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## Potential of Antibacterial Activity in Natural Ingredients (Betel Leaves, Basil Leaves, Avocado Seeds, Lemongrass, Garlic) As Natural Hand Sanitizers: Journal Review

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### ABSTRACT

*In an effort to reduce the use of alcohol-based hand sanitizers because of their quite dangerous impact on the skin, natural hand sanitizers are an alternative. The antibacterial substances in betel leaves, basil leaves, avocado seeds, lemongrass, and garlic can be used as materials for natural hand sanitizers. The purpose of this study was to collect, identify, and compare journals about the effectiveness of antibacterial agents in natural ingredients that are used as natural hand sanitizers. Using the journal review method through reviewing the contents of journals that discuss an antibacterial activity, the results show that the five phytochemical compounds contain active antibacterial compounds so that they have the potential to make natural hand sanitizers, as well as the antibacterial inhibition zone of these five ingredients showing high results with strong to very strong categories. It was concluded that the five ingredients could be used as natural hand sanitizer materials. However, betel leaves are preferred because of their largest inhibition zone, 3.1-4.0 cm.*

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### 1. Introduction

During the COVID-19 pandemic, everyone is required to maintain cleanliness by using hand sanitizer as a substitute for soap at certain times. Hand sanitizers in the market are still predominantly made from alcohol. 70% alcohol and 0.05% triclosan formulation is the most optimal in its manufacture. This is evidenced by its inhibitory effect on the growth of *S. aureus* bacteria which reached 72.45% for 30 seconds. The inhibition of bacterial growth in alcohol is due to the active substance that acts as an antiseptic. This inhibitory effect goes hand in hand with the higher the alcohol concentration, but this high alcohol concentration has a harmful impact on the skin. One of them can cause dryness of the skin so that microbes can easily stick to it [1].

Natural ingredients are highly recommended to reduce the use of hand sanitizers made from alcohol, especially those with high concentrations. In addition to its abundant and safe ingredients, natural ingredients also contain active antibacterial substances applied as hand sanitizers. Plants classified as antibacterial compounds are terpenoid, flavonoid, saponin, alkaloid, tannin, and phenol [1]. Some of these compounds are present in betel leaves [2], basil leaves [3], avocado seeds [1], lemongrass [4], and garlic [5]. Research [6] showed that eugenol compounds in methanol extract of betel leaves could inhibit the growth of gram-positive bacteria: *Bacillus sp*; *E. Faecalis*; *S. aureus*; *S. agalactiae* and gram-negative bacteria: *A. hyrophila*; *E. coli*; *K. pneumoniae*; *P. aeruginosa*; *V. alginolyticus* with an inhibition zone of 0.5-2.0 cm. In 200 mg/ml of ethanolic extract of basil leaves, *S. aureus* and *E. coli* can be inhibited with inhibition zones of 21 mm against *E. coli* and 16

mm against *S.aureus* [3]. Then, the antibacterial properties of the essential oil of avocado stems and seeds showed that *S. aureus* and *E. coli* growth could be inhibited with an inhibition zone of 20 mm against *E. coli* and 6 mm against *S.aureus* [7].

The purpose of this study is to collect, identify, and compare journals on the effectiveness of antibacterial on several natural ingredients, including betel leaves, basil leaves, avocado seed, lemongrass, and garlic which have the potential to be used as natural hand sanitizers.

## 2. Methods

### 2.1. Research Design

This research was designed in a literature study by collecting, identifying, and comparing related research journals. The research object is the antibacterial activity of betel leaves extract, basil leaves, avocado seeds, lemongrass, and garlic. The research subject is the comparison of antibacterial effectiveness.

### 2.2. Research Methods

Journal searches were done using Google Scholar, PubMed, and BioMed databases based on related keywords, namely antibacterial in natural ingredients (betel leaves/basil leaves/avocado seed) and natural hand sanitizer. Selection or retrieval of source journals is based on the following conditions: the journal year ranges from 2015-2020 in national and international journals. The source journal contains data on the antibacterial activity of the research object. The data were analyzed in the form of antibacterial and phytochemical activity on the research object.

## 3. Result and Discussion

### 3.1. Natural Hand Sanitizer

Hand sanitizer is a substitute for soap to clean hands when outside the home. Hand sanitizer during the pandemic is very much needed. However, the raw material for hand sanitizer on the market still uses alcohol, which is limited in existence during this pandemic. The effects of alcohol are quite dangerous on the skin if used continuously.

Abundant natural ingredients that are environmentally friendly are a solution to the limited alcohol during this pandemic as a raw material for hand sanitizers because natural ingredients contain lots of phytochemical compounds classified as antibacterial, such as phenols and alkaloids, saponins, flavonoids, tannins, and terpenoids [8,9]. In addition to functioning as antibacterial, flavonoids also act as anti-inflammatory, antibiotic, and other drugs [10]. According to research [11], the inhibition of 15 mm against *Streptococcus sp* and *Staphylococcus sp* bacteria indicates the inhibitory ability of natural hand sanitizers can be comparable to the inhibitory power of commercial hand sanitizers.

### 3.2. Extraction Method and Bacterial Inhibition

From the literature review on the Google Scholar, PubMed, and BioMed databases based on the keyword antibacterial on natural ingredients, there are references to  $\pm 7000$  articles of international journals and  $\pm 700$  articles of national journals. These results fulfill the first requirement for selecting the source journal because it includes the year range of the article during the search process, namely from 2015 to 2020. Then the search is narrowed through other conditions that have been determined. The journals under study discuss the antibacterial activity of each natural ingredient in this study, indicated by the inhibition zone data in most journals and several other antibacterial test data. A summary of the journal review in the extraction method and the results obtained are presented in table 1.

**Table 1.** The extraction method and test results

Method	Result	Reference
<ul style="list-style-type: none"> <li>Ethanol extraction of betel leaves</li> <li>Phytochemical Screening</li> <li>In Vitro Antibacterial Test:</li> </ul>	Antibacterial test on green betel leaves extract with a concentration of 5% ethanol solvent was in line with the results of the LSD test and antibacterial test on <i>Staphylococcus aureus</i> bacteria in vitro	[12]

Method	Result	Reference
Microdilution		
<ul style="list-style-type: none"> <li>LSD test</li> </ul>		
<ul style="list-style-type: none"> <li>Betel leaves extraction with 95% Ethanol</li> <li>Phytochemical Analysis</li> <li>Observation of Candida Albicans. inhibitory activity</li> </ul>	From the results of the Candida Albicans inhibition test, betel leaves extraction has stronger antibacterial activity than liquid antiseptics	[13]
<ul style="list-style-type: none"> <li>Betel leaves extraction</li> <li>Antioxidant activity test for NO radicals</li> <li>Antioxidant activity test for ferrous ion RPA</li> </ul>	The results of the antioxidant activity test on betel leaves extraction showed less antioxidant activity than eugenol	[14]
<ul style="list-style-type: none"> <li>Extraction of basil leaves with 96% ethanol solvent</li> <li>Adhesion test</li> <li>Spreadability test</li> <li>Stability test</li> <li>Antibacterial activity test</li> </ul>	This test used the concentration of Sodium-Carboxymethyle Cellulose as a gel agent, increasing the concentration of the gel; if the concentration increases, the adhesion will take longer, and vice versa if the concentration decreases, the adhesion will be smaller, and the antibacterial activity will decrease. The adhesion test for four weeks resulted in $3.21 \pm 0.22$ , the stability test for the size of the diameter of the globules resulted in the greatest 3.93 m, and the antibacterial activity test resulted in the greatest inhibition zone diameter of $25.45 \pm 0.42$ mm.	[15]
<ul style="list-style-type: none"> <li>Basil leaves extraction</li> <li>Antibacterial activity test by good method</li> </ul>	Extraction of basil leaves was made in various concentrations of 20%, 40%, 60%, 80%, and 100% with the average inhibition zone diameter for each concentration was 6; 6.68; 8.17; 9.80; 12.60 mm	[16]
<ul style="list-style-type: none"> <li>Basil leaves extraction</li> <li>Antibacterial activity test</li> </ul>	This study tested the antibacterial activity using the <i>Enterococcus faecalis</i> ATCC 29212 bacterium using four concentrations, namely 80%, 60%, 40%, and 20%. The most effective concentration of basil leaves is 80%, with an inhibitory zone of 16.7 mm	[17]
<ul style="list-style-type: none"> <li>Extraction using a soxhlet extractor</li> <li>Inhibition ability test</li> <li>Test gel formula</li> </ul>	Terpenoid and flavonoid compounds function to inhibit <i>E.coli</i> bacteria without causing harmful effects on the skin	[8]
<ul style="list-style-type: none"> <li>Extraction using maceration method</li> <li>Paper disc diffusion test</li> </ul>	Ethanol and dichloromethane solvents showed a low spectrum on <i>Streptococcus agalactiae</i> bacteria with 7-9.5 mm (ethanol) and 10.93 mm (dichloromethane) inhibition, respectively.	[18]
<ul style="list-style-type: none"> <li>Extraction using maceration method</li> <li>Paper disc diffusion test</li> <li>Phytochemical Analysis</li> </ul>	Avocado stems and seeds produced inhibition of 12 mm against <i>E.coli</i> and 14 mm against <i>S. aureus</i> using n-hexane as solvent.	[19]
<ul style="list-style-type: none"> <li>Extraction using maceration method</li> <li>Phytochemical analysis using Harabone and Colorimetric methods</li> <li>Paper disc diffusion test.</li> </ul>	From the extraction of avocado seeds, the inhibitory power of 11-20 mm against <i>P. mirabilis</i> , <i>S.aureus</i> , and <i>P. aeruginosa</i> bacteria and against <i>Candida albican</i> was $32 \pm 0.14$ mm. This inhibition is quite strong.	[9]
<ul style="list-style-type: none"> <li>Extraction using maceration method</li> <li>Phytochemical analysis</li> <li>Proximate test</li> <li>Mineral content test</li> </ul>	Avocado leaves contain high phytochemical compounds, while the seeds contain high protein compounds and minerals Fe and Zn.	[20]
<ul style="list-style-type: none"> <li>Extract lemongrass with hot water</li> <li>Antibacterial effectiveness test</li> <li>Phytochemical and proximate analysis</li> <li>Cytogenotoxicological analysis</li> <li>Statistic analysis</li> </ul>	Lemongrass extract was more effective against <i>Staphylococcus saprophyticus</i> ( $21.33 \pm 1.20$ mm) and <i>E.coli</i> ( $4.0 \pm 1.15$ mm), <i>Proteus mirabilis</i> ( $3.33 \pm 0.88$ mm), <i>Klebsiella pneumoniae</i> ( $13.00 \pm 1.15$ mm).	[4]
<ul style="list-style-type: none"> <li>Extraction</li> </ul>	Lemongrass extract was more effective against <i>S.typhi</i>	[21]

Method	Result	Reference
<ul style="list-style-type: none"> <li>Antibacterial effectiveness test</li> <li>Phytochemical analysis</li> </ul>	bacteria with a moderate inhibition zone (10 mm) using methanol as a solvent.	
<ul style="list-style-type: none"> <li>Extraction</li> <li>Antibacterial effectiveness test</li> <li>Phytochemical analysis</li> </ul>	The essential oil obtained from lemongrass contains phytochemicals that can inhibit the growth of pathogenic bacteria, especially <i>E.coli</i> and <i>S.Aureus</i> bacteria, with an average inhibitory power of 9.30 mm ( <i>E.coli</i> bacteria) and 10.6 mm ( <i>S.coli</i> bacteria). Aureus).	[22]
<ul style="list-style-type: none"> <li>Extraction of garlic using distilled water and methanol</li> <li>Antibacterial effectiveness test</li> <li>Phytochemical analysis</li> </ul>	The use of methanol as a solvent to inhibit the activity of pathogenic bacteria is more efficient than aquadest	[5]
<ul style="list-style-type: none"> <li>Extraction</li> <li>Microorganism test</li> <li>Antibacterial activity</li> </ul>	There were differences in the antibacterial effects of the types of garlic and each of its extracts. The solvent of distilled water was stronger than methanol, with various inhibition zones ranging from a diameter of 9.6 to 23.7 mm	[23]
<ul style="list-style-type: none"> <li>Extraction</li> <li>Phytochemical screening</li> <li>Inductively coupled ICPOES</li> <li>Fourier transform infrared spectrophotometer (FTIR)</li> </ul>	Phytochemical screening of garlic extract with hexane, ethyl acetate, methanol, and water as solvents showed the presence of steroids, saponins, alkaloids, flavonoids, glycosides, phenolic compounds, terpenoids, and carbohydrates.	[24]

Table 1 shows the extraction method used to obtain active compounds that are potentially antibacterial substances, namely extraction with a soxhlet extractor and extraction with the maceration method. The solvents used were distilled water, ethanol, methanol, hexane, and ethyl acetate. Phytochemical analysis showed that using different solvents resulted in extracts with different content of active substances. Each extract has a different antibacterial effect. Extracts from several natural ingredients effectively inhibited the growth rate of bacteria with different inhibitory powers, with moderate to very strong inhibition zone variations. The use of extracts of natural ingredients with a greater concentration will increase the value of the inhibition zone.

### 3.3. Phytochemical Analysis

The phytochemical analysis identifies active compounds that have the potential as antibacterial substances in natural extracts [25]. A literature review of several journals (listed in Table 2) found that some of the phytochemical content contained in betel leaves, basil leaves, avocado seed, lemongrass, and garlic [1,4,5,12,17].

**Table 2.** Phytochemical test results

Natural Ingredients	Sample Test	Solvent	Result
Betel leaves	Alkaloids	Ethanol	+
	Flavonoids	Ethanol	+
	Saponins	Ethanol	+
	Tannin	Ethanol	+
Basil leaves	Alkaloids	Mayer + HCl	+
	Flavonoids	Amyl alcohol + Mg + HCl	+
	Saponins	Air + HCl	+
	Quinone	NaOH	-
	Tannins	FeCl <sub>3</sub> 1%	+
	Steroid/ Triterpnoid	H <sub>2</sub> SO <sub>4</sub> + As.asetat	+
Avocado Seeds	Alkaloids	Methanol	+
	Flavonoids	Methanol	+
	Saponins	Methanol	+
	Phenol	Methanol	-
	Tannins	Methanol	+
	Steroids/Triterpenoids	Methanol	+
Lemongrass	Akaloids	Alcohol	-
	Glycoside	Alcohol	+
	Flavonoids	Alcohol	+
	Steroids	Alcohol	-
	Triterpenoid	Alcohol	-



Natural Ingredients	Sample Test	Solvent	Result
Garlic	Tannins	Alcohol	+
	Saponins	Aquadest	+
	Flavonoids	Aquadest	+
	Carbohydrate	Aquadest	+
	Phenol	Aquadest	-
	Tannins	Aquadest	-
	Steroids	Aquadest	+
	Anthraquinone	Aquadest	-
	Terpenoids	Aquadest	+
	Alkaloids	Aquadest	+
Description :			
+ contains the compound tested			
- does not contain the compound tested			

Table 2 proves that betel leaves, basil leaves, avocado seeds, lemongrass, and garlic contain phytochemical compounds in alkaloids, flavonoids, saponins, phenols, tannins, and terpenoids. The content of flavonoids has an antibacterial effect against *E. coli*, *S. aureus*, and *K. pneumonia* [15]. These phytochemical compounds are needed to make natural hand sanitizers [8,9].

### 3.4. Antibacterial Activity

One of the antibacterial tests is the inhibition zone test. Solvents play an important role in antibacterial testing [26]. The zone of inhibition was measured in cm or mm, with categories: weak with inhibition zone (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm) [27]. The following presents the antibacterial activity of several natural ingredients.

#### a. Betel Leaf

Betel leaf contains active antibacterial substances that inhibit the growth of pathogenic bacteria. The following are data on antibacterial activity on betel leaves with various solvents.

**Table 3.** Leaves Extract Antibacterial Activity

Solvent	Bacteria	Gol. Bacteria	Obstacles	Category	Reference
Methanol	<i>Basil sp.</i>	(+) gram positive	3.1-4.0 cm	Very strong	[6]
	<i>E. faecalis</i>		3.1-4.0 cm		
	<i>S. aureus</i>		1.1-2.0 cm		
	<i>S. agalactiae</i>		1.1-2.0 cm		
	<i>A. hydrophila</i>	(-) gram negative	1.1-2.0 cm	Very strong	
	<i>E. coli</i>		2.1-3.0 cm		
	<i>K. pneumoniae</i>		2.1-3.0 cm		
	<i>P. aeruginosa</i>		2.1-3.0 cm		
Ethanol	<i>V. alginolyticus</i>	(+) gram positive	3.1-4.0 cm	Strong	[28]
	<i>S. aureus</i>		16 mm		

Table 3 shows that the betel leaves extract with two solvents has a good inhibition zone. The inhibition zone of ethanol is in a strong category, while methanol is very strong against various gram-positive and negative bacteria. Betel leaves contain high inhibitory power antibacterial compounds hydroxycaviol and eugenol [6]. Eugenol itself is a phenolic substance that inhibits bacterial growth, and eugenol is also included in the aromatic substances usually found in cloves and betel leaves [29]. The number of substances contained in the leaves causes the betel leaves to have the potential to be used as a natural hand sanitizer.

#### b. Basil Leaf

This leaf is not only useful as a complement to dishes; basil leaf also contains antibacterial compounds that are useful in inhibiting bacterial growth. The antibacterial activity of basil leaf is listed in table 4.

**Table 4.** Antibacterial Activity Basil Leaf Extract

Solvent	Bacteria	Gol. Bacteria	Obstacles zone	Category	Reference
Alcohol	<i>S. aureus</i> ATCC 2592	(+) gram positive	12.60 mm	Strong	[15]
Ethanol	<i>Streptococcus mutans</i>	(+) gram positive	10.26 mm	Strong	[16]
Ethanol	<i>S. aureus</i>	(+) gram positive	18.90 mm	Strong	[8]
	<i>E. coli</i>	(-) gram negative	10.26 mm	Strong	
Ethanol	<i>E. coli</i>	(-) gram negative	9.91-14.85 mm	Strong	[30]
	<i>B. cereus</i>	(+) gram positive	9.91-14.85 mm	Strong	

Although all solvents showed a strong category, ethanol solvent showed the highest yield of basil leaf extract against *S. aureus* bacteria at 18.90 mm.

### c. Avocado Seeds

Although classified as waste, avocado seeds are rich in benefits. Avocado seeds are commonly used as antidiabetic drugs [31]. Another benefit of avocado seeds is their antibacterial content in inhibiting pathogenic bacteria. The following is the antibacterial activity of avocado seeds through their inhibition zone.

**Table 5.** Antibacterial Activity of Avocado Seed Extract

Solvent	Bacteria	Bacteria Class	Obstacles zone	Category	Reference
Ethanol	<i>Enterobacter aerogenes</i>	(-) gram negative	14 mm	Strong	[23]
	<i>Klebsiella sp</i>	(-) gram negative	14 mm	Strong	
Ethyl Acetate	<i>Vibrio cholera</i>	(-) gram negative	3.23 mm	Weak	[21]
Methanol	<i>S. aureus</i>	(+) gram positive	7.22 mm	Medium	[32]
N- Heksana	<i>E. coli</i>	(-) gram negative	7.53 mm	Medium	

Ethanol solvent showed the highest yield of other solvents against *Enterobacter aerogenes* and *Klebsiella sp* bacteria with an inhibition zone of 14 mm each, exceeding the previously larger methanol solvent in Table 5.

### d. Lemongrass

In addition to being abundant and easy to obtain, lemongrass also has the potential as a hand sanitizer with antibacterial properties. The following is lemongrass's antibacterial activity, which is presented through its inhibition zone.

**Table 6.** Lemongrass Extract Antibacterial Activity

Solvent	Bacteria	Group of Bacteria	Obstacles zone	Category	Reference
Acetone	<i>E. coli</i>	(-) gram negative	7 mm	Medium	[21]
	<i>S. typhi</i>	(-) gram negative	10 mm	Medium	
	<i>Shigella sp.</i>	(-) gram negative	9 mm	Medium	
	<i>S. aureus</i>	(+) gram positive	8 mm	Medium	
Acetonitrile	<i>E. coli</i>	(-) gram negative	9.30 mm	Medium	[22]
	<i>S. enteritidis</i>	(-) gram negative	10.02 mm	Medium	
	<i>S. aureus</i>	(+) gram positive	10.60 mm	Medium	

### e. Garlic

Garlic tends to be used as a spice in cooking. However, its antibacterial content is quite promising in inhibiting the growth of pathogenic bacteria. The following is the antibacterial activity of garlic.

**Table 7.** Garlic Extract Antibacterial Activity

Solvent	Bacteria	Group of Bacteria	Obstacles zone	Category	Reference
Methanol	<i>S. aureus</i>	(+) gram positive	16.0 mm	Strong	[23]
	<i>P.aeruginosa</i>	(-) gram negative	12.0 mm	Strong	
	<i>E.coli</i>	(-) gram negative	16.7 mm	Strong	
	<i>S. typhi</i>	(-) gram negative	23.7 mm	Very Strong	
Ethanol	<i>S. aureus</i>	(+) gram positive	7.6 mm	Medium	
	<i>P.aeruginosa</i>	(-) gram negative	11.5 mm	Strong	
	<i>E.coli</i>	(-) gram negative	8.4 mm	Medium	

Bacteria that are often found on human hands vary depending on their activity. Angga et al. [33] stated that the bacteria on the hands of nurses at Ulin Hospital were *S. aureus*, *S. epidermidis*, *E. coli*, and *Bacillus sp.* Tests conducted on food vendors identified five types of bacteria, namely *E. coli* (20%), *Klebsiella spp.* (28%), *Proteus spp.* (8%), *S. aureus* (20%), and *S. epidermidis* (24%) [34]. Tables 4, 5, 6, and 7 show extracts from betel leaves, basil leaves, avocado seeds, lemongrass, and garlic extracted with various solvents capable of inhibiting several bacteria, including *S. aureus*, *E. coli*, *Klebsiella spp.* The antibacterial activity of lemongrass was the weakest, while the antibacterial activity of betel leaves was the strongest. Cicaningsih and Cahyono [35] compared the reduction in germ numbers with a natural hand sanitizer containing basil leaves extract and aloe vera gel with a hand sanitizer from the market. Natural hand sanitizers can reduce the number of germs on the hands by 69.60%, while market hand sanitizers reduce the number of germs by 53.19%. Hand sanitizers with active ingredients from betel leaves extract are more effective at inhibiting *S. aureus* bacteria than hand sanitizers from the market. The organoleptic test stated that both types of hand sanitizer, natural hand sanitizer and hand sanitizer from the market, had less than 1 minute drying time and were not sticky on the hands [36].

#### 4. Conclusion

From the studies that have been carried out, it can be concluded that natural ingredients in the form of betel leaves, basil leaves, avocado seeds, lemongrass, and garlic have the potential as ingredients for making natural hand sanitizers due to the antibacterial substances contained in them. Hand sanitizer from natural ingredients is more effective at reducing the number of germs on the hands than hand sanitizer from the market. From the inhibition zone data, it can be concluded that betel leaves are superior and have more potential to make hand sanitizers because the high inhibition zone is even categorized as very strong at 3.1-4.0 cm. Apart from the inhibition zone data, betel leaves are easy to obtain, abundant, and cheap. Other ingredients tend to be more useful in several other aspects, such as basil leaves being used as food additives, lemongrass, and garlic cooking spices.

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