ISSN No:-2456-2165

Formulation and Antibacterial Activity Test on Ethanol Extract Gel of Guava Leaf (*Psidium guajava* L.)

Sholichah Rohmani[1], Jean Priscila BR Sebayang[2]
[1,2] Pharmacy Department, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Indonesia

Abstract:- Guava leaf (Psidium guajava L.) contains flavonoids and tannins which have bacterial activity against Staphylococcus aureus. The use of extract as an antibacterial is not practical to use so it is made in gel form. The purpose of this study was to determine the effect of variations in the concentration of guava leaf ethanol extract on the physical properties of the gel and determine the inhibitory zones produced from the ethanol extract of guava leaves. The extract was obtained from maceration with 70% ethanol, and then it was made in gel form. The gel formula was made on the basis of CMC-Na and the ethanol extract content of guava leaves used 1, 5, and 7%. Gel evaluation includes organoleptic, homogeneity, adhesion, viscosity, pH, dispersal, and cycling tests which are then carried out with antibacterial tests using the bacteria of Staphylococcus aureus. The concentration of guava leaves ethanol extract has a significant effect on gel stability and antibacterial activity. The higher concentration of ethanol extract of guava leaves shows the higher zone of bacterial inhibition, adhesion, viscosity, and decreased pH and gel dispersion.

Keywords:- *Gel, Guava Leaves, Antibacterial, Staphylococcus aureus*

I. INTRODUCTION

The use of guava leaves as a natural antibiotic because guava leaves (Psidium guajava L.) contain active substances that act as anti-bacterial substances. These chemical compounds include tannins, saponins, ethanol, polyphenols, flavonoids, essential oils (eugenol), malic acid, ursolic acid, psidiolic acid, cratogolic acid, oleanolic acid, guajaverin acid and others (Afifi and Erlin, 2017)). Minimum inhibitory concentration of ethanol extract of guava leaves on Staphulococcus aureus (0.1mg / mL), L. monocytogenes (0.1 mg / mL), Aeromonas hydrophilia (4.0 mg / mL). Staphylococcus aureus is one of the normal flora which can cause skin infections such as acne and ulcers. The process of acne is when loose keratinin builds up in the skin. Blockage occurs due to one bacterium that causes acne, namely Staphylococcus aureus, causing inflammation. (Sarlina et al., 2017).

Gel, sometimes called jelly, is a semisolid system consisting of two colloidal phases namely, water and solid and penetrated together in a liquid. The gel leaves a thin layer when applied, does not clog pores, and has a high elastic adhesion so that it gives good skin respiration. The drug dispersion is very good in a short time and is almost perfect so that it increases the effectiveness of using the gel (Bokti and Saputri., 2018).

The quality of physical properties and physical stability of gel preparations are affected by the composition of the ingredients used. Gelling agent is one part that is very influential on the physical quality of the gel preparation. CMC-Na has a short time to develop into a good gel structure (Candradireja, 2014). Humectants play an important role in maintaining the water content of gel preparations so that the physical properties and stability of the gel can be maintained. Humectants are also able to maintain the water content on the surface of the skin by binding water from the environment. Glycerol is the most commonly used humectant but tends to cause a heavy and wet feeling that can be covered by combining with other humectants. Propylenglycol has a smaller molecular weight, lower viscosity and high evaporation compared to glycerol so that the physical properties and stability of the preparation during storage can be maintained (Anggraeni, 2008).

The study aims to determine the effect of variations in the concentration of ethanol extract of guava leaves (Psidium guajava L.) on the physical and chemical stability of gel preparations and determine the concentration of ethanol extract of guava leaves in a good gel preparation in providing antibacterial activity on the growth of Staphylococcus aureus.

II. MATERIAL AND METHOD

➢ *Equipment :*

The equipments used in this study include, analytical scales (Ohaus, Shanghai, China, accuracy of 0.1 mg), rotary evaporator (STUART RE300DB, Staffordshire, United Kingdom), pH meter (LUTRON PH-208, Taipei, Taiwan), thermometer, water bath (MASPION S-302, Jakarta, Indonesia), viscosimeter (RION VT-04/03, Tokyo, Japan), a set of modified sticky test equipment, and other supporting tools.

➢ *Material :*

The main material used is guava leaves taken from the Kemuning, Ngargoyoso, Karanganyar, Karanganyar Regency, Central Java, Indonesia. The material used for maceration of guava leaf powder is 70% ethanol. As well as the ingredients used to make gel preparations are Na CMC (Dow Walocel, Canada), glycerin (Dow chemical pacific,

Singapore), Propylene glycol (Dow chemical pacific, Singapore), and water.

This research is an experimental laboratory study to obtain results data. This study consisted of 4 stages, namely the extraction stage, gel formulation, physical and chemical properties test of the gel preparation, and the antibacterial activity test of the gel.

Guava leaves as much as 2 kg are dried in the sun. Simplisia then crushed using a blender and obtained guava leaf powder. Extraction was carried out by maceration method using 70% ethanol solvent. Guava leaf powder (Psidium guajava L.) soaked with 70% ethanol in a ratio of 1: 5 for 5x24 hours. The maserate is filtered, then the extracted substances are evaporated with a rotary evaporator at 60ºC until a thick extract is obtained and evaporation of the remains of the spider on the water bath.

The gel making used 3 formulas with different concentrations of guava leaf ethanol extract in each formula, the gel formula can be seen in table I.

Material	Function	Formula I (gram)	Formula II (gram)	Formula III (gram)
Ethanol Extract of Guava Leaves	Active substances	1	5	7
Na –CMC	Gelling agent	2	2	2
Glycerin	Humectant	10	10	10
Propylene glycol	Humectant	5	5	5
Water	Solvent	82	78	76

Table 1:-Formulation of Ethanol Extract Gel of Guava Leaf (*Psidium guajava* L.)

The physical and chemical properties consist of organoleptic, viscosity, pH determination, adhesion test and dispersion test. Organoleptic test is done by observing qualitatively including consistency, color and odor. The viscosity test was carried out with a Rion VT-04 viscometer to determine the thickness of the gel. The pH test was carried out using a pH meter LUTRON PH-200. Scattering and adhesion measurements using a set of modification tools. Antibacterial activity testing was carried out antiseptically by the diffusion method of wells using MHA (Mueller Hinton Agar) media.

Data collection techniques are carried out by testing physical and chemical properties, and testing for antibacterial activity. Data analysis was performed with a theoretical approach with parameters from library sources and performed with a statistical approach using One Way Anova and Paired T-Test.

III. RESULT AND DISCUSSION

➢ *Organoleptic Test*

Organoleptic test is done by observing the gel, namely observing the color, odor and consistency. The results of observations made can be seen in table II.

Formula	Organoleptic Patameter	Before Cycling Test	After Cycling Test
Formula I	Consistency	A little thick	Slightly Liquid
	Color	Brown	Brown
	Smell	Typical Guava Leaves	Typical Guava Leaves
Formula II	Consistency	Thick	Thick
	Color	Brown	Brown
	Smell	Typical Guava Leaves	Typical Guava Leaves
Formula III	Consistency	Very Thick	Very Thick
	Color	Dark Brown	Dark Brown
	Smell	Typical Guava Leaves	Typical Guava Leaves

Table 2:- Organoleptic Test Results of Ethanol Extract Gel of Guava Leaf

From the results of organoleptic testing, it can be seen that the gel in formula I underwent organoleptic changes after cycling test on consistency parameters. Differences in consistency before and after the cycling test can occur due to the influence of temperature changes and also suspected syneresis, so that the consistency changes. The difference in the concentration of guava leaves ethanol extract affects the level of consistency of the gel, where in formula I with the consistency of 1% guava leaves ethanol extract has a slightly thick consistency, formula II which with a concentration of guava leaves 5% ethanol extract has a thick consistency, and the formula III with a concentration of ethanol extract of guava leaves 7% has a very thick consistency. The difference in consistency of each preparation is due to the consistency of the thick ethanol extract of guava leaves so that it affects the consistency of the gel.

➢ *Homogeneity Test*

Homogeneity testing is done before and after cycing test which is then seen whether there is a change in homogeneity in each preparation. A preparation is said to be homogeneous if there are particles that separate the preparation. Homogeneity test results of guava leaves ethanol extract showed that formula I, formula II, and formula III were homogeneous and did not change after cycling test.

ISSN No:-2456-2165

➢ *pH Test*

The pH test aims to determine the acidity of the gel preparation to ensure the gel does not cause irritation to the skin. pH of preparations that meet the skin's pH criteria is in intervals of 5 to 10 (Suhery et al., 2016). The results of pH testing can be seen in Table III.

Formula	Average before Cycling Test	Average after Cycling Test
Formula 1	6,82 ± 0,015	7,72 ± 0,010
Formula II	6,75 ± 0,010	7,11 ± 0,015
Foemula III	6,24 ± 0,010	6,84 ± 0,015

Table 3:- Average pH of Ethanol Extract Gel of Guava Leaves Before and After Cycling Test

Increasing the pH value in each formula after cycling test, this might be due to the storage of the preparation at low and high temperatures so that the pH can change. The test results showed p <0.05 which means that there is an effect of doing a cycling test on the pH value of formulas I, II, and III and a significant difference in the pH value between formulas.

➢ *Adhesion Test*

Adhesion testing aims to determine the bond between the gel and the skin. The higher adhesion strength of the gel indicates the stronger bond between the gel and the skin so as to allow higher absorption of the drug by the skin. Conversely, if the bond between the gel and the skin is less than optimal the drug will be easily erased from the skin (Yati et al., 2018). The results of the Adhesion test can be seen in Table IV.

Formula	Average before Cycling Test (seconds)	Average after Cycling Test (seconds)
Formula 1	1,43 ± 0,021	1,40 ± 0,015
Formula II	1,77 ± 0,021	1,77 ± 0,015
Foemula III	2,03 ± 0,020	1,98 ± 0,025

Table 4:- Average Adhesion of Ethanol Extract Gel of Guava Leaves Before and After Cycling Test

Adhesion is directly proportional to viscosity, that is, the greater the viscosity or thickness of a preparation, the greater the adhesion. The test results show that p> 0.05, which means that there is no effect of a cycling test on the value of the adhesion of formula I, II, and the adhesion between formulas has a significant difference because the value of p <0.05. The higher concentration of ethanol extract of guava leaves, the greater the adhesion power obtained.

➢ *Dispersion Test*

The result of good gel dispersion is 5-7 cm or 5.54-6.08 cm (based on SNI standards). The greater the dispersal power of preparations shows the ability of active substances to spread and skin contact more widely (Yati et al., 2018). The results of the dispersion test can be seen in Table V.

Formula	Average before Cycling Test (cm)	Average after Cycling Test (cm)
Formula 1	6,90 ± 0,020	6,90 ± 0,015
Formula II	6,34 ± 0,010	6,46 ± 0,015
Formula III	6,23 ± 0,025	6,32 ± 0,020

Table 5:- Average Dispersion of Ethanol Extract Gel of Guava Leaves Before and After Cycling Test

The high viscosity value of the preparations results in a low spreadability and vice versa. This is because high viscosity makes it difficult for the gel to flow so that the area produced is small. The results of the three formulas can be seen that the difference in the concentration of ethanol extract of guava leaves gives the effect of decreasing the spread of gel power between formulas, the higher the concentration of ethanol extract, the smaller the scatter power produced. The test results show p> 0.05 which means there is no effect of the cycling test on the spread value of formula I and III while the effect of cycling test on the spread value of formula II and the spreadability between formulas has a significant difference because the value of p <0, 05.

➢ *Viscosity Test*

Viscosity testing aims to determine the viscosity value of a substance. The higher the viscosity value, the higher the viscosity level of the preparation. Viscosity affects the rate of absorption of the drug, the thicker it will cause the longer the absorption of the drug. Good viscosity of gel preparations is 200-400 dPas. Viscosity test results can be seen in Table VI.

Formula	Average before Cycling Test (dPas)	Average after Cycling Test (dPas)
Formula 1	160	100
Formula II	300	300
Foemula III	310	310

Table 6:- Average Viscosity of Ethanol Extract Gel of Guava Leaves Before and After Cycling Test

Viscosity test results show that formula III has the highest viscosity value and formula I has the smallest viscosity value. The results of the viscosity test showed that the greater the concentration of ethanol extract of guava leaves, the greater the value of viscosity. The consistency of the thick ethanol extract affects the viscosity of the gel. Formula I does not meet the requirements of good gel viscosity. Formula I after the cycling test the viscosity decreases, it can be caused by gel preparations showing the characteristics of syneresis which is the process of releasing the entangled liquid in the gel so that it allows the liquid to move towards the surface, therefore the preparation has decreased viscosity. The reduced thickness of the gel can also be caused by high temperatures. High temperatures will increase the distance between particles so that the force between particles will decrease. Increasing distances cause viscosity to decrease.

IV. CONCLUSION

The conclusion of this research are:

1. Based on research that has been done, it can be concluded that the increase in the concentration of ethanol extract of guava leaves as an active substance causes an increase in adhesion and viscosity as well as a decrease in the spreadability and pH value.
2. Based on antibacterial testing against Staphylococcus aureus bacteria, it can be concluded that formula III, gel with a concentration of 7% guava leaf ethanol extract provides the biggest inhibition zone compared to the other two formulas, but the inhibitory zone produced is still in the weak category.

REFERENCES

[1]. Afifi, R., Erlin, E., 2017, Uji Antibakteri Ekstrak Daun Jambu Biji (*Psidium guajava L)* Terhadap Zona Hambat Bakteri Jerawat Propionibacterium acnes Secara Invitro, *Jurnal Kesehatan Bakti Tunas Husada*, 17(2): 321-330.

[2]. Sarlina., Razak,A.R., Tandah,M.R., 2017, Uji Aktivitas Antibakteri Sediaan Gel Ekstrak Daun Sereh (*Cymbopogon nardus* L. Rendle) terhadap Bakteri *Staphylococcus aureus Penyebab Jerawat*, *Jurnal Farmasi Galenika*, 3(2): 143-149.

[3]. Bokti, S,B,K., Saputri,F,A., 2018, Formulasi dan Evaluasi Sediaan Gel Dari Ekstrak Seledri (*Apium graveolens Linn.)* Sebagai Anti inflamasi, *Farmaka*, 16(1): 63-71.

[4]. Candradireja, K,C,M., 2014, Pengaruh Penambahan Konsentrasi CMC-Na Sebagai *Gelling Agent* Pada Sediaan *Sunscreen* Gel Ekstrak Temugiring (*Curcuma heyneana* Val.) Terhadap Sifat Fisik Dan Stabilitas Sediaan Dengan Propilen Glikol Sebagai *Humectant*, *Skripsi*, Fakultas Farmasi,

[5]. Anggraeni, T,M,R., 2008, Optimasi Formula Gel *Sunscreen* Ekstrak Kering Polifenol The Hitam (*Camellia sinensis* L.) Basis Carbopol Dengan Humektan Gliserol dan Propilenglikol Menggunakan Metode Desain Faktorial, *Skripsi*, Fakultas Farmasi, Universitas Sanata Dharma, Yogyakarta, 2-3.

[6]. Suhery,W.N., Fernando,A., Has, N., 2016, Uji Aktivitas Antioksidan dari Ekstrak Bekatul Padi Ketan Merah dan Hitam (*Oryza sativa* L. var. glutinosa) dan Formulasinya dalam Sediaan Krim, *Jurnal Pharmacy*, 13(01): 101-115.

[7]. Yati, K., Jufri, M., Gozan, M., Mardiastuti., Dwita, L.P., 2018, Pengaruh Variasi Konsentrasi *Hidroxy Propyl Methyl Cellulose.*