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Use of *abelmoschus esculenta* gum in the formation of wound healing device containing extract from *vernonia amygdalina*

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ABSTRACT

The present study evaluated the wound healing activity of gel formulation of *Albemoshus esculenta* gum and *Vernonia amygdalina* Del. (Astereaceae) leaf extract. Cicatrin® antibiotic powder was used as standard wound healing formulation. Gels containing different proportions of *A. esculenta* gum: *V. amygdalina* leaf extract were prepared and all the formulations were evaluated using the excision wound model. Gel preparations were smeared on the wound of known diameter inflicted on the dorsum of albino rats. To determine the rate of wound healing, the diameter of each wound was measured daily for 15 days. The wound healing effects of the preparation were compared within the batches to find the proportion that healed faster and to that of Cicatrin®. In all the formulations, there was a progressive reduction in the wound diameter with time and there was a complete wound closure in all the gels formulated with the extracts by the 15th day. It was concluded that the gel formulations of *V. amygdalina* leaf extract with *A. esculenta* gum possessed wound healing effect which was comparable to effect produced by Cicatrin® alone.

KEYWORDS

Albemoschus esculenta gum;
Cicatrin® Gel;
Vernonia amygdalina Leaf extract;
Wound healing.

INTRODUCTION

Wound healing generally implies reconstruction of the integrity of the damaged skin. It involves a chain of well organized biochemical and cellular events leading to growth and regeneration of wounded tissues in a special manner[1]. Immediately after wounding, tissue repair begins with haemostasis. Healing then involves a complex chain of events coordinated by key cells, principally macrophages, which use an array of polypeptide growth factors. These newly identified factors act to induce migration and multiplication of cells, as well as the production of other growth factors. The interrelated processes of epithelialisation, angiogenesis, fibroplasias and collagen synthesis occur only when appropriate cells receive growth factor encoded signals. Antibiotics form the mainstay of wound management by eliminating infection thus allowing the natural tissue repair processes to start. With the increasing emergence of multiple antibiotics resistance, wound isolates are posing enormous public health concerns thus making the need for exploring possible alternatives a

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necessity.

The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine for health programs. This is because plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development[2]. It was estimated that about 80% of the world inhabitants rely on traditional medicine for their primary health care and play an important role in the health care system of the remaining 20% of the population[3]. The potential of higher plants as a source of new drugs is still largely unexplored; hence last decade witnessed an increase in the investigation on plants as sources of new biomolecules for human disease management[4,5]. A large number of the plants are used for various medicinal purposes including wound management. Traditional treatment of circumcision wounds, other wounds and chronic skin ulcers with locally prepared herbs and other natural occurring substances has been known for generations[6]. The leaf extracts of *Garcinia kola*, *Vernonia amygdalina*[6,7] and honey[8] are extensively employed.

Vernonia amygdalina Del. (Astereaceae) popularly known as bitter leaf is widely used for its therapeutic and nutritional purposes. It is a shrub of 2-5 m tall with petiolate leaves of about 6.0 cm wide[9]. It is native to the South Eastern part of Nigeria where it is commonly used for preparing soup and has been widely used in folk medicine as anti-malaria, purgative, antiparasitic or in the treatment of eczema and for maintaining healthy blood glucose levels[10]. Other effects reported for the plant include anthelminthic, antitumorigenic fever, hiccups, and gastric discomfort[11-13] and treatment of multi-drug resistant infection[4] and wound management[6].

Gums are polysaccharide complexes formed from sugar and uronic acid units[14]. Gums may be natural polymers such as proteins and carbohydrates, derived polymers obtained from natural polymers by chemical modification such as methylcellulose; or synthetic polymers obtained by synthesis from low molecular weight substances such as polyvinyl alcohol[15]. Being polymers, gums find extensive use in many fields such as thickeners, adhesives, gelling agents. In pharmaceutical preparations, they are used as emulsifiers, stabilizers, binders, disintegrants, suspending agents etc. The synthetic and semi-synthetic gums are not only expensive but not always available to the local industries especially in developing countries[16]. These add to the cost of pharmaceuticals and other industrial products which are produced with these gums. The natural gums are easily available and hence cheaper and some of them have been characterized[16]. The plants sources are described briefly. *Albemoschus esculenta* (Malvaceae) is a hibiscus-like flower that produces capsules resembling elongated boils. The gum from the plant is widely used in Nigeria and acts as a thickening agent in soups. The rheological properties of the gum mucilage of *A. esculenta* have been studied[17].

Gels are preparation in which the base is usually a carbohydrate polymer (starch, pectin, methylcellulose, tragacanth, sterculli gum) and water[18]. They are semisolid systems consisting of suspension of small inorganic particles or large organic molecules[19]. There are two classes of gels-hydrophobic gels where the bases are usually liquid paraffin with polyethylene or fatty oils gelled with colloidal silica while hydrophilic gels usually consist of water, glycerol or propylene glycol gelled with suitable gelling agents. Gel formulations have been reported to promote wound healing[19].

Though the leaf extracts of *V. amygdalina* have been used traditionally as wound healing agent, a systematic investigation of its gel formulation on the wound healing activity is lacking. In the present study, the wound healing of the gel formulated with *A. esculenta* gum and loaded with leaf extract from *V. amygdalina* was investigated.

MATERIALS AND METHODS

Extraction of gum

The extraction of gum followed the procedure reported by Uzor *et. al*[16]. The *A. esculenta* fresh pods were purchased from Ogige market, Nsukka, Nigeria. The plant materials were washed thoroughly with distilled water after which the barks were peeled and cut into pieces with a knife. The materials were soaked in freshly prepared double strength chloroform water for 24 hours for the gums to ooze out. Sodium metabisulphite at a concentration of 0.1%w/v was added as an antioxidant to prevent atmospheric oxidation of

the gums. The gums were expressed from the plant materials and precipitated from the aqueous medium using acetone in the proportion of one part to three parts of gums. Furthermore, the gums were washed daily for one week by soaking in fresh acetone each day. They were then dried in the desiccators for 24 hours after which they were pulverized using mortar and pestle and further sieved with sieve number 125.

Plant materials

The fresh leaves of *V. amygdalina* Del. were collected from the natural habitat in Nsukka, Enugu State, Nigeria. Their botanical identity was confirmed by Mrs. Eze of Botany Department, University of Nigeria, Nsukka. A voucher specimen of the plant has been deposited in the University of Nigeria, Nsukka.

Preparation of extract

The extracts were prepared following the procedure earlier reported[9]. The leaves were sorted out to obtain only the fresh leaves and washed with distilled water. They were shade dried for three days and dried leaves pulverized with a manual blender. A portion (150 g) of the powdered leaves was cold macerated with distilled water for 24 hours and filtered to obtain the *V. amygdalina* aqueous extract (VAE; 35 g) which was concentrated in a freeze dryer and stored for gel preparation.

Preparation of gel

Three batches of gel were prepared containing different proportion of *A. esculenta* gum and *V. amygdalina* as shown below:

Batch 1: 5% gel of *A. esculentus* gum + 1.0g of VAE;

Batch 2: 10% gel of *A. esculentus* gum + 1.0g VAE;

Batch 3: 15% gel of *A. esculentus* gum + 1.0g of VAE.

Gels were prepared according to an earlier described[19]. The powdered gum was added to 10 ml of distilled water and stirred properly to allow uniform mixing, and then 1 g each of *V. amygdalina* was added gradually to each gum dispersion and stirred vigorously until homogenous mucilage was produced. The gel was allowed to stand for 24 h for complete hydration.

Animals

Adult albino rats (200-300 g) of both sexes were purchased from the Department of Pharmacology and Toxicology, 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. The animals were divided into five groups of four animals each. Each group received different treatment as follows:

Group 1 received Batch 1 gel;

Group 2 received Batch 2 gel;

Group 3 received Batch 3 gel;

Group 4 received Cicatrin® powder (positive control) while

Group 5 received no medication.

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.

Evaluation of wound healing rate

The animals were anaesthesized with diazepam injection (0.5 ml, *i. p.*) after which their lower back and left flank were clipped with clippers. After thoroughly swabbing with cotton wool soaked in methylated spirit and allowing to dry, full-thickness wounds (2 cm in diameter) were produced by excising the skin and subcutaneous tissue down to the level of the muscle fascia using surgical scissors and forceps. The wounds were immediately dosed with a superficial smear of the gel preparation. Test sample was administered topically to the wounded area to the respective animals every day starting from the day of wound infliction (Day 0). 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 $p < 0.05$.

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TABLE 1 : Effect of *A. esculenta* gel containing *V. amygdalina* leaf extract on wound diameter

Group	Description	Wound healing diameter (cm) on day post surgery[a]									
		Day 0	Day 2	Day 5	Day 7	Day 9	Day 11	Day 12	Day 13	Day 14	Day 15
1	0.5 g AEG +1.0 g VAE	2.00 ± 0.00	1.65 ± 0.05	0.90 ± 0.10	0.65 ± 0.15	0.50 ± 0.25	0.35 ± 0.15	0.15 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2	1.0 g AEG +1.0 g VAE	2.00 ± 0.00	1.70 ± 0.10	1.10 ± 0.10	1.05 ± 0.00	0.85 ± 0.15	0.75 ± 0.15	0.45 ± 0.25	0.20 ± 0.00	0.10 ± 0.00	0.00 ± 0.00
3	1.5 g AEG +1.0 g VAE	2.00 ± 0.00	1.65 ± 0.05	1.10 ± 0.10	0.90 ± 0.00	0.60 ± 0.15	0.45 ± 0.25	0.25 ± 0.05	0.10 ± 0.01	0.05 ± 0.00	0.00 ± 0.00
4	10 g Cicatrin® powder	2.00 ± 0.00	1.75 ± 0.05	1.30 ± 0.10	1.00 ± 0.15	0.70 ± 0.10	0.50 ± 0.10	0.30 ± 0.10	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
5	No medication	2.00 ± 0.00	1.85 ± 0.05	1.15 ± 0.15	1.10 ± 0.10	1.00 ± 0.00	1.00 ± 0.00	0.95 ± 0.05	0.85 ± 0.00	0.80 ± 0.00	0.75 ± 0.05

***a*Wound diameter±SEM, n=3; *Statistically different from Cicatrin® ($p<0.05$);AEG=*A. esculentus* gum; VAE=*V. amygdalina* leaf extract.**

RESULTS AND DISCUSSION

Results of the study shows that in all the formulations of the *A. esculenta* gel and *V. amygdalina* leaf extract, there was a steady decline in the wound diameter with time (TABLE 1). This is also presented in Figure 1 which shows the percentage reduction in the wound diameter with time. Interestingly, there was a complete wound closure in all the three gel formulations of the *V. amygdalina* extracts by the observation period of 15 days. There was however, a very slow reduction in the wound diameter in Group 5 animals which received no medication, suggesting that the extracts and the gum possess wound healing activity. Animals in Group 1 that received 5% of the gel containing 1% of VAE (batch 1 gel) exhibited the fastest wound healing rate as there was a 100% wound closure by the 13th day post wound infliction. The wound closure by batch 1 gel was better than that of Cicatrin® (Group 4), a standard drug for wound dressing, which produced a 100% wound closure by the 14th day. Based on the LSD analysis, there was no significant difference ($p>0.05$) between Groups 1, 2, 3, and 4 in the mean wound diameter on day 2 while on other days there were marked differences in the wound diameter in the various groups. The results suggest that though Group 1 produced the fastest rate of healing while the other

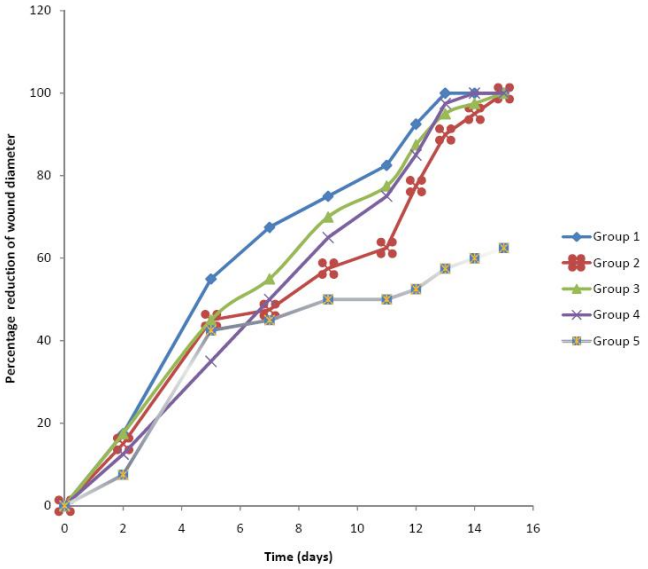

Figure 1: Wound reduction rate in animals treated with *V. amygdalina* leaf extract formulated in *A. esculenta* gels compared with Cicatrin®

formulations of gel and *V. amygdalina* leaf extract had comparable wound healing efficacy similar to Cicatrin®. The gel containing leaf extract may have facilitated chains of haemostatic processes to effect the wound healing. Haemostasis is important for initiation of tissue repair processes proceeds through a cascade of reactions to cause blood coagulation and wound healing[19]. Additionally, the wound healing effect of the formulations could be attributed to the inherent ability of gels to cover wound surface thereby eliminating infection and allow-

ing the natural tissue repair process to take place[19,20].

The wound healing property of *A. esculenta* gel containing *V. amygdalina* leaf extract may be attributed to the phytoconstituents present in the plant, which may be either due to their individual or additive effect that facilitated the process of wound healing. At this stage, it is difficult to say which component(s) of the extracts or the gum were responsible for the wound healing activity. However, compounds such as steroid, glycoside and sesquiterpene lactones like vernodalin which possess antimicrobial properties have been isolated from the *V.amygdalina* plant[21]. The antimicrobial activity of *V. amygdalina*[4] suggests that the leaf extract could facilitate wound healing by preventing infection or eradicating already established infection thereby allowing the natural wound healing process to take place. Wound healing activity of medicinal plants has been associated with their antioxidant properties[19-20, 22-23]. Phytochemical studies are underway in our laboratory to isolate the active components responsible for these pharmacological activities.

CONCLUSION

The results of the present investigation have shown that the leaf extract of *Vernonia amygdalina* formulated as gel with the gum from *Albemoschus esculenta* possessed wound healing effect that was comparable to a standard drug Cicatrin®. This has confirmed the traditional use of *V. amygdalina* leaf extract for wound dressing and sore treatment.

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