Original Research Article

Zanthoxylum species in Uganda: a novel wound healing alternative

ABSTRACT

Aim: The study evaluated the efficacy and possible mechanism of the stem bark of Zanthoxylum species (plant X) used by communities for wound healing in South Western Uganda.

Study design: Experimental controlled

Place and Duration: Departments of Pharmacy, Pharmacology and Pharmaceutical Sciences, Faculty of Medicine, Mbarara University of Science and Technology between August 2016 and February 2017.

Methodology: Excision wounds were humanely made on the bark of healthy albino rats and then randomly divided into four groups i.e Group I (Plant X water extract) n=9, Group 2 (control herbal drug) n=6, Group 3 (distilled water)n=9 and Group 4 (neomcycine antibiotic) n=3. Treatments were applied twice a day for 15 days. The wound areas determined at baseline (day 1), then at day 6 and day 15 for each of the animals in groups 1, 2 and 3. Percentage reduction in wound areas was determined on day 6 and 15 and statistically compared. On day 7 the rats in group 4 and three rats randomly picked by a blinded laboratory technician from groups 1 and 3 were humanely sacrificed for histology examination of wound tissues. Phytochemical analysis of the water extract and effect of solvent were also evaluated.

Results: The Plant X water extract was found to significantly reduce wound areas better than distilled water on day 6 and 15 , (55.93±2.845) Vs (35.06±3.508),p=0.0312 and (93.18±1.721) Vs (74.89±5.604), p=0.0097 , and marginally better than herbal control drug on day 6, (55.93±2.845) Vs (39.55 **±** 6.524) , p=0.0799. Five alkaloids were identified by Nuclear magnetic Resonance and Mass spectrometry in the plant X water extract as possible active compounds in wound healing. No significant difference was observed in the solvent effects.

Conclusion: Plant x shows great potential for stimulation of collagen formation and promoting natural wound healing mechanisms and therefore offers an alternative for wound treatment.

Key words: Zanthoxylum spp, Plant X, Wounds, Alternative.

1.0 INTRODUCTION

Injury is one of the leading causes of death in children and working-aged adults in almost every country and there are more than five million injury-related deaths every year, as well as a tremendous burden of disability [1]. The injury healing process involves a complex series of interactions between different cell types, cytokine mediators, and the next extracellular matrix [2]. It also occurs naturally in four phases namely; hemostasis (coagulation), inflammation, proliferation and remodeling [3]. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelization and

wound contraction. Alterations in any of these steps can lead to healing delay or even the inability to heal completely [4]. Severe injuries lead to formation of visible wounds on the skin or other parts of the body most of which are difficult to heal and even where they are healed, significant scars are left on the affected part of the body.

Almost 25 to 40% of the active components of the synthetic allopathic medicine had origins from higher flowering plants of the world and the clues to discover them came from folklore medicines of various cultures [5, 6]. Some of these plants have immense potential in management of wounds especially for people living in resource limited nations [7]. Despite deliberate efforts to treat wounds, some specific ones due to influence of some disease processes like diabetes mellitus, HIV and varicose ulcers among others have been unhealable and have continued to be entry point of disease causing organisms that can eventually lead to amputation or death[8]. According to **S**asidharan et al., (2010) nearly 6 million people suffer from chronic wounds worldwide and the prevalence of chronic wounds in the community was reported as 4.5 per 1000 population, whereas that of acute wounds was nearly double, at 10.5 per 1,000 population [9]. In an effort to address this challenge, interventions like stem cell treatment have been considered but this is too expensive for the ordinary patients especially in developing countries like Uganda. Also administration of oral and topical antibiotics has been other options but is rarely successful in treating non-healing wounds [10]. In a bid to find a sustainable therapy, locals of Budibugyo (South Western Uganda) have discovered the usefulness of Plant X stem bark powder (suspected to be a Zanthoxylum spp, Rutaceae) in management of both acute and chronic wounds. This present research work aimed at validating the wound healing activity of this Ugandan medicinal plant so as to establish a scientific evidence for the observed community use of the plant to treat non healing wounds of various causes.

2.0 MATERIALS AND METHODS

2.1 Plant Material

The fresh stem bark of Plant X were supplied by the herbalist under Medical Research Center, Wandegeya Kampala and received at Mbarara University Pharmaceutical Analysis Laboratory. The plant material were washed, shade dried and crushed in to a course powder using electric grinder. It was then stored in a well closed and dry container at room temperature till use in the experiment.

2.2 Preparation of the Extracts

A portion (500g) of the raw powder was hot macerated using distilled water. The mixture was filtered using a muslin cloth followed by Whatman's filter papers. The filtrate was evaporated using rotary evaporator (RV 10 D S99) at 40°C and low pressures followed by oven drying at 50°C to obtain a constant weight extract. The phytochemical groups were analyzed using the methods previously described [11]. The remaining raw powder portion (500g) was serially extracted as follows: It was first subjected to Petroleum ether using Soxhlet apparatus, the resulting residue was cold macerated in ethanol (96%) and finally hot maceration in distilled water. The different filtrates were treated as for water extract to obtain dry fractions of petroleum ether, ethanol and water.

2.3 Preparation of the treatments and controls

A 5% extract solution of each extract was made by dissolving 5g in 100mls of its extraction solvent for application on the excision wounds. A herbal drug for wound treatment on the Ugandan market used a positive control was previously tested for wound healing while distilled water served as a blank control in wound treatment phase[12].

2.4 Creation of wounds and application of treatments

Fifty seven (57) inbred Wistar albino rats (150 – 200g) of either sex and of approximately the same age were obtained from the same colony at the animal research facility of department of Pharmacology, Mbarara University of Science and Technology. They were housed in clean cages with access to clean water and standard laboratory pellet diet *ad libtum* throughout the experimentation period as per National Institutes of Health (NIH) guidelines for animal handling in teaching and research.

Excision wounds were created on the barks of rats after shaving and application of analgesia and local anesthesia using diclofenac, lignocaine and adrenaline by injection into the site for wound excision [13]. The animals were then randomly picked without replacement by a blinded Laboratory Technician and placed into groups: group 1 (n=9) for plant X aqueous extract, group 2 (n=6) for the herbal wound healing drug, group 3 (n=9) for the distilled water, group 4 (n=3) for the neomycine group, group 5 (n=10) for the petroleum fraction, group 6 (n=10) for the ethanol fraction and group 7 (n=10) for the distilled water fraction group. The fresh wounds were left for 24hours before starting the topical applications of the treatments twice a day for 15 days. The wound diameters were measured using a digital Vernier caliper in

diagonal way as 'a' and 'b' which were used to determine wound area (mm ²) using the formula (π a*b)/4 for each animal at baseline and then at day 6 and day 15. Wound contraction was calculated as percentage of the reduction in wound area of the day of measurement from the baseline value i.e. Percentage of wound contraction = [(Initial wound area – Specific day wound area) / Initial wound area] x 100 equation [6]. A sample of plant X powder was sent to department of Chemistry, Wits University South Africa for active compound identification using Nuclear Magnetic Resonance and Mass spectroscopy.

2.5 Statistical Analysis

The data obtained was analyzed using GraphPad Prism software version 7.03. One way ANOVA was used for determining the statistical significant difference in the group means. The inter group significance was analyzed using Turkey's multiple comparison test and a *P* value < .05 was considered to be statistically significant. All the values are presented as Mean ± SEM with their corresponding P values.

3.0 RESULTS AND DISCUSSION

The crude aqueous extract revealed the presence of various phytochemical groups with alkaloids being abundant as shown in table I.

Table I: Phytochemical groups identified in the crude aqueous stembark extract of Plant X.

Phytochemical group	Presence	Phytochemical group	Presence
Terpenoids	+	Saponins	+
Tannins	+	Anthroquinone glycosides	+
Flavonoids	-	Alkaloids	++
Amino acids	+	Phenols	+
Glycosides	+	Steroids	+

Presence (+) Absent (-) Abundant (++)

Plant X crude water extract demonstrated better wound size reduction effect than the control treatments (Table 2).

Table 2: Percentage wound reduction effect of Plant X compared with controls.

	Percentage Mean ± SEM, n=6		
Time	**Distilled water**	**Plant X. extract**	**P-values**
Day 6	35.06 **±** 3.508	55.93 **±** 2.845	0.0312*
Day 15	74.89 **±** 5.604	93.18 **±** 1.721	0.0097**
	Distilled water	**Control herbal drug**	
Day 6	35.06 **±** 3.508	39.55 **±** 6.524	0.7950

Day 15	74.89 ± 5.604	86.75 ± 2.498	0.0784	112
	Plant X extract	**Control herbal drug**		
Day 6	55.93 ± 2.845	39.55 ± 6.524	0.0799	
Day 15	93.18 ± 1.721	86.75 ± 2.498	0.4228	

There was no statistical significant difference between the healing effects produced by ethanol, petroleum ether, ethanol and aqueous fractions (Table 3).

Table 3: Comparison of wound area reduction effect of plant X extracted in various solvents

	% wound reduction (mean ± SEM, N=10)		
	Petroleum ether fraction	**Ethanol fraction**	P-value
Day 6	13.03 ±1.988	1.675 ± 6.18	0.5434
Day 15	70.7 ± 5.579	56.09 ± 3.893	0.3274
	P. ether fraction	**Aq. fraction**	
Day 6	13.03 ±1.988	11.76 ± 10.82	0.9926
Day 15	70.7 ± 5.579	57.03 ± 9.794	0.3742
	Ethanol fraction	**Aq. fraction**	
Day 6	1.675 ± 6.18	11.76 ± 10.82	0.5974
Day 15	56.09 ± 3.893	57.03 ± 9.794	0.9948

Histology analysis revealed that Plant X stimulated collagen formation more than the control treatments (neomycin or water) and the wound tissue had fewer inflammatory cells indicative of better healing effects and possible anti-inflammatory effects (Figure 1-3).

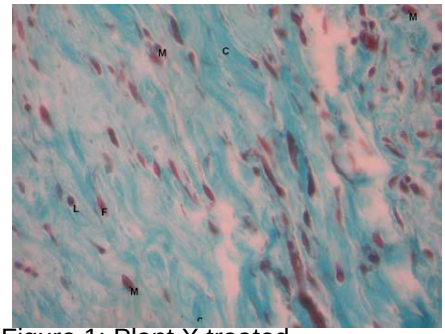

Figure 1: Plant X treated

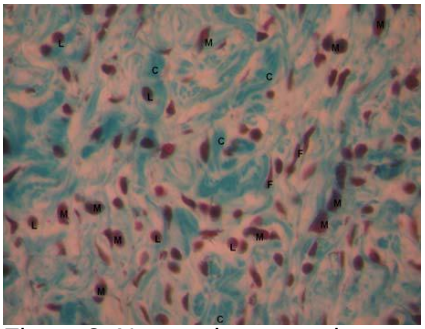

Figure 2: Neomycine treated

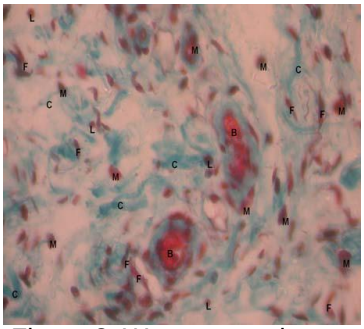

Figure 3: Water treated

M= Macrophages; L= Lymphocytes: C= Collagen fibers: F= Fibroblasts; B=Blood vessel

Five previously known alkaloids were detected in the plant X samples (Figures 4 to 8).

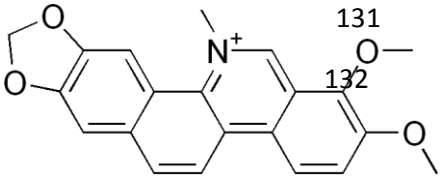

Figure 4: Chelerythrine

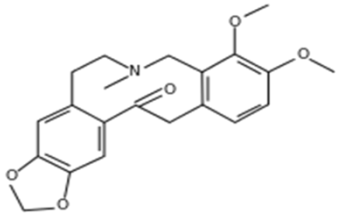

Figure 5: Fagarine

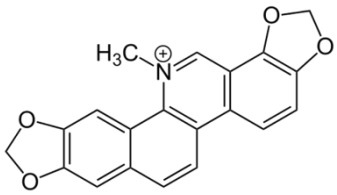

Figure 6: Sanquinarine

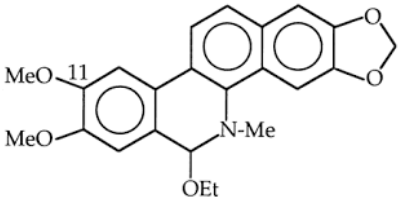

Figure 7: Dihydrionitidine

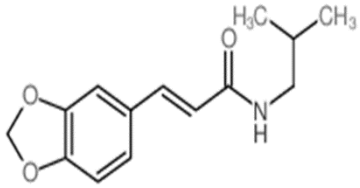

Figure 8: Trans fagaramide

4.0 DISCUSSION

Wound healing is a complex process involving a series of physiological and biochemical changes, but these steps can be shortened by herbs which possess antiseptic, antioxidant and anti-inflammatory activities [14]. Our findings indicate that the aqueous extract significantly had better wound healing effect than the herbal drug on the market by day 6. This could be attributed to the phytochemical groups like phenols and alkaloids previously implicated in wound healing [15] and identified in the plant X extract. According to Sunita et al., (2017), the wound healing potential of natural phytomedicines can be explained by the presence of saponins (anti-oxidant and antimicrobial activity), tannins (antimicrobial) and triterpenoids (promotes wound contraction and the rate of epithelization)[16] and these group of compounds were present in the plant X extract (table 1). Five alkaloids (Figures 4-8) were also identified in the aqueous extract and some of these compounds are known to have unique effects important for wound healing particularly Sanquinarine is a tissue regenerator with anti-inflammatory effects [17, 18]. Chelerythrine has antimicrobial and antitumor properties [19].

The histology pictures (Figures 1, 2 and 3) indicate significant differences in collagen formation (blue color) with the plant X extract having the highest accumulation compared to the neomycin and water

treated groups. This could be attributed to amino acids in the plant extract (Table 1) as source of hydroxy proline which is a constituent of collagen [20] or it could due to other compounds in the extract causing induction of collagen formation by a mechanism that remains to be established. The low levels of infiltration by inflammation promoting cells in the histology picture of Plant X treated wounds indicates the possible anti-inflammatory effects of the plant X. The histology also shows abundant tissues of well-organized building materials like fibroblasts, grand substance and fibrous tissue associated with excessive angiogenesis all seen in the plant X extract group indicating that plant X appears to accelerate the natural wound mechanism. This is further demonstrated by the shorter time taken for the wounds to heal again in plant X treated groups (Table 2).

5.0 CONCLUSION

Plant x shows great potential for use in stimulation of collagen formation, shortening wound healing time and promoting natural wound healing mechanisms that mimic stem cell stimulation. This mechanism offers great hope for a cheaper alternative for healing of difficult to heal wounds and needs further exploration for possible development into a drug for wider clinical application as a low cost alternative.

ETHICAL APPROVAL

The Ethical clearance TREC007/17 was obtained from THETA Uganda Research Ethics Committee, accredited by Uganda National Council for Science and Technology with a focus on traditional medicine research approval and the study was conducted in accordance with the national and international institutional rules concerning animal experiments and biodiversity rights. The experimental animals were humanely treated throughout the study and at the end of the study were sacrificed under general anesthesia and incinerated.

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