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Evaluation of the wound healing activity of gel formulations of leaf extract of *Aspila africana* Fam. Compositae

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ABSTRACT

The methanol leaf extract of Aspila africana formulated as gels was studied for its potency on experimentally-induced wound in rats. Wounds were inflicted on Wistar rats using excision model. The extract was formulated as hydrogel and xerogel. The wound healing effects of the formulations were compared to that of a standard antibiotic, Cicatrin® together with the gel bases. In all cases, there was a progressive decrease in wound area with time. A 100% wound closure was observed by the 17th day post wound day in both gel formulations of the extract and the standard. It was concluded that the extract formulated in gel forms were effective in healing wounds.

Keywords: *Aspila africana*. Gel formulation. Hydrogel. Wound healing. Xerogel.

INTRODUCTION

A wound is described as ‘a break in the continuity of tissue, from violence or trauma’ and is regarded as healed if there is a restoration of the wounded or inflamed tissue to normal condition [1]. Wound healing is an important biological process involving tissue repair and regeneration and it involves a chain of well organized biochemical and cellular events leading to growth and regeneration of wounded tissues in a special manner [2-3]. Several drug classes have been used in the management of wounds. Among these are the antibiotics. Penicillin and streptomycin have been widely employed in combating post-operative infections in man and animals [4]. The

antibiotics are chosen based on their ability to destroy or inhibit the growth of pathogenic organisms, while the tissue is left unharmed. In addition, the wound healing activities of plants have since been explored.

Several medicinal plants are used in folklore for wound treatment. One of such plants is *Aspilia africana* C.D. Adams (Compositae), a herb about 1 m tall covered with bristles and commonly known as "haemorrhage plant" due to its ability to stop bleeding from fresh wounds [5-7]. The plant is widespread in Africa [6-7]. The bruised leaves and flowers of *A. africana* are used to clean the surfaces of sores which subsequently heal [8-9]. In addition, it is used for the treatment of rheumatic pains [5]. The plant of *A. africana has* been reported to possess haemostatic [10, 7], antibacterial [11], membrane stabilization [12] and anti-inflammatory [13] activities. The leaf extract has also been shown to cause extracellular Ca^{2+} dependent increase in vascular tone [14]. Phytochemical analysis of the plant leaf extract revealed the presence of a number of terpenoids [15], saponins and tannins [16].

Beside the drug, the formulation vehicle is known to affect the rate of delivery and the efficacy of the therapeutic agent. One of such delivery medium is the gel which finds extensive use in sustained and controlled release drug delivery systems. Gels are semi-solid systems consisting of suspension of small inorganic particles or large organic molecules. Though previous study had demonstrated the wound healing activities of *A. africana* leaf extract [7], the present work was designed to assess the wound healing activities of the plant extract formulated in two different gels: hydrogel and xerogel.

EXPERIMENTAL SECTION

Plant materials

The fresh leaves of *A. africana* were collected in July from a farmland in Nsukka, South-Eastern Nigeria and was identified at the Botanical Garden, Department of Botany, University of Nigeria, Nsukka. The leaves were cut into smaller pieces, air-dried for two days and pulverized to powder using a blender. The powdered sample (250 g) was weighed and extracted with 500 ml of 80% methanol by cold maceration for 48 h. The methanol extract was concentrated in a rotary evaporator, lyophilized and thereafter preserved for further use.

Animals

Adult albino rats (200-300 g) were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were maintained in normal and standard laboratory conditions and fed with commercial diet (Vital Feed Nig. Ltd.) and water, *ad libitium*. They were maintained in normal and standard laboratory conditions of temperature (28±2 □C) and relative humidity (46±6%) with 12-hour light-dark cycle and adequate ventilation.

Permission for the use of animals and animal protocols was obtained from the Animal Ethics Committee of the University of Nigeria, Nsukka, prior to experimentation.

Preparation of gels of the extract

Two gels (hydrogels and xerogels) of the extract were prepared according to the formula previously described [17].

Hydrogels

A preservative, methylhydroxybenzoate (0.1 g) was dissolved in water using heat and maintained at 70°C. A solution of sodium carboxyl methyl cellulose (SCMC), glycerin and the extract was then added in small amounts in a beaker containing the preservative solution and stirred vigorously using electric stirrer till a clear gel was formed. This was packed in a collapsible tube.

Xerogels

The procedure for the preparation of hydrogels was repeated and the gel formed was heated in a petri dish for 3 h and stored in a collapsible tube.

Preparation of wound site

The wound site was prepared following the excision wound model [2, 18-19]. The animals (20) were anaesthetized with diazepam and the hairs on the dorsal skin shaved with sterilized electric clipper. A circle of diameter 25 mm was marked on the skin. Circular incision was then made on the marked area of the skin surface and the skin carefully dissected out. The area was measured immediately by tracing out the wound area using a transparent tracing paper and the squares counted.

Determination of wound healing rate

The animals were divided into five groups of four animals each. Each group received different treatments thus: Group 1 received 1% w/w of extract in hydrogel, Group 2 received 1% w/w of extract in xerogel, Group 3 received Cicatrin powder (positive control), Group 4 received hydrogel (vehicle only) while Group 5 received xerogel (vehicle only). Test sample was administered topically to the wounded area every 2 days starting from the day of wound creation to the respective animals. Wound diameter was measured every 2 days and wound healing was calculated as the number of days required for wound to close.

Statistical analysis

Results were analyzed using one way analysis of variance (ANOVA) and results expressed as Mean ± SEM. Data was further subjected to LSD post hoc test and differences between means were accepted as significant at $p < 0.05$.

RESULTS AND DISCUSSION

The yield of methanol extract of *A. africana* following removal of solvent and dry freeze was 3.92%. The results of the wound healing effects of the formulations of the extract in various gels are shown in Table 1. There was a general decrease in wound area upon application of the test samples and also with time. By the 15^{th} day, the wound area of the animals that received the extract in both gel types (Group 1 and 2) were almost zero and by the 17^{th} day, the wounds were completely healed. The wound healing activities of the extract in the various gel formulations are comparable and not significantly different ($p>0.05$) to that of Cicatrin®, a known antibiotic containing preparation with wound healing properties, by the 17^{th} day. Results also show that the

gel bases alone (Group 4 and 5) produced some degree of wound healing activities though xerogel appeared to be better than hydrogel.

The observed decrease in the wound area on the application of the gel formulations extract of the extract of *A. africana* indicates that the plant extract possesses wound healing properties even when formulated in gels. The reduction in epithelization time of experimentally-induced wound by the extract of the plant has been described [7]. *A. africana* was also reported to possess antimicrobial and haemostatic activities [10, 16], which are necessary in wound healing process. Since wound provide environment for microbial growth, the antimicrobial activity of the extract may partly contribute to the wound healing effect by eliminating infection thus allowing the natural tissue repair processes to start. It also suggests that the leaf extract may play a useful role in accelerating the healing of old wounds by eradicating already established infection. The antimicrobial activity of honey is believed to be responsible for its usefulness in wound healing [20-21]. Cicatrin® is a topical antibiotic preparation which contains bacitracin and neomycin which are very effective against Gram positive and Gram negative bacteria as well as viral and fungal infections. The comparative wound healing effect of the gel formulation of the leaf extract and Cicatrin® suggests that antimicrobial effect of the extract plays a major role in its wound healing activity.

Table 1. Effect of gels containing *A. africana* and Cicatrin® on wound diameter

Group	Description	Wound diameter (mm) on day post surgery[a]								
		Day 0	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
1	1% w/w of Extract in hydrogel	25.00 ±0.23	20.75 ±0.67	17.00 ±2.13	13.75* ±0.34	6.50 ±0.05	5.63 ±0.04	3.25* ±0.81	1.38 ±0.09	0.00
2	1% w/w of Extract in Xerogel	25.00 ±0.45	21.25 ±1.09	17.63 ±1.19	12.00 ±1.00	8.13 ±0.09	5.00 ±0.11	2.25 ±0.01	0.50 ±0.00	0.00
3	Cicatrin powder	25.00 ±0.56	20.25 ±1.10	16.25 ±0.34	9.13 ±0.39	5.70 ±0.12	5.66 ±0.12	1.75 ±0.02	0.13 ±0.01	0.00
4	Hydrogel (vehicle)	25.00 ±1.03	22.13 ±2.05	19.75 ±2.23	15.38* ±0.45	12.25* ±1.01	10.00* ±0.98	7.00* ±0.97	3.38* ±0.02	1.00 ±0.01
5	Xerogel (vehicle)	25.00 ±1.21	21.75 ±1.04	18.75 ±0.99	13.75 ±0.01	12.13* ±0.09	10.25* ±0.23	5.12* ±0.34	2.38* ±0.02	0.50 ±0.00

*[a]Wound diameter±SEM; *Statistically different from Cicatrin® ($p<0.05$)*

Haemostasis involves the spontaneous arrest of bleeding from damaged blood vessels [22], which is important for initiation of tissue repair processes and prevention of tissue death through haemorrhage. The haemostatic process proceeds through a cascade of reactions, which starts with vascular spasm of the ruptured vessels [23], formation of platelet plug through platelet aggregation, and coagulation of the blood [23]. The gel formulated leaf extract may have facilitated these chains of haemostatic processes to effect the wound healing.

Interestingly, the gels alone showed some degree of wound healing effect indicating a possible synergy of the extract and the gels. The exact mechanism of such interaction is not clear. However, the interaction may have led to increased collagen formation since collagen is the principal component of any repaired tissue [24]. In the tissue repair process, inflammatory cells promote the migration and proliferation of endothelial cells, leading to neo vascularisation of connective tissue cells which synthesize extracellular matrices including collagen, and of

keratinocytes resulting to the re-epithelialisation of the wounded tissue [25]. Additionally, the wound healing effect of the gel bases could be attributed to the inherent ability of gels to cover wound surface thereby eliminating infection and allowing the natural tissue repair process to take place. Moreover, xerogel was found to have better wound healing activity due possibly to the fact that xerogel was thicker than hydrogel and covered the wounded surface better.

Furthermore, the wound healing activity of medicinal plants has been associated with their antioxidant properties [26]. Tannins, the main components of many plant extracts, act as free radical scavengers [19, 27-30] and have been reported as partly responsible for the wound healing activity of *A. africana* [7]. Other secondary metabolites such as saponins could be involved. However, the identification and elucidation of the structure of the actual compound(s) are underway.

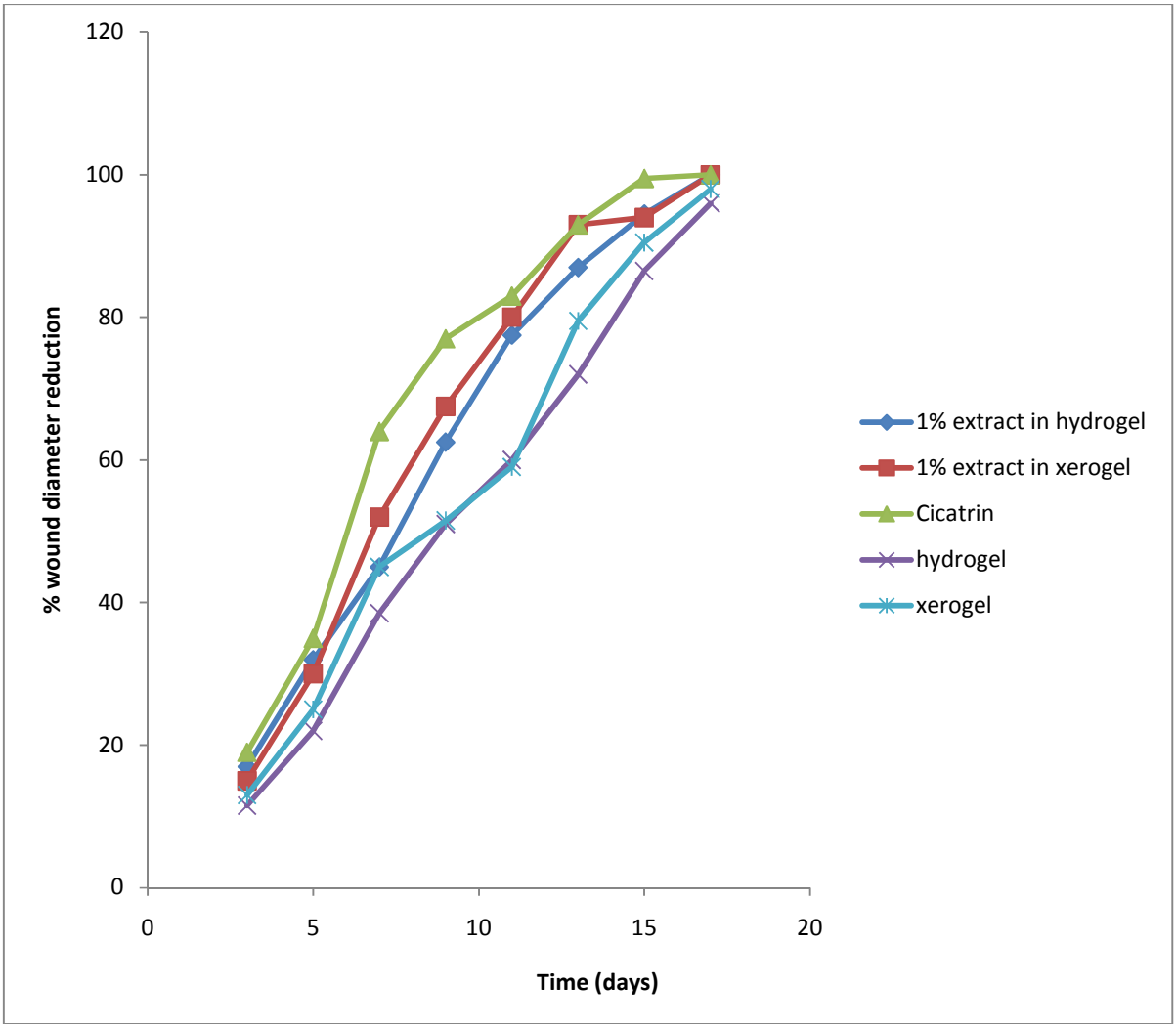

Figure 1: Wound reduction rates in animals treated with *A. africana* extract formulated in various gels compared with Cicatrin® and the vehicles

CONCLUSION

The present study had demonstrated that the leaf extract of *Aspila africana* posseses wound healing activity and this is better still when formulated as a xerogel. The use of the plant extract is therefore important as an alternative therapy in wound care.

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