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Antibacterial Activity NaDES *Zwitterion Melastoma malabathricum* L. Leaves *Microwave Assisted extraction* (MAE) results Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Background: Increasing antibiotic resistance is a challenge in controlling *Staphylococcus aureus* and *Escherichia coli* infections, so it is necessary to search for new antibacterial candidates from local medicinal plants. Indonesia has high biodiversity, one of which is the senduduk plant (*Melastoma malabathricum* L.) which has been used as a traditional medicine by the community.
Aims : To evaluate the antibacterial activity of senduduk leaves extract (*Melastoma malabathricum* L.) obtained through environmentally friendly extraction (*Green Extraction*).
Methods: The extraction process used the Microwave-Assisted Extraction (MAE) method with a solvent made from a Zwitterion-based Natural Deep Eutectic Solvent (NaDES).This solvent is made by mixing betaine, which acts as a Hydrogen Bond Acceptor (HBA), with lactic acid, which acts as a Hydrogen Bond Donor (HBD). The amounts of these two components were tested in different ratios: 1:1, 1:3, and 1:5. Antibacterial tests were done against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method, and each test was repeated three times.
Result: The extract prepared using the 1:5 molar ratio exhibited the highest antibacterial activity with a very strong inhibition zone category in both test bacteria, with an average value of inhibition zone diameter of 22.46 mm in *Staphylococcus aureus* and an average value of inhibition zone diameter of 20.66 mm in *Escherichia coli*. This result was higher compared to the molar ratios of 1:1 and 1:3, which only reached the category of strong inhibition zones.
Conclusion: The MAE method with NaDES solvent is an effective method in developing the potential of senduduk leaves as a natural antibacterial agent.

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INTRODUCTION

Staphylococcus aureus *infection cases* are still a significant public health problem. The main challenge in the control of *Staphylococcus aureus* today is the increasing antibiotic resistance, including the emergence of *the Methicillin-Resistant Staphylococcus aureus* (MRSA) strain, which has led to a decline in the effectiveness of therapy (Grozdanova *et al*., 2020). In Indonesia, especially in Jambi province, several clinical reports show that *Staphylococcus aureus* is one of the bacteria that is often found in cases of osteomyelitis, wound infections, and skin infections, and exhibits a concerning pattern of antibiotic resistance (Lipinwati *et al*., 2023).

In Indonesia, cases of food poisoning show an increasing trend that warrants strict monitoring. Data from the Ministry of Health recorded an increase in the number of food poisoning cases from 3,514 in 2022 to 4,792 cases until mid-October 2023, where the Food and Drug Control Agency (BPOM) identified *E. coli* as one of the main causative microorganisms (Budiman *et al*., 2025). E. coli *contamination* in food occurs due to being influenced by various interrelated factors including the quality of clean water used, individual hygiene, sanitation of cooking utensils, to unqualified food processing and storage methods (Sihaloho *et al*., 2025).

Indonesia is famous for its very high diversity of plants and some of them are used as traditional medicinal plants. One potential plant is senduduk (*Melastoma malabathricum* L.), which is traditionally used to treat stomach pain, coughs, and skin infections (Barung *et al*., 2023). Senduduk leaves (*Melastoma malabathricum* L.) contain flavonoids, tannins, saponins, terpenoids, alkaloids, and phenolics, which function as antibacterial agents (Mayasari *et al*., 2022). In identification using LC-MS/MS, senduduk leaves contain major flavonoid compounds such as quercetin, quercitrin, as well as alkaloids, lignans, amides and terpenoid compounds that have antioxidant activity and other pharmacological potentials (Yulion *et al*., 2023).

NaDES is a mixture of natural components such as organic acids, sugars, or amino acids that are able to form liquid solvents with high extraction ability, safer, and are environmentally friendly. The use of NaDES in plant extraction can increase antimicrobial and antioxidant activity, as well as improve the stability of the extract (Kulinowska *et al*., 2025). NaDES formulations have antimicrobial capabilities and are able to increase the effectiveness of plant extracts against both Gram-positive and Gram-negative bacteria (Bedair *et al*., 2024).

In a past study, the antibacterial effect of senduduk leaves, which are from the plant Melastoma malabathricum L., was tested using the ethanol maceration method.The biggest area where bacteria were stopped grew only 19mm, and this was considered a strong inhibition zone (Sapitri *et al*., 2020). Conventional extraction methods for *Melastoma malabathricum* L. leaves, such as maceration, often require prolonged extraction times and large volumes of hazardous organic solvents, which can lead to thermal degradation of active compounds. To address these limitations, *Microwave-Assisted Extraction* (MAE) is utilized in this study as a green technology that offers rapid heating and higher extraction efficiency. Furthermore, the integration of *Natural Deep Eutectic Solvents* (NaDES) provides an eco-friendly alternative due to their biodegradability and low toxicity. Specifically, a zwitterionic NaDES formulation using a betaine-lactic acid combination is selected. Betaine, acting as a *zwitterionic* hydrogen bond acceptor, possesses unique charge properties that enhance the solubility and stabilization of diverse secondary metabolites within the NaDES matrix compared to conventional ionic components.

Therefore, this study fills this gap by integrating a green extraction technique via *Microwave-Assisted Extraction* (MAE) with a novel *zwitterionic* NaDES solvent system. This approach explicitly explores the optimization of molar ratios to enhance both the extraction yield and the antibacterial performance of *Melastoma malabathricum* L. leaves against *Staphylococcus aureus* and *Escherichia coli.*

METHOD

Research Design

This study is an experimental type of research that has multiple planned steps. The first step is collecting fresh leaves, and then they check the samples to decide what to do next. Then, the process of extracting compounds from senduduk leaves, which are known scientifically as *Melastoma malabathricum* L., was done using a solvent called Zwitterion Natural Deep Eutectic Solvent, or NaDES. After that, antibacterial tests were done against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. With a chloramphenicol disc used as a positive test control and an empty disc used as a negative test control. To control the test treatment, a disc with an extract from senduduk leaves (*Melastoma malabathricum* L.) was used, with a molar ratio of 1:1, 1:3, and 1:5.The data analysis was then performed.

Sampling

The sample used in this study was the top 10 parts of the senduduk leaves (*Melastoma malabathricum* L.) which was taken before sunrise in Mayang Mangurai Village, Alam Barajo District, Jambi Province.

Sample determination

Determination of the senduduk leaves plant (*Melastoma malabathricum* L.) was carried out at the Plant Taxonomy Laboratory, Department of Biology, FMIPA UNPAD.

NaDES Solvent Preparation

Preparation of *Natural Deep Eutectic Solvent* (NaDES) solvent is carried out by making mole calculations of each solvent. In this study, the NaDES solvent used was a comparison of *Hydrogen Bond Acceptor* (HBA) and *Hydrogen Bond Donor* (HBD), respectively:
HBA = Betaine ($C_5H_{11}NO_2$) 117,15 g/mol
HBD = Lactic Acid ($C_3H_6O_3$) 90,08 g/mol

- HBA : HBD (1:1)
  Betaine ($C_5H_{11}NO_2$) 117,15 g/mol : Lactic Acid ($C_3H_6O_3$) 90,08 g/mol
- HBA : HBD (1:3)
  Betaine ($C_5H_{11}NO_2$) 117,15 g/mol : Lactic Acid ($C_3H_6O_3$) 270,2 g/mol
- HBA : HBD (1:5)
  Betaine ($C_5H_{11}NO_2$) 117,15 g/mol : Lactic Acid ($C_3H_6O_3$) 450,4 g/mol

With a ratio of 1:1, 1:3, and 1:5 respectively with a total volume of 15 ml. It is done with the addition of distilled water by 25%. Each solvent component is then stirred using a magnetic hotplate at a temperature of 80°C for 60 minutes at a speed of No.3 until a clear and homogeneous NaDES solvent is obtained (Putra *et al*., 2024).

Microwave NaDES Extraction

Preparation of NaDES (*Natural Deep Eutectic Solvent*) extraction uses the MAE (*Microwave Assisted Extraction*) method. This method was chosen because of the fast and efficient extraction process. And it is expected to get optimal results. Extraction is carried out using a 3-minute time variant and the power used is 100 watts. A comparison of the various solvents used is the top 10 parts of Senduduk leaves (*Melastoma malabathricum L.*) fresh that has been processed is put into 100 parts of NaDES solvent and then extracted using MAE (*Microwave Assisted Extraction*) (Indawati *et al*., 2023). The resulting extract is filtered using filter paper to separate the maserate from its filtrate. The extracts obtained are stored in glass bottles and tightly sealed (Indawati *et al*., 2023).

Equipment Sterilization

The equipment to be sterilized is washed and dried first. Glass tools (beakers, erlenmeyers, test tubes) are covered with sterile cotton wrapped in sterile gauze and aluminum foil, then wrapped in parchment paper (Athaillah & Sugesti, 2020). To make the appliance heat-resistant, wrap the appliance in aluminum foil. Fill the autoclave with enough water, then insert the tool wrapped in aluminum foil into the autoclave. All culture media are sterilized by autoclave at 121ºC and 1 atm pressure for 15 minutes (Hayati *et al*., 2022). Tweezers and needles are sterilized by flaming by aligning them on a bunsen fire (Bankole *et al*., 2022).

Creation of Agar Nutrient Media (NA)

Preparation of Nutrient Agar (NA) media begins by dissolving 6 grams of NA base media into 300 ml of sterile distilled water inside erlenmeyer. Then heat it on a hot plate while stirring until boiling and stirring until it is clear yellow. Then the erlenmeyer is covered with gauze and cotton and then sterilized in an autoclave at 121ºC for 15 minutes (Athaillah & Sugesti, 2020). Then the sterilized NA base media is poured into a sterile petri dish with a uniform surface level, then as much as 5 ml is poured into the sterile test tube in warm conditions, then tilted, at the mouth of the test tube is closed using a cotton swab wrapped in sterile gauze. Media manufacturing is carried out aseptically in *Laminar Air Flow* (LAF) (Athaillah & Sugesti, 2020).

Test Bacteria Rejuvenation

Bacteria are grown in the Nutrient Agar (NA) agar medium by scraping the bacteria from pure cultures using an inoculating loop on the slanted agar surface that has been streriled. Furthermore, the bacteria that have been scratched on the oblique agar are then incubated in the incubator device at a temperature of 37ºC for 24 hours (Athaillah & Sugesti, 2020).

Preparation of Test Bacterial Suspension

The bacterial suspension test is prepared by suspending some of the rejuvenated bacterial ose in 5 ml of 0.9% NaCl solution in a sterile test tube. Next, the mixture is homogenized until the suspension is cloudy (Yulion e*t al*., 2023).

Standardization of bacterial suspension using UV-Vis spectrophotometry

For percent transmittance measurement, bacterial suspensions that had been homogenized with 5 ml of 0.9% NaCl solution were measured for turbidity using a UV-Vis spectrophotometer with a wavelength of 580nm with a percent transmittance value close to 25% (Yulion *et al*., 2023).

Antibacterial Activity Test of Senduduk Leaves Extract (*Melastoma malabathricum* L.)

In this study, antibacterial activity was tested using the disc diffusion method. Bacterial suspension inoculated on Nutrient Agar (NA) media in a petri dish (Athaillah & Sugesti, 2020). Then, use a hockey stick cotton swab to level out the suspension and let it dry (Nurhayati *et al*., 2020). The prepared blank disc paper is taken using flame-sterilized forceps and then dipped slightly at a concentration of 1:1, 1:3, 1:5 respectively. The negative control uses a blank disc and for the positive control uses a chloramphenicol disc and is then placed on the surface of the agar medium. Using tweezers 2-3 cm from the edge of the cup, then incubated for 1x24 hours at 37ºC (Marliza *et al*., 2025).

Observation and Measurement of Inhibition Zones

Observations were made on the media after 24 hours during the incubation period. The inhibition zone, also known as the clear zone, around the disc paper indicates the sensitivity of the bacteria to the antibacterial substance being tested. This inhibition zone is measured by calipers with vertical and horizontal measurements in millimeters.
The diameter of the inhibition zone is measured by the formula:

$$\frac{(DV - DC) + (DH - DC)}{2}$$

Description:
DV: Diameter vertical
DH: Horizontal diameter
DC: Disc diameter (Marliza *et al*., 2025).

The test was carried out 3 times. Whether or not there is a inhibition zone formed around the disc, then the sample used is proven to inhibit the growth of bacteria, if there is no inhibition zone on the sample disc or the substance used then it cannot inhibit the growth of bacteria (Marliza *et al*., 2025).

Table 1. Criteria for bacterial growth inhibition activity

No	Category	Inhibition Zone Diameter
1.	Weak	< 5 mm
2.	Medium	5-10 mm
3.	Strong	11-20 mm
4.	Very Strong	> 20 mm

Source : (Ulfah *et al*., 2022).

Destruction

After all stages of antibacterial activity testing are completed, the remaining media and equipment that have been contaminated with test microorganisms are immediately destroyed to prevent environmental pollution. The destruction process is carried out using an autoclave at a pressure of 15 psi for 20 minutes. This procedure aims to deactivate or kill all surviving bacterial

cells before the laboratory waste is discharged into the final sewer or further managed (Aviana & Ni'am, 2020).

RESULTS AND DISCUSSION

Results

Using green-synthesized Natural Deep Eutectic Solvents (NaDES) along with microwave radiation, the leaves of senduduk were extracted, resulting in a very thick, dark brown liquid. The bright color and thick consistency of the extract mostly show that there are a lot of plant chemicals, like flavonoids and polyphenols, which are commonly found in *Melastoma malabathricum* L. Using Microwave-Assisted Extraction (MAE) greatly improved the breakdown of the plant's cell walls by applying focused heat, which sped up the movement of desired compounds into the NaDES system in a quick amount of time. Keeping the power at 100 Watts helped stop the heat from breaking down these active ingredients, which helped keep the extract's ability to fight bacteria intact.

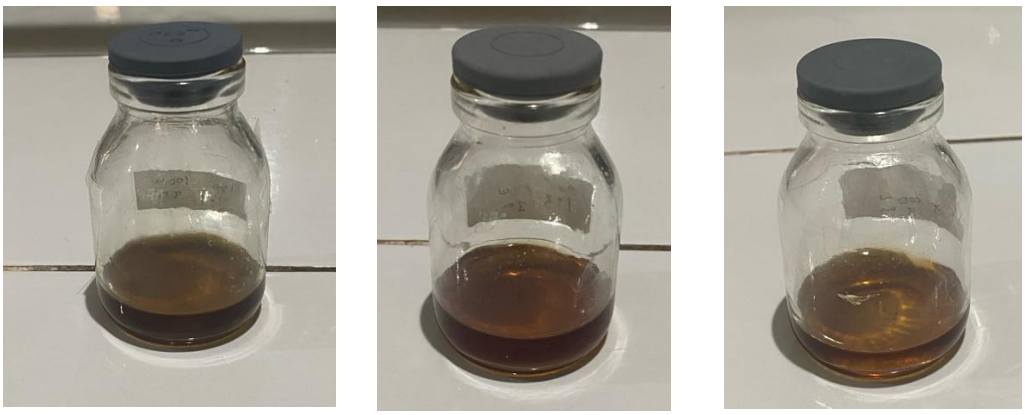


Figure 1. Results of NaDES Extract of Senduduk Leaves

The results of percent transmittance tests on *Staphylococcus aureus* and *Escherichia coli* bacteria using a wavelength of 580nm.

Table 2. Percent transmittance Test Results Based on Wavelength

No	Sample	Wavelength	% Transmit
1.	*Staphylococcus aureus*	580	28.5%
2.	*Escherichia coli*	580	25.7%

The antibacterial test was done using the disc diffusion method.A chloramphenicol disc was used as the positive control, and an empty disc was used as the negative control.The test treatment used a disc made from senduduk leaves (*Melastoma malabathricum* L.) with a molar ratio of 1:1, 1:3, and 1:5. The results from the antibacterial test against Staphylococcus aureus using the extract disc showed that a 1:1 molar ratio fell into the strong inhibition zone, a 1:3 molar ratio also fell into the strong inhibition zone, and a 1:5 molar ratio was in the very strong inhibition zone. In Escherichia coli, on the extract disc, the ratio of 1:1 falls into the category of strong inhibition zone, the ratio of 1:3 also falls into the category of strong inhibition zone, and the ratio of 1:5 falls into the category of very strong inhibition zone.

Table 3. Results of Measurement of Diameter of Seating Leaf Inhibition Zone Against Staphylococcus aureus and Escherichia coli

No.	Test Bacteria	Treatment	Jamming Zone Diameter (mm)			Avarage	SD	Avarage ± SD	Category
			I	II	III				
1	*Staphylococcus aureu*	K(-)	0	0	0	0	0	0 ± 0	Weak
		K(+)	23,25	20	23,85	22,3666667	2,07143268	22,36 ± 2,07	Very Powerful
		1:1	18,05	16,45	14,15	16,2166667	1,96044213	16,21 ± 1,96	Strong
		1:3	22,7	19,05	16,4	19,3833333	3,16319986	19,38 ± 3,16	Strong
		1:5	22,95	23,05	21,4	22,4666667	0,92511261	22,46 ± 0,925	Very Powerful
2	*Escherichia coli*	K(-)	0	0	0	0	0	0 ± 0	Weak
		K(+)	25,7	20,45	23,3	23,15	2,62821232	23,15 ± 2,62	Very Powerful
		1:1	18	7,95	11,5	12,4833333	5,09664923	12,48 ± 5,09	Strong
		1:3	23	15,6	15,8	18,1333333	4,21584313	18,13 ± 4,21	Strong
		1:5	27,6	12,35	22,05	20,6666667	7,71853829	20,66 ± 7,71	Very Powerful

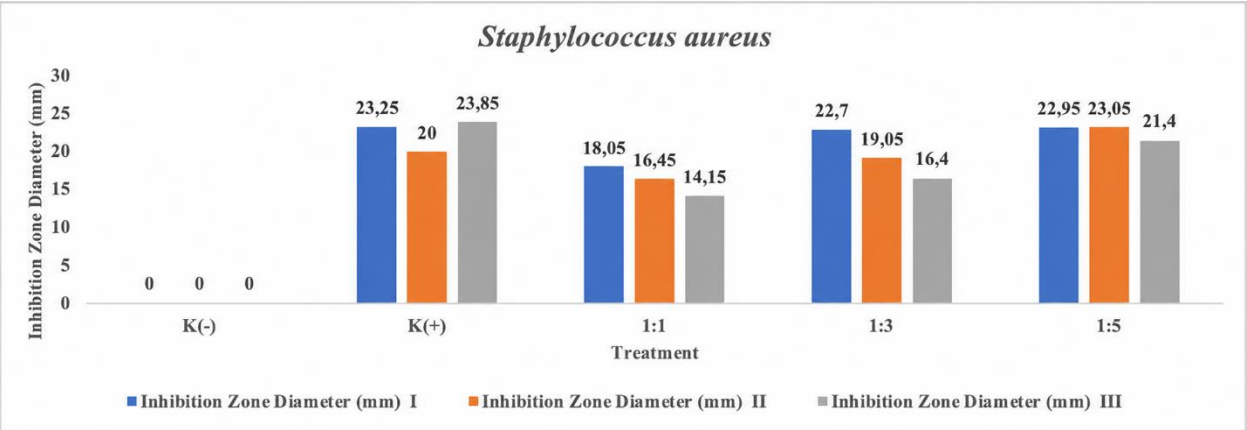


Figure 2. Antibacterial Activity of Senduduk leaves(*Melastoma malabathricum* L.) Against Staphylococcus aureus

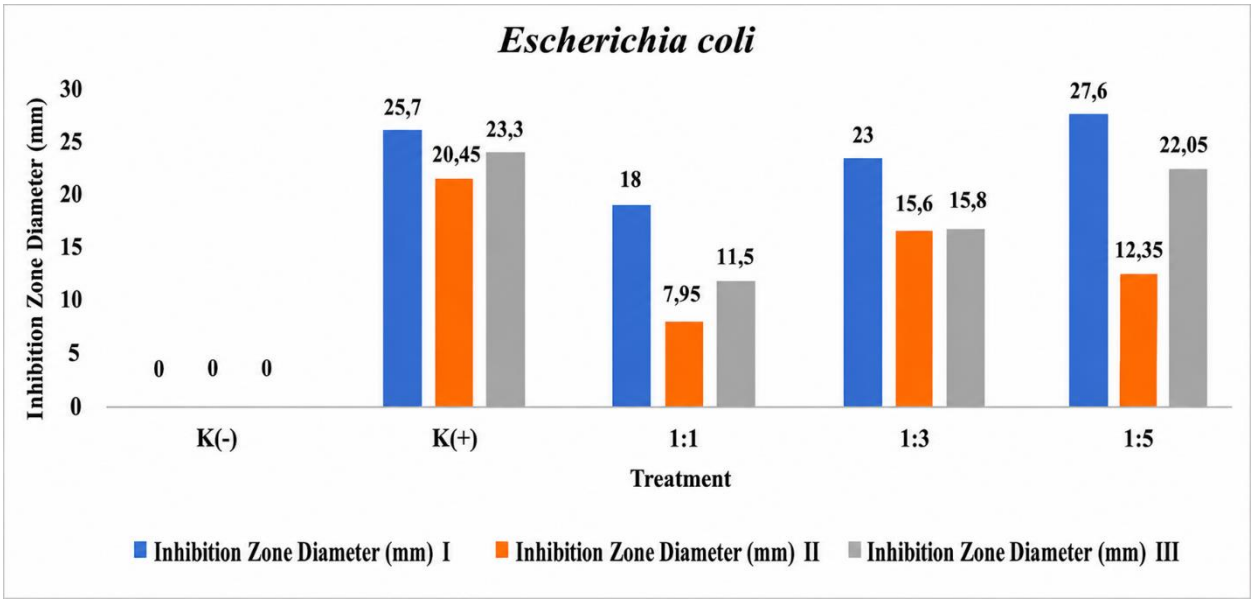
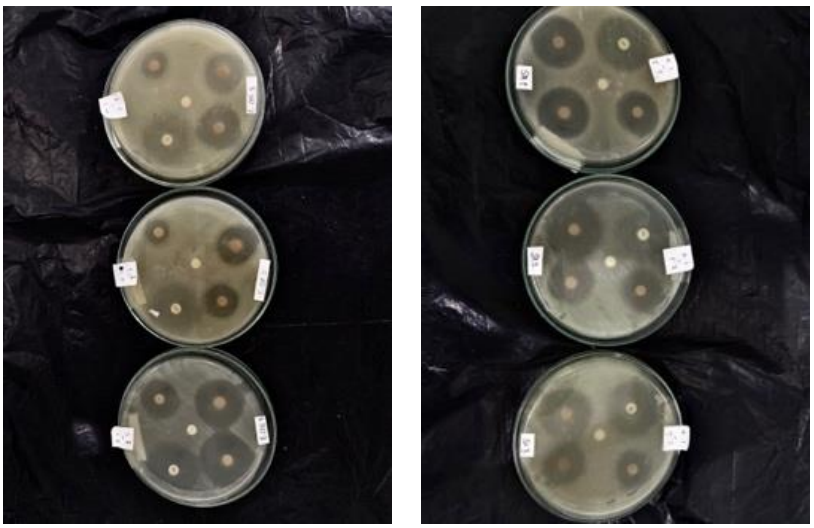


Figure 3. Antibacterial Activity of Senduduk leaves (*Melastoma malabathricum* L.) Against Escherichia coli



Escherichia coli antibacterial test results	*Staphylococcus aureus antibacterial test results*

Figure 4. Antibacterial Test Results

Discussion

In this study, leaves from the senduduk plant, which is called *Melastoma malabathricum* L., were used to make extracts.These extracts were then tested to see if they have antibacterial properties against two types of bacteria, Staphylococcus aureus and Escherichia coli. These two bacteria were chosen as examples to show both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) types. Furthermore, both are notorious human pathogens frequently associated with severe infections, and as highlighted by their increasing antibiotic resistance, finding new plant-derived antibacterial candidates against them is highly critical. Senduduk leaves (*Melastoma malabathricum* L.) were obtained from Mayang Mangurai Village, Alam Barajo District, Jambi Province. The results of the determination carried out at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Sciences, UNPAD, are listed with identification number 93/HB/01/2026. The sample used in this study is indeed the leaf of the species (*Melastoma malabathricum* L.) which belongs to the family Melastomataceae. The determination is carried out with the aim of providing the truth of the identity of the plants used and avoiding errors in the collection of materials and the mixing of plants to be studied with other plants (Klau & Hesturuni, 2021).

The extraction process in this study uses *the Microwave-Assisted Extraction* (MAE) method with *Natural Deep Eutectic Solvent* (NaDES). Based on the research that has been carried out the extraction process for 3 minutes and using 100 watts of power using MAE produces significant physical changes in the NaDES solvent which is initially in the form of a clear colorless liquid and after the extraction process ends, the liquid turns brown. This discoloration indicates that the secondary metabolite compounds of the senduduk leaves (*Melastoma malabathricum* L.) have been maximally dissolved into the NaDES solvent system. In addition to color changes, extraction results are obtained with a thick and homogeneous concentration. This proves that the microwave successfully breaks down the plant cell wall and attracts chemical components without causing clumping or damage to the NaDES extract. This efficiency is much superior to conventional methods (Hikmawati *et al*., 2021). In addition to being superior in time efficiency, NaDES is formed from a mixture of plant primary metabolites such as sugars, amino acids, organic acids, and choline derivatives that are naturally present in living organisms (Hikmawati *et al*., 2021).

In this study, the measurement of the density of bacterial suspensions was carried out using a UV-Vis spectrophotometer at a wavelength of 580nm. The selection of this wavelength is based on the efficiency of light scattering by bacterial cells over the visible spectrum range, which allows for

accurate detection of cell density. Based on the measurement results, the suspension *of Staphylococcus aureus* and *Escherichia coli bacteria* was regulated until it reached a percent transmittance value of 25% (Yulion *et al*., 2023).

In this antibacterial activity test, it was carried out to test the effectiveness of senduduk leaves extract (*Melastoma malabathricum* L.) on NaDES solvent resulting *from Microwave-Assisted Extraction* (MAE) using betaine as *a Hydrogen Bond Acceptor* (HBA) and lactic acid as a *Hydrogen Bond Donor* (HBD) used a variation of extracts with molar ratios of 1:1, 1:3, 1:5, chloramphenicol discs for positive (+) control and empty discs for negative (-) control against *Staphylococcus aureus* and *Escherichia coli.* Based on the results of the study, the results obtained in the *Staphylococcus aureus antibacterial test* on disc extract were a molar ratio of 1:1 from the category of strong inhibition zone with a diameter of 16.21 ± 1.96 mm, a molar ratio of 1:3 from the category of strong resistance zone with a diameter of 19.38 ± 3.16 mm, a molar ratio of 1:5 from the category of a very strong inhibition zone with a diameter of 22.46 ± 0.925 mm, and in the control of positive (+) tests the chloramphenicol disc of the category of the inhibition zone was very strong with a diameter of 22.36 ± 2.07 mm. Then in *Escherichia coli bacteria, the* inhibition zone category in the extract disc ratio was 1:1 in the category of strong inhibition zone with a diameter of 12.48 ± 5.09 mm, the molar ratio of 1:3 in the category of strong inhibition zone with a diameter of 18.13 ± 4.21 mm, the molar ratio of 1:5 in the category of very strong inhibition zone with a diameter of 20.66 ± 7.71 mm, and the positive test control (+) of the chloramphenicol disc in the category of the very strong inhibition zone with a diameter of 23.15± 2.62 mm. This advantage of the 1:5 ratio is hypothesized to be closely related to a potential decrease in viscosity and solvent density. It is known that the viscosity of the NaDES solvent is influenced by the hydrogen bond network between its components, where high viscosity causes the mobility of the molecules to be limited (Rahman *et al*., 2022). With the increase in the concentration of lactic acid as a Hydrogen Bond Donor (HBD) at a molar ratio of 1:5, the interaction between molecules is expected to become more tenuous, potentially decreasing the viscosity. This assumeddecrease in viscosity may enhance the diffusion efficiency and solvent activity against the target metabolite compounds (Schuh *et al*., 2023). This allows solvents at a molar ratio of 1:5 to attract a much more abundant amount of antibacterial active compoundscompared to molar ratios of 1:1 and 1:3 which tend to be thicker.

This efficiency is also supported by the use of *the Microwave Assisted Extraction* (MAE) method. The MAE mechanism involves selective and directed heating so that heat does not escape to the circle and accelerates the extraction process (Datu *et al*., 2019). The process triggers the rupture of the cell wall effectively so that the release of active compounds such as flavonoids and polyphenols takes place optimally in a relatively short time (Schuh *et al*., 2023). In a similar study using the ethanol maceration method with the highest inhibition zone diameter of 19mm with the category of strong inhibition zone (Sapitri *et al*., 2020). And in other similar studies using ethanol solvents, the highest inhibition zone diameter of 14.8mm was obtained with the category of strong inhibition zone (Barung *et al*., 2023). The results of this study show the advantage with the diameter of the inhibition zone (>20mm). This advantage is likely attributed to the use of NaDES solvent technology with a molar ratio of 1:5. Previous studies suggest that a lower viscosity can increase the penetration capacity of the solvent into the plant cell matrix under the influence of microwaves (MAE), which may enhance the extraction of active antibacterial compounds. The synergy between NaDES and MAE exhibits a strong potential to optimize the extraction of active compoundscompared to conventional maceration methods, thereby potentially contributing to a greater disruptive effect on the cell membrane integrity of Staphylococcus aureus and Escherichia coli.

One weakness of this study is that the negative control used was just a blank disc, and they didn't check how well the NaDES solvent itself works against bacteria. Some past research has found that specific NaDES mixtures might naturally have the ability to kill microbes. So, even though the observed inhibition zones show that the NaDES extract has antibacterial activity, it's possible that the NaDES solvent itself might also be contributing to this effect. Future research should include a control group that uses only NaDES to more clearly understand how much each part the solvent and the plant extract contributes.

Implication

The findings of this study demonstrate that the combination of a zwitterionic Natural Deep Eutectic Solvent (NaDES) and Microwave-Assisted Extraction (MAE) represents a promising green extraction approach for obtaining antibacterial compounds from *Melastoma malabathricum* L. leaves. The strong antibacterial activity observed against *Staphylococcus aureus* and *Escherichia coli* suggests that this extraction method has the potential to be applied in the development of environmentally friendly herbal antibacterial products. Furthermore, the use of biodegradable and low-toxicity solvents supports sustainable extraction practices while reducing the reliance on conventional organic solvents.

Research Contribution

This study helps move forward green extraction methods by showing that using a zwitterionic NaDES system along with MAE can better increase the antibacterial properties of the leaf extract from *Melastoma malabathricum* L. The study also gives scientific proof about how well different NaDES ratios work to kill bacteria, especially showing that the 1:5 ratio is the best. These results could help guide future research on using green solvents, improving methods for extracting herbs, and creating natural antibacterial products.

Limititations

This study was limited to the evaluation of the antibacterial activity *of Melastoma malabathricum* L. leaf extract obtained using zwitterionic NaDES and Microwave Assisted Extraction under the extraction conditions applied in this study. Antibacterial assessments are only performed against Staphylococcus aureus and Escherichia coli, which may not fully represent the spectrum of activity against other clinically relevant microorganisms. Further, the study did not optimize for additional extraction parameters, such as microwave power, extraction time, or solvent composition beyond the selected NaDES molar ratio. Therefore, the results obtained must be interpreted within the scope of the experimental conditions used.

Suggestion

Future studies should look into how well the extracts work against a wider variety of Gram-positive and Gram-negative bacteria, including those that are resistant to many antibiotics. It is also suggested to adjust factors like microwave power, how long the extraction takes, the type and amount of solvent used, and the ratio of solid material to solvent, in order to get the most bioactive compounds possible. Also, more research is needed to check the safety, how well it holds up over time, and how it can be made into a product to help in making it a natural antibacterial for use in medicine or healthcare.

CONCLUSION

When leaves from *Melastoma malabathricum* L. were extracted using Microwave-Assisted Extraction with a natural deep eutectic solvent made from betaine and lactic acid, the resulting extract showed the ability to fight off bacteria like *Staphylococcus aureus* and *Escherichia coli*. Among the tested formulas, the NaDES system with a 1:5 ratio made the biggest area where bacteria couldn't grow and was labeled as having a "Very Strong" antibacterial effect. Based on previous reports, this enhanced activity may be related to favorable physicochemical characteristics of the NaDES system that improve extraction efficiency; however, these mechanisms were not directly investigated in the present study. Because only a blank disc was used as the negative control, the possible contribution of the NaDES solvent to the observed antibacterial activity cannot be completely excluded. Future studies should include NaDES-only controls, phytochemical characterization, and direct physicochemical measurements to clarify the mechanisms underlying the observed antibacterial activity.

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AUTHOR CONTRIBUTION STATEMENT

MKA is responsible for research design, experimental implementation, data collection, data analysis, manuscript preparation, and article revision. N contributes to research conceptualization, research supervision, method validation, data interpretation, and manuscript review. MKA and N contributes to research supervision, evaluation of results, critical review of manuscripts, and final approval of manuscripts for publication. All authors have made significant contributions to the research and approved the final published manuscript.

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