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Karamunting Leaf Extract (Rhodomyrtus tomentosa (Aiton) Hassk.) as antibiofilm on *Escherichia coli*

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Abstract – Escherichia coli is a bacterium that causes many serious infections such as digestive tract, urinary tract and bloodstream infections. One of the main challenges in treating this bacterial infection is the formation of biofilm, which increases bacterial resistance to antibiotics. As an alternative to overcome antibiotic resistance, karamunting has been identified as having antibacterial potential against Gram-negative bacteria, including E. coli. The active compounds in these leaves have been shown to inhibit bacterial growth and biofilm formation. This research describes the activity of karamunting leaf extract against the anti-biofilm E. coli. The thick karamunting leaf extract was then made into three variations of concentration, namely 125µg/mL, 250µg/mL and 500µg/mL. The parameter measured for the eradication test is the thickness of the biofilm which is read using the Elisa Reader 590nm. In this study, the eradication percentages for the three concentrations were obtained -92.15% at a concentration of 125 µg/mL, -187.24% at a concentration of 250 µg/mL and 52.78% at a concentration of 500 µg/mL. Karamunting leaf extract has activity as an antibiofilm against E. coli bacteria. Karamunting leaf extract with a concentration of 500 µg/mL is the Minimum Inhibitory Concentration (MIC) which is effective as an antibiofilm on E. coli.

Kata kunci: *Karamunting, extract, Eshcerichia coli, antibiofilm*

INTRODUCTION

Escherichia coli (*E. coli*) is one of the most researched bacteria and is the main cause of various infections, including gastrointestinal, urinary tract, and bloodstream infections (Bria et al., 2022; Jang et al., 2017; Vila et al., 2016). *E. coli* is naturally part of the gut microbiome, but some strains are pathogenic. Infections caused by *E. coli* have a significant impact on global health, especially in developing countries, with high incidence and mortality rates (Freeman et al., 2009; Pratiwi, 2017). Biofilm formation by *E. coli* increases the bacteria's protection against antibiotics and the immune system, thereby exacerbating infections and antibiotic resistance (Kobayashi et al., 2021; Martinson & Walk, 2020; Sharma et al., 2016).

Antibiotic resistance is a major challenge in the treatment of bacterial infections. The Centers for Disease Control and Prevention (CDC) records two million cases of resistant bacterial infections each year in the United States, with 23,000 deaths. The WHO report in 2020 shows an increase in antibiotic resistance, including in *E. coli*, *Klebsiella pneumoniae*, and *Acinetobacter spp*. Biofilm-related infections, particularly those involving medical devices, are also on the rise. In addition, irrational use of antibiotics also triggers bacterial resistance (Hutomo et al., 2023; Purnama et al., 2022; Tjampakasari & others, 2021).

The novelty and originality of this research lies in testing the activity of karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk) leaf extract as an antibiofilm agent against *E.coli*, a pathogenic bacterium that has the ability to form biofilms as a protective mechanism against antibiotics and the immune system. Although the karamunting plant is known to have antibacterial potential against Gram-positive and Gram-negative bacteria, its use as an anti-biofilm agent has not been widely explored (Dwicahmi, 2015; Hujjatusnaini et al., 2022; Paramanandana et al., 2019; Sabrina et al., 2021).

This research makes an important contribution in overcoming antibiotic resistance which continues to increase globally, especially in infections involving biofilms. This research was carried out by utilizing abundant local natural resources in Indonesia. This research not only supports the development of natural ingredient-based therapies but also becomes a reference strategy for alternative treatments that are more effective in dealing with resistant bacterial infections.

MATERIALS AND METHODS

This study used a descriptive method to test the antibiofilm activity of karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk) leaf extract against *Escherichia coli* bacteria. This research was conducted at the Microbiology, Biotechnology and Biochemistry Laboratory, Faculty of Medicine, Sriwijaya University. The resulting karamunting leaf extract was then made in three concentration variations, namely 125 µg/mL, 250 µg/mL, and 500 µg/mL (Sabrina et al., 2021). The positive control uses the antibiotic meropenem, while the negative control uses DMSO solution. The parameter observed in this study was the thickness of the bacterial biofilm which was measured using an Elisa Reader at a wavelength of 590 nm.

Before the biofilm eradication test is carried out, the *E. coli* bacteria that can produce biofilm will be identified first. Bacteria that have been rejuvenated using Nutrient Agar are then put into a test tube containing Tryptic Soy Broth (TSB) media. The test tube filled with bacteria and TSB media will be incubated at 37°C for 24 hours. After the incubation process, the tube media was rinsed and stained using 0.1% gentian violet for 15 minutes. The results of the biofilm activity test using this test tube showed positive results if purple film lines formed on the bottom and walls of the test tube.

E. coli bacteria were placed in wells filled with TSB media, then incubated for 2x24 hours. The thick extract of karamunting leaves was diluted using Dimethyl Sulfoxide (DMSO) to a concentration of 250 µg/mL, 500 µg/mL and 1000 µg/mL. Each concentration is put into a well that is already filled with bacteria. In addition, a positive control was prepared containing the antibiotic meropenem, TSB media and bacteria, as well as a negative control containing TSB media, bacteria and DMSO solution (Kırmusaoğlu, 2019).

RESULT AND DISCUSSION

The extraction results of karamunting leaf extract in this study were 30.70 grams. The percentage yield of karamunting leaf extract in this study was 15.35%. Based on test results, the lowest concentration of karamunting leaf extract which was able to inhibit the growth of *E. coli* bacteria was 125 µg/mL.

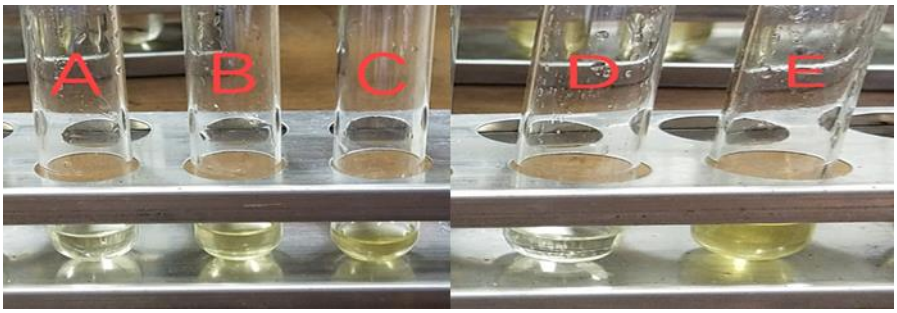

Figure 1. Variations in concentration of karamunting leaf extract. (A) 125 µg/mL, (B) 250 µg/mL, (C) 500 µg/mL (D) Positive control in the form of the antibiotic meropenem (E) Negative control in the form of DMSO solution

From this figure 1, it showed the differences in each test tube ranging from clear, cloudy and very cloudy.

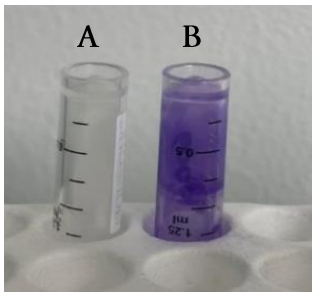

Figure 2. Results of Biofilm Identification Formed by *Escherichia coli*
A. Control (-) Empty tube; B. Biofilm formation is indicated by purple color in the tube

Figure 2 shows that a purple color appears in the entire tube from 0.1% gentian violet dye, which indicates the formation of biofilm in the test tube on the right.

Figure 3. Biofilm eradication results

Figure 3 shows the results of eradication of *E. coli* bacterial biofilm after being given karamunting leaf extract. The purple color appears fading, indicating that karamunting leaves are effective in destroying the *E. coli* bacterial biofilm. The thickness of the biofilm formed at the bottom of the microplate well was read using a 590 nm Elisa Reader and expressed in optical density units.

The results of descriptive analysis of *E. coli* bacterial biofilm eradication are presented in the table below using the formula:

$$\% \text{ eradication} = \frac{\textit{Mean}\ \text{OD control} - \textit{Mean}\ \text{OD treatment}}{\textit{Mean}\ \text{OD control}} \times 100\ \%$$

Table 1. Biofilm eradication test results

Group of treatment (µg/mL)	Mean of Optical Density (nm)	Percentage of inhibition (%)
Positive control	0.2536	33.68
Negative control	0.3824	-
Concentration 250	1.0984	-
Concentration 500	0.1806	52.77
Concentration 1000	0.2536	33.68

Table 1 shows the average value of optical density and percentage of biofilm eradication for the concentration of karamunting leaf extract on *E. coli* bacterial biofilms. A concentration of 250 µg/mL has an average optical density value of 1.0984. A concentration of 500 µg/mL has an average optical density value of 0.1806 and an eradication percentage value of 52.77%. A concentration of 1000 µg/mL has an average optical density value of 0.2536 and an eradication percentage value of 33.68%.

The concentration listed in table 1 has a different concentration from the concentration previously used as a reference, namely, there is an additional concentration of 1000 µg/mL. This is because, during the eradication test, concentrations of 125 µg/mL and 250 µg/mL which were used as initial references, only had antibacterial activity, but not as antibiofilm. This was proven when the biofilm eradication test was carried out at concentrations of 125 µg/mL and 250 µg/mL which had negative eradication percentage values.

Extraction is an important first step in the analysis of medicinal plants to extract active compounds. This research uses the maceration method, which is simple and efficient, although it takes longer than other methods such as percolation, infusion, or dekokta. Maceration is carried out by immersing the sample in a solvent and changing it periodically, so as to extract maximum active compounds. This method is more practical because it does not require special equipment and produces an extract with a lower water content than infusion or decoction.

In this research, the solvent used was 96% ethanol, which is capable of extracting polar, semipolar and nonpolar active compounds. Ethanol was chosen because it is volatile, non-toxic, and effective in attracting active compounds such as alkaloids, flavonoids, steroids, saponins, tannins, and quinones. Compared to 70% ethanol, 96% ethanol is more effective in attracting nonpolar compounds, resulting in a more optimal extract. Dilution was carried out using Dimethyl Sulfoxide (DMSO) solution.

The results of extracting karamunting leaves showed a yield percentage of 15.35%, higher than previous research which reached 14.22% with a similar method. The extraction process involved the use of 500 grams of karamunting leaves soaked in 96% ethanol and dried using an incubator, showing the effectiveness of the maceration method in this research

Minimum Inhibitory Concentration (MIC) is the lowest concentration of a compound, such as an antibiotic or plant extract, that is able to inhibit bacterial growth. MIC is used to determine the minimum effective dose in the treatment of bacterial infections. Based on this research, the MIC of karamunting leaf extract against *E. coli* bacteria is 125 µg/mL, which is determined by the clarity of the media in the test tube. This concentration is interpreted as the minimum point at which the extract is able to inhibit the growth of Gram-negative bacteria, in line with previous research which showed the antibacterial activity of caramunting leaves against Carbapenemase Resistance *Klebsiella Pneumoniae* (CRKP) at the same concentration.

In this study, the extract concentrations tested included 125 µg/mL, 250 µg/mL, and 500 µg/mL. The highest concentration, namely 500 µg/mL, showed antibiofilm activity with an eradication percentage of 52.77%. However, lower concentrations, such as 125 µg/mL and 250 µg/mL, did not have a significant effect on biofilm inhibition. This is thought to be because the active compound content at this concentration is not sufficient to optimally disrupt the biofilm structure. These findings indicate that the antibacterial and antibiofilm activity of karamunting leaf extract tends to increase linearly with increasing concentration until it reaches a certain threshold.

The eradication of *E. coli* biofilm was conducted using the Microtiter Plate Biofilm Eradication Assay method, which demonstrated significant effectiveness in disrupting biofilms. At concentrations of 125 µg/mL and 250 µg/mL, karamunting leaf extract acted only as an antibacterial agent and was not effective as an antibiofilm agent, as shown by negative eradication percentages of -92.15% and -187.24%, respectively. These results indicate that the secondary metabolites at these concentrations were insufficient to disrupt biofilm structure. However, concentrations of 500 µg/mL and 1000 µg/mL showed significant antibiofilm activity, with eradication percentages of 52.77% and 33.68%, respectively, comparable to the positive control (meropenem). From this results, the lower concentration (500 µg/mL) has higher eradication percentage (52.77%) than 1000 µg/mL. This can occur for several reasons, including the hormetic response of bacteria. Exposure to antibiotics at sub-inhibitory concentrations (sub-MIC) can stimulate biofilm formation, modulate virulence, stress adaptation, and even transfer resistance genes, indicating an adaptive bacterial response at low doses. High concentrations can trigger bacterial defenses such as increased extracellular polymeric substance (EPS) secretion, adaptive genetic compensation, or activation of efflux pumps, which strengthen the biofilm structure (Iavicoli et al., 2021; Song et al., 2016). This suggests the potential of karamunting leaf extract as a new antibiotic candidate.

Supporting findings align with previous studies showing rhodomyrtone's antibiofilm activity, similar to vancomycin, against *Staphylococcus aureus* biofilms. Phytochemical screening confirmed the presence of secondary metabolites such as alkaloids, flavonoids, triterpenoids/steroids, saponins, and tannins, which contribute to the extract's antibacterial and antibiofilm properties. Alkaloids disrupt bacterial cell walls by targeting peptidoglycan, flavonoids inhibit quorum sensing to prevent biofilm formation, steroids damage bacterial membranes, saponins disrupt membrane integrity leading to leakage of proteins and enzymes, and tannins cause cell lysis by targeting cell walls.

This study highlights the effectiveness of karamunting leaf extract, particularly at higher concentrations, in combating *E. coli* biofilms and its potential for further development as an antibacterial and antibiofilm agent.

CONCLUSION

Minimum Inhibitory Concentration (MIC) of karamunting leaf extract with antibacterial activity against *Escherichia coli* is 125 µg/mL and karamunting leaf extract exhibits anti-biofilm activity against *Escherichia coli* biofilms, with an eradication percentage of 52.77% at a concentration of 500 µg/mL and 33.68% at a concentration of 1000 µg/mL.

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