



# Assessing the wound healing effects of *Erythrina abyssinica* crude aqueous stem bark extract on mechanically induced wounds in wistar rats

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## Abstract

Wounds tend to affect a large proportion of the population both in developing and developed countries. They can result from accidents both at work and home but most commonly road accidents contribute a large percentage of road accidents. Wounds can impact on the victim's social life and as well as reducing the productivity of the affected individuals, hence reducing the work output. However, little attention is always given to wounds and their impact in society. Wounds can be open or closed depending on the kind and extent of injury. Therefore the above study sought to find a cheaper and readily available alternative to wound healing especially for the local communities who may find it hard to access or even afford modern alternatives for wound management. In this study, the aqueous stem bark extract of *Erythrina abyssinica* was used for phytochemical analysis, assessing the wound healing effects and antibacterial properties. The wounds were induced by cutting off a circular part of full thickness from the skin on the dorsal lateral region of each rat by using a sterile surgical blade and the diameter of each wound was measured using a Vanier caliper. The extract and the standard drug for wounds (povidone iodine ointment) were applied topically on the wounds for 9 days

and the percentage wound contraction was determined by measuring with a vanier caliper. A negative control of the animals which were induced with wounds but received no intervention was also used for comparison with the results from the standard control. The antibacterial effects of the extract against *S. aureus* and *E. coli* was also determined on the same wounds using the same aqueous extract.

The results showed that the percentage wound contraction achieved for *Erythrina abyssinica* extract was  $75.5\pm 2.2\%$ ,  $48.6\pm 6.0\%$  for Povidone iodine and  $29.4\pm 2.6\%$  in the untreated wounds. For in vivo antimicrobial activity, Povidone showed higher activity than *Erythrina abyssinica* crude aqueous extract. It was therefore concluded that the aqueous crude extract of *Erythrina abyssinica* was better than the standard drug on wound healing but for antimicrobial effects, the standard drug for wound healing (Povidone) showed better results than the extract. Therefore in conclusion, povidone and *Erythrina abyssinica*, extract can be used concomitantly for wound healing since most of the wounds tend to be septic and infected by bacteria. The combination can provide additive effects on wounds resulting in rapid wound healing rates.

**Key words:** Wounds, Herbal medicine, *Erythrina abyssinica*, Healing, Wistar rats

## Introduction

A wound is defined as a disruption to the continuity of the normal epithelial lining of the skin or mucosa which can either be acute or chronic depending on the etiology (Dorai, 2012). Furthermore, the skin may be torn, cut or punctured by the piercing or cutting object which is commonly termed as an open wound, or in some other cases, a force from a blunt object may cause injury to the body but does not cause an opening to the body which is termed as closed wound. Acute wounds are commonly caused by accidents like trauma or burns and normally heal in a short time given the right treatment or even sometimes they heal on their own without any intervention depending on the extent of damage. While chronic wounds on the other hand take a longer time to heal and can even recur due to the underlying pathology like diabetes (Dorai, 2012). Recent estimates indicate that close to 6 million people most of which are children and rural dwellers tend to experience chronic and open wounds worldwide (Tan *et al.*, 2012). Wounds can be a source of infection and this infection prolongs the recovery of injured patients and causes progression of wounds from acute to chronic and septic wounds. This may result into organ failure of the affected patients which may eventually lead to death of the patients (Dimitrai & Tesseromati, 2013).

Since most of the wounds especially the open wounds tend to occur in the poor communities especially among the hunter-gatherer communities, grazers and diggers, these people usually tend to use medicinal plants since they are always readily available in case of such injuries instead of the modern drugs which may not be readily available or are usually expensive.

Herbal preparations from medicinal plants have been used since time immemorial for the management of wounds in humans and different species of animals and have proven to be effective in the treatment of various external wound infections. However, there is limited experimental evidence regarding the efficacy and safety of some of these plants in wound infections (Mummed *et al.*, 2018).

Some of the plant species which have been proven to have wound healing ability include: *Ocimum kilim* and *Ocimum scharicum*, *Rubia cordifolia*, *Tephrosia purpurea*, *Sphaeranthus indicus*, *Allium cepa*, *Alternanthera brasiliana*, *Cantharanthus roseus* and *Carica papaya*, among others (Alam & Singh, 2014). *Erythrina abyssinica* was mentioned by Bushenyi Medical and Traditional Healers Association (BUMETHA) in Bushenyi district, Western Uganda to have wound healing properties but with no experimental evidence. *Erythrina abyssinica* is a common plant in sub-Saharan Africa where it is used in the management of inflammation, gonorrhoea, wounds, stomach problems, diarrhea and viral infections (Nkeh-chungag *et al.*, 2013). Some of the medicinal plant species like as *Erythrina* species are usually employed in the management of various bacterial infections especially on wounds that have become septic (Lagu & I.B., 2012). The plant was found to contain phytochemical constituents like alkaloids, tannins, saponins and flavonoids among others that tend to possess a wide range of effects such as antimicrobial effects, anti-inflammatory effects, antioxidant effects, antipruritic effects, antihypertensive effects, hypoglycemic effects, and analgesic effects which always play an essential role in the treatment and healing of wounds (Kone *et al.*, 2011).

The people of Bushenyi district western Uganda reported using herbal remedies like *Bidens pilosa*, and *Erythrina abyssinica* among others for the management of wounds. The major driver for the use of these herbal products could be poverty and inaccessibility of modern drugs. According to the 66<sup>th</sup> edition of the British National Formulary, the way a wound is treated will depend on the type of wound, whether closed or open wound and at which stage of healing process the wound has reached.

The colonization of wounds by microorganisms especially bacteria significantly leads to wounds becoming septic and greatly delays the healing process (Bessa *et al.*, 2015). Antibiotic drug resistance in skin wound infections is a growing concern in wound management. Therefore there is need for alternative therapy to curb the increased antibiotic resistance (Jacobsen *et al.*, 2011). It is believed that the cell walls of gram positive bacteria are easily penetratable by plant extracts hence they are more susceptible to plant extracts as compared to gram negative bacteria whose cell walls tend to resist penetration by plant extracts. Therefore plant species like *Erythrina abyssinica* which has been shown to have anti-multidrug-resistance activity against various bacterial species can be used as a very good agent in the fight against MDR-bacterial infections especially in sub-Saharan Africa (Kone *et al.*, 2011).

The prevalence of wound infection globally, ranges from 5% to 34% (Hemant, 2018). In Africa, the prevalence of infection most especially on surgical wound sites can be classified to range from 6.5% to 20.2% in clean wounds, 10.1% to 23.8% in clean-contaminated wounds, 13.3% to 51.9% in contaminated wounds and 44.1% to 83.3% in dirty wounds (Nejad *et al.*, 2011). In Mbarara, Western Uganda, the incidence of surgical site infection was estimated to be 16.4% according to the study carried out in 2017 (Lubega *et al.*, 2017). The data regarding wounds and wound incidences in Bushenyi district (The study area) could not be readily obtained from secondary sources since there was no published data regarding the above. Therefore, the above wound incidence was the driver for trying to get a better and cheaper alternative to modern drugs regarding wound healing, hence the choice to study the effects of *Erythrina abyssinica* on wound healing and also its antimicrobial properties.

The results from the study revealed that the aqueous crude bark extract of *Erythrina abyssinica* contained wound healing effects as well as antibacterial effects against *E coli* and *S aureus*.

## Materials and Methods

### Plant collection, identification and drying

*Erythrina Abyssinia* stem bark was collected from Rukararwe (BUMETHA) botanical garden in Bushenyi district which lies at a latitude:  $-0^{\circ} 32' 24.6''$  and longitude:  $30^{\circ} 11' 25.93''$  in Bushenyi district.

The stem bark was then transported to the KIU-WC pharmacology laboratory and dried under the shade to avoid degradation of phytochemical constituents and occasionally turned to avoid fermentation and rotting for a period of two weeks. After that, drying was done under direct sun light until the samples dried completely. During drying exercise, the material was repeatedly weighed until constant weight was attained, which proved complete dryness. The dried samples were then crushed to obtain powder using a mortar and pestle, then with an electric grinder to obtain the fine powder. The fine powder was then weighed using an analytical scale and kept in air tight container prior to use. The plant was also identified by an authentic Taxonomist from Mbarara University of Science and Technology and a voucher specimen was deposited in the herbarium of Mbarara University of Science and Technology.

### Sample processing

After the materials were completely dry, they were ground to make a powder. Grinding was done using a mortar and pestle and then using an electric grinder to obtain the fine powder. After pulverizing, the powder was stored in air tight containers prior to use. The aqueous extract was prepared by boiling 100g of the powder in 500ml of water in a round bottomed flask using an electro mantle for 1 hour. The mixture was then allowed to cool and filtered using a clean white piece of cloth, and then with a Whatman filter papers to generate a very fine filtrate. The filtrate obtained was then concentrated using the water bath at  $50^{\circ}\text{C}$  to form a 12.153g semi-solid paste

which was used for the wound healing effects and antibacterial effects. The other fine solution was used for phytochemical analysis.

### **Experimental animals**

A total of 15 male young adult albino wistar rats (*Ratus norvigicus*) were used for the study. The rats were purchased from the Animals house of pharmacology department from Kampala International University western campus. The animals were acclimatized to the rat cages in the pharmacology laboratory for one week prior to the study. These rats were eight weeks old and weighing between 100 to 150gm, this age was selected because it was more likely to respond to the wound healing and treatment more than the very young or vey old ones. Using randomization, the rats were assigned to three different groups each comprising of 5 rats. The first group was used for positive control and the animals were dosed with the standard drug, the second group was used for negative control where animals received no intervention or treatment and the third group was the treatment group which received the extract.

### **Inclusion and exclusion criteria**

All animals which were male, 8 weeks old and weighing between 100 to 150g were included in the study. All animals which met the above criteria and were not diseased were also included.

All animals which were younger than 8 weeks and older than eight weeks of age were excluded from the study. Also animals weighing less than 100 and more than 150g were excluded from the study. Female animals and those which were diseased were also excluded.

### **Induction of wounds**

The wounds were induced by first shaving the mid-dorsal region of each rat using a sterile clipper, the shaved area was then disinfected using 0.3% w/v Chlorhexidine gluconate. After shaving and disinfecting the shaved area, a circular piece of full skin thickness was cut off from shaved area on the dorsal lateral region of each rat using a sterile surgical blade. Bleeding from the induced wounds was stopped by cleaning using cotton wool and the initial transverse and longitudinal diameter of each wound was measured using a Vanier caliper after 12 hrs. The wounds had the average diameter of 3mm.

Prior to shaving and induction of wounds, all the rats in each group were anesthetized using diethyl ether and diclofenac (100mg/kg) used as an analgesic to prevent the animals from feeling the pain during induction of wounds.

### **Treatment of wounds**

After wound induction, the animals in the groups 1 and 2 were treated with povidone iodine and the extract to act as a positive control and treatment respectively. The two treatment interventions (standard drug and the extract) were applied topically on the wounds making sure that the entire surface of the wound area is covered.

This was done twice daily for 9 days. The third group of animals received no treatment and it was used as a positive control.

The rate at which wounds were contracting in percentage was determined using the formula: Wound contraction percentage = [(original wound area - unhealed area)/original wound area] × 100%.

Treatment stopped on day nine but the animals were observed and monitored until complete healing was attained. This occurred at 19 days for the extract, 23days for the standard drug/ positive control and 30days for the positive control when complete healing was observed.

For the microbiological aspect, 9 samples were obtained from 3 animals per group from which swabs were taken at days 0, 3, 6 and the method of micro-dilution was employed in the study of both bactericidal and inhibitory effects of the extract against the two pathogens of *S. aureus* and *E. coli* that were identified in the samples under light microscope used after gram staining. After swabbing the swabs were inserted into peptone water, and then transported aseptically to the Microbiology laboratory of Kampala international university western campus. -3 dilution was made from which 1000µl were inoculated on MSA (for *Staphylococcus aureus*) and VRBA (for *Escherichia coli*) by surface spreading then incubated for 24 hours (Hussein *et al.*, 2012). It was observed that the *Erythrina abyssinica* aqueous stem bark extract inhibited the growth of bacterial colonies for both *Escherichia coli* and *Staphylococcus aureus* indicating its antibacterial effects.

### Phytochemical screening

The plant extract was screened for phytochemicals like tannins, alkaloids, anthraquinones, flavonoids, saponins, reducing sugars, anthracenocides, flavonoids, coumarins, phenols and steroid glycosides among others using the methods described by Trease and Evans, 1996.

### Data collection and storage

A data collection sheet was used in the collection of wound longitudinal and transverse diameters on days 0, 3, 6 and 9 of treatment and microbial count on the wounds on days 0,3 and 6 (Kyakulaga *et al.*, 2011).

The gathered data were recorded in a laboratory book, stored on both computer hard disk and also on a compact disk, which were kept in the Pharmacology Department of Kampala International University-Western Campus

### Data analysis and presentation

The surface areas of the wounds for all the used rats were calculated on days 0, 3, 6 and 9 of treatment and these were used to calculate the percentage at which the wounds were closing. Data was expressed as Mean±SEM and analyzed using STATAv15 software followed by t-test at 95% level of confidence interval which was used to compare the means and standard deviations of treated groups to that of controls and p values of ≤0.05 considered significant (Kyakulaga *et al.*, 2011)

### Ethical considerations

Consent to use the animals was sought from the animals ethics committee of KIU-WC and the experimental procedure was carried out according to the guidelines which are spelt out by the Institutional Animal Care and Use Committee (IACUC) and ethical guidelines for investigation of experimental pain in conscious animals (Brown & Smiler, 2012). The experimental animals were handled with care in accordance to the guidelines for the care and handling of laboratory animals which are in accordance with the guidelines for laboratory biosafety, 2004 (Kyakulaga *et al.*, 2011).

### Quality control

Measures to ensure quality of the results were adhered to so as to achieve the best results. These included following:

1. The regulations and principles of laboratory safety, like putting on protective gear and avoiding unnecessary injuries
2. Recording all the events of the study and storing them safely on a daily basis
3. Strictly using analytical grade chemicals and reagents
4. All the laboratory equipment were disinfected using absolute ethanol at the beginning and end of each experimental procedure to avoid contamination.
5. All experimental animals were given food and water *adlibitum* throughout the experimental period

### Results

**Table 1**

A table showing results for Phytochemical analysis

TEST	RESULTS
TERPENOIDS	+
TANNINS	+
FLAVONES	+
PHENOLS	+
ALKALOIDS	+

From the above table, the results show that terpenoids, tannins, flavones and phenols plus alkaloids were present but in different amounts while others like starch and reducing sugars were absent. The presence and absence of some phytochemical constituents was attributed to the plant part which was harvested since different plant parts tend to contain different chemical constituents or the same phytochemicals but in different amounts and concentrations. The other factor could be the method and the solvent used for extraction because different extraction solvents tend to extract different chemical compounds, hence water could have only extracted the polar phytochemicals only and left out the non-polar solvents. Another factor could be the place where the plant material was harvested from. This is because, soil type and topography also influence the amount and type of phytochemicals found in a given plant material.

**Table 2**

**A table showing the Longitudinal diameter analysis 1**

Day of measurement	Mean $\pm$ SEM (mm)			P value	
	Group 1 (n=5)	Group 2 (n=5)	Group 3 (n=5)	Interday	Intergroup
<b>Day 0 (baseline)</b>	18.76 $\pm$ 3.25	14.84 $\pm$ 0.94	16.45 $\pm$ 1.15		0.5434
<b>Day 3</b>	16.18 $\pm$ 2.64	12.11 $\pm$ 1.20	15.75 $\pm$ 1.07	<b>0.0002</b>	0.2484
<b>Day 6</b>	14.85 $\pm$ 2.67	9.81 $\pm$ 0.90	15.10 $\pm$ 1.06	<b>&lt;0.0001</b>	0.0905
<b>Day 9</b>	13.86 $\pm$ 2.68	7.30 $\pm$ 0.46*	13.95 $\pm$ 1.14	<b>&lt;0.0001</b>	<b>0.0262</b>

\*this shows a significantly different mean

**Table 3**

**A table of transverse diameter analysis 1**

Day of measurement	Mean $\pm$ SEM (mm)			P value	
	Group 1 (n=5)	Group 2 (n=5)	Group 3 (n=5)	Interday	Intergroup
<b>Day 0 (baseline)</b>	16.66 $\pm$ 2.22	13.15 $\pm$ 0.45	15.39 $\pm$ 0.58		0.0652
<b>Day 3</b>	15.04 $\pm$ 2.36	10.76 $\pm$ 0.45*	14.51 $\pm$ 0.62	<b>&lt;0.0001</b>	<b>0.0299</b>
<b>Day 6</b>	13.43 $\pm$ 2.50	8.66 $\pm$ 0.39	13.52 $\pm$ 0.72	<b>&lt;0.0001</b>	0.0697
<b>Day 9</b>	12.08 $\pm$ 2.65	6.40 $\pm$ 0.25*	12.83 $\pm$ 0.70	<b>&lt;0.0001</b>	<b>0.0293</b>

\*this shows a significant mean



**Table 4****A table showing wound contraction results***Table of analysis of change in wound areas with time1*

Day of measurement	Wound area, Mean $\pm$ SEM (mm <sup>2</sup> )			P value	
	Group 1 (n=5)	Group 2 (n=5)	Group 3 (n=5)	Interday	Intergroup
<b>Day 0</b>	268.18 $\pm$ 83.99	154.98 $\pm$ 13.72	201.20 $\pm$ 21.05		0.3263
<b>Day 3</b>	209.48 $\pm$ 64.80	104.77 $\pm$ 14.66	181.66 $\pm$ 18.8	<b>0.0002</b>	0.0805
<b>Day 6</b>	176.15 $\pm$ 62.33	68.23 $\pm$ 9.00	162.86 $\pm$ 18.10	<b>&lt;0.0001</b>	<b>0.0299</b>
<b>Day 9</b>	152.91 $\pm$ 59.92	37.60 $\pm$ 3.70	143.10 $\pm$ 18.36	<b>&lt;0.0001</b>	<b>0.0178</b>

*Table of Percentage wound contraction 1*

Experimental group	Mean $\pm$ SEM		
	Original wound area (mm <sup>2</sup> )	Unhealed wound area (mm <sup>2</sup> )	Wound contraction (%)
<b>Group 1</b>	268.18 $\pm$ 84.21	152.91 $\pm$ 59.92	48.6 $\pm$ 6.0
<b>Group 2</b>	154.98 $\pm$ 13.75	37.60 $\pm$ 3.70	75.5 $\pm$ 2.2
<b>Group 3</b>	201.20 $\pm$ 21.05	143.10 $\pm$ 18.36	29.4 $\pm$ 2.6

Mean wound area at the beginning and end of the experiment for the different groups with the corresponding percentage contraction.

**The antimicrobial effect; microbial enumeration***On Staphylococcus aureus**Table for analysis of S. aureus count 1*

Day of measurement	Mean $\pm$ SEM (CFU/ml)			P value	
	Group 3 (n=3)	Group 2 (n=3)	Group 1 (n=3)	Interday	Intergroup
<b>Day 1</b>	U.C	U.C	U.C		
<b>Day 3</b>	U.C	122.7 $\pm$ 37.8	60.3 $\pm$ 9.0		0.5653
<b>Day 6</b>	U.C	103.7 $\pm$ 15.2	15.7 $\pm$ 1.2	<b>0.0208</b>	<b>0.0078</b>

U.C; Uncountable and NA; Non applicable

On *E. coli*

Table 4.7 analysis of *E. coli* count 1

Day of measurement	Mean ± SEM (CFU/ml)			P value	
	Group 3 (n=3)	Group 2 (n=3)	Group 1 (n=3)	Interday	intergroup
Day 1	U.C	U.C	U.C		
Day 3	U.C	202.0±3.1	13.0±1.2		<b>0.0043</b>
Day 6	U.C	47.1±26.3	1±0.6	<b>0.0182</b>	<b>0.0007</b>

Morbidity rate due to wounds is high worldwide and studies have shown that about one million wound patients die every year worldwide and of which at least 10,000 patients die due to microbial infections of wounds (Wong *et al.*, 2013). This finding prompted our study to find an alternative to the problem of wounds and wound infections both locally and internationally.

In this study, an aqueous crude extract of *Erythrina abyssinica* which was obtained from Rukararwe Botanical Garden was tested for its wound healing capabilities including antimicrobial activity in vivo against a standard Povidone iodine 10% and a negative control with no treatment.

A total of 15 wistar rats were used with 5 animals per group and 3 animals within each group used to determine in vivo antimicrobial activity.

According to the different treatment choices used, *Erythrina abyssinica* crude aqueous stem extract exhibited a much greater percentage of wound healing and scar formation, followed by Povidone iodine and then lastly the negative control which exhibited the slowest rate of wound contraction.

At the end of the study, animals treated with *Erythrina abyssinica* extract showed a significantly different Mean±SEM in longitudinal and transverse wound diameters (7.30±0.46mm and 6.40±0.25mm) compared to those treated with Povidone iodine 10% (13.86±2.68mm and 12.08±2.56mm) and the untreated animal wounds (13.95±1.14mm and 12.83±2.56mm). P values obtained on the last day (day 9) were 0.0262 and <0.0001 intergroup and inter day respectively for longitudinal wound diameter, for transverse, 0.0293 and <0.0001 intergroup and inter-day respectively showing that there was significant difference in the diameters as per different groups but also significant reduction of these wound diameters with time determined by the inter-day P values.

The wound areas at the different days of measurement i.e. 3, 6 and 9 were compared to the baseline (day 0) and significant P values were obtained showing that the areas were different and reducing every time the

measurement was done. This implied that there was wound reduction in all groups but difference in the rates as per the different groups.

The percentage of wound contraction capacity was obtained by the use of the initial wound area and final wound area. The percentage wound contraction achieved for *Erythrina abyssinica* extract was  $75.5 \pm 2.2\%$ ,  $48.6 \pm 6.0\%$  for Povidone iodine and  $29.4 \pm 2.6\%$  for the untreated wounds (negative control).

As for in vivo antimicrobial activity, Povidone showed more activity than *Erythrina abyssinica* crude aqueous extract. The microbial count of the untreated group were uncountable throughout the investigation. *E. coli* was more susceptible to the treatment than *S. aureus*.

The P values obtained for the antimicrobial activity against *S. aureus* showed that there was significant difference in the microbial count between the animals treated with the extract and those with Povidone iodine (intergroup) and on different days (Interday). Lesser counts with Povidone iodine  $60.3 \pm 9.0$  than with the extract  $103.7 \pm 15.2$  with a P value of 0.0078 on the final day and significant decrease in the counts with time obtaining a P value of 0.0208.

Antimicrobial activity against *E. coli* also showed significant difference in the microbial count between the animals treated with the extract and those with Povidone iodine (intergroup) and on different days (Interday). Lesser counts with Povidone iodine  $1 \pm 0.6$  than with the extract  $47.1 \pm 26.3$  with a P value of 0.0007 on the final day and significant decrease in the counts with time obtaining a P value of 0.0182.

According to the experiment, the extract's possible mechanism of action is epithelization, a process where keratinocytes, a major cellular component of the epidermis restore the epidermis after injury (Pastar *et al.*, 2014).

The rapid wound healing effects exhibited by the extract from the study plant could also be attributed to its ability to inhibit the development of the two bacteria, *Escherichia coli* and *Staphylococcus aureus*. The two species of bacteria were selected because research shows that these two species of bacteria are the commonest in wounds. The group which was treated with the extract also exhibited better wound healing properties more than the standard drug and this was attributed to the fact that there were various chemical constituents present in the single extract which could have worked synergistically to produce the better results which were observed in the above experiment. Yet the standard drug may be containing a single compound which may not be very strong, hence the observed slow wound healing rate.

## Conclusion

The results from the study showed that an aqueous crude stem extract of *Erythrina abyssinica* is an effective therapy for the treatment of wounds and would work best used in combination with an antibiotic especially in

chronic and septic wounds. This is because the extract was less effective in vivo on microbes *S. aureus* and *E. coli*, the commonest bacteria species mostly found in wounds.

## Recommendations

More studies should be done on *Erythrina abyssinica* to determine its safety profile especially the LD50. Other toxicity studies should also be carried out on the same plant part especially the chronic toxicity studies. This is because a compound may appear to be safe in a short run yet toxic on a chronic basis.

More phytochemical screening should be carried out using different extraction solvents to isolate the specific constituents which are responsible for wound healing and also those phytochemicals responsible for antibacterial effects.

More ethnopharmacological studies should be done on plants with promising medicinal properties against wounds so as to widen the medicinal base and provide cost effective alternatives for wound healing to patients.

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