

Antibacterial potential of four herbal plants (*Syzygium cumini*, *Piper ornatum*, *Anredera cordifolia*, and *Alpinia galangan*) against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Introduction: The lack of new antibiotics and the increasing rate of resistance on pathogens requires the discovery of bioactive compounds with antibacterial activity. Using ethnopharmacology knowledge, several Indonesian herbs, in particular Juwet (*Syzygium cumini*), Sirih merah (*Piper ornatum*), Binahong (*Anredera cordifolia*) and Laos (*Alpinia galangan*) had been shown possess wound-healing, anti-inflammatory and gastroprotective activities. It was postulated that these plants would also have antibacterial activity.

Method: This study aims to assess the potential for antibacterial activity of these plants, in which decoction, methanolic and chloroform extraction was used against *Staphylococcus aureus* and *Escherichia coli* growth by measuring and comparing zone of inhibition (ZOI), minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Results: All plants tested had some activity against *S. aureus*, but none were found to be active against *E. coli*. Furthermore, various extraction methods of *S. cumini* and *A. galangan* had antibacterial activity against *S. aureus* with a ZOI of 10 – 15 mm and 12 – 25 mm, respectively, with increased antibacterial activity found in non-polar extracts. Methanolic extract of *S. cumini* acts as a bactericidal at 0.391 mg/ml, whilst other extracts had a bactericidal activity at 6.25 mg/ml.

Conclusion: From four plants tested, methanolic extracts of *S. cumini* and *A. galangan* has medium to strong antibacterial activity against clinical *S. aureus* strains.

Keywords: Juwet, Sirih Merah, Binahong, Laos, Antibacterial, Antibiotic

INTRODUCTION

Infectious diseases, particularly respiratory tract infections (for example, pneumonia and tuberculosis), is one of the major cause of deaths in Indonesia [1]. The high prevalence of lung infection in Indonesia is caused by the presence of various risk factors found in Indonesia, e.g. high population and density, low sanitation, low nutrition status, and high air pollution [2; 3]. A complication for respiratory tract infection treatment is the emergence of antibiotic resistant strains,

both hospital acquired (nosocomial) and community acquired, which is partly caused by the non-compliance or low-compliance of patients in Indonesia and the ease to buy antibiotics from local pharmacies, with or without prescription [4].

The antibiotic crisis faced today requires the discovery of novel antibiotic compounds, whether those having a different mode of action compared to existing drugs (e.g., biofilm inhibitors) or having better efficacy [5; 6]. Most

antibiotics used (80%) today was derived from different kinds of microorganisms (e.g. actinomycetes, particularly *Streptomyces* and many kinds of fungi) and several different plants [7; 8]. However, efforts to discover antibiotics with a different mode of action or target is slowing down, whether due to lack of investment in the industrial side [9], or from difficulty in the laboratory side [10]. Furthermore, antibiotic discovery from a plant source is relatively unexplored [11], although several researches has shown the potential for plant-based antibiotic screening in the discovery of novel compounds [12 – 15].

Many plants in different areas in Indonesia is shown to have the potential as bioactive drugs, anti-hypertension, anti-oxidants, anti-diabetic, and so on. However, the rich biodiversity of Indonesian natural sources results in many plants unknown for its antibiotic bioactivity. The four plants explored in this study, i.e. Juwet (*Syzygium cumini*), Sirih merah (*Piper ornatum*), Binahong (*Anredera cordifolia*) and Laos (*Alpinia galangan*), has been shown to have anti-inflammatory and wound healing properties, or is gastroprotective. These may indicate that these plants would also have antibacterial properties; and therefore, this study aims to explore the potential for these plants to be used as an antibacterial agent and whether these plants can be used to treat infectious diseases.

MATERIAL AND METHODS

Plant extraction

Plant samples were obtained from Batu Materia Medika in a dried powder form. The extraction of these plants, as well as all following tests, were conducted in Laboratorium Herbal Biomedik in Medical Faculty, Universitas Islam Malang. Three extraction methods were

conducted on these samples, namely decoctation, methanolic maceration and chloroform maceration. For each extraction method, plant dry powder was first weighed to 20 g, and added with a solvent in a 1:10 ratio. In decoctation method, the dry powder was added with 200 ml of distilled water and boiled at 90°C for 30 minutes. In maceration methods, the dry powder was added with the corresponding solvent at 200 ml and inserted in a water-bath shaker with a speed setting of approximately 100 to 125 rpm for 24 hours. After extraction process, the yield was calculated by removing the solvent (water-based extraction was inserted in an oven at 60°C for 24 – 48 hours, whilst solvent-based extraction was evaporated using a rotary evaporator until semi-dry, followed by a drying in an oven at 60°C for 24 hours). The yield was weighed and reconstituted using distilled water or the corresponding solvents to 1 mg/ml, and stored in 4°C until further evaluation.

Bacterial stock suspension preparation

Bacterial stocks (*Escherichia coli* and *Staphylococcus aureus*) used were clinical strains purchased from the Microbiology department of Universitas Brawijaya, Malang, Indonesia. The samples were inoculated in Nutrient Agar (NA) (HiMedia Laboratories®; composition: Peptic digest of animal tissue 5 g/L, NaCl 5 g/L, Beef extract 1.5 g/L, Yeast extract 1.5 g/L, Agar 15 g/L) and incubated at 37°C for 20 to 24 hours. The bacteria stocks were stored at 4°C until further use and was reinoculated and refreshed every 2 weeks.

Determination of Zone of Inhibition (ZOI) using disc-diffusion assay

Zone of inhibition was measured using a Kirby-Bauer method. From bacteria stock plates, a colony was transferred to a 0.9% NaCl solution and

compared with a McFarland Standard, which equates to 3×10^8 CFU/ml. Using a sterile cotton bud, the bacteria solution was thoroughly streaked on a NA plate. To test the extract, blank assay discs was first submerged in different concentrations of the extract for 30 minutes before placing on top the inoculated agar. A standard antibiotic disc and solvents were used as a method control. The plates were the incubated at 37°C for 20 to 24 hours. The diameter of the resulting clear zone was measured in millimeters using a ruler to determine antibacterial inhibition

Determination of Minimal inhibitory concentration (MIC)

MIC determination was only conducted on extracts showing a ZOI of over 10 mm in any bacteria by using a microdilution method in a 96 well-plate. The concentration tested was an initial concentration of 1 mg/ml followed by a serial dilution (by removing half of the initial concentration into another half of media) until 1/2048 of the initial concentration. The media used for dilution and the growth of the tested bacteria was Nutrient broth (HiMedia Laboratories®; composition: Peptone 10 g/L, Beef extract 10 g/L, NaCl 5 g/L). Aside from a blank (media only) control, each well was then added with a bacterial suspension having a final concentration of 1.5×10^5 CFU/ml, then incubated at 37°C for 20 to 24 hours. Bacterial growth was then measured using a spectrophotometer in a wavelength (λ) of 600 nm. Minimal inhibitory concentration was defined as the minimal concentration in which growth was observed to be equal blank control.

Determination of minimum bactericidal concentration (MBC)

Minimal bactericidal concentration was defined as the smallest extract concentration in which bacteria does not grow in normal medium after 24-hour

incubation. From each well in the 96 well-plate, 10 μ l was inoculated on NA medium, and incubated at 37°C for 24 hours. After 24 hours, the smallest concentration in which no colonies were observed was determined to be the MBC of the extract tested.

RESULTS AND DISCUSSION

Zone of inhibition (ZOI) determination shows that *S. cumini* and *A. galangan* to be potential against *S. aureus*

Results of ZOI of decocta, methanolic extraction and chloroform extraction on *S. cumini*, *P. ornatum*, *A. cordifolia* and *A. galangan* against *S. aureus* and *E. coli* is shown in table 1. On tested extracts, ZOI against *E. coli* was shown to have a maximum of 8 – 9 mm, showing weak inhibition. On the other hand, all plants tested was able to inhibit *S. aureus* with various strengths.

Tabel 1. ZOI measurement on four tested plants against *S. aureus* and *E. coli* (-) denotes maceration solvent

From table 1, the inhibition against *S. aureus*, from largest to smallest, was shown to be by chloroform maceration of *A. galangan*, methanolic maceration *A.*

Plant	Extract ion method	Bacteria	
		<i>S. aureus</i>	<i>E. coli</i>
<i>S. cumini</i>	Decocta	15	0
	*MeOH	18	0
	*CHCl ₃	10	9
<i>P. ornatum</i>	Decocta	0	9
	*MeOH	8	0
	*CHCl ₃	0	0
<i>A. galangan</i>	Decocta	12	0
	*MeOH	20	8
	*CHCl ₃	25	0
<i>A. cordifolia</i>	Decocta	0	0
	*MeOH	10	8
	*CHCl ₃	0	0

galangan, methanolic maceration of *S.*

cumini, decoction *S. cumini*, decoction of *A. galangan*, and chloroform maceration of *S. cumini*. Weak inhibition was observed against *S. aureus* on methanolic maceration of *A. cordifolia* and *P. ornatum*. These findings show that the active compound in *S. cumini* is likely a compound with was more easily extractable using polar solvent, indicative of a polar compound, whilst the active compound of *A. galangan* was more likely to be a non-polar compound. In *S. cumini*, methanolic maceration had the best result (18 mm), which can be categorized as medium inhibition. On *A. galangan*, chloroform maceration showed strong inhibition (25 mm).

E. coli inhibition was found in several different extracts, albeit, as mentioned previously, only weakly. Some theories suggest that Gram negative bacteria would be more resistant to certain compounds compared to Gram positive bacteria, due to differences in cell wall structure (peptidoglycans, lipids, cross-bonding, etc) and enzymatic activities which determine the penetration, binding and antibacterial activity [16].

MIC and MBC determination on plant extract showing some inhibition against *S. aureus* with *S. cumini* having the lowest MBC compared to all plants tested

Comparison of absorbance under spectrophotometer to measure MIC of each extract yielded inconclusive results. This is due to the presence of pigments from all extracted samples (as shown in Figure 1). The solvent itself was shown to have a negligible effect on bacterial growth (data not shown). Chloroform samples proved difficult to measure, as the evaporation rate of chloroform occurred to quickly to have an effective dilution.



Figure 1. MIC measurement on 96 well plate on 6 samples and 1 control sample. Extracted pigments obstructed effective reading for a quantitative determination of MIC.

Results of MBC (shown in table 2) indicate that most tested samples show some bactericidal activity on a high concentration (1/16 of the stock concentration, or equal to 6.25 mg/ml). Methanolic extraction from *S. cumini* however, showed the lowest MIC of 1/256, or equal to 0.391 mg/ml. This indicates that *S. cumini* has a potent active compound which can inhibit *S. aureus*, which is also potent in a small dosage, or that the compound mainly works as a bactericidal. Decoction of *S. cumini* did not yield the same results, having an MBC of 1/8th of the stock concentration (or equivalent to 12.5 mg/ml), and therefore the bioactive compound from *S. cumini* was less extractable using decocta method.

Previous studies in Indonesia have examined the chemical content of *S. cumini*. Sudarmi (2013) notes that ethanolic extraction of *S. cumini* leaves contains different compounds, such as alkaloids, phenolics, saponins and steroids [17]. Taher (2011) in Sudarmi (2013) adds that the leaves also contain flavonoids and various essential oils [17]. Similar data was obtained by Gowri (2010) who also compared the phytochemicals between water extract and methanolic extract [18]. From this study, it can be speculated that the bioactive compound is a steroid, as steroid yield was found to increase in methanolic extraction and decrease in water extraction. According to Gowri (2010), other compounds aside from steroids seem to have an opposite pattern and mostly be acquired via water extraction compared to methanolic extraction [18].

Methanolic extraction of *A. galangan* shows strong inhibitory effect against *S. aureus* and weak inhibition against *E. coli*, while chloroform and decoctation of *A. galangan* was only able to inhibit *S. aureus*. These findings was

agents, inhibiting bacterial growth and in some cases fungal growth. Different flavonoids is known to act as anti-allergens, anti-inflammatories, antimicrobial, and anti-cancer agents. Similarly, phenolics may also act as anti-oxidants, anti-cancer, anti-allergens, anti-mutagenics, and anti-diabetics. The potential for phenolics to act as antibacterial agents may be by damaging protein bonds in the cell wall, hydrophobic bonds in the cell membranes, and inactivate metabolic enzymes in a bacteria [20]. Therefore, it can be speculated that phenolic is responsible as an antibacterial agent against *S. aureus* and *E. coli*.

Decocta of *A. cordifolia* was not found to have any antibacterial effect, which is also in line with what has been done by other researchers [21 – 25], thus indicating that a water solvent was unable to pull out any antimicrobial compounds. However, methanolic extraction of *A. cordifolia* was able to weakly inhibit *S. aureus* and *E. coli* growth. According to Hasri (2017), ethanolic extraction was able

Table 2. Determination of minimal bactericidal concentration on four plant extracts

Plant	Extraction method	Serial dilution (compared to initial concentration 1 mg/ml)								
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
<i>S. cumini</i>	Decocta	-	-	-	+	+	+	+	+	+
<i>S. cumini</i>	*MeOH	-	-	-	-	-	-	-	-	+
<i>A. galangan</i>	*MeOH	-	-	-	-	+	+	+	+	+
<i>P. ornatum</i>	*MeOH	+	+	-	-	+	+	+	+	+
<i>A. cordifolia</i>	*MeOH	-	-	-	+	+	+	+	+	+
Control	MeOH	-	+	+	+	+	+	+	+	+

Positive (+) indicates growth in solid medium; negative (-) indicates no growth.

concurrent with Rani et al. (2016), which states that ethanolic extraction was able to obtain flavonoids, phenolics and terpenoids compound, whilst chloroform extraction was able to pull out chlorides, flavonoids, and alkaloids [19]. Water extraction yielded flavonoids, terpenoids, and chlorides. All these active compounds may act as antioxidants and antimicrobial

to pull out alkaloids, steroids, flavonoids, and phenolic compounds [26]. As previously mentioned, phenolics would be able to denature protein and solubilize fatty acids, and therefore damaging the cell wall and cell membrane [27]. Alkaloids destroys cell walls via peptidoglycan wall of the bacterial cells causing cell death [27].

Methanolic extraction of *P. ornatum* was able to obtain flavonoids which can inhibit bacterial growth. *P. ornatum* has a derivate of phenols named cavicoles and cavibetoles which can denaturate bacterial proteins [28]. According to Kartasaputra [28], *P. ornatum* active compounds has five times the activity of other phenolics against *S. aureus*. The inactivity of *P. ornatum* against *E. coli* (as also found in this study) is well documented in previous research [29]. This study contradicts other findings in which only low activity was observed, which either indicates that the extraction method used was unsuccessful in obtaining active compounds, or the plant batch used had only low bioactive compounds production.

CONCLUSION

From the four plants tested in this research, *S. cumini* and *A. galangan* extract shows antibacterial potential against *S. aureus*, while only weak antibacterial effects were found against *E. coli* on all plants tested. Methanolic extraction showed highest success in antibacterial yield on all four plants tested. Methanolic extract of *S. cumini* was able to act as a bactericidal (against *S. aureus*) with a low concentration, equivalent to 0.391 mg/ml, while most plants which showed antibacterial activity against *S. aureus* had an MBC of 6.25 mg/ml.

FUTURE DIRECTIONS

Future directions for this research include the isolation of active compounds from *S. cumini* and *A. galangan* using a bio-guided assay, followed by identification using LC-MS or NMR techniques.

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