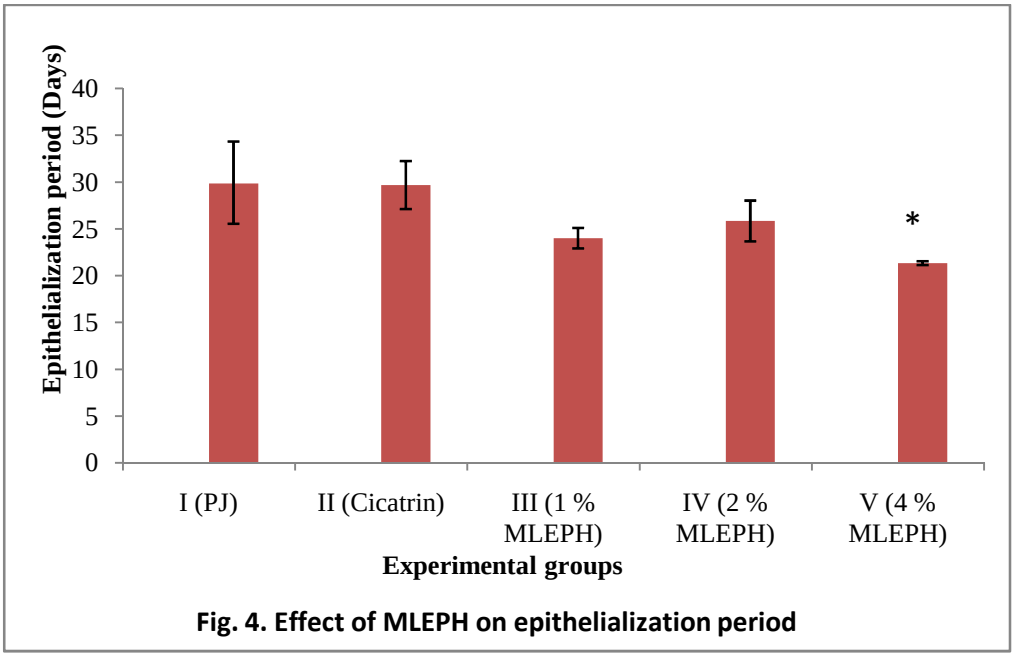

Fig. 4. Effect of MLEPH on epithelialization period

In the agar diffusion assay however, MLEPH induced no inhibition zones against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, an indication of no activity against these microorganisms. This was contrary to the work of Anaga *et al.* [8] where MLEPH showed narrow spectrum antibacterial

activity against *Staphylococcus aureus* and *Streptococcus pyogenes.* This may be due to differences in the age of the plant and the time and season of the year when it was harvested [22]. Stages of maturity, season and soil type/content are factors that have been known to influence the phytochemistry of the active principles in plants thereby affecting their activity [22].

In conclusion, methanol leaf extract of *Palisota hirsuta* significantly improved all wounding parameters evaluated, thereby confirming the wound healing potential of the extract, thus, justifying the folkloric use of the plant as a wound healing agent. On the basis of these findings, the plant extract is recommended for use in the clinical management of wounds. Further studies may be needed to isolate the active principle in the extract that is responsible for its wound healing activity.

REFERENCES

1. Badri, P. N. and Renu, S. (2011). Role of medicinal plants in wound healing. *Research Journal of Medicinal Plants*, 5: 392 - 405.
2. Kumar, B., Vijayakumar, M., Govindarajan, R. and Pushpangadan, P. (2007). Ethnopharmacological approaches to wound healing-exploring medicinal plants of India. *Journal of Ethnopharmacolology,* 114: 103 - 113.
3. Menke, N.B., Ward, K. R., Witten, T. M., Bonchev, D. G. and Diegelmann, R. F. (2007). Impaired wound healing. *Clinics in Dermatology,* 25: 19 - 25.
4. Roberts, P. R., Black, K. W., Santamauro, J. T. and Zaloga, G. P. (1998). Dietary peptides improve wound healing following surgery. *Nutrition,* 14: 266 - 269.
5. Dorai A.A. (2012). Wound care with traditional, complementary and alternative medicine. *Indian Journal of Plastic Surgery,* 45 (2): 418-424.
6. Akobundu, I. O. and Aggakwa, C. W. (1987). *A Handbook of West African Weeds.* International Institute of Tropical Agriculture, Ibadan, 1-521.
7. Burkill, H. M. (1985). *The useful plants of west tropical Africa*. Vol 1, Royal Botanic Gardens, Kew.1-960.
8. Anaga, A. O., Eke, I. G. and Chah, K. F. (2009a).Some pharmacological properties of methanolic extract of *Palisota hirsuta* leaves. *Tropical Veterinaria,* 27(4): 37 - 47.
9. Anani, K., Hudson, J. B., De-Souza, C., Akpagana, K., Tower, G. H. N., Arnason, J. T. and Gbeassor, M. (2000). Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. *Pharmaceutical Biology,* 38(1): 40 - 45.
10. Hudson, J. B., Anani, K., Lee, M. K., De Souza, C., Arnason, J. T. and Gbeassor, M. (2000). Further investigations on the antiviral activities of medicinal plants of Togo. *Pharmaceutical Biolology,* 38: 46 - 50.
11. Boakye-Gyasi, E., Woode, E., Ainooson, G. K., Obiri, D. D., Ansah, C., Duwejua, M. and Donkoh, A. (2008). Anti-Inflammatory and antipyretic effects of an ethanolic extract of *Palisota hirsuta* K. Schum roots. *African Journal of Pharmacy and Pharmacology*, 2: 191 - 199.
12. Anaga, A. O., Ndukwe, C. P. and Shoyinka, S. V. O. (2009b). Hepatoprotective effect of the methanolic leaf extract of *Palisota hirsuta* against CCl4 –induced hepatic damage in mice. *Tropical Veterinarian,* 27 (1): 17 - 27.
13. Woode, E., Boakye-Gyasi, E., Ainooson, G. K., Ansah, C. and Duwiejua, M. (2009). Anti-nociceptive effects and the mechanism of *Palisota hirsuta* K. Schum leaf extract in murine models, *International Journal of Pharmacology,* 5: 101 - 113.
14. Department of Health and Human Services (1985). *Guide for the care and use of laboratory animals* (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council. National Academy, Washington DC.
15. Murray, P. R., Baron, E. J., Pfallar, M. A., Tenover, F. C. and Yolke, R. H. (1995). *Manual of Clinical Microbiology.* 6th ed., ASM Press, Washington DC. Pp.15 - 18.

16. Morton, J. J. and Malone, M. H. (1972). Evaluation of vulneray activity by an open wound procedure in rats. *Archives Internationales de Pharmaco dynamie et de Therapie*, 196 (1): 117 – 126.
17. Bairy, K. L. and Rao, C. M. (2001). Wound healing profile of *Ginko biloba. Journal of Natural Remedies*, 1: 25 - 27.
18. Bhat, R. S., Shankrappa, J. and Shivakumar, H. G. (2007). Formulation and evaluation of polyherbal wound treatments. *Asian Journal of Pharmaceutical Sciences*, 2(1): 11 – 17.
19. Gabbaiani, G., Hirschel, B. J., Ryan, G. B., Statkov P. R. and Majno G. (1972). Granulation tissue as a contractile organ. *Journal of Experimental Medicine*, 135 (4): 719-34.
20. Fossum, T. W. (2007). *Small Animal Surgery.* 3rd Edn., Mosby, St. Louis, Missouri. ISBN-10: 0-323-04439-5. 1-1610.
21. Himesh, S. and Akhlesh, K. S. (2012). A recent update of botanicals for wound healing activity. *International Research Journal of Pharmacology*, 3(7): 1-7.
22. Lampe, K. F., McCann, M. A. (1985). *AMA Handbook of poisonous and injurious plants.* American Medical Association, Chicago, Ill., USA. Pp. 1-432.