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Antimicrobial activity of polyisoprenoids of sixteen mangrove species from North Sumatra, Indonesia

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Abstract. *Sumardi, Basyuni M, Wati R. 2018. Antimicrobial activity of polyisoprenoids of sixteen mangrove species from North Sumatra, Indonesia. Biodiversitas 19: 1243-1248.* Mangroves including those that are distributed in the coast of North Sumatra contain polyisoprenoid with varying levels of polyprenol and dolichol constituents. Differences in polyisoprenoid levels were closely related to the salinity of sea but the information about their biological activities is scarce. The present study aimed to describe the biological activities, antimicrobial, antioxidant, and antifungal effects of polyisoprenoid extracts from sixteen mangrove species of North Sumatra, Indonesia. Polyisoprenoids were isolated from mangrove leaves and tested for their antimicrobial activity against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans*. Meanwhile, their antioxidant activity was represented by their capacity in scavenging DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical agents. The most predominant polyisoprenoids found in sixteen mangrove leaf extracts was dolichols (75%). Polyprenols-dominant species, *Acacia auriculiformis, Hibiscus tiliaceus, Pongamia pinnata,* and *Ricinus communis,* and dolichols-dominant species, *Avicennia lanata, Av. marina, Av. officinalis, Barringtonia asiatica, Bruguiera gymnorrhiza, Calophyllum inophyllum, Nypa fruticans,* and *Pandanus odoratissimus,* inhibited the growth of *E. coli* and *S. aureus*. However, the antioxidant activity of those sixteen mangrove species was of a weak category. Surprisingly, all other mangroves polyisoprenoid extracts did not inhibit *C. albicans* growth. This study suggested that polyisoprenoids in mangroves have potential antibacterial properties to be developed further.

Keywords: Antioxidant, antibacterial, antifungal, mangrove leaves, nonsaponifiable lipid

INTRODUCTION

Mangroves exist widely in the tropics and subtropics where 75% of them are distributed in 15 countries while 22.6% of the global mangrove was found in Indonesia (Giri et al. 2010). Mangrove forest in North Sumatra mainly existed in the eastern coast of Sumatra island and occupied 37,132.62 ha in 2015 (Basyuni and Sulistiyono 2018) Mangroves play an important role in the socio-economic development of surrounding communities, especially in the region of North Sumatra (Basyuni et al. 2018a, 2018b). Mangroves are used as firewood and charcoal, as well as for traditional medicine (Bandaranayake, 1998). Mangroves are well known to produce secondary metabolites that have promising health benefits, lipid input into estuarine, and biomarkers for organic matters (Basyuni et al. 2007; Patra and Thatoi 2010; Koch et al. 2011).

The enormous potential of mangrove' bioactive compounds is not fully utilized. In this context, mangroves can be used for pharmaceutical, especially antimicrobial (Patra and Thatoi 2010). The antimicrobial development has a role of important because of the crisis of antibiotic resistance. They have played in medicine, surgery and chemotherapy. Beside that continuous use of them cause side effects on the degenerative therapy (Ventola 2015).

Polyisoprenoid alcohols were a group of compounds that have an essential role in living things, including in mangrove plants that are firmly related to the biosynthesis of secondary metabolites of isoprenoid (Basyuni et al. 2007, 2016, 2017a, 2018c). Isoprenoid and polyisoprenoid compounds are the most diverse plant secondary metabolites (Basyuni et al. 2007, 2012, 2016, 2017a; Skorupinska-Tudek et al. 2008; Koch et al. 2011). These compounds were encoded by oxidosqualene cyclase (OSC) gene (Basyuni et al. 2012; Lodeiro et al. 2007). The OSC and salt tolerance genes play an important role in adaptation to salinity tolerance (Basyuni et al. 2012; Basyuni and Sumardi 2017c). The content of isoprenoids and polyisoprenoids have opposed seawater stress in mangrove leaves (Basyuni et al. 2012, 2017d; Basyuni and Sumardi 2017b).

Mangrove plants are commonly used for medical purpose, but there is still little information about the biological activity of the polyisoprenoid of the plants. Study on mangrove polyisoprenoid compounds, especially in light of their function as antimicrobial, antioxidant, and antifungal agents, is required for pharmaceutical and medicinal purpose. We are interested in providing complete information for the utilization of the compounds in the field of medicine, food, industry, and others. Therefore, our current study aimed to describe the antimicrobial, antioxidant and antifungal activities of polyisoprenoid extract from sixteen mangrove species in North Sumatra, Indonesia.

MATERIALS AND METHODS

Sample collection

Leaves from sixteen mangrove species were collected from Lubuk Kertang and Pulau Sembilan mangrove forest, Langkat, North Sumatra, Indonesia. These species are *Acacia auriculiformis* (Fabaceae), *Avicennia alba* (Acanthaceae), *A. lanata* (Acanthaceae), *A. marina* (Acanthaceae), *A. officinalis* (Acanthaceae), *Barringtonia asiatica* (Lecythidaceae), *Bruguiera gymnorrhiza* (Rhizophoraceae), *Calophyllum inophyllum* (Calophyllaceae), *Ceriops tagal* (Rhizophoraceae), *Hibiscus tilliaceus* (Malvaceae), *Nypa fruticans* (Arecaceae), *Pandanus odoratissimus* (Pandanaceae), *Pongamia pinnata* (Fabaceae), *Ricinus communis* (Euphorbiaceae), *Rhizophora mucronata* (Rhizophoraceae), and *Stachytarpheta jamaicensis* (Verbenaceae). The leaves were dried at 60-75°C for two days for further analysis.

Polyisoprenoid extraction

Polyisoprenoid extraction method was performed as described previously (Sagami et al. 1992; Basyuni et al. 2016, 2017a, 2018c). The dried leaves (3-7 g) were first ground and extracted with 30 mL of chloroform-methanol (2: 1, v: v) for 48 h. The insoluble cell wall debris was removed following the method of Basyuni et al. (2016). The lipid extract of leaves was saponified with 4 mL 2 M KOH in 50% ethanol solution at 60°C for 24 h. The nonsaponifiable lipids (NSL) were then regarded as polyisoprenoids and partitioned into n-hexane. The extracted polyisoprenoids were applied for antimicrobial, antifungal, and antioxidant activity tests.

Antimicrobial and antifungal activities

The antibacterial and antifungal activity tests were done as previously reported by Okigbo and Mmeka (2008). Tests were done on two organisms, *S. aureus* and *E. coli*. The antibacterial test was performed on nutrient agar plates prepared by dissolving 28 g of nutrient agar in 1 L of water. The antifungal test against *C. albicans* was done on sabour and dextrose agar prepared by dissolving 64 g in 1 L of water. The media culture to be poured into a petri dish and made wellbore with a diameter 6 mm. Media were sterilized in an autoclave at 121°C for 15 min (Okigbo and Mmeka, 2008).

One hundred (100) mg/mL extracts were prepared in dimethyl sulfoxide (DMSO): ethanol (1: 2, v: v) solution. The extract was placed on culture plates in triplicate (Okigbo and Mmeka, 2008). The culture was incubated in an incubator for 24 h for antibacterial assay and 48 h for the antifungal test at 37°C, respectively. The zone of inhibition of plant extracts was observed and measured. The solvent was used as negative control.

Antioxidant activity

Antioxidant activity assay was carried out based on the modification of standard DPPH (2,2-diphenyl-1-picryllhydrazyl) method (Mustarichie et al. 2017). DPPH solution was prepared with a concentration of 60 μg/mL. DPPH maximum wavelength and the specified operating time of DPPH in methanol were determined. Its absorbance was measured using UV-Vis spectrophotometer every min for two h. Each of polyisoprenoid was prepared in methanol: n-hexane (4: 1, v/v) and made in three various concentrations. Each 0.2 mL of test solution was mixed with 1 mL DPPH and 3.8 mL methanol: n-hexane (4: 1, v/v). The mixture was incubated for two h at room temperature, then measured using UV-Vis spectrophotometry at maximum wavelength 517 nm. Percentage of inhibition, regression curves, and the linear equation were applied to calculate the IC_{50} as previously reported (Sebaugh 2011).

RESULTS AND DISCUSSION

In this study, isolation of polyisoprenoid from 16 species of mangrove plants was performed using a previously described protocol (Basyuni 2016, 2017a, 2018c). As displayed in Table 1, polyisoprenoid in the form of polyprenol is predominantly detected in four species of mangroves, *Ac. auriculiformis, H. tilliaceus, P. pinnata,* and *R. communis*. Meanwhile, dolichols predominantly (more than 90%) compose the polyisoprenoids in the 12 mangrove species tested.

The result of polyisoprenoid extracts inhibitory test against *E. coli* and *S. aureus* as representatives of gram-negative and gram-positive bacteria, respectively, were shown in Table 2 and Figure 1. *N. fruticans* and *C. tagal* had the largest and smallest diameter of inhibition area against *E. coli*, respectively. Meanwhile, *C. tagal* and *R. communis* had the largest and smallest diameter of inhibition area against *S. aureus*, respectively. Determination of antioxidant activity of the mangrove extract followed a previously reported method (Marjoni and Zulfisa, 2017). Meanwhile, determination of antimicrobial effectivity followed a method described by Herni et al. (2016). Interestingly, the mangrove species containing polyprenols, *Ac. auriculiformis, H. tiliaceus, P. pinnata*, and *R. communis*, and eight species with predominant dolichols content, *Av. lanata, Av. marina, Av. officinalis, B. asiatica, B. gymnorrhiza, C. inophyllum, N. fruticans*, and *P. odoratissimus*, inhibited the growth of *E. coli* and *S. aureus* (Table 2).

The inhibitory test against *C. albicans* fungi was shown in Table 2. All the mangroves did not show an inhibitory effect on *C. albicans* culture growth. Antioxidant activity of polyisoprenoid extracted from each mangrove was shown in Table 3. The antioxidant assay for a natural substance using DPPH radical agents has been established (Milardovićet et al. 2006). A high degree of DPPH scavenging indicates an excellent antioxidant activity.

Acacia auriculiformis showed weak antioxidant activity with an IC_{50} of 17100 μg/mL, but the polyisoprenoid from this species was able to inhibit the growth of *E. coli* and *S. aureus* with inhibitory diameter 13.7 and 12.29 mm, respectively, while no activity against *C. albicans*. This result suggests that the polyisoprenoid of *Ac. auriculiformis* may not have antifungal property. Polar extract such as saponins in *Ac. auriculiformis* has been reported to inhibit *Bacillus megaterium, Salmonella typhimurium*, and *Pseudomonas aeruginosa* (Mandal et al. 2005).

Avicennia alba is one of the medicinal plants traditionally used by communities. Polar extract of *Av. alba* Bark are able to inhibit the culture of bacterial bacteria but that n-hexane extract of the plant was not active against the bacteria (Vadlapudi and Naidu 2009). Polyisoprenoid of *Av. alba* moderately impedes the growth of *S. aureus* with a diameter of inhibition of 9.24 mm (Table 2). In contrast, dolichols family compounds did not show significant inhibitory effect against to *E. coli* and *C. albicans* (Table 1).

Table 1. Polyisoprenoid profile of sixteen mangroves species

Species	Polyisoprenoid (%)	
	Polyprenol	Dolichol
Ac. auriculiformis[c]	100	0
Av. alba[b]	0	100
Av. lanata[b]	0	100
Av. marina[a]	4.2	95.8
Av. officinalis[b]	0	100
B. asiatica[c]	8	92
B. gymnorrhiza[a]	0	100
C. inophyllum[c]	0	100
C. tagal[b]	0	100
H. tiliaceus[a]	100	0
N. fruticans[b]	0	100
P. odoratissimus[c]	0	100
P. pinnata[c]	100	0
R. communis[c]	100	0
R. mucronata[b]	9.8	90.2
S. jamaicensis[c]	0	100

Note: [a] Basyuni et al. (2016), [b] Basyuni et al. (2017), [c] Basyuni et al. (2018)

Table 2. Antibacterial and antifungal activities of polyisoprenoid in 16 mangrove leaves

Sampel	Diameter of Inhibition (mm)		
	E. coli	*S. aureus*	*C. albicans*
Ac. auriculiformis	13.17±4.63	12.29±0.87	na
Av. alba	na	9.24±0.22	na
Av. lanata	10.19±0.01	13.25±0.32	na
Av. marina	10.85±0.58	10.52±0.38	na
Av. officinalis	12.66±0.71	8.85±0.56	na
B. asiatica	12.83±0.58	9.6±0.51	na
B. gymnorrhiza	10.16±1.02	11.645±0.67	na
C. inophyllum	9.65±0.74	8.66±0.38	na
C. tagal	9.14±0.01	13.48±3.10	na
H. tiliaceus	10.52±0.59	11.57±1.31	na
N. fruticans	14.48± 1.17	11.66±0.33	na
P. odoratissimus	12.49±0.58	12.38±0.72	na
P. pinnata	10.19±0.01	10.96±0.10	na
R. communis	10.16±1.02	8.23±0.06	na
R. mucronata	na	11.09±0.86	na
S. jamaicensis	9.99±0.80	11.35±0.18	na

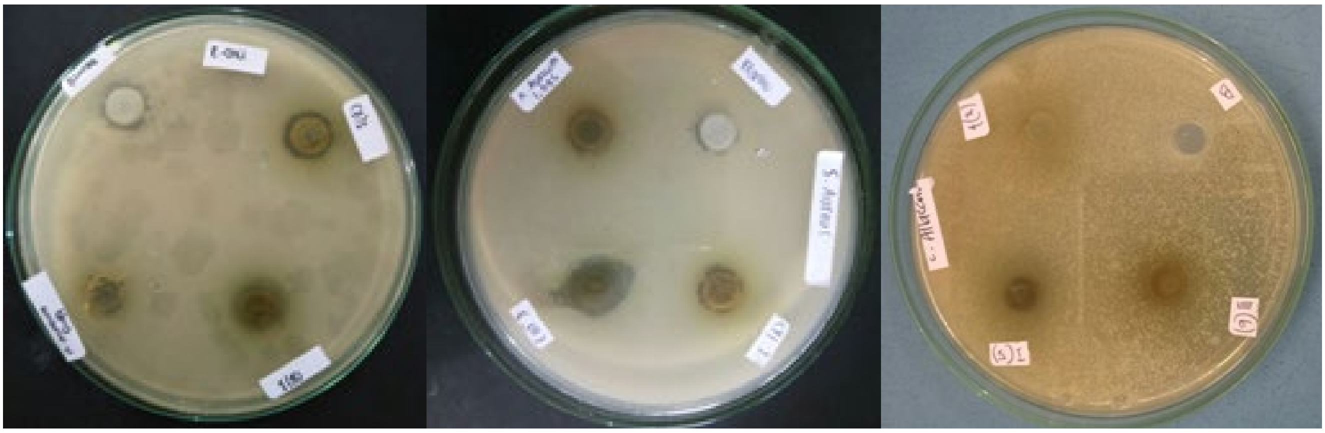
Note: na: not active

Even though the polyisoprenoid of *Av. lanata* showed a weak scavenging activity toward DPPH but the extract inhibited the growth of the pathogenic bacteria (Table 2 and 3). *S. aureus* was inhibited more strongly than *E. coli* by the *Av. lanata* extract. The polar extract of Avicennia has been reported to have antimicrobial, antidiarrhoeal, analgesic and antipyretic, antiulcer, antinociceptive, anti-inflammatory, diuretic and neuropharmacological activities (Thatoi et al. 2016).

Table 3. Antioxidant activity of polyisoprenoid from 16 mangrove leaves

Sample	Conc. (μg/mL)	% Inhi-bition	Regression equation	IC_{50} (μg/mL)
Ac. auriculiformis	4800	25.75	y = 0.002x + 15.80	17100
	480	17.92		
	48	14.89		
Av. alba	2720	32.53	y = 0.002x + 25.50	12250
	272	26.78		
	27.2	25.07		
Av. lanata	3460	17.28	y = 0.003x + 5.958	14681
	346	8.56		
	34.6	4.75		
Av. marina	2800	17.03	y = 0.003x + 9.316	13561
	280	18.46		
	28	1.86		
Av. officinalis	3260	46.37	y = 0.004x + 31.94	4515
	326	33.44		
	32.6	32.05		
B. asiatica	6000	59.14	y = 0.003x + 36.72	4427
	600	41.03		
	60	35.08		
B. gymnorrhiza	4860	11.62	nc	nc
	486	14.17		
	48.6	8.22		
C. inophyllum	2260	60.05	y = 0.012x + 32.44	1463
	226	35.15		
	22.6	32.77		
C. tagal	3240	45.41	y = 0.002x + 38.64	5680
	324	40.21		
	32.4	37.92		
H. tiliaceus	3240	56.83	y = 0.005x + 39.91	2018
	324	40.43		
	32.4	41.14		
N. fruticans	2680	54.51	y = 0.015x + 13.43	2438
	268	18.85		
	26.8	12.67		
P. odoratissimus	4580	0.72	nc	nc
	458	1.45		
	45.8	4.43		
P. pinnata	7660	64.83	y = 0.003x + 34.70	5100
	766	39.00		
	76.6	33.85		
R. communis	4860	42.23	y = 0.002x + 31.61	9195
	486	32.92		
	48.6	31.50		
R. mucronata	6920	31.35	y = 0.001x + 22.38	27620
	692	22.00		
	69.2	23.62		
S. jamaicensis	200	30.06		
	20	25.25	y = 0.026x + 24.75	971
	2	24.84		

Note: nc: not calculated



A B C

Figure 1. Zones of microbe growth inhibition produce by polyisoprenoid in leaves of the mangrove. A. *S. aureus,* B. *E. coli* , C. *C. albicans*

Avicennia marina had an IC_{50} of 13.561 µg/mL indicating the antioxidant activity of its polyisoprenoid was of a weak category. The polyisoprenoid extract of Av. Marina inhibited *E. coli* and *S. aureus* with an inhibition area diameter of 10.85 mm and 10.52 mm, respectively while no inhibition zone observed in *C. albicans* culture.

Avicennia officinalis is a mangrove and medicinal plant that is distributed in Bangladesh, India, Indonesia, Malaysia, Brunei, Myanmar, Vietnam and southern Papua New Guinea (Thatoi et al. 2016)). The extract of ethyl acetate from *Av. officinalis* leaves was able to inhibit the growth of *E. coli* and *S. typhi* and has good IC_{50} in reducing DPPH free radical (Khushi et al. 2016; Bhimba et al. 2010). In line with those previous reports, in this study, polyisoprenoids extracted from *Av. officinalis* inhibited the growth of *E. coli* and *S. aureus* even though its antioxidant activity was weak. This difference in activity was due to the solubility of active phenol compounds dissolved in a polar or semi-polar solvent, i.e., lipids tend to dissolve in a nonpolar solvent.

Barringtonia asiatica extract had a stronger inhibition effect towards *E. coli* than against *S. aureus*. However, it did not inhibit the growth of *C. albicans*. Furthermore, its ability to reduce free radical DPPH was relatively weak with an IC_{50} of 4427 µg/mL. In a previous study conducted, it has been reported that *B. asiatica*'s crude methanol extract and its fractions had high antibacterial and antifungal activities (Khan and Omoloso 2002). Our result showed that the nonpolar portion of *B. asiatica* has less potential antimicrobial and antioxidant activity that compared with its polar fraction

Bruguiera gymnorrhiza is one of mangroves plants that have economically valuable fruits (Rudiyanto 2016). Polyisoprenoids of *B. gymnorrhiza* moderately inhibited the growth of *E. coli* and *S. aureus* whereas it did not exhibit scavenging activities towards DPPH free radical. In a study conducted, it has been reported that the ethanol extract, but not the chloroform extract, of *B. gymnorrhiza*, had inhibitory activity against pathogenic bacteria (Haq et al. 2011).

Calophyllum inophyllum is a medicinal plant for skin diseases including itches, skin allergy, burns and mild wounds (Girardi et al. 2015). *C. Inophyllum* seeds extract in chloroform: methanol (2:1) was able to inhibit the growth of *S. aureus* culture (Léguillier et al. 2015). In this study, we showed that *C. inophyllum* was able to inhibit the growth of *E.coli* and *S. aureus* cultures but not that of the *C. albicans* (Table 2).

Ceriops tagal is a medicinal plant that has been reported to have efficacy to treat infected wounds, obstetric, hemorrhagic, sores, malignant ulcers and malaria (Zhang et al. 2005; Bamroongrugsa 1999; Wang et al. 2012; Yang et al. 2015;). In this study, we found that polyisoprenoid compounds of *C. tagal* inhibited the growth of gram-positive bacteria *S. aureus* better than that of the gram-negative bacteria *E. coli*, but not that of the *C. albicans* (Table 2). Previously, have reported that the ethanol extract of *C. tagal* and its partition did not show antimicrobial and antifungal activities. Lipid compounds in *C. tagal* have an antimicrobial property in comparison to the polar component (Bulbul et al. 2017).

Hibiscus tiliaceus has been used as a traditional medicine by Indonesian and Bangladeshi as a cough, bloody/slimy diarrhea, and tonsilitis medicines. In addition to having an inhibitory effect against pathogenic bacteria, surprisingly, the polar extract of the plant shows good cytotoxicity against cancer cell culture (Shaikh et al. 2009; Wong et al. 2010; Ramproshad et al. 2012). The polyisoprenoid of *H. tiliaceus* in this study showed a moderate inhibitory effect against *E. coli* (inhibition zone diameter 10.52 mm), and *S. aureus* (11.57 mm) while has a relatively weak antioxidant activity (Table 2 and 3).

Polyisoprenoid of *N. fruticans* showed an IC_{50} of 2438 µg/mL, indicating a weak antioxidant activity. At a concentration of 100 mg/mL, *N. fruticans* extract could inhibit the growth of *E. coli* and *S. aureus* but not that of the fungus *C. albicans* (Table 2). Yusoff et al. (2015) reported the ethyl acetate-soluble part of *N. fruticans* showed a potent antioxidant activity whereas the water extract was able to improve blood sugar levels in streptozotocin-induced diabetic rats.

Pandanus odoratissimus has been traditionally known as one of the Indian Ayurvedic medicines for a headache, rheumatism, spasm, cold, flu, epilepsy, wounds, boils, scabies, leucoderma, ulcers, colic, hepatitis, smallpox, leprosy, syphilis, cancer, dysuric, as well as a cardiotonic, antioxidant, and aphrodisiac (Adkar et al. 2014). The ethanol extract from leaves of *P. odoratissimus* contains glycosides, flavonoids, alkaloids, saponins, flavonoids dan polyphenol were considered responsible for the effect of therapy (Gurmeet and Amrita. 2015). The polyisoprenoid extract from *P. odoratissimus* in this study showed moderate inhibition of *E. coli* and *S. aureus* (Table 2). However, the extract did not show any antioxidant activity (Table 3).

Pongamia pinnata has been used as a medicinal plant by the Indian for fever, ulcer, skin diseases, piles, bronchitis, etc. (Duke 1983). The extract of petroleum ether and ethyl acetate from leaves of *P. pinnata* did not show an inhibitory effect on *E. coli*, *S. aureus*, and *C. albicans* (Ujwal et al. 2007). In this study, polyisoprenoids from the leaves of *P. pinnata* moderately inhibited the growth of *E. coli* and *S. aureus*, but not that of the *C. albicans* (Table 2). This study suggests that the polyisoprenoid may act as an antibacterial agent and the difference in solvent's polarity may affect the solubility of the active compound.

Ricinus communis is a traditional medicinal plant, and its leaves and seeds were reported to have pharmacological effects, such as anti-inflammatory, immunomodulator, antidiabetic, antiulcer, etc. (Kumar. 2017). Polar extract from *R. communis* leaves and seeds actively inhibited the growth of pathogenic gram-negative and gram-positive bacteria (Naz and Bano. 2012). We found that the polyisoprenoid extract of *R. communis* showed a weak antioxidant activity (Table 3). Nevertheless, it inhibited the growth of *E. coli* and *S. aureus* but not that of the *C. albicans* (Table 2). The polar and non-polar extract of *R. communis* showed synergistic activity in biological effects.

Rhizophora mucronata has been traditionally used to treat diarrhea, dysentery, fever, angina, diabetes, hematuria, and bleeding (Batool et al. 2014). Hexane, chloroform and methanol extracts of leaves and roots of *R. mucronata* have been reported to have high antibacterial and antifungal activity (Kusuma et al. 2011). However, in this study, we found that the polyisoprenoid extract of the plant did not have inhibitory activity against *E. coli* and *C. albicans* while moderately inhibited the growth of *S. aureus*. *S. jamaicensis* has been reported to contain secondary metabolites alkaloids, flavonoids, phenols, steroids, and terpenoids; and has traditionally been used for medicinal purpose in various countries (Putera and Shazura 2010). The active compounds have pharmacological effects as antacid, analgesic, anti-inflammatory, hypotensive, antihelminthic, diuretic, laxative, lactagogue, purgative, sedative, spasmogenic, vasodilator, vulnerary, and vermifuge properties (Liew and Yong 2016). Our study showed that the polyisoprenoid of *S. jamaicensis* had moderate inhibitory effects on the growth of *E. coli* and *S. aureus*.

Overall, this study identifies mangroves whose polyisoprenoid content have the inhibitory effect to *E. coli* and *S. aureus* bacteria, they are *Ac. auriculiformis, Av. lanata, Av. marina, Av. officinalis, B. asiatica, B. gymnorrhiza, C. inophyllum, H. tiliaceus, N. fruticans, P. odoratissimus, P. pinnata* and *R. communis*. Information of polyprenol and dolichol compounds that have biological activity is still limited. Some mangroves species have been reported to produce polyprenyl acetone, polyprenol, and dolichol content. They were in different variations of proportion in each mangrove (Basyuni et al. 2017a). The dominant polyprenol content in polyisoprenoid has a significant inhibitory tendency against bacterial growth. The data of the antimicrobial activity of polyisoprenoid compounds was that of the polyprenol only.

In conclusion, we reveal that the polyisoprenoid extracts from the leaves of the twelve mangroves species showed significant antibacterial activities that are potential for antimicrobial drugs development.

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