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Analysis of Wound Healing After Tooth Extraction from Ethanol Extract of Mangosteen Fruit Peel (Garcinia Mangostana L.) in Wistar Rats (Rattus Norvegicus)

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Abstract: **One of the natural ingredients that have the potential to serve as such an alternative is mangosteen peel. Mangosteen peel is a shell that is discarded by consumers or can be called agricultural waste. So far, the utilization of mangosteen peels is only for tanning leather, traditional medicine, and the making of anti-rust substances and textile dyes. The utilization of mangosteen rind for treatment in Indonesia is still not much, especially as a wound healing. Therefore, researchers are interested in exploring the wound-healing effects of mangosteen peel extract. This experimental laboratory study used a complete randomized design with a post-test-only control group design pattern. The experimental animals used in this study were 32 male Wistar rats, physically healthy, 2-3 months old, with body weight between 200-250 grams. The sample size was determined by the Federer formula, namely: (t - 1) (r - 1) ≥ 15, and the minimum sample size for each treatment was 16 rats. The results showed a significant relationship between the number of fibroblast tissue per visual field in Wistar rats after tooth extraction with the administration of Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50% concentration and Ethanol Mangosteen fruit peel (Garcinia mangostana L) 100% concentration, p=0.008 (p<0.05). In conclusion, Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50% and 100% are effective in accelerating wound healing time after tooth extraction of Wistar rats. Ethanol Mangosteen fruit peel (Garcinia mangostana L) 100% is more effective than Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50% in accelerating wound healing time after tooth extraction of Wistar rats because the flavonoid content in Ethanol Mangosteen fruit peel (Garcinia mangostana L) 100% which helps accelerate wound healing is higher than Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50%.**

Keywords: **Garcinia mangostana L, Wound healing, Tooth Extraction, Ethanol.**

I. INTRODUCTION

The World Health Organization (WHO) recommends using traditional medicine, including herbs, in public health maintenance, disease prevention, and treatment, especially for chronic diseases, degenerative diseases, and cancer (1). Tooth extraction will cause an open wound in the oral cavity; the severity of the injury depends on the amount of trauma the tissue receives (2). Routine wound healing is a complex and dynamic process. The wound healing process can be divided into three main phases, namely, the inflammatory phase, the proliferation phase, and the remodeling phase. These phases continue from the onset of the wound until wound closure. The inflammatory phase is the body's reaction to the damage that starts after a few minutes and lasts about three days after the injury. The proliferation phase is characterized by the

appearance of new blood vessels resulting from reconstruction and occurs within 3-24 days. The maturation phase is the final stage of the wound-healing process. This process can take up to 1 year, depending on the depth and extent of the wound (3).

Fibroblasts are stem cells that form and lay down fibers in the matrix, especially collagen fibers. They secrete small tropocollagen molecules that combine with the primary substance to form collagen fibers. Collagen will provide strength and integrity to any well-healed wound. Fibroblasts more actively synthesize matrix components in response to the damage by proliferating and increasing fibrinogenesis. Therefore, fibroblasts are the leading agents in the wound-healing process (4). Modern research also shows that herbal medicine is effective for health and does not cause side effects like chemical drugs. From the facts above, developing herbal medications that tend to have lower side effects and be safer becomes essential. One of the natural ingredients that have the potential to serve as such an alternative is mangosteen peel. Mangosteen peel is a shell that is discarded by consumers or can be called agricultural waste. So far, the utilization of mangosteen peels is only for tanning leather, traditional medicine, and the making of anti-rust substances and textile dyes (5); (6). The utilization of mangosteen rind for treatment in Indonesia is still not much, especially as a wound healing. Therefore, researchers are interested in exploring the wound-healing effects of mangosteen peel extract.

II. RESEARCH METHODS

This experimental laboratory study uses a randomized controlled design with a post test only control group design pattern. This research will be conducted at the Pharmacology Laboratory & Traditional Medicine Laboratory, Faculty of Pharmacy, University of North Sumatra, and the Anatomical Pathology Laboratory, Faculty of Medicine, the University of North Sumatra, from September to December 2023. The experimental animals used in this study are Wistar rats, 32 males, physically healthy, 2-3 months old, with a body weight between 200-250 grams. The rats will be divided into two groups, namely, 16 treated with 50% Ethanol Mangosteen fruit peel (Garcinia mangostana L) and 16 treated with 100% Ethanol Mangosteen fruit peel (Garcinia mangostana L) to see the comparison of accelerated wound healing after tooth extraction. The sample size was determined by the Federer formula, namely: (t - 1) (r - 1) ≥ 15. Where t = several treatments; (2 treatments) r = several replications. Thus, the minimum sample size for each treatment was 16 rats.

$= (t-1)\ (r-1) \geq 15$

$= (2-1)\ (r-1) \geq 15$

$= (r-1) \geq 15$

$= (r-1) \geq 15$

$= r \geq 15 + 1$

$= r \geq 16$

Tools

Tools used in research :

1. Number-coded experimental animal cages.
2. Diagnostic set (mouth glass, sonde, tweezers).
3. Nierbeken.
4. Dental extraction forceps (in this case a needle holder is used) under sterile conditions.
5. Syringe.
6. Gloves.
7. Mask.
8. Petri dish of jaw preparation.
9. A set of tools for making histology preparations.
10. Microscope.

Material

Materials used in the study:

1. Ethanol Mangosteen fruit peel (Garcinia mangostana L) Extract 50%
2. Ethanol Mangosteen fruit peel (Garcinia mangostana L) Extract 100%
3. Ketamine.
4. Formalin 10%.
5. Histology preparation material with Hematoxylin Eosin (HE) staining.
6. 70% alcohol as sterilization material.
7. Cotton pellet.

Data Type

The type of data collected in this study is primary data obtained from the results of measurements (scoring) on the histological picture of the process of accelerating wound healing after tooth extraction by administering Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50% and Ethanol Mangosteen fruit peel (Garcinia mangostana L) 100%.

Extraction on Ethanol Mangosteen fruit peel (Garcinia mangostana L)

Collecting 3 kg of turmeric, the turmeric was washed and divided into two parts to take the inner flesh to obtain the gel. After washing, the turmeric flesh was dried in an incubator at 500 for 72 hours. The dried turmeric meat was then pulverized using a blender to form a powder. The turmeric meat that has become powder is then extracted by maceration with stirring. The extraction process uses water solvent. The powder is put into a maceration vessel or impermeable lid container and then filtered using filter paper; the pulp is macerated up to 2 times. The obtained maceration results were collected and evaporated using a rotary vacuum evaporator at 50oC until there was no more solvent condensation on the condenser. After the solvent is evaporated using a rotary vacuum evaporator, evaporation is continued using a 70-degree temperature water bath to obtain a pure extract. The turmeric extract was then diluted with water to get 50% and 100% extract concentrations.

Treatment of Wistar Rats

1. Before treatment, 32 rats were divided into 50% turmeric extract and 100% turmeric extract. After that, all rats were adapted for one week. Then, animals were put into cages, with five rats in each cell in the same environmental conditions, given the same food, and monitored for health.

2. Rat tooth extraction will be performed using a modified needle holder under the anesthetic effect of ketamine 1000 mg/10 ml at a dose of 20 mg/kg bw intraperitoneally.

3. One incisor tooth will be extracted from every five rats daily.

4. After tooth extraction, observe the extraction wound and apply a tampon (cotton pellet) to stop bleeding in the wound for 5 minutes.

5. Dropped Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50% in treatment group I and dropped Ethanol Mangosteen fruit peel (Garcinia mangostana L) 100% in treatment group II shortly after tooth extraction as much as 0.05 ml every day.

6. After extraction and treatment, the test animals (rats) were fed fine porridge with attention to the health of the test animals.

7. On the 5th day after tooth extraction, rats from each group were physically sacrificed by neck dislocation. The rat's tail was held and then placed on a surface it could reach. The rat will stretch its body; when the rat's body extends, a holder held by the left hand is placed on the nape of the neck. The right hand pulls the tail hard so the rat's neck will be dislocated. Then the jaw of the rat is taken out.

8. Then the tissue was fixed with 10% formalin for 24 hours at room temperature, then the decalcification process was carried out using Ethylene Diamine Tetra Acetic Acid (EDTA 10%) solution at room temperature.

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9. Tissue dehydration was then performed using alcohol. First, the specimen was put into toluol alcohol solution (1:1) using pure toluol, then into a paraffin-saturated toluol solution.

10. The following process is infiltration in the oven by inserting the specimen into liquid paraffin.

11. The embedding process is carried out (inserting the tissue into paraffin) and then labeled/coded. After the embedding stage, the tissue is sliced in series with a thickness of approximately 6 microns using a microtome.

12. Evaluating fibroblast cell response using Hematoxylin Eosin (HE) staining. The procedure that must be done is deparaffinization using xylol and alcohol solution, then continued with the rehydration process with alcohol. After that, it is washed with running water, rinsed with distilled water, and then wiped. The glass slide was then placed in Meyer's hematoxylin solution, washed with running water, and then rinsed with distilled water, after which the staining was assessed under a light microscope. If the staining has been considered good, proceed to the next step, namely the dehydration process with alcohol in stages, and then wipe.

13. The next step, put it into xylol solution, and the object glass was covered with deck glass and observed using a light microscope.

14. Fibroblast density was assessed by counting the fibroblasts in 5 fields of view.

Histopathology Scoring Parameters for Fibroblast Counts

Histopathology scoring parameters to determine the distribution of fibroblast tissue is done based on the field of view is:

1. (-) = no fibroblast tissue found
2. (+) = small number of fibroblasts (less than 10% per field of view)
3. (++) = moderate amount of fibroblast tissue (10%-50% per field of view)
4. (+++) = large amount of fibroblast tissue (50%-100% per field of view) 4.

Data Analysis Method

Data analysis using the SPSS 16 program. Research using a pure experiment with a nonparametric Chi-Square Test, after testing, showed that ($p < 0.05$) means there is a significant difference between groups.

III. RESULTS AND DISCUSSION

Table 1. Distribution and Frequency Data of Fibroblast Tissue Counts Per Field of View After Tooth Extraction

NO	Number of Fibroblasts	Ethanol extract of mangosteen fruit peel (Garcinia mangostana L)			
		Concentration 50%		Concentration 100%	
		n	%	n	%
1	No fibroblast tissue found	0	0%	0	0%
2	A small number of fibroblasts (less than 10% per field of view)	7	22%	4	13%
3	Moderate amount of fibroblast tissue (10%-50% per field of view)	5	16%	6	19%
4	A large amount of fibroblast tissue (50%-100% per field of view).	4	13%	6	19%

From Table 1, it can be seen that all samples found fibroblast tissue in the administration of 50% and 100% Ethanol extract of mangosteen fruit peel (Garcinia mangostana L) after tooth extraction of Wistar rats. The number of fibroblasts found in the small category (less than 10% per field of view) on the administration of 50% Ethanol extract of mangosteen fruit peel (Garcinia mangostana L) after tooth extraction of Wistar rats was 7 (22%) and on the administration of 100% Ethanol extract of mangosteen fruit peel (Garcinia mangostana L) was 4 (13%). The number of fibroblasts found in the moderate category (10%-50% per field of view) on the administration of mangosteen fruit peel Ethanol extract (Garcinia mangostana L) 50% after tooth extraction of Wistar rats as many as 5 (16%) heads and on the administration of mangosteen fruit peel Ethanol extract (Garcinia mangostana L) 100% as many as 6 (19%) heads. The number of fibroblasts found in the many categories (50% - 100% per field of view) on the administration of mangosteen fruit peel Ethanol extract (Garcinia mangostana L) 50% after tooth extraction of Wistar rats as many as 4 (13%) heads and on the administration of mangosteen fruit peel Ethanol extract (Garcinia mangostana L) 100% as many as 6 (19%) heads.

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Table 2. Relationship between the number of tissue fibroblasts per field of view in Wistar rats after tooth extraction with turmeric extract concentrations of 50% and 100%

Number of Fibroblasts	Ethanol extract of mangosteen fruit peel (Garcinia mangostana L)		
	Concentration 50%	Concentration 100%	p
1. No fibroblast tissue was found	0	0	0,012*
2. A small number of fibroblasts (less than 10% per field of view)	7	4	
3. Moderate amount of fibroblast tissue (10%-50% per field of view)	5	6	
4. A Large fibroblast tissue (50%-100% per field of view).	4	6	

Significant p<0.05. Chi Square Test

From Table 2. it can be seen that there is a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by administering Ethanol Mangosteen fruit peel (Garcinia mangostana L) with a concentration of 50% and Ethanol Mangosteen fruit peel (Garcinia mangostana L) with a concentration of 100%, p = 0.012 (p <0.05).

IV. DISCUSSION

This study compares the effectiveness of 50% mangosteen fruit peel Ethanol extract (Garcinia mangostana L) and 100% mangosteen fruit peel Ethanol extract (Garcinia mangostana L) in accelerating wound healing time after tooth extraction of Wistar rats. The samples used in this study were Wistar rats. Wistar rats are known to have a physiological body similar to human physiology and have a short average age of 1-2 years, so it is appropriate to be used as an experimental object. The number of research samples taken was 32 Wistar rats that were physically healthy and 2-3 months old with body weight between 200-250 grams. The samples were divided into two groups, namely 16 (50%) for the group treated with 50% mangosteen (Garcinia mangostana L) Ethanol extract and 16 (50%) for the group treated with 100% mangosteen (Garcinia mangostana L) Ethanol extract.

Rat tooth extraction will be performed under the anesthetic effect of ketamine 1000 mg/10 ml dose of 20 mg/kg bw intraperitoneally. After extraction, the post-extraction wound will be observed, and a tampon (cotton pellet) will be applied to stop bleeding in the damage for 5 minutes. The ethanol extract of mangosteen fruit peel (Garcinia mangostana L) 50% was given to treatment group I, and the ethanol extract of mangosteen fruit peel (Garcinia mangostana L) 100% to treatment group II shortly after tooth extraction as much as 0.05 ml daily by dropping. On the 5th day, the rat jaw was taken and fixed with 10% formalin for 24 hours at room temperature; then, the decalcification process was carried out using Ethylene Diamine Tetra Acetic Acid (EDTA 10%) solution at room temperature. The tissue was then dehydrated in toluol alcohol solution (1:1) using the pure tool. The fibroblast cell response evaluation process used Hematoxylin Eosin (HE) staining. Fibroblast density was assessed by counting the number of fibroblasts in 3 fields of view. The sample test was conducted on the fifth day because fibroblasts are known to start growing during the third to the seventh day of the wound healing process, so researchers took the average day, namely on the fifth day (6).

The results of this study we obtained that all samples found fibroblast tissue in the administration of Ethanol extract of mangosteen fruit peel (Garcinia mangostana L) 50% and 100% after tooth extraction of Wistar rats. Furthermore, the number of fibroblasts found in the small category (less than 10% per field of view) on the administration of 50% mangosteen fruit peel Ethanol extract (Garcinia mangostana L) after tooth extraction of Wistar rats as many as seven heads (22%) and on the administration of 100% mangosteen fruit peel Ethanol extract (Garcinia mangostana L) as many as 4 (13%) heads.

Based on Chi-Square data analysis, there is a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by administering 50% mangosteen fruit peel Ethanol Extract (Garcinia mangostana L) and 100% mangosteen fruit peel Ethanol Extract (Garcinia mangostana L), p=0.012 (p<0.05). This is seen in the distribution of data on the number of fibroblasts that are many (50%-100% per field of view) in 100% mangosteen fruit peel Ethanol (Garcinia mangostana L) as many as five samples and in 50% mangosteen fruit peel Ethanol (Garcinia mangostana L) only six pieces. The number of fibroblasts (less than 10% per field of view) was also found to be more in 50% mangosteen fruit peel Ethanol (Garcinia mangostana L) in as many as seven samples. In comparison, only four pieces were found in 100% mangosteen fruit peel Ethanol (Garcinia mangostana L).

The results of this study are supported by Ayati et al. (2019), entitled The Effect of Watermelon Skin Extract Gel Combination (Citrullus lanatus (Thunb.) And Mangosteen Skin Extract (Garcinia Mangostana L.) Against Healing Burns In Rabbits (Effect of Watermelon Skin Extract Gel Combination (Citrullus lanatus (Thunb.)) And Mangosteen Skin Extract (Garcinia Mangostana L.) Against Healing Burns In Rabbits). He stated that giving rabbits a combination of watermelon rind and mangosteen skin gel can affect the diameter of burn wound closure. The most effective formula in healing rabbit burns is formula 3, combining 75% watermelon rind and 25% maggie peel. The gel preparation of various watermelon rind and mangosteen rind extracts for healing burns in rabbits provides reasonable quality control (7).

Also supported research by Kharani (2020) states that mangosteen fruit peel extract cream (Garcinia mangostana L.) has an activity to accelerate burn healing in white male rats. The optimum concentration of extract cream to accelerate burn healing in white male rats is shown in 15% extract (8). Using mangosteen peel extract topically is safe to use and can reduce inflammation and accelerate ulcers' healing process due to scratches (9) and promote angiogenesis in wound healing (6).

Ethanol mangosteen fruit peel (Garcinia mangostana L) stimulates the formation of new fibroblast cells and accelerates wound healing due to glucomannan content. This complex polysaccharide can stimulate fibroblasts to increase rapidly in the wound area. Active substances such as mannose, glucomannan, chrysophane acid, acemannan, flavonoids, saponins, tannins, vitamin A, vitamin C, vitamin E, and enzymes contained in Ethanol mangosteen fruit peel (Garcinia mangostana L) are beneficial in the wound healing process (5). Saponins are steroids or triterpenoid glycosides that are essential to human and animal health. Saponins can trigger vascular endothelial growth factor (VEGF) and increase the number of macrophages migrating to the wound area, thus increasing the production of cytokines that will activate fibroblasts in the wound tissue. In addition, saponins will increase the action of TGF-β on fibroblast receptors, and TGF-β will stimulate fibroblast migration and proliferation (7).

From the results of this study, it can be seen that 100% Ethanol extract of mangosteen fruit peel (Garcinia mangostana L) is more effective in the wound healing process than 50% Ethanol extract of mangosteen fruit peel (Garcinia mangostana L) because the higher the concentration of the section, the content in the Ethanol extract of mangosteen fruit peel (Garcinia mangostana L) is also higher, so that the wound healing process is faster. However, some difficulties in this study are the teeth of Wistar rats that easily fracture when extracted. This is because the anatomy of the Wistar rat teeth is long in the socket and crooked, so when the fracture, the researcher must remove the remaining teeth by slightly tearing the soft tissue from the socket. Another difficulty during the study was finding a comparator substance to check vitamin C levels, so the researcher did not check vitamin C levels and only checked the total flavonoid levels in the turmeric extract 50% with 100%.

V. CONCLUSION

Based on the results and discussions that have been carried out in this study, it can be concluded:

1. Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50% and 100% are effective in accelerating wound healing time after tooth extraction of Wistar rats.

2. Ethanol Mangosteen fruit peel (Garcinia mangostana L) 100% is more effective than Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50% in accelerating wound healing time after tooth extraction of Wistar rats because the flavonoid content in Ethanol Mangosteen fruit peel (Garcinia mangostana L) 100%, which helps accelerate wound healing is higher than Ethanol Mangosteen fruit peel (Garcinia mangostana L) 50%.

REFERENCES

[1] Hu X, Logue M, Robinson N. European Journal of Integrative Medicine Antimicrobial resistance is a global problem – a UK perspective. Eur J Integr Med [Internet]. 2020;36(December 2019):101136. Available from: https://doi.org/10.1016/j.eujim.2020.101136

[2] Abedi N, Sajadi-javan ZS, Kouhi M, Ansari L, Khademi A. Antioxidant Materials in Oral and Maxillofacial Tissue Regeneration : A Narrative Review of the Literature. 2023.

[3] Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S. Skin Wound Healing Process and New Emerging Technologies for Skin Wound Care and Regeneration. 2020;

[4] Mollo N, Esposito M, Aurilia M, Scognamiglio R, Accarino R, Bonfiglio F, et al. Human trisomic ipscs from down syndrome fibroblasts manifest mitochondrial alterations early during neuronal differentiation. Biology (Basel). 2021;10(7).

[5] Kusmita L, Puspitaningrum I, Famasi F, Tinggi Ilmu Farmasi S, Pharmasi Y. UJI AKTIVITAS GEL EKSTRAK ETANOL KULIT BUAH MANGGIS (Garcinia mangostana L.) SEBAGAI PENYEMBUH LUKA BAKAR PADA KULIT PUNGGUNG KELINCI ACTIVITY OF GEL MANGOSTEEN RIND AETHANOL EXTRACT (Garcinia mangostana L.) TOWARD BURNS HEALING ON SKIN RABBIT. Media Farm Indones [Internet]. 2014;9(1):606–15. Available from: https://mfi.stifar.ac.id/MFI/article/view/75

[6] Biran AR, Chairani S, Rusdiana S, Dewi P, Studi P, Gigi K, et al. EFEK EKSTRAK KULIT MANGGIS (Garcinia Mangostana L.) TERHADAP PEMBENTUKAN PEMBULUH DARAH BARU PADA LUKA GINGIVA TIKUS WISTAR. J Aisyiyah Med. 2019;3(2):199–207.

[7] Ayati YVP, Setiawan I, Ariani NR, Hastuti DD. Pengaruh Gel Kombinasi Ekstrak Kulit Semangka (Citrullus Lanatus(Thunb.)) Dan Ekstrak Kulit Manggis (GarciniaMangostana L.) Terhadap Penyembuhan Luka Bakar Pada Kelinci (Effect of Watermelon Skin Extract Gel Combination (Citrullus Lanatus (Thunb.)) And. Indones J Med Sci. 2019;5(2):149–55.

[8] Khairani TN, Rumanti RM, Manao A, Farmasi D, Farmasi F, Helvetia IK, et al. FORMULASI SEDIAAN KRIM EKSTRAK ETANOL KULIT BUAH MANGGIS (Garcinia mangostana L .) SEBAGAI OBAT LUKA BAKAR PADA TIKUS PUTIH JANTAN CREAM FORMULATION OF ETHANOL EXTRACT OF MANGOSTEEN PEEL (Garcinia mangostana L .) AS A BURN HEALING IN WHITE MALE RATS Ala. 2020;4(2):53–8.

[9] Sunarjo L, Salikun, Rimbyastuti H. Peranan Pasta Manggis terhadap Kesembuhan Ulkus Akibat Luka Gores (Pemeriksaan Histo Patologi Anatomi) Lanny Sunarjo Salikun Hermien Rimbyastuti Jurusan Keperawatan Gigi Poltekkes Kemenkes Semarang. J Ris Kesehat. 2015;4(2):779–85.