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Antibacterial activity of several Indonesian plant extracts and combination of antibiotics with *Syzygium malaccense* extract as the most active substance

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ABSTRACT

Natural products have an important role in the development of drugs, especially as an antibacterial. Some plant extracts in Indonesia such as Syzygium (Myrtaceae) and Graptophyllum (Acanthaceae) have been used empirically for the treatment of non-infectious diseases, and are also for management infections such as throat, mouth and vaginal infections, dysentery and diarrhea. The objectives of this study are to investigate antibacterial activity of 11 extracts from ten plants which were used in traditional medicine in Indonesia to treat infection disease and to evaluate interaction between potential antibacterial extract and antibiotics. Antibacterial activity and combination extract with antibiotics were evaluated using agar diffusion method on *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa*. Among the ten plants, *Syzygium malaccense* leaves extract, and *Nephelium lappaceum* peel and leaves extracts showed significant antibacterial activity. They have antibacterial activity against Gram positive and Gram negative. *S. malaccense* extract had better antibacterial activity against *P. aeruginosa* compared to the other tested bacteria and provided better activity compared to *N. lappaceum* leaves extract. Phytochemical screening showed that *S. malaccense* leaves extract contained flavonoid, tannin, quinone, phenol and triterpenoid/steroid. The combination of *S. malaccense* extract with antibiotics showed that the usage of *S. malaccense* extract together with ciprofloxacin gave antagonistic result.

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INTRODUCTION

Antibiotic resistance has increased since the last few years. This phenomenon has an impact on health costs, recovery time and increasing mortality. Many factors can contribute to antimicrobial resistance. In developing countries, inadequate access to antimicrobial agents and health care systems, poverty and malnutrition and antibiotic abuse are factors that play a role in increasing the incidence of antibiotic resistance (Vila and Pal, 2010). Antibiotic resistance that often occurs caused by an enteric pathogen such as *Escherichia coli and Salmonella sp;* Gram-

negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii, Staphylococcus aureus,* and *Mycobacterium tuberculosis* (Vila and Pal, 2010; Theuretzbacher, 2013). Development of new drug to treat bacterial resistance is urgently required. Plant-derived antimicrobials are considered as alternatives drugs to control infection (Srivastava *et al.*, 2014). The mechanism of plant-derived antimicrobials to control the resistance of microbes are reducing pH, increasing membrane permeability and altering efflux pumping (Srivastava *et al.*, 2014). Combination antibiotic with plant-derived antimicrobials can minimise resistance and give synergistic of antimicrobial effect (Srivastava *et al.*, 2014; Sibanda and Okoh, 2007).

Indonesia has a biodiversity that is used as herbal medicine. It has been used empirically to treat various infectious diseases. To evaluate efficacy to treat infection, we aim to a screening of some plants for their antibacterial activity. The plant was selected from literature based on practical use to treat infection disease like throat and mouth infections, dysentery, diarrhoea, cough and bronchitis. In this study, we also evaluated the in vitro of a combination of extracts when used together with antibiotics. Interactions between herbal medicines and synthetic drugs can have serious clinical consequences. Interaction between herbs and drugs can be beneficial (synergist or additive), not influence the effect of each other (indifference) or give a detrimental effect (antagonist).

MATERIALS AND METHODS

Plant Material

Ten plants were collected from several places in Indonesia, from West Java and Kalimantan. From West Java, we collected: *Syzygium polycephalum* (Miq.) Merr. & L.M. Perry from Sumedang, *S. polyanthum* (Wight) Walp. and *S. malaccense* (L.) Merr. & L.M. Perry from Soreang, *S. samarangense* (Blume) Merr. & L.M. Perry from Kopo Sayati, *S. myrtifolium* Walp. from Sarijadi, *Psidium cattleianum* (Sabine) from Garut, *Graptophyllum pictum* (L.) Griff. from Bogor, *Nephelium lappaceum* (L.) from Subang, *Plectranthus scutellarioides* (L.) R.Br. from Lembang. *Kleinhovia hospita l* (L.) collected from Kalimantan. The identity of plants material was confirmed at Herbarium of Jatinangor, Department of Biology, Padjadjaran University, Bandung, Indonesia. The plant materials were washed, dried and powdered for extraction.

Extraction

Powdered plant materials were macerated with 96 % ethanol for 24 hr. Maceration was repeated three times. The ethanol extracts were dried using vacuum rotary evaporator. Maceration method was used for the extraction of all plants material in this study. The reflux method also was used for extraction *S. malaccense* leaves. The leaves are immersed in 96% ethanol for 3 hr at the boiling point of ethanol and repeated three times.

Test Organism

The bacteria (Thermo scientific) used were *Bacillus subtilis* ATCC 6633, *Stapylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 25922. The bacteria cultured were maintained in Mueller Hinton Agar, MHA (Oxoid).

Phytochemical analysis of the plant extract

Syzygium malaccense leaves extract was subjected to phytochemical screening for detecting secondary metabolites, tannins, saponins, steroid/triterpenoid, alkaloids, flavonoid and phenolic compound and quinone according to standard methods (Parekh and Chanda, 2007).

Screening extract for antibacterial activity

The inoculum size of bacteria was prepared, according to (CLSI, 2015). The bacteria were inoculated into Mueller Hinton Broth, MHB (Merck) and incubated for 18-24 hr. Each bacteria was suspended in MHB and diluted until the turbidity equal to 0.5 McFarland (1.5 x 10^8 cfu/ml) standards. The ethanol extract was dissolved in dimethyl sulphoxide (DMSO). DMSO was used as a negative control. Ciprofloxacin and tetracycline were used as a positive control. Antibacterial activity was tested by disc diffusion method, but 20 μl of extracts did not give antibacterial effect. Then 20 μl extracts were tested by agar well diffusion technique, and the extracts gave the inhibition zone diameter. According to this result, the research was determined by the agar-well diffusion method. The antibacterial assay was determined by a modified agar well diffusion method (Parekh and Chanda, 2007). 100 μl of standardised inoculum was added aseptically to 20 ml melted Mueller Hinton Agar, MHA (Oxoid) to give solid plate (9 cm in diameter). Wells (8 mm) were prepared in the seeded agar plate. The ethanol extract (40 μl) was pipetted into well. The plates were placed at room temperature for 30 min to allow diffusion of extract into the agar and then incubated at 37°C for 18-24 hr. Inhibition zone diameter was measured around each well. The tests were conducted in triplicate. Determination of Minimal Inhibitory Concentration (MIC) was used by the agar well diffusion method that has been described pre-

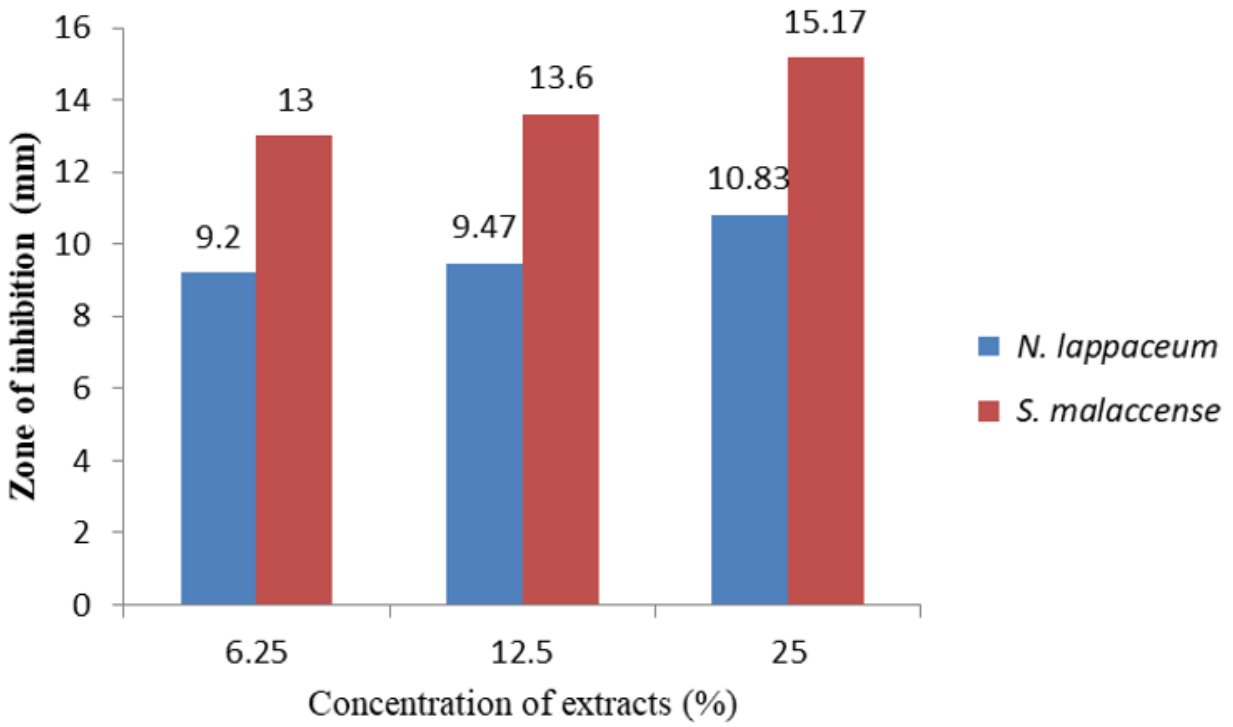
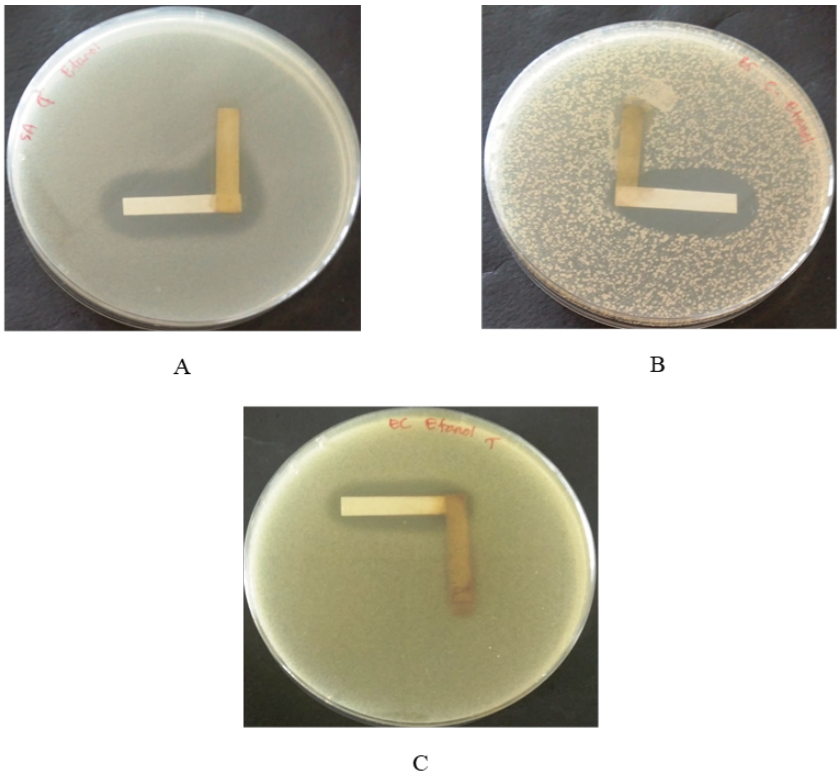


Figure 1: Antibacterial activity of *S. malaccense* and *N.lappaceum* leaves extracts against *Pseudomonas aeruginosa*



A. ethanol extract with tetracycline on *S. aureus* show synergistic effect; B. combination ethanol extract with ciprofloxacin on *B. subtilis* showed antagonistic effect; C. ethanol extract with tetracycline on *E.coli* showed indifference effect

Figure 2: Various inhibition patterns in combination antibiotics and *S. malaccense* leaves extracts

Table 1: Inhibition zone diameter of Syzygium extracts

Extract	Percent conc.	Inhibition zone diameter (mm) against bacteria			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
S. samarangense	25	9.13 ± 0.68	-	-	-
	12.5	8.87 ± 0.15	-	-	-
	6.25	8.63 ± 0.25	-	-	-
S. polyanthum	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
S. polycephalum	25	-	-	-	9.87 ± 0.58
	12.5	-	-	-	9.23 ± 0.21
	6.25	-	-	-	8.70 ± 0.26
S. malaccense	25	10.9 ± 0.56	12.17 ± 0.9	12 ± 0.6	11.13 ± 0.55
	12.5	10.37 ± 0.4	10.80 ± 0.36	10.53 ± 0.42	10.20 ± 0.3
	6.25	9.03 ± 0.06	9.40 ± 0.46	9.30 ± 0.36	9.77 ± 0.12
S. myrtifolium	25	8.83 ± 0.59	11.50 ± 1.14	10.37 ± 0.71	9.97 ± 0.47
	12.5	8.30 ± 0.10	9.60 ± 0.52	9.30 ± 0.36	9.30 ± 0.3
	6.25	-	9.30 ± 0.17	9.00 ± 0	8.93 ± 0.12
DMSO		-	-	-	-
Tetracycline	100 μg/ml	-	10.38 ± 0.45	12.08 ± 0.9	-

viously.

The combination of *Syzygium malaccense* extract and antibiotics

The combination of *Syzygium malaccense* extract and antibiotics was evaluated by agar diffusion method using paper strips (Lorian and Fodor, 1974) with modification. 100 μl of standardised inoculum of bacteria (1.5 x 10^8 CFU/ml, 0.5 McFarland) was added aseptically to the molten Mueller Hinton Agar (MHA) into sterile Petri dishes to give a solid plate. Two paper strips, each containing antimicrobial agents and extract, were placed on plates on Mueller-Hinton Agar to permit antibiotic and extract to enter the agar. After 18-24 h of incubation at 37° C, specific growth patterns were indicative of additive, indifference, synergistic, or antagonistic effects of the drug combination used.

RESULTS AND DISCUSSION

Antibacterial activity screening of medicinal plants was performed on Syzygium genus, which was *Syzygium samarangense*, *Syzygium polyanthum*, *Syzygium polycephalum*, *Syzygium malaccense* and *Syzygium myrtifolium*. The results were presented in Table 1. The results from the five tested Syzygium extracts, *S.malaccence* gave the best activity against all four tested bacteria, both Gram-positive and negative bacteria, which the inhibitory zone diameter was 9-12 mm. *S. malaccense* leaves extract was more susceptible to *B. subtilis* than the other test bacteria. When the dosage level increased, the inhibitory effect of the extract also increased. Empirically, *S. malaccense* was used for treating infectious disease such as mouth lesion, sore throats, thrush, dysentery, cough, pulmonary disease (Cheenickal and Sheela, 2016; Whistler and Elevitch, 2006). According to (Yusoff *et al.*, 2013), *S. malaccense* has potential effect as an antimicrobial preservative agent for topical application product at concentration 0.25% (w/v) and 0.5 % (w/v). *S. aureus* growth was inhibited on day seven and *Candica Albicans* on day 21 (Yusoff *et al.*, 2013). This study provided scientific evidence of practical use of *S. malaccense* as an antibacterial. The previous reports

Table 2: Inhibition zone diameter of several plants extract

Extract	Percent concentration	Inhibition zone diameter (mm) against bacteria		
		S. aureus	*B. subtilis*	*E. coli*
Kleinhovia hospita	25	-	10.43 ± 1.31	-
	12.5	-	10.4 ± 1.4	-
	6.25	-	10.17 ± 1.26	-
Psidium cattleianum	25	-	14.17 ± 0.47	-
	12.5	-	13.33 ± 0.21	-
	6.25	-	13 ± 0.5	-
Graptophyllum pictum	25	-	-	-
	12.5	-	-	-
	6.25	-	-	-
Leaves of Nephelium lappaceum	25	18.40 ± 0.26	11.93 ± 0.49	11.23 ± 0.05
	12.5	15.57 ± 0.38	9.97 ± 1.15	10.00 ± 0
	6.25	11.77 ± 0.25	-	-
Peel of Nephelium lappaceum	25	11.47 ± 0.46	11.83 ± 0.38	13.57 ± 0.06
	12.5	10.13 ± 0.23	10.60 ± 0.36	11.43 ± 0.49
	6.25	8.97 ± 0.15	-	12.57 ± 0.49
Plectranthus scutellarioides	25	11.00 ± 0	12.17 ± 0.15	-
	12.5	10.37 ± 0.32	12.00 ± 0.06	-
	6.25	9.47 ± 0.47	11.3 ± 0.52	-
DMSO		-	-	-
Ciprofloxacin	200μg/ml	25.7 ± 3.47	28.83 ± 1.38	22 ± 0.28

Table 3: Comparison of inhibition zone of S. malaccense using two extraction methods

Test bacteria	Method of extractior	Inhibition zone (mm) at concentration		
		25 %	12.5%	6.25%
P. aeruginosa	Reflux	10.8 ± 0.17	9.70 ± 0.61	9.03 ± 0.4
	Maceration	15.17 ± 0.21	13.60 ± 0.4	13.01 ± 0
E. coli	Reflux	-	-	-
	Maceration	12.10 ± 0.61	10.33 ± 0.49	-
B. subtilis	Reflux	11.83 ± 0.38	10.6 ± 0.36	-
	Maceration	11.83 ± 0.4	10.53 ± 0.61	
S. aureus	Reflux	16.17 ± 0.25	14.13 ± 0.12	12.77 ± 0.25
	Maceration	16.20 ± 0.1	14.07 ± 0.21	12.5 ± 0.46

Table 4: Minimum inhibitory concentration of S. malaccense ethanol extract

Test bacteria	Inhibition zone (mm) at concentration						
	7 %	6 %	5 %	4 %	3 %	2 %	1 %
P. aeruginosa	15.30 ± 0.40	13.23 ± 0.29	13.07 ± 0.49	10.70 ± 0.95	10.70 ± 0.46	9.77 ± 0.00	9.03 ± 0.38
E. coli	12.47 ± 0.51	12.43 ± 0.12	12.17 ± 0.46	11.7 ± 0.26	9.90 ± 0.56	8.97 ± 0.71	-
B. subtilis	9.03 ± 0.84	-	-	-	-	-	-
S. aureus	12.33 ± 0.42	12.13 ± 0.70	11.37 ± 0.23	10.87 ± 0.31	9.67 ± 1.01	-	-

Table 5: Effect of combination antibiotics and S. malaccense ethanol extract

Antibiotics	Bacteria	Combination Effect
Amoxicillin	Bacillus subtilis	Synergistic
	S . aureus	Indifference
	Escherichia coli	Indifference
	P . aeruginosa	-
Ciprofloxacin	Bacillus subtilis	Antagonistic
	S . aureus	Antagonistic
	Escherichia coli	Antagonistic
	P . aeruginosa	Antagonistic
Tetracycline	Bacillus subtilis	Indifference
	S . aureus	Synergistic
	Escherichia coli	Indifference
	P . aeruginosa	-

indicated that plant material from genus Syzygium (*S. polyanthum*, *S. samarangense*) had antibacterial activity against *P. aeruginosa*, *E.coli* and *S. aureus* and others microbes (Ramli *et al.*, 2017; Ratnam and Raju, 2008). *S. polyanthum* leaves extract at concentration 10 mg/ml (w/v) inhibited foodborne pathogen, *E. coli, S. aureus, Vibrio cholerae, Vibrio parahaemolyticus,Klebsiella pneumonia, Proteus mirabilis, Salmonella typhimurium,Listeria monocytogenes,* and *P. aeruginosa* ranged from 6.67 to 9.67 mm of inhibition zone. Methanol fruit extracts of *S. samarangense* exhibited significant inhibition against *S. aureus, E. coli*, *P. aeruginosa, K. pneumonia* and *Bacillus cereus* at concentration 25-50 mg/ml. In this study, *Syzygium polyanthum* did not show activity against tested bacteria and *S. samarangense* only active against *S. aureus.* Differences in place of growth can affect the contents of chemical compounds and the biological activity; therefore, in this study showed different results (Karahan *et al.*, 2016). There was no scientific report regarding the antibacterial activity of *S. myrtifolium* and *S. polycephalum*.The other materials were six extracts from five plants that were *Kleinhovia hospita*, *Graptophyllum pictum*, *Psidium cattleianum, Plectranthus scutellarioides* and *Nephelium lappaceum*. They were chosen because of its practical use as an anti-infection in Indonesia and limited scientific evidence as an antibacterial. Leaf oil of *Kleinhovia hospita* showed activity against *Bacillus subtilis, Bacillus licheniformis*, *Escherichia coli* and *Acinetobacter junii* with MIC value 61.75 μg/ml, 60.02 μg/ml, 35.75 μg/ml and 38.04 μg/ml, respectively (Dey *et al.*, 2017). *Graptophyllum pictum* has been used in Asian folk medicine as anthelmintic and syphilis infection. A study by (Jiangseubchatveera *et al.*, 2015) demonstrated that *G. pictum* inhibited *S. aureus* and *E. coli* with MIC values of 11.75 and 35.25 μg/disc (Jiangseubchatveera *et al.*, 2015). The ethanol extract of *P. cattleianum* showed higher antimicrobial activity against Gram-positive and harmful bacteria tested when compared to the aqueous extract (Scur *et al.*, 2016). Traditional used of *Plectranthus scutellarioides* in Indonesia used traditionally for treat dysentery and tuberculosis (Romulo *et al.*, 2018).

The results of this research were shown in Table 2. It was confirmed that leaves and peel of *Nephelium lappaceum* have highest potential antibacterial activity with inhibition zone diameter 8.9 -18.4 mm. *Nephelium lappaceum* leaves extract exposed the highest activity against *S. aureus* with inhibition zone diameter 12-18 mm. According to a study by (Taylor, 2013), methanol peel extracts of *N. lappaceum* has antibacterial activity against *P. aeruginosa*, *V. cholerae*, *Enterococcus faecalis*, *S. aureus* and *S. epidermidis* with MIC value in the range of 2.00-62.5 mg/ml. The previous research by (Sulistiyaningsih *et al.*, 2017) revealed that ethanol leaves extract of N . *lappaceum* had antibacterial activity against *P. aeruginosa* multiresistant at MIC value 2.5% (w/v).

The results of 11 tested extracts demonstrated that *S. malaccense* leaves, *S. myrtifolium* leaves, N . *lappaceum* leaves and peel extracts had antibacterial activities on both Gram-positive and Gram-negative. Antibacterial activity of broad-spectrum is important because many of them are used clinically by most of the general medical practitioners for bacterial infections of unknown causes (Wood *et al.*, 2007). *S. malaccence* and *N. lappacceum* more potential antibacterial than the others based on their activity against all tested bacteria and their inhibition zone. The antibacterial activity against *P. aeruginosa* strains is an important feature because of the limited antibiotics available for this infection.

P. aeruginosa is one of the bacteria that often cause nosocomial infections, namely Hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) and can cause high morbidity and mortality (Chung *et al.*, 2011). *S. malaccense* and N *.lappaceum* leaves extracts gave antibacterial activity against *P. aeruginosa* with inhibition zone in the range of 9.2 – 15.17 mm. *S. m alaccense* leaves extract provided better activity against *P. aeruginosa* compared to *N. lappaceum* leaves extract (Figure 1).

Based on its activity against *P. aeruginosa*, then *S. malaccense* was extracted by using different extraction methods (maceration and reflux), to optimise extraction condition and related with a chemical compound which responsible for antibacterial activity. Extraction was carried out using 96 % ethanol. The results were given in Table 3. The influence of temperature can affect extraction efficiency (Ćujić *et al.*, 2016). In this study, maceration methods can attract chemical compounds that responsible for antibacterial activity, whereas the activity of reflux extract was decreased and inactive to *E. coli*. Based on the result in the present study it can be concluded that the reflux method can reduce biological activity because thermolabile compounds in *S. malaccense* will damage due to temperature of extraction (Sarker *et al.*, 2006).

Determination of MIC of *S. malaccense* ethanol extract against each tested bacteria was done by microdilution method. The results were shown that the MIC value of ethanol extract of *S. mallacense* > 4000 μg/ml. Then the MIC value was determined using agar well diffusion method. The results were shown in Table 4. The MIC values of *S. malaccense* ethanol extract against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E.coli* were 7 %, 3 %, 1 % and 2 %, respectively. A disadvantage of the agar-well diffusion method in determining MIC is depended on quantifying the amount of antimicrobial agent diffused into the agar medium (Balouiri *et al.*, 2016).

The phytochemical screening results presented that ethanol leaves extract of *S. malaccense* contained flavonoids, tannins, quinones, phenols and triterpenoid/steroids. An earlier report revealed that *S. malaccense* also contain alkaloid and saponins content (Cheenickal and Sheela, 2016). These variation of a chemical compound are due to environmental factor such as light, temperature and soil (Karahan *et al.*, 2016; Yang *et al.*, 2018). Flavonoids and phenolic content might cause antibacterial activity of *S. malaccense* extract. Flavonoid and phenol content in *S. malaccense* leaves have been reported as much 1 % and 6 % respectively (Cheenickal and Sheela, 2016). The earlier reports showed that flavonoids and phenolic compounds contributed as antimicrobial (Kumar and Pandey, 2013).

Besides using as an anti-infectious disease, *S. malaccense* empirically was used for diabetic therapy, anti-inflammation, headache, emetic, emmenagogue (promoting menstrual function) (Whistler and Elevitch, 2006). Sometimes these herbs are used in conjunction with medications so that drug interactions can occur. Interaction herbal medicine with antibiotics can occur when the antibiotic was used together with herbal medicine. Determination effect of combination antibiotics and extracts was evaluated using diffusion agar using paper strips because of this method easy enough to be used and provided an overview of the pattern of combination (Lorian and Fodor, 1974). The results indicated that *S. malaccense* extract gave various patterns when combined with antibiotics (Table 5). The growth patterns are shown in Figure 2. A synergistic effect was shown by a zone of no growth inside the angle formed by the two strips. Ethanol extract of *S. malaccense* showed synergistic effect when combined with amoxicillin against *B. subtilis*. The synergistic effect also showed by combination ethanol extract with tetracycline against *S. aureus*. Other inhibition patterns of combination extract and amoxicillin or tetracycline gave indifference; zone of inhibition does not interfere with each other.Meanwhile, a combination of ethanol extract with ciprofloxacin against four tested bacteria showed antagonistic tendencies. An antagonistic effect was shown by an indentation of growth inside the angle. The results stated that it needs caution when the usage of *S. malaccense* extract to maintain health or as a traditional remedy together with antibiotics to treat an infection. Concurrent use of extract with ciprofloxacin can decrease ciprofloxacin effects to inhibit the growth of bacteria. Amoxicillin and tetracycline against Pseudomonas in this study cannot be determined because of the limited sensitivity of bacteria against antibiotics.

CONCLUSIONS

S malaccense and *N. lappaceum* leaves extract exhibited better antimicrobial activity against Gram-positive and Gram-negative bacteria compared to the other plants. Ethanol leaves extract of *S. malaccense* showed potential activity against *P. aeruginosa,* meanwhile *N. lappaceum* leaves extract against *S. aureus.* Phytochemical screening of *S. malaccense* extract indicated the presence of flavonoid and phenol compounds that might be responsible

for antimicrobial activity. This research proved the practical use of *S. mallacense* and *N. lappaceum* to treat infectious diseases and can be used as a basis for an approach in the search for novel bioactive compounds. Combination *S. malaccense* with antibiotics can decrease the effect of ciprofloxacin, so concurrent use with antibiotics needs caution.

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Conflict Of Interest

The authors declare no conflict of interest.

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