Antibacterial Activity of *Pluchea indica* and *Piper betle* Ethanol Extract on *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*

Nela[1], Umi Yuniarni[1], Diki Prayugo[2]

[1] Department of Pharmacology, Sekolah Tinggi Farmasi Indonesia, Bandung, West Java, Indonesia
[2] Department of Biology Pharmacy, Sekolah Tinggi Farmasi Indonesia, Bandung, West Java, Indonesia

Abstract

Medicinal plants are widely used for the treatment of different infectious diseases. This study was aimed to investigate antibacterial activity of *Pluchea indica* (*P. indica*) and *Piper betle (P.betle)* ethanol extract on *Staphylococcus epidermidis* (*S. epidermidis*) and *Pseudomonas aeruginosa (P. aeruginosa)* using agar disk diffusion method. Ethanol extract of *Piper betle* showed more potent antibacterial activity than *P. indica* against *S. Epidermidis* with the highest inhibiton zone at 30.71 mm and 21.73 mm from 1 mg/ml concentration, respectively. In contrast, against *P. aeruginosa*, the ethanol extract of *P. indica* was more potent than *P. betle* with 21.44 mm and 20.12 mm of inhibition zone on 1 mg/ml concentration, respectively. There was no increased effect from the combination of these two extracts against these bacteria. When comparing the antibacterial activity of these extract with tetracycline as the standard, we found that antibacterial activity of *P. indica* at the concentration of 0.9 mg/ml was comparable with that of tetracycline at concentration of 12.52 µg/ml, while *P. betle* needed 0.3 mg/ml concentration to had similar activity with 10.51 µg/ml of tetracycline. In conlusion, the antibacterial activity of ethanol extracts of *P. indica* and *P. betle* indicated that these extract had sufficient potential to warrant further examination and development as a new antibacterial agent.

Keywords: *Pluchea indica, Piper betle*, antibacterial, agar disk diffusion

Introduction

Inappropriate use of antimicrobial drugs contributes to the emergence and spread of drug resistant. This situation has encouraged the development of new antimicrobial substances from various sources, such as medicinal plants.[1-3] Medicinal plants are widely used empirically for the treatment of infectious disease. Investigation of safety and efficacy of these plants as the candidate of potential antimicrobial agent is considered necessary.[4,5]

Indonesia has enormous variety of medicinal plants, including *Piper betle (P. betle)* and *Pluchea indica (P. indica)*. *P. betle* is usually used for the treatment of mouth ulcers, hemorrhoids, stomach disorders, *etc. P. indica* is known as body deodorizing agent. It is usually consumed as fresh vegetables, decoction, and topical medication.[6-10]

Corresponding author: Nela. Department of Pharmacology, Sekolah Tinggi Farmasi Indonesia, West Java, Indonesia. Email: nelasimanjuntak89@gmail.com

Staphylococcus epidermidis (S. epidermidis) and *Pseudomonas aeruginosa (P. aeruginosa)* are two most prevalent bacterial pathogens infecting respiratory disease, urinary tract, eyes, and burn wounds. Their presence was associated with poor patient outcomes.[11,12]

Previous studies showed that *P. indica* extract could inhibit the growth of several gram-positive bacteria, including *B. cereus, B. subtilis, and S. aureus*. *P. betle* also had potential antibacterial activity against *S. aureus, E. coli, S. typhi, and S. dysenteriae*.[13,14] However, there is a lack of study investigating antibacterial activity of these extracts against *S. epidermidis* and *P. aeruginosa*. Therefore, we conducted this study to investigate antibacterial activity of ethanol extract of *P. indica* and *P. betle* against *S. epidermidis* and *P. aeruginosa*.

Methods

Materials and instruments

The materials used in this study included *P. betle* leaves, *P. indica* leaves, *S. epidermidis* and *P. aeruginosa*. For phytochemical screening, the reagent of Mayer, Dragendorff, Liebermann-Burchard, ether, amyl alcohol, HCl 2N, 5% KOH, $FeCl_3$, vanillin 10% in concentrated H_2SO_4, 1% gelatine, ammonia, magnesium powders and distilled water were used. Extraction was performed using 95% ethanol solvent (Brataco). Antibacterial activity test was conducted using growth media of agar nutrient (Deffco), broth nutrient (Deffco), physiological NaCl (Otsuka), ethanol 70% (Brataco), disinfectant, and distilled water. Tools used in this study included soxhlet extractor, rotary evaporator, and laboratory glass tools.

Antibacterial activity test procedure

All materials and instruments, including agar nutrient and distilled water were sterilized using an autoclave at 121 °C for 15 minutes. It was dried in the oven for 15 minutes. The bacteria colonies were rejuvenated and incubated at 37°C for 18-24 hours. The colonies were then put in a sterilized physiological NaCl solution. It was measured at spectrophotometer with λ=580 nm to obtain T=25%.

Agar nutrient solution was obtained by dissolving it with distilled water and stirring it on water bath until a clear solution was obtained. 20 ml sterilized agar nutrient solution was put into petri dishes. 50 µl bacterial suspension was added into petri dish. It was then homogenized until hardened agar formed. Using perforator, a hole was formed on each agar with a diameter of 10 mm. 50 µl of various concentration of *P. indica* and *P. betle* extracts were put in each

Table 1. Phytochemical screening of *P. betle*

Secondary Metabolite	Crude materials	Ethanol Extract	MMI
Alkaloid	+	+	+
Flavonoid	+	+	+
Tannin	+	+	+
Phenolic acid	+	+	+
Monoterpene dan Sesquiterpene	+	+	+
Steroid dan Triterpenoid	-	-	-
Quinone	+	+	+
Saponin	-	-	-
	-	-	-

Table 2. Phytochemical screening of *P. indica*

Secondary Metabolite	Crude materials	Ethanol Extract	MMI
Alkaloid	+	+	+
Flavonoid	+	+	+
Tannin	+	+	+
Phenolic acid	+	+	+
Monoterpene dan Sesquiterpene	+	+	+
Steroid dan Triterpenoid	-	-	-
Quinone	+	+	+
Saponin	+	+	+
	-	-	-

agar nutrient (0.2 mg/ml; 0.4 mg/ml; 0.6 mg/ml; 0.8 mg/ml; 1 mg/ml).

Each petri dish was incubated at 37 °C for 18-24 hours. After incubation, the clear zone formed around the hole was measured in diameter by using a vernier caliper. The concentrations which gave inhibiton zone more than 20 mm in the first experiment were selected and combined. The similar antibacterial testing was then performed for these combination.

Antibacterial activity compared to tetracycline

Comparation of antibacterial activity between these extracts and tetracycline aimed to understand the extent to which antibacterial

Table 3. Antibacterial activity of *P. indica* and *P. betle* against *S. epidermidis* and *P. aeruginosa*

Extract	Bacteria	Inhibition Zone (mm)					
		Control	0.2 mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	1 mg/ml
P. indica	*S. epidermidis*	9	10.11	14.71	16.56	19.16	21.57
		9	10.49	14.61	16.85	19.57	21.87
		9	10.38	14.81	16.34	18.98	21.75
	Mean	**9**	**10.32**	**14.71**	**16.58**	**19.23**	**21.73**
	P. aeruginosa	9	9.78	10.78	12.61	14.56	21.60
		9	9.87	10.87	12.37	14.78	21.17
		9	9.88	10.97	12.35	14.91	21.55
	Mean	**9**	**9.84**	**10.87**	**12.44**	**14.75**	**21.44**
P. betle	*S. epidermidis*	9	16.71	22.61	24.55	27.17	30.57
		9	16.87	21.83	24.81	27.56	30.72
		9	16.75	21.32	24.33	27.81	30.81
	Mean	**9**	**16.77**	**21.92**	**24.56**	**27.51**	**30.71**
	P. aeruginosa	9	11.65	13.81	15.71	17.12	20.13
		9	11.87	13.12	15.68	17.87	19.74
		9	11.98	13.51	15.19	17.41	20.51
	Mean	**9**	**11.83**	**13.51**	**15.52**	**17.46**	**20.12**

diameter of perforator: 9 mm

Table 4. Antibacterial activity of combination of *P. indica* and *P. betle* against *S. epidermidis* and *P. aeruginosa*

Extract	Bacteria	Inhibition Zone (mm)			
		Control	1	2	3
P. indica	*S. epidermidis*	9	20.12	19.85	20.36
		9	20.91	20.15	20.39
		9	19.87	19.78	19.97
	Mean	**9**	20.11	20.48	19.87
	P. aeruginosa	9	18.78	17.85	18.36
		9	18.67	18.15	18.39
		9	17.99	18.78	18.97
	Mean	**9**	18.48	18.26	18.57
P. betle	*S. epidermidis*	9	21.52	20.85	21.87
		9	22.51	21.52	20.81
		9	19.78	21.81	20.11
	Mean	**9**	21.41	21.61	20.56
	P. aeruginosa	9	22.52	22.85	22.87
		9	22.51	21.52	22.81
		9	22.78	21.81	22.11
	Mean	**9**	22.60	22.04	22.59

diameter of perforator: 9 mm

activity of the extract was similar with that of standard treatment.The score was obtained by comparing inhibition zone of the extract and the standard. The result of comparative observation was then illustrated in a curve with concentration log data on x-axis and inhibiton zone diameter (mm) on y-axis. This curve was used to compare the substance which had the highest antibacterial activity.

Results and Discussion

Phytochemical screening result showed that both extracts contained alkaloids, flavonoids, tannins, phenolics, monoterpenes, steroids, triterpenoids and quinones (Table 1 and 2). Table 3 shows the result of antibacterial activity from *P. indica* and *P. betle* leaves ethanol extract against *S. epidermidis* and *P. aeruginosa* bacteria. We found that mean inhibiton zone diameter of *P. indica* extract against *S. epidermidis* was 21.73 mm at 1 mg/ml concentration and against *P. aeruginosa* was 21.44 mm with the same concentration. *P. betle* could produce inhibitory zone with diameter of 21.92 mm at concentration 0.4 mg/ml against *S. epidermidis*, while against *P. aeruginosa*, it gave 20.12 mm inhibitory zone with the same concentration. Table 4 shows the inhibitory zone of combination of these extracts. There was a difference in inhibitory diameter for both bacteria. For *S. epidermidis*, combination 1 (0.15 mg / ml *P. betle* and 0.45 mg/ml *P. indica)* gave a mean inhibitory diameter of 20.15 mm, while against *P. aeruginosa* it could produce a mean inhibitory diameter of 18.43 mm. Combination 2 gave inhibitory diameter of 21.19 mm and 22.41 mm, against *S. epidermidis* and *P. aeruginosa* respectively. There was no increased effect of antibacterial acitivity of combined extract of *P. indica* and *P. betle.*

Various concentration of tetracycline were

Table 5. Antibacterial activity of tetracycline

Bacteria		Inhibition Zone (mm) Concentrations (µg/ml)					
		Control	10	7.5	5	2.5	1.25
P. aeruginosa	1	9	21.87	20.54	19.72	18.11	17.65
	2	9	21.81	20.59	19.71	18.21	17.59
	3	9	21.78	20.45	19.68	18.12	17.55
Mean		**9**	21.82	20.52	19.70	18.17	17.59
S. epidermidis	1	9	23.64	22.78	21.38	20.48	19.16
	2	9	23.72	22.68	21.42	20.52	19.24
	3	9	23.66	22.74	21.42	20.60	19.14
Mean		**9**	23.67	22.73	21.41	20.53	19.18

diameter of perforator: 9 mm

tested for their antibacterial activity against *S. epidermidis* and *P. aeruginosa* using the agar diffusion method. The results of the test can be seen in Table 5. The result of tetracycline standard curve with log concentration data on x-axis and inhibitory diameter (mm) on y-axis was illustrated in figure 1. Based on linear regression equation with $y = 4.558 x + 16.76$ $R^2 = 0,942$ in Figure 1, for *P. aeruginosa bacteria*, activity produced by *P. indica* at concentration of 1 mg/ml was comparable with that of tetracycline at the concentration of 9.89 µg/ml. The antibacterial activity produced by *P. betle* at the concentration of 1 mg/ml was comparable with that of tetracycline at 11.49 µg/ml. The combination 1 (0.3 mg/ml *P. betle* and 0.45 mg/ml *P. indica*) was comparable to 14 µg/ml tetracycline while combination 2 (0.5 mg/ml *P. betle* and 0.5 mg/ml *P. indica*) was equivalent to 10 µg/ml (Figure 1).

Based on linear regeression equation with $y = 4,554x + 17,84$ with $R^2 = 0.940$ in Figure 2, for *S. epidermidis* bacteria, antibacterial activity of *P. indica* at concentration of 0.9 mg/ml was comparable with that of tetracycline at 12.52 µg/ml, and the antibacterial activity produced by *P. betle* at 0.3 mg/ml was comparable with 10.51 µg/ml tetracycline.

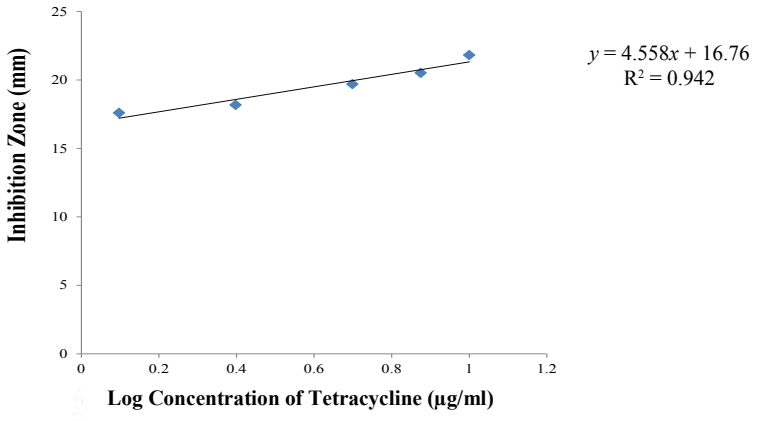

Figure 1. Standard curve of tetracycline antibacterial activity against *P. aeruginosa*

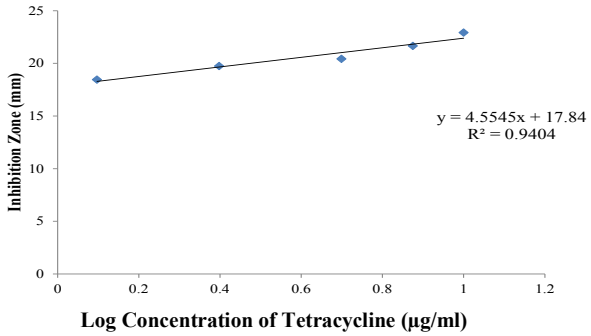

Figure 2. Standard curve of tetracycline antibacterial activity against *S. epidermidis*

The activity produced by combination 1 was comparable with 12.92 µg/ml tetracycline and combination 2 was equivalent to the activity of tetracycline at the concentration of 9.98 µg/ml.

Our finding was comparable with previous studies.[14,15] Bioactive compounds which might contribute in the antibacterial activity of these plants were dependent on solvent system. Previous study showed that organic solvent could retain more antibacterial compunds compared to water solvent. Since our study used ethanol solvent for extraction, the result was comparable. Several studies reported that terpenoid, flavonoid, alkaloid, and tannin were responsible for the antibacterial acitivity.[16]

Conclusion

The ethanol extract of *P. betle* showed more potent antibacterial activity against *S. epidermidis* compared to *P. indica*, while against *P. aeruginosa*, *P. indica* was more potent than *P. betle*. There was no increased effect from the combination of these extracts against two bacteria. *P. indica* and *P. betle* had sufficient potential to warrant further examination and development as a new antibacterial agent. Further research might be needed to identify and isolate bioactive substances which contribute in antibacterial activity from these plants.

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