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Gel Formulation of Ethanol Extract of Mangosteen Peel (Garcinia mangostana L.) as A Medication for Burns in Wistar Rats

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ABSTRACT

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Background: Mangosteen peel (Garcinia mangostana L.) has been shown to stimulate the regeneration of damaged body cells and have an anti-microbial activity that can be beneficial in healing burns. Therefore, it is necessary to make it into a gel dosage form that has the advantages of being easily washed with water, high adhesion, cooling of the skin, and good drug release.

Objective: To determine the effect of varying concentrations of ethanol extract of mangosteen peel in a gel formulation on wound healing in burns and the physical properties of the gel.

Methods: Mangosteen peel extract was obtained by maceration method using ethanol 70%. This extract was formulated in a gel dosage form with variations of extract concentration, namely F I (5%), F II (10%), and F III (15%). The gels were then tested for their physical properties (organoleptic, homogeneity, pH, spreadability, adhesiveness, and physical stability) and their activity against burns experimentally using mice as test animals. Burns were made by using a hot plate with an area of 2 cm x 2 cm.

Results: The increase of extract concentration could increase spreadability ($p>0.05$), adhesiveness ($p>0,05$), and percentage of healing activity against burns as shown by a significant difference between FI and FIII. On the other hand, the difference in the concentration of the extract did not affect pH (all formulations have pH of 3.5); all formulas also remained homogenous and stable after centrifugation.

Conclusion: The best physical properties and wound healing activity for burns was shown by the gel with the ethanol extract of mangosteen peel at a concentration of 15%.

Latar Belakang: *Kulit buah manggis telah terbukti mempunyai aktivitas menstimulasi regenerasi sel tubuh yang rusak dan sebagai antimikroba sehingga dapat digunakan untuk penyembuh luka bakar. Oleh karena itu perlu dibuat sediaan gel yang memiliki keuntungan mudah dicuci dengan air, daya lekat tinggi, memberikan rasa dingin pada kulit, dan pelepasan obatnya baik.*

Tujuan: *Mengetahui pengaruh variasi konsentrasi ekstrak etanol kulit buah manggis dalam formulasi gel terhadap daya penyembuhan luka bakar dan sifat fisik gel.*

Metode: *Ekstrak kulit buah manggis diperoleh dengan metode maserasi menggunakan pelarut etanol 70%. Ekstrak diformulasi dalam bentuk sediaan gel dengan variasi konsentrasi ekstrak meliputi F I (5%), F II (10%), dan F III (15%). Gel kemudian diuji sifat fisiknya (organoleptik, homogenitas, pH, daya sebar, daya lekat dan stabilitas fisik) dan aktivitasnya terhadap luka bakar secara eksperimental menggunakan*

tikus sebagai hewan uji. Luka bakar dibuat dengan menggunakan lempeng panas dengan luas 2 cm x 2 cm.

Hasil: *Hasil menunjukkan bahwa peningkatan konsentrasi ekstrak dapat meningkatkan daya sebar dengan nilai p sebesar 0,383 (p>0,05), daya lekat dengan nilai p sebesar 0,000 (p<0,05) dan kemapuan menyembuhkan kulit yang mengalami luka bakar menunjukkan adanya perbedaan signifikan antara FI dan FIII. Disisi lain perbedaan konsentrasi tidak mempengaruhi pH (semua formulasi memiliki pH 3,5) semua formulasi homogen dan tetap stabil setelah mengalami perlakuan sentrifugasi.*

Kesimpulan: *Konsentrasi ekstrakyang memberikan sifat fisik gel dan daya sembuh luka bakar terbaik adalah gel dengan konsentrasi ekstrak etanol kulit buah manggis sebesar 15%.*

INTRODUCTION

Burns are a form of damage or loss of tissue caused by contact with heat sources, such as fire, hot water, chemicals, electricity, and radiation. Damage caused by burns varies, ranging from mild pain and red skin to charring of the victim's body. Considering these varied abnormalities, burns are classified based on the severity of damage, namely first, second, and third degree burns. The depth of the burn depends on the temperature of the heat and the duration of contact with the high temperature.[1] Nowadays, a lot of natural ingredients are studied to determine their efficacy as medications for burns. One of these natural ingredients is the peel of mangosteen fruit.

The main compound contained in the peel of mangosteen fruit is xanthone, which is, in fact, responsible for some of its pharmacological activities. Inside the peel of mangosteen fruit, there is an important element that is beneficial in wound healing, called gamma-mangostin. This contained gamma-mangostin is very important in stimulating the formation of collagen in structure maintenance and wound healing actions.[2] Moreover, mangosteen peel also contains other compounds with anti-inflammatory activities, such as flavonoids, vitamin B1, B2, C, saponins, and tannins that can accelerate wound healing as well.[3]

Based on the above description, it is necessary to conduct a research to formulate a gel preparation of the extract of mangosteen peel in various concentrations in order to determine the concentration that presents the best physical properties and ability to heal skin that suffers from burns.

METHODS

Types of research

Tools and materials

The tools used in this research consist of rotary evaporator (Heidolph), glassware, heat inducer, rat fur shaver, scale (Ohaus), water bath (memmert), scaled glass plate, spreadability test kit, adhesiveness test kit, pots for gels, and pH paper.

The material used in this research consist of mangosteen peel (Garcinia mangostana L.) obtained from Beringharjo Market, Yogyakarta with the condition of the fruit being ripe and extracted using 70% ethanol. Gel materials used pharmaceutical quality, namely carbopol, glycerin, propylene glycol, and aquadest. Bioplacenton^{R} was used as positive control, whereas male rats weighing between 150-200 grams and aged between 2-3 months were used as test subjects for burns.

Methodology

Preparation of Ethanol Extract of Mangosteen Peel

Four hundred grams of mangosteen peel powder was macerated with 1.6 L of 70% ethanol solvent for 24 hours along with stirring. It was filtered afterward using vacuum and filter paper to separate the precipitate from the filtrate. The precipitate was further macerated and the result was combined with another solution of maceration product before evaporated using a rotary evaporator at 60°C. The evaporated condensed extract was poured into a container and stored with aluminum foil covering.[4] The yield of the ethanol extract of the mangosteen fruit peel obtained was 27,13%.[5]

Formulation of Mangosteen Fruit Peel Gels

The extract was formulated into gel dosage

forms with varying concentrations of the extract, namely F I (5%), F II (10%), and F III (15%) as presented in Table 1 in reference to the results of a previous study.[6]

Table 1. Gel Formulations in the Study

Formulations (in grams)	Base Gel	F I	F II	F III
Ethanol extract of mangosteen peel	-	6	12	18
Carbopol	6	6	6	6
Glycerin	12	12	12	12
Propylene glycol	6	6	6	6
Methylparaben	0,18	0,18	0,18	0,18
Aquadest ad	120	120	120	120

Annotations:
Base Gel : Gel without ethanol extract of mangosteen peel
F I : Gel with concentration of ethanol extract of mangosteen peel at 5%
F II : Gel with concentration of ethanol extract of mangosteen peel at 10%
F III : Gel with concentration of ethanol extract of mangosteen peel at 15%

Preparation of the gel began with the development of carbapol in 10 ml of water at 70oC with the addition of the extract, creating mixture 1. Methylparaben was dissolved in a little water with the addition of a mixture of glycerin and propylene glycol, creating mixture 2. The two mixtures were combined and stirred as 20 grams of water was added, then mixed homogeneously.

Evaluation of the Physical Properties of Mangosteen Peel Extract Gels

The three gels were evaluated for their physical properties. The evaluation consists of organoleptic, homogeneity, pH, spreadability, and physical stability tests.

Organoleptic Test

Observations are performed directly on the shapes, colors, and smells of the gels. The gels were mostly clear with half-solid consistency.[7]

Homogeneity Test

Samples of the gels were applied to a piece of glass or other suitable transparent material. The preparation was required to show a homogeneous construction with no visible crude grains.[8]

PH Test

Universal pH sticks were dipped into diluted samples of the gels. Once they were completely immersed, the change in the color of the universal pH was observed and matched with the universal pH standard.

Spreadability Test

A sample of 0.5 grams was taken from each gel to be placed on a 15 cm diameter round glass with another glass placed on top of it then left for 1 minute. The diameter of the gel spread was measured. An extra 150 grams of load was added afterward before it was allowed to stand for another 1 minute and measured for its constant diameter.[9]

Adhesiveness Test

Gel weighing 0.25 g was placed between object glasses then pressed by a weight of 1 kg for 5 minutes. After that, the load was lifted and another load of 80 g was given to the adhesiveness test tool, then the time required for the 2 object glasses to separate was recorded.[10]

Physical Stability Test

Physical stability was observed based on the separation between the gelling agent and its carrier, which was water. The test was performed using centrifugal test where the samples of the gels were centrifuged at 3800 rpm for 5 hours then observed for any changes in their physical attributes.[11]

Test for Healing Activities against Burns

Test of the healing activities of the mangosteen peel extract gels was performed experimentally using mice as test animals. The test was conducted by injuring the mice on their backs using a heat inducer in the form of a hot metal plate with an area of 2 cm x 2 cm at a temperature of 80°C for 8 seconds. The mice were divided into 7 groups for this test with each group receiving treatments as presented in table 2. This study used 28 male Wistar rats and each treatment group had 4 replications.

Table 2. Treatment Groups for Healing Activity Test in Burns

Groups	Treatment	N
Negative Control	Induced with burns and given no gel	4
Positive Control	Induced with burns and given Bioplacenton^{R}	4
Extract	Induced with burns and given pure extract	4
Basis	Induced with burns and given base gel	4
Formula I	Induced with burns and given formula I	4
Formula II	Induced with burns and given formula II	4
Formula III	Induced with burns and given formula III	4

Each wound created was smeared with a test preparation that weighed 350 mg. The smearing was done twice a day (once in the morning and once in the evening) until the wound was healed (the diameter of the wound is considered zero when the wound is completely covered by new tissue). Measurement of burned area was performed every week for 1 month.

STATISTICAL ANALYSIS

Data of statistical test and wound healing activities in burns were analyzed using One-way ANOVA with 95% confidence level and continued with T-test.

RESULTS

Results of Test for Physical Properties of Ethanol Extract of Mangosteen Peel

Results of the test for physical properties are presented in Tables 3 and 4.

Table 3. Results of Organoleptic Test of Ethanol Extract of Mangosteen Peel Gel

	Base Gel	F I	F II	F III
Consistency	Homogenous	Homogenous	Homogenous	Homogenous
Color	Clear	Brown +	Brown ++	Brown +++
Odor	Gel	Specific +	Specific +	Specific +

Results of the organoleptic test showed that the color and odor of the four formulas were different. The base gel had a different color from that of the one containing the ethanol extract of mangosteen fruit peel. The base gel appeared clear because it did not contain the ethanol extract of the mangosteen peel, whereas the color of the gel F I, F II, and F III appeared brown because it contained the ethanol extract of the mangosteen peel. Higher concentration of the extract also increased the intensity of the color.

Table 4. Results of Physical Properties Test of Ethanol Extract of Mangosteen Peel in Different Formulas

Test	Base Gel	F I	F II	F III
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
pH	2.5	3.5	3.5	3.5
Spreadability (cm2)	5.53	4.65	4.68	5.35
Adhesiveness (seconds)	12.50	6.00	14.26	37.18
Physical stability	Stable	Stable	Stable	Stable

Results of Test for Healing Activities Against Burns

The results of calculation of the percentage of burn injury in the 4th week is presented in Figure I. The data obtained was tested statistically afterward using paired T-test to know the

differences between the percentages wound healing areas in Wistar rats among treatment groups as presented in Table 5.

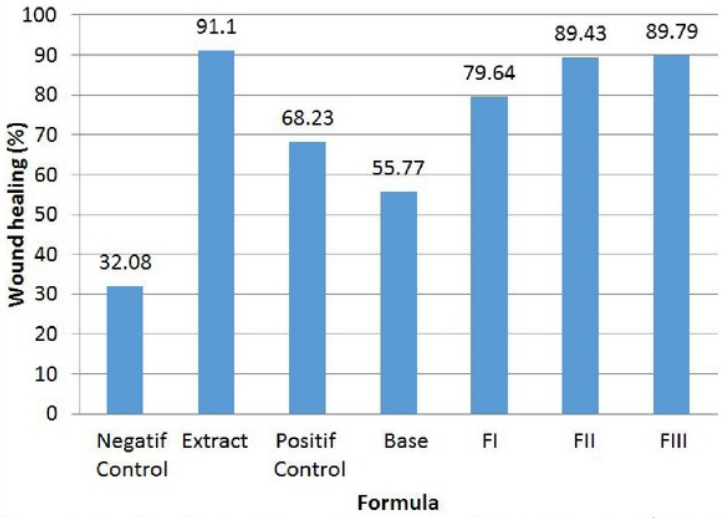

Figure I. Results of Calculation of Percentage of Burn Injury in 4th Week

Table 5. Results of Statistic T-test for Healing Efficacy against Burns

Treatment	Significancy	Conclusion
Negative Control – F I	0.042	Negative Control < F I
Negative Control – F II	0.025	Negative Control < F II
Negative Control – F III	0.031	Negative Control < F III
F I – F II	0.144	F I = F II
F I – F III	0.010	F I < F III
F II – F III	0.589	F II = F III
Extract – F I	0.005	Extract < F I
Extract – F II	0.104	Extract = F II
Extract – F III	0.057	Extract = F III
Positive Control– F I	0.031	Positive Control < F I
Positive Control – F II	0.001	Positive Control < F II
Positive Control – F III	0.020	Positive Control < F III

DISCUSSION

The results of physical properties of gel showed that the base gel appeared clear because it did not contain the ethanol extract of the mangosteen peel, whereas the color of the gel F I, F II, and F III appeared brown because it contained the ethanol extract of the mangosteen peel. Higher concentration of the extract also increased the intensity of the color. Results of the homogeneity test showed that all of the preparations produced, namely base gel, F I, F II and F III, were homogeneous as indicated by the absence of any visible crude grains on the observation glass.

In this study, the pH of the base gel was 2.5, whereas the pH of F I, F II and F III was 3.5. The addition of the extract into the base can make the pH more alkaline because the pH of the extract is

5.3012. This explains why the gel that contained the extract of the mangosteen peel was more alkaline than the base. The pH of the preparations did not meet the requirements to match the pH of the skin preparation that is between 4.5-6,513. In this study, the concentration of the extract added was up to 15%. However, it turned out to not affect the pH of the gel. Further research is needed to determine the concentrations of extract that can produce a pH close to the pH of the skin.

Results of the spreadability test showed that the spreadability of mangosteen peel extract gel has an area of the spread between 4.65-5.35 cm^2. The range of area of good gel spreadability is between 5-7 cm.[14] Among the preparations used in this study, F III was the only one that fulfilled this requirement. In comparison to that of the base, the addition of extract tends to reduce the spreadability of the gel. Increased concentration of the extract added into the base increased the consistency of the gel and thus, decreased the spreadability. On the other hand, increased concentration of the extract increased the spreadability, but statistic test result showed no significant difference as shown by the p value of 0.383 ($p>0.05$). This means that the addition of mangosteen peel extract does not affect the gel power.

The adhesiveness test was performed in order to determine the ability of the ethanol extract formula of mangosteen peel to stay attached to the skin upon application. The longer the gel is attached to the skin, the more effective it will be for healing burns. However, if attached for too long to the skin, the gel will be difficult to remove. Increased concentration of extracts increases the adhesiveness of the gel. This is because the greater consistency of the gel, the stronger its adhesiveness will be. Statistically, the adhesiveness of the gel formulations showed significant differences as indicated by the p value of 0.000 ($p<0.05$). This means the addition of mangosteen peel extract in various concentrations affects the adhesiveness of the gel. The results of this adhesiveness test are in accordance with the results of other studies showing that the addition of mangosteen peel extract in the base gel with carbopol as the gelling agent could increase its adhesiveness.[15]

The purpose of the physical stability test of the gels is to determine the stability of mangosteen peel extract gel. The results of the test on the four formulas showed that they were stable or did not undergo any physical changes or separations after centrifugation at 3800 rpm for 5 hours. This means that all of the gel preparations were not affected by gravity.

In this study, a test on the healing effects of the mangosteen peel ethanol extract gel against burns was carried out to determine the formula that provides the fastest healing effects against burns. The results of the test showed that the gel formula of ethanol extract of mangosteen peel containing 15% concentration of the extract had a rapid healing effect against burns as indicated by the rapid formation of new tissue.

Results of the T-test showed a significant difference between the negative controls and F I, F II, F III. This demonstrates the potential of ethanol extract of mangosteen peel in healing burns. As for the mechanism, the healing of burns is due to the presence of gamma-mangostin in mangosteen peel that stimulates the formation of collagen which plays an important role in the actions of structural maintenance and wound healing.[2] This is supported by the data of which mangosteen peel extract showed the highest percentage of wound healing in burns.

The formulation of the extract in gel form does not affect the activity of the extract as a healer for burns. This is indicated by the insignificant differences between extract groups and F II as well as F III. This proves that the release of the active substances from the base gel happened smoothly. Although there is an insignificant difference between extract groups and FI, this was actually due to the low level of active substances contained in the gel so that the active substances released were also small in amount and thus, presented a smaller percentage of healing area in comparison to that of the extract groups. This is supported by the statistical test results between the positive controls (healing

products for burns on the market) and F I, F II, F III that resulted in a significant difference with a wider percentage of the healing area. This means that the healing ability of F I, F II and F III against burns is better than that of the positive control groups.

Different concentrations turned out to give significant differences in the comparison between F I and F III. This means that the varying concentrations of the extract affect the percentage of wound healing of burns in Wistar rats. Increased concentration also increase the percentage of wound healing area. The concentration already provided healing capacity against burns that equals to that of the positive controls or products that have been available on the market.

Related studies on the formulation of mangosteen peel extract as a medication for burns showed that healing capacity were best demonstrated by gel with carbapol as the gelling agent.[15] Likewise, this study used gels that were formulated with carbapol as the gelling agent. Related studies on the effect of active substance concentration on healing activities in burns was conducted in a research on gel formulation of Chinese petai leaf extract as a medication for burns. Increased concentration of Chinese petai leaf extract increased the healing power of the gel against burns as indicated by the shorter healing time of burns. The extract concentration of 30% showed a healing time that was not significantly different from that of the positive control.[16]

CONCLUSIONS

1. Higher concentration of ethanol extract of mangosteen peel in the gel causes organoleptic differences, greater spreadability ($P>0.05$), and longer adhesiveness ($P<0.05$), but it do not affect the homogeneity, pH, and stability of the ethanol extract of mangosteen peel gel.

2. Higher concentration of ethanol extract gel of the mangosteen peel provides better healing efficacy against burns.

3. The highest healing activity was shown in the formula containing a concentration of ethanol extract of mangosteen peel of 15%.

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