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Antimicrobial Activity of Endemic Herbs from Tangkahan Conservation Forest North Sumatera to Bacteria and Yeast

KIKI NURTJAHJA[1*], TATA BINTARA KELANA[2], DWI SURYANTO[1], NUNUK PRIYANI[1], GINTA RIO[1], DEDI PRIMA PUTRA[3], DAYAR ARBAIN[3]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Sumatera Utara University, Jalan Bioteknologi No.1, Kampus USU, Medan 20155, Indonesia
[2]Faculty of Biology, Medan Area University, Jalan Kolam No.1, Medan 20223, Indonesia
[3] Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Andalas University, Limau Manis, Padang 25163, Indonesia

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Tangkahan Conservation Forest in Karo County, North Sumatera has high biodiversity of endemic herbs. Many species of the wild herbs are well known used as traditional medicine not only by local people but also by people out of the area. The methanol extract of the medicinal wild herbs in Tangkahan Conservation Forest, Karo County to relief skin diseases caused by bacteria and fungi never been studied medically. The antimicrobial activity leave extract of the medicinal herbs to pathogenic microorganisms are studied. The leaves extract of kembu-kembu (*Callicarpa candicans*), rintih bulung (*Piper muricatum*), cep-cepan (*Castanopsis costata*), and sereh kayu (*Eugenia grandis*), has antimicrobial to bacteria (*Bacillus* sp., *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*) and yeast (*Candida albicans*). Toxicity assay of these plants by brine shrimp method using *Artemia salina* indicates that cep-cepan dan sereh kayu have lethal concentration higher than kembu-kembu and rintih bulung.

Keywords: antimicrobial activity, methanol extract, brine shrimp, *Artemia salina*

INTRODUCTION

Indonesia is one of tropical countries in South East Asia that has high plant biodiversity. Tropical rainforest with high gene pool of plants is a potential source for technology development of forestry, agriculture, pharmacy, etc. The exploration of plant biodiversity especially for medicinal herbs is required to maintain natural resources for human use.

Tangkahan Conservation Forest, Langkat, North Sumatera is one of the areas in Sumatera that has high biodiversity of medicinal herbs. The Conservation is located in Karo County. Wild plants in the site have been used for traditional herbs by local people and this tradition was inherited from their ancestor (Yuharmen *et al*. 2002). Two examples of such plants are *Callicarpa candicans* Burm.f. (local name: kembu-kembu) which being use for skin rash and skin inflammation caused by bacteria and *Piper muricatum* Blume (local name: rintih bulung) for bones medicine.

The effectiveness of plants as traditional medicine to microorganism infection related diseases has been extensively studied (Pisutthanan *et al*. 2004; Nazliniwaty 2006; Suryanto *et al*. 2006; Hasanah *et al*. 2006; Ajizah *et al*. 2007; Ćirić *et al*. 2008; Selvamohan *et al*. 2012; Prasannabalaji *et al*. 2012). However, limited data is currently available about the antimicrobial effectiveness of kembu-kembu (*Callicarpa candicans*), rintih bulung (*Piper muricatum*), cep-cepan (*Castanopsis costata* Blume), and sereh kayu (*Eugenia grandis* Wight.), to microorganisms such as *Bacillus* sp., *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus,* and *Candida albicans*.

In this experiment we investigated the antimicrobial substances' activity of endemic wild herbs from Tangkahan Conservation Forest to bacteria and yeast (*Candida albicans*).

MATERIALS AND METHODS

Plant Materials and the Extracts. Plant materials for this experiment were chose based on their use in traditional medicine by local people in Karo County. One kilogram fresh leaves of kembu-kembu, rintih bulung, cep-cepan, and sereh kayu were obtained from Tangkahan Conservation Forest, Karo County. The forest has an altitude of 150-800 m above the sea level. All sample were air-dried for 48 hours and then sliced into 1 x 1 cm^2. Each sample was powdered separately using a waring

*Corresponding author. Phone: +62-61-8223564, Fax: +62-61-8214290, E-mail: 62kiki@gmail.com

blender for about 15 min. Each powdered material (645 g kembu-kembu, 116.6 g rintih bulung, 450 g cep-cepan, and 351 g sereh kayu,) was extracted with methanol in dark sterile vial for 3 days at room temperature or 30 °C. Each methanolic extract was filtered 5 times with methanol using Whatman filter paper No. 54 and concentrated by rotary evaporator at 50 °C. The concentrated extract (10.8 g kembu-kembu, 4.8 g rintih bulung, 40.2 g cep-cepan, 32.1 g sereh kayu,) were then stored in a desiccator.

The methanolic extract (0.3 g) of each plant in dark sterile vial was mixed with 2 mL dimethilsulfoxyde (DMSO) and 15% extract was obtained. Each extract solution was diluted to become 10, 5, and 1%.

Bacterial and Yeast Culture. Bacterial species, *Bacillus* sp., *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*, and yeast *Candida albicans* isolates were obtained from Medical Faculty, Sumatera Utara University. Each species of the bacteria was inoculated and cultured in nutrient agar (NA), and yeast was cultured in potato dextrose agar (PDA). The stock cultures were incubated for 24 h at 37 °C. Each culture from stock cultures was transferred aseptically using ose needle and suspended in 3 mL 0.9% sodium chloride solution. The suspended culture was later on homogenated by vortex, and the turbidity of the suspension was compared to Mc Farland standard at 108 cfu mL^{-1}.

Treatment of Plant Extracts to Bacteria and Yeast. Kirby-Bauer disc diffusion method was used in this experiment. Oxoid paper discs (5 mm diameter) in 9 cm diameter sterile Petri dishes were added with 10 µL plant extract of 15, 10, 5, 1, and 0% (control) and were being kept for 1 h until the extract was fully diffused into the paper discs. Each species of bacteria was spread plated on mueller hinton agar (MHA) medium and yeast on PDA. Paper disc containing 30 µg mL^{-1} chloramphenicol, 10 µg mL^{-1} penicillin, and 100 mg ml^{-1} antifungi ketoconazole were used as control. All bacterial cultures were incubated at 37-38 °C for 24 h and yeast was at 32 °C for 24 h. Each treatment was replicated three times. The inhibition zone was then measured which can be indicated by clear zone surrounding the paper disc.

Brine Shrimp Lethality Test. The toxicity of methanolic extract was tested using brine shrimp lethality test method according to Pisutthanan *et al*. (2004). The methanolic extract of each plant was applied to *Artemia salina* (brine shrimp). Twenty mg extract, was mixed with 2 mL DMSO as a stock solution. Solution at 1000, 100, and 10 ppm concentration were prepared by adding 500, 50, and 5 µL with 5 mL of stock solution to 5 mL sterile sea water, respectively. Each concentration was filled into small open vial (2 cm diameter), and latter on ten *A. salina* were added into the vial. The experiment was replicated three times. The mortality of the *A. salina* was observed after 24 h and the lethal concentration (LC50) of each solution was determined by Finney's Probit analysis program.

RESULTS

Antimicrobial Activity of the Methanolic Extract. At the highest extract concentration (15%) the antimicrobial activity of kembu-kembu affected all species of bacteria, but not the yeast (*C. candicans*). The strongest antimicrobial activity of kembu-kembu extract was found for *Bacillus* sp. and *S. aureus* (Table 1). In the case of rintih bulung, it inhibited *Serratia marcescens* and *Bacillus* sp. and had a little effect on the growth of yeast (Table 2).

Similar results were also found for the extract of cep-cepan (Table 3) and sereh kayu (Table 4), *E. coli* and *C. albicans* were the most resistant to all leaves extracts. The comparison between antibiotics (chloramphenicol, penicillin), and antifungi (ketoconazole) with leaves extract showed that

Table 1. The diameter of inhibition zone (mm) for kembu-kembu methanolic extract on bacteria and yeast (after 24 hours incubation)

	Extract concentration (%)/inhibition zone (mm)				
	0	1	5	10	15
Bacillus sp.	5.0g	10.2d	13.6b	15.3a	18.7a
Escherichia coli	5.0g	5.5fg	5.8fg	5.4fg	5.8fg
Serratia marcescens	5.0g	5.3fg	5.9fg	7.8e	8.8d
Staphylococcus aureus	5.0g	7.0f	10.4c	11.1c	12.4b
Candida albicans	5.0g	5.0g	5.0g	5.0g	5.0g

The different letters on the same columns and rows show significant differences 5% according to Duncan New Multiple Range Test.

Table 2. The diameter of inhibition zone (mm) for rintih bulung methanolic extract on bacteria and yeast (after 24 hours incubation)

	Extract concentration (%)/inhibition zone (mm)				
	0	1	5	10	15
Bacillus sp.	5.0g	5.6cd	7.3ab	7.1bc	8.2a
Escherichia coli	5.0g	5.7cd	5.6cd	5.7cd	5.2cd
Serratia marcescens	5.0g	5.5cd	6.4bcd	8.8a	9.8a
Staphylococcus aureus	5.0g	5.3cd	6.0cd	6.3cd	6.6bc
Candida albicans	5.0g	6.5cd	5.5cd	6.4cd	6.8bc

The different letters on the same columns and rows show significant differences 5% according to Duncan New Multiple Range Test.

antibiotics and antifungi had a wider inhibition zone than the leaves extracts (Table 5).

Brine Shrimp and LC50. Brine shrimp method to examine the toxicity of leaves extracts indicated all extracts had an effect to the mortality of *A. salina*. All extracts showed a close-dependent response to mortality of *A. Salina*, where, higher extract's concentration gave the higher percent of mortality. The LC50 of kembu-kembu (Table 6), rintih bulung (Table 7), cep-cepan (Table 8). sereh kayu (Table 9) were 383.9, 114.0, 7.9, and 5.7 µg mL^{-1} respectively.

Table 3. The diameter of inhibition zone (mm) for cep-cepan methanolic extract on bacteria and yeast (after 24 hours incubation)

	Extract concentration (%)/inhibition zone (mm)				
	0	1	5	10	15
Bacillus sp.	5.0g	8.0e	8.7e	11.9ab	11.9a
Escherichia coli	5.0g	5.0f	5.0f	5.0f	5.0f
Serratia marcescens	5.0g	7.5e	8.5e	11.0abcd	11.1abc
Staphylococcus aureus	5.0g	6.9e	7.8e	8.3e	8.3e
Candida albicans	5.0g	5.0f	5.0f	5.0f	5.0f

The different letters on the same columns and rows show significant differences 5% according to Duncan New Multiple Range Test.

Table 4. The diameter of inhibition zone (mm) for sereh kayu methanolic extract on bacteria and yeast (after 24 hours incubation)

	Extract concentration (%)/inhibition zone (mm)				
	0	1	5	10	15
Bacillus sp.	5.0g	6.7f	9.5c	11.0ab	11.7a
Escherichia coli	5.0g	5.0g	5.0g	5.0g	5.0g
Serratia marcescens	5.0g	6.2f	6.6f	8.50cde	9.4cd
Staphylococcus aureus	5.0g	6.2f	6.7f	7.3ef	5.0g
Candida albicans	5.0g	5.0g	5.0g	5.0g	5.0g

The different letters on the same columns and rows show significant differences 5% according to Duncan New Multiple Range Test.

Table 5. The comparison of inhibition zone (mm) among chloramphenicol, penicillin, and antifungi (ketoconazole) on the growth of microorganisms

	Inhibition zone (mm)		
	Chloramphenicol (30 µg/ml)	Penicillin (10 µg/ml)	Ketoconazole (100 mg/ml)
Bacillus sp.	21.65	5.00	-
Escherichia coli	28.65	5.00	-
Serratia marcescens	31.05	5.00	-
Staphyloccus aureus	29.98	13.63	-
Candida albicans	-	-	15.45

- = the antibiotics or antifungi is not tested.

Table 6. The toxicity and LC50 of leaves extract of kembu-kembu to *A. salina*

	Number of mortality			
	Kembu-kembu extract concentration (ppm)			
	10	100	1000	Control
Mortality	2	8	20	0
Number of *A. salina*	30	30	30	0
% mortality	6.67	26.67	66.67	0
LC50	383.9 µg mL^{-1}			

Table 7. The toxicity and LC50 of leaves extract of rintih bulung to *A. salina*

	Number of mortality			
	Rintih bulung extract concentration (ppm)			
	10	100	1000	Control
Mortality	6	8	27	0
Number of *A. salina*	30	30	30	0
% mortality	20	26.67	90	0
LC50	114.0 µg mL^{-1}			

Table 8. The toxicity and LC50 of leaves extract of cep-cepan to *A. salina*

	Number of mortality			
	Cep-cepan extract concentration (ppm)			
	10	100	1000	Control
Mortality	17	20	28	0
Number of *A. salina*	30	30	30	0
% mortality	56.7	66.7	93.4	0
LC50	7.9 µg mL^{-1}			

Table 9. The toxicity and LC50 of leaves extract of sereh kayu to *A. salina*

	Number of mortality			
	Sereh kayu extract concentration (ppm)			
	10	100	1000	Control
Mortality	16	25	27	0
Number of *A. salina*	30	30	30	0
% mortality	53.4	83.4	90	0
LC50	5.7 µg mL^{-1}			

DISCUSSION

Chemical substances and secondary metabolites that presence in the plant cells such as alkaloids, flavonoids, terpenoids, tannins, glycosides, and phenolic have antimicrobial effect on Gram positive (G+) and Gram negative (G-) bacteria. However, the plant chemical substances have less effect on yeast such as *Candida albicans* (Rajakaruna *et al*. 2002). Study on the effect of *Lantana camara* (Famili

Verbenaceae) to G+ bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and G- bacteria (*Escherichia coli* and *Pseudomonas*) had been conducted by Rajakaruna *et al*. (2002) and Pasqua *et al*. (2005). Kembu-kembu, a member of Family Verbenaceae, has similar effect to inhibit the growth of *Bacillus* sp., *E. coli*, and *S. aureus*, as previously reported for *L. camara* extract. In addition, kembu-kembu inhibits *Serratia marcescens*, and *C. albicans*. The ability of the leaves extract to inhibit the growth of the microorganisms is caused by the presence of secondary metabolites in the plant cells. Secondary metabolites in plants such as alkaloid, flavonoid, terpenoid, and phenolic can affect bacteria. Several factors also influence the effectiveness of inhibitory activity to microorganisms such as the ability of the antimicrobial substance to diffuse into medium, the number of microorganisms, and the sensitivity of microorganisms to antimicrobial activity. In addition, pH stability, and the solubility of the antimicrobial substance in medium also influence the antimicrobial effectiveness. Substances that soluble in medium are able to diffuse and make contact with the wall of microorganisms. G+ bacteria (*Bacillus* sp. and *S. aureus*) are the most inhibited by the leaves extracts followed by G- (*E. coli* and *S. marcescens*). The cell wall structure of G+ is different to G- bacteria, thus this result indicated that the cell wall is the main target of antimicrobial substances. G- bacteria contain large quantity of lipids (lipoprotein, lipopolysaccharide, and other lipid) that might become an important factor that cause lipid-insoluble extracts unable to penetrate G- cell wall bacteria. It was also found in this research that the leaves extracts has no effect on *C. albicans*.

Rintih bulung inhibits not only G+ and G- bacteria but also inhibits the growth of *C. albicans*). Sereh kayu (Family Myrtaceae) and cep-cepan (Family Fagaceae) has no inhibition effect on the growth of *E. coli* and *C. albicans*. Soniya *et al*. (2013) reported that methanol plant extracts of *Syzygium aromaticum*, and *Piper nigrum* were more effective against *S. aureus*, *E. coli,* and *Bacillus subtilis* compared to ethanol plant extracts. Whereas, Andyana *et al*. (2004) stated that leaves extract of *Psidium guajava* (Family Myrtaceae) was able to inhibit diarrheic bacteria such as *E. coli* and *Shigella dysenteriae*. However, each leaves extract contains chemical compounds such as alkaloid, flavonoid, and terpenoid that can inhibit the growth of the bacteria and yeast. In fact in this study, the raw extract that might contain other compounds, had a lower activity performance compare to pure antimicrobial-compounds (chloramphenicol, penicillin) and antifungi (ketoconazole) as shown by Table 5. Both of these antimicrobial compounds have higher inhibition zone than that of medicinal plant extracts. The LC50 of the brine shrimp test by using *A. salina* showed that methanolic extract of cep-cepan and sereh kayu have higher value than of kembu-kembu and rintih bulung.

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REFERENCES

Adnyana IK, Yulinah E, Sigit, Fisheri JI, Insanu M. 2004. The effect of white and red guava leaf extract as an antidiarrhea. *Acta Pharm Indones* 29:20-28.

Ajizah A, Thihana, Mirhanuddin. 2007. *In vitro* the potential of ulin wood extract (*Eusideroxylon zwagery*) to inhibit the growth of *Staphylococcus aureus*. *Bioscientiae* 4:37-42.

Ćirić A, Vinterhalter B, Šavikin-Fodulović K, Soković M, Vinterhalter D. 2008. Chemical analysis and antimicrobial activity of methanol extracts of celandine (*Chelidonium majus* L.) plants growing in nature and cultured *in vitro*. *Arch Biol Sci Belgrade* 60:7P-8P. http://dx.doi.org/10.2298/ABS0801169C

Hasanah N, Soesatyo M, Mustofa. 2006. The effect of methanolic extract of *Eurycoma longifolia* Jack.) to the phagocytic activity peritoneal macrophage of mouse to the infection of *Listeria monocytogenes*. *J Sains Kes* 19:255-270.

Nazliniwaty. 2006. The use of herb and root extract of *Acalypha indica* as an antibacteria. *J An Indones Pharm* 14:66-67.

Pasqua RD, Feo FD, Villani F, Mauriello G. 2005. *In vitro* antimicrobial activity of essential oil from Mediterranian Apiaceae, Verbenaceae, and Lamiaceae against foodborne pathogens and spoilage bacteria. *Ann Microbiol* 55:139-143.

Pisutthanan S, Pliangbanchang P, Pisutthanan N, Ruangruay S, Muanrit O. 2004. Brine shrimp lethality activity of Thai medicinal plants in the Family Meliaceae. *Naresuan Univ J* 12:13-18.

Prasannabalaji N, Muralitharan G, Sivanandan RN, Kumaran S, Pugazhvendan SR. 2012. Antibacterial activities of some Indian traditional plant extracts. *Asian Pac J Trop Dis* 2:291-295. http://dx.doi.org/10.1016/S2222-1808(12)60168-6

Rajakaruna N, Harris CS, Towers GHN. 2002. Antimicrobial activity of plants collected from Serpentine Outcrops in Sri Lanka. *J Pharm Biol* 40:235-244. http://dx.doi.org/10.1076/phbi.40.3.235.5825

Selvamohan T, Ramadas V, Kishore SSS. 2012. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Adv Appl Sci Res* 3:3374-3381.

Soniya M, Kuberan T, Anitha S, Sankareswari P. 2013. *In vitro* antibacterial activity of plant extracts against Gram positive and Gram negative pathogenic bacteria. *Int J Microbiol Immunol* 2:1-5.

Suryanto D, Kelana TB, Munir E, Nani N. 2006. Brine shrimp and methanolic leaves extract of *Psychothria stipulacea* Wall. (Rubiaceae) to microbes. *J An Indones Pharm* 14:85-89.

Yuharmen, Eryanti Y, Nurbalatif. 2002. The antimicrobial activity of eteris oil and methanolic extract of *Alpinia galanga*. *J Natur* 4:1-8.