Sains Indonesia Vol. 8, No.3 (2003) 5-9

Investigation on Antibacterial Activity of Fermented *Pangium Edule* Reinw. Seed Against Several Bacterial Cause Respiratory and Skin Infections

Effionora Anwar [a] dan Atik Soemiati [b]

[a] Dept. of Pharmaceutical Science, University of Indonesia, Depok, Indonesia

[b] Dept. of Food Tech. and Human Nutrition, Bogor Agricultural University, Bogor Indonesia

Abstract

Nowadays, the importance thing is antimicrobial phytotherapy, because of a lot of strains of pathogenic microorganisms that have become resistant to the action of antibacterial substances in concentrations with in a therapeutic range. To overcome the problem the antimicrobial of phytotherapy can be used as a natural medicine. One of them that has been investigated is the fermented *Pangium edule* Reinw. seed kernels (kluwak). The objective of this research was to investigation the activity of oil, which was extracted from the seed kernels by methanol against three bacteria that had been known cause skin infections; *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa*; and the activity of ethanol extract fermented seed kernels against three bacteria cause respiratory infections; *Staphylococcus aureus* ATCC 25923, *Streptococcus β-hemolyticus* Group A, and *Pseudomonas aeruginosa* ATCC 27853. Oil and ethanol extract of fermented seed kernels showed antibacterial action against both of the above bacteria.

Keywords: *Pangium edule* Reinw, *kluwak*, antimicrobial of phytotherapy

1. INTRODUCTION

Pangium edule Reinw., is a tropical tree that grows in Indonesia, especially in Sumatra and Jawa, it has various names according the area, e.g Sumatra: simaoeng /kapetjong, Jawa: picung/pakem. The fruit had been used as a skin traditional medicine; e.g. ulceration, and leprosy [1,2].

The fruit of *Pangium edule* Reinw., contains cyanide acids, ginocardine (cyanogenic glucoside) phenol/ polyphenol, hidnocarpat acid, and choulmograt acid, and some organic acid [3,4]. In west Sumatra the fruit was used as fish preservative, beside that it had been used as a skin medicine [2], and after fermentation process, the fermented product called *kluwak*, which are used as spices for *rawon* soup [1]. Fermented process of *kluwak* is specific way. Some interesting research on kluwak have been concerning about antioxidant activity, and the chemical structure of the antioxidant is 1-hydroxyphenyl-7-aminoheptane (a phenolic compound) [5-7], and the phenolic antioxidant has been proved as antibacterial agent [8,9]. Another component which dominant in the kernel is lipid or oils, and the oils content common cyclic fatty acids in the *floucortiaceae* family, and γ-tocotrienol a predominant tocol [10,11]. The oils and the other compounds of the kernel can be extracted by organic solvent, and may be used as antibacterial phytotherapy for skin infection.

The importance thing is the antibiotic resistance among bacteria, has been concerned in recent years. The special concern is the increased incidence of infections caused by methicillin *or* penicillin derived - resistant *Staphylococcus aureus* derived [12,13], but it's strains to be equally susceptible to phenol compounds [14]. Another information explained, *P.aeruginosa* and other non fermentative gram negative *bacilli* are resistance to many commonly used antibiotics [15,16]. Thus it is possible the *kluwaks* can be used as antimicrobial in traditional drugs. To many kind of diseases are caused by bacteria. For example, the genera of bacteria, *Staphylococcus, Streptococcus, and Pseudomonas* are frequent causes of skin-related diseases, and respiratory infections in people [17].

In the developing country like Indonesia, a lot of people suffering skin and respiratory infections, so the country have to utilization the plant that growth as the effective drug for solving the problem..

The objective of this research is to investigate microbial activity of lipid and dry extract ethanol from *kluwak* as antibacterial skin and respiratory infections.

2. MATERIAL AND METHODS

2.1. Bacterial strains

The bacterial used in this research were selected from the culture collection of the microbiology laboratories of The Microbiology Department of Medical Faculty, University of Indonesia. Three strains isolates from the patient skin infection; *S. aureus, S. epidermidis* and *P. aeruginosa,* and other is *S. aureus* ATCC 252923, *P. aeruginosa* ATCC 27853, *S. β-hemolyticus* Group A.

2.2. Preparation of *kluwak*

The ripe fruits of *Pangium edule* Reinw. had been harvested from Bogor, Indonesia, October 15, 1999 and leached out the seed from fruit and placed in the field for 10-14 days until the brown colour [1]. Then, wasted and boiled for 3 hours, cooled, placed in a hole in the ground (in doors), and covered by ash. After 40 days, the fermented seeds are cleaned.

2.3. Preparation of lipids

Lipid/oil of *kluwaks* was obtained by 8 hr sohxlet extraction, using methanol as solvent, then evaporated the extract with vacuum evaporator until produce oil which equivalent 49,8 % of dry weight basis. For determination of minimum inhibitory concentration (MIC), the oil was made emulsion with polyethylene glycols 400, and concentration of the emulsion were 250,00 mg/ml (O1); 125,00 mg/ml (O2); 62,50 mg/ml (O3); 31,25 mg/ml (O4); 15,62mg/ml (O5); 7,81 mg/ml (O6); 3,91 mg/ml (O7) respectively, and emulsifying agent as a control (C). For determination of diameter of zone inhibition (min), the oil was dissolved with methanol, and concentration were 7,5 µg/µl (Oa), and 5, 0 µg/µl (Ob).

2.4. Preparation of extract

Extraction, using ethanol 70% as solvent, then evaporated under reduced pressure until produce extract which equivalent 9,6% of dry weight basis. For dilution method (MIC), the extract was dissolved by water, and concentration of liquid; 129,00 mg/ml (E1); 64,50 mg/ml (E2); 32,25 mg/ml (E3); 16, 12 mg/ml (E4); 8,06 mg/ml (E5), 4,03 mg/ml (E6), 2,15 mg/ml (E7) respectively and solvent as a Extract from *kluwak* was obtained by 8 hr sohxlet control (C). For determination of diameter of zone inhibition (min), the extract was dissolved by water, and concentration were 30 µg/µl (Ea) and 25 µg/µl (Eb).

2.5. Investigation of antimicrobial activity

a). Broth the Minimum Inhibitory Concentration (MIC) [18,19]. Concentration both dilution to determine of bacteria are 10^6 /ml in 0,9% NaCl solution.

b). Cylinder diffusion to determine the diameter of growth inhibition zone around the cylinder [18,19]. Concentration both of bacteria are 10^7/ml in 0,9% NaCl solution.

3. RESULTS AND DISCUSSION

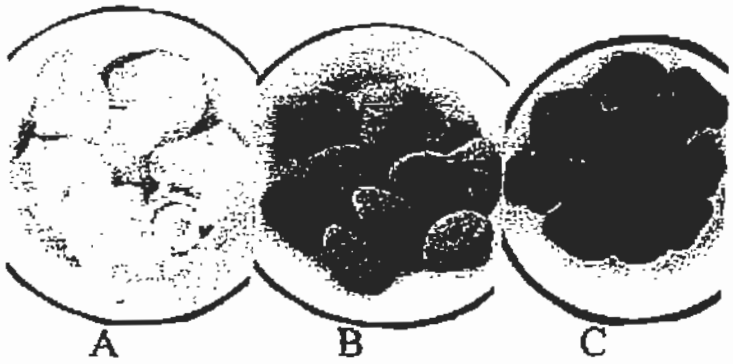
The fermented *Pangium edule Reinw.* seed (*kluwaks*) and the kernels have dark and brown colour respectively (Fig 1). This brown colour could be from browning non enzymatic reaction products, might have occurred when seeds were boiled and during fermentation. The seeds content sufficient levels of protein, and reducing sugar [10], heat treatment of the seeds could be resulted browning colour because of Maillard reaction [20,21]. Fermentation of the seeds has been carried out to remove cyanide from cyanogenic glucoside (ginocardine), which is highly poisonous [1].

Assay of sensitivity of antibacterial agents is carried out in the usual method; successive dilutions of the substance are made with in broth, and the minimum inoculated with a standard amount of the test bacteria. The activity of the oils is measured by the dilution method at the concentration between 7,81 mg/ml and 15,62mg/ml, which the bacterial growth does not occur (Table1). Inhibition of the bacteria growth, because of material constitute in the oils [10,11].

The simplest assay and most commonly used; a plate is inoculated with the microorganism to be tested, and small discs impregnated with different concentrations of the various samples are placed upon the surface with sterile forceps. After incubation 10 discs showed the relative sizes of zones of growth inhibition around all of discs, it was indicated the sensitivity of the bacteria against the sample (Table 2). The result was mesured as on everage with deviation standard 0,25.

The oil showed that it could inhibit growing of the bacteria isolates in low concentration (5,0 µg/.µl). Thus, in the future, oil of *kluwaks* can be used as antibacterial against bacteria cause skin infection, especially for resistance bacteria. The recent report explained, 100% ubiquitous on the basis of the testing of 195 clinical *S. aureus* isolates from various countries, and 58 (29,7%) of these isolates were methicillin resistance [22].

Assay of sensitivity of the extract *kluwak* which is measured by the highest dilution at the same concentration (16,06 mg/ml), showed that growth does



Gambar 1. *A. Pangium edule Reinw. seed and the kernels[V] before fermentation*
B. Pangium edule Reinw. seed after fentetation
C. The seed kernels after fermentation

Table 1. *Antibacterial activities as Minimum inhibition concentration (MIC) of oil from kluwak[a]*

Bacteria	Minimum inhibition concentration							
	O1	O2	O3	O4	O5	O6	O7	C
S. aureus	+	+	+	+	+	+	-	-
S. epidermidis	+	+	+	+	+	-	-	-
P. aeruginosa	+	+	+	+	+	-	-	-

[a]O_{1-7} = emulsion of oil 250,00 mg/ml (O1); 125,00 mg/ml (O2); 62,50 mg/ml (O3); 31,25 mg/ml (O4); 15,62mg/ml (O5); 7,81 mg/ml (O6); 3,91 mg/ml (O7) respectively, and (C) as a control. + no inhibitation

Table 2. *Antibacterial activities of oil and extract of kluwak[a]*

Bacteria	Diameter of zone of inhibition (mm)			
	Oa	Ob	Ea	Eb
S. aureus	22,52	21,43	n.t	n.t
S. epidermidis	29,28	28,45	n.t	n.t
P. aeruginosa	25,41	23,57	n.t	n.t
S. aureus ATCC 25923	n.t		20,04	18,40
S.β-hemolitycus Group A	n.t		21,15	19,13
P. aeruginosa ATCC 27853	n.t		16,79	14,95

[a] Values are the mean of seven replicates; concentration of the oils 7,5 µg/µl (Oa), 5, 0 µg/µl (Ob), and the extracts 30 µg/µl (Ea) and 25 µg/µl (Eb); n.t= not tested.

Table 3. Antibacterial activities as Minimum inhibition concentration (MIC) of extract kluwaks[a]

Bacteria	Minimum inhibition concentration							
	E1	E2	E3	E4	E5	E6	E7	C
S. aureus ATCC 25923	+	+	+	+	+	+	-	-
S.β-hemolitycus Group A	+	+	+	+	+	-	-	-
P. aeruginosa ATCC 27853	+	+	+	+	+	-	-	-

[a]E_{1-7} = extract of *kluwaks* 129,00 mg/ml (E1); 64,50 mg/ml (E2); 32,25 mg/ml (E3); 16, 12 mg/ml (E4); 8,06 mg/ml (E5), 4,03 mg/ml (E6), 2,15 mg/ml (E7), , and (C) as a control. + no inhibitation

not occur for all bacteria (Table3). Inhibition of the bacteria growth, because of the phenolic compound in the extract [5,6]. The mechanism of action for phenolic compound as antimicrobials can be classified into reaction with the cell membrane, inactivity of essential enzymes and destruction or functional inactivity of genetic material [17,23]. On the basis of MIC assays can be predicted the amount of extract is effective as antibacterial traditional drug. This is very importance thing to avoid drug-resistance. Drug resistance because of the improper amount has been used of antibiotics and other antibacterial agents drug resistant micro organisms The development of resistance to drug is depend upon both dose of drug and the species of the bacteria.

Both of the bacteria that have been used in this assay showed zone of inhibitation by the extract (Tablel 2). There is indication that extract of *kluwaks* effective as antibacterial agent. Thus, for the future have to design the extract as antibacterial tradisional drug, especially for respiratory infections.

4. CONCLUTIONS

On the basis of the assay both oil and ethanol extract of the fermented *Pangium edule* ReinW. seeds or *kluwaks* showed activity as antibacterial agent against some bacteria that have been reported resistance to many antibiotics. Especially to bacteria cause skin and respiratory infections in people.

ACKNOWLEDGEMENT

We thank director of The Microbiology Department of Medical Faculty, University of Indonesia for providing the bacteria.

REFERENCES

[1] Heyne K. De Nuttige Planten Van Indonesie. Bandung: N.V. Uitgeverrij.van Hoeve's-Gravenhege, 1950.

[2] Hilditch TP and Wiilliams PN. The Chemical Constitution of Natural Fats. London: Chapman and Hall, 1964.

[3] Burkil I.H. A Dictionary of the Economic Products of Malay Peninsula. Vol2. London: Crown agents & Co, 1935.

[4] Brill HC. In : Cox, Alfin J. editors. The Philippines Journal of Science (Section A, Chemical and Geologcal Sciences and the Industry) 1917;17:37-46.

[5] Fardiaz,D., and Eti Romlah. In: Liang OB, Buchanan A, Fardiaz D, editors. Development of food Science and Technology in ASEAN. Proceedings of the Fourth ASEAN Food Conference, February 17-21, Jakarta 1992:814-819.

[6] Anwar E, Fardiaz D and Wijaya CH. In: LiangOB, Buchanan A, Fardiaz D, editors. Development of food Science and Technology in ASEAN. Proceedings of the Fourth ASEAN Food Conference, February 17-21, Jakarta 1992:820-825.

[7] Nuri A, Fardiaz D, Wattimena GA, Kalidas S. J.Agric Food Chem. 1999;47:3158.

[8] Davidson PM and Branen AL. J. Food Sci. 1980a; 45:1603.

[9] Davidson PM and Branen AL. J.Food Prot. 1981; 44:623.

[10] Nuri A, Fardiaz S, .Anton A, Purwiyatno H., Kalidas S. J. Process Biochem. (Elsevier).1999;35:197.

[11] Nienaber PNL., Aitzemuller A, Werner, G. Langfassung Publikation Stand 20.12.94 (poster unter Nr.98025),1994.

[12] Barber M. J. Clin.Pathol 1961;14:385-393
[13] Hurlimanndalel RL, Ryffel C, Kayser FH, Berger-Bachi B. Antimicrob Agents Chemother 1992;36:2617-2621
[14] Rutala WA. AJIC AMJ.Infect Control 1996;24: 313-324
[15] Maloney J, Rimland D, Stephens DS, TerryP. Whitney AM. Arch Intern. Med. 1989;149:630-634.
[16] Then RL,Angehrn P. Antimicrob Agents Chemother 1982;21:711-717.
[17] Gerard JT, Berdell RF, Christine LC, editors. Microbiology An Introduction, 5th ed. San Juan: The Benjamin/Cummings Publishing Co., Inc, 1999.
[18] Lorian, V. Antibiotic in Laboratory Medicine. Baltimor: The Wiilliam & Wilkins Co, 1980.
[19] Freeman, B.A., Burrows Textbook of Microbiology, 22nd ed. Saunders Internasional. 1985.
[20] Bailey RG, Ames JM and Mann J. J.Agric.Food Chem. 1999:47:438-443
[21] Patton S: a review J. Diary Sci.1955;38:457-478
[22] Martineau F, Picard FJ, Roy PH, Ouellette M, and Michel MG. J.Clin. Microbiol. 1998; 36:618.
[23] Davidson PM. Phenolic Compounds. In A.L. Branen AL and P.M. Davidson PM. editors. New York and Basel Antimicrobial in Food. Marcel Dekker, 1983.