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THE POTENCY OF PSIDIUM GUAJAVA LEAVES AS A NATURAL DISINFECTANT AND ANTISEPTIC INGREDIENT

Potensi Daun Jambu Biji Sebagai Bahan Disifektan dan Antiseptik Alami

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ABSTRACT
Preventing the spread of nosocomial infection are generally carried out by disinfection process of the tools to be used with chemical disinfectant such as phenol, alcohol, chlorine, iodine or sublimate. Excessive use of chemicals can cause health problems. Guava leaves (*Psidium guajava* L.) have long been empirically used to treat diarrhea and acne. Scientific data shows that guava leaves have positive activity against several types of pathogenic bacteria due to its tannin content. Guava leaves have the potential to be developed as a disinfectant and antiseptic material. This study aims to determine the disinfection activity of guava leaves on surgical instruments using the total plate count method, skin irritation test using patch test method and quantify the tannin content in young and old guava leaves using spectrophotometry method. The results showed that the tannin content in old and young guava leaves was 68.73 ± 0.067 mg/g (6.873%) and young 61.87 ± 0.046 mg/g (6.187%). At concentrations of 10, 20, 30 and 40% guava leaf extract were able to reduce the total plate count in surgical instrument samples by 6.85; 37; 80.82; and 83.56%. The primary irritation index of 0.05 only occurs at a concentration of 40%, classified as non-irritant. It can be concluded that guava leaves have the potential to be further developed as a natural disinfectant or antiseptic.

Keywords: *Antiseptic, Disinfectant, Psidium guajava, Tannin*

ABSTRAK
Pencegahan penyebaran infeksi nosokomial umumnya dilakukan dengan proses desinfeksi alat-alat yang akan digunakan dengan desinfektan kimia seperti fenol, alkohol, klorin, iodium atau sublimasi. Penggunaan bahan kimia yang berlebihan dapat menimbulkan gangguan kesehatan. Daun jambu biji (*Psidium guajava* L.) telah lama digunakan secara empiris untuk mengobati diare dan jerawat. Data ilmiah menunjukkan bahwa daun jambu biji memiliki aktivitas positif terhadap beberapa jenis bakteri patogen karena kandungan taninnya. Daun jambu biji berpotensi untuk dikembangkan sebagai bahan desinfektan dan antiseptik. Penelitian ini bertujuan untuk mengetahui aktivitas desinfeksi daun jambu biji terhadap instrumen bedah menggunakan metode total plate count, uji iritasi kulit menggunakan metode patch test dan mengukur kadar tanin pada daun jambu biji muda dan tua menggunakan metode spektrofotometri. Hasil penelitian menunjukkan bahwa kadar tanin pada daun jambu biji tua dan muda masing-masing 68,73 ± 0,067 mg/g (6,873%) dan muda 61,87 ± 0,046 mg/g (6,187%). Pada konsentrasi 10, 20, 30 dan 40% ekstrak daun jambu biji mampu menurunkan total plate count pada sampel instrumen bedah masing-masing sebesar 6,85; 37; 80,82; dan 83,56%. Indeks iritasi primer sebesar 0,05 hanya terjadi pada konsentrasi 40% yang tergolong non-iritan. Dapat disimpulkan bahwa daun jambu biji berpotensi untuk dikembangkan lebih lanjut sebagai desinfektan atau antiseptik alami.

Kata Kunci: *Antiseptik, Desinfektan, Psidium guajava, Tanin*

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INTRODUCTION

Infectious diseases remain one of the leading causes of morbidity and mortality worldwide with up to three million children and adolescents dying from infectious diseases each year, equivalent to one death every 10 seconds (Global Burden Diseases, 2019). According to data from the World Health Organization (WHO), around 3-21% or an average of 9% experience nosocomial infections. Recent research confirmed that the rate of nosocomial or hospital acquired infection (HAIs) in the world is increasing by 0.06 percent annually (Raoofi, 2023).

Preventing the spread of infection are generally carried out by disinfection process of the tools to be used with disinfectant fluids. Disinfectant materials are various chemicals such as phenol, alcohol, chlorine, iodine or sublimate (Centre For Food Security & Public Health, 2023; Fijan et al. 2007). The use of various disinfectant chemicals is quite effective in reducing the incidence of infection, but has negative effects on health. Chlorine gas can cause respiratory disorders and irritate the skin and mucous membranes (Hoyle & Svendsen, 2016; Li et al., 2015). Chemical phenol disinfectants are cytoplasmic poisons and if used for a long period of time will cause multisystem organ failure (Downs & Wills, 2024). In addition to having negative effects on body health, the chemicals in disinfectants also cause microorganism resistance (Rozman et al., 2021).

Natural ingredients can be an alternative disinfectant that is safer for health and more environmentally friendly. Guava leaves (*Psidium guajava* L.) are medicinal plants that are easily obtained and have long been empirically used as a natural antidiarrheal drug. Previous study of Gupta and Birdi (2015) states that *Psidium guajava* leaf extract shows the capacity to prevent intestinal colonization of *Citrobacter rodentium* in the mouse model. This data is supported by the results of other studies which confirmed that guava leaf decoction can speed up the healing of acute diarrhea without side effects (Birdi et al., 2020). In addition to disinfection activities, guava leaves are also expected to have antiseptic properties which can be used on the surface of living tissue according to study of Jayawera et al. (2021) which has confirmed the ability of guava leaves to suppress the growth of acne-causing bacteria. To predict the potential of guava leaves as an antiseptic, it is necessary to conduct a skin irritation test using a closed sample test method on human skin (human patch test) by observing the symptoms that arise such as redness (erythema), swelling (oedema) and itching after the skin is treated for 24 hours (OECD, 2015).

The antimicrobial activity of guava leaves is most likely related to its tannin and other polyphenol content. Guava leaves are known to have high tannin and polyphenol content (Huynh et al., 2025). Tannins and polyphenol compounds are confirmed to have antimicrobial activity (Huang et al., 2024; Daglia, 2012) with a various mechanism such as directly destroying bacterial membrane and cell wall, inhibiting the synthesis and expression of bacterial proteins and enzyme activity, damaging bacterial metabolism, and inhibiting the formation of bacterial biofilms and toxins (Jing, et al., 2022). The levels of secondary metabolites differ in each part of the plant, so it is necessary to test the part of the plant with the highest tannin content to obtain efficient raw materials.

MATERIAL

The tools were glassware (Pyrex®), scalpel (property of pharmacology practical lab. Universitas Pakuan), autoclave (Hirayama), incubators, ovens (memmert®), rotary evaporator (IKA RV3V), 40 mesh sieve (PT Pharmeq), brown glass bottles, petri dishes, vortex, filter paper (Whatman®), sterile gauze.

The materials used were aquadest (H_2O), 70% alcohol (C_2H_6O), guava leaves (*Psidium guajava* L., determination number 996/IPH.1.08/If30/I/2024 issued by PT Palapa Muda Perkasa), Folin-Ciocalteu reagent, Na_2CO_3, Plate Count Agar (PCA media, Oxoid CMO 325)

METHODS

Preparation of Guava Leaves Extract

Each fresh old and young guava leaf was dried in an oven at 50 ^{O}C and ground to obtain dry powder. To prepare extract for

determination of tannin content, 50 g of old and young guava leaf powder were macerated with 500 mL of 70% ethanol as a solvent. Each filtrate was dried to obtain a thick extract using a rotary evaporator.

Determination of Tannin Content

Tannin content was measured in young guava leaf samples and old guava leaves using the spectrophotometric method.

A set of standard tannic acid reference solutions was prepared to obtain a standard curve for the measurement reference were prepared using a concentration series of tannic acid (10, 15, 20, 25, 30, 35 ppm). About 1 mL of each concentration were mixed with 1 mL of Folin-Ciocalteu reagent then left for 3 minutes. The mixture was added with 1 mL of saturated Na2CO3 and incubated in a dark place for 40 minutes. The absorbance was measured using visible UV/VIS spectrophotometry at a wavelength of 700 nm. The value of absorbances were plotted to obtain a standard curve.

About 1 mg of young and mature guava leaves extracts were weighed and dissolved with distilled water to 10 ml (100 ppm). Each was pipetted as much as 9 mL and dissolved with distilled water to 10 mL (90 ppm). 1 mL of folin-Ciocalteu reagent was added, left for 3 minutes, 1.0 ml of saturated Na2CO3 solution was added and incubated for 40 minutes. The absorbance was read at a wavelength of 700 nm. The tannin content was calculated by entering the absorbance value into the linear regression equation obtained from the standard curve. The leaves with the highest tannin content used as the raw material for making disinfectant fluid

Disinfection Activity Test

Old leaves are selected as raw materials for disinfectant solution based on previous test of tannin content. Guava leaf extract solution was prepared at a concentration of 10, 20, 30, 40% using decoction method with distilled water as a solvent. Sterile distilled water and 70% alcohol were used as negative and positive controls. A plate count agar media was prepared by dissolving 18 gr PCA in 1 L aquadest and sterilized by autoclaving at a pressure of 15 lbs (121°C). The test material were a scalpels in the pharmacology laboratory of Pakuan University. The scalpels has been stored without sterilization for 1 month after the last use.

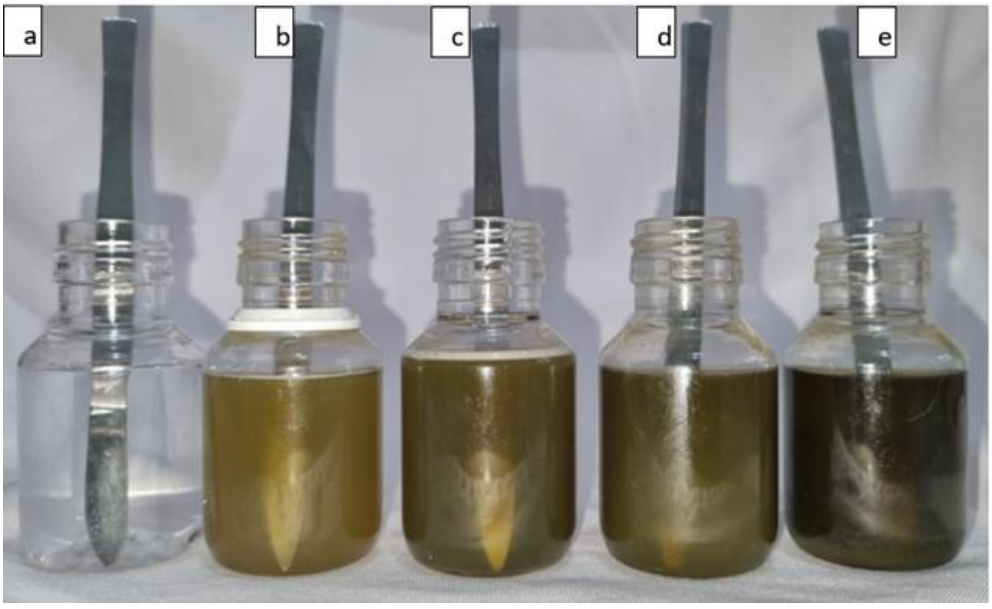

Figure 1. Disinfection process of surgical instruments with guava leaf extract solution
Note: a. Aquadest b. 10% concentration c. 20% concentration
d. 30% concentration e. 40% concentration

The sampling procedure was carried out with a modified contact plate method, total plate count test and calculations refer to the manual published by Unites States Food and Drug Administration (Maturin and Peeler, 2001). 3 replications were prepared for all tests. Disinfection process was carried out by soaking the scalpel in the guava leaf extract solution for 30 minutes (Figure 1). After the disinfection process, the scalpel instrument was transferred into a physiological NaCl solution (10 mL in a test tube), the tube was then vortexed for 10 seconds to remove microorganisms from the surface of the surgical instrument into the physiological NaCl solution. About 1 ml of solution from each test tube was taken and put in a petri dish containing 20 mL of warm PCA media. The plates were incubated at 37^{o}C for 24 hours. The number of bacterial colonies that grew on each plate was counted to determine the total plate count. The best disinfectant concentration is indicated by the fewest number of total plate count in each plate.

Skin Irritation Test

The irritation test (OECD 404) aims as a preliminary study to determine the potential of guava leaves as an antiseptic The test was conducted on 20 volunteers aged 18-40. Before the test procedure, volunteers have agreed and signed an informed consent, which explains the purpose of the test. During the test period, volunteers did not use lotion, steroid drugs, and antihistamines. Guava extract (0.2 mL at a concentration of 40%) was placed on a sterile gauze patch measuring 2x2cm (Figure 2), attached to the volunteer's upper arm for 4 hours, then the patch was removed. Skin irritation examination was carried out at 24, 48, and 72 hours after the patch was removed. The skin irritation degree was asses by giving a score of 0 to 4 based on the oedema and erythema reactions in the skin area according to Draize dermal irritation scoring system (DDISS) as presented in Table 1.

Figure 2. Gauze patch containing guava leaf extract at a concentration of 40%

Table 1. Primary Skin Irritation Scoring System

Erythema Formation	Value	Oedema Formation
No erythema	0	No oedema
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)
Well-defined erythema	2	Slight oedema (edge of area well defined)
Moderate to severe erythema	3	Moderate Oedema (raised ± 1 mm)
Severe Erythema	4	Severe Oedema (raised > 1 mm, accompany by extending area)

RESULTS AND DISCUSSION

Tannin Content

Determination reveal that the tannin content in old and young guava leaves are 68.73 ± 0.067 mg/g dry weight (6.87%) and 59.84 ± 0.046 mg/g dry weight (5.98%) respectively. The tannin content of old guava leaves is higher than the tannin content of young guava leaves. Similar research on tea leaves shows the similar pattern where old leaves contain higher tannin than young leaves (Mufti et al., 2009; Widarta and Wiadnyani, 2019).

Previous studies on tannin levels in guava leaves showed varying results ranging from very low levels of 0.2351% (2.351mg/g) (Mailoa et al., 2014) to quite high levels reaching 17.76% (177.6 mg/g) (Rosnani and Mariane, 2014). The tannin content of tea leaves in this study was classified as the average tannin content contained in medicinal plants (Siqueira, 2011). Sample preparation, plant varieties, growing locations, solvents used, and extraction methods are the causes of differences in the content data produced from each study. Based on tannin content measurement data, old guava leaves were selected as the raw material to prepare disinfectant solution.

Disinfection Activity

Based on the test results, guava extract can reduce the total plate count on the surface of the test material. The disinfection activity of guava leaf extract at various concentrations can be seen in Figure 3.

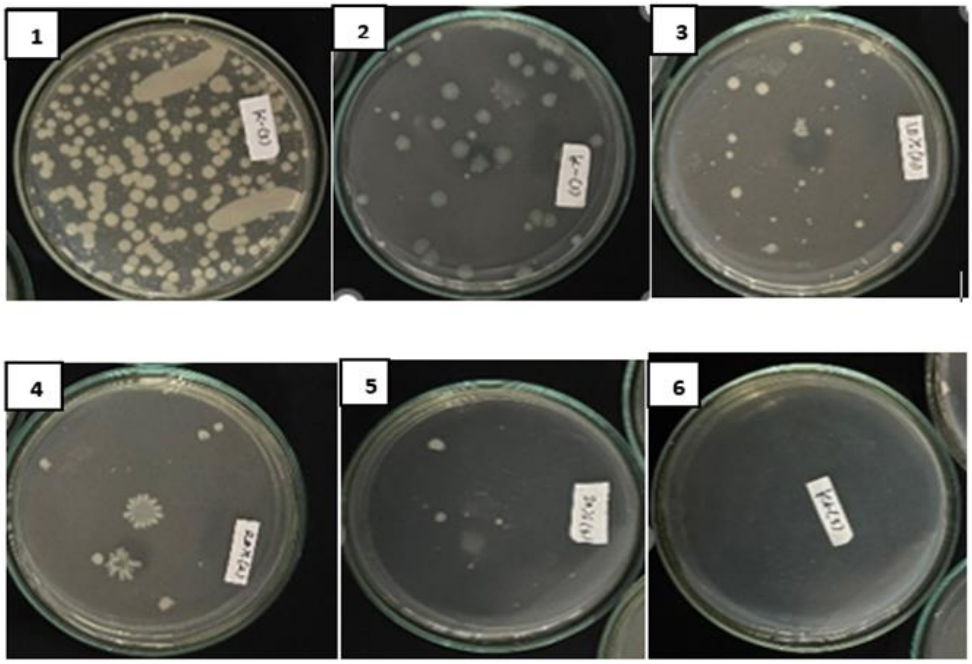

Figure 3. Total plate count after disinfection process with guava leaf extract solution

1. Control (-)
2. 10%
3. 20%
4. 30 %
5. 40 %
6. Control (+)

The percentage of total plate count reduction is quite high as seen in Table 2. The total plate count on the scalpel after the disinfection process of guava leaf solution at a concentration of 40% decreased by 83.56%. In this study, increasing the concentration of guava leaf extract above 40% did not increase its disinfection power, a concentration of 40% was stated as the optimal concentration to reduce the total plate count on the surface of surgical instruments as test samples.

Table 2. Total Plate Count After Disinfection Process with Guava Leaf Extract Solution

Concentration of Guava Leaves Extract	Total Plante Count (CFU/cm)	Percentage of Total Plate Count Decrease Against Negative Control (%)
Aquadest (-)	73	-
10 %	68	6.85
20 %	46	37
30 %	14	80.82
40 %	12	83.56
Ethanol 70 % (+)	0	100

The ability of guava leaves to reduce the number of microorganisms is proven by other studies where the use of guava leaf ethanol extract at a concentration of 1.5 mg/L was able to eliminate fecal coliform by 97% (El-Sesy and Mahran, 2020). Guava leaf extract at high concentrations was also confirmed to be able to reduce the total plate count by 89% in the chicken egg incubator (Marlina, 2017).

Another previous studies showed that methanol extract and ethanol extract of guava leaves at a concentration of 50 µ L had activity against *Bacillus cereus* and *Staphylococcus aureus* with an average inhibition zone of 8.27 and 12.3 mm, and 6.11 and 11.0 mm and other test results showed that guava leaf extract at a concentration of 250 ppm-3250 ppm has the potential as an antibacterial against Aeromonas hydrophila bacteria with an inhibition zone diameter ranging from 6.5-11.5 mm Biswas et al., 2013). Ethanol extract of guava leaves was also confirmed to have inhibitory activity against *Shigella dysentriae* with an inhibition zone of 22 mm at a concentration of 200 mg/mL (Sule et al., 2022), had a minimum inhibitory concentration value of 30 mg/mL against *S. aureus* bacteria (Azizan et al., 2020), and was able to inhibit the growth of *Escherichia coli* with an inhibition zone diameter of 6.43-8.17 mm at a concentration of 10% (Qonita et al., 2019).

This disinfection activity is predicted to be related to the tannin compounds found in guava leaves. Tannins are known to have strong antibacterial properties with the ability to eliminate microorganisms through different mechanisms of action including iron chelation, inhibition of cell wall synthesis, disruption of the cell membrane, and inhibition of fatty acid biosynthetic pathways (Guofeng et al., 2018). Tannins can act as inhibitors to prevent the formation of certain virulence factors such as biofilms, enzymes, adhesins, motility, and toxins. With these characteristics, tannins can be a promising alternative as an antibacterial agent (Arakkaveettil et al., 2020; Salunkhe et al., 2021; Chung et al., 1998). Overall, the results of this study indicate the potential of guava leaves as a natural ingredient for controlling microorganisms, but optimization is needed because the total plate count value at a concentration of 40% is still below the positive control (+).

Skin Irritation Test

The results of the skin irritation test was displayed in table 3. Based on the table 3, it was found 1 volunteer out of 20 experienced mild erythema at a solution concentration of 40%, test time of 48 and 72 hours as shown in Table 3.

Table 3. Skin Irritation Test of Several Concentrations of Guava Leaf Extract Solution

Concentratiof Guava leaves (%)	**Number of Volunter**	**Duration of Test 24 hours**		**Duration of Test 48 hours**		**Duration of Test 72 hours**		**Primary Iritation Index**
		Erythema	Oedema	Erythema	Odema	Erythema	Odema	0
10	20	0	0	0	0	0	0	0
20	20	0	0	0	0	0	0	0
30	20	0	0	0	0	0	0	0
40	20	0	0	1	0	1	0	0.05*

The primary irritation index (PII) value was 0.05, included in the non-irritant category based on the skin irritation index classification (Table 4).

Table 4. Classification of Primary Irritation Index

Irritation Classification	Primary Irritation Index (PII)
Non irritant	0 – 0,4
Mild	0,5 – 1,9
Moderate	2 – 4,9
Severe	5,0 – 8,0

The result of skin irritation test in this study was similar to previous studies on the formulation of cosmetic preparations based on guava leaf extract which confirmed that there was no irritation after using the cosmetic (Salunkhe et al., 2021; Archana et al., 2022). In addition to being safe for the skin, previous studies have shown that guava leaf extract has antibacterial activity (Pereira, et al., 2023) so it can be concluded that guava leaf extract does not cause skin irritation, safe to use and has the potential to be developed both as a disinfectant and antiseptic ingredients.

CONCLUSION

The data from this study showed that old guava leaves contain tannins with a level of 68.73 ± 0.067 mg/g (6.873%), higher than young jamu biji leaves with a tannin content of 61.87 ± 0.046 mg/g (6.187%). The tannin content of guava leaves is classified as moderate. A disinfectant solution made from guava leaves with a concentration of 40% showed good ability to reduce the total plate count by 83.56% on the surface of surgical instruments. The results of the irritation test confirmed that the guava leaf solution did not irritate the skin. In conclusion, guava leaves have the potential to be further developed as a natural disinfectant and antiseptic.

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