Excision wounds healing activity of *Centella Asiatica* (Gotukola) extract on laboratory rats

Mgonja Frida^{1*}, Lusekelo Mwangengwa¹ and Mohamed Ally¹

¹College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture P.O. Box 3015 Morogoro, Tanzania.

Abstract

Background: Skin wound cases are increasing in hospitals requiring efficient treatment. The dependence on antimicrobial has been expensive and sometimes less effective hence requiring alternatives. Medicinal herbs with wound healing properties could be among the alternatives.

Methods: The current study assessed the wound healing efficacy of *Centella sciatica* (Gotukola leaves) ethanolic extract using Laboratory rats as a model. A total of 32 animals were divided into 8 groups (n=4). G1 (Control group (nothing), G2 (Dexamethasone + 1% Gotukola extract), G3 (0.1% Gotukola extract), G4 (0.5% Gotukola extract), G5 (1% Gotukola extract), G6 (Grounded fresh Leaves of Gotukola), and G7 (Silver sulphadiazine). Excision wounds were made on the skin. The plant extract solution was applied to the wound and results were observed on days 3, 7, and 10. Assessed parameters included wound contraction percentages, wound epithelialization time, duration taken for complete wound healing, and gross appearance of wounds.

Results: wounds treated with 1 or 0.5 percentages of Gatukola leaf extract had relatively higher contraction percentages, shorter epithelialization time, and shorter duration for complete healing compared to wounds of rats treated with the lower concentration of the extract and those of the control rats. Visual assessment of excision wounds in the current study revealed corroborative results in that wounds of rats under Gotukola extract at 1% and 0.5 appeared to be recovering faster similar to that of the positive control compared to the wounds treated with a lower concentration of the extract, leaf juice, dexamethasone incorporated extracts and wounds of the negative control.

Conclusion: The extracts of *C*. *Asiatica* at the dosage of 1 or 0.5 %, promoted wound healing at a rate similar to that of the conventional silver sulphadiazine suggesting its potential use in wound management.

Keywords: Excision Wounds, C. Asiatica, Gotukola extracts, silver sulphadiazine, Wistar rats, Healing.

Corresponding author, Mgonja Frida: fmgonja@gmail.com

Introduction

Skin wounds are among the most prevalent disease cases presented routinely in health facilities (Sohal and Moshy, 2019, da Rosa et al., 2017). It is reported from some studies that, the most common wound cases presented in hospitals include laceration wounds, dehiscence of old wounds, surgical wound complications (Zabaglo and Sharman, 2021), puncture wounds (Baldwin and Colbourne, 1999) and fistulas, and other miscellaneous wounds (Lyimo and Mosha, 2019).

Wounds management in patients is a vital medical procedure to relieve the patients from intense pain and avoid further lethal health complications. However, in the treatment of skin wounds, numerous underlying factors may affect the healing rate of wounds and sometimes make the wound not heal at all. Such factors which influence the efficacy, speed, and manner of wound healing fall under two types: local and systemic factors (Clark, 1991). Local factors include moisture content, edema, a technique of wound closure, ischemia and necrosis, foreign bodies, low oxygen tension, and perfusion (Clark, 1991). Systemic factors include inflammation, malnutrition/nutrition, metabolic diseases such as diabetes, immunosuppression, connective tissue disorders, and age (Clark, 1991, Stadelmann *et al.*, 1998).

Nevertheless, some documented histological studies report that well-managed wounds heal smoothly under the processes characterized by an increasing fibroplasia, angiogenesis, and wound re-epithelization (Clark, 1991, Maquart *et al.*, 1999). Moreover, a smoothly healing wound shows an increased migration and proliferation of cells such as fibroblasts, endothelial cells, and

epithelial cells also a deposition of connective tissue leading to enhanced contraction of the wounds (Clark, 1991, Maquart *et al.*, 1999, Nguyen *et al.*, 2009). When animals have fresh wounds on the skin, the recovery rate needs to be quick since prolonged healing or incompletely healed wounds may proceed to chronic wounds (Ingold, 1993).

Therefore, research efforts in wound management protocols are focusing more on discovering procedures and drug agents that can promote quick healing of wounds. Early efficient treatment of any wound type is important not only to relieve the patient from suffering but also to reduce the cost of hospitalization and save the patient from further severe complications. Antimicrobial ointments such as silver sulfadiazine, mafenide, silver nitrate, povidone-iodine, mupirocin, and bacitracin, have been the most reliable drugs in wound management (Leaper & Gottrup, 1998). However, all these topical antimicrobials are expensive to the majority of the lower-earning end-users in developing countries. Moreover, there are cases where conventional therapeutic agents have been less effective in curing some of the wounds. Therefore exploration of more novel therapeutic agents of wound management that are cheap, effective, and safe to supplement or replace the currently used conventional wound healing drugs is still inevitable (Stadelmann *et al.*, 1998). Medicinal herbs have been used since ancient times in the curation of various diseases including wounds.

One of the important medicinal herbs is *Centella. asiatica* (Li *et al.*, 2009). *C. Asiatica* is a perennial plant that belongs to the family Apiaceae. The plant has small fan-shaped green leaves with white or light purple flowers and it possesses small oval fruit, odorless, tasteless, and thrives around the water (Gohil *et al.*, 2010; Flora, 2014). Studies done elsewhere repots that, the whole plant of *C. Asiatica* (gotu kola) has been very useful in traditional medicine for treatments of wounds and other skin diseases, treatment of high blood pressure, purifying blood, and enhancement of memory (Jamil *et al.*, 2007; Wangchuk 2018).*C. Asiatica* is a perennial plant, commonly found in tropical and subtropical countries such as India, Sri Lanka, China, Indonesia, Malaysia, South Africa, Madagascar and East Africa (GBIF, 2014, USDA-ARS, 2014). Whether the extract of *C. Asiatica* (gotu kola) found in Tanzania will be efficacious enough in enhancing the wound healing processes as the plant species reported elsewhere need to be studied. Currently, there are no studies in Tanzania reporting the wound healing activity of the locally found *C. Asiatica* (gotu kola) in domesticated animal cases. Hence, the current study was carried out to evaluate the efficacies of the locally found *C. Asiatica* in Tanzania in excision wound healing in animals using rats as a study model.

METHODOLOGY

Study area

The study was carried out in Morogoro urban (6° 50' 42.66" S, 37° 39' 29.14"E), Tanzania. Studied plant materials were collected around the Sokoine University of Agriculture (SUA), the Main campus in Morogoro Municipal. Plant extraction was carried out in the laboratory of toxicology in the Department of Physiology, Biochemistry, and Pharmacology. Experimental setup and animal treatment were done at the small animal research unit in the College of Veterinary Medicine and Biomedical Sciences at the SUA. The study design was experimental and permission to conduct this study was granted by the Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy (DPRTC). This study was done to establish the wound healing efficacy of *Centella sciatica* (Gotukola leaves) ethanolic extract as a potential supplement to the conventional drug ointments involved in wound management, using Laboratory rats as a study model.

Preparation of the Ethanolic Extract of C. Asiatica

The plant leaves of *C*. *Asiatica* were harvested, collected, and kept in bags then transported to the laboratory for extraction. Harvested leaves were firstly dried under the shade until they were

breakable. The dry leaves were pulverized mechanically by using an electrical grinder to give 732.9g (14.21%) of ground to powder. The powder was macerated by using alcohol (98% ethanol) at a ratio of 1:3 for 5 days. The macerate was filtered using filter paper and the extract was concentrated at 85°C using a rotary evaporator to obtain a viscous solid. This was then left in a water bath to evaporate the remaining ethanol finally producing 90.7 (1.75%) of extract. Three different solution of varying concertation 0.1% (1mg/ml), 0.5% (5mg/ml) and 1% (10 mg/ml) was prepared by dissolving extract paste in distilled water as per Azis *et al.* (2017) with some modifications.

Research animals and Experimental setup

A total of 32 male laboratory rats weighing 250–300g obtained from the small animal unit in the College of Veterinary Medicine and Biomedical Sciences (CVMBS), Sokoine University of Agriculture (SUA), Tanzania were used in this study. These rats were allowed to acclimatize for 7-days to copy with the laboratory environment. The rats were caged in special rooms with a temperature of 27°C, free access to a commercial pellet diet, and water ad libitum. The allocation of animals and their respective treatments have shown in (table 1). All surgical procedures were carried out under chloroform anesthesia and local analgesia at the site of incision using lignocaine.

| GROUP | NUMBER OF RATS | TREATMENT |
|-------|----------------|---|
| T1 | 4 | Control group (nothing) |
| T2 | 4 | Dexamethasone + 1% Gotukola extract |
| T3 | 4 | 0.1% Gotukola |
| Т4 | 4 | 0.5% Gotukola |
| Т5 | 4 | 1% Gotukola |
| Т6 | 4 | Grounded fresh leaves juice of Gotukola |
| T7 | 4 | Silver sulphadiazine |

Table 1. Treatment allocations in different groups of rats were used in the research.

Full-thickness, completely transdermal circular wounds were made on the pre-shaved, 70% alcohol sterilized dorsal surface of the animal with the help of forceps, a scissor, and a skin marker. Gotukola extract solution (0.5 ml/wound) was applied topically in concentrations of 0.1%, 0.5%, and 1% once daily until complete wound healing was attained. The control group received nothing. Other groups of rats received either topical silver sulphadiazine as the positive control, dexamethasone (0.5mg/kg) together with 1% Gotukola extract solution, or the fresh leaf juice of Gotukola.

Test for acute dermal toxicity

Four rats were assigned to this group. The site of application (mid-dorsal region) was shaved and cleaned with distilled water, following disinfection. The extract solution of the herb was applied to the skin. Normal saline was applied to the first shaved rat as a control. Gotukola extract solution of 0.1%, 0.5%, and 1% was applied to second, third, and fourth shaved rats respectively. The shaved area was inspected within 24 hours for any cutaneous changes such as erythema, swelling, and development of vesicular eruptions.

Excision wound preparation and measurements

Excision wounds were prepared using a scissor and tissue forceps with the rats under general anesthesia of chloroform. The size of wounds was measured by a meter rule on days 0, 3, 7, and 10.

Determination of wound contraction percentages

The diameter of the wound was recorded on days 0, 3, 7, and 10 was used to calculate the wound contraction rate. Then the percentages of wound contraction (wound reduction rate) were obtained by dividing the extent to which wound contract (area) on a specific day by that of day zero and multiplying by 100 (Somboonwong *et al.*, 2012).

wound contraction = $\frac{A-A1}{40} \times 1$

Whereby: $A_1 = area$ of the wound on a specific day, & $A_0 = area$ of the wound at day zero.

Determination of epithelialization and complete wound healing duration

Epithelialization simply refers to the process of covering denuded epithelial surfaces that are necessary for the successful closure of the wound (Pastar *et al.*, 2014). And it is a defining parameter of successful wound healing. In the current study, the falling off of the old scar signifying its replacements by the newly formed epithelial tissues marked the completion of epithelialization time (Pastar *et al.*, 2014). Complete wound healing time was the time taken for complete restoration of the epidermis on the excision wound (Pastar *et al.*, 2014).

Visual or gross assessment of excision wounds

Visual assessments of the wounds followed the criteria of Somboonwong *et al.* (2012) whereby the excision wound was evaluated for size, wound bed, color, exudates, swelling of the wound surface, and the consistency of tissues surrounding the wounds.

Data Analysis

Data storage and cleaning were performed using Microsoft Excel. Data analysis for means and standard error of means was done using SPSS version 20 software. Statistical significant differences between groups were tested by the Analysis of variance (ANOVA) at P < 0.05.

RESULTS

Acute dermal toxicity test

Observation from the acute dermal test showed no skin reaction on the control rats under normal saline treatments and on the experimental rats under Gotukola leaf extracts at concentrations of 0.1%, 0.5%, and 1%. That is because both the control and treated rats revealed no evidence of edema, erythema, and evident irritation following the application of their respective treatment materials on their intact skin.

Wound contraction percentages

Figure 1. indicates the wound reduction rates (percentages) among the different groups of studied rats. It is shown in Figure 1 that wound contraction percentages increased proportionately with the Gotukola (*C. Asiatica*) leaf extract treatment duration. Wound measurements on days 3, 6, and 10 of treatment revealed a higher contraction rate of wounds in the 1% and 0.5 % dosage of Gotukola extract treatments relative to other comparative groups (figure 1). The wound contraction rate was lowest in the 0.1 % of Gotukola extract-treated and the negative control rats (figure 1).



Figure 1. Excision wound contraction rate (percentage) in different treatment groups after different treatment duration (3, 6, and 10 days) with different concentrations of Gotukola (*Centella Asiatica*) leaf extract. ANOVA from repeated measure analysis indicated significantly differing excision wound contraction rates in-within subjects (treatment duration-dependent) (P< 0.0001) and in-between subjects due to different treatments (P<0.0001).

The figure above indicates a significantly differing wound reduction rate amongst the treatment groups of rats. Observations in figure 1 were supported further by the Analysis of Variance (ANOVA) from repeated measure analysis which revealed a significantly differing wound shrinkage percentage which differed within the subjects with treatment duration (P<0.0001) and between the groups due to different treatment types (P<0.0001). Bonferonni test (Table 2) revealed further that, a differing wound contraction percentage between the Gotukola extract at 0.5 % treated, Gotukola extract at 1% treated, fresh Gotukola leaf juice treated, and the dexamethasone + 1% Gotukola extract-treated rats in comparison to the Gotukola extract 0.1 % treated and the negative control rats were statistically significant (P<0.05). No significant differences (table 2) in wound contraction percentage existed between the rats under Gotukola extract treatment at 1 % and those under Gotukola extract treatment at 0.5 %. Also, no significant differences (table 2) in wound reduction percentage existed between the rats treated with Gotukola at 0.1% in comparison to the negative control rats.

| Table2. Pair-wise Comparisons (Bonferonni test) of wound contraction percentages among the studied |
|---|
| groups of rats under Dexamethazone+ 1% Gotucola, Gotukola leaf juice, Gotukola extract 1%, Gotukola |
| extract 0.5 %, Gotukola extract 0.1, silver + sulfadiazine, and negative control. |

| Comp | ared groups | Mean Difference (I-J) | Std. Error | P-value |
|---------------------------------------|--|----------------------------------|------------|---------|
| Dexamethazone +1% Gotucola extract | Gotukola leaf juice | -6 . 509950 [*] | 1.0573034 | 0.000 |
| | Gotukola extract 1% | -12 . 751752 [*] | 1.0573034 | 0.000 |
| | Gotukola extract 0.5 % | -10.816959 [*] | 1.0573034 | 0.000 |
| | Gotukola extract 0.1% | 4.978266* | 1.0573034 | 0.003 |
| | Negative control | 7.290261* | 1.0573034 | 0.000 |
| | Postive control + silver sulfadiazine | -2.673784 | 1.0573034 | 0.410 |
| Gotukola leaf juice | Gotukola extract 1% | -6 . 241802 [*] | 1.0573034 | 0.000 |

| | Gotukola extract 0.5 % | -4 . 307009 [*] | 1.0573034 | 0.011 |
|------------------------|--|---------------------------------|-----------|-------|
| | Gotukola extract 0.1% | 11.488216* | 1.0573034 | 0.000 |
| | Negative control | 13.800210* | 1.0573034 | 0.000 |
| | Postive control + silver sulfadiazine | 3.836166* | 1.0573034 | 0.033 |
| Gotukola extract 1% | Gotukola extract 0.5 % | 1.934793 | 1.0573034 | 1.000 |
| | Gotukola extract 0.1% | 17.730018 [*] | 1.0573034 | 0.000 |
| | Negative control | 20.042012* | 1.0573034 | 0.000 |
| | Postive control + silver sulfadiazine | 10.077968* | 1.0573034 | 0.000 |
| Gotukola extract 0.5 % | Gotukola extract 0.1% | 15.795226 [*] | 1.0573034 | 0.000 |
| | Negative control | 18.107220 [*] | 1.0573034 | 0.000 |
| | Postive control + silver sulfadiazine | 8.143175* | 1.0573034 | 0.000 |

Wound epithelialization time

Wound epithelialization durations among the studied groups of rats are represented in figure 2. It is shown in figure 2 that the completion of wound epithelialization took much shorter in rats treated with Gotukola extracts at 1%, Gotukola extracts at 0.5 % relative to those under Gotukola extract at 0.1%, those under dexamethasone+ 1% Gotukola treatment, and the negative control rats. This was supported further by the ANOVA, from univariate analysis which indicated a significantly differing (P< 0.0001) excision wound epithelialization time (Days) among the treatment groups of rats.





Tukey test (table 3) confirmed further that wound epithelialization time differed significantly between the following groups of rats; Gotukola leaf juice treated rats against the negative control, Gotukola extract 1% treated against Gotukola extract 0.1% treated, and the negative control rats. Also, a significant difference in wound epithelialization time was revealed by

the Tukey test between the rats under Gotukola extract treatment at 0.5 % against the rats under 0.1 Gotukola extract treatment.

Table 3 Pairwise comparisons (Tukey test) on wound epithelialization time among the studied groups of rats under Dexamethazone+ 1% Gotucola, Gotukola leaf juice, Gotukola extract 1%, Gotukola extract 0.5 %, Gotukola extract 0.1, silver + sulfadiazine, and negative control.

| | | Mean Difference | | |
|------------------------|--|-----------------|------------|---------|
| Compared groups | | (I-J) | Std. Error | P-value |
| Dexamethazone | Gotukola leaf juice | 1.00 | 0.617 | 0.672 |
| +1% Gotucola extract | Gotukola extract 1% | 2.25* | 0.617 | 0.022 |
| | Gotukola extract 0.5 % | 2.00 | 0.617 | 0.051 |
| | Gotukola extract 0.1% | -1.00 | 0.617 | 0.672 |
| | Negative control | -2.25* | 0.617 | 0.022 |
| | Postive control + silver sulfadiazine | .25 | 0.617 | 1.000 |
| Gotukola leaf juice | Gotukola extract 1% | 1.25 | 0.617 | 0.429 |
| | Gotukola extract 0.5 % | 1.00 | 0.617 | 0.672 |
| | Gotukola extract 0.1% | -2.00 | 0.617 | 0.051 |
| | Negative control | -3.25* | 0.617 | 0.001 |
| | Postive control + silver sulfadiazine | 75 | 0.617 | 0.881 |
| Gotukola extract 1% | Gotukola extract 0.5 % | 25 | 0.617 | 1.000 |
| | Gotukola extract 0.1% | -3.25* | 0.617 | 0.001 |
| | Negative control | -4.50* | 0.617 | 0.000 |
| | Postive control + silver sulfadiazine | -2.00 | 0.617 | 0.051 |
| Gotukola extract 0.5 % | Gotukola extract 0.1% | -3.00* | 0.617 | 0.001 |
| | Negative control | -4.25* | 0.617 | 0.000 |
| | Postive control + silver sulfadiazine | -1.75 | 0.617 | 0.114 |

Duration for Complete wound healing

Figure 3 represents the duration (Days) taken for excision wound healing completion. The figure indicates that wound healing to completion was markedly faster (took much few days) in the Gotukola extract 1% and Gotukola extract 0.5 % treated rats as compared to the Dexamethasone + 1% Gotukola extracts treated, Gotukola extracts 0.1 % treated and the negative and positive control rats (figure 3). It is indicated further in the figure that the duration for complete wound healing varied only marginally between the Gotukola extract 0.1% in comparison to the Dexamethasone + 1% Gotukola extracts treated, Gotukola extract 0.1% in comparison to the Dexamethasone + 1% Gotukola extracts treated, Gotukola leaf juice treated, and negative control rats.



Figure 3. Time is taken for complete excision wound healing among different treatment groups of rats. ANOVA, from the univariate analysis, indicated a significant difference (P< 0.0001) in the complete excision wound healing time (Days) among the treatment groups.

Consistent findings revealed by the analysis of Variance from the univariate analysis indicated a significantly (P<0.0001) varying number of days taken for completion of wound healing among the experimental rats. Pairwise comparison by the Tukey test confirmed further that the duration for complete wound healing differed significantly between the following groups of rats; The dexamethasone + 1% Gutukola extracts treated rats against the Gotukola extract 1% and Gotukola extracts 0.5 % treated rats (table 4), Gotukola leaf juice treated against the Gotukola extract 0.1% treated, and the negative control rats (table 4).

The Tukey test revealed a significant difference in duration taken for complete wound healing between the Gotukola extract 1% treated against the 0.1% Gotukola extract-treated rats, the negative and the positive control rats (table 4). Moreover, the duration taken for complete wound healing differed significantly between the 0.5 % Gotukola extract-treated rats when compared to the 0.1 % Gotukola extract-treated and the negative control rats.

Table 4 Pairwise comparisons (Tukey test) on complete wound healing time among the studied groups of rats under Dexamethazone+ 1% Gotucola, Gotukola leaf juice, Gotukola extract 1%, Gotukola extract 0.5 %, Gotukola extract 0.1, silver + sulfadiazine, and negative control.

| Compa | and droups | Mean Difference | Std Error | Pvaluo |
|---------------------------------------|------------------------|-----------------|------------|---------|
| Compared groups | | (-) | Stu. LITUI | F-value |
| Dexamethasone +1% Gotucola extract | Gotukola leaf juice | 1.50 | 0.954 | 0.700 |
| | Gotukola extract 1% | 3.75* | 0.954 | 0.012 |
| | Gotukola extract 0.5 % | 3.25* | 0.954 | 0.036 |
| | Gotukola extract 0.1% | -1.75 | 0.954 | 0.542 |

| | Negative control | -2.25 | 0.954 | 0.264 |
|------------------------|--|-----------------------------|-------|-------|
| | Postive control + silver sulfadiazine | .50 | 0.954 | 0.998 |
| Gotukola leaf juice | Gotukola extract 1% | 2.25 | 0.954 | 0.264 |
| | Gotukola extract 0.5 % | 1.75 | 0.954 | 0.542 |
| | Gotukola extract 0.1% | -3.25* | 0.954 | 0.036 |
| | Negative control | -3.75 [*] | 0.954 | 0.012 |
| | Postive control + silver sulfadiazine | -1.00 | 0.954 | 0.936 |
| Gotukola extract 1% | Gotukola extract 0.5 % | 50 | 0.954 | 0.998 |
| | Gotukola extract 0.1% | -5.50* | 0.954 | 0.000 |
| | Negative control | -6.00* | 0.954 | 0.000 |
| | Postive control + silver sulfadiazine | -3.25* | 0.954 | 0.036 |
| Gotukola extract 0.5 % | Gotukola extract 0.1% | -5.00* | 0.954 | 0.001 |
| | Negative control | -5 . 50 [*] | 0.954 | 0.000 |
| | Postive control + silver sulfadiazine | -2.75 | 0.954 | 0.105 |

The findings on wound shrinkage percentages, epithelialization time, and total time for complete wound healing were supported further by visual wound assessment which revealed corroborative results (figure 4). General visual assessment of wound status revealed that would recovery was relatively faster in rats on 1% of Gotukola extract treatment followed by the animals on 0.5 % of Gotukola treatment and was relatively slow in the negative control rats (figure 4).

Visual or gross assessment of excision wounds

On day 3 of treatments, the wounds of rats under Dexamethasone + 1% of Gotukola extracts, Gotukola leaf juice, Gotukola extract at 0.1%, and those of the negative control were relatively more swollen, less contracted, and were relatively wet and reddish-brown (figure 4). However, the wounds of rats under Gotukola extract at 1% and 0.5 and were relatively drier, less swollen, appeared to be more contracted with a brownish scar, and were comparable to the positive control wounds(figure 4).



Figure 4. Shows the different rates of wound healing recorded at an interval of days 0, 3, 7, and 10 of treatments. 1% represent Gotukola extract 1%, 0.5 % represent Gotukola extract at 0.5 %, sulfadiazine represents Positive control treated with silver and sulfadiazine, and control represents the negative control group.

The excision wounds of rats were smallest with complete shrinkage or with only a small scar on days 7 and 10 days of treatments in the 1 % and 0.5 % Gotukola extract-treated rats and were comparable to the positive control wounds recovery rate (figure 4). However, there were some delays in wound shrinkage in rats receiving diluted Gotukola extract (0.1%), the negative control, and those receiving Gotukola leaf juice.

DISCUSSION

It was revealed in the current study that, the ethanolic extract of *C. Asiatica* (Gatukola) leaves accelerated the healing process of excision wounds after the extracts were applied at various concentrations to the wound-inflicted experimental Wistar rats. The current study revealed that the wounds treated with 1 or 0.5 percentages of Gatukola leaf extract had relatively higher contraction percentages, shorter epithelialization time, and shorter duration for complete healing compared to wounds of rats treated with the lower concentration of the extract and the control rats.

Also, the incorporation of dexamethasone into the leaf extracts of Gotukola appeared to have delayed the contraction rate of the excision wound, wound epithelialization time, and the duration of complete wound healing. This was expected because dexamethasone has antiinflammatory and immunosuppressant effects which may delay the wound healing process while preventing wound inflammation (Shetty *et al.*, 2006).

Visual assessment of excision wounds in the current study revealed corroborative results in that wounds of rats under Gotukola extract at 1% and 0.5 appeared to be recovering faster similar to that of the positive control compared to the wounds treated with a lower concentration of the extract, leaf juice, dexamethasone incorporated extracts and wounds of the negative control.

The current results correlated well with those of Sunilkumar *et al.* (1998) who reported an increased cellular proliferation and collagen synthesis at the wound site of the *C. Asiatica* extracts treated rats more evidenced by an increased collagen content and tensile strength of the wound. Results from the current study were also in line with the results of (Somboonwong *et al.* 2012) who revealed a significantly increased degree of healing in both the burn and incision wounds treated with the hexane, ethyl acetate, and methanol extracts of *C. Asiatica* when compared to similar wounds on the control rats.

Other documented studies report that the active ingredients contained in the *C. Asiatica* plant are the ones responsible for enhanced wound healing. It is reported that the presence of active compounds in *C. Asiatica* such as saponins and triterpenes particularly the Asiatic acid, madecassic acid, asiaticoside, and madecassoside, enhance wound healing through their antioxidants activities (Shukla *et al.*, 1999 a&b; Maquart *et al.*, 1999, Liu *et al.*, 2008 a&b), microbial inhibitory properties (Gohil *et al.*, 2010) and promotion of angiogenesis and wound epithelialization (Shukla *et al.*, 1999).

Conclusion

The results revealed that the locally available *C. Asiatica* in Morogoro region in Tanzania enhanced rapid wound healing when the plant ethanolic crude extracts were applied to the excision wounds of rats. It was a concentration of 1 or 0.5 % of the *C. Asiatica* extract in the current study which showed to promote wound healing at a rate similar to that of the conventional silver sulphadiazine. However, since using *C. Asiatica* extracts in wound management can be cheaper than conventional drugs, we recommend further studies on wound healing efficacies of various organs of *C. Asiatica*. Also, we suggest for investigation of the efficacies of higher dosages of the plant extracts in wound healing and the efficacies of the plants on chronic wounds.

Acknowledgment: We extend our sincere gratitude to Dr. Rajabu Awami, Dr. Shaban Mshamu, and Mr. Ignatus kiiza for laboratory assistance.

Ethical issues: The ethical approval for the study was granted by the Ethical Committees of Sokoine University of Agriculture, Tanzania.

Competing interests: The authors declare that there is no competing interest.

References

- Aziz HA, Taher M, Ahmed AS, SulaimanWMW, Susanti D, Chowdhury SR and Zakaria ZA. 2017. In vitro and In vivo wound healing studies of a methanolic fraction of *Centella Asiatica* extract. South African Journal of Botany. (108): 163-174
- Baldwin G and Colbourne M. 1999. Puncture Wounds. Pediatrics in Review 20 (1), 21-23.
- Clark R.A.F. 1991.Cutaneous Wound Repair. Oxford University, New York, p. 576.
- da Rosa Silva CF, Santana RF, de Oliveira BGRB. 2017. High prevalence of skin and wound care of hospitalized elderly in Brazil: a prospective observational study. *BMC Res Notes* 10, 81.
- Flora Z. Kew Databases. 2014. Richmond, UK: Royal Botanical Gardens Kew. http://apps.kew.org/efloras/fz/families.htm.
- GBIF. Global Biodiversity Information Facility. 2014 http://www.gbif.org/species
- Gohil KJ, Patel JA and Gajjar AK. 2010. Pharmacological review on Centella Asiatica: a potential herbal cure-all. *Indian Journal of Pharmaceutical Sciences* 72(5):546-556.
- Ingold W.M. 1993. Wound therapy: growth factors as agents to promote healing. Trends in Biotech 11: 387–392.
- Jamil SS, Qudsia N and Mehboobus S. 2007.Centella asiatica (Linn.) Urban a review. Natural Product Radiance 6(2): 158-170.

- Karpal SS and Jeremiah RM. 2019. Etiology, pattern, and outcome of management of facial lacerations in Dar es Salaam, Tanzania. *Tanzania Journal of Health Research*. 21(2),8p.
- Leaper DJ and Gottrup F. 1998. Surgical wounds. In Wounds: Biology and management. Edited by Leaper DJ, Harding KG. Hong Kong: Oxford University Press: 23–40.
- Li HGX and Zhang L. 2009. Madecassoside attenuates inflammatory response to collagen-induced arthritis in DBA/1 mice. *Phytomedicine*16(6-7): 538-546.
- Liu MDYand Li Y. 2008. Madecassoside isolated from Centella Asiatica herbs facilitates burn wound healing in mice. *Planta Med* 74(8): 809-815.
- Liu MD.Y and Yao X. 2008. Anti-rheumatoid arthritis effect of madecassoside on type II collageninduced arthritis in mice. Int Immunopharmacol 8 (11):1561-1566.
- Lyimo MA, and Mosha IH. 2019. Reasons for delay in seeking treatment among women with obstetric fistula in Tanzania: a qualitative study. BMC Women's Health. 19, 93.
- Maquart FX, Chastang F, Simeon A, Birembaut P, Gillery P and Wegrowski Y. 1999. Triterpenes from Centella Asiatica stimulate extracellular matrix accumulation in rat experimental wounds. *Eur J Dermatol*, 9: 289–296.
- Nguyen DT, Orgill DP and Murphy GT. 2009."The Pathophysiologic Basis for Wound Healing and Cutaneous Regeneration". Biomaterials for Treating Skin Loss. Elsevier. pp. 25–57. Orgill DP, Blanco C (editors).
- Pastor I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Patel SB, Khalid L, Isseroff RR and Tomic CM. 2013. Epithelialization in Wound Healing: A Comprehensive Review. *Adv Wound Care* (New Rochelle). 2014 Jul 1;3(7):445-464.
- Shetty BS, Udupa SL, Udupa AL and Somayaji SN. 2006. Effect of Centella Asiatica L (Umbelliferae) on normal and dexamethasone suppressed wound healing in Wistar Albino rats. *Int J Low Extreme Wounds* 5:137–144.
- Shukla A, Rasik AM and Dhawan BN. 1999. Asiaticoside induced elevation of antioxidant levels in the healing wound. *Phytotherapy Res* 13:50–54.
- Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK and Dhawan BN. 2007. In vitro and in vivo wound healing activity of asiaticoside isolated from Centella Asiatica. *J Ethnopharmacology* 65: 1–11.
- Somboonwong J, Kankaisre M, Tantisira B and Tantisira MH. 1999.Wound healing activities of different extracts of Centella Asiatica in the incision and burn wound models: an experimental animal study. BMC complementary and alternative medicine, 12: 103, 2012.
- Stadelmann WK, Digenis AG and Tobin GR. 1998."Physiology and healing dynamics of chronic cutaneous wounds". American J Surg. 176: 265–385.
- Sunilkumar P, Parameshwaraiah S, Shivakumar HG. 1998. Evaluation of topical formulations of aqueous extract of Centella Asiatica on open wounds in rats. *Indian J Exp Biol* 36: 569–572.
- USDA-ARS. 2014. Germplasm Resources Information Network (GRIN). Online Database. Beltsville, Maryland, USA: National Germplasm Resources Laboratory, https://npgsweb.arsgrin.gov/gringlobal/taxon/taxonomysearch.aspx.
- Wangchuk P. 2018. Therapeutic applications of natural products in herbal medicines, biodiscovery programs, and biomedicine. *Journal of Biologically Active Products from Nature* 1:1-20.
- Zabaglo M, Sharman T. 2021. Postoperative Wound Infection. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560533.