ANTISEPTIC EFFECTIVENESS OF GUAVA LEAVE EXTRACT (*Psidium guajava L.*), TURMERIC EXTRACT (*Curcuma longa L.*) AND BIOPLACENTON AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

Iis Kurniati[1)], Nina Marliana[1], Linda Triana[2], Asep Dermawan[1], Deny Rudiansyah[1], Hafizah Ilmi Sufa[1]*
[1*)]Jurusan Teknologi Laboratorium Medik (TLM)
Politeknik Kesehatan Bandung, Jl. Pajajaran No. 56 Bandung 40171
[2]Jurusan Kesehatan Lingkungan (Kesling)
Politeknik Kesehatan Pontianak, JL. DR. Sudarso, Pontianak
kurniaisti@yahoo.co.id[1]

Abstract, Background: Methicillin-resistant Staphylococcus aureus (MRSA) against various antimicrobials, especially based on the insertion of mobile genetic elements, is called staphylococcal cassette chromosome mec (SCCmec) on chromosome *Staphylococcus aureus*. Antiseptic is an anti-bacterial that fights pathological flora mechanically, chemically, or a combination of both, with the aim of killing, inhibiting, or reducing the number of microorganisms such as *Staphylococcus aureus*. Antibiotic resistance has been one of the major public health problems humans face since the discovery of antibiotics. *S. aureus* has been resistant to methicillin and other -lactam antibiotics such as penicillin, cephalosporin, monobactam, and carbapenem. This group of S. aureus is known as Methicillin-Resistant *Staphylococcus aureus* (MRSA). Infections caused by MRSA have spread rapidly and are found in almost all countries. Guava leaves (*Psidium guajava L*) and turmeric (*Curcuma longa L*) are plants in journals showing therapeutic effects such as anti-inflammatory, antioxidant, antiviral, anti-bacterial, and antifungal activities and other activities. This research was conducted to determine whether guava leaf extract and turmeric can be used as an anti-bacterial against MRSA and the minimum guava and turmeric leaf extract concentration, effectively inhibiting MRSA.

Methods: this study used guava leaf extract 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% and 20% respectively and turmeric 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10%. Bioplacenton is used as a control which is commonly used as an antiseptic. The design of this study used an experimental laboratory design. The treatment group (dilution of guava leaf extract and turmeric) is contacted against MRSA, equivalent to the standard Mc Farland 0.5. MRSA planted in the media Mueller Hinton Agar (MHA) were incubated at 37 ° C for 24 hours in the hole method.

Conclusions: the results of the observations were analyzed using the ANOVA test. The minimum concentration of guava leaf extract (*Psidium Guajava L*), effective against MRSA, is 18%. In comparison, turmeric (*Curcuma longa L*) is 10%, controlling bioplacenton inhibition of 15 mm in diameter.

Keywords: Antiseptic, Psidium guajava L., Curcuma longa L., Bioplacenton, MRSA (Methicillin-Resistant Staphylococcus aureus)

Background

Methicillin-resistant Staphylococcus aureus (MRSA) resistance against various antimicrobials, primarily based on mobile genetic elements, is called *staphylococcal cassette chromosome mec* (SCCmec) on chromosome *Staphylococcus aureus*. SCCmec comprises recombinase gene (ccr), mec complex gene, additional resistance gene, and insertion sequences. The structure of the recombinase gene allows SCCmec to pass from one bacterium to another. Identification and analysis of SCCmec are needed to determine the genetic basis of resistance and estimate the spread of this bacterium. *(1.6)*

Staphylococcus aureus is a Gram-positive bacteria that live on human mucous membranes. Bacteria can perform nasal colonization, resulting in an increased risk of infection during an injury (Champoux, 2004). One of the antibiotic-resistant strains of *S. aureus* is *Methicillin-Resistant Staphylococcus aureus* (MRSA). The strain was resistant to *methicillin* and □-lactam antibiotics. Resistance occurs due to the expression of a *penicillin-binding protein* (PBP2a) with a low affinity for □-lactam antibiotics.

Noviana reported that the prevalence of MRSA at Atmajaya Hospital Jakarta in 2003 reached 47%. In 1998, in the United States, it was found that community-acquired MRSA or community-associated MRSA (CA-MRSA) did not show the multiresistant phenomenon and was more virulent than HA-MRSA because it carried an additional virulent factor, namely the Pantone valentin leukocidin (PVL) protein. (4,5)

Indonesia is a country that has a biodiversity that can be processed into various kinds of drugs. The rapid development of medicinal plant production is influenced by increasing public awareness about the benefits of medicinal plants. Society increasingly aware will be the importance of returning to nature (*back to nature*) by utilizing drugs of natural, for example, guava leaves, leaf cottonwoods, and turmeric. (7)

Leaves of guava seeds (*Psidium Guajava L*) contain an anti-bacterial agent. These chemical compounds include tannins, saponins, ethanol, polyphenols, flavonoids, essential oils (eugenol), malic acid, ursolic acid, psionic acid, kratogolic acid, oleic acid, guajaverin acid, and others. People use this guava leaf, among others, for diarrhea medicine and is also used as a face wash to prevent or treat skin infections. About guava leaf users, guava leaves can act as a natural anti-bacterial. (8,9,10)

Turmeric (*Curcuma longa L*) is a tropical plant widely found on the Asian continent and is extensively used as a food coloring agent and fragrance. Turmeric is also used as a dye, medicine, and flavoring since 600 BC. Turmeric is considered one of the most valuable herbs for humans. In the history of Indian folk medicine, turmeric is considered the best antibiotic ingredient. At the same time, turmeric is also used to ease the digestive process and improve the intestinal passage.

Wound treatment must be done as soon as possible. For example, burns to avoid complications were mild to severe. Burn treatment usually uses a commercial topical medication such as Bioplacenton®. The high cost of commercial drugs such as Bioplacenton® can increase the cost of burn care for patients with extensive burns. Bioplacenton®, which is used as a burn therapy, is less able to absorb the exudate formed due to burns. Bioplacenton in this study was used as a control.

Based on previous research, it can be seen that the higher the concentration of guava leaf extract, the wider the inhibition zone formed. The analysis of variance showed that the heating treatment did not make any difference to the results of the precise zone measurements. That means that the heating process carried out does not affect the work of active substances contained in the leaves of guava seeds. The formation of the zone inhibition is due to the guava leaves being active antimicrobial substances. The active substances contained in guava leaves include essential oils, tannins, and flavonoids. (11)

The results of research by Dwitiyanti (Faculty of Pharmacy and Science, University of Muhammadiyah Prof. DR. Hamka) concluded that 70% ethanol extract of guava leaves had cytotoxic activity against T47D cells anticancer breast of 27.54 g/ml. (17)

In China, turmeric is used for stomach ailments and jaundice [2]. Studies on the anti-bacterial properties of aqueous extracts of C. longa rhizome demonstrated MIC (minimum inhibitory concentration) from 4 to 16 g/L and MBC (minimum bactericidal concentration) from 16 to 32 g/L against *S. epidermis* ATCC 12228, *Staph. aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 10031, and E. coli ATCC 25922. (4)

Methods

This type of research is experimental laboratory research. Researchers will conduct data

collection activities, conduct experiments by instilling MRSA by adding guava leaf extract (*Psidium guajava L*) and turmeric (*Curcuma longa L)* at concentrations of 1%, 2%,3%, 4 %, 5%, 6%, 7%, 8%, 9% and 10% by *Hole method.* on MHA media as the experimental group. The control group was experimented with adding a bioplacenton to MHA media planted with MRSA.

The independent variables in this study were guava leaf extract (*Psidium guajava L.*), turmeric (*Curcuma longa L.)*. in contrast, the dependent variable is the MRSA inhibition zone on MHA. Data were collected from observations and presented in tables and graphs. Data analysis and processing were carried out statistically with SPSS using the ANOVA statistical test.

Results

There was no inhibition (ineffective) based on the preliminary test of 1-10% guava leaf extract. There was no inhibition (ineffective). So, the concentration of guava leaf extract was concentrated from 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% and 20%. For turmeric extract, a 1-10% concentration is used with an interval of 1%. The test substance was declared effective if the diameter of the inhibition zone was not significantly different from the positive control (in this case, a bioplacenton was used). For this reason, the range test (*post hoc*) is used as a tool to detect the minimum concentration of the test substance capable of inhibiting the growth of MRSA.

Table 1. Test results of the antiseptic effectiveness of guava leaf extract against MRSA

Treatment	Inhibition zone diameter (mm)			Information
Control	**P1**	**P2**	**P3**	
a. 95% alcohol	11	11	11	Negative control
b. Aquadest	8	8	8	Negative control
c. DMSO	8	8	8	Dimetil sulfoksida
d. Bioplasenton	15	15	15	Positive control
Guava Leaves	P1	P2	P3	
a. 11%	8	9	10	
b. 12%	10	10	10	
c. 13%	11	11	11	
d. 14%	12	12	12	
e. 15%	13	12	13	
f. 16%	13	13	13	
g. 17%	14	13	14	
h. 18%	15	15	15	
i. 19%	18	17	18	
j. 20%	19	19	19	

Note: P1, P2 and P3 = Repeat 1-3

From table 1, it can be seen that the minimum concentration of guava leaf ethanol extract on MRSA is a concentration of 14%. While the effect is guava leaf ethanol extract with a concentration of 18%.

From table 2, it can be seen that the minimum concentration of turmeric ethanol extract on MRSA is a concentration of 7%. While the effect is turmeric ethanol extract with a concentration of 10%.

Table 2. Test results of the antiseptic effectiveness of turmeric extract against MRSA

Treatment	Inhibition zone diameter (mm)			Information
Control	**P1**	**P2**	**P3**	
a. 95% alcohol	11	11	11	Negative control
b. Aquadest	8	8	8	Negative control
c. DMSO	8	8	8	Dimetil sulfoksida
d. Bioplasenton	15	15	15	Positive control
Turmeric	**P1**	**P2**	**P3**	
a. 1%	8	8	8	
b. 2%	9	9	9	
c. 3%	9	9	9	
d. 4%	10	9	9	
e. 5%	11	11	11	
f. 6%	11	11	11	
g. 7%	12	12	12	
h. 8%	13	13	13	
i. 9%	14	14	14	
j. 10%	15	15	15	

Note: P1, P2 and P3 = Repeat 1-3

The study began with preparing fresh guava and turmeric leaves using the ethanol extraction method. The media used in this study used Mueller Hinton Agar (MHA). MHA was selected according to CLSI (Clinical and Laboratory Standard Institute) used as a standard medium for sensitivity testing because it can show optimal reproducibility results and can remove inhibitors in the media due to the presence of casein hydrolysate and starch. (Oxoid, 2021). It grows well in growing non-fastidious bacteria. Many research data have been published regarding the use of these media. [26] MHA contains beef extract, casein hydrolysis acid provides nitrogen content such as carbon, sulfur, and other nutrients. Starch acts as a protective colloid to prevent bacteria from being affected by toxic materials, while hydrolyzed starch produces dextrose, which serves as an energy source. Because reviewing the content. [12,13,14]

Methicillin-resistant *Staphylococcus aureus* (MRSA) is still a significant health problem because its frequency increases globally to increase morbidity and mortality. MRSA is the leading cause of nosocomial infections, namely infections in hospitals in postoperative infections, respiratory infections, urinary tract infections, and circulatory infections (Madigan, 2012). MRSA infections have spread worldwide with increasing numbers over the last ten years. According to Green (2012), the prevalence of MRSA in Asian regions such as Japan and Singapore is more than 50%, while in the Americas, Australia, and several European countries, it is between 25-50%. The prevalence in Southeast Asia, including Indonesia, is not widely known because research on MRSA is still limited.

MRSA is a form of antibiotic resistance, especially -lactam antibiotics. MRSA bacteria are the leading cause of infection in humans. This resistance ability comes from *the mecA gene* found in the bacterial chromosome. Therefore, it is vital to know the role of *the mecA gene* on antibiotic resistance that occurs. The method used is to browse journals that describe MRSA bacteria, *the mecA gene,* and their relationship to cause resistance. [18] In this study, MRSA was used as a test bacterium to see the antiseptic effectiveness of guava and turmeric leaf extracts. As a control, a bioplacenton was used. Bioplacenton can be used as an anti-bacterial. In people infected with skin (ulcers) that ooze from MRSA infection. Antiseptics are anti-bacterial that pathological fight flora mechanically, chemically, or a combination of both with the aim of killing, inhibiting/reducing the number of bacteria.

Leaves of guava seeds (*Psidium Guajava L*) and turmeric (*Curcuma longa L*) have active substances, which are anti-bacterial agents. Guava leaves contain chemical compounds such as tannins, saponins, ethanol, polyphenols, flavonoids, essential oils (eugenol), malic acid, ursolic acid, psionic acid, kratogolic acid, oleanolic acid, guajaverin acid. Meanwhile, in addition to turmeric essential oil, turmeric contains other compounds such as curcumin, resin, desmethoxycurcumin, oleoresin, and bisdemethoxycurcumin.

The method used in this research is Kirby Bauer. The method with modification of suitable method based on CLSI standard. CLSI recommended this method to support the sensitivity test results by diffusion. [19] It was characterized by the presence of an inhibition zone around the wells that had been planted with MRSA, and anti-bacterial substances were added to the wells, namely guava leaf extract, and turmeric. The diameter of the inhibition zone indicates the sensitivity of MRSA to anti-bacterial substances.

Furthermore, the guava leaf extract and turmeric were tested on the growth of MRSA. In the research test results listed in table 1, it can be seen that the minimum concentration of guava leaf ethanol extract against MRSA is a concentration of 14%. While the effect is guava leaf ethanol extract with a concentration of 18%. In table 2, it can be seen that the minimum concentration of turmeric ethanol extract on MRSA is a concentration of 7%. While the effect is turmeric ethanol extract with a concentration of 10%.

The ability of guava and turmeric leaf extracts to inhibit the growth of these bacteria is thought to be due to the active compounds in guava and turmeric leaves. The anti-bacterial activity shown by guava leaf extract and turmeric in this study had active substances, namely tannins, flavonoids, and essential oils. The mechanism of tannins as an anti-bacterial is related to the ability of tannins to inactivate microbial cell adhesion (molecules attached to host cells) on the cell surface. Tannins have a target against cell wall polypeptides that cause damage to the cell wall, which ends with the death of the bacterial cell. [15] The mechanism of action of flavonoids is by denaturing bacterial cell proteins and damaging the cytoplasmic membrane. Prajitno (2007) in Retnowati et al. (2011) explained that flavonoid compounds could damage the cytoplasmic membrane, which can cause leakage of essential metabolites and inactivate bacterial enzyme systems. This damage allows nucleotides and amino acids to seep out and prevent the entry of active ingredients into cells; this situation can lead to bacterial death. In destroying the cytoplasmic membrane, H+ ions from phenolic compounds and their derivatives (flavonoids) will attack the polar group (phosphate group). The phospholipid molecule will decompose into glycerol, carboxylic acid, and phosphoric acid. That results in phospholipids not maintaining the shape of the cytoplasmic membrane. As a result, the cytoplasmic membrane will leak, and the bacteria will experience growth inhibition and even death. [16]

Based on the study results, the minimum concentration of guava leaf extract, which was effective as an anti-bacterial, was higher (18%) than turmeric (10%), which could inhibit MRSA. The active substances in turmeric are more

numerous and varied, like curcumin, resin, desmethoxycurcumin, oleoresin, and bisdemethoxycurcumin, which function not only as anti-bacterial but also as an antioxidant and anti-inflammatory.

Conclusions

There is the anti-bacterial substance of guava leaves extract (*Psidium Guajava L*), turmeric (*Curcuma longa L*), namely 18%-20% and 10%, respectively, with inhibitory control diameter of bioplacenton was 15 mm against *Methicillin-Resistant Staphylococcus aureus* (MRSA). The minimum concentration of guava leaf extract (*Psidium Guajava L*) against *Methicillin-Resistant Staphylococcus aureus (*MRSA) was 18%. In comparison, turmeric (*Curcuma longa L*) was 10%, with the inhibitory control diameter of the bioplacenton being 15 mm. Suggestions for further research can be used other extracts besides ethanol extracts such as n-hexane and ethyl acetate. It is advisable to use guava leaf extract (*Psidium guajava L*) at least 18% and turmeric (*Curcuma longa L*) 10% in the form of cream or ointment on the outside of the body/ wounded skin.

Competing Interests

There is no conflict of interest in this study.

Author's Contributions

Iis Kurniati has made substantial contributions to the conception and design, data acquisition, or data analysis and interpretation and has given final approval for the version to be published. Nina Mariana, Linda Triana, and dan Asep Dermawan contributed to the data's conception, analysis, and interpretation. Deny Rudiansyah and Hafizah Ilmi sufa were involved in drafting the manuscript or revising it critically for important intellectual content.

Acknowledgment

The researcher thanked the Director of Health Polytechnic, Ministry of Health, Bandung, for agreeing to this research and helping finance this research with a PPSDM Research DIPA.

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