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Gel Formulations of Guava Leaves (*Psidium Guajava* L) Leaves Ethanol Extract As a Wound Healing Of Burns in Rabbits

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Abstract

The ethanol extract of guava leaves (*Psidium guajava L*) in previous studies was known to have antiburn, antiinflammatory, antidiarrhea and antibacterial activity. The preparations made are gel preparations which are preferred by the public. The purpose of this study was to determine the activity of guava leaf ethanol extract gel and its effective concentration in healing burns in rabbits. The research method uses rabbits 4 tails made 5 wounds on the back, consisting of negative control, comparison (Bioplacenton®), gel preparations 3%, 5%, and 11%. Wound diameter measurements were carried out on days 3, 6, 9, 12, 15 and 18. The parameters observed were the size of the wound diameter, wound healing such as wound drying, scab formation, closure of the wound and the growth of new hair around the wound. An inflammatory process occurs for 3 days after injury and not processed wound healing. Fibroblasts appeared on day 6 and peaked on day 9, so that on day 9 all treatment and control groups experienced linear healing. On the 9th day the fibroblasts began to peel off on the skin indicating the group has reached the peak of the proliferation phase and wound contractions occur gradually urgent. The results of the guava leaf extract gel activity test (*Psidium guajava L*) showed a concentration of 11% gave the best effect.

Keywords: *Psidium Guajava*, Guava Leaf Extract Gel, Wound Healing Activity

1. Introduction

Indonesia is known as one of the countries with the greatest biodiversity in this world. Biodiversity can be seen from various types of plants can traditionally be used as a medicine for disease. One of the plants known as medicine and which is utilized by the community is the guava plant (Ajizah, 2004). Based on previous research, guava leaves are known to have benefits as an anti-diarrhea (Ansel and Allen, 2014), increases blood platelet levels (Ansel and Stockton, 2017), antioxidant (Biswas, *et al*. 2013), antiinflammatory (Burkill, 1997) and antibacterial (Chisholm-Burns, *et al*., 2016) and antispasmodic (Cushnie and Lamb, 2005).

Guava leaves contain compounds tannins, flavonoids, alchloids and saponins (Desiyana, *et al.*, 2016). The leaves of guava contain eugenol, fat, cineol, malic acid, triterpenes, resin, cellulose, chlorophyll, mineral salts, and a number of other fixed substances (Dipiro, *et al*., 2015; Ekom and Tamokou, 2018). The content of guava leaves can help wound healing process because it functions as an antioxidant and antimicrobial affect wound healing and accelerate epithelialization. Flavonoids serves to inhibit bleeding, saponins have a function as a stimulant. Tannins function as anti-bacterial and antiseptic in wounds thus preventing infection of the wound. Alkaloids work to fight back microbial infection.

Burns are a form of damage and or loss of tissue that is caused contact with sources of extremely high temperature (e.g. fire, hot water, chemicals, electricity and radiation) or extremely low temperatures (Farnsworth, 1966). Burns are classified into three zones based on the degree of tissue damage and changes in blood flow. In the middle it is known as a wound coagulation zone (Fortin and Maynart, 1990). Outside the zone coagulation there is a stasis zone or ischemic zone which is characterized by a decrease tissue perfusion (Katzung, 2004). The stasis zone is a potential zone to do network rescue (Kao, *et al*., 2010).

A gel formulation that is particularly suitable for local ailments with the advantage of being easier to wash (Karl, *et al*., 1995). Gel is a semi-solid preparation consists of a suspension material small inorganic particles or molecules large organic matter penetrated by a fluid (Ministry of Health Republic Indonesia, 2008).

Based on data from research previously so that researchers were interested in making ethanol extract gel dosage forms Guava leaves heal burns. The purpose of this study was to determine the activity of guava leaf ethanol extract gel and the effective dose in treating burns.

2. Materials and Methods

This research is a type of experimental research in the laboratory, this research will also evaluate the effect of giving guava leaf extract gel on animals. The experiment was a rabbit, this research included making simplicia, gel formulation and inducing burns. The research stages started from collecting guava leaf material. The seeds will be obtained in the yard of the Sukabumi area, West Java later phytochemical determination, characteristics, screening and extraction were carried out.

2.1. Materials

2.1.1. Animal

The animal used is a rabbit (Oryctolagus cuniculus) weighing 2-4 kg, 3-4 months old and healthy animal condition. Before doing research, rabbits adapted to the room with an room temperature of 20-25ºC (Ethical Approval: 402/UN6.KEP/EC/2020).

2.1.2. Tools

A macerator set, beaker, analytical scale, bathtub water, vaporizer cup, test tube, drip pipette, vortex, tube rack, animal enclosure, equipment shavers, metal plates, containers, stoves, thermometers, markers. Strirer, beaker, measuring cup, pH meter, stir bar, preparation container and pipette. The ingredients used in this research are guava leaves, alcohol 96%, lidocaine spray, batis cloth, cotton, aquadest, bioplacenton®, dreagendrof reagent, mayer reagent, magnesium metal, amyl alcohol, iron (III) chloride, toluene, solutions galatin, chlorophome, ether, liberman-buchardat, sulfuric acid p, and sodium hydroxide.

2.1.3. Determination

Plant determination was carried out at Padjadjaran University, Bandung Herbarium Jatinangor, Biology Department, Faculty of Mathematics and Natural Sciences. The determination is made to find out the correctness of the test plants by means of compare specified material with data from literature.

2.2. Methods

2.2.1. Characterization of Simplicia

Determination of water content, determination of water soluble extract content, determination of ethanol soluble extract content, determination of total ash content, determination of acid insoluble ash content, and tetermination of drying shrinkage (Mulisa *et al.*, 2015).

2.2.2. Phytochemical Screening

Examination of alkaloids, flavonoids, saponins, tannins, quinones, steroids / triterpenoids (Morales, *et al*., 1994).

2.2.3. Extraction

Extraction was carried out by maceration method using ethanol 96% solvent room temperature for 3 times 24 hours, where every 24 hours filtered using filter paper, then the solvent is replaced with a new solvent. Then the extract was collected and concentrated using a rotary vaporator until. The viscous extract obtained was then calculated as a percent yield.

2.2.4. Gel Formulation

Guava leaf gel formulation can be seen in Table 1.

Table 1: Gel Formulations (Ncube, *et al.*, 2008)

Number	Ingredient Name	Formulation and Composition (b/b%)			
		F1	F2	F3	F4
1.	Guava leaf extract	-	11	5	3
2.	Carbopol	2	2	2	2
3.	TEA	2	2	2	2
4.	Glyserin	10	10	10	10
5.	Methyl paraben	0.2	0.2	0.2	0.2
6.	Water ad	100	100	100	100

2.2.5. Gel Processing

100 grams of gel preparations are made. Carbopol is developed with aquadest until it expands and is stirred while gradually adding triethanolamine to form a gel mass. Add glycerin and extract and finally methyl paraben, stir until it forms a gel.

2.2.6. Physical Evaluation of Gel Preparations

the preparation was stored at room temperature for 15 days. on days 0, 3, 6, 9, 12, and 15. organoleptic evaluation, of ph, homogeneity and viscosity were evaluated (Nadkarni amd Nadkarni, 1999).

2.2.7. pharmacological Test

Rabbits were randomly selected and then divided into 5 groups namely, 1: Gel wound Bioplacenton® (comparison), 2: The wound was given gel base (negative control), 3: The wound was given gel from guava leaf extract 11%, 4: Wounds given 3% gel extract gel, 5: Wounds given a base of 3% extract gel. Giving guava leaf extract gel and medicine the comparison was given 3 times a day for 18 days. The data obtained were then analysed statistically using this method ANOVA. The parameters observed were measuring the diameter of the wound using a ruler. Healing wounds by drying the wound, scabs form, closure of the wound and the growth of new hair around the wound (Panjaitan, *et al*., 2012).

3. Results and Discussion

The determination was made at the Jatinangor Herbarium Laboratory Plant Taxonomy, Department of Biology, FMIPA - Padjadjaran University. Result the determination of plants shows that the plants used are correct guava leaf plant (Psidium guajava NO.115/HB/01/2020).

This study used 5 kg of guava leaves. Wet filtering is carried out to separate from dirt or other foreign material. Wash is complete with running water to remove dirt that is still attached to the leaves which can interfere with the next process. A total of 1 kg of guava leaves is made into dry simplicia for use in phytochemical screening processes and standard parameter testing. Guava leaves that have been washed, then chopped simplify the drying process. The smaller the size of the material to be made dry, the faster the evaporation of water and the faster the processing time drying. Drying is done using a drying cabinet. After obtaining dry simplicia, dry sorting is carried out to separate them dirt that may remain. Simplicia of dried guava leaves obtained 500g. The results of the simplicia characteristic test were water content determination (9%), water soluble extract content determination (19.9%), ethanol soluble extract content determination (14.4%), total ash content determination (13.19%), ash content determination insoluble acid (1.29%), and shrinkage drying (14.8%).

The results of phytochemical screening (table 4) on dry and fresh leaves showed the presence of alkaloids, flavonoids, saponins and tannins. Flavonoids are known to have analgesic and anti-inflammatory properties. Tannins act as an astringent that can shrink pores on the skin, harden the skin, stop exudates and bleeding. Steroids as anti-inflammatory which prevent stiffness and pain.

Table 2: The Results of Phytochemical Screening

Compound group	Simplicia	**Extract**
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+

(+) : Présent; (-): Absent

Gel preparations were chosen because they have high water content and dispersibility which is better than other topical preparations. Carbopol is used as gelling agent or gelling agent because it can form a gel on low concentration, namely 0.5%, carbopol produces a clear base and transparent with a better texture than NaCMC and sodium alginet. Gel bases are used in preparations gel is Carbopol which is a derivative of cellulose synthesis and is included in the base hydrophilic. Use a hydrophilic gel base because of its good dispersibility on the skin, the effect cools, does not clog skin pores, is easy to wash with water and the drug release is good.

Organoleptic gel test was carried out by observing the shape and color visually and the smell of the gel. The organoleptic results for the four preparations, the high concentration obtained, resulted in a darker green color compared to the results of the ingredients contains extracts. This is possible due to temperature and storage may affect the dosage form.

The pH test results (Table 2) have a range of skin pH requirements, namely 4.5-6.5 (Dipiro, *et al.*, 2015). Based on the pH test results above indicate that the guava leaf extract gel has met the requirements pH requirements for skin.

Table 3: pH Test Results for Gel Preparations

Days	pH			
0	5.8	5.8	5.7	5.7
3	5.6	5.6	5.8	5.5
6	5.4	5.6	5.5	5.5
9	5.2	5.3	5.3	5.4
12	5	5.2	5.2	5.3
15	5	5.1	5.2	5.3

The results of measuring the viscosity of guava leaf extract gel preparations (Table 3) the viscosity of the resulting gel preparation showed a higher concentration dosage extract, the viscosity of the preparation decreases. Based on the results measurement of viscosity up to day 15, this can be due to gel preparations shows the characteristic of synersis, namely the discharge process entangled in the gel so that the liquid moves towards it surface. Therefore, the preparation has decreased in viscosity. Less Gel viscosity can also be caused by surface area factors, such as temperature and how to save.

Table 4: The Results of the Viscosity Test for Guava Leaf Gel Preparations.

Days	Viscosity			
	F1	F2	F3	F4
0	2566.67	1566.67	1859.50	2246.67
3	2566.67	1566.67	1859.50	2245.00
6	2566.00	1533.33	1859.50	2245.00
9	2566.00	1483.33	1859.50	2221.60
12	2555.50	1416.67	1789.79	2220.00
15	2555.50	1250.00	1799.50	2220.00

Healing burns using guava leaf extract gel (Psidium guajava L) (Table 5) with treatment 1 as a comparison (bioplacenton). Treatment 2 as a negative control (gel base). Treatment 3 (with the concentration of guava leaf extract 11%). treatment 4 (with a concentration of guava leaf extract 5%). and treatment 5 (with a concentration of 3% guava leaf extract).

Table 5: Average Diameter of Burns Shrinkage

Tretment	Average Wound Area				
	Day 6	Day 9	Day 12	Day 15	Day 18
Control (-)	2.88±0.05	2.75±0.05	2.53±0.05	2.33±0.05	1.60±0.14
Control (+)	0.65±0.05	2.48±0.12	1.85±0.56	0.95±0.05	0.50±0.14
GFGL11%	2.50±0.08*	2.15±0.17*#	1.48±0.56*	0.75±0.19*	0.38±0.17*
GFGL 5%	2.73±0.05	2.58±0.05	2.2±0.14	1.48±0.34*#	1.00±0.21*#
GFGL 3%	2.78±0.09	2.53±0.05	2.23±0.17	1.63±0.22*#	1.05±0.17*#

Information: *differed significant from the negative control # different significant by comparison
DFGL: Gel formulations of guava leaves

The results of the data that have been obtained are obtained from the results of the average data ($\pi \pm SD$; $n = 3$). Based on the results, the average diameter of wound healing above shows between control (-). 11% concentration and Extract Test based on one-way ANOVA test data Statistics show that this value is significantly different from the control (+) (comparison). This shows that with a concentration of 3%. 5% is less able to stimulate burns healing faster than the comparator (bioplasenton). Neither (negative) contains only gel. Nor does it able to provide a significant effect by comparison. a lower effect versus Bioplacenton®. While at a concentration of 11% the table above yields One-way ANOVA test. Showed a significant difference with this negative control this means that the gel with a concentration of 11% is able to stimulate wound healing with better than the concentration of guava leaf extract. Respectively 5% and 3% the overall effect of optimal burn healing is provided with the gel preparation the content of guava leaves is 11% which causes stimulation 6th day. With healing effect equivalent to bioplaston and administration wound healing that is almost the same but not as effective as bioplasenton®. In this study it was found that guava leaf extract gel is one of them therapy that is as effective or as good as bioplastone against heal burns in

rabbits. And the most effective is the gel with the concentration of guava leaf extract is 11%. This is due to several compounds contained in guava leaf extract has the ability to accelerate regeneration tissue, re-epithelialization, stimulates fibroblasts and collagen formation in the skin who has burns.

Picture Index	Day	Concentration	Picture
A	6	Negative control	
B	6	Bioplacenton (Positive control)	
C	6	Gel formulations of guava leaves 11%	
D	18	Bioplacenton (Positive control)	
E	18	Gel formulations of guava leaves 11%	

Table 6: Healing Burns in Rabbits

The 3rd and final phase is the renovation phase. This phase is the final phase and longest wound healing process. There is a dynamic process in form wound contraction, and scar ripening. During this phase new tissue is formed will be arranged in such a way as the original network on the 15th and 18th day indicates that it has entered the renovation stage.

Tannins act as anti-bacterial and antiseptic in wounds to prevent them the occurrence of infection in wounds, alkaloids function against microbial infections, Its working mechanism is to disturb its constituent components peptidoglycan in bacterial cells, the cell wall is not formed completely causes cell death (Rowe, 2009). Flavonoid compounds Such as quercetin and quersitrin have anti-inflammatory by inhibiting effects signaling in microglia cells resulting in unusable disruption of nitric oxidsynthase (iNOS) and decreased levels of nitric oxide (NO). This produces an analgesic effect and neuroprotection that causes pain in the part of the skin that has been traumatized by a burn minus (Susilawati, *et al*., 2018).

4. Conclusion

Based on the results of the study it can be concluded that guava leaves have activity against burn healing and based on statistical results with One of the ANOVA dosage methods that is effective in healing burns is by concentration 11%. This is due to several compounds contained in guava leaf extract has the ability to accelerate regeneration tissue, re-epithelialization, stimulates fibroblasts and collagen formation in the skin who has burns.

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