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Inhibition Activity of Ultrasonic-Assisted Extract of *Sonneratia caseolaris* Mangrove Leaves (UCESc) against *Escherichia coli*

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ABSTRACT

Mangrove Sonneratia caseolaris's leaf contains bioactive compounds such as alkaloid, flavonoid, phenolic, and tannin. Previously, crude extract of S. caseolaris leaf was reported has potential as antibacterial. However, information on antibacterial activity profile of ultrasonic-assisted extract of S. caseolaris leaf against Escherichia coli was limited. Therefore, the aim of this study was to determine the effect of ultrasonic-assisted extraction on S. caseolaris leaf extract's antibacterial activity against E. coli. The extraction was performed using ultrasonicator bath at 40 KHz, 40 °C for 25 minutes using 96% ethanol as a solvent. The phytochemical analysis was indicated that the crude extract Sonneratia caseolaris (UCESc) contains flavonoid, saponin, steroid, and tannin, but lack of alkaloid. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of UCESc were performed with microdilution with MIC of 24 mg/mL and MBC is 48 mg/mL. The antibacterial assay using disc dilution assay indicated that the best activity was at a concentration of 96 mg/mL with an inhibition zone of 4.88 mm. This study suggested that ultrasonic assisted extraction is potential approach to extract S. caseolaris bioactive compounds with a potential to be used as an antibacterial.

INTRODUCTION

With a total area of approximately 3.36 million hectares, Indonesia has the largest mangrove forest ecosystem in the world (Goldberg *et al.*, 2020). In East Java, according to Hidayah and Rachman (2023), the mangrove forest area in the Madura Strait, located between Surabaya and Sidoarjo, spans 3,956.77 hectares. This ecosystem consists of various types of mangroves, including *Sonneratia caseolaris*. This mangrove species generally contributes 5–10% to the total composition of Indonesia's natural mangroves (~15 million hectares in total), though the exact percentage varies by region (Sasson, 2000). *S. caseolaris* has high bioactive potential with various parts of this plant are utilized by local communities, including dry stem as wood and fruit for syrup. In addition, its leaves contain various secondary metabolites, including flavonoids, tannins, alkaloids, and phenolic compounds, which exhibit antibacterial activity (Hasibuan & Sumartini, 2021).

The constituent of bioactive compounds of *S. caseolaris* leaf was reported to be potential as functional foods and pharmaceutical ingredients (Ghina *et al.*, 2024). To obtain the bioactive compounds several extraction methods can be used, including maceration and Soxhlet extraction. These methods are simple, however both methods are often time-consuming and inefficient for heat-sensitive metabolites (Geetha, 2025). Advanced approaches, such as microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE), have been developed to improve yield and reduce solvent use (Haque *et al.*, 2022; Singh *et al.*, 2020). However, ultrasound-assisted extraction (UAE) has distinct advantages over these methods because it is highly efficient, has a short extraction time, requires relatively little energy, and uses minimal solvent (Shen et al., 2023). Through acoustic cavitation, UAE disrupts plant cell walls and enhances solvent penetration. This leads to improved recovery of thermolabile bioactives that might otherwise degrade at high temperatures. These features make UAE a particularly suitable and preferred method for extracting antimicrobial and antioxidant compounds from mangrove leaves (Shen *et al.*, 2023).

The antibacterial activity of bioactive compounds extracted from mangrove leaves can be applied to inhibit pathogenic and spoilage bacteria in fishery sector. One of the important potential pathogenic bacteria in the fisheries is *Escherichia coli*. It is often found as a major contaminant in fishery products and especially prevalent in fresh fish (Fauzi *et al.*, 2024). According to a report from the Fish Quarantine Agency of the Ministry of Maritime Affairs and Fisheries (2022), *E. coli* was often found in samples of fish consumed in traditional markets and fish auctions in Indonesia, exceeding the threshold value set by the Indonesian National Standard (SNI). Therefore, the aims of this study were to analyze the application of UAE in extraction of *S. caseolaris* leaf bioactive compound and its antibacterial activity against *E. coli in vitro*. It could serve as basic information for obtaining potential natural preservative additive which might be incorporated into a microbial control strategy in the seafood-based food supply chain.

LITERATURE REVIEW

Sonneratia caseolaris, locally known as Pedada Merah, is an evergreen mangrove tree from the Sonneratiaceae family (Ahmad et al., 2018). It was reported that S. caseolaris leaves extracted with maseration method contains secondary metabolites, including flavonoids, phenolic compounds, terpenoids, steroids, and alkaloids. These secondary metabolites exhibit significant biological activities, including antioxidant, antibacterial, anticancer, and anti-inflammatory properties, rendering them highly promising for medical applications (Ahmad *et al.*, 2018). It was reported that secondary metabolite is a natural product which possesses beneficial bioactivities for humans including antibacterial, antiviral, and antiinflammation; therefore, further study on this field is essential to identify the bioactive compounds contained in these natural products and method to obtain it.

In the screening and analysing of bioactive compounds extracted from plants, phytochemical screening is one of the initial step to describe the compound classes present in the plant under study (Yanti & Vera, 2019). The key factors in obtaining phytochemicals are including the choice of solvents and extraction method. Using inappropriate solvents or method can result in failure on the active compounds extraction (Ningsih and Nurrosyidah, 2020).

The most common method in bioactive compounds extraction from plants is maceration because of it simple. Ethanol, especially at 70% and 96% concentrations, is effective for extracting phenolic compounds that provide antioxidant activity. Among them, the 96% ethanol extract of *S. caseolaris* fruits showed the strongest radical scavenging activity (80.21%) (Sukeksi *et al.*, 2024). Using maceration method, the ethanol-derived extract of *S. caseolaris* leaves exhibited the strongest antioxidant and anti-methicillin resistant *Staphylococcus aureus* activities (Audah et al., 2022). On the other hand, Haslina et al. (2020) reported that ultrasonic assisted extraction (UAE) of *Sonneratia alba* using ethanol 70% has successfully extracting bioactive compounds including phenolic compounds and alkaloids.

METHOD

This study was performed by an experimental method employing a completely randomized design (CRD) with four *S. caseolaris* mangrove leaf extract concentration treatments (12, 24, 48, and 96 mg/mL) and four replications. There are three steps performed in this study, extraction, phytochemical analysis, and antibacterial assay as indicated in Figure 1.

Time and Places

This study was conducted from December 2024 to April 2025 in the Chemistry and Analysis Laboratory and the Fisheries Microbiology Laboratory at the Faculty of Fisheries and Marine Sciences at Airlangga University. The FTIR analysis was conducted at Airlangga University's Institute of Life Sciences, Engineering & Technology (LIHTR).

Mangrove *Soneratia caseolaris* leaves Sample collection

S. *caseolaris* mangrove leaves, counted from the third to fifth order from the top of the plant branch with no indication of fungi or insect infestation were collected from Wonorejo Mangrove Ecotourism in Surabaya, East Java, at the coordinates 7°18′29.21″ S, 112°49′18.86″ E. The leaves were collected randomly and collected two times in a polyethylene plastic.

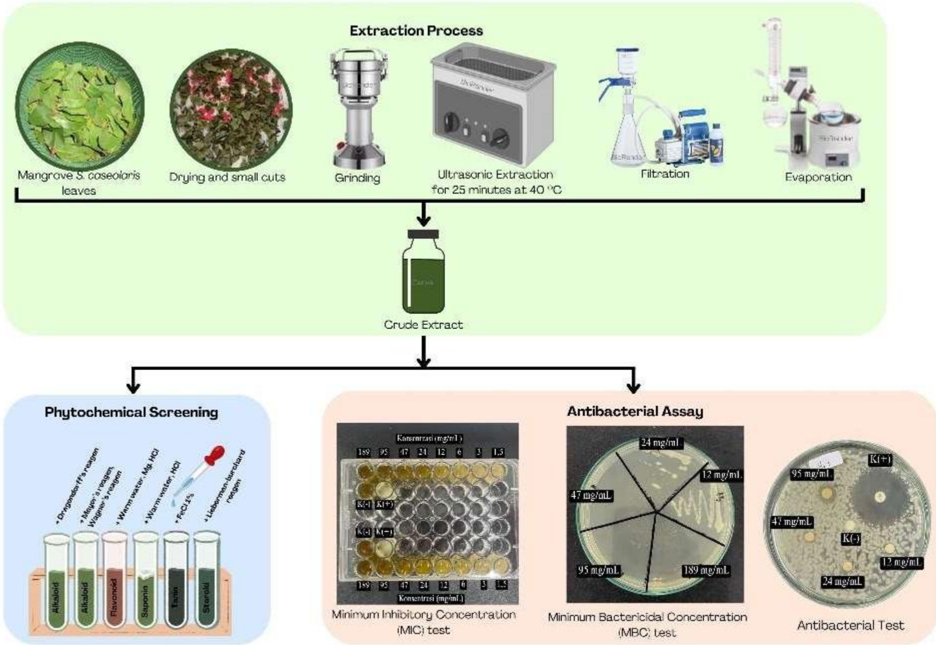

Figure 1. Extraction method and antibacterial activity assay

Dry Simplicia preparation and ultrasonic assisted extraction (UAE) extraction

S. caseolaris mangrove leaves samples were washed with tap water to remove debris and dried for five to seven days with air drying in a place protected from direct sunlight. The dried leaf samples were then reduced in size using a grinder (further called simplicium), and their water content was measured to ensure that the material has low moisture content (< 10 %) (Wijaya & Noviana, 2022). The simplicium was then extracted using the UAE method. Briefly, the simplicium was weighed, and then 96% ethanol was added to the sample at a ratio of 1:10 (w/v) in an Erlenmeyer flask. The sample was then extracted using ultrasonic bath at a 40 kHz, 40 °C for 25 min (Haslina *et al.*, 2023). The extract was filtered using Whatmann filter paper no. 1 (pores ±11 µm), assisted by a vacuum pump, to produce a filtrate. The filtrate was concentrated using a rotary vacuum evaporator at 40°C with a rotation speed of 60 rpm until a thick, dark green extract was obtained. The resulting extract was weighed to determine the yield, then placed in a container for further testing.

Yield

The yield is calculated by weighing the dried extract. Then, the weight of the extract is divided by the initial sample weight and multiplied by 100% (Faisal *et al.*, 2022) following this equation:

$$\%yield = \frac{Extract\ mass\ (g)}{Simplicia\ mass\ (g)} x100\%$$

Phytochemical analysis

The detection of alkaloids, flavonoids, saponins, tannins, and steroids was performed. To detect alkaloids, the extract was treated with 1 mL of HCl, followed by the addition of Mayer's, Wagner's, and Dragendorff's reagents in separate tubes. The presence of alkaloids was indicated by the formation of a white or yellowish-white precipitate (Mayer's), a brown precipitate (Wagner's), or an orange-red precipitate (Dragendorff's) (Amir et al., 2019). Flavonoids were tested by adding 10 mL of hot water to the extract, followed by 0.25 g of magnesium powder and five drops of HCl. A positive reaction was indicated by orange coloration (Yeti & Yuniarti, 2021). Saponins were identified by mixing the extract with warm distilled water and shaking vigorously for ten seconds. The presence of stable foam (one to ten centimeters in height) that persisted for at least ten minutes and was unaffected by the addition of one drop of HCl confirmed the presence of saponins (Kusumawati et al., 2017). Tannins were detected by adding 10 drops of a 1% $FeCl_3$ solution. The appearance of a dark blue or greenish-black precipitate indicated a positive result (Kusumawati et al., 2017). Steroid detection was conducted by adding 2 mL of Liebermann-Burchard reagent to the extract. After gentle shaking, the mixture was left to stand for several minutes. The development of a green coloration confirmed the presence of steroids (Yeti & Yuniarti, 2021).

Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

In order to analyse the inhibition activity of UCESc, *Escherichia coli* was used as indicator bacteria. A single loop of *E. coli* bacteria was taken from Tryptic Soy Agar (TSA) medium and rejuvenated in 1 mL of Tryptic Soy Broth (TSB) medium. Then, it was incubated in an incubator for 24 hours at 37 °C. After incubation, the bacterial culture had reached the exponential phase and was centrifuged at 5,000 rpm for 10 minutes to separate the cells from the medium. The cells were washed with a normal saline solution (0.9% NaCl) to remove residual media. Next, the bacteria were transferred to a test tube, and their microbial density, or turbidity, was measured using a 0.5 McFarland standard (1.5×10^8 CFU/mL).

The minimum inhibitory concentration (MIC) value was tested using a serial dilution method on a microplate to determine the lowest concentration of extract capable of inhibiting bacterial growth. The extract was diluted into eight serial concentrations prepared in Mueller-Hinton broth (MHB) media: 1.5, 3, 6, 12, 24, 48, 96, and 192 mg/mL. The MIC value was determined based on wells showing no turbidity. Extracts from microplate wells that showed bacterial growth in the previous MIC test were taken and grown in Mueller-Hinton agar (MHA) to determine the minimum bactericidal concentration (MBC). Bacterial colonies that did not grow in the media were considered the MBC value, which is the lowest concentration of the extract capable of killing all bacteria (Behbahani *et al.*, 2018).

Antibacterial assay confirmation using Kirby-Bauer Assay

For Kirby-Bauer disc diffusion assay, previously prepared *E. coli* was swabbed on MHA agar and then disc impregnated with 1.5, 3, 6, 12, 24, 48, 48, and 189 mg/mL (20 µL/disc) UCESc and positive control (chloramphenicol) and negative control (1% DMSO) was put on surface of the media. The plate was stored at 4 ºC for 1-2 h followed incubation at 37 ºC for 24 h. The antagonistic activity, indicated by formation of clear zone around disc, was measured with calliper.

Data Analysis

The inhibition zone diameter data from the concentration and control treatments will be analyzed statistically. First, the normality and homogeneity tests will be performed. Then, a one-way ANOVA (analysis of variance) will be performed. If there is a significant difference, a Duncan test will be performed.

RESULT

Simplicium characterization

Water content measurements showed that the *S. caseolaris* mangrove leaf sample contained 4.40 % water. The yield of the *S. caseolaris* mangrove leaf extract (UCESc) was calculated as a percentage of weight per weight (% w/w) based on the weight of the resulting extract compared to the initial weight of the dry sample. The UCESc yield value was 16.58 ± 0.86% with a dark green color as showed in Figure 2.

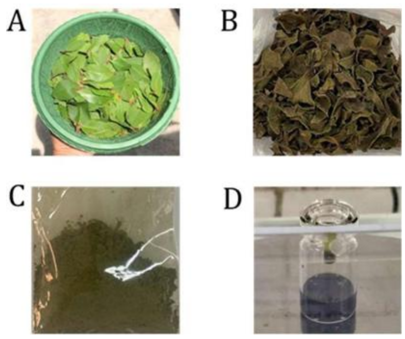

Figure 2. Preparation of *Sonneratia caseolaris* Mangrove Leaves simplicium. Fresh leaves (A); Leaves after air drying (B); Leaf simplicia (C); Crude Extract obtained with UAE (UCESc)(D).

Phytochemical analysis

To analysis the bioactive compounds of UCESc, a qualitative phytochemical screening test was performed. The results of the phytochemical test are presented in Table 1.

Table 1. Phytochemical analysis of Sonneratia caseolaris mangrove leaves extracted with ultrasonic assisted extraction

Test	Result	Explanation	Documentation
Alkaloid			
Mayer	Negative	Positive alkaloid indicated by white sediment on tube	
Wagner	Negative	Positive alkaloid indicated by brown color	
Dragendorff	Negative	Positive alkaloid indicated by orange color	
Flavonoid	Positive	Positive flavonoid indicated by red or orange color	
Saponin	Positive	Positive saponin indicated by formation of stable bubble after shaking	
Tanin	Positive	Positive tanin indicated by blue-black precipitate	
Steroid	Positive	Positive steroid indicated by dark green color	

FTR Profilling

The FTIR test results in the above image show several wave peaks, indicating the presence of active compounds in the extract. The peak around 3300 cm^{-1} indicates the presence of a hydroxyl (–OH) group, which is typically found in phenol or alcohol compounds. The peak around 2900 cm^{-1} indicates the presence of a C–H group from an aliphatic compound, or a carbon chain. The peak around 1700 cm^{-1} indicates the presence of a carbonyl group (C=O), which is typically found in acids or esters. Another peak around 1600–1500 cm^{-1} indicates an aromatic ring. The peak in the 1000–1300 cm^{-1} range indicates a C–O or C–N group, which is typically found in alcohols, esters, or amines. These results suggest that the extract contains active compounds, such as phenols, flavonoids, tannins, and esters, that play a role in antibacterial activity.

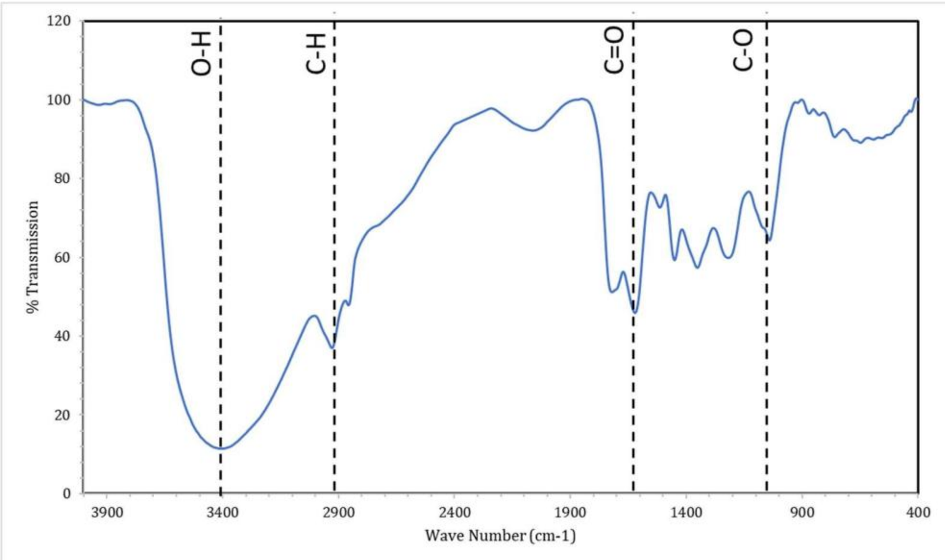

Figure 3. Profile of Ultrasonic assisted crude extract of *Sonneratia caseolaris* leaves (UCESc)

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC test results of the crude extract of S. caseolaris mangrove leaves (UCESc) were observed by checking if the solution in the microplate was clear or cloudy before and after 24-hour incubation at 37°C. A clear solution indicates a lack of bacterial growth, while a cloudy solution indicates ongoing bacterial growth. The MIC value is the lowest concentration of the extract that can prevent bacterial growth. Figure 4 shows the results of this observation. The MIC test results show that the antibacterial activity of UCESc is evident in solutions with concentrations of 189 mg/mL, 48 mg/mL, 48 mg/mL, and 24 mg/mL, which appear clear after 24 hours of incubation, indicating no growth of E. coli bacteria. Therefore, 24 mg/mL, the lowest concentration that can still inhibit bacterial growth, is determined as the MIC value.

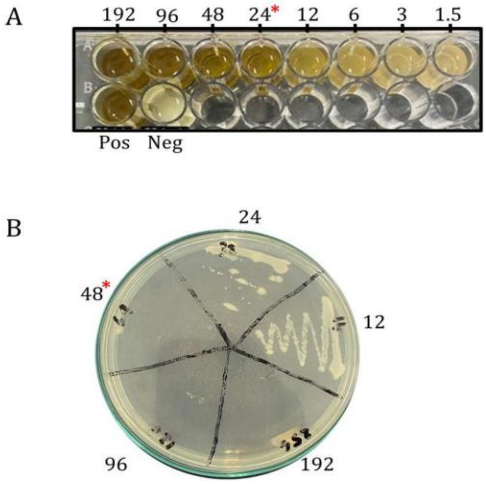

Figure 4. Minimum inhibition concentration and minimum bactericidal activity of ultrasonic assisted extract of *S. caseolaris mangrove.* MIC indicated by red asterisk (A), MBC indicated by red asterisk (B)

The extract from the microplate is planted on MHA media at a concentration that can inhibit bacteria to determine the MBC value. The MBC results are observed by checking for growth of *E. coli* bacteria on Mueller-Hinton agar (MHA) media. The presence of bacterial growth on the MHA media indicates that the extract is ineffective. Conversely, the absence of bacterial growth on the media indicates that the extract is effective in killing *E. coli* bacteria. The MBC test results can be seen in Figure 4. The results show that UCESc is more effective at killing *E. coli* bacteria at concentrations of 192 mg/mL, 96 mg/mL, and 48 mg/mL, as indicated by the absence of bacterial growth on MHA media.

Antibacterial activity confirmation using Kirby-Bauer Assay

Antibacterial tests were carried out using UCESc at concentrations of 48, 48, 24, and 12 mg/mL against *E. coli* bacteria. The results are shown in Table 2. As shown in Table 2, the 96 mg/mL extract produced the largest inhibition zone diameter against *E. coli* bacteria, with an average diameter of 4.88 ± 0.37 mm, compared to other concentrations. For comparison, chloramphenicol, which was used as a positive control in this test, had the largest inhibition zone diameter of all the treatment concentrations.

Table 2. Antibacterial activity of ultrasonic assisted extract of *Sonneratia caseolaris* leaf

Concentration	Inhibitory zone (mm)
12 mg/mL	0,09a±0,02
24 mg/mL	0,26a±0,05
48 mg/mL	1,23b±0,25
96 mg/mL	4,88c±0,51

Concentration	Inhibitory zone (mm)
Positive control	24,44d±0,46
Negative control	0

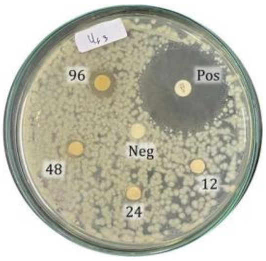

Figure 5. Inhibition activity of ultrasonic-assisted extract of *Sonneratia caseolaris* leaf. Positive control (chloramphenicol), negative control (DMSO 1%). Number representing concentration (12, 24, 48, 96 mg/mL).

Statistical tests using ANOVA show that there is a significant difference in the diameter of the inhibition zone based on the extract concentration ($\alpha < 0.001$). These results indicate that UCESc concentration significantly affects the ability to inhibit *E. coli* growth; the higher the concentration, the larger the resulting inhibition zone.

DISCUSSION

The antibacterial activity test of the crude extract of *Sonneratia caseolaris* mangrove leaves (UCESc) revealed an inhibition zone against *Escherichia coli* bacteria. The MIC and MBC tests determined the concentrations to be used in the antibacterial test: 12, 24, 48, and 96 mg/mL. UCESc has the ability to inhibit *E. coli* growth, with the highest inhibition occurring at a concentration of 96 mg/mL (4.88 ± 0.51 mm) and the lowest at a concentration of 12 mg/mL (0.09 ± 0.02 mm). This ability is evident in the formation of an inhibition zone around the paper disc. The antibacterial activity test showed that, at a concentration of 96 mg/mL, UCESc formed the largest inhibition zone, with an average diameter of 4.88 ± 0.51 mm; however, it was classified as weak. Previous study, Saad *et al.* (2012) reported that *Soneratia alba* extracted by Soxhlet extraction has inhibition activity of 17.5 mm *in vitro*. Interstigly, maceration extract of mangrove (*Rhizophora mucronata*, *Avicennia marina*, *Rhizophora apiculata*, and *Sonneratia alba*) has no antibacterial activity against Gram negative bacteria, including *E. coli* (Wijaya & Indraningrat, 2021) which indicated that different method has different antibacterial activity. The results of the study show that antibacterial activity increases with higher concentrations because more bioactive compounds are available (Pramesti *et al.*, 2024). For comparison, a 1% DMSO solution produced no inhibition zone as a negative control, while the antibiotic chloramphenicol produced an inhibition zone of 24.44 ± 0.46 mm as a positive control.

The formation of the inhibition zone is due to the presence of flavonoids, saponins, tannins, and steroids in the extract, as indicated by a color change during phytochemical screening. Alkaloids were not detected, likely because of their low concentrations and polar and nonpolar properties. These results are supported by the FTIR spectrum, which shows various functional groups indicating active compounds in the extract. The presence of these functional groups suggests that the extract contains bioactive compounds with antibacterial potential. Observations of the extract revealed a clear zone, suggesting antibacterial properties. Although no alkaloids were detected, this is likely the result of the four compounds working together. Because the test was qualitative, it is not yet known which compound contributes most to inhibiting bacterial growth.

The water content analysis of *S. caseolaris* mangrove leaf simplicia revealed a value of 4.40%, which is below the maximum limit of 10% established by the Ministry of Health of the Republic of Indonesia (2017) and in accordance with the generally applicable water content requirements (Wijaya & Noviana, 2022). The extraction process used the ultrasound-assisted extraction (UAE) method. This method uses high-frequency ultrasonic waves to produce a cavitation effect, which involves the formation of small bubbles that break down cell walls and increase the release of active compounds (Ardianti & Kusnadi, 2014). In this study, the extraction process was carried out at 40°C for 25 minutes to maintain the stability of heat-sensitive compounds. The extraction process uses a 96% ethanol solvent due to its polarity properties, which allow it to dissolve various bioactive compounds. It is also relatively safe, volatile, environmentally friendly, and highly effective at extracting active compounds of various levels of polarity (Lee *et al.*, 2024).

An MIC test was conducted to determine the concentration of ECC that can inhibit the growth of *E. coli* bacteria. The results showed that, at concentrations ranging from 24 to 189 mg/mL, the solution in the well remained clear, indicating no bacterial growth. In contrast, at concentrations ranging from 1.5 to 12 mg/mL, the solution appeared cloudy. Thus, the MIC value of the extract was determined to be 24 mg/mL. To determine whether the bacteria were inhibited or killed, an MBC test was conducted. For this test, the suspension from the MIC tube was planted in MHA media and observed after 24 hours of incubation. The results showed that, at a concentration of 24 mg/mL, colony growth was present; however, at a concentration of 48 mg/mL, no colonies grew. This indicates that the extract began to exhibit bactericidal properties at a concentration of 48 mg/mL. However, the results of the MIC and MBC tests can vary depending on several factors, such as the type of plant part, the extraction method, and the type of bacteria used. Determining MIC and MBC values is important for establishing the most effective concentration for therapy or treatment (Haerazi *et al.*, 2014).

This study shows that mangrove leaves from *S. caseolaris* have the potential to act as a natural antibacterial against *E. coli* bacteria. This research opens up opportunities to utilize local natural ingredients as alternative antibacterial agents that can be further developed into herbal products in the future. These results can inform further research, including the isolation of specific active compounds, the formulation of natural pharmaceutical preparations, and in vivo testing to determine biological effectiveness and toxicity. For instance, Barman *et al.* (2021) investigated the antioxidant and antidiabetic properties of ethanol extracts from *S. caseolaris* leaves, which inhibited the diabetogenic activity of alloxan by regulating blood glucose levels in treated mice. This research could support the development of modern traditional medicine and the sustainable management of mangrove resources in the health sector and nature-based pharmaceutical industry.

CONCLUSION

Sonneratia caseolaris mangrove leaf extract, obtained via the ultrasonic extraction method, exhibited antibacterial effectiveness against *Escherichia coli*, influenced by its active compound content. The best results were achieved at a concentration of 96 mg/mL, with an average inhibition zone diameter of 4.88 ± 0.51 mm. Increasing the concentration of *S. caseolaris* mangrove leaf extract revealed that higher concentrations exhibit greater antibacterial activity.

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