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Wound Healing Potentials of *Ocimum gratissimum* L. (Lamiaceae) Methanol Extract in Experimental Wistar Albino Rats

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ABSTRACT

The potency of *Ocimum gratissimum* L. as a wound healing agent was investigated with a view to develop a drug that is readily available, affordable and accessible to the rural farmers .Thirty (30) Albino Wistar rats weighing 180-200g were grouped into six (A-F). Each animal in the group was anaesthetised using chloroform. The part of their hind leg (thigh) was shaved using a very sharp and sterilised surgical blade. Thereafter, a surgical blade and a forceps were employed to create an external nosa (wound) of a known diameter. Group A was the standard control treated with 0.5g of penicillin ointment, while the remaining groups were treated with 0.25, 0.50, 0.75 and 1.00g/ml of the extract respectively and group F was the negative control treated with distilled water. The treatment was carried out on daily basis for a period of 3 weeks. All doses (except 50mg/ml) showed a significant reduction ($P<0.05$) in the wound diameter on the third day of the extract administration/application. *Occimum gratissimum* can be packaged and employed as a phyto-agent against external nosa in farm animals.

Keywords: External nosa, *Occimum gratissimum,* Ethnobotanical survey, Phyto-agent,

Introduction

Wound is a circumscribed injury which is caused by an external force and it can involve any tissue or organ. While injury on the other hand is caused by external noxa that causes cellular and/or tissue trauma and dysfunction. External noxa refers to wound inflicted due to mechanical, chemical, radiation or combination of them. Wound healing or wound repair, is the body's natural process of regenerating dermal and epidermal tissue (Györgyi, 2016)

Wound infliction through external nosa (Mechanical in particular) is more pronounced with both the small and large ruminants. This wound infliction is more prevalent in Africa and Asia with Europe and Americas recording less than 3% of all the fatalities recorded (FAO, 2015). An estimated $204.6M loss is incurred globally as result of death and/or depreciation in value due to wound inflicted on both small and large ruminants, with Europe and Americas accounting for only 2.8% of the loss.(Orwa and Lakhshmir, 2017).More to the afore mentioned, current estimates indicate that, worldwide nearly 6 million people suffer from chronic wounds. Unhealed wounds constantly produce inflammatory mediators that produce pain and swelling at the wound site. Chronic wounds may even lead to multiple organ failure of death of the patients (Kumar *et al,* 2007).

The mechanism of wound healing is initiated by the leakage of Adenosine diphosphate from the damaged cells. The leaked ADP activate the secretion of adhesive type 1 glycoprotein that in turn attract platelets and their aggregation. As reported by Broughton *et al.*, (2006) the

damaged cells also secrete factors that interact with and stimulate the intrinsic clotting cascade through the production of thrombin, which in turn initiates the formation of fibrin from fibrinogen. The fibrin mesh strengthens the platelet aggregate into a stable haemostatic plug. The platelets on the other hand promote the secretion of growth factors like transforming growth factor beta (TGF-ß), platelet derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF) whichcollectively play a vital role in the movement of monocytes and neutrophils to wound site to begin the inflammatory phase.

Intensive Ethno-botanic research in wound healing has not yielded economic and efficacious pro-healing agents that could alleviate the long hospitalization of patients following surgery and wound infliction attended by frequent deaths and wanton losses.(Olukemi*et al.*, 2012)

The plant: *Ocimumgratissimum*also known as African Basil is a plant belonging to the order *Lamiales*, and family *Lamiaceae*, Genus: In Nigeria it is commonly called, Ncho-anwuorAhuji (Igbo), Efinrin (Yoruba), Aramogbo (Edo) and Daidoya (Hausa) (Olukemi*et al.*, 2012).Different tribes of Nigeria use the leaf extract in treatment of diarrhoea, while the cold leaf infusions are used for the relief of stomach upset and haemorrhoids (Kabir*et al.*, 2005)

The main thrust of the research work is to find out, through ethno-botanical survey, plant resources with wound healing effect and to scientifically validate such claims in experimental animals

Materials and Methods

Methodology

Plant sample was collected through ethno-pharmacological survey in the surrounding villages of Awuru, FarinDutse, Amfani, Nassarawa, Garafini, Wawa, GadaOli, Leshigbe, Lubararu and Babanna all in Borgu Local Government Area of Niger State.

The whole plant (Herb) except the root was washed and dried at room temperature. It was then pulverised into a powdered form using a pestle and mortar. Thereafter, sample was cold extracted by placing 100g of the pulverised sample in a conical flask to which was added 400 mL of 70% methanol and securely sealed with tin foil. It was occasionally shaken for 24 hours before being filtered with a clean muslin cloth into a 1000mL capacity beakeras described by Garba*et al.* (2018). The Marc was discarded and the procedure repeated thrice to have sufficient quantity of the filtrate. The solvent was recovered using the rotary evaporator while the extract was concentrated and dried using steam bath set at 40^{o} C. Spatula was used to scrap the dried extract into sample bottle and stored in the refrigerator at 4^{o} C until required for use.

Animals

Thirty (30) Albino Wistar rats weighing 180-200g were purchased from the animal house of the Biochemistry Department, of the Federal University of Technology, Minna, Niger State, Nigeria. Access to rat pellets (Vital feed^{R}) and clean drinking water was provided/made *ad libitum.*Wood shavings was used to provide a litter bed of about 50mm thickness. Periodic replacement of the litter bed was carried to avoid mould growth and to maintain a cleaner and safe environment.

Wound healing activity of crude extract.

Infliction of external nosa (Mechanical injury)

Each animal in the group was Anaesthetised using Chloroform. The anaesthetised animals were then brought out and placed on a sterilised dissecting tray and had the upper part of their hind leg (thigh) shaved using a very sharp and sterilised surgical

blade. A uniform circular mark (the same diameter) was made on the shaved portion of each of the anaesthetised animal. Thereafter, a surgical blade and a forceps were employed to cut and subsequently removed the surface skin layer to create an external nosa (injury)of a known diameter.

The animals were grouped thus:

Group A: External nosa was and treated with 0.5g penicillin ointment (Standard control)
Group B: External nosa was treated with 0.25g/5ml extract
Group C: External nosa was treated with 0.50g/5ml extract
Group D: External nosa was treated with 0.75g/5ml extract
Group E: External nosa was treated with 1.00g/5ml extract
Group F: External nosa was treated with Distilled water (Negative control)

Treatment and Measurement

The treatment was carried out on daily basis for a period of 3 weeks by disinfecting the wound surface with Hydrogen peroxide (H_2O_2) solution prior to the application of the various dosages in the respective groups. It was thereafter covered with adhesive plaster to prevent licking of the spot.
Prior to the application of the extract on the second day of the treatment circle, the wound surface diameter was measured with Vernier callipers and recorded (i.e at two days interval).

Statistical Analysis

The data obtained were subjected to one way analysis of variance (ANOVA) in a completely randomized design (CRD) arrangement. The significant means was separated and compared using Duncan multiple range test (DMRT) incorporated in the application software.

Results

The wound healing potency displayed by the plant as shown from the result in Table 1 confirms its efficacyand therefore its subsequent classification as plant with wound healing effect. All the doses (except 50mg/ml) showed a significant reduction ($p < 0.05$) in the wound diameter on the third day of the extract administration/application. However, the dose of 50mg/ml turned out to be the most effective in the subsequent days having promoted the wound surface closure earlier than the standard drug. The extract at the dose of 0.5g/ml competes favourably with the standard drug and showed significant ($p<0.05$) higher efficacy than the other concentrations. Additionally, the same dosage of 0.5g/ml effected the complete wood healing within nine (9) days of the treatment of the external nosa compared to the 13, 15 and 19^{th} days into the treatment recorded in the 0.25, 0.75, 1.00g/ml, standard and negative controls respectively. The rate at which the diameter of the external nosa reduces in the group treated with 0.50g/ml of the extract is significantly higher ($p>0.05$) compared to the standard group (Table 1)

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Table 1. Effect of application of various dose concentrations of *Occimumgratissimum* methanol extract on an external nosa

Dosage	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
		Diameter of the external nosa in millimetre (mm)									
0.25g/ml	14.00±0.00	10.00±1.15^{a}	7.33±0.35^{b}	5.33±0.33^{c}	4.00±0.80^{bc}	2.00±1.00^{d}	1.00±0.58^{d}	0.30±0.00	0.10±0.00	0.10±0.00	0.00±0.00
0.50 g/ml	14.00±0.00	13.00±1.53^{a}	9.33±1.86^{b}	4.50±3.56^{c}	1.33±0.88^{d}	0.67±0.67^{e}	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.75g/ml	14.00±0.00	7.67±0.67^{a}	6.67±0.67^{b}	5.33±0.33^{c}	3.33±0.33^{d}	3.00±0.58^{d}	2.33±0.33^{e}	0.33±0.33^{f}	0.00±0.00	0.00±0.00	0.00±0.00
1.00g/ml	14.00±0.00	7.67±0.67^{a}	6.67±0.67^{ab}	6.35±0.88^{b}	3.33±0.33^{c}	3.00±0.58^{c}	2.33±0.33^{d}	0.33±0.33^{e}	0.00±0.00	0.00±0.00	0.00±0.00
STD control	14.00±0.00	6.67±1.20^{a}	5.00±1.73^{b}	4.33±1.45^{c}	2.33±1.20^{d}	1.67±1.20^{e}	0.67±0.67^{f}	0.33±0.33^{f}	0.00±0.00	0.00±0.00	0.00±0.00
Negative control	14.00±0.00	10.33±1.45^{a}	5.67±1.20^{b}	4.00±1.15^{c}	2.33±2.08^{d}	1.67±1.53^{e}	0.67±1.15^{f}	0.40±0.00^{f}	0.21±0.00	0.10±0.00	0.00±0.00

***Values on the same row with different superscripts are significantly (p> 0.05) different**

Discussion

The wound healing displayed by the plant as shown from the result in Table 1 confirms its efficacy and therefore buttress the authenticity of its classification as plant with wound healing effect by Olukemi *et al*.(2012) and Sawadogo *et al.* (2012) in their separate reports on ethno-botanical surveys. Worthy of note is the delayed in the onset of healing effect/activity shown by the 50mg/ml dosage, on the third day of the extract application, there was only a slight reduction in the size of the external nosa by just 7.14% when compared to the remaining doses and the standard control. The slow healing trend was however reversed in the subsequent days with the 0.5g/ml extract concentration competing favourably with the standard drug and showing significant ($p<0.05$) higher efficacy than the other concentrations. Additionally, the same dosage of 0.5g/ml effected the complete wood healing within nine (9) days of the treatment of the external nosa compared to the 13, 15 and 19th days into the treatment recorded in the 0.25, 0.75, 1.00g/ml standard and negative controls respectively.

The rate at which the diameter of the external nosa reduces in the group treated with 0.50g/ml of the extract is significantly higher ($p>0.05$) compared to the standard group (Table 1)

The mechanisms involved in wound healing are quite elaborate, therefore, *O. gratissimum* methanol extract most probably exerts its action through one or interaction of these mechanisms. The possible reason that can be adduced to the effectiveness of this extract could be due to the presence of high level of total phenol and flavonoid content (218.3255 and 51.9572 mg/ml respectively) in the leaf juice of the plant as determined by Olukemi *et al.*, (2012) and the quantitative antioxidant activity determined by the same authors (74.974%) was found to correlate positively with both phenol (R^2=0.388) and flavonoid (R^2=0.461) content. Since whenever an injury occur there is interaction of leukocytes and stromal cells during an acute inflammatory response which revolves around the inflammatory focus (Buckley, 2011; Salamone, 2016), this event leads to the mobilisation of neutrophils which are known for expressing many pro-inflammatory cytokines and a large quantity of highly active antimicrobial substances, such as reactive oxygen species (ROS),supero oxide (SÖ), cationic peptides, and proteases at the location of the lesion (Shaw and Martin, 2009)). This cascade of events at the inflammatory stage of wound healing should be brief (24-48 hours) (Kumar *et al*., 2007). Therefore, the high antioxidant capacity coupled with high levels of total phenols and flavonoid in the extracts possibly reduced the ROS and SÖ species which paved way for the proliferative phase to set in. Additionally, Polyphenols have astringent properties that hasten the healing of wounds and the inflamed mucous membrane through the promotion of angiogenesis and fibroblast proliferation (collagen deposition) (Njoku and Akemefula, 2007).

Therefore, considering the mentioned phyto-components of *O. gratissimum* and their established role in promoting wound healing, it could be argued with a degree of certainty that the higher efficacy observed in the extract compared to the standard drug (Penicillin ointment) (Table 1) is due to the presence in the extract, of phyto-components (Phenols, Flavonoids and saponins) which have both strong antioxidant activity and astringent properties that also promote the proliferative stage of wound healing. In the case of the standard drug used (Penicillin ointment) whose composition is mainly penicillin and Potassium, its mechanism of action was found to be mainly promoting the secretion

of neutrophils through a role played by "Potassium" in vasodilation and the antibacterial role played by the "Penicillin" a known antibiotic substance (Eming*et al*., 2009).

When the rate of wound contraction is taken into consideration across the various doses and the standard drug, it is pertinent to state that, the extract at all concentrations greatly improves the proliferative phase in the wound healing processes ofangiogenesis, fibroblast proliferation (collagen deposition), granulation tissue formation, wound contraction and re-epithelialisation were seen to proceed faster than in the negative control group. This observation is in line with a report by Stadelmalmann *et al*. (1998). Within the concept of this research work, the actual role of this extract with regards remodelling and realignment of the collagen tissue to produce greater tensile strength comparable to that of normal skin could not be stated with certainty as Diegelmann and Evans, (2004) and Iba *et al*. (2004) have reported that remodelling can take up to 2 years after wounding. From the outcome of this research it is clear that the methanol extract of *O. gratissimum* can be quite a reliable and cheaper substitute the conventional pharmaceutical drugs particularly in the management of acute wounds.

Conclusion

The results obtained from this study clearly revealed the potency of this extract as a promising wound healing agent which can be employed as an alternative to the orthodox cream/ointment (Penicillin) and which will be quite affordable, accessible and available to rural herders in particular**.** The crude extract from this plant when further purified might probably yield a lead compound that would be more efficacious and safer than the hitherto available agents that are both expensive and discredited by the resistance to their action by the wound contaminating microbes. Moreso, the drug candidate may, due to its multiple secondary plant metabolites components counter such resistance thereby, drastically reducing the estimated $204.6M loss incurred globally as result of death and/or depreciation in value due to wound inflicted on both small and large ruminants in agricultural sector and incapacitation and disabilities due to loss of organs in humans.

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