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Natural Sources Screening for Antimicrobial Agent of Herbs, Spices, and Extracts: A Semi-Qualitative Study

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Abstract. Indonesia has been recognized for its rich natural ingredients such as spices,herbs, and extracts for decades. Furthermore, these components also have been used as an herbal medicine for a long time. Meanwhile, the apparent capability comparison of several components of antimicrobial activity has not been updated yet. This study was conducting the comparison of antimicrobial activity for several materials such as noni (*Morinda citrifolia*), garlic (*Allium sativum*), celery (*Apium graveolens*), galangal (*Alpinia galangal*), ginger (*Zingiber officinale*), yellow turmeric (*Curcuma longa*), lime (*Citrus aurantifolia*), papaya (*Carica papaya*) leaf, betel (*Piper betel*) leaf, and cutcherry (*Kaempferia galangal*) using agar dilution method. Two types of bacteria are used for the test, namely gram-negative bacteria and gram-positive bacteria, with a total of seven bacteria. The media used were TSA (Trypticase Soy Agar) media for gram-negative bacteria and MRSA (Methicillin-Resistant Staphylococcus Aureus) media for gram-positive bacteria. This study had been conducted by using a fast screening method, which is referred to as a semi-qualitative method. Several components, such as noni, lime, and betel leaf, showed a significant result of antimicrobial activity. Otherwise, other compounds, surprisingly, could not suppress bacterial growth.

Keywords: *herbs, spices, extracts, antimicrobial activity, and agar dilution method*

1. Introduction

The abundance of natural resources in Indonesia has not been mapping accordingly, to antimicrobial agent capability. As known as a tropical country, Indonesia has several beneficial plants, bushes, shrubs, and even animals and derivative components such as herbs, spices, and extracts from leaves, bulbs, rhizomes, and flowers. For decades, researchers have been interested in studying natural herbs from Indonesia as famous as “Jamu” [1]. With advancing technology recently, the mapping of antimicrobial capability from several resources should be

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started to valorize the product value. Wherein after the initial study of the antimicrobial screening process would be a basis for conducting further research to find suitable substances in nature as antimicrobial agents.

Many researchers have studied antimicrobial activity from many natural resources [2–4]. Antimicrobial activity is the capability of substances to suppress or kill a single or combination of microorganisms such as fungi, mold, microbe, and algae. The antimicrobial attributes are useful properties that have broad application in many industries such as textile, food, beverage, medicine, and so forth. Mostly, screening of substances from nature was initially conducted on the macroscopic scale and followed by microscopic identification of responsible chemicals as antimicrobial agents. There are some limitations for continuous study from macroscopic to microscopic scale due to the abundance of chemicals in an originally natural source. Mostly, the screening of natural herbs is presented by Minimum Inhibitory Concentration (MIC) [5]. As a rapid detection, MIC would be beneficial for comparing the strength of several prospective antibacterial agents. Usually, the comparison is made with the antibiotic concentration [5].

This study mainly discusses the screening process of several ingredients such as noni (*Morinda citrifolia*), garlic (*Allium sativum*), celery (*Apium graveolens*), galangal (*Alpinia galangal*), ginger (*Zingiber officinale*), yellow turmeric (*Curcuma longa*), lime (*Citrus aurantifolia*), papaya (*Carica papaya*) leaf, betel (*Piper betel*) leaf, and cutcherry (*Kaempferia galangal*) for prospectus antimicrobial agents. The selection of ingredients is mostly caused by the medical story behind the ingredients. Their availability is also the factor to be selected because of mass production if the ingredients are capable of antimicrobial agents. The study also used positive control using antibiotics and negative control without any addition to giving a comparison of the antimicrobial agent capability. So, the results were analyzed semi-qualitatively with a comparison with positive control. This study was conducted because to minimum data existed in Indonesia, mostly the natural components screening process conducted from another country.

2. Materials and Methods

2.1 Materials

Materials consisted of three types of substances, namely antimicrobial agents, bacteria colonies, and growth medium. Antimicrobial agents consisted of antibiotics as the positive control, and natural ingredients for the screening process of antimicrobial activity were studied, as shown in Table 1. Meanwhile, the bacteria used in this study can be seen in Table 2. The growth medium such as TSA (Trypticase Soy Agar) and MRSA (Methicillin-Resistant Staphylococcus Aureus) Merck was obtained from Nugen Bioscience Indonesia laboratory. All works were conducted in Nugen Bioscience Indonesia Laboratory, Jakarta.

Table 1. Natural ingredients as prospective antimicrobial agent

Plant species	Description	Origin
Morinda citrifolia	Juice	Jakarta, Indonesia
Allium sativum	Bulb	Traditional market, Jakarta, Indonesia
Apium graveolens	Leaves	Traditional market, Jakarta, Indonesia
Alpinia galangal	Rhizome	Traditional market, Jakarta, Indonesia
Zingiber officinale	Rhizome	Traditional market, Jakarta, Indonesia
Curcuma longa	Rhizome	Traditional market, Jakarta, Indonesia
Citrus aurantifolia	Juice	Traditional market, Jakarta, Indonesia
Carica papaya	Leaves	Traditional market, Jakarta, Indonesia
Piper betle	Leaves	Traditional market, Jakarta, Indonesia
Kaempferia galangal	Rhizome	Jakarta, Indonesia
Doxysol (2 g/L)	Antibiotic	Sumber Hidup Satwa -International
Comoxy 500 (2 g/L)	Antibiotic	Sumber Hidup Satwa-International
Amcol (2 g/L)	Antibiotic	Sumber Hidup Satwa-International

2.2 Natural ingredients extraction

Besides *Morinda citrifolia* and *Citrus aurantifolia*, which only were heated to sterilization, the extraction of natural ingredients was conducted with a Soxhlet extractor using distilled water as the solvent. The extraction was conducted at 70°C with Feed-Solvent Ratio (1:2) for 6 hours. The solution of the extraction would be used as the solution sample in the antimicrobial screening test.

2.3 Antimicrobial screening agent

The method was agar dilution method using Trypticase Soy Agar (TSA) growth media for gram-negative bacteria and Methicillin-Resistant Staphylococcus Aureus (MSTA) growth media for gram-positive bacteria which was adopted from Baker et al. (1991). The selection of bacteria shown in Table 2 was the occurrence in the hatchery. The study had been carried out the screening process of several ingredients from nature. The screening process was conducted in two steps. The first step was to evaluate the inhibitory activity of each ingredient. The last step was a deepened comparison of the promising antimicrobial agent. Positive control with the addition of antibiotics, namely Doxysol, Comoxy, and Amcol was also conducted in this study. Meanwhile, a negative control was conducted as a bacterial growth basis comparison with another treatment. Bacterial growth score was used on a scale of 0 – 4. This scale was used to determine the sufficient growth of bacteria despite the inhibition zone diameter. Thus, this study was referred to as a semi-qualitative study despite the qualitative study.

Table 2. Bacteria used in this study

Bacteria species	Code	Source
Salmonella spp.	4054-36P306* 3912-36P297*	Chicken yolk Chicken air sac
Escherichia coli	4014-36P304* 3958-36P301* 4098-36P308*	Chicken bone Chicken heart Chicken liver
Pseudomonas aeruginosa	4032-36P306* 3909-36P296*	Chicken lung Chicken navel
Lactobacillus plantarum	N1A1	Microbiology Department
Lactobacillus rhamonsus	MD4[b]	Microbiology Department
Enterococcus faecium	-	Microbiology Department
Pediococcus pentosaceus	-	Microbiology Department

3. Results and Discussions

The first screening process resulted in only noni, lime, and betel leaves were adequate to suppress the bacteria growth, as seen in Table 3. It is shown by less amount of numbers in Table 3 with a green column. Other substances are not strong enough to suppress bacteria growth, as shown by numbers 3 and 4 in the screening test. Hypothetically, negative control had prolific bacterial colonies. *Allium sativum*, *Apium graveolens*, and *Kaempferia galangal* had a higher growth score than the negative control. The occurrence of total sugar and carbohydrates in those natural substances was believed to support bacterial growth. *Allium sativum*, *Apium graveolens*, and *Kaempferia galangal* have a total sugar content of as much as 14.8 mg/g [7], 338.8 mg/g [8], and 19.0 mg/g [9], respectively.

Meanwhile, positive control is mostly very effective as expected, but Comoxy and Amcol seem to lose against *Salmonella spp.*, which was isolated from the chicken yolk. It is, believed, caused by bacterial resistance against the antibiotic, which has become a recent issue in the hatchery.

The successive components, as antimicrobial agents at the first screening, were hereafter evaluated to find the optimum condition. As seen in Table 4, lime seemed to be the most potent antimicrobial agent compared to others with a lower concentration, which could be achieved to suppress microbial growth at 10%. This condition would be the optimum condition for this study. This concentration seemed could be optimized further to find concentration economically in the application. Meanwhile, noni was sufficient to be used at 50% concentration with the growth media, and piper betel was effective at 40% concentration with the growth media.

The mapping of this study can be seen in Table 5. Another study was also presented to give additional information on tropical natural resources, which could be used as an antimicrobial agent. Microscopic-scale research should be conducted in the future to give a perspective on responsible substances in lime. Meanwhile, another abundance of natural sources in Indonesia should be conceivably evaluated macroscopically to give a large map of Indonesia's natural source potential as microbial agents.

The initial study strongly is believed very useful for another implementation and application in both industry and society. The dense map of Indonesia's natural sources could enhance the possibility of antimicrobial products. Otherwise, natural sources that do not have potential could not be studied further to minimize the research sustainability. The application of each natural resource should be matched with the nature of its.

Further study needs to be conducted to find the chemical substances that take responsibility as an antimicrobial agent with the microscopic study. Meanwhile, the optimum condition was obtained with the addition of 1:10 lime to the growth media. This research is beneficial in a broad application such as food ingredients, natural medicine for both human beings and animal husbandry, and antimicrobial application addition in natural dyes.

Table 3. Bacterial growth in every treatment

Treatment	Extract in Growth Media	*[a]Salmonella spp.*		*[a]Escherichia coli*			*[a]Pseudomonas aeruginosa*		*Lactobacillus Plantarum*	*Lactobacillus rhamonsus*	*Enterococcus faecium*	*Pediococcus pentosaceus*
		(-)		(-)			(-)		(+)	(+)	(+)	(+)
		TSA							MRSA			
NC	-	3	3	3	3	3	3	3	3	3	3	3
NO	50%	0	0	0	0	0	2	2	0	0	0	0
GL	50%	4	3	3	3	3	3	3	2	1	0	0
CE	50%	4	3	3	3	3	4	4	3	3	3	3
GA	50%	2	1	3	3	3	3	3	0	2	2	2
GI	50%	3	3	3	3	3	3	3	3	3	3	3
YT	50%	3	3	3	3	3	3	3	3	3	3	3
LI	50%	0	0	0	0	0	0	0	0	0	0	0
PL	50%	3	3	3	3	3	3	3	3	3	3	3
BL	50%	0	0	0	0	0	0	0	0	1	0	1
CU	50%	4	3	3	3	3	4	4	2	2	2	2
DO	50%	0	0	0	0	0	0	0	0	0.5	0	0
CO	50%	3	0	0	0	0	0	0	0	0.5	0	0
AM	50%	3	0	0	0	0	0	0	0	0.5	0	0

4	extensive growth
3	good growth
2	moderate growth
1	weak growth
0	no growth

[a] bacterial code followed Table 2; **NC**: Negative Control; **NO**: Noni (Morinda citrifolia); **GL**: garlic (Allium sativum); **CE**: celery (Apium graveolens); **GA**: galangal (Alpinia galangal); **GI**: ginger (Zingiber officinale); **YT**: yellow turmeric (Curcuma longa); **LI**: lime (Citrus aurantifolia); **PA**: papaya (Carica papaya) leaf; **BL**: betel (Piper betle) leaf, **CU**: cutcherry (Kaempferia galangal); **DO**: Doxysol; **CO**: Comoxy; and **AM**: Amcol.

Table 4. Bacterial growth for prospectus antimicrobial agent comparison and optimization

Treatment	Extract in Growth Media	[a]*Salmonella spp.*		[a]*Escherichia coli*			[a]*Pseudomonas aeruginosa*		*Lactobacillus Plantarum*	*Lactobacillus rhamonsus*	*Enterococcus faecium*	*Pediococcus pentosaceus*
		(-)		(-)			(-)		(+)	(+)	(+)	(+)
		TSA							MRSA			
NO	50%	1	0	0	0	0	0	0	1	1	1	1
	40%	1	1	0	0	0	1	1	1	1	1	1
	30%	2	1	2	2	2	4	4	2	2	2	2
	20%	3	2	3	3	3	4	4	3	3	3	3
	10%	3	2	3	3	3	4	4	4	4	4	4
LI	50%	0	0	0	0	0	0	0	0	0	0	0
	40%	0	0	0	0	0	0	0	0	0	0	0
	30%	0	0	0	0	0	0	0	0	0	0	0
	20%	0	0	0	0	0	0	0	0	1	0	0
	10%	0	0	0	0	0	0	0	2	2	1	0
BL	50%	0	0	0	0	0	0	0	2	2	2	2
	40%	0	0	0	0	0	0	0	3	3	3	3
	30%	2	2	3	3	2	2	2	3	3	3	3
	20%	3	3	3	3	3	3	3	4	4	4	4
	10%	4	4	4	4	4	4	4	4	4	4	4

4	extensive growth
3	good growth
2	moderate growth
1	weak growth
0	no growth

[a] bacterial code followed Table 2; **NO**: Noni (Morinda citrifolia); **LI**: lime (Citrus aurantifolia); and **BL**: betel (Piper betle) leaf.

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Table 5. Recent work and references for tropical antimicrobial agents

Common name	Scientific name	Compound	Class	Activity	Relative toxicity [a]	Bacteria growth [b]	Reference(s) [c]
Aloe	*Aloe vera*	Latex	Complex mixture	Corynebacterium, Salmonella, Streptococcus, S. aureus	2.7		[10]
Betel pepper	*Piper betel*	Catechols, eugenol	Essential oils	General	1.0	0.0 & 0.5	[3], TS
Chili, peppers, paprika	*Capsicum annuum*	Capsaicin	Terpenoid	Bacteria	2.0		[11–13]
Clove	*Syzygium aromaticum*		Terpenoid	General	1.7		[3]
Garlic	*Allium sativum*	Allicin, ajoene	Sulfoxide, Sulfated terpenoids	General	-		[14–17],TS
Ginseng	*Panax notoginseng*		Saponins	E. coli, Sporothrix schenckii, Staphylococcus, Trichophyton	2.7	3.1 & 0.8	[3]
Mountain tobacco	*Arnica montana*	Helanins	Lactones	General	2		[3]
Papaya	*Carica papaya*	Latex	Mix of terpenoids, organic acids, alkaloids	General	3		[18–20], TS
Noni	*Morinda citrifolia*	-	-	General	-	3.0 & 3.0	TS
celery	*Apium graveolens*	-	-	Bacteria	-		TS
galangal	*Alpinia galangal*	-	-	Bacteria	-	0.6 & 0.0	TS
ginger	*Zingiber officinale*	-	-	Bacteria	-	3.4 & 3.0	TS
yellow turmeric	*Curcuma longa*	-	-	Bacteria	-	2.6 & 1.5	TS
lime	*Citrus aurantifolia*	-	-	Bacteria	-	3.0 & 3.0	TS
Cutcherry	*Kaempferia galangal*	-	-	Bacteria	-	3.0 & 3.0	TS

[a]Data from reference Duke (2002); [b]gram-negative & gram-positive (scale 0 – 4); [c]Table is based on data compiled from references [21–22]; TS: This Study

4. Conclusion

The screening process of several ingredients as an antimicrobial agent has been successfully conducted. Most of the ingredients did not give a glance to the antimicrobial activity. Only noni, lime, and betel extract showed inhibitory properties against bacteria, both gram-negative and positive in other words; these three materials could be antimicrobial agents derived from nature. Lime, betel leaf extract, and noni are the strongest components with the order higher level, respectively.

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