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Full Length Research Paper

Wound healing potential of the ethanolic extracts of *Bidens pilosa* and *Ocimum suave*

Kyakulaga .A. Hassan[1,2], Olila Deogratius[2], Jane .F. Nyafuono[3], Omujal Francis[3] and Ogwang .P. Engeu[3]*

[1]Department of Pharmacology and Therapeutics, Makerere University, College of Health Sciences, P. O. Box 7062, Kampala, Uganda.
[2]Department of Physiological Sciences, Faculty of Veterinary Medicine, Division of Pharmacology, Makerere-University P. O. Box 7062, Kampala, Uganda.
[3]Natural Chemotherapeutics Research Institute, Ministry of Health Uganda, P. O. Box 4864, Kampala, Uganda.

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The ethanolic extracts of *Bidens pilosa* and *Ocimum suave* were evaluated for their wound healing potential on excision wound models in Wistar albino rats. A total of nine (n=9) rats per group were used to assess the wound healing potential of the extracts. There were four groups of animals treated topically twice a day with either of the following: *O. suave, B. pilosa*, neomycin sulfate or distilled water. The rate of wound contraction, epithelialization, and complete healing were assessed over the experimental period. Wounds treated with extracts of *O. suave*, *B. pilosa* and neomycin sulfate had faster rates of wound contraction ($p<0.05$) on days 3, 6 and 9 than negative control. Histological examination of wound on day 7 also revealed better collagenation, angiogenesis and organization of the wound tissue. Epithelialisation and total healing time for *B. pilosa* and *O. suave* were comparable to neomycin sulfate ($p>0.05$). In conclusion, extracts of O. suave and *B. pilosa* show potential for use as alternative to neomycin for treatment of wounds.

Key words: *Bidens pilosa, Ocimum suave*, wound healing, epithelialization, ethanolic extract.

INTRODUCTION

Wound healing is a multifactorial physiological process of repairing injured tissues that involves several cell types that result into restoration of a physical barrier (Nayak et al., 2007). Impaired wound healing due to infection or metabolic complications can result into severe morbidity leading to long hospitalization of patients. The aim of treating wounds is to shorten the time taken for healing and to reduce risks of undesired complications.

Plants have been found to synthesize compounds that are useful in the process of wound healing (Kalyon et al., 2009). The use of plants and plant products in the management of diseases has particularly been fueled by the rising cost of synthetic drugs. Plants have immense potential in management of wounds especially for people living in resource limited nations (Raina et al., 2008).

In Uganda, *Bidens pilosa* (Asteraceae) and *Ocimum suave* (Labiatae) are used in traditional medicine for treatment of wounds however, there is no data to enable their development into clinically useful products. The present study was undertaken to determine the wound healing potential of the ethanolic extracts of *B. pilosa* and *O. suave* in male Wistar albino rats to generate preclinical data a step towards development of formulations and trial in clinical setting.

METHODS

Preparation of plant extracts

The leaves of *B. pilosa* and *O. suave* were collected from the animal farm at Faculty of Veterinary Medicine, Makerere University

*Corresponding author. E-mail: pe7321@gmail.com. Tel: 256779617612, +256-414-250488.

in Kampala and authenticated by a botanist and voucher specimen number NCJ 268 and NCJ 270 respectively, deposited at the Natural Chemotherapeutics Research Laboratory, Ministry of Health herbarium. The plants were shed dried at Natural Chemotherapeutics Research Laboratory (NCRL) for a week and pulverized mechanically using a grinder. The powder (250 g) was exhaustively extracted by cold maceration in 90% ethanol (750 ml) for 72 h. The macerate was filtered using Whatman filter paper and concentrated at 55°C using a rotary evaporator (BIBBY STERLIN LTD Model RE 100) to obtain a viscous solid (extractive) with yield of 11 and 14% for *B. pilosa* and *O. suave* respectively. 25 g of the extractive was dissolved in 250 mls of distilled water to make a concentration of 100 mg/ml for application to wounds. The solutions were kept in a refrigerator at temperature of 2 to 8°C throughout the study period.

Experimental animals

50 healthy male inbred Wistar albino rats of approximately 8 weeks old and weighing (150 to 200 g) were obtained from the same colony at the animal house of Faculty of Veterinary Medicine, Makerere University. The animals were housed in clean cages with access to clean water and standard laboratory pellet diet *ad libtum*.

Experimental design

The study was an experimental controlled study on excision wound models on Wistar albino rats.

Study outcomes

The primary outcomes were (1) rate of wound contraction and (2) rate of epithelialisation. The secondary outcomes were; complete epithelialisation time and complete healing time.

Experimental

36 male Wistar albino rats were randomly selected and divided into 4 groups (n=9, df=8). Excision wounds were made on each rat by first injecting Ketamine (50 mg/kg b.w) and diclofenac (100 mg/kg) to provide pre and post operative analgesia. The mid-dorsal region of each rat was then shaved using a clipper and disinfected using 70% alcohol.

A circular piece of full thickness was cut off from predetermined area on the back of the rat using a surgical blade and scissors (Rajabi et al., 2007; Suntar et al., 2009). Bleeding was arrested using cotton wool and initial diameters (a=transverse diameter and b=longitudinal diameters) were measured using a Vanier caliper after 12 h from excision time.

Treatment of the wounds

The animals were treated as follows; group I: Distilled water, group II: neomycin cream, group III: *B. pilosa* extract and group IV *O. suave* extract. The extracts were applied on the wounds in the amounts sufficient to cover the entire area and this was done twice a day till complete healing of the wounds.

At 7 days of treatment, 3 rats per group were humanely sacrificed under ether anaesthesia. The wound tissue of the 3 animals randomly sampled per group were excised and fixed in 10% formaldehyde. The tissues were processed using an automatic histokinette and sectioned using a microtome. The slides were stained using haematoxylin and Eosin routine stain and examined under a light microscope.

Data collection

A data recording sheet was used for every rat to collect information on wound diameters (a and b) on days 0, 3, 6 and 9 of treatment. Time for complete epithelialization (defined as the time taken for the residual scar to fall off the wound) and time for complete healing of the wounds were documented.

Statistical analysis

Area of wound was calculated as (abπ)/4 for all the animals on days 0, 3, 6 and 9 of treatment. These were used to estimate the rate of wound closure by considering the area of wounds at day 0 as reference for all the subsequent areas. Data is presented as Mean±SD and the differences between the means of the various groups was analyzed by SPSS version 12.0 using One way ANOVA followed by Student's t-test at 95% level of confidence. P-values less than 0.05 were considered statistically significant.

Ethical issues

The study was approved by the Research and Ethics Committee of Makerere University Faculty of Veterinary medicine Kampala, Uganda and the Ethics Committee of Natural Chemotherapeutics Research Laboratory-Ministry of Health. The study registration number is NCRL/08-5. Study animals were handled in conformity with guidelines for the care and handling of laboratory animals provided by the two Institutions and in accordance with guidelines for laboratory biosafety Guidelines, 2004.

RESULTS

General observations

No death was observed for any of the rats in the study groups and there were no remarkable changes in general appearance or animal behavior. The animals treated with *B. pilosa* and *O. suave* extracts had less scar formation at the wound sites and also had hair growing at the wound sites. The rats treated with neomycin and distilled water had prominent scars and no hair at the wound sites.

Wound contraction, complete epithelialisation and complete healing

O. suave extract had highest percentage wound closure followed by *B. pilosa* extract and then neomycin sulfate cream at day 3, 6 and 9 of treatment (Table 1). *O. suave* also had the shortest complete epithelialisation time of 8.33 ± 0.84 days, followed *B. pilosa* 8.67 ± 0.42 days and neomycine sulfate 9.50 ± 0.34 days. *O. suave* had shortest compete healing time of13.67 ± 0.21days followed by neomycin sulfate 14.17 ± 0.65 days and then *B. pilosa* 15.00 ± 0.37 days. These time periods were significantly shorter than those for the controls at $p<0.05$. *O. suave* demonstrated better cell differentiation and organization of the epithelial tissue at day 7 (Figure 1).

Table 1. Percentage wound closure in various groups of treatment.

Group	Day 3	Day 6	Day 9
D.W	23.35 ± 5.11	39.89 ± 2.25	63.18 ± 2.27
B.P	33.33 ± 4.07*(11.70,29.84)	56.32 ± 4.96*(6.77, 21.64)	74.31 ± 3.70*(3.01, 19.25)
O.S	36.12 ± 2.23**(7.31,28.65)	58.39 ± 3.53*(9.62, 27.39)	75.52 ± 4.52*(0.12, 24.80)
N.S	23.35 ± 5.11(-7.96, 23.95)	42.43 ± 3.57(-9.54, 14.63)	68.66 ± 3.51(-3.36, 14.32)

Means *p<0.05, ** Means p<0.001, DW: distilled water, B.P: *B. pilosa*, O.S: *O. suave*, N.S: neomycin sulfate.

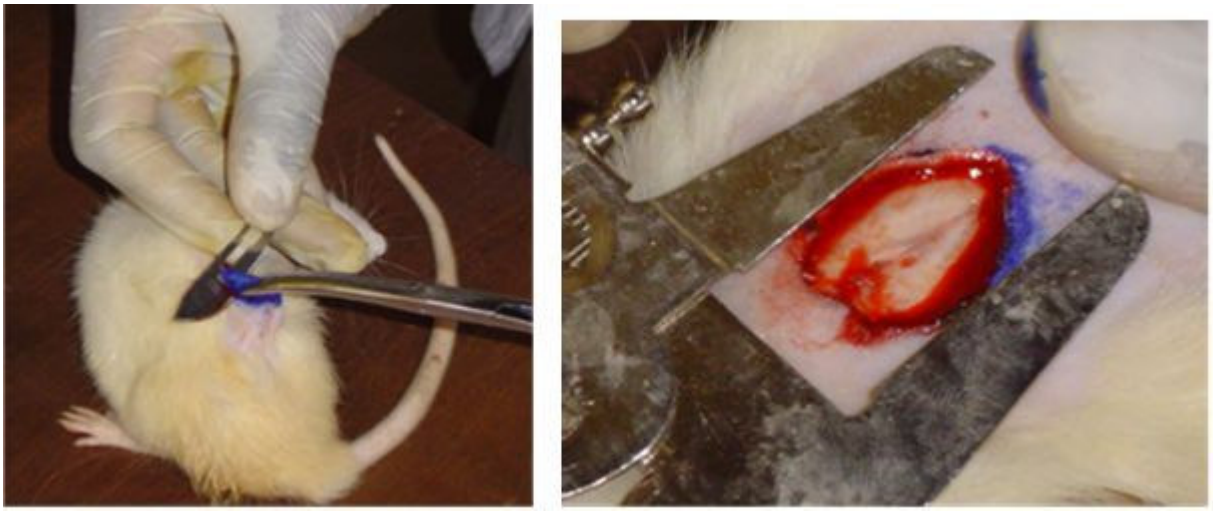

Figure 1. Creation of excision wounds on the mid-dorsal region.

Table 2. Showing the epithelialization time and time for total healing of the wounds.

Group	Epithelialization time (Days)	Total healing time (Days)
DW	11.67 ± 0.92	17.17 ± 0.65
B.P	8.67 ± 0.42 (-4.48, -1.52)*	15.00 ± 0.37*(-3.71, -0.62)
O.S	8.33 ± 0.84(-5.60, -1.07)*	13.67 ± 0.21*(-4.95,- 2.05)
NS	9.50 ± 0.34(-4.09, -0.24)*	14.17 ± 0.654*(-4.48,-4.52)

*p<0.05, DW: distilled water, B.P: *B. pilosa*, O.S: *O. suave*, N.S: neomycin sulfate.

Only *O. suave* had a higher rate of healing than neomycin sulfate at day 6 (19.3 ± 7.8, p=0.03). Histopathology of the tissue on day 7 (H and E routine stain, Light microscope, X40) are shown in Figures 2 to 5.

DISCUSSION

In this study we demonstrate for the first time that *B. pilosa* and *O. suave* extracts accelerate the process of wound healing *in vivo* and these findings corroborate the basis for using these plants in traditional treatment of wounds. Extracts of *B. pilosa* and *O. suave* significantly increased the rate of wound closure compared to negative control (distilled water) although *O. suave* showed higher rates. At day 6 *O. suave* had significantly higher rate than neomycine sulfate cream an antibiotic commonly used in Uganda for management of wounds. Epithelialization time and total healing time were also significantly reduced (Table 2). Histopathological microscopic examination of the wound tissue under the light micros-cope revealed better remodeling of the wound tissue especially in *O.suave* treated group. In the histological sections, it was evident that wound tissue from animals treated with *O. suave* had properly organized collagen fibers, less infiltration with macrophages and lymphocytes (Figure 3). *B. pilosa* treated rats also showed collagen organization which was less than that observed in *O. suave* treated rats. In Figure 5, wounds treated with distilled water were poorly remodeled, with infiltration of tissue by macrophages and lymphocytes which were indicative of delayed healing.

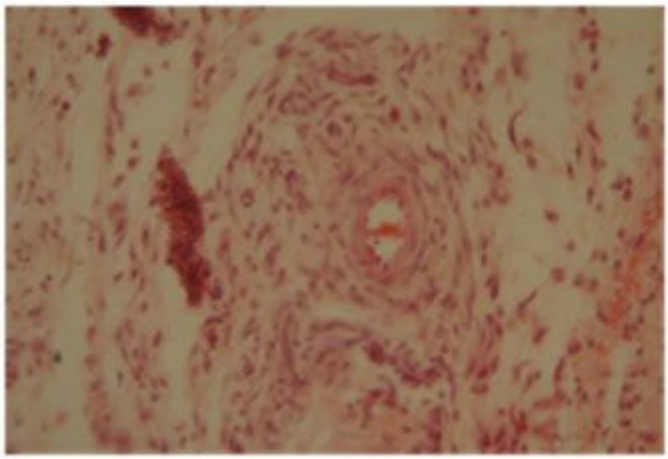

Figure 2. Wounds treated with *B. pilosa*. Tissue shows more collagen fibers and less infiltration of tissue.

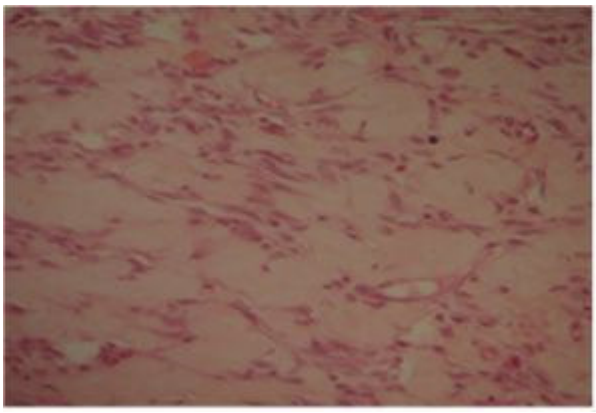

Figure 3. Wounds treated with *O. suave*. Show proper tissue remodeling, more organized collagen fibers a sign of faster healing.

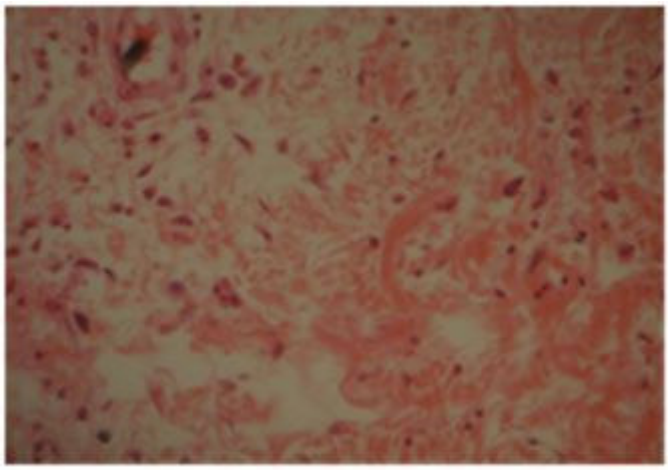

Figure 4. Wounds treated with neomycin sulfate. Shows collagen formation, tissue Infiltration with macrophages and lymphocytes.

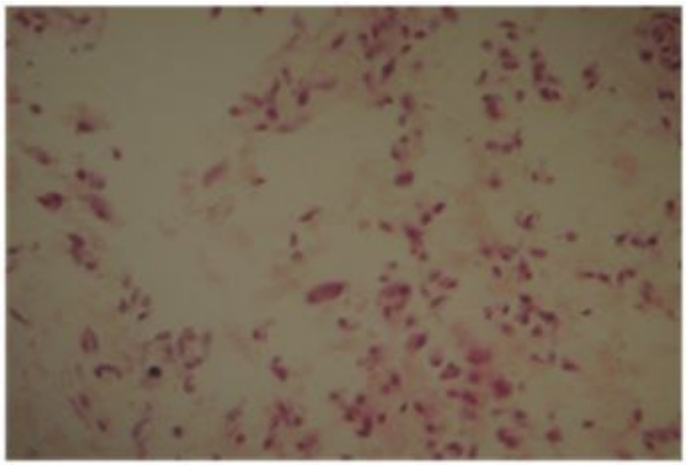

Figure 5. Wounds treated with distilled water. Shows less collagenation, more macrophages and lymphocytes and poor remodeling of the wound tissue.

This ability of plants to accelerate wound healing has been demonstrated for other plants (Rain, 2008; Suntar et al., 2009; Kalyon et al., 2009; Abdulla et al., 2009; Akkol, 2009; Nayak et al., 2007) but not for *B. pilosa* and *O. suave*. In Uganda a tube of neomycin costs about $1 and yet 30% of Ugandans live on less than $1 a day (UBOS, 2008). B. *pilosa* and *O. suave* are widely available in Uganda and therefore could be easily formulated and made affordable to most people who cannot afford neomycin and other expensive products for wound management. On the basis of previously reported pharmacological activities, the plants have significant antibacterial activity (Ashafa and Afolayan, 2009), antioxidant and anti-inflammatory activity (Abajo et al., 2004) which are all important in the process of wound healing. *B. pilosa* has previously been found to contain alkaloids, favonoids, essential oils and polyacetylenes (Geissberger, 1991; Deba, 2008). Alkaloids and flavonoids have been demonstrated to play key role in promoting wound healing (Suntar et al., 2009; Kalyon et al., 2009; Raina et al., 2008), therefore we hypothesize that they could be responsible for the wound healing activity of the *B. pilosa* extracts. The essential oils of four *Ocimum* species grown in Rwanda have been shown to possess antimicrobial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Trichophyton metagrophytes* (Janssen et al., 1989). It is probable that the antimicrobial activity of the oils from *O. suave* may be helpful in keeping away microor-ganisms especially *S. aureus* which is a major bacterial burden in chronic wounds (Gardner et al., 2004) and therefore enhance wound healing. The plant extracts left no prominent scar at the wound sites while neomyicin sulfate treated groups had prominent scars. This property which is lacking in neomycin and other synthetic drugs used for wound treatment in general is of cosmetic importance

especially to people who are prone to scar formation.

Conclusion

B. pilosa and *O. suave* ethanol extracts accelerated wound healing and left no prominent scar in rat models used. We recommend formulation of extracts in to stable dosage forms that can be clinically tried and promoted as affordable wound healing medicines.

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