Effectiveness of Mangosteen Rind (*Garcinia mangostana* L.) Ethanol Extract Ointment Against The Cut Wounds of Mice

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Abstract: Mangosteen rind (*Garcinia mangostana* L.) contains xantones flavonoids which have an anti-inflammatory effects by triggering the formation of collagen. Collagen plays an important role in structure maintenance and wound healing on skin. Other compounds such as saponin, phenol and tannin in mangosteen rind was also reported to accelerate the healing process of cut wounds in mice. The aim of this study was to determine the activity of the mangosteen rind ethanol extract ointment on the mice's wounds, at concentrations 10%, 20% and 30%, respectively, in terms of wound healing time. The progress of wound healing was observed until day-7. The results showed that the 10% extract ointment group was have 80% of cut wound healing, the 20% extract ointment group was have 85%, the 30% extract ointment group was have 95%, meanwhile the positive control group had an average percentage of cut wound healing 75%. The optimal wound healing process on day 7 was seen in the 30% of mangosteen rind ethanol extract ointment group, where a significant reduction in the diameter of the incision was compared to the other groups.

INTRODUCTION

Wounds occur as a result of continuous cellular, anatomical and functional disturbances in the tissue [1]. The disturbance comes from physical, chemical, heat, or microbial disturbances. Wounds on the skin that are torn, cut, or punctured are called open wounds, bruises hit by a blunt object are called closed wounds, while burns are wounds caused by fire, heat, radiation, chemicals, electricity, or solar heat [2]. Wound healing occurs in three phases, namely the inflammatory phase, the proliferative phase, and the maturation phase [3]. Many plants are reported to be efficacious in healing wounds and are often used by the community, one of which is mangosteen.

Mangosteen (*Garcinia mangostana* L.) is widely cultivated in Southeast Asian countries such as Indonesia, Malaysia, Philippines, Myanmar and Thailand. Indonesia's mangosteen fruit production was reported at 85538 tons in 2010, the weight of the mangosteen fruit consumed was only 20-30%, the rest was the weight of the mangosteen rind, so that 59000-67000 tons of mangosteen rind was wasted. Mangosteen rind has secondary metabolites of the flavonoid group, namely xanthones, which have anti-inflammatory, antibacterial, antiallergic and anticancer activities [4]. Xanthones in mangosteen rind were found as much as 107.76 mg per 100 grams of rind [5].

The antioxidant activity of mangosteen rind has been reported to accelerate the cell recovery process by accelerating the process of fibroblast proliferation [6]. Research conducted by Mutmainah and Maulina in Mardiana [7], showed that the formulation of a mangosteen rind extract gel preparation was able to heal burns on the back skin

of rabbits. However, the effectiveness of mangosteen rind has not been confirmed in mice cut wounds. Therefore, this study aims to measure the effectiveness of the ethanol extract of the mangosteen peel ointment against the cut wounds of mice.

MATERIAL & METHODS

Experimental research was carried out in the laboratory. The stages of the research included collecting mangosteen rind, extracting mangosteen rind using maceration method (96% ethanol as solvent), and formulation of mangosteen rind extract ointment. Furthermore, evaluation of mangosteen rind extract ointment was also carried out including organoleptic test, homogeneity test, spreadability test, pH test, irritation test and effectiveness test of the ointment on the healing of mice's cut wounds which were observed from day 0 to day 7.

Materials

Vacuum rotary evaporator, blender, analytical balance, stir bar, porcelain cup, maceration vessel, mortar & stamper, water bath, beaker glass, spatula, mesh size 60, measuring cup, buchner funnel, sterile disposable scalpel no. 11, universal pH, mangosteen rind (Garcinia mangostana L), 96% ethanol, vaseline flavum, adeps lanae, aquadestilata, betadine ointment, ethyl chloride.

Animals

The study used 25 healthy male mice (each group n = 5), with an average weight of 20-30 grams. Previously, mice were acclimatized for 7 days, with cycles of 12h light / 12h dark, access to food and drink was given freely. The research group was divided into 5 groups including the positive control group (betadine ointment), the negative control group (ointment base), and the treatment group were given mangosteen rind extract ointment with concentrations of 10%, 20%, and 30% respectively.

Mangosteen Rind Extraction

Mangosteen rind (*Garcinia mangostana* L.) was obtained from the Karang Pule Fruit Market, Mataram. Mangosteen rind was separated from the fruit and then cleaned and chopped. Furthermore, the mangosteen rind was air-dried for 7 days, then powdered and sieved with a mesh sieve No. 60.

Next, 300 grams of dried mangosteen rind powder was extracted by maceration method, using 1.5 liters of 96% ethanol as a solvent. Maceration was carried out for three days and stirred every 24 hours. After three days, the extract was filtered using a Buchner funnel and concentrated using a rotary evaporator, then evaporated over a waterbath at 60°C to obtain a thick mangosteen rind extract [8].

Preparation of Mangosteen Rind Extract Ointment

Mangosteen rind extract ointment was made using a fatty (hydrocarbon) ointment base with a total weight of 30 grams. The standard formulation of mangosteen rind extract ointment is shown in Table 1 [9],

TABLE 1. Formulation of mangosteen rind extract ointment at various concentrations

Formula Component	Mangosteen rind extract ointment 10%	Mangosteen rind extract ointment 20%	Mangosteen rind extract ointment 30%
Mangosteen rind extract	3 grams	6 grams	9 grams
Adeps lanae	4.5 grams	3 grams	1.5 grams
Vaseline flavum	22.5 grams	21 grams	19.5 grams

The mortar and stamper are preheated in the oven at 50ºC. Take out the mortar and stamper, then add the adeps lanae and grind until melted, add the vaseline flavum and stir the mixture at constant speed until it is homogeneous. Next, add the mangosteen rind extract according to the amount in each formula, then stir until a homogeneous ointment mass was formed.

Evaluation of Mangosteen Rind Extract Ointment

1. Organoleptic Test
   Organoleptic tests were carried out by observing the mangosteen rind extract ointment including shape, smell and color. Ointments must have a semisolid form, an appropriate color, and a non-rancid odor [10].
2. Homogeneity Test
   The ointment was applied to a glass or other suitable transparent material. A homogeneous ointment was characterized by the absence of lumps of particles after being smeared, homogeneous ointment and has a uniform color from the starting point to the end of application. Samples were taken in three places including the top, middle and bottom of the container [10, 11].
3. Spreadability Test
   A total of 0.5 grams of ointment was placed between two transparent round glass plates with a diameter of 15 cm, and a load of 100 grams on it. The diameter of the spread was measured after the ointment didn't spread again or 1 minute after the load was applied. A good spreading diameter is 5 - 7 cm [11].
4. pH test
   The pH value was determined using a universal pH stick dipped in 0.5 gram of ointment (previously diluted with 5 ml of distilled water). A good pH is 4.5 - 6.5 in ointment [12].
5. Test Irritant Effects
   The irritating effect of the ointment on the skin of volunteers was tested using an open patch test. An open patch test was carried out by applying an ointment to the volunteer's forearm, waiting for 5 minutes and observing the reaction that occurred. There was a positive irritation reaction marked by redness, itching, or inflammation of the skin that was smeared with ointment [13].
6. Induction and Measurement of Mice Wounds
   The back hair of mice was shaved and cleaned with 70% alcohol. Mice were anesthetized using ethyl chloride by spraying it on the skin and waiting for 10 to 30 seconds. Furthermore, the mice skin on the back was injured lengthwise with a wound diameter of ±2-3 cm [14]. The ointment was applied once a day thinly and evenly to the wound area of mice. Measurement of the wound area was carried out every day from day 0 to 7. The wound heals if the wound diameter reaches 0 cm [15]. The percentage of wound healing can be calculated using the equation:

$$P\% = \frac{do - dx}{do} \times 100\%$$

P% : Healing Percentage
do : Wound diameter before treatment
dx : Wound diameter after treatment

Data Analysis

The results of this study were analyzed using SPSS 24.0 by first conducting normality and homogeneity tests. The results of measuring the diameter of the mice's wound after treatment were analyzed using a one-way ANOVA test, followed by a post hoc test (Tukey HSD). The results were considered significant for each group if the p value <0.05 (95%).

RESULTS AND DISCUSSION

Simplicia's Yield

A total of 2.5 kilograms of wet mangosteen rind simplicia (Garcinia mangostana L.) was processed and dried, then 1 kilogram of dried mangosteen rind simplicia was obtained. The dried simplicia was crushed into 600 grams of powdered simplicia. A total of 300 grams of simplicia powder was taken for extraction. Furthermore, 135 grams of viscous extract was obtained, with a yield value of 45%.

Results of Mangosteen Rind Extract Ointment Evaluation

1. Organoleptic Test Results
   The results of organoleptic observations of mangosteen rind extract ointment have a distinctive odor of mangosteen rind. The smell of each concentration of ointment has a different level, an ointment with an extract concentration of 30% has a stronger smell than a concentration of 10% and 20%. While the color of the mangosteen rind extract ointment was almost same as brownish yellow, but the mangosteen peel extract ointment with a concentration of 30% has a more intense yellow color.
2. Homogeneity Test Results
   The three formulations of mangosteen rind extract ointment (concentrations of 10%, 20% and 30%) showed a homogeneous ointment. The results showed that there were no coarse grains or clustered particles after applying the ointment to glass objects, a homogeneous structure and a uniform color from the starting point to the end point.
3. Spreadability Test Results
   The test results showed that mangosteen rind extract ointment had met the spreadability requirements for an ointment, which was between 5 - 7 cm [11]. Each ointment formula with an extract concentration of 10%, 20% and 30% has an average spreadability of 5.1 cm, 5.0 cm and 5.0 cm.
4. pH Value
   The ideal ointment pH ranges from 4.5-6.5 [12]. It was important to know the pH value of topical preparations, so their use does not irritate the skin. Ointments with an acidic pH can irritate the skin, while an alkaline pH makes the skin scaly. The test results showed that all ointment formulas had a pH value within the range of pH requirements of topical preparations. Ointments with mangosteen rind extract concentrations of 10%, 20% and 30% had a pH value of 5.0; 4.7; and 4.5, respectively. The higher concentration of mangosteen rind extract used, the lower pH of the ointment obtained, but the pH value of three ointment formulas was not significantly different.
5. Volunteer Irritation Test
   The irritation test of mangosteen rind extract ointment was carried out on three volunteers in each group. The results showed that mangosteen rind extract ointment did not have an irritating effect on the skin. This was due to the absence of erythema, edema, itching, and burning sensation on the skin of the volunteers.

Effect of Mangosteen Rind Extract Ointment on Mice Cut Wounds Healing

The results of the effectiveness test showed that the mangosteen rind extract ointment had a positive effect on the wound healing of mice. This can be seen from the reduced diameter of the incision wound in mice that were given mangosteen rind extract ointment, at concentrations of 10%, 20% and 30% compared to the negative control group. The results can be seen in Table 2.

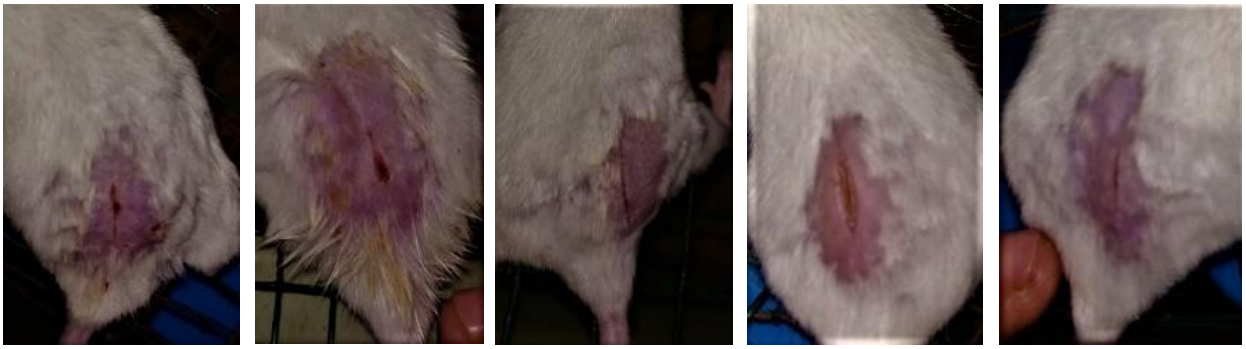
TABLE 2. Results of measuring cut wounds length in mice from days 0 to 7

Day	Diameter of the incision (cm)				
	10% Concentration Extract Group	20% Concentration Extract Group	30% Concentration Extract Group	Negative control	Positive control
D0	2	2	2	2	2
D1	1.9	1.9	1.8	2	1.9
D2	1.7	1.7	1.6	1.9	1.8
D3	1.6	1.5	1.2*	1.9	1.6
D4	1.4	1.2*	1.1*+	1.8	1.4
D5	1,2	1.1*+	0.8**+	1.7	1.3
D6	1+	0.9*+	0.6**+	1.5	1.1+
D7	0.4+	0.3+	0.1*++	0.7+	0.5+

* $p < 0.05$; ** $p < 0.01$ vs negative control group
+ $p < 0.05$; ++ $p < 0.01$ vs day 0
Kontrol (-) : cut wounds given an ointment base (vaseline flavum)
Kontrol (+) : cut wounds given betadine ointment

The normality and homogeneity test results showed data was normally distributed and homogeneous. Analysis using ANOVA showed positive results, the diameter of cut wound in the treatment group that was given mangosteen rind extract ointment decreased, especially in the 20% and 30% treatment group. In the 20% treatment group, there was a significant reduction in wound diameter seen from day 4 when compared to the negative control group, meanwhile in the 30% treatment group was significant starting from day 3. The wound then gradually became smaller until day 7. These results indicate that the difference concentration of the mangosteen rind extract ointment was quite influential, where better wound healing results are seen with the higher concentration of the mangosteen rind extract used (Figure 1).

The wound healing process occurs in three phases, namely the inflammatory phase, the proliferative phase, and the maturation phase. The inflammatory phase occurs on days 1 to 3, the proliferative phase occurs on days 4 and 5, while the maturation phase when the wound diameter decreases occurs on days 6 to 7. During the maturation phase, there are dynamic processes including contraction in the wound and maturation of scar tissue. Furthermore, new skin tissue that resembles the initial tissue is also formed [3, 16]. Treatment of wounds in this study used mangosteen rind extract ointment by giving it once a day. The healing of the wound is indicated by the tightening of the skin, the wound dries up and the scab around the wound begins to peel off by itself [17].



(a) (b) (c) (d) (e)

FIGURE 1. Measurement of the diameter of cut wounds in mice treated with mangosteen rind extract ointment with various concentrations on the 7th day, (a) 10% concentration extract ointment group, (b) 20% concentration extract ointment group, (c) 30% concentration extract ointment group, (d) negative control group, (e) positive control group

The results also showed that treatment with mangosteen rind extract ointment affected the speed of wound healing in mice skin. Healing time was significantly different from day 3 to day 7 when compared to day 0 (Table 2, Figure 1). The percentage of wound healing for the ointment extract group with concentrations of 10%, 20% and 30% were 80%, 85% and 95%, respectively. Meanwhile, the negative and positive control groups had healing percentages of 65% and 75%, respectively. The percentage of wound healing showed that mice treated with mangosteen rind extract ointment with a concentration of 30% had a faster wound healing time than the control group.

The percentage of wound healing in mangosteen rind extract ointment for 7 days of treatment has reached 50%, because mangosteen rind contains lots of flavonoids as active wound healing components namely xanthone, α-Mangostin and 8-Deoxygartanin which function as anti-inflammatory, antibacterial and antioxidant. These flavonoid components also support the occurrence of angiogenesis in the wound healing process and can accelerate the cell recovery process by accelerating the process of fibroblast proliferation [18, 19, 20].

CONCLUSION

In this study, mangosteen rind extract ointment (Garcinia mangostana L.) proved effective in accelerating the healing of cut wounds in mice. The most optimal ointment with the ability to heal cut wounds in mice for 7 days is mangosteen rind extract ointment with a concentration of 30%.

REFERENCES

[1] Nagori B.P. and Solanki R., 2011, Role of Medicinal Plants in Wound Healing, Research Journal of Medicinal Plant, 5 (4), 392–405.
[2] Thakur R., Jain N., Pathak R. and Sandhu S.S., 2011, Practices in Wound Healing Studies of Plants, Evidence-based Complementary and Alternative Medicine, 2011 (438056), 1–17.
[3] Prasetyono T.O.H., 2009, General Concept of Wound Healing, Revisited, Medical Journal of Indonesia, 18 (3), 208–216.
[4] Magallanes. B. O., Perez, D. E., Chaverri, J. P. 2017. Medicinal Properties of Mangosteen (*Garcinia mangostana* L.): A Comprehensive Update. Food and Chemical Toxicology 109, 102-122.
[5] Negi, J. S., Bisht, V. K., Singh, P., Rawat, M. S. M., Joshi, G. P. 2013. Naturally Occurring Xanthones: Chemistry and Biology. Journal of Applied Chemistry vol. 2013, 1-9
[6] Wulandari, P.A.C., Ilmi, Z.N., Husen, S.A., Winarni, D., Alamsjah, M.A., Awang, K., Vastano, M., Pellis, A., MacQuarrie, D. and Pudjiastuti, P., 2021. Wound Healing and Antioxidant Evaluations of Alginate from Sargassum ilicifolium and Mangosteen Rind Combination Extracts on Diabetic Mice Model. *Applied Sciences*, *11*(10), p.4651.
[7] Mardiana, Lina. 2011. Ramuan dan Khasiat Kulit Manggis. Penebar Swadaya. Jakarta.
[8] Kurniawan, D. W. and Sulaiman, T. N. S. 2009. Teknologi Sediaan Farmasi. Yogyakarta: Graha Ilmu.
[9] Agoes, Goeswin. 2006. Pengembangan Sediaan Farmasi Edisi Revisi. Bandung: ITB Bandung.
[10] Kementerian Kesehatan RI. 2020. Farmakope Indonesia Edisi VI. Jakarta: Kementerian Kesehatan Republik Indonesia.
[11] Astuti, I. Y., Hartanti, D., Aminiati, A. 2010. Peningkatan Aktivitas Anti Jamur *Candida albicans* Salep Minyak Atsiri Daun Sirih (*Piper bettle*) melalui Pembentukan Kompleks Inklusi dengan β-siklodekstrin. *Majalah Obat Tradisional.* Vol. 15: 94-99.
[12] Naibaho, D. H., Yamkan, V. Y., Weni, W. 2013. Pengaruh Basis Salep Terhadap Formulasi Sediaan Salep Ekstrak Daun Kemangi (*Ocinum sanchum* L.) Pada Kulit Punggung Kelinci yang dibuat Infeksi *Staphylococcus aureus*. Jurnal Ilmiah Farmasi UNSRAT. Vol. 2(2).
[13] Laras, A.A.I.S., Swastini, D.A., Wardana, M., Wijayanti N.P.A.D. 2014. Uji Efek Iritasi Ekstrak Etanol Kulit Buah Manggis (*Garcinia mangostana* L.). *Jurnal Farmasi Udayana*. Vol. 3(1): 74-77.

[14] Esimone, C.O., Ibezim, E.C., Chah, K. 2006. The wound healing effect of herbal ointments formulated with Napoleona imperialis. *J Pharmaceutical and Allied Science.* Vol. 3(1): 294-299.
[15] Pongsipulung G. R., Yamlean, P. V. Y., Banne, Y. 2012. Formulasi dan Pengujian Salep Ekstrak Bonggol Pisang Ambon (*Musa paradisiaca* var. *sapientum* (L.)) Terhadap Luka Terbuka Pada Kulit Tikus Putih Jantan Galur wistar (*Rattus norvegicus*). *Pharmacon*. Vol. 1(2): 7-13.
[16] Magallanes. B. O., Perez, D. E., Chaverri, J. P. 2017. Medicinal Properties of Mangosteen (Garcinia mangostana L.): A Comprehensive Update. *Food and Chemical Toxicology*. Vol.109: 102-122.
[17] Chaverri, Jose Pedraza, et al. 2008. Medicinal Properties of Mangosteen. *Food and Chemical Toxicology*. Vol. 46: 3227-3239.
[18] Sampah P and Vijayaragavan K. 2008. Ameliorative prospective of alpha-mangostin, a xanthone derivative from Garcinia mangostona against beta-adrenergic cathecolamine-induced myordial toxicity and anomalous cardial TNF-alpha and COX-2 expressions in rats. *Exp Toxicol Pathol*. Vol. 60: 357-367.
[19] Maulina, L., Sugihartini, N. 2015. Formulasi Gel Ekstrak Kulit Buah Manggis (*Garcinia manggostana L)* dengan Variasi *Gelling Agent* Sebagai Sediaan Luka Bakar. *Pharmaqiana*. Vol. 5(1) : 43-52.
[20] Wulandari PAC, Ilmi ZN, Husen SA, Winarni D, Alamsjah MA, Awang K, Vastano M, Pellis A, MacQuarrie D, Pudjiastuti P. Wound Healing and Antioxidant Evaluations of Alginate from *Sargassum ilicifolium* and Mangosteen Rind Combination Extracts on Diabetic Mice Model. *Applied Sciences*. 2021; 11(10):4651