ANTIBACTERIAL PROPERTIES OF SELECTED PLANTS CONSUMED BY PRIMATES AGAINST *ESCHERICHIA COLI* AND *BACILLUS SUBTILIS*

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Abstract. Bacterial antimicrobial resistance is a major health problem worldwide. Plants consumed by non-human primates are potentially safe for humans. In this study, we examined the potential antibacterial properties of plants consumed by non-human primates in Indonesia. We studied the antibacterial properties of the leaf extracts of 34 primate-consumed plants against *Escherichia coli* and *Bacillus subtilis in vitro*. The plants were collected from the Pangandaran Conservation Area, West Java Province, Indonesia. The leaves were dried and then powdered by crushing and the potential active ingredients were extracted with 95% ethanol at room temperature for 24 hours. The obtained solvent was then dried at 50ºC under reduced pressure. The antibacterial properties of each product were then tested to determine the minimum inhibitory and minimum bactericidal concentrations using the broth microdilution technique and a disc diffusion test was also performed. The results show *Kleinhovia hospita, Dillenia excelsa* and *Garcinia celebica* had the best antibacterial properties against *Escherichia coli* and *Ficus benjamina*, *Ficus altissima*, and *Elaeocarpus glaber* had the best antibacterial properties against *Bacillus subtilis.* Some of the studied leaf extracts in our study have the potential to be developed into antibacterial medications and need to be studied further.

Keywords: medicinal plants, antibacteria, *Escherichia coli, Bacillus subtilis*

INTRODUCTION

Bacterial antimicrobial resistance is a worldwide health problem, especially in developing countries where there is uncontrolled access to antibiotics (Ansari, 2001; Orrett, 2001; Chukwuani *et al*, 2002; Hu *et al*, 2003; Pradipta *et al*, 2015). Antimicrobial resistance is mainly due to inappropriate, irrational, excessive or profligate use of antibiotics combined with poor patient compliance (Abdulah, 2012). The lack of a nationwide health insurance system may also play a significant role in the irrational use of antibiotics in Indonesia (Hidayat *et al*, 2004).

Solving this problem of inappropriate antimicrobial use is only part of the solution. It is also important to search for new antimicrobials (Livermore, 2011). In

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the past 30 years there has been a 90% decrease in approval of new antimicrobials (Spellberg *et al*, 2008; Shlaes *et al*, 2013; Spellberg, 2014).

Some foods consumed by primates are good sources of hexose, cellulose, hemicellulose, pectin, vitamin C, minerals, essential fatty acids and protein and may be a potential safe source to be developed for human health (Milton, 1999, 2000). Several studies have evaluated the potential antibacterial effect of foods consumed by primates (Huffman, 2003; Krief *et al*, 2005, 2006).

In the present study, we evaluated the antibacterial properties of plants consumed by primates in Indonesia.

MATERIALS AND METHODS

Plant materials

Thirty-four plants consumed by non-human primates in the Pangandaran Conservation Area, West Java Province, Indonesia were collected and identified by the Department of Biology, Faculty of Mathematica and Natural Sciences, Universitas Padjadjaran, Indonesia. The leaves of these plants were then rinsed with tap water and air dried out of direct sunlight.

Extract and fraction preparation

The dried leaves were then powdered by crushing. The leaf powder was then soaked in 95% ethanol for 24 hours at room temperature to extract the potential active ingredients. The solvent was then evaporated at 50°C under reduced pressure. This process was repeated 3 times for each leaf powder sample. The plants extracted with ethanol were then tested for antimicrobial activity against *Escherichia coli* and *Bacillus subtilis*. Those showing antimicrobial activity were then fractionated using freshly obtained leaf power with n-hexane ethyl acetate, and methanol for 24 hours each time, 5 times. These new fractions were then tested for their antimicrobial properties against *Escherichia coli* and *Bacillus subtilis*.

Microorganisms and growth conditions

We test the antimicrobial effects of the tested plants against *Escherichia coli* ATCC 25922 and *Bacillus subtilis* ATCC 6633. The bacteria were obtained from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Padjadjaran Bandung, Indonesia. The tested bacterial strains were maintained on Mueller-Hinton agar (MHA; Oxoid, Hampshire, UK) at 37°C until used. A purity test was performed on each strain prior to use (Tille, 2013).

Determination of minimum inhibitory and minimum bactericidal concentrations

The broth microdilution technique was used to test the minimum inhibitory and minimum bactericidal concentrations following the method described reviously (CLSI, 2009) using Mueller-Hinton broth (MHB; Oxoid). The extract was prepared in dimethyl sulfoxide and placed in microplate wells with sterile water at a concentration of 100 μg/ml in each well. Loopfuls of the bacterial colonies were collected from solid media and inoculated into sterile 0.85% saline solution. The bacterial cell concentration was diluted 1:100 to a final concentration of approximately 5×10^5 CFU/ml. The bacterial suspension was added to each of the wells. The plant extract fractions in the wells varied from 1,000 μg/ml to 3.9 μg/ml. The plates were then incubated at 37°C for 24 hours. After the incubation period each well was examined for the presence of turbidity indicating bacterial growth. The bacterial viability and minimum inhibitory concentration (MIC) were determined by measuring

the turbidity by measuring absorbance. The lowest concentrations of plant extract in a well having a clear suspension was presumed to be the MIC. The bacterial suspension was also plated onto MHA and incubated at 37°C overnight. The lowest concentration of extract producing no growth was considered to be the minimum bactericidal concentration (MBC).

Antimicrobial susceptibility testing

The bacterial sensitivities were determined using the Kirby-Bauer agar diffusion method for susceptibility testing with modifications as described previously (CLSI, 2012). An inoculum was prepared via overnight culture of the test strain. The colonies were suspended in 0.85% saline and visually compared with a 0.5 McFarland standard against a white background with contrasting black lines to produce bacterial suspensions of 2×10^8 CFU/ml. The suspensions were inoculated uniformly over the entire surface of sterile MHA. Wells were then aseptically cut into each agar plate using a 9-mm sterile cork borer. Each well was then filled with 50 µl of the studied solution, with the highest plant fraction concentration being 50% m/v, and incubated at 37°C for 16-18 hours. The tests were performed in triplicate. Control plates were prepared for each test organism by excluding the addition of the test solution. The inhibition zone diameter was read to the nearest millimeter at the point at which a sharp reduction in growth was present.

Statistical analysis

The disc diffusion result data were analyzed using the Statistical Package for Social Science (SPSS), version 16 (SPSS, Chicago, IL). The data are presented as means and standard deviations (SD). Whenever possible, two or more groups were compared with the Mann-Whitney test. Statistical significance was set at $p<0.05$.

RESULTS

MIC and MBC values

In total, 34 plant extracts were tested for their antimicrobial effects against *Escherichia coli* and *Bacillus subtilis*. Extracts with a stronger antimicrobial effect against the studied bacteria (a MIC value <75 µg/ml and a MBC value <500 µg/ml) are shown in Table 1. The 3 extracts with the strongest inhibitory effect against *Escherichia coli* were: *Garcinia celebica, Dillenia excelsa,* and *Kleinhovia hospita*. Meanwhile the 3 extracts with the strongest inhibitory effect against *Bacillus subtilis* were *Ficus altissima*, *Ficus benjamina*, and *Elaeocarpus glaber*.

Sensitivities of *E. coli* to the studied extract

The antimicrobial activity of *Garcinia celebica, Dillenia excelsa, Kleinhovia hospita* against *Escherichia coli* using the disc diffusion method were tested. The extracts with the greatest activity against *E.coli* were the methanol fractions of *Kleinhovia hospita, Dillenia excelsa*, and *Garcinia celebica* (Table 2).

Sensitivities of *B. subtilis* to the studied extracts

The antimicrobial activity of *Ficus altissima*, *Ficus benjamina* and *Elaeocarpus glaber* against *Bacillus subtilis* using the disc diffusion method were tested. The extracts with the greatest activity against *Bacillus subtilis* were the ethyl acetate fractions of *Ficus altissima* and *Ficus benjamina* and the methanol fraction of *Elaeocarpus glaber* (Table 3).

DISCUSSION

Some primate-consumed plants have been studied to determine their potential

Table 1
MIC and MBC values for the 34 studied plant extracts against *Escherichia coli* and *Bacillus subtilis*.

Name of plants	*Escherichia coli*[a]		*Bacillus subtilis*[b]	
	MIC values (μg/ml)	MBC values (μg/ml)	MIC values (μg/ml)	MBC values (μg/ml)
Acronychia laurifolia	500	n.d.	250	n.d.
Amoora aphanamixis	250	n.d.	250	n.d.
Antidesma bunius	250	n.d.	500	n.d.
Ardisia humilis	250	n.d.	250	n.d.
Barringtonia macrocarpa	250	n.d.	250	n.d.
Buchanania arborescens	1,000	n.d.	250	n.d.
Cynometra ramiflora	250	n.d.	125	n.d.
Dalbergia latifolia	250	n.d.	250	n.d.
Decaspermum fruticosum	250	n.d.	125	n.d.
Dillenia excelsa	62.5[c]	250	125	n.d.
Elaeocarpus glabra	250	n.d.	62.5[c]	500
Eugenia aquea	250	n.d.	250	n.d.
Ficus altissima	125	n.d.	62.5[c]	250
Ficus annulata	250	n.d.	500	n.d.
Ficus benjamina	250	n.d.	62.5[c]	250
Ficus pubinervis	250	n.d.	125	n.d.
Ficus septica	250	n.d.	125	n.d.
Flacourtia rukam	125	n.d.	125	n.d.
Garcinia celebica	31.25[c]	250	125	n.d.
Heritiera littoralis	250	n.d.	125	n.d.
Hernandia peltata	125	n.d.	250	n.d.
Kleinhovia hospita	62.5[c]	250	125	n.d.
Litsea mappaceae	250	n.d.	250	n.d.
Lygodium circinnatum	250	n.d.	125	n.d.
Melastoma polyanthum	250	n.d.	250	n.d.
Neonauclea calycina	250	n.d.	125	n.d.
Pandanus tectorius	250	n.d.	125	n.d.
Phanera fulva	250	n.d.	250	n.d.
Psychotria valentonic	250	n.d.	250	n.d.
Pterospermum diversifolium Bl.	250	n.d.	250	n.d.
Pterospermum javanicum	125	n.d.	125	n.d.
Rhodamnia cinerea	250	n.d.	250	n.d.
Schleitsera oleosa	125	n.d.	125	n.d.
Vitex heterophylla	250	n.d.	250	n.d.

[a]*Escherichia coli* ATCC 25922; [b]*Bacillus subtilis* ATCC 6633; [c]the most active extracts. n.d., not detected until the highest concentration tested (1,000 μg/ml).

Table 2
Sensitivities of *Escherichia coli* to the extracts of 3 studied plants using the disc diffusion method.

Name of plants	Extract solvent	Concentration (mg/ml)	Inhibition zone ± SD (mm)
Garcinia celebica	Hexane	50	n.d.
		40	n.d.
		30	n.d.
		20	n.d.
	Ethyl acetate	50	23.6 ± 0.2
		40	20.5 ± 0.3
		30	18.2 ± 0.2
		20	15.1 ± 0.3
	Methanol	50	25.2 ± 0.5
		40	19.1 ± 0.4
		30	19.4 ± 0.2
		20	16.7 ± 0.1
Dillenia excelsa	Hexane	20	n.d.
		10	n.d.
		5	n.d.
		2.5	n.d.
	Ethyl acetate	20	25.2 ± 0
		10	18.8 ± 0
		5	16.5 ± 0.1
		2.5	13.2 ± 0.1
	Methanol	20	33.8 ± 0
		10	23.7 ± 0.1
		5	18.8 ± 0.1
		2.5	15.1 ± 0.1
Kleinhovia hospita	Hexane	40	n.d.
		30	n.d.
		20	n.d.
		10	n.d.
	Ethyl acetate	40	n.d.
		30	n.d.
		20	n.d.
		10	n.d.
	Methanol	40	17.7 ± 0.3
		30	14.2 ± 0.7
		20	13.1 ± 0.8
		10	11.8 ± 0.6

Escherichia coli ATCC 25922; n.d., not detected.

to be used to treat human disease. In our study, we found some plants had antibacterial effects (Table 1). The methanol fractions of *Kleinhovia hospita*, *Dillenia excelsa*, and *Garcinia celebica* had antibacterial properties against *Escherichia coli* (Table 2) and the ethyl acetate fractions of *Ficus altissima* and *Ficus benjamina* and the methanol fraction of *Elaeocarpus glaber* had antibacterial properties against *Bacillus subtilis* (Table 3).

Alexander Fleming isolated the anti-

Table 3
Sensitivities of *Bacillus subtilis* to the extracts of 3 studied plants using the disc diffusion method.

Name of plants	Extract solvent	Concentration (mg/ml)	Inhibition zone ± SD (mm)
Ficus benjamina	Hexane	50	11.7 ± 0.4
		40	10.8 ± 0.2
		30	10.5 ± 0.1
		20	10.9 ± 0.1
	Ethyl acetate	50	42.1 ± 0
		40	33.6 ± 0
		30	36.2 ± 0
		20	32.7 ± 0.5
	Methanol	50	35.9 ± 0
		40	26.8 ± 3.2
		30	27.1 ± 0.9
		20	26.6 ± 0.1
Ficus altissima	Hexane	50	12.8 ± 0.2
		40	10.8 ± 0.4
		30	9.6 ± 0.2
		20	n.d.
	Ethyl acetate	50	41.5 ± 0
		40	41.2 ± 0.1
		30	35.2 ± 2.7
		20	31.6 ± 0.7
	Methanol	50	23.0 ± 1.6
		40	22.7 ± 0.3
		30	20.7 ± 0.9
		20	19.6 ± 0.3
Elaeocarpus glaber	Hexane	40	n.d.
		30	n.d.
		20	n.d.
		10	n.d.
	Ethyl acetate	40	15.4 ± 0.4
		30	14.6 ± 0.1
		20	13.4 ± 0.2
		10	10.7 ± 0.3
	Methanol	40	22.7 ± 1.0
		30	20.9 ± 0.9
		20	20.3 ± 0.3
		10	18.0 ± 1.3

Bacillus subtilis ATCC 6633; n.d., not detected.

bacterial compound from *Penicillium* sp that was later developed into penicillin (Alharbi *et al*, 2014). This was followed by the discovery of other antimicrobials, such as streptomycin, aureomycin and chloromycetin (Trease and Evans, 1972).

Plants have been studied for their antibacterial effects (Cowan, 1999; Kurek *et al*, 2011), including those consumed by primates (Huffman, 2003; Krief *et al*, 2005, 2006). Cousins and Huffman (2002) found *Aframomum danielli*, a plant consumed by

primates, had potent bactericidal activities to *Escherichia coli, Pseudomonas aeruginosa, Yersinia enterocolitica, Bacillus subtilis, Proteus vulgaris, Klebsiella pneumoniae,* and *Serratia marcescens* (Adegoke and Skura, 1994). Krief *et al* (2005) evaluated the antibacterial properties of several plants, including *Chaetacme aristata* Planch, *Diospyros abyssinica*, *Ficus exasperata* Vahl, *Phytolacca dodecandra* L'Herit, and *Trichilia rubescens* Oliv (Taniguchi *et al*, 1978; Krief, 2003; Krief *et al*, 2004). Krief *et al* (2005) also studied this effects of *Mimusops bagshawei*, *Chrysophyllum albidum*, and *Pancovia pedicellaris* against *Escherichia coli* (Krief *et al*, 2006).

Our preliminary study showed some studied plants had antimicrobial activity and need further study to identify the active compounds of the extracts and test them for safety and efficacy.

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Conflicts of interest. The authors declare that they have no conflicts of interest.

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