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## IN VITRO TESTING OF CLOVE EXTRACTS IN INHIBITING AND KILLING *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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### ABSTRACT

**Background:** Diarrhea that could be caused by *Staphylococcus aureus* and *Escherichia coli* can be prevented by increasing hand hygiene using alcohol-based hand rub, but frequent use might cause skin irritation. Replacing alcohol with herbs could avoid this side effect. Cloves have been proved to have antibacterial properties. However, most researchers used complex extraction methods that might not be applicable on a household scale.

**Objective:** To prove that clove extracts have ability to inhibit and to kill *S. aureus* and *E. coli*. **Methods:** This is an experimental study by using post-test only control group design. Clove extract was obtained with simple aqueous maceration. Samples were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 allocated into six groups: clove extracts at concentrations of 12.5%, 25%, 50%, 100%; 70% alcohol as a positive control; aquadest as a negative control. Each group was given 5 repetitions of intervention. MIC was measured with dilution method, while MBC with streak method. Negative controls were used to monitor the quality of work. **Results:** MIC of *S. aureus* was started at 50% concentration of clove extract, while MBC of *S. aureus* was started at 25 % concentration of the same extract. MIC and MBC of *E. coli* was both started at 25% concentration of clove extract. MIC and MBC of 100% concentration of clove extract were equal with 70% alcohol. **Conclusion:** Concentration of 100% clove extracts have similar ability with 70% alcohol to inhibit and to kill *S. aureus* and *E. coli*.

**Keywords:** Clove Extracts, *Escherichia coli*, MBC, MIC, *Staphylococcus aureus*

### INTRODUCTION

Diarrhea in Indonesia is still the main cause of death in children under 5 years old.<sup>1</sup> According to Baseline Health Research 2018, the prevalence of diarrhea in Indonesia for the past five years has increased from 4.5% to 6.8%. Until now, only a few cases of national diarrhea have been treated.<sup>2</sup> *Staphylococcus aureus* and *Escherichia coli* are transient flora found in human hands and can cause diarrhea by fecal-oral transmission.<sup>3</sup> This could be prevented by maintaining good hand hygiene. Hand-rubbing with antiseptic is a method that is often done by the community due to its practical implementation. Alcohol based hand rub can contain 60% to 95% of alcohol. This high concentration can cause dryness and irritation of the skin.<sup>4-6</sup> Alcohol is also dangerous when applied to wounds or skin laceration because it can increase the risk of injury severity.<sup>7</sup> Changing the base of antiseptic to herbs could avoid this side effect. Cloves (*Syzygium aromaticum*) are used in this study as the base of antiseptic because it has been proved to have antibacterial properties.

Besides, cloves are easy to obtain, affordable, and suitable for application on a household scale.

Antibacterial properties that are found in cloves are flavonoid, tannin, alkaloid, and eugenol.<sup>8</sup> The antibacterial activity of cloves has been quite extensively studied and tested for its effects on various bacteria that colonize the skin. However, previous researches used complex method to obtain clove extracts, such as by using essential oil and ethanolic extraction, so it would be difficult to be applied in the household.

Based on this background, it is necessary to further investigate the antibacterial activity of clove extracts that are obtained by a simple extraction method that is thought to be able to inhibit the growth of *S. aureus* and *E. coli*. The outcome of this study is to prove that clove extracts have the ability to inhibit and kill *S. aureus* and *E. coli*. This study also would like to provide information about Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal



Concentration (MBC) of clove extracts against *S. aureus* and *E. coli*.

## METHOD

### Research Design and Study Variables

This is an experimental study with post-test only control group design. It was held in the Microbiology Laboratory, Faculty of Medicine, Diponegoro University from May to June 2019. The dependent variable of this research was the growth of *S. aureus* and *E. coli*. While the independent variable of this research was the concentration of clove extracts.

### Samples

Samples used in this research were *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. In this research, samples were divided into 6 groups: clove extracts at concentrations of 12.5%, 25%, 50%, 100%; a positive control (70% alcohol); and aquadest as a negative control. Based on the calculation of sample size, the repetition needed for each group was 5 times. Negative controls were only used to monitor the quality of work.

### Materials

Materials that were used in this research were cloves, aquabidest, 70% alcohol, 0.9% sodium chloride, Mueller Hinton Agar (MHA), Blood Agar (BA), Mac Conkey Agar (MCA), aquadest.

### Procedure

Clove extracts were prepared by grinding cloves into powder (5 g) and dissolving it in aquabidest (50 ml) and left for 24 hours at 5°C.<sup>9</sup> Extracts were filtered by filter paper to obtain clear infusion. Infusion then stored at 5°C. Extracts were subjected to various chemical tests for phytochemical constituents.

Various concentrations of clove extracts (12.5%, 25%, 50%, and 100%) were obtained by serial dilution. Concentration of 100% clove extract represents 5 g of cloves powder in 50 ml aquabidest or equals to 100 mg/mL. Positive control tube was filled with 70% alcohol (1 ml) and negative control tube was filled with aquadest (1 ml). Bacterial suspensions of *S. aureus* and *E. coli* were obtained by homogenized bacterial colony into 0.9% sodium chloride (5 ml) using vortex mixer. Then the bacterial suspension turbidity was adjusted to a McFarland 0.5 standard. One ml of bacterial suspension was

added to each intervention and control tube, then incubated at 37°C for 24 hours.

MIC was determined by observing clarity of the intervention tubes after incubation and was defined as the lowest concentration of clove extracts that produced similar clarity with a positive control. MBC was obtained by streaking preparations on MHA and then incubated at 37°C for 24 hours. MBC was defined as the lowest concentration of clove extracts where no colony found on the media.

### Ethical Approval

Prior to data collection, ethical approval was obtained from the Medical Research Ethical Committee of Kariadi. The Ethical Clearance of this research was No. 41/EC/H/KEPK/FK-UNDIP/V/2019.

## RESULT

### Phytochemical Testing of Clove Extracts

**Table 1.** Phytochemical Testing of Clove Extracts

Parameter	Result
Flavonoid	+ (Positive)
Alkaloid	+ (Positive)
Tannin	+ (Positive)

Clove extract at concentration of 100% was subjected to various chemical tests for phytochemical constituents with positive result for flavonoid, alkaloid, and tannin.

### MIC and MBC Testing of Clove Extracts against *S. aureus* and *E. coli*

The MIC testing with *S. aureus* showed that clove extracts at concentrations of 12.5% and 25% did not show clear results, which meant that bacterial growth could not be inhibited at this concentration. Clove extract at concentration of 50% showed clear results only on 2 repetitions, whereas at 100% concentration bacterial growth was inhibited on all repetitions.



**Table 2.** MIC and MBC Testing of Clove Extracts (CE) against *S. aureus* and *E. coli*

Group	N	<i>S. aureus</i>				<i>E. coli</i>			
		MIC		MBC		MIC		MBC	
		Clear	Turbid	Sterile	Not Sterile	Clear	Turbid	Sterile	Not Sterile
Alcohol 70%	5	5	0	5	0	5	0	5	0
CE Concentration 12.5%	5	0	5	0	5	0	5	0	5
CE Concentration 25%	5	0	5	4	1	1	4	1	4
CE Concentration 50%	5	2	3	4	1	3	2	4	1
CE Concentration 100%	5	5	0	5	0	5	0	5	0

The MBC testing with *S. aureus* showed that clove extracts at concentration of 12.5% did not show sterile results which meant that *S. aureus* could not be killed at this concentration. Clove extracts at concentrations of 25% and 50% showed that *S. aureus* was killed only on 4 repetitions, whereas at concentration of 100% showed that *S. aureus* was killed on all repetitions.

The MIC testing with *E. coli* showed that clove extract at concentration of 12.5% did not show clear results, which meant that bacterial growth could not be inhibited at this concentration. Clove extract at concentration of 100% showed that bacterial growth was inhibited on all repetitions. The MBC testing with *E. coli* showed that clove extract at only concentration of 100% could kill *E. coli* on all repetitions.

## DISCUSSION

This research showed that clove extracts have the ability to inhibit and kill *S. aureus* and *E. coli*, in which the MIC of clove extracts against *S. aureus* and *E. coli* was 100%. This result matches with previous research done by Noreen et al in 2018 where the MIC of clove extracts from aqueous maceration against *E. coli* was 100%.<sup>9</sup> Previous research done by Xu et al in 2016 showed that clove extracts had the ability to inhibit the growth of *S. aureus* with the MIC 0.625 mg/mL, while MIC obtained in the author's research was 100% or equals to 100 mg/mL. These different findings of MIC can be caused by the difference in extraction methods. Methods used in this research were maceration extraction and dilution, while the previous research used distillation extraction method and micro-dilution with Resazurin method.<sup>10</sup>

Another study also showed that clove extracts with *aqueous* solvent could inhibit the growth of *S. aureus* and *E. coli*, but the extraction methods used in this research were decoction and distillation.<sup>11</sup> Extraction method by distillation has been proved to be more effective because the extract contained higher and more stable active compound than the extracts obtained from maceration and decoction methods.<sup>12</sup>

This study showed that the MBC of clove extracts against *S. aureus* and *E. coli* was 100%. This result is in line with other study which showed that clove extracts have the ability to kill *S. aureus*. MBC of clove extracts against *S. aureus* in the previous study was 4 mg/mL, while MBC obtained in the author's research was 100% or equals to 100 mg/mL. These different findings of MBC can be caused by the difference of extraction methods. The extraction method in the previous research used maceration and evaporation method with 96% ethanol as solvent.<sup>13</sup> Ethanol is often used as a solvent because of its relatively high solubility. It is also inert therefore reaction with other components could be avoided.<sup>14</sup> This research used maceration method with aqueous solvent because it is more applicable on a household scale. There are no other studies that discuss MBC of clove extract against *E. coli* therefore it could not be compared with other studies.

This research has limitation in the maceration method with aqueous solvent that needs much more cloves compared to previous methods. However, within this method it will be more applicable to be used on a household scale production. Another limitation of this



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research is that to obtain MIC, dilution method was used. This method lacks of objectivity and precision because the interpretations of this method obtained by visual assessment.<sup>15</sup>

## CONCLUSION

This research shows that clove extracts have the ability to inhibit and to kill *S. aureus* and *E. coli* in-vitro. MIC and MBC obtained against *S. aureus* and *E. coli* in this research are 100%.

Further research is needed on the antibacterial effectiveness of clove extracts against *S. aureus* and *E. coli* by using different methods. Besides, further research is also needed on the antimicrobial effectiveness of clove extract against another microorganisms. Synergistic effect of combined clove extract with other herb is probably also needs to be investigated.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest to disclose.

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