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Efficacy of wound cleanser 25% based on Indonesian medicine plants on chronic wound healing

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Abstract

Background: Chronic wounds are a challenge for wound care professionals and a global economic burden that has increased morbidity and mortality in patients worldwide.

Purpose: This study aims to determine the efficacy of wound cleanser with guava leaf extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) 25% on the inhibition of bacterial growth in chronic wounds.

Methods: The research conducted was laboratory experimental in nature. Guava leaf extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) were formulated in the form of liquid soap with a concentration of 25% then evaluation of the physical preparations, allergy tests and bacterial tests were carried out in the form of the inhibition of liquid soap against *Pseudomonas aeruginosa* bacteria, *Staphylococcus aureus*, *Escherichia coli* in chronic wounds, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus epidermidis* and Yeast *Candida Albicans*. Data collection is carried out after passing the ethical test.

Results: This proves that liquid soap preparations of ethanol extract of guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) have medium, strong and very strong inhibition against *Pseudomonas aeruginosa* (12.28 mm), *Staphylococcus aureus* (19.48 mm), *Escherichia coli* (13.94 mm), *Bacillus subtilis* (27.58 mm), *Bacillus cereus* (11.11 mm), *Staphylococcus epidermidis* (14.44 mm) and category of respond medium inhibition on Yeast *Candida Albicans* (8.13 mm).

Conclusion: Based on the results of the study, it can be concluded that the formulation of liquid soap preparations of ethanol extract of guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) with a concentration of 25% fulfills the quality requirements for liquid soap preparations. The results of this liquid soap antibacterial test had a strong response in inhibiting the growth of the bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus epidermidis*.

Keywords: Wound Cleanser, *Psidium guajava* Linn, *Piper betle* L, Bacteria

Abstrak

Latar belakang: Luka kronis merupakan tantangan bagi profesional perawatan luka dan beban ekonomi global yang meningkatkan morbiditas dan mortalitas pada pasien di seluruh dunia.

Tujuan: Penelitian ini bertujuan untuk mengetahui khasiat pembersih luka dengan ekstrak daun jambu biji (*Psidium guajava* Linn) dan ekstrak daun sirih (*Piper betle* L) 25% terhadap penghambatan pertumbuhan bakteri pada luka kronis.

Metode: Penelitian yang dilakukan bersifat eksperimental laboratoris. Ekstrak daun jambu biji (*Psidium guajava* Linn) dan ekstrak daun sirih (*Piper betle* L) diformulasikan dalam bentuk sabun cair dengan konsentrasi 25% kemudian dilakukan evaluasi sediaan fisik, uji alergi dan uji bakteri berupa penghambatan sabun cair terhadap bakteri *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* pada luka kronis, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus epidermidis* dan Yeast *Candida Albicans*. Pengumpulan data dilakukan setelah lolos uji etik.

Hasil: Hal ini membuktikan bahwa sediaan sabun cair ekstrak etanol daun jambu biji (*Psidium guajava* Linn) dan ekstrak daun sirih (*Piper betle* L) mempunyai daya hambat sedang, kuat dan

sangat kuat terhadap *Pseudomonas aeruginosa* (12,28 mm), *Staphylococcus aureus* (19,48 mm), *Escherichia coli* (13,94 mm), *Bacillus subtilis* (27,58 mm), *Bacillus cereus* (11,11 mm), *Staphylococcus epidermidis* (14,44 mm) dan kategori media respon penghambatan pada *Yeast Candida Albicans* (8,13 mm).

Kesimpulan: Berdasarkan hasil penelitian dapat disimpulkan bahwa formulasi sediaan sabun cair ekstrak etanol daun jambu biji (*Psidium guajava* Linn) dan ekstrak daun sirih (*Piper betle* L) dengan konsentrasi 25% memenuhi syarat mutu. persyaratan pembuatan sabun cair. Hasil uji antibakteri sabun cair ini memiliki respon yang kuat dalam menghambat pertumbuhan bakteri *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus epidermidis*.

Kata kunci: Pembersih Luka, *Psidium guajava* Linn, *Piper betle* L, Bakteri

INTRODUCTION

Wounds can be treated optimally if the wound is handled properly. Inappropriate wound handling can result in the wound healing process taking longer and sepsis spreading to other parts and can even lead to amputation(1). Several methods are used in the treatment of diabetic ulcers including wound cleansing, debridement, and dressing(2).

Soap production has developed so that it becomes softer and can be used for bathing. The development of technology and knowledge so that there are many types of liquid soap. Liquid soap is produced for various purposes such as for bathing, washing hands, washing dishes or household appliances and so on. The characteristics of liquid soap are different for each need, depending on the composition of the ingredients and the manufacturing process. The advantages of liquid soap include being easy to travel with and more hygienic because it is usually stored in a tightly closed container(1,3). Wound washing is an important component and is a standard goal during acute and chronic wound care. The very important role of microorganisms in the process of wound infection makes *Streptococcus mutans* the main target in efforts to prevent wound infections(4). Research using natural materials that aim to produce drugs has been widely carried out, this is considered very useful because since ancient times people have long used drugs derived from natural ingredients to treat various diseases (4,5).

In addition, the use of natural materials in the form of 25% Wound Cleanser also

supports the government's efforts to manage and empower natural resources because Indonesia is a rich country. with biodiversity and natural resources(6). The benefits of Wound Cleanser in Indonesia are still few scientific studies. According to Putri (7) shows that betel leaf extract provides a boundary area of inhibition with an average diameter of 2.3 mm at an extract concentration of 1%, an average diameter of 3.3 mm at an extract concentration of 2%, an average diameter of 4.3 mm at a concentration of 4%, an average diameter of 5.6 mm at a concentration of 6%, an average diameter of 7.3 mm at a concentration of 8%, an average diameter of 9 mm at a concentration of 10% to inhibit the growth of *Pseudomonas aeruginosa* bacteria.

The results of pharmacological tests showed that betel leaf infusion could inhibit the growth of the *Pseudomonas aeruginosa* bacteria(2). The phenol content contained in green betel is believed to have more phenol content than phenol in general. Phenol can inhibit bacterial activity(5). In Putri study(7), extracts of green betel leaves and guava leaves obtained with ethanol solvent had antibacterial activity against several gram-positive and gram-negative bacteria, one of which was *Pseudomonas aeruginosa*(1).

Betel leaf and guava contain active compounds that have the potential to have antibacterial and antioxidant activity. Green betel leaves contain active compounds such as eugenol, cineole, and safrole which have antibacterial activity against gram-positive and gram-negative bacteria(1). In addition, green betel leaves also contain flavonoid compounds that have potential as

antioxidants. Meanwhile, guava seeds contain active compounds such as gallic acid, ellagic acid, and tannins which have antibacterial activity against gram-positive and gram-negative bacteria(8). In addition, guava seeds also contain flavonoid compounds that have potential as antioxidants. However, to find out the advantages of betel leaf and guava compared to other natural ingredients in terms of antibacterial and antioxidant activity, the researchers were interested in conducting this research(9).

Based on what has been explained above, it is necessary to carry out further research to determine the antibacterial activity of leaf extract Guava leaf extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) are formulated in the form of liquid soap preparations with a concentration of 25% against bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* in chronic wounds, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Yeast Candida Albicans* Fungus

METHODS

Time and Place of Research

This research was conducted at the Wound Care Laboratory of the Yakpermas Polytechnic Banyumas and also the microbiology laboratory at Muhammadiyah University, Purwokerto. Starting from June – November 2022.

Type of research design

Bacterial Test The research conducted was laboratory experimental in nature. Guava leaf extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) were formulated in the form of liquid soap with a concentration of 25% then evaluation of the physical preparations, allergy tests and bacterial tests were carried out in the form of the inhibition of liquid soap against *Pseudomonas aeruginosa* bacteria, *Staphylococcus aureus*, *Escherichia coli* in chronic wounds, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Yeast Candida Albicans*.

Tools and materials

The tools used in this study were a blender (WARING), filter paper, aluminum foil, jars, sieves, analytical balances (AE

Adam), glassware, funnels, magnetic hot plates (ACIS), loop needles, Bunsen burners, stir bar, stirrer, pH meter (CP-505), burette and stand, micropipette, spatula, scale ruler, reservoir, autoclave (ALP), oven (MMM group), laminary air flow (LAF), incubator (EcoCell). The materials used were guava leaf extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L), *Pseudomonas aeruginosa* bacteria, *Staphylococcus aureus*, *Escherichia coli* in chronic wounds, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Yeast Fungus Candida Albicans*, Nutrient Agar media, 96% ethanol, Carboxymethylcellulosum Sodium (CMC-Na), olive oil, potassium hydroxide (KOH), stearic acid, Beta Hydroxy Acid (BHA), Sodium Lauryl Sulfate (SLS), flavoring (peppermint), phenolphthalein, distilled water, sulfuric acid (H₂SO₄) 1%, Barium Chloride (BaCl₂.2H₂O) 1.175%, NaCl, X soap.

Sampling Research Procedures

The samples used in this study were guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) which were taken from the vicinity of Jumbo Kulon Village, Sokaraja District, Banyumas Regency.

Sample Preparation

Guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) were taken directly from the tree as much as 3 kg and then wet sorted to separate from impurities and plant parts that were not used in research which were carried away during the process of collecting guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L). Then guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) are then washed thoroughly using running water. After that, chopping is done so that the leaves dry quickly. The drying process is carried out by aerating at room temperature which is not exposed to direct sunlight. This is so that the chemicals contained in the leaves are not damaged by exposure to direct sunlight.

After dry sorting, guava leaf extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) were mashed using a blender and then sieved. Sieving is carried out to reduce the size of the sample particles so as

to widen the contact angle between the solvent and the sample in order to facilitate the process of withdrawing the active substance when it is extracted. The simplicial powder obtained was 340 grams.

Guava leaf extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) were extracted by cold method, namely the maceration method. The media used in this test is nutrient agar media which consists of beef extract, peptone and agar. The most important compositions in this medium are carbohydrates and proteins contained in bovine extracts and peptones according to the needs of most bacteria(4,5)). In this study we were conducted as Texapone weighed as much as 200 grams mixed with 100% NaCl as much as 30 grams. then all stirred until it becomes surfactant. Then mixed with water little by little to become a solution. When it becomes a solution, mix the extracts of the two ingredients as much as 100 ml each and stir until they become a compound solution. then add food coloring and perfume, pour the ingredients into the container and leave for 24 hours so that it becomes clear soap and loses its foam. The diameter of the drag is measured in millimeters (mm) using a ruler scale. Measurements are made horizontally and vertically. The results obtained horizontally and vertically are summed then divided by two, then subtracted by 7 (mm) which is the diameter of the well then reduced

by 2.5 which is the diameter of the inhibition produced by K- or soap base.

RESULTS

The results show the results of testing the antibacterial effect of ethanol extract of guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* in chronic wounds, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus epidermidis* and Yeast *Candida Fungus Albicans* at a concentration of 25%. Colony counts were performed at all concentrations, negative control and positive control.

Based on Table 1, it shows that the antibacterial activity test was carried out on liquid soap preparations of ethanol extract of guava leaves (*Psidium guajava* L.) and betel leaves with a concentration of 25% with various concentrations as test samples, aquadest liquid as a negative control, and 500 mg of antibiotics (25 %) as a positive control. The method used is the diffusion method by means of wells(3,5,6,9). The well method has advantages compared to other methods such as the disc, which is easier to measure the inhibition zone formed and more sensitive. This is because the sample does not only move above the media, but also below.

Table 1. Wound Cleanser Bacteria Test

Bacteria	Sampel	Diameter Zona Hambat (mm)					
		Cawan 1		Cawan 2		Cawan 3	
		Diameter Horizontal	Diameter Vertical	Diameter Horizontal	Diameter Vertical	Diameter Horizontal	Diameter Vertical
Bacteria <i>Escherichia coli</i>	aquadest						
	Ciprofloxacin 25%	19,13	19,32	21,86	19,04	18,83	18,64
	Soap sample	12,64	12,41	10,92	11,42	13,94	13,97
Bacteria <i>Pseudomonas aeruginosa</i>	aquadest						
	Ciprofloxacin 25%	22,43	27,53	34,15	36,45	39,29	38,31
	Soap sample	10,17	9,97	12,10	12,43	12,28	12,68
Bacteria <i>Staphylococcus aureus</i>	aquadest						
	Ciprofloxacin 25%	38,17	38,58	16,93	16,71	16,70	15,59
	Soap sample	19,48	21,45	10,97	10,56	11,37	11,40
Bacteria <i>Staphylococcus epidermidis</i>	aquadest						
	Ciprofloxacin 25%	19,89	19,52	21,40	20,72	23,02	22,02
	Soap sample	12,25	13,05	11,60	12,39	12,83	14,44
Bacteria	aquadest						

Bacillus subtilis	Ciprofloxacin 25%	36,65	36,92	21,70	36,48	35,75	33,68
	Soap sample aquadest	27,58	23,90	22,38	21,36	24,04	21,23
Bacteria Bacillus cereus	Ciprofloxacin 25%	56,05	48,65	38,98	10,30	39,98	40,29
	Soap sample aquadest	9,16	10,53	10,82	11,11	10,37	10,69
Yeast Candidas albicans	Ciprofloxacin 25%						
	Soap sample	8,13	7,41	7,87	6,74	6,93	6,19

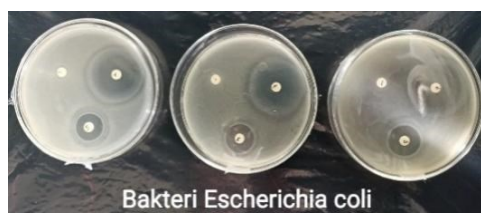


Figure 1. Bacteria Escherichia coli



Figure 2. Bacteria Pseudomonas aeruginosa



Figure 3. Bacteria Bacillus subtilis



Figure 4. Bacteria Staphylococcus aureus

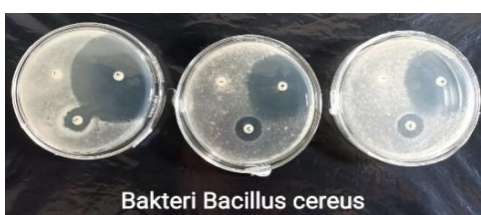


Figure 5. Bacteria Bacillus cereus



Figure 6. Bacteria Staphylococcus epidermidis



Figure 7. Yeast Candidas albicans

DISCUSSION

Each concentration was replicated 3 times and then the average was calculated. In the media given the ethanol extract of guava leaves (*Psidium guajava* L.) and betel leaf with a concentration of 25% and the positive control showed the same results in

each replication in the range / ml or sterile, which means that after planting in NA media and incubated for 24 hours at 37°C temperature, there was no growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* in chronic wounds, *Bacillus subtilis*, *Bacillus cereus*,

Staphylococcus epidermidis and Yeast Candida Fungi(10).

An active substance is said to have high potential as an antibacterial if at low concentrations it has great inhibition. Antibacterial inhibition test according to Dimpidus et al (11), categorized based on the diameter of the inhibition formed, namely the diameter of the inhibition of 5 mm or less is categorized as weak, the inhibition of 5-10 (mm) is categorized as moderate, the inhibition of 10-20 (mm) is categorized strong and a resistance of 20 (mm) or more is categorized as very strong(3,5,12).

The media used in this test is nutrient agar media which consists of beef extract, peptone and agar. The most important composition in this media. The media used in this test is nutrient agar media which consists of beef extract, peptone and agar. The most important composition in this media is carbohydrates and proteins found in bovine extracts and peptones according to the needs of most bacteria(1). The diameter of the drag is measured in millimeters (mm) using a ruler scale. Measurements are made horizontally and vertically. The results obtained horizontally and vertically are summed then divided by two, then subtracted by 7 (mm) which is the diameter of the well then reduced by 2.5 which is the diameter of the inhibition produced by K- or soap base.

The results of counting the number of colonies (as Figure 1-7) at a concentration of 25% with a negative control guide found that the bacterial colonies of the type Bacillus subtilis had a very strong criterion response to inhibiting their growth both in replicating bacterial preparations 1, 2, 3 and at a concentration of 25% with a negative control guideline found that the bacteria type Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus cereus, Staphylococcus epidermidis had an average growth-inhibiting response in the strong category, while responses that had moderate criteria occurred in Yeast Candida.

This proves that liquid soap preparations of ethanol extract of guava leaves (Psidium guajava Linn) and betel leaf extract (Piper betle L) have medium, strong and very strong inhibition against Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli bacteria in chronic

wounds. , Bacillus subtilis, Bacillus cereus, Staphylococcus epidermidis and category of response medium inhibition on Yeast Fungus Candida Albicans. In line with research by Nurul et al (2019), namely using 5 concentrations, namely 100%, 50%, 25%, 12.5%, and 6.25% with 4 repetitions each. Antibacterial effectiveness testing using disk diffusion method. The results of the study using the Kruskal Wallis statistical test showed that there were significant differences at various concentrations on the growth of Streptococcus mutans. The conclusion of this study is that guava leaf infusion has an antibacterial effect against Streptococcus mutans in vitro with an effective concentration of 100%.

This is in accordance with the opinion of Wong et al (4) that the higher the concentration of an antibacterial substance, the stronger the antibacterial activity will be. The existence of antibacterial activity from liquid soap preparations of ethanol extract of soursop leaves shows the effectiveness of the compounds contained in guava extract(7). The active compounds that are thought to provide antibacterial activity against Staphylococcus aureus bacteria are flavonoid compounds. The mechanism of action of flavonoids inhibits bacterial growth by damaging the cell walls, deactivating enzymes, binding to adhesins, and damaging cell membranes. The beta ring and the -OH group in flavonoids are thought to be structures responsible for antibacterial activity(12).

In this study, researchers also used positive controls and negative controls. The use of a positive control serves as a control for the test substance by comparing the diameter of the inhibition area formed. The positive control used was ciprofloxacin 500 mg with a concentration of 25%.

CONCLUSION

Based on the results of the study, it can be concluded that the formulation of liquid soap preparations of ethanol extract of guava leaves (Psidium guajava Linn) and betel leaf extract (Piper betle L) with a concentration of 25% fulfills the quality requirements for liquid soap preparations. The results of this liquid soap antibacterial test had a strong response in inhibiting the

growth of the bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus epidermidis*.

SUGGESTION

Based on the results of research on liquid soap preparations of ethanol extract of guava leaves (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) concentration of 25%, it is recommended to conduct further research for other physical tests that have not been carried out in this study and to conduct research by making extracts Guava leaf ethanol extract (*Psidium guajava* Linn) and betel leaf extract (*Piper betle* L) used other extraction methods and higher concentrations.

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