Formulation and Antibacterial Tests of Serum Preparation of Ethanol Extract of Guava Leaves (*Psidium Guajava* L) as an Anti-Acne

Neni Sri Gunarti(✉), Shintia, Farhamzah, Eko Sri Wahyuningsih, and Putri Agustina

Department of Pharmacy, Universitas Buana Perjuangan Karawang, Indonesia
neni.gunarti@ubpkarawang.ac.id

Abstract. The guava leaf plant (*Psidium guajava* L) is a plant that can be found in almost every region in Indonesia. The part of the guava leaf that has been extensively researched and contains anti-acne properties is the leaf. In previous studies, it was known that guava leaves have antibacterial activity against *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermis* which are the causes of acne. Purpose of this study is to determine the antibacterial activity of serum preparations of guava leaf extract against *Propionibacterium acnes*. The extraction method used was maceration using 96% ethanol. Phytochemical screening was carried out to determine the content of secondary metabolites in the guava leaf extract. Furthermore, the ethanol extract from guava leaves was used as an active substance in the manufacture of serum with various concentrations of 3.75, 2.5, and 1.25%. Physical evaluation of serum preparations was carried out by organoleptic tests, homogeneity, pH tests, spreadability, and viscosity. The research results found that formula III had the best inhibition where the diameter of the resulting inhibition zone was 19.0 mm and included in the strong category.

Keywords: Serum Preparation, Ethanol Extract of Guava Leaves, Antibacterial Tests

1 Introduction

Propionibacterium acnes is a skin microbe that is usually found on skin rich in sebaceous glands, such as the skin on the head and face [1]. Acne is a major problem for teenagers, especially at the age of puberty. One of the causes of acne that often occurs in this age group is a bacterial infection, such as *acne vulgaris* sp. After an increase in hormones, the fat glands become active so the growth of bacteria in these glands increases. To know the best treatment for acne sufferers, it is important to understand the various causes. One of the factors that cause acne is bacteria. Therefore, intense treatment is needed against it, and of course naturally. One of them is using the right cosmetic series according to the condition of the facial skin. The cosmetic preparation that is currently being developed is serum.

Z. B. Pambuko et al. (eds.), *Proceedings of the 4th Borobudur International Symposium on Humanities and Social Science 2022 (BIS-HSS 2022)*, Advances in Social Science, Education and Humanities Research 778,
https://doi.org/10.2991/978-2-38476-118-0_16

The serum is classified as emulsion preparation since it has a low viscosity. The serum has a high concentration of active substances that allows the skin to absorb this effect more quickly. Due to the low viscosity, it is able to provide a more comfortable effect and can be spread evenly over the surface of the skin. It's not so high in viscosity which may give it a more comfortable effect and easier spreading on the surface of the skin. There are various types of serums, such as antiacne, brightening, aging, eyelash and so on. At present, there is also the development of a serum derived from natural ingredients, one of the plants that can be used in making serum for anti-acne guava leaf plant (*Psidium guajava* L).

The guava leaf plant which can be found in nearly all areas of Indonesia is called Psidium gua ljava. The leaf is part of the guava leaf, which has been extensively studied and contains antiacne properties [3]. Guava leaves in studies have antibacterial activity against *propionibacterium acne* [4], *staphylococcus aureus* and *staphylococcus* which are the causes of acne. The results showed that the best quality and antiacne activity in skin cleansing preparations is obtained by formulations containing 2.5% leaf extract. Based on the background above, the researchers initiated a serum preparation from the ethanol extract of guava leaves (*Psidium guajava* L) for facial treatment as an anti-acne made from natural ingredients. The choice of this serum preparation was made due to its several advantages, e.g. that it is well distributed in the skin and can be applied with ease releasing three active substances into the blood stream.

Synthetic substances and natural ingredients, such as Guava leaves, which are known for their antibacterial properties, may prevent the growth of *Propionibacterium acnes* bacteria. Guava leaves are antibacterial against *Propionibacterium acnes*, Staphylococcus *aereus* and *Staphylococcus epidermis* because of their antibacterial action against *Propionibacterium acnes*, *Staphylococcus aereus* and *Staphylococcus epidermis* [5]. The presence of tannins, triterpenoids and flavonoid glycosides in the leaves has been reported to have an influence on the antibacterial activity of Guava leaf extract [6].

2 Methods

2.1 Extraction

The leaves of the guava are cleaned and then cut into pieces and dried in the sun for drying. Once the guava leaves are dried, they are ground into a powder. The powder is then extracted by the maceration process, which involves a mixture of 96% ethanol. After that, the guava leaf extract powder was soaked in 96% ethanol for three days and stirred every 1x24 hours for 5 minutes. The filtrate solution shall be obtained after the process of maceration is completed and subsequently filtered in order to separate the dregs from the filtrate. The filtrate was then evaporated by a rotary evaporator, the extract obtained was condensed in a porcelain cup over a water bath, until a thick extract was obtained.

2.2 Phytochemical screening

Alkaloid test. The extract was added 1 ml of 2N HCL, heated for ± 2 minutes, filtered, added to a test tube, and then added with Dragendorf and Mayer reagent. The orange precipitates indicate dragendorf, while the white clusters of phytochemicals show there is a presence of Mayer [7].

Flavonoid test. The extract was heated with a mixture of 0.1 g of Mg powder, 1 ml of HCl, and 2 ml of amyl alcohol and a red, yellow, or orange color formed indicating the presence of flavonoids [7].

Tanin test. The extract is heated ± 5 minutes and cooled. Into the test tube, drop the FeCl3 solution to form a blue-black color indicating the presence of tannins [8].

Saponin test. Dropping the extract into a test tube, it was boiled in water bath for 20 ml. The filtrate was shaken and left for ten minutes. The foaming shows the presence of saponins [8].

Serum Formulation Production. Serum preparation begins with weighing each ingredient. Guava leaf ethanol extract, Carbomer, Glycerin, Triethanolamine, Na benzoate, Disodium EDTA, and distilled water. Then calibrate the 100 ml aquadest ad, then heat the aquadest sufficiently. Add carbomer then add enough hot water to grind until homogeneous. Add glycerin and mix until homogeneous. Add triethanolamine, grind ad condensed. Dissolve with aquadest Na benzoate and disodium EDTA, then add Na benzoate to the homogeneous mortar, and add the ad-homogeneous gerus disodium EDTA. Add little by little the ethanol extract of guava leaves, and grind it until it is strong. Then add the remaining homogeneous mixed aquadest. Lastly, put the preparation into the serum bottle. The formulation of guava leaf extract serum preparations can be seen in Table 1.

Table 1. Formulation of Guava Leaf Extract Serum

Ingredient	Function	Concentration (%)			
		F1	F2	F3	F0
Guava Leaf Extract	Active substance	1,25	2,5	3,75	0
Carbomer	Gelling agent	1	1	1	1
Gliserin	Humectan	5	5	5	5
Na Benzoat	Preservative	0,15	0,15	0,15	0,15
TEA	Neutralizer	3	3	3	3
Dinatrium EDTA	Chelating Agent	0,2	0,2	0,2	0,2
Aquadest	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

2.3 Physical Evaluation of Serum Preparation Ethanol Extract of Guava Leaves

Organoleptic Test. In this way, changes in the physical form, color, smell, and texture of the serum preparation were observed. After that note the changes

Homogenic Test. The preparations were tested using two glass slides where the sample was placed on one of the slides and placed evenly. A good preparation must be homogeneous and free from clumping particles [9].

pH Test. The pH value of the Guava Leaf Ethanol Extracts Serum has been measured using a pH meter. The measurement was done by dropping a pH meter into the preparation and measuring the indicated acidity of the solution. Measurements were carried out in three replications at room temperature (approximately 25^0C) and the average pH results met the facial pH requirements, which were in the range of 4.5-6.5 [10].

Spread Power Test. The serum preparation shall be placed in the middle of a scaled watch glass, above the serum, and another watch glass shall be placed. Stand for one minute, record how much power is spreading. A spreadability test was conducted for 48 hours after serum production, replication three times and the desired spreadability of 5 to 7 cm has been achieved [11].

Viscosity Test. Observation of the pointer from the viscometer leads to a number on the viscosity scale and then records it (Adnan, 2016). The constant number shown by the viscometer is the viscosity value of the preparation with units of cP = 1 mPa·s. The viscosity requirements are 2000cPs – 50,000cPs (SNI 16-4399-1996) [11].

2.4 Antibacterial Activity Test of *Propionibacterium acnes* Serum Preparation of Ethanol Extract of Guava Leaves (*Psidium guajava*)

The media is perforated with wells, enter each concentration according to a predetermined formula. Store the media in the incubation container at 37^0C for 2x24 hours.

3 Results And Discussion

3.1 Extraction

The ethanol extract of guava leaves was obtained from the Spice and Medicinal Plants Research Institute (Balitro) as much as 12.60 gr. Green viscous extract 126.0 gr with a yield value of 12.60%.

3.2 Phytochemical Screening

From the screening results, it was found that the ethanol extract of guava leaves has secondary metabolites of alkaloids, flavonoids, tannins, and saponins. The results of the phytochemical screening of the ethanol extract of guava leaves can be seen in Table 2.

Table 2. Results of phytochemical screening of ethanol extract of guava leaves

Secondary Metabolites	Result
Alkaloid	+
Flavonoid	+
Tanin	+
Saponin	+

3.3 Physical Test of Serum Preparation of Guava Leaf Extract

Organoleptic Test. Based on the results of the organoleptic test that has been carried out F0 is a preparation base without extract having a white viscous gel form. Whereas in FI; FII; and FIII in the form of an army green-colored liquid with a distinctive plant smell. It can be concluded that the 4 formulas have good and stable criteria based on smell, shape, and color.

Homogeneity Test. Based on the results of the homogeneity test carried out on Serum preparations of ethanol extract of guava leaves (*Psidium guajava* L) as an anti-acne, it has good homogeneity and meets the criteria for serum preparations.

Serum pH Test. Based on the results of the pH test that has been carried out, the serum preparation of ethanol extract of guava leaves (*Psidium guajava* L) as an anti-acne has a different pH, namely F0 6.45; FI 6.15; FII 6.06; FIII 5.75. These results indicate that the pH value of Serum ethanol extract of guava leaves (*Psidium guajava* L) as an anti-acne is by the provisions of previous studies, namely in the criteria of 4.5-6.5. So it can be seen that the four formulas for serum preparations meet the serum criteria by the provisions of previous research journals [12].

Viscosity Test. Based on the results above, the viscosity of the ethanol extract serum preparation of guava leaves (*Psidium guajava* L) showed that the four formulas tested met good viscosity standards. If the serum is made too high (thick) it will be difficult to remove from the packaging container, whereas if the viscosity is too low (dilute) the serum will decrease the length of time it stays on the skin when used.

Spread Power Test. Based on the results of the spreadability test of the ethanol extract serum preparations of guava leaves (*Psidium guajava* L) that the four formulas tested

had good spreadability values because they met the spreadability requirements of the serum preparations, namely 5-7 cm [11].

3.4 Antibacterial Activity Test of *Propionibacterium acnes* Serum Preparation of Ethanol Extract of Guava Leaves (*Psidium guajava*)

In this study FI (extract concentration 12.5%), FII (extract concentration 2.5%), and FIII (extract concentration 3.75%) were tested for bacteria against Propionibacterium acnes. The F0 used is a serum formulation without extract to see whether the serum base used has antibacterial activity or not. F0 (positive control) and aquadest (negative control). The test results can be seen in Table 3.

Table 3. Antibacterial test results for serum preparations of ethanol extract of guava leaves (*Psidium guajava*)

Formula	Replication			Average	Category
	I	II	III		
FI	15,6	16,0	15,4	15,0 ± 0,02	High
FII	16,4	17,0	16,0	16,0 ± 0,02	High
FIII	17,9	21,0	17,0	19,0 ± 0,09	High
Control (+)	15,7	13,3	15,9	15,0 ± 0,02	High
Control (-)	0	0	0	-	-

The table above shows that the results of antibacterial testing on anti-acne serum preparations of guava leaf extract on FI have a clear zone value of 15.0 mm, the inhibition zone indicates that it is in a strong category. From the clear zone in the wellbore area, FII has an inhibition zone value of 16.0 mm with a strong category and FIII has an inhibition zone value of 19.0 mm with a strong category. The three formulas suggest that the best clear zone is produced in FIII, which has been classified as strong and contains a higher concentration of guava leaf extract, thereby resulting in greater inhibition. The clear zone produced by the strong positive control is 15.0 mm, the clear zone around the good holes is caused by the presence of active substances in guava leaf extract, flavonoids, alkaloids, tannins, and saponins. Flavonoid compounds have antibacterial properties with a working mechanism that damages the permeability of bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids [13]. Saponin compounds can interfere with the permeability of microbial cell membranes which results in damage to cell membranes and cause their release as important components from inside microbial cells, namely nucleic acids, proteins, and others [13]. Due to their inability to form a complete wall layer and lead to cell death, alkaloid compounds may have an effect on the constituents of peptidoglycan in bacteria. In bacterial cells, alkaloid compounds may interfere with the components of peptidoglycan and cause cell death, so that the cell wall layer is not fully formed [14]. In order to enhance its efficacy and activity in inhibiting growth of bacteria, the tannin compound has a mechanism whereby each of these compounds can be integrated into one another [1]. Psidium guava leaves also contain an anti-inflammatory activity, which

may serve to reduce the inflammation process in acne lesions due to a decrease of serum chemokines like interleukin 8 and estradiol caticolic protein [5].

Inhibition of the growth of Propionibacterium acnes bacteria may be achieved with serum preparations in this study. The viscosity of the serum may explain this, making it easier to release active substances and easy to penetrate the skin in a more dilute way than with other types of serums that are highly concentrated [14]. From the results of the analysis of antibacterial test data, the serum preparation of ethanol extract of guava leaves (*Psidium guajava* L) as an anti-acne was stated to be significant with a value of> 0.05.

4 Conclusion

Based on the results of the antibacterial activity test, serum extract preparations have been carried out Guava leaf ethanol (*Psidium guajava* L) can be used as an antibacterial activity which has the best activity against *Propionibacterium acnes* with an inhibition zone of 19.0 mm this shows the antibacterial activity of the serum preparation as an anti-acne is included in the strong category.

References

1. S. Marselia, M. Agus Wibowo, S. Arreneuz, and J. H. Hadari Nawawi, "AKTIVITAS ANTIBAKTERI EKSTRAK DAUN SOMA (Ploiarium alternifolium Melch) TERHADAP Propionibacterium acnes," vol. 4, no. 4, pp. 72–82, 2015.
2. S. W. Raharjeng, C. Ikhda, N. Hamidah, and Z. Pangestuti, "FORMULASI DAN EVALUASI SERUM ANTI JERAWAT BERBASIS MINYAK ATSIRI Curcuma zedoaria," 2021.
3. E. A. Maulana, I. A. R. Astiti Asih, and M. Arsa, "ISOLASI DAN UJI AKTIVITAS ANTIOKSIDAN SENYAWA FLAVONOID DARI EKSTRAK DAUN JAMBU BIJI PUTIH (Psidium guajava Linn)," *J. Kim.*, no. band I, pp. 161–168, 2016, doi: 10.24843/jchem.2016.v10.i01.p22.
4. N. S. Gunarti, "PEMANFAATAN EKSTRAK DAUN JAMBU BIJI (Psidium guazava) SEBAGAI GEL FACIAL WASH ANTIJERAWAT," 2018. doi: 10.36805/farmasi.v3i2.492.
5. F. Qa'dan, A. J. Thewaini, D. A. Ali, R. Afifi, A. Elkhawad, and K. Z. Matalka, "The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms," *Am. J. Chin. Med.*, vol. 33, no. 2, pp. 197–204, 2005, doi: 10.1142/S0192415X05002783.
6. R. Yulianti, "FORMULASI KRIM ANTI JERAWAT KOMBINASI EKSTRAK DAUN SIRSAK (Annona muricata L.) DAN DAUN JAMBU BIJI (Psidium guajava L.)," *J. Kesehat. Bakti Tunas Husada J. Ilmu-ilmu Keperawatan, Anal. Kesehat. dan Farm.*, vol. 14, no. 1, p. 158, 2015, doi: 10.36465/jkbth.v14i1.125.
7. H. M. Rumagit, M. R. J. Runtuwene, S. Sudewi, J. Kimia, and F. U. Manado, "UJI FITOKIMIA DAN UJI AKTIVITAS ANTIOKSIDAN DARI EKSTRAK ETANOL SPONS Lamellodysidea herbacea Program Studi Farmasi Fakultas MIPA UNSRAT Manado," *PHARMACONJurnal Ilm. Farm. – UNSRAT*, vol. 4, no. 3, pp. 2302–2493, 2015.

8. P. S. Manongko, M. S. Sangi, and L. I. Momuat, “Uji Senyawa Fitokimia dan Aktivitas Antioksidan Tanaman Patah Tulang (Euphorbia tirucalli L.),” *J. MIPA*, vol. 9, no. 2, p. 64, 2020, doi: 10.35799/jmuo.9.2.2020.28725.
9. N. A. Sayuti, “Artikel Riset Formulasi dan Uji Stabilitas Fisik Sediaan Gel Ekstrak Daun Ketepeng Cina (Cassia alata L .) Formulation and Physical Stability of Cassia alata L . Leaf Extract,” *J. Kefarmasian Indones.*, vol. 5, no. 2, pp. 74–82, 2015.
10. K. Kartini, L. A. D. Putri, and M. A. Hadiyat, “FTIR-based fingerprinting and discriminant analysis of Apium graveolens from different locations,” *J. Appl. Pharm. Sci.*, vol. 10, no. 12, pp. 62–67, 2020, doi: 10.7324/JAPS.2020.101208.
11. I. D. K. Irianto, P. Purwanto, and M. T. Mardan, “Aktivitas Antibakteri dan Uji Sifat Fisik Sediaan Gel Dekokta Sirih Hijau (Piper betle L.) Sebagai Alternatif Pengobatan Mastitis Sapi,” *Maj. Farm.*, vol. 16, no. 2, p. 202, 2020, doi: 10.22146/farmaseutik.v16i2.53793.
12. S. Ojha, H. Chadha, B. Aggarwal, S. Sinha, S. Das Chaudhuri, and S. Mahor Jain, “Formulation and Evaluation of Face Serum Containing Bee Venom and Aloe Vera Gel,” *World J. Pharm. Res.*, vol. 8, no. 2, pp. 1100–1105, 2019, doi: 10.20959/wjpr20192-14104.
13. L. Fikayuniar *et al.*, “Formulasi dan Uji Efektivitas Antibakteri Sediaan Serum Antijerawat Ekstrak Etanol Daun Kemangi (Ocimum x africanum Lour.),” *J. Buana Farma*, vol. 1, no. 4, pp. 15–17, 2021.
14. A. Maghfiroh, *Formulasi dan Evaluasi Sediaan Serum Ekstrak Daun Sirih Hijau (Piper betle L.) Terhadap Bakteri Propionibacterium acnes Secara In Vitro*. 2019.