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ANTI-STAPHYLOCOCCAL COMPARATIVE STUDY OF *PHALERIA MACROCARPA* (SCHEFF). BOERL FRUIT AND RED GUAVA ((*PSIDIUM GUAJAVA*) LEAF ETHANOLIC EXTRACT

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ABSTRACT

Objective: This study was purposed to compare the anti-staphylococcal strength between *Phaleria macrocarpa* (Scheff). Boerl fruit ethanolic extract and red guava leaf ethanolic extract against *Staphylococcus aureus ATCC* 25923, using chloramphenicol as antibiotic standard. **Methods:** The comparative test was conducted by evaluating the concentration of each extract and chloramphenicol to generate the same diameter of inhibition against *S. aureus* ATCC 25923. The comparative study was carried out using the agar diffusion method by utilizing perforation method. The diameter data, then plotted to form an inhibition curve against log concentration of the extract or chloramphenicol. The anti-staphylococcal activity of each extract, then calculated and compared with the chloramphenicol strength to obtain the comparative value. **Results:** The antibacterial activity of chloramphenicol was the strongest then both extracts. However, the *P. macrocarpa* fruit extracts showed stronger anti-staphylococcal against *S. aureus* ATCC 25923 that of red guava leaf extract. To produce the same inhibitory diameter, chloramphenicol only needed 1 unit, whereas *P. macrocarpa* fruit extracts and red guava leaf extract needed 51.408 and 1,002.37 units, respectively. **Conclusion:** The fruit extract of *P. macrocarpa* fruit extract is more promising to be a medicinal candidate for staphylococcal infections than red guava leaf extract.

KEYWORDS: Comparative**,** *Phaleria macrocarpa* (Scheff). Boerl, *Staphylococcus aureus* ATCC 25923, red guava, chloramphenicol.

INTRODUCTION

Staphylococcus aureus is a normal flora of human skin and mucous membrane that may cause several infections.[1,2] *S. aureus* are naturally colonized on adults for 30 to 50%.[3,4] *S. aureus* is the important pathogen that responsible for several infections, such as skin infections, damaged, chronic and recurrent airway infections, osteomyelitis, and mastitis.[5-8] The pathogenesis of *S. aureus* are because of its capability to spread in the tissue and producing the extracellular substances as its virulence factors, such as toxins and enzymes.[9] Beside that, *S. aureus* is a well know pathogen as nosocomial infections at hospital.[10]

The staphylococcus diseases have been effectively treated by several antibiotic agents.[11] But in the recent years, *S. aureus* has a trend in antibiotic resistant states, and this trend is predicted to continue.[12,13] Therefore, new alternate antibiotics are needed to overcome the future infections caused by *S. aureus*. The use of medicinal plants based compounds can be used as an option.

Indonesia is a country that has abundant recipes of traditional medicine, in accordance with Indonesia's wealth of biodiversity. For the same diseases, Indonesians sometimes uses different plants to cure their disease based on their believed. Among those medicinal recipes, many Indonesians also believe in red guava (*Psidium guajava*) leaves and crown god (*Phaleria macrocarpa)* fruit to treat diseases, including infectious diseases.

P. macrocarpa fruit and red guava leaf are available in abundance in Indonesia at a low price so that it can be reached by the community. The *P. macrocarpa* fruit is known to treat dysentery, antitumor and skin pain medication.[14] Meanwhile, *P. guajava* leaf usually used to treat various diseases such as gastroenteritis, dysentery, diarrhea, malaria, ulcers, vomiting, toothache, coughs, sore throat, wounds, and inflamed gums.[15-18] All of these pharmacological activities cannot be separated from the phytochemical content they have. However, the concentration of each chemical content in the extract, which acts as an antibacterial, will also affect the effectiveness of the extract in inhibiting *S. aureus*. Therefore, in this study, the antibacterial strength of crown god fruit extract and red guava leaf extract was compared, with chloramphenicol as the comparison antibiotic.

MATERIALS AND METHODS

Plant Materials

The plant used in the study was ripe *P. macrocarpa* fruit and red guava leaf. The fruit was obtained from the Manoko plantation, Lembang, Bandung, West Java, Indonesia. Meanwhile the red guava source from guava plantation at Cikutra, West Java, Indonesia.

Bacterial Strains

The antibacterial activity of *P. macrocarpa* fruit extracts was tested against the chloramphenicol sensitive and resistant-*S. aureus*, obtained from a culture collection of microbiology laboratory of Faculty of Pharmacy, Padjadjaran University, Indonesia.

Chemical Materials

The chemicals used were 95% ethanol, chloramphenicol (PT. Kimia Farma), normal saline solution, Mayer reagent, Dragendorf reagents, Lieberman - Burchard reagent, sulfuric acid solution (Merck), vanillin (Merck), barium chloride solution (Merck), n-butanol, technical toluene (Brataco), ferric chloride reagent (Merck), and distilled water. The Mueller Hinton Broth (MHB-Oxoid) and Mueller Hinton Agar (MHA-Oxoid), were used as *S. aureus* growth media.

Extract Preparation

P. macrocarpa fruits and the red guava leaf were washed using clean water. Before further process, the seeds of *P. macrocarpa* fruits were discarded. Meanwhile the leaves of red guava were screened to select the broken leaf by insects. The fruits and the leaves, then were cut into 1 mm in width and dried in the open air. The dried materials were then powdered and weighed. The extraction process of *P. macrocarpa* fruits and red guava leaves were carried out using a maceration method with 95% ethanol as the solvent. The powder of *P. macrocarpa* fruits and the red guava leaves were soaked in 95% ethanol in the separate macerator. Each of the process need solvent replacement every 24 h for 3 d. Each macerates was collected every 24 h and mixed before evaporated. Each macerates was evaporated until the extract achieved a constant weight extract.

Phytochemical Screening

The phytochemical components of *P. macrocarpa* fruit extracts were conducted to ensure the presence of flavonoids, alkaloids, tannins, saponins and polyphenols using a standard method.[19]

Inoculum Preparation

S. aureus colonies from slant agar were taken using Ose and then suspended in 0.95% sterile normal saline. The cell amount of bacterial suspension was adjusted to get the equal turbidity as the 0.5 McFarland standard.

Comparison Analysis of Antibacterial Activity

The comparison analysis test was done using the agar diffusion method. Each of tested extract and chloramphenicol as a comparator antibiotic was tested in the same plate. The tested concentration of chloramphenicol was desain as follows: 50.000; 25.000 and 12.500 µg/ml. To achieve the first tested concentration, a concentration of 100.000 µg/ml was made in 0.1 N hydrochloric acid and serially diluted using sterile distilled water to produce a 50.000 µg/ml chloramphenicol solution. The same dilution method was applied to the lower concentration, both chloramphenicol and extract. For the extract, the used concentration were 500.000; 250.000 and 125.000 µg/ml. Then the medium was prepared by mixing a 20 µL of bacterial suspension with 20 mL MHA (40-45 ^{0}C) and homogenized then the medium was allowed to solidify. The solid medium was perforated to make 6 holes in each plate. Then a 50 µL of each concentration was put into the hole and incubated for 18-24 h at 37 ^{0}C. The inhibitory diameter of tested each concentration was observed, measured and compared. Data inhibition zones were plotted to produce curve inhibitory against log concentration.[20]

RESULTS AND DISCUSSION

Phytochemical Screening Result

The phytochemical screening result of *P. macrocarpa* fruits and the red guava leaves could be compared and seen in the table 1. From the table, we can seen that the phytochemical content of *P. macrocarpa* fruit extracts were more complete than that of red guava leaves extract. *P. macrocarpa* fruit extracts contain of flavonoids, alkaloids, tannins, saponins and polyphenols. In the extract of red guava leaves only contain of polyphenols, tannins, and flavonoid. The difference in chemical content will influence the strength of the antibacterial activity produced by each of these extracts against *S. aureus*.

Table 1: Phytochemical Content of The Extract.

Phytochemical content	Extract	
	***P. macrocarpa* fruit**	**Red guava leaves**
flavonoids	+	+
alkaloids	+	-
tannins	+	+
saponins	+	-
polyphenols	+	+

Notes: (+) presence; (-) absence.

Comparative Analysis Result

Comparative test was carried out to determine the value of comparative antibacterial activity of *P. macrocarpa* fruits and the red guava leaves to chloramphenicol by comparing the needed concentration to generate the same inhibition diameter against *S. aureus*. The diameters of inhibition zone can be seen in Table 2 and 3. Each of these diameters was plotted into the equation to obtain equation using linear regression methods. The line equation of chloramphenicol against *P. macrocarpa* fruit was y = 0,0114x + 1,574; as for the *P. macrocarpa* fruits extract was y = $8{,}6.10^{-3}$ x + 4,034 1,574. Based on the line equation, it was found that the needed of chloramphenicol concentration to produce a diameter inhibition of 27.6 mm was 50.000 µg / mL while in the *P. macrocarpa* fruits, to produce the same diameter required a concentration of 2,570,395.8 µg/mL. Thus, to produce a 27.6 mm of inhibition zone, it took 1 unit of chloramphenicol but the *P. macrocarpa* fruit extract needed 51.408 units. In compared to the red guava extract, the different result were shown when the extract was challenged with chloramphenicol. The line equation of chloramphenicol against *P. macrocarpa* fruits was y = 0,186 x - 0,535; as for the red guava leaf extract was y = 0,222 x + 1,39. Based on the line equation, it was found that the needed of chloramphenicol concentration to produce a diameter inhibition of 28.3 mm was 50.000 µg/mL, while in the red guava leaf extracts, to produce the same diameter required a concentration of 50,118,723.36 µg/mL. Thus, to produce a 28.3 mm of inhibition zone, it took 1 unit of chloramphenicol but the red guava leaf extract needed 1,002.37 units. Based on the comparative value of both extracts against chloramphenicol, it was found that the *P. macrocarpa* fruits needed more smaller unit concentration than that of red guava leaf extracts.

Table 2: Comparison of inhibition zone diameter of *P. macrocarpa* fruit extracts to Chloramphenicol.

Materials	Concentration (µg/ml)	Inhibitory Diameter (mm)
Extracts	125.000	13.35±0.0025
	250.000	14.70±0.0025
	500.000	19.65±0.0004
Chloramphenicol	12.500	22.40±0.0000
	25.000	24.47±0.0025
	50.000	27.60±0.0001

Note: Perforator diameter = 6 mm.

Table 3: Comparison of inhibition zone diameter of Red Guava Leaf extracts to Chloramphenicol.

Materials	Concentration (µg/ml)	Inhibitory Diameter (mm)
Extracts	125.000	19.50±0.0000
	250.000	17.85±0.0025
	500.000	19.50±0.0000
Chloramphenicol	12.500	25.20±0.0000
	25.000	26.20±0.0020
	50.000	28.30±0.0004

The phytochemical content of both extracts play important roles in antibacterial activity against *S. aureus*. The crude extract of *P. macrocarpa* fruits had an inhibiting strength that was closer to the ability of chloramphenicol, compared to red guava. It was supported by the complete of phytochemical substances in *P. macrocarpa* fruits than in red guava leaf extracts.

The mechanism of flavonoid as antibacterial agent is inducted the permeability of bacterial membrane then interfered the protein membrane existed in the bacterial cell wall.[21] The antibacterial of both extracs wwere improved by the presence of tannins which capable to inactivated microbial adhesin and the protein transport of cell envelope.[22] The existence of saponin in the extract could supported the cidal activity of the extracts with the mechanism of bacterial cell membrane damaging.[23]

CONCLUSION

It could be concluded that The fruit extract of *P. macrocarpa* fruit extract is more promising to be medicininal candidate for staphylococcal infections than red guava leaf extract.

REFERENCES

1. Jawetz M, Adelberg. Mikrobiologi Kedokteran. Ed 23. Translated by: Huriawati H, Chaerunnisa R, Alifa D, Aryana D. Jakarta; EGC, 2007; 149-160, 169-170, 225-231, 266-268.
2. Steven YCT, Joshua SD, Emily E, Thomas LH, Vance GFJ. (*Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management). Clin Microbiol Rev, 2015; 28(3): 603-61.
3. Noble WC, Valkenburg HA, Wolters CHL. (Carriage of *Staphylococcus aureus* in random samples of a normal population). J Hyg (Lond), 1967; 65: 567–73.
4. Casewell MW, Hill RLR. (The carrier state: methicillin-resistant *Staphylococcus aureus).* J Antimicrob Chemother, 1986; 18(A): 1-12.
5. Brady A, Loughlin R, Gilpin D, Kearney P, Tunney M. (In vitro activity of tea-tree oil against clinical skin isolates of methicillin resistant and -sensitive *Staphylococcus aureus* and coagulase negative staphylococci growing planktonically and as biofilms). J Med Microbiol, 2006; 55: 1375–80.
6. Psaltis AJ, Ha KR, Beule AG, Tan LW, Wormald PJ. (*Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis*). Laryngoscope, 2007; 117: 1302–6.
7. Tu QPH, Genevaux P, Pajunen M, Savilahti H, Georgopoulos C, Schrenzel J, *et al*. (Isolation and characterization of biofilm formation-defective mutants of *Staphylococcus aureus*). Infect Immun, 2007; 75(3): 1079–88.
8. Kania RE, Lamers GEM, Vonk MJ, Dorpmans E, Struik J, Tran Huy P, *et al*. (Characterization of mucosal biofilms on human adenoid tissues). Laryngoscope, 2008; 118: 128–4.
9. SAF Kusuma. *Staphylococcus aureus*. Scientific Article. Bandung; Pustaka Ilmiah Universitas Padjadjaran, 2009.
10. Pavillard R, Harvey K, Douglas D, Hewstone A, Andrew J, Collopy B, *et al.* (Epidemic of hospital-acquired infection due to methicillin-resistant *Staphylococcus aureus* in major Victorian hospitals). *Med J Aust*, 1982; 1: 451-4.
11. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. (*Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: a multicentre study*). *Indian J Med Microbiol*, 2006; 4(1): 34-8.

12. Hiramatsu K, Aritaka N, Hanaki, Kawasaki H, Hosoda, Hori Y, *et al*. (Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin). Lancet, 1997; 350(9092): 1670–3.
13. Peterson DL. (Vancomycin-Resistant *Staphylococcus aureus*). Infect Med, 1999; 16: 235–8.
14. Djumidi, Sutjipto, Gotama, Sugiarso S, Nurhadi M, Widyastuti Y, *et al*. Inventaris Tanaman Obat Indonesia Ed 5. DepKes RI; Jakarta, 1999.
15. Novick RP. (*Penicillinase plasmids of Staphylococcus aureus). Fed Proc*, 1967; 26: 29-38.
16. Richmond MH. The plasmids of *Staphylococcus aureus* and their relation to other extrachromosomal elements in bacteria vol. In A. H. Rose and J. F. Wilkinson (ed.), Advances in Microbial Physiology, 2nd. Academic Press Inc; New York, 1968.
17. Sabath LD, Gerstein DA, Lode PB, Finland MF. Independent segregation of chloramphenicol resistance in Staphylococcus aureus, In G. L. Hobby (ed.), Antimicrobial agents and chemotherapy-1967. American Society for Microbiology; Ann Arbor, Mich, 1968.
18. Miyamura S. (Inactivation of chloramphenicol by chloramphenicol resistant bacteria). J Pharm Sci, 1964; 53: 604- 7.
19. Harborne JB. *Phytochemical Methods* 3th ed. Chapman and Hall Int, New York, 1998.
20. Kusuma SAF, Herawati IE, Novianti. (Comparative study on antibacterial activity of *Jatropha curcas* Linn. leaves extract and neomycin sulfate against *Staphylococcus aureus* ATCC 25923). Int J Sci Eng Appl Sci, 2017; 3(4): 114-9.
21. Davidson PM, Naidu AS. Natural food antimicrobial systems 1st ed. CRC Press, Florida, 2000.
22. Saura-calixto F, Perez JJ. Tannins: bioavailability and mechanism of action. In: Knasmuller S, DeMarini DM, Johnson I, Gerhauser C, editors. Chemoprention of Cancer and DNA Damage by Dietaryfactors. Wiley-VCH, Weinheim, Germany, 2009.
23. Ciocan D, Bara I. Plant products as antimicrobial agents. *Genet Si Biol Mol*, 2007; 8: 151-6.