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Evaluation of wound healing activity of alpha mangostin ointment in rats

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ABSTRACT

This study was aimed to evaluate the wound healing effects of alpha mangostin ointment using excision wound model. Twenty rats were divided into four groups of five rats each; group I was treated with ointment base (control), whereas group II, group III and group IV were treated with 10% (w/w) povidone-iodine (standard), alpha mangostin 1% (w/w), and alpha mangostin 2 % (w/w) respectively for wound healing evaluations. The size of the wound area was measured, and the reduction in the wound size was calculated, and the tissues examined histologically. The significant difference in the wound size reduction between the control and treated group (p<0.05) was observed in wound healing activity. Histopathological studies showed a lesser number of chronic inflammatory cells, lesser oedema and increased collagenisation in the test than control. This study showed that the alpha mangostin topical ointment could be a promising candidate for the betterment of wound care.

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INTRODUCTION

Wound healing is the body's natural process of regenerating dermal and epidermal tissues. When an individual is wounded, a set of events predictably takes place to repair the damage. Following injury, an inflammatory response occurs, and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated (Iba *et al.*, 2004). Current methods used to treat wounds include debridement, irrigation, antibiotics, tissue grafts, proteolytic enzymes, and corticosteroids, which possess significant drawbacks and unwanted side effects. Although there are various wound healing therapies, they are only partially effective. Therefore, there is a need for new cost-effective therapies with better efficacy. Medicinal plants are important sources of a new chemical substance that have a beneficial therapeutic effect.

Mangosteen (*Garcinia mangostana*) is a tropical evergreen fruit tree from Malaysia, India and Sri Lanka with a long history of uses as a source for traditional medicine for the treatment of chronic diarrhoea, infected wounds, skin infections and dysentery (Pedraza-Chaverri *et al.*, 2008). The major

bioactive secondary metabolites of mangosteen are xanthone derivatives. These xanthone derivatives have displayed potent pharmacological activities, including antibacterial, antifungal, antioxidant, antitumoral, anti-inflammatory and anti-allergy properties (Obolskiy *et al.*, 2009). Alpha-mangostin is the most abundant xanthone existed in mangosteen pericarp. It has been confirmed to have antiproliferative and apoptotic effects in various types of human cancer cells (Akao *et al.*, 2008). No report has been published in the literature regarding the wound healing potential of this compound. The present study demonstrates the wound healing effect of alpha mangostin using a rat's model.

MATERIALS AND METHODS

Selection of animals

Male rats weighing 150–200 grams were used in this study, the experimental animal procedure complied with the Institute's Animal Ethics Committee, International Medical University, Malaysia (approval code no. BP I-01-12 (39) 2015). Animals were fed on a standard pellet diet and water ad libitum and maintained at 24–28°C temperature and relative humidity (30% - 70%). Animals marked as fasted were deprived of food for 16 hours but had free access to water.

Preparation of ointment

Scanning of lambda (λ) max for alpha mangostin

10 mg of alpha mangostin powder was dissolved with methanol in a 100 mL volumetric flask. Then, 10 ml of the solution was diluted with methanol in a 50 mL volumetric flask. Aliquots of samples at different concentrations were further diluted into 10 mL volumetric flask using methanol. The samples were scanned to determine the lambda (λ) max value using a UV spectrophotometer. The standard calibration curve plotted at 283 nm.

Preparation of alpha mangostin ointments

The composition of mangostin ointments was shown in Table 1. The PEG 400 and 6000 bases were melted to 70°C, and 0.5 g of alpha mangostin was incorporated into the molten base while stirring. Stirring was continued until it cools down to form ointment. The consistency and colour of ointment were recorded (Panda *et al.*, 2009; Majumder and Majumder, 2013).

Drug loading capacity of alpha mangostin ointments

One gram of the ointment transferred to 100 mL beaker and added methanol gradually up to 100 mL. Then, the solution was filtered, and about 10 mL of the filtered solution was transferred to 50 mL volumetric flask and diluted with methanol. A UV spectrophotometer analysed the absorbance of each formulation at 283 nm, and the percentage weight by weight was calculated using the formula:

$$\frac{Practical\ value}{Theoretical\ value} \times 100\% = \_\_\_\_\_\% \ w/w$$

Evaluation of *in vitro* drug release of alpha mangostin ointments

The amount of drug released from each ointment was determined with the Franz diffusion cell using the cellophane membrane along with phosphate buffer solution (PBS) at 32°C. At precise time intervals, serial samplings were performed after 0.05, 0.10, 0.15, 0.30, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 hours respectively. The absorbance of the withdrawn samples was analysed at 283 nm using the UV spectrophotometer. The cumulative percentage of drug release profile was plotted (Panda *et al.*, 2009).

Preliminary evaluation of the formulations

pH

One gram of ointment was dissolved in 100 mL of distilled water. The ointment was stored for two hours. The measurement for pH was done in triplicate, and the average was recorded.

Spreadability

One gram of ointment was weighed and placed between the two glass slides. An object weighing 100 g was kept on the upper slide for five minutes to distribute the ointment evenly. Then, a 250 g object was added to the pan (Panda *et al.*, 2009; Majumder and Majumder, 2013). The separation time of the two glass slides was recorded to calculate the spreadability based on the formula given below:

$$S = m \times \frac{l}{t}$$

where, m = weight on the upper slide in gram

l = length of the glass slide in cm

t = time in seconds

Thermal cycling

One gram of ointment was transferred to a small tube and kept in a hot water bath (37°C) for two weeks. The ointment's organoleptic and physicochemical properties were tested.

Animal studies

Acute Dermal Toxicity Test

Based on the OECD guideline number 402, the rats were randomly selected and evenly distributed to treatment and control groups. Fur from the back of each rat (weighing about 200g to 300g) was shaved

approximately 24 hours before the test. The application of formulation (2000 mg/kg) covered about 200 mm^2 of the total surface area for the treatment groups and the application of ointment base alone on the control groups. The treated area was instantly protected using the non-irritating adhesive plaster. The rats were individually caged for 24 hours after application. Then, cage-side observations were done for 14 days. Changes in skin and fur, eyes, salivation, diarrhoea, lethargy, sensory stimuli, respiratory, level of activity, urination, erythema, oedema, behaviour patterns, tremor, gait and posture, convulsion as well as death were examined. The individual weight of each rat was measured on the first day and last day of observation. All the test and control rats were sacrificed and weighed liver, heart, and kidneys, and histopathological studies were done in the wound area.

Wound healing activities

Group I: Control group (only ointment base)

Group II: Standard group received povidone-iodine ointment (10%)

Group III: Drug treated grouped received 1% w/w of alpha mangostin ointment

Group IV: Drug treated grouped received 2% w/w of alpha mangostin ointment

Wound Area and Wound Contraction

Twenty adult rats divided into four groups of five rats; each was used for the study. Excision wounds were created after shaving the left dorsal thoracic region 1 cm away from the vertebral column. The animals were anaesthetised, and 3 cm in diameter excision wound was created. The wound was monitored, and transparent tracing paper was used to mark the border of healing of the wound. An average of 3 readings of the wound was taken to minimise potential human error. The marked tracing paper was placed on a graph paper, and the area of the wound was calculated. The area of the wound was measured every day before the application of the ointment, and the percentage of wound closure was determined using the following formula (Süntar *et al.*, 2010).

$$Percentage\ of\ wound\ closure = \frac{wound\ area\ on\ day\ 0 - wound\ area\ on\ day\ n}{wound\ area\ on\ day\ 0} \times 100$$

Where n =number of days 4th, 8th, 12th, 16th and 20th day.

Histopathology

On the same day of sacrificing the animal, a piece of skin from the site of the wound was collected and fixated using 10% neutral buffered formalin. The tissue was processed in a systematic way for histological evaluation. Five micrometre thick sections were stained with haematoxylin and eosin. The samples were then observed under a light microscope for detection of fibroblast proliferation, blood vessel formation, collagen deposition, re epithelisation, and keratinisation.

Statistical analysis

The means of wound area measurement and percentage closure of wounds between groups at different time intervals were compared using one-way analysis of variance (ANOVA), followed by Dunnet's test. A p-value of <0.05 was significant.

RESULTS

The standard calibration curve of alpha mangostin

From Figure 3, the absorbance of alpha mangostin ointment at 283 nm was measured. This implied that the absorbance obtained has corresponded to Beer-Lambert's Law.

Preparation of alpha mangostin ointments

In this experiment, alpha mangostin ointments were prepared with the same ingredients but with different proportion of PEG 400 and 4000 (Table 1). The ointment preparation was carried out using the hot plate method. PEG was used as the ointment base because they act as a drug vehicle for a lipophilic drug. Alpha mangostin is a lipophilic drug. Besides, PEGs are stable, hydrophilic, and has a good release potential compared to other ointment bases. The possibility of a hydrophilic vehicle to cause irritations are minimal.

The consistency of the prepared ointments, as shown in Table 2 revealed that formulation 1 has a smooth texture and good spreadability compared to other formulations. However, formulation 2 was comparably lower than formulation 1. Conversely, formulation 3 had a rough texture and was challenging to spread upon application on the skin. All three ointments were present in the form of pale yellow (Table 3).

Evaluation of *in vitro* drug release of alpha mangostin ointments

In vitro drug release was conducted to determine the percentage of an active compound being released into the skin membrane for biological action. Based on Figure 4, formulation 2 was found to be better compared to the other two formulations. However, formulation 1 and 2 were increasing over a more

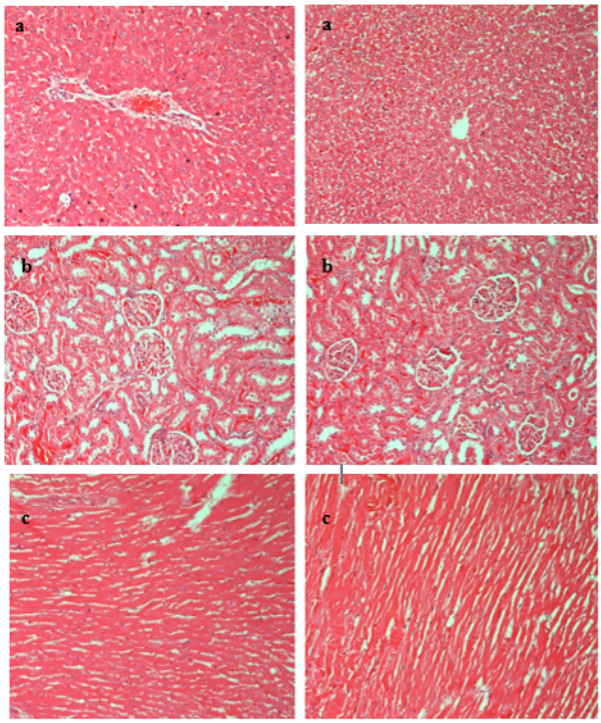

Figure 1: Acutetoxicity studies: Histology of liver (a), kidney (b), and heart (c) of controland alpha mangostin

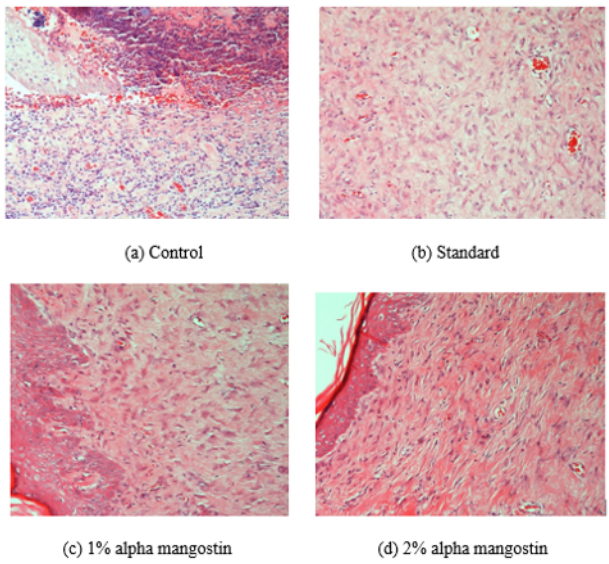

Figure 2: Effect of alpha mangostin on excision wound model in rats(a–d)

Table 1: Compositions alpha mangostin ointments

Ingredients	Quantity % w/w		
	F1	F2	F3
PEG 400	35 g	30 g	25 g
PEG 4000	15 g	20 g	25 g
Alpha mangostin	0.5 g	0.5 g	0.5 g

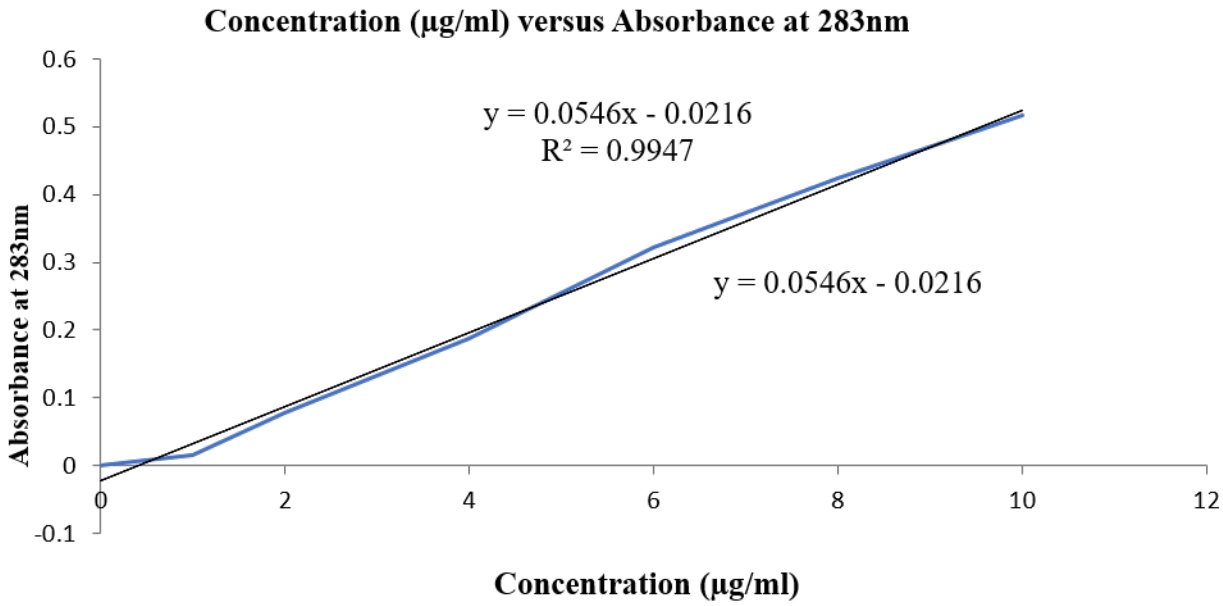

Figure 3: Calibration curve graph of alpha mangostin

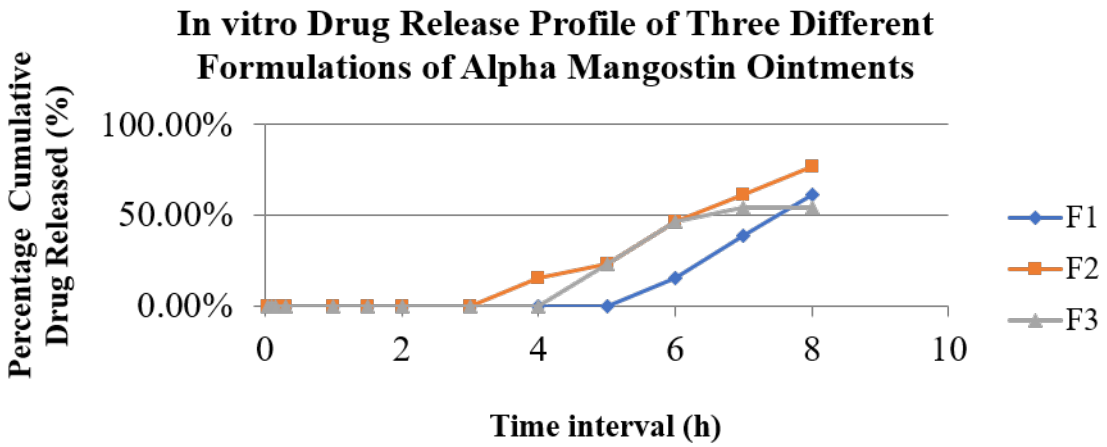

Figure 4: *In vitro* cumulative percentage of drug release profile for three formulations

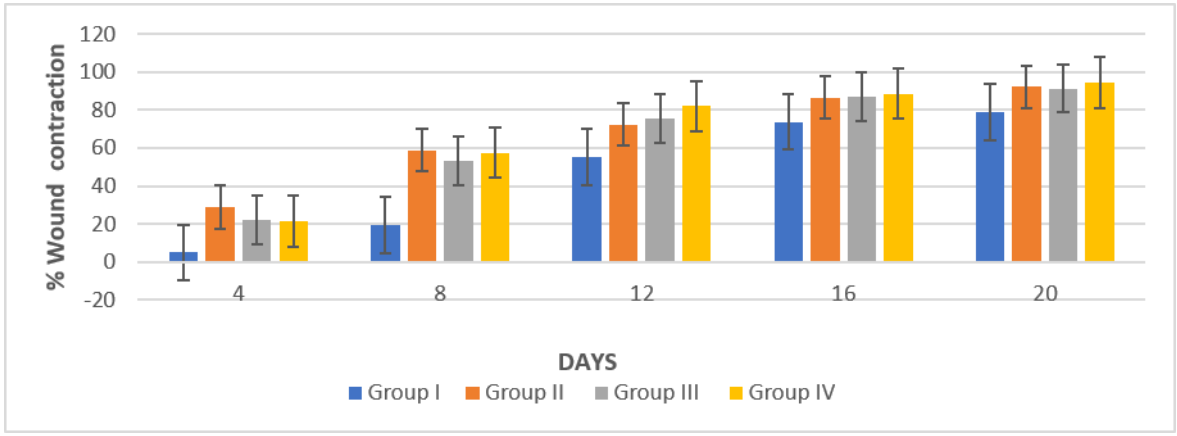

Figure 5: Effect of alpha mangostin on excision wound model in rats

Table 2: Consistency of alpha mangostin ointments

Ointment colour: pale yellow	
Formulation	Consistency
1	Smooth
2	Slightly smooth
3	Rough

Table 3: Physicochemical properties of the three formulations

Formulation	Drug content (% w/w)	Colour	pH	Spreadability (gcms-1)	Thermal cycling (37°C)
1	88.75	Pale yellow	6.56	633.3	Normal
2	86.25	Pale yellow	6.17	380.0	Normal
3	93.75	Pale yellow	6.20	190.0	Normal

Table 4: Relative organs weight of treated and control groups

Group	Treatment	Control
Liver (g)	3.4625 ± 0.234	3.2250 ± 0.163
Heart (g)	0.3075 ± 0.013	0.3100 ± 0.014
Right kidney (g)	0.3025 ± 0.022	0.2850 ± 0.050
Left kidney (g)	0.3225 ± 0.025	0.3000 ± 0.057

Table 5: Body weight of treated and control groups

Group	BW (g) Day 0	BW(g) Day 7	BW(g) Day 14
Treatment	227.35 ± 21.75	232.72 ± 27.28	236.89 ± 29.63
Control	237.11 ± 40.33	251.37 ± 44.71	261.86 ± 52.95

extended time. Formulation 3 was increasing as well, but at the 7^{th} and 8^{th} hour, their release profile was constant. There was only a slight variation in the release profile at the particular time for all the three formulations. The full release of formulation 1, 2, and 3 was 61.54%, 76.92%, and 53.85% respectively for 8 hours.

Physicochemical properties of the three formulations

The physicochemical properties of pharmaceutical formulations are essential tests in which quantifiable measurements are made according to the manufacturer's specification. The drug content determines the amount of active compound readily present in the formulation while the pH denotes the suitability of formulation to be applied on the skin. The spreadability implies the extent of the area the formulation spreads on the skin upon application, whereas the viscosity measures the thickness and strength of the formulation. Based on Table 3, the drug content of F1, F2, and F3 was 88.75 % w/w, 86.25 % w/w, and 93.75 % w/w respectively. The pH of formulation 1 was 6.56, while the pH of formulation 2 and 3 was 6.17 and 6.20, respectively. All three formulations were within the normal pH range of human skin. The spreadability results indicated that as the spreadability increases, the torque and shear stress were lower, and hence, the viscosity decreases and vice versa. For stability testing at 37°C, three formulations had shown no changes in any parameters after keeping them for two weeks. All three formulations have exhibited good ointment properties. However, only formulation 1 was best for acute toxicity testing.

Acute Toxicity Test

A median lethal dose (LD_{50}) of 2000 mg/kg using a 2% of alpha mangostin ointment of formulation 1 was applied once on the rats' wound. All rats did not show any signs of toxicity or death. There were no changes in behaviour patterns or other physiological activities. Bodyweight and the weight of the internal organs were tabulated and recorded in

Table 4 and Table 5, respectively. A decrease in body weight would have indicated the presence of toxicity. However, all rats showed an increase in their body weight. The treatment group that received the maximum dose had a slightly lower weight gain compared to the control group. There was no significant difference ($p < 0.05$) in the body weight and relative organs weight between treated and control groups. Therefore, the results implied that the internal organs such as liver, kidney, and heart were not harmfully affected during the treatment.

Excision Wound Model

Since 9th day, 1% and 2% alpha mangostin treated groups showed significantly higher wound contracting ability compared to control groups. The significantly higher percentage of wound closure was noticed on 20th day in 2% alpha mangostin treated groups (94.3700±2.59%) followed by 1 % alpha mangostin and standard treatments groups (91.31±1.82%, 92.08±2.76% respectively). The entire epithelisation period was also found to be significantly faster in case of 2% alpha mangostin treated groups followed by 1% alpha mangostin and the standard ointment groups than the control group. The rate of wound contraction and complete epithelisation period was almost in 2% w/w and 1% w/w alpha mangostin higher than control groups (Figure 5)

Histopathological Observations

The alterations in tissues and organs cell structure were examined via pathological analysis. No morphological changes were observed between the control and treated groups, as shown in Figure 1 — histopathology of various groups of rat skin stained with hematoxylin and eosin (HE) at day 20. Figure 2(a) shows the section of skin of rat in the control group with ointment base showed minimum fibroblast production and collagen deposition with a huge presence of inflammatory cells, suggesting infection and incomplete wound healing. Figure 2(b, c, d). shows the section of skins of rat in group 2 treated with povidone-iodine ointment as well as the skin of wounded rats in group 3 and 4 treated with alpha mangostin with 1% w/w and 2% w/w concentration showed good fibroblast proliferation, collagen deposition and minimum blood vessel formation.

DISCUSSION

Many previous studies have investigated the effects of herbal ointments on the healing time of wounds in laboratory animals (Mahomoodally, 2013; Hamman *et al.*, 2014). One such plant is *Garcinia mangostana* Linn or simply known as mangosteen, it contains xanthone, which holds the most important biological activities in wound healing, which are antibacterial and anti-inflammatory.

Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavanols, catechins, anthocyanins, and proanthocyanins could delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals (OH), or superoxide radical (O_2) scavengers. Mangostin is a xanthone derivative; it may be responsible for wound healing activity (Sen *et al.*, 2002).

Reactive oxygen species are involved in several degenerative diseases such as atherosclerosis, cancer, cirrhosis, and diabetes, including wound healing. Antioxidants enhance the healing of infected and noninfected wounds by reducing the damage caused by oxygen radicals. Mechanisms of wound healing may be contributed to stimulate the production of antioxidants in the wound site and provides a favourable environment for tissue healing (Shukla *et al.*, 1999). Alpha mangostin has shown antioxidant activity. It has been reported that antioxidants may play a significant role in the wound healing process and maybe a crucial contributory factor in the wound healing property (Shukla *et al.*, 1999). Antioxidants have been reported to play a significant role in the wound healing process and significantly improve wound healing and protect tissues from oxidative damage (Martin, 1996).

In the wound healing studies, the wound closure time and wound contraction were taken as parameters. In both betadine and alpha, mangostin treated groups, 90% of wound healing was recorded on 20^{th} day. Results obtained in this study confirmed the wound healing activity of alpha mangostin.

CONCLUSION

From this research, it is shown that alpha mangostin has wound healing activity by measuring the wound area daily and wound contraction and compared it against the control group. Thus, alpha mangostin could be a promising showed wound healing property, as shown in the experiment conducted.

Conflict of Interest

The authors declare that they have no conflicts of interest for this study.

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REFERENCES

Akao, Y., Nakagawa, Y., Nozawa, Y. 2008. Anti-Cancer Effects of Xanthones from Pericarps of Mangosteen. *International Journal of Molecular Sciences*, 9(3):355–370.

Hamman, J., Fox, L., Plessis, J., Gerber, M., Zyl, S., Boneschans, B. 2014. In Vivo skin hydration and anti-erythema effects of Aloe vera, Aloe ferox and Aloe marlothii gel materials after single and multiple applications. *Pharmacognosy Magazine*, 10(38):392–403.

Iba, Y., Shibata, A., Kato, M., Masukawa, T. 2004. Possible involvement of mast cells in collagen remodeling in the late phase of cutaneous wound healing in mice. *International Immunopharmacology*, 4(14):1873–1880.

Mahomoodally, M. F. 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence Based Complement Altern Med*, 2013:1–14.

Majumder, P., Majumder, S. 2013. Preparation and characterization of some herbal ointment formulations with evaluation of antimicrobial property. *Indian Journal of Research in Pharmacy and Biotechnology*, 1(3):385–390.

Martin, A. 1996. The use of antioxidants in wound healing. *Dermatol. Surg*, 22(2):156–160.

Obolskiy, D., Pischel, I., Siriwatanametanon, N., Heinrich, M. 2009. Garcinia mangostanaL.: a phytochemical and pharmacological review. *Phytotherapy Research*, 23(8):1047–1065.

Panda, P., Nayak, S. S., Mohanty, A., Panda, D. P., Panda, P. K. 2009. Formulation and evaluation of topical dosage form of Pandanus fascicularis Lamk. and their wound healing activity. *J Pharm Res*, 2(4):630–635.

Pedraza-Chaverri, J., Cárdenas-Rodríguez, N., Orozco-Ibarra, M., Pérez-Rojas, J. M. 2008. Medicinal properties of mangosteen (Garcinia mangostana). *Food and Chemical Toxicology*, 46(10):3227–3239.

Sen, C. K., Khanna, S., Gordillo, G., Bagchi, D., Bagchi, M., Roy, S. 2002. Oxygen, Oxidants, and Antioxidants in Wound Healing. *Annals of the New York Academy of Sciences*, 957(1):239–249.

Shukla, A., Rasik, A. M., Dhawan, B. N. 1999. Asiaticoside-induced elevation of antioxidant levels in healing wounds. *Phytotherapy Research*, 13(1):50–54.

Süntar, I. P., Akkol, E. K., Yılmazer, D., Baykal, T., Kırmızıbekmez, H., Alper, M., Yeşilada, E. 2010. Investigations on the in vivo wound healing potential of Hypericum perforatum L. *Journal of Ethnopharmacology*, 127(2):468–477.