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THE IDENTIFICATION OF ANTIBACTERIAL COMPOUNDS IN CLOVE STEM EXTRACT (*Syzygium aromaticum*) AND ITS EFFECTIVENESS IN INHIBITING THE GROWTH OF *Escherichia coli*

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ABSTRACT
Clove plants usage in daily life is very diverse, such as kitchen spices, mixtures of various drinks, and medicines. Clove is a spice plant that contains clove oil which is known as an antibacterial agent. The clove stem has a fairly high clove oil content, but this is not widely known by the public. Clove oil is known to have the ability as a fungicide, bactericide, insecticide, and nematicide. The purpose of this study is to identify antibacterial compounds and the effectiveness of clove stem extract on the growth of Escherichia coli. We used GCMS (Gas Chromatography-Mass Spectrometry) analysis to identification antibacterial compounds. Antibacterial activity test using paper disc method by measuring the diameter of the inhibition zone formed from clove stem extract at doses of 30%, 50%, and 70% against Escherichia coli. The results of the study were analyzed using ANOVA with significance (p<0.05). The identification of the active compound showed 13 kinds of compounds were found with the dominant compound was alpha-pinene. The results of the antibacterial test showed the antibacterial activity of clove stem extract at doses of 30%, 50% and 70% with inhibition zones formed 12 mm, 12.7 mm, and 14.1 mm, respectively. The conclusion of this study showed the clove stem extract was proven to have antibacterial activity by inhibiting the growth of Escherichia coli, and the identification of GCMS showed that alpha-pinene, hexylene glycol, betha myrcene, eucalyptol and alpha terpineol were compounds in clove stem which were known to have antibacterial properties.

Introduction

Indonesia is one of the largest spice-producing countries in the world. Based on the Directorate General of National Export Development/MJL/XXVIII/04/2018, from the various kinds of spices produced, clove is one of the spice commodities that is traded on the international market (Directorate General, 2018). The use of clove plants in daily is very diverse, from kitchen spices, mixtures of

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various drinks, and medicines. Clove is also one of the main ingredients in the manufacture of cigarettes. In recent years, clove has also begun to be used in the manufacture of cosmetics and pesticides (Nurdjannah, 2004).

Clove has a spicy taste that contains clove oil which is known as its antibacterial agent (Pandey & Singh, 2011). Clove oil is distributed in various parts of the clove plant, including flowers (10-20%), stems (5-10%) and leaves (1-4%) (Nurdjannah, 2004). The usage of clove plants is usually limited to clove flowers, while the usage of clove stems and leaves is very rare.

The clove stem has a quite high clove oil content, but this is not widely known by the public. Clove oil is known to have the ability as a fungicide, bactericide, insecticide, and nematicide (Bustaman, 2011). Previous research that has been carried out shows that clove stems have antibacterial activity against *Staphylococcus aureus* with a quite sensitive classification when compared to clove flowers which have a sensitive classification (Safitri & Purnamawati, 2021).

In Indonesia, *Escherichia coli* is one of the pathogenic bacteria that causes various kinds of infections. These bacteria cause food contamination, diarrhea, and urinary tract infections (Kudinha, 2017; Hutasiot, 2020). The use of antibiotics as antibacterial agents will increase a risk of resistance if the usage is not appropriate, therefore the use of natural ingredients as alternative drugs is considered safer in inhibiting infections caused by bacteria (Slipranata et al., 2016).

In this study, we tested the antibacterial activity of the clove stem extract against *Escherichia coli* and identified the antibacterial compounds in the clove stem using the Gas Chromatography-Mass Spectrometry (GCMS) method.

Materials and Methods

1. Preparation of clove stem extract

Dried clove stems were purchased from clove traders in Trenggalek, East Java. Clove stem as much as 100 grams were crushed into a coarse powder, then extracted by soaking in 750 mL of 80% methanol for 48 hours at room temperature and dark conditions (Pandey & Singh, 2011). The mixture was then filtered using Whatman filter paper number 42, then evaporated using a rotary evaporator to produce a concentrated extract that was ready for use.

2. Antibacterial activity test of clove stem extract

Escherichia coli ATCC 25922 were obtained from the Central Health Laboratory (BBLK), Surabaya. Bacterial preparation was carried out by culturing *E. coli* for 24 hours to be used as an antibacterial test. The antibacterial test method that we used is the Kirby–Bauer diffusion method which refers to Mirpour et al. (2017) research. We used paper discs and measured the inhibition zone formed by bacteria after 24 hours of incubation.

Escherichia coli was planted on nutrient agar media using sterile cotton buds, then some paper disc contained clove stem extract at a dose of 30%, 50%, and 70% were placed on the surface of the media. In this study, Amoxicillin at a dose of 0.5 mg/ml was used as a control comparison. The plates then incubated at 37 °C for 24 hours, and the inhibition zone formed was measured.

3. The analysis of antibacterial agent of clove stem using GCMS

GC-MS analysis was carried out at the Microanalysis Laboratory, University of Muhammadiyah Purwokerto, Central Java.

Results and Discussion

1. Antibacterial compounds on clove stem extract

The results of the analysis of the compound test in the clove stem extract obtained a total of 13 peaks in the GCMS chromatogram analysis (Table 1). Based on the results of the GCMS chromatogram, from 13 compounds found, there was one dominant compound with the highest peak compared to the others.

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Table 1. The results of antibacterial compounds on clove stem extract analyzed by GCMS

The name of compounds	Retention time (Rt)	Area (%)	Formula	Molecule mass (g/mol)
Hexylene Glycol	-	-	$C_6H_{14}O_2$	118.17
Alpha-Pinene	4.240	84	$C_{10}H_{16}$	136.23
Bicyclo[3.1.1]Heptane, 6,6-Dimethyl-2-Methylene-, (1S)	-	-	$C_{10}H_{16}$	136.23
Beta Myrcene	-	-	$C_{10}H_{16}$	136.23
Delta.3-Carene	-	-	$C_{10}H_{16}$	136.23
Benzene, 1-Methyl-2-(1-Methylethyl)-	-	-	$C_{10}H_{14}$	134.22
Bicyclo[2.2.1]Hept-2-Ene, 1,7,7-Trimethyl-	-	-	$C_{10}H_{16}$	136.23
Eucalyptol	-	-	$C_{10}H_{16}O$	154.25
Gamma-Terpinene	-	-	$C_{10}H_{16}$	136.23
Naphthalene, Decahydro-2-Methyl-	-	-	$C_{11}H_{20}$	152.27
Menthol	-	-	$C_{10}H_{20}O$	156.26
Alpha Terpineol	-	-	$C_{10}H_{18}O$	154.25
Dodecane	-	-	$C_{12}H_{26}$	170.33

Based on the results of the screening of GCMS compounds, 13 compounds were obtained, namely hexylene glycol; alpha-pinene; bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1s); beta myrcene; delta.3-carene; benzene, 1-methyl-2-(1-methylethyl)-; bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl-; eucalyptol; gamma-terpinene; naphthalene, decahydro-2-methyl-; menthol; alpha terpineol; dodecane. A total of 13 compounds were found in the GCMS chromatogram, alpha-pinene was the dominant compound which had a retention time of 4,240 with an area percentage of 84% (Figure 1).

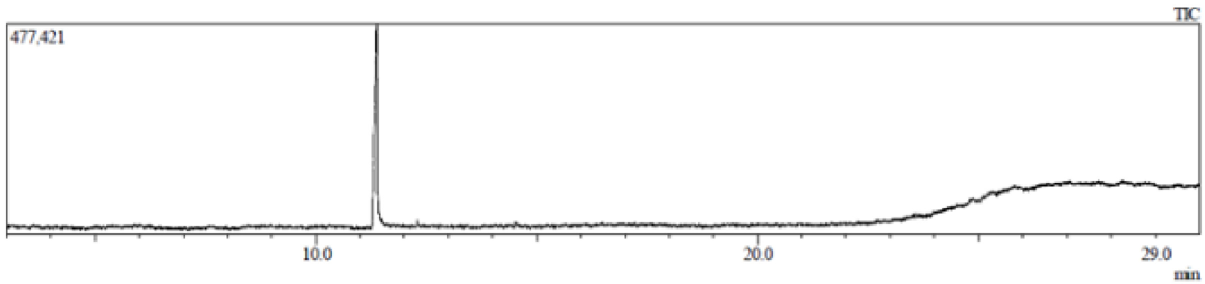

Figure 1. GCMS chromatogram of clove stem extract

2. The antibacterial activity of clove stem extract

The results showed that there was antibacterial activity in the clove stem extract at doses of 30%, 50%, and 70% against *Escherichia coli*. The diameter of the inhibition zone that were formed against *Escherichia coli* at doses of 30%, 50%, and 70%, respectively, was 12 mm, 12.7 mm, and 14.1 mm in average.

Inhibition zone diameter classification has 4 kinds of categories, namely insensitive (diameter < 8 mm), quite sensitive (8.0 mm < diameter < 14 mm), sensitive (14 mm < diameter < 20 mm), and very sensitive (> 20 mm). mm) (Xiao et al., 2019). Based on the classification table, it is known that the antibacterial activity of the clove stem at a dose of 70% is equivalent to the positive control antibiotic amoxicillin 0.5 mg/ml, which is sensitive to the growth of *Escherichia coli*. Meanwhile, doses of 30% and 50% were still in the different categories with positive control of 0.5 mg/ml amoxicillin, which was quite sensitive.

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Tabel 1. The diameter of inhibition zone of clove stem extract against *Escherichia coli*

Treatments	Diameter of inhibition zone (mm)	Classification
K + (Amoxicillin 0,5 mg/ml)	18,7	Sensitive
K – (Empty disc)	0	Not sensitive
G-30%	12	Quite sensitive
G-50%	12,7	Quite sensitive
G-70%	14,1	Sensitive

Based on statistical analysis using SPSS, the comparison of the inhibition zone of the clove stem extract on the growth of Escherichia coli at a dose of 30% was not significantly different from that of the clove stem extract at a dose of 50% but was significantly different at a dose of 70%. Based on these results, it is known that doses of 30% and 50% have the same ability to inhibit the growth of *Escherichia coli*, while the clove stem extract at a dose of 70% has the high ability to inhibit the growth of *Escherichia coli* compared to the positive control Amoxicillin 0.5 mg/ml (Figure 2).

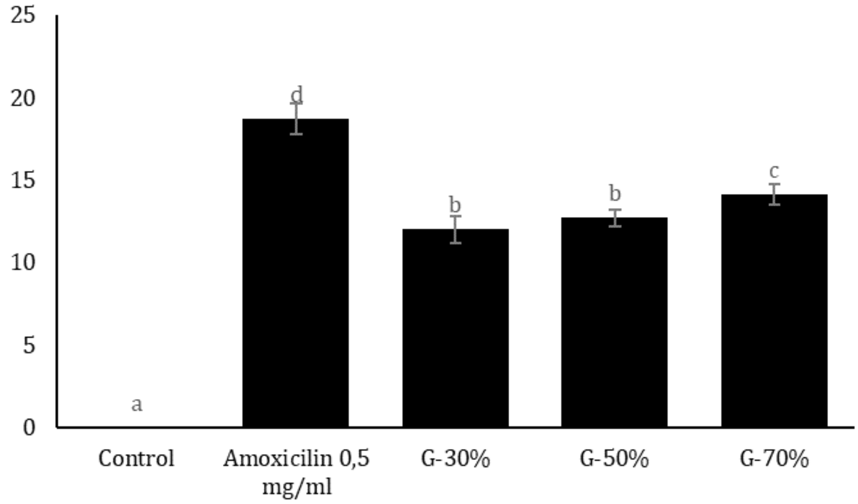

Figure 2. The comparison of the inhibition zone of clove stem extract on the growth of *Escherichia coli* based on SPSS version 16.0 analysis

The main compound of the clove plant is clove oil which is known to have antibacterial properties. The clove stem has a clove oil content of 5-10% where the main component of clove oil is eugenol (Nurdjannah, 2004). Eugenol is known to have antibacterial activity against gram-positive and negative bacteria (Pandey & Singh, 2011). Moreover, cloves also contain flavonoids, the antibacterial compounds by interfere with the metabolism and synthesis of genetic material in bacteria, while eugenol kills bacteria by changing the permeability of bacterial cell membranes (Cortés-Rojas et al., 2014; Safitri & Purnamawati, 2021). The use of amoxicillin as positive control is due to this antibiotic having a broad spectrum and able to kill bacteria by destroying the structure of the bacterial cell wall (Kapoor et al., 2017).

Based on the results of the GCMS screening, 13 compounds were found in the clove stem. Alpha-Pinene is the predominant compound found in clove stems. These compounds have antibacterial activity against gram-positive and gram-negative bacteria, particularly *Escherichia coli* and Staphylococcus (Leite et al. 2007; Ghavam et al., 2020). In addition to having antibacterial activity, alpha-pinene has the potential as an anti-inflammatory, antifungal, and antioxidant (Mohamed et al., 2013).

The analysis of GCMS also found several compounds that have the potential as antibacterial. These compounds include

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hexylene glycol and mycrene where these compounds can kill and inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* by damaging the cell membranes of these bacteria (Kinnunen & Koskela, 1991; Inoue et al., 2004). Eucalyptol and terpineol are also known to have antibacterial activity, where alpha terpineol compounds can change the size of bacterial cells, damage bacterial cell walls and membranes, and damage the composition of the bacterial cell nucleus (Hendry et al., 2009; Li et al., 2014).

In the antibacterial test, it was found that the methanol extract of the clove stem was able to inhibit the growth of *Escherichia coli* in the in vitro test. This research is in accordance with the results of other studies which showed that the clove stem was able to effectively inhibit the growth of gram-positive and gram-negative bacteria (Safitri & Purnamawati, 2021). These finding are also supported by the results of the compound screening using GCMS. The GCMS analysis discovered several antibacterial compounds such as alpha pinene, hexylene glycol, beta mycrene, eucalyptol, and alpha terpineol which contribute to the ability of clove stem extract to inhibit the growth of *Escherichia coli*.

Conclusions

Identification of compounds using GCMS obtained 13 kinds of compounds which are some of them such as alpha-pinene, hexylene glycol, beta mycrene, eucalyotol, and alpha terpineol have antibacterial activity. Clove stem methanol extract was proven to have antibacterial activity by inhibiting the growth of *Escherichia coli.*

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