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Optimization microwave-assisted extraction (MAE) to obtain total phenol from *Ampelocissus thyrsiflora* (Blume) Planch leaves for antibacterial activity response of *Staphylococcus Epidermidis*

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Abstract *Ampelocissus thyrsiflora* (Blume) Planch. is a traditional North Sumatra medicinal plant that can be used to cure various ailments, such as wounds, diarrhea, infections, and as a stamina enhancer. *A. thyrsiflora* leaves contain secondary metabolite compounds such as alkaloids, flavonoids, tannins, and saponins, which have antibacterial properties. The extraction method used was microwave-assisted extraction (MAE) using ethanol solvent and different times and power extractions. The extraction process was optimized to obtain the optimum extraction conditions. Optimum extraction conditions can be developed as standardized herbal medicines. The method started with phytochemical screening and characterization of simplicia powder, extraction using MAE with 96% ethanol solvent with power variations of 180 watts, 300 watts, 450 W, and time variations of 3 min, 7 min, 15 min, determination of total phenol, and testing of antibacterial activity from optimization results using paper discs. The optimum extract yield was 20,20% at 450 watts of power and 15 min of time. Total phenol with optimum power and time of 232,0088±4,54 mg GAE/g sample was obtained from an ethanol extract of *A. thyrsiflora* leaves with 300 watts and 7 minutes is 6.25 mg/mL has an inhibition zone is 7,93±0,81 mm as bacterial minimum inhibitory concentration (MIC) and does not have minimum bacteria concentration (MBC) *of Staphylococcus epidermidis* bacteria. Ethanol extracts of *A. thyrsiflora* leaves with different extraction powers and times affected the yield of the extracts and total phenol. The antibacterial activity of the optimization result of ethanol extract of *A. thyrsiflora* leaves with the highest total phenol had MIC, but did not have MBC.

Keywords: *Ampelocissus thyrsiflora*, MIC, MBC, MAE, phenol

1. Introduction

Infectious diseases are a major health concern in developing and industrialized nations worldwide (Scott, 2020). Bacteria are microorganisms that can cause infections, such as *Staphylococcus epidermidis* (Garcia et al., 2020). *S. epidermidis* is a gram-positive bacterium (Otto, 2009) and the most common bacterial colonizer of healthy human skin (Brown, 2020). Gram-positive bacteria have a thick peptidoglycan, to which the cytoplasmic membrane is attached (Tavares et al., 2020). Although the pharmaceutical industry has produced several new antibiotics, there has been an increase in the resistance of microorganisms to these drugs. Therefore, there is an urgent need for alternative medicines (Nascimento et al., 2000).

One of the local plants from North Sumatra used in traditional medicine is *Ampelocissus thyrsiflora* (Blume) Planch.) It belongs to the *Vitaceae* family. Traditionally, the leaves of A. can treat various diseases such as diarrhea, stamina enchancer, wound infection, and diabetes (Silaban et al., 2015). Alkaloids, flavonoids, tannins, and saponins are secondary metabolites found in the leaves of *A. thyrsiflora* (Syamsul et al., 2022). These leaves exhibit antibacterial activity. Phenol is the most abundant secondary metabolite in plants. Phenol compounds in plants can be in the form of phenols, phenolic acids, tannin lignins and flavonoids (Oliver et al., 2001). Phenol has demonstrated broad-spectrum antibacterial activity against both gram-negative and gram positive bacteria. At high levels, phenol can penetrate and disrupt the bacterial cell wall. At lower levels, phenols in bacterial cells can activate important enzymes (Godstime et al., 2014).

Previous research from Directorate General of Intellectual Property Pranoto et al, 2022 the antibacterial activity of *Vitis gracilis* Wall ethanol extract with the results of the extract's inhibition zone against *Staphylococcus aureus* germs respectively being 16.63 mm (100 mg/mL), 15.96 mm (80 mg/mL), 14.30 mm (60 mg/mL), 23.36 mm (40 mg/mL) into the range of strong

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to extremely strong. This indicated that the extract has the potential to act as an antibacterial agent. The antibacterial activity of *A. thyrsiflora* extract against Staphylococcus epidermidis was analyzed to determine the MIC and MBC. Although previous studies may have assessed the antibacterial properties of the same plants, this study relates the influence of extraction parameters to quantitative analysis of total phenols and specific antibacterial activity.

One type of withdrawal of active compounds is by extraction using appropriate solvents such as microwave-assisted extraction (MAE) (Chung, et al., 2011). This method has advantages in comparison to other extraction methods that reduce extraction time and use less solvent to obtain a fast extraction result (Ondruschka & Asghari, 2006). The goal of optimization is to obtain the best extraction conditions, which can be affected by factors such as temperature, extraction time, type of solvent, microwave power, solvent, solvent concentration, ratio, and material size [Achmad & Mahfud, 2021), (Rahmawati, 2021). The optimum extraction conditions can be developed as a standardized herbal medicine that can be consumed by people (Lopez et al., 2023).

The power used in this research was 180, 300, and 450 watts with times of 3, 7, and 15 min, respectively. Based on previous research, the higher the microwave power used, the higher the extraction yield; however, extended time duration will cause the degradation of bioactive compounds (Alishlah, et al., 2018). The novelty of this research is that the use of MAE with optimal parameters through variations in power and time is very relevant and innovative to accelerate and increase the efficiency of extracting high quality bioactive compounds with maximum results, especially in less explored plant species such *as A. thyrsiflora*. Researchers are interested in optimizing the extraction process using the MAE method and antibacterial activity testing of the optimized results of the ethanol extract of *A. thyrsiflora.*

2. Materials and Methods

2.1. Material

The materials used included Clindamycin DA2 (Oxoid CT0064B), Dimethyl Sulfoxide (KGaA Darmstadt, Germany), Folin-Ciocalteu's phenol (KGaA Darmstadt, Germany), Gallic Acid (Sigma-Aldrich, USA), Nutrient Agar (KGaA Darmstadt, Germany), Nutrient Broth (KGaA Darmstadt, Germany), and Staphylococcus aureus bacterial culture. The equipment used in this study was a rotary evaporator, Microwave (Samsung), Spectrophotometer uv-vis (Shimadzu), Vernier Calipers (Tuffware), Ose Needle, Micropipette (Eppendorf), microscope (Primovert), and laminar airflow cabinet (Astec HlF 1200n L).

2.2. Plant materials

A. thyrsiflora leaves are medicinal plants collected from Telagah, Sei Bengai, Langkat, North Sumatera, Indonesia (National Research and Innovation Agency number B-1431/II.6.2/IR.01.02/5/2024, Scientific Collection Management Directorate, Central Jakarta, Indonesia).

2.3. Macroscopical and microscopicale

Macroscopic examination was performed by observing the shape, size, surface, color, odor, and taste *A. thyrsiflora* leaves. Microscopic investigation was carried out by observing the leaves of *A. thyrsiflora* under a microscope using chloralhydrate and warming gently over the bunsen flame (Shewale et al., 2022)

2.4. Characterization of Ampelocissus thyrsiflora

The dried samples were characterized to determine their quality. Characterization includes the determination of water-soluble essence, water, moisture, acid-insoluble ash , ethanol-soluble ash, and total ash contents (Depkes, 1995).

2.5. Phytochemical screening of simplicia and extract A. thyrsiflora

Phytochemical screening was performed to determine the biologically active secondary metabolites. A reagent containing amyl alcohol, magnesium powder, and concentrated hydrochloric acid (concentrated) was used to detect the flavonoids. Alkaloids were discovered using the reagents provided by Mayer, Bouchardat, and Dragendorf. Saponins were identified using foam tests. The iron (III) chloride reagent helped identify tannins, whereas the Liebermann-Burchard reagent identified the presence of steroids or terpenoids (Anyanwu et al., 2022) (Doss, 2009).

2.6. Plant collection and extraction using MAE of A. thyrsiflora

A drying cabinet was used to chop and dry the leaves. The sample was weighed, dried, mashed into a powder, and stored (Khaerunnisa, 2021). In this research, 300 grams simplicia powder of *A. thyrsiflora* put into a round bottom flask then add ethanol solvent 96% as much as 300 mL and put into a microwave. The power used are 180 watts, 300 watts, and 450 W, with the time needed to extract 3, 7, and 15 min. This variation is important to assess the relationship between power and time parameters on extraction results by evaluating the optimum conditions that provide the best extraction results and do not damage bioactive compounds. The device was run until the specified time, and automatically stopped after the extraction

was complete. The round-bottomed flask was then removed from the microwave and left to stand until it reached room temperature. The *first* solution was filtered to obtain the first filtrate and residue. The residue was re-soaked in a new solvent and extracted thrice. After completion, the filtrates were pooled at each power and time variation, thickened using a rotary evaporator, and kept in an oven at 50°C (Alam et al., 2019).

2.7. Total phenol content

Using Folin-Ciocalteu reagent and gallic acid for comparison, the total phenol content of the ethanol extract of *A. thyrsiflora* leaf was measured spectrophotometrically. The principle of this method is the reaction between phenolic compounds in the sample and Folin-Ciocalteu reagent in the presence of sodium carbonate to form a blue-colored complex (Steve et al., 2017).

2.8. Determination of maximum wavelength of gallic acid

Put 0.2 mL was added to a 5 mL volumetric flask and diluted with methanol pro analysis until a concentration of 20 µg/mL was obtained. Then a solution of 0.5 mL was after that adding 0.5 mL of Folin-Ciocalteu reagent vortexed for 1 minute, and leave for 5 minutes. Subsequently, 1 mL of 10% sodium carbonate was added. The wavelength was measured using a visible spectrophotometer in the range of 400 nm-800 nm (Soekamto & Syah, 2021).

2.9. Gallic acid calibration curve

The standard solution was pipetted into a 5 mL flask to prepare concentrations of 10, 20, 30, 40, and 50 µg/mL. Next, 0.5 mL of the solution was transferred to a test tube, followed by the addition of 0.5 mL of Folin-Ciocalteu reagent. The mixture was vortexed for 1 minute and allowed to sit for 5 minutes. Afterward, 1 mL of 10% sodium carbonate was added, and the solution was shaken until homogeneous. It was then incubated for a set period, after which absorbance was measured at the maximum wavelength. A calibration curve was constructed using the regression equation $y=bx+a$, based on the maximum absorption wavelength of nm (Soekamto & Syah, 2021).

2.10. Total phenol content of ethanol extract of A. thyrsiflora

Ten milligrams of the extract were dissolved in 1 mL of methanol and subsequently diluted with distilled water to a final volume of 10 mL, resulting in a concentration of 1000 µg/mL. From this solution, 1 mL was taken and further diluted with 5 mL of water to achieve a concentration of 200 µg/mL. A 0.5 mL aliquot of the sample was mixed with 0.5 mL of Folin-Ciocalteu reagent in a test tube. The mixture was vortexed for 1 minute and left to stand for 5 minutes. Then, 1 mL of 10% sodium carbonate was added, and the solution was left to react. Absorbance was measured using a visible spectrophotometer at a specific wavelength (Ezez & Tefera, 2021). All tests were conducted in triplicate. Each sample was measured twice, and the average was calculated. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of the sample (Baek et al., 2021).

2.11. Minimum inhibitory concentration test (MIC)

This antibacterial activity test was performed using paper discs using the diffusion method (Humphries et al., 2018) Pipetted 0.1 mL the Staphylococcus epidermidis inoculum and then put in a Petri dish. each. Then, 15 mL of sterile Mueller hinton that had been thawed (45–50°C) was added. 50°C). Next, the petri dish was homogenized, and after the media had solidified, paper discs containing positive control were saturated with the concentration of ethanol extract and negative control. The positive control used was a clindamycin disc 2 mcg, and the negative control used was DMSO. The cells were incubated at 37°C for 18-24 hours under aerobic conditions. After the incubation period, the diameter of the inhibitory zone was measured to determine the minimum inhibitory concentration. All experiments were independently repeated three independent times (Salem et al., 2021).

2.12. Minimum bactericidal concentration (MBC)

This test was carried out using the solid dilution method by scratching the clear zone in the MIC test using a sterile ose, then scratched into new media, and incubated for 18 to 24 h to observe the presence or absence of bacteria. MBC is characterized by the absence of bacterial growth as a result of scratches in a Petri dish (Parvekar et al., 2020).

3. Results

3.1. Macroscopic and microscopic examination

Macroscopic examination results can be seen by observation of the fresh leaves (Figure 1), which have an elliptical oval shape with tapered and serrated leaf tips, shiny dark green color on the upper surface, light brown and smooth haired leaf on the lower surface, 20-26 cm long and 8-10 cm wide, smells typical of leaves, bitter taste, and feel itchy. The microscopic results

of the samples showed the presence of epidermis, palisade, parenchym, anomocytc stomata, xylem, phloem, essential oils, and unicellular trichomes (non-glandular).

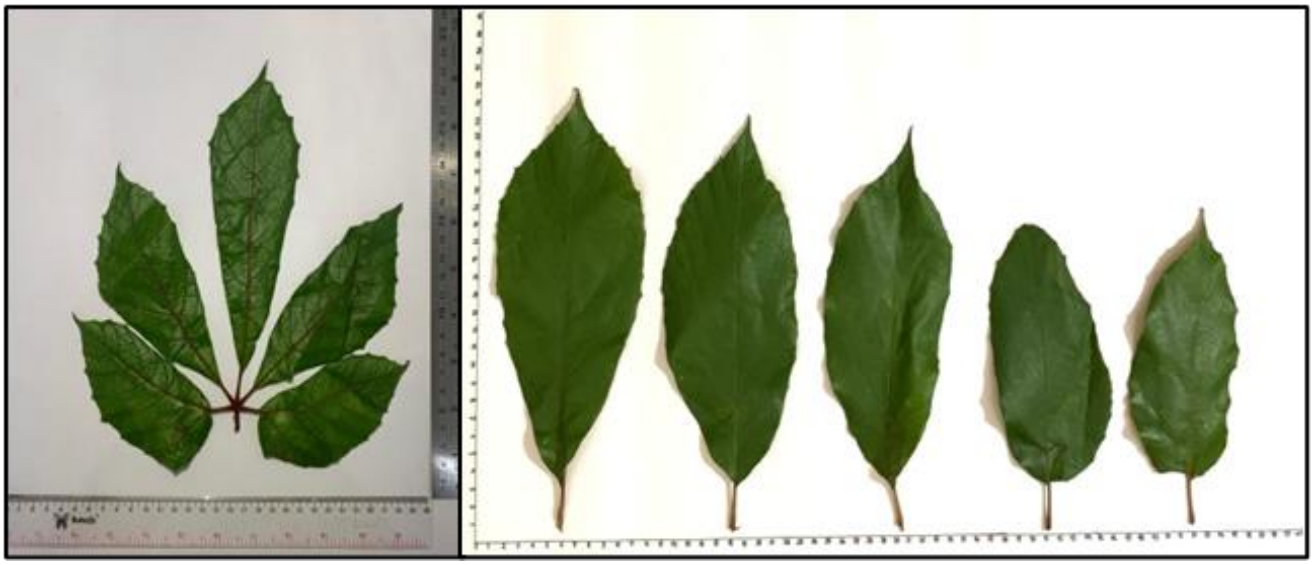

Figure 1 *Ampelocissus thyrsiflora* (Blume) Planch. Leaves.

3.2. Characterization result

Characterization of *A. thyrsiflora* (Blume) Planch. simplicia powder is ethanol water-soluble, water soluble, extractive, insoluble in acid ash, and total ash. The characterization results are presented in Table 1.

Table 1 Simplicia powder sample characteristics *Ampelocissus thyrsiflora* (Blume) Planch of leaves.

Parameters	Results (%)
Ethanol water soluble content	8.38
Water content	6.60
Water soluble extractive	11.21
Insoluble in acid ash	1.52
Total ash	3.82

3.3. Phytochemical screening of simplicia powder and extracts

Secondary metabolites were determined by phytochemical screening, which showed biological activity in the simplicia powder and ethanol extracts of *A. thyrsiflora* (Blume) Planch leaves. The screening results are shown in Table 2.

Table 2 Phytochemical Screening Results of Simplicia Powder and Ethanol Extracts.

Compound Group	Simplicia powder	Ethanol Extract (450 power, 15 minutes)
Flavonoids	+	+
Alkaloids	+	+
Steroids	+	+
Saponins	+	+
Glycosides	+	+
Tannins	+	+

Description:

(+) = Positive (contains a class of compounds).

(−) = Negative (does not contain a compound class)

The table shows that simplicia powder and ethanol extract contain secondary metabolites such as flavonoids, alkaloids, glycosides, tannins, saponins, and steroids.

3.4. Extraction of A. thyrsiflora

After extraction of 300 g of simplicia powder in 300 mL of ethanol solvent, the results in Figure 2 were concentrated in a rotary evaporator and in a water bath at 40-50^{o}C to produce a thick extract. The optimum yield was 20.2% at 450 watts of power and 15 min of time. The high power and high temperature cause many cells to be damaged and broken. The longer

extraction time, the longer the contact of the material with the solvent so that more active compounds are obtained (Tatke & Jaiswal, 2011).

3.5. Results of the maximum wavelength of gallic acide

The maximum wavelength measured using the gallic acid standard was 738 nm, and the operating time was obtained–54-59 minutes. The operating time is the time at which the solution begins to produce stable absorbance values. Before measurement, it was incubated during the operating time.

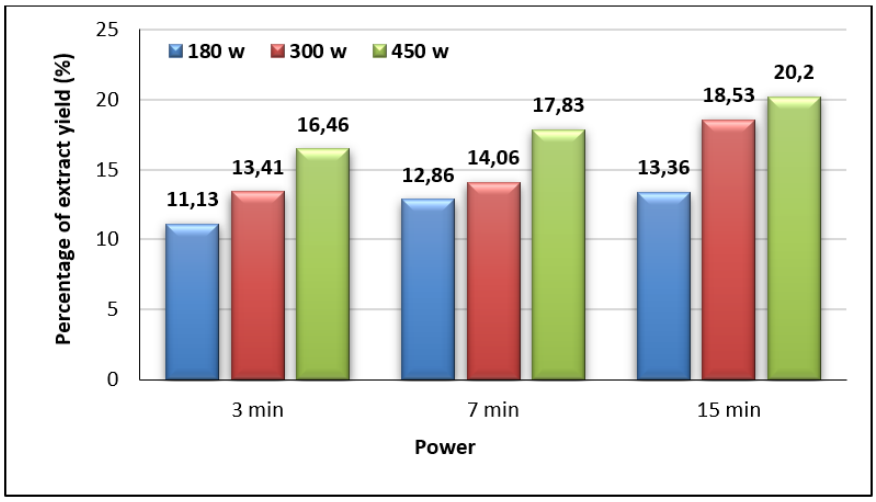

Figure 2 Percentage of extract yield of *A. thyrsiflora* leaves.

3.6. Gallic acid calibration curve results

The absorbance of the solutions at concentrations of 10, 20, 30, 40, and 50 µg/mL at a wavelength of 738 nm was used to create a calibration curve can be seen in Figure 3. A calibration curve was obtained from the relationship between various gallic acid levels and absorbance can be seen in Table 3. The r^2 value was obtained from a calibration curve. The r^2 value is between 0 and 1, which indicates the closeness of the value of the estimates for the regression analysis that represent the actual data. From the calibration curve, the regression equation was y = 0.0174x + 0.0551 with r^2 = 0.9926.

Table 3 Absorbance of Gallic Acid.

Concentration (µg/mL)	Absorbance
0	0
10	0.2571
20	0.4407
30	0.5924
40	0.7698
50	0.8808

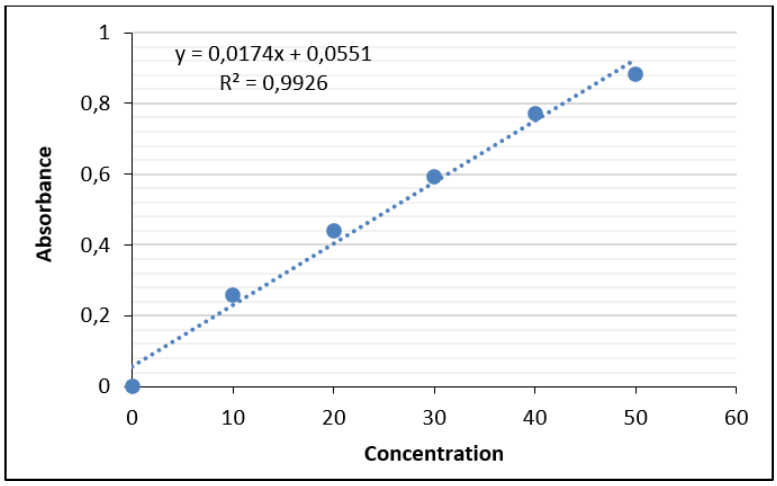

Figure 3 Curve Calibration of Gallic Acid.

3.7. Result of total phenol content of ethanol extract of A. thyrsiflora

The linear regression equation y = ax + b from the gallic acid calibration curve was used to calculate the total phenol content, and the concentration (x) was obtained by substituting the absorbance value (y) of the ethanol sample extract at the maximum wavelength. The value of x was then substituted into the formula to calculate the total phenol content can be seen in Table 4.

Table 4 Total Phenol Content of Ethanol Extract of *A. thyrsiflora*

Sample	Total Content Phenol (mg GAE/g sample) ± SD
180 watt, 3 minutes	155.1743 ± 1.97
180 watt, 7 minutes	167.0218 ± 1.52
180 watt, 15 minutes	137.5247 ± 2.20
300 watt, 3 minutes	172.7502 ± 2.01
300 watt, 7 minutes	232.0088 ± 4.54
300 watt, 15 minutes	167.5690 ± 2.85
450 watt, 3 minutes	177.2727 ± 3.94
450 watt, 7 minutes	194.0312 ± 2.88
450 watt, 15 minutes	132.1606 ± 1.23

The data analysis using SPSS 22 software by carrying out a normality test using *Shapiro-Wilk* showed that the data was not normally distributed for the 180 watt (p=0.200), 300 watt (p=0.003) and 450 watt (p=0.031) groups and extraction time with 3 minutes (p=0,090), 7 minute (p=0.131), 15 minute (p=0.009). Therefore, the analysis was continued with the *Kruskal-Wallis* non-parametric test. The results of the *Kruskal-Wallis* test show to retrieved that difference between the effect power on total phenol content with significantly 0.022 ($p<0.05$). The effect of time on total phenol content was 0.001 ($p<0.05$). According to these findings, the power and extraction time have a significant impact on the total phenol content.

3.8. Results of minimum inhibitory concentration of ethanol extract A. thyrsiflora (EEATB)

Antibacterial activity tests were carried out on EEATB with an optimum power and time of 300 watts and 7 min, respectively. A high total phenol value will produces bioactive compounds with better antibacterial activity (Johari & Khong, 2019). The product yields are listed in Table 5.

Table 5 MIC of Ethanol Extract *A. thyrsiflora.*

Concentration (mg/mL)	Diameter of inhibitory zone (mm)	Category
400	15,13±0,81	Strong
200	12,97±0,49	Strong
100	11,97±0,31	Strong
50	11,83±0,21	Strong
25	11,66±0,64	Strong
12.5	10,47±0,85	Strong
6.25	7,93±0,81	Medium
Clindamycin disc 2 mg (control +)	19,6 ± 0,68	Strong
Dimetyl sulfoxide (control -)	-	No activity

Data analysis using SPSS 22 software by carrying out the normal distribution test (*Shapiro-Wilk*) then One Way Anova statistical test to revealed the effect of various concentrations of *extract A. thyrsiflora* leaves on the diameter of the inhibitory zone. The results were significant 0.000 ($p<0.05$), showing a significant difference between various concentrations of the ethanol extract *A. thyrsiflora* for the diameter of the inhibitory zone.

3.9. Results of minimum bactericidal concentration (MBC) of ethanol extract A. thyrsiflora

The minimum bactericidal concentration is the lowest level or concentration of antimicrobials that can kill 99.9% of the microorganisms after incubation for 24 h under appropriate conditions. The results are presented in Table 6.

Table 6 MBC of ethanol extract *A. thyrsiflora.*

Concentration (µg/mL)	Results
400	Bacterial growth
200	Bacterial growth
100	Bacterial growth
50	Bacterial growth
25	Bacterial growth
12.5	Bacterial growth
6.25	Bacterial growth

4. Discussion

Based on the results of the research conducted, it was found that extract *A. thyrsiflora* which has an optimum total phenol content of 232.01 ± 4.54 mg GAE / g sample uses 300 watts of power and 7 minutes. According to previous research, Purbowati et al., (2018) stated that the phenol content will increase from 100, 175, 250, 325 W of power but will decrease at 400 W of power due to increasing temperature. According to previous research, Magdalena and Kusnadi (2015) stated that the phenol content will increase along with the increasing power used, namely 320 W, 560 W but will decrease at 800 W of power because the treatment of power that is too high can cause excessive heat energy so that the phenol compound is damaged. Based on the table 5, the optimum total phenol using 300 watt of power and 7 min of time is 232.0088 ± 4.54 mg GAE/g sample. The results demonstrated that microwave power influenced the total amount of phenol in the sample. The higher the energy used, the higher the microwave intensity, which produces more heat and increases the temperature. Too much energy also lowers the phenol content because the temperature is too high, which could damage the components of the sample (Antony & Farid, 2022). According to previous research, high-temperature heating can cause damage to components that cannot resist heat, including phenol compounds (Gunalan et al., 2022). Phenol compounds are sensitive to temperature changes; therefore, processing at different temperatures from the power used will affect phenol content. The phenol content will increase with a long extraction time, but at a certain point, it will decrease (Zin et al., 2020).

According to the data of antibacterial activity testing, extract *A. thyrsiflora* has antibacterial activity with a MIC at a concentration of 6.25 mg/mL with the diameter of inhibitory zone diameter of 7.93 mm with moderate strength. MIC is the concentration of a microbial agent that can prevent the accretion of microorganisms (Kowalska et al., 2021). The category for antibacterial power based on the diameter of the inhibitory zone formed was divided into four categories: < 5 mm (weak), 5–10 mm (medium), 10–20 mm (strong), and > 20 mm (very strong) (Rakatama et al., 2018). The results showed that the diameter of the inhibitory zone was directly proportional to the increase in the extract concentration. The higher concentration of extracts will produce a larger diameter of the inhibitory zone because more extracts that are antibacterial accumulate on the growth medium so that they can interfere with the process of bacterial growth (Haghgoo et al., 2017). Dimethyl sulfoxide was used as the research is dimethyl sulfoxide (DMSO). DMSO was also used as a solvent to dissolve various concentrations of the test solutions. DMSO does not prevent the accretion of bacteria; therefore, it will not affect the observations when conducting antibacterial activity tests (Saad et al., 20212). The positive control used clindamycin disc 2 mg. Clindamycin was used to compare whether the ethanol extract at various concentrations can be used as a test solution with antibacterial activity. In this study, a clear zone was obtained around the paper, blocking the positive control against Staphylococcus epidermidis bacteria by 19.6 mm with a strong category. This indicates that clindamycin effectively inhibits bacterial growth (Jubeh et al., 2020).

The MBC in the antibacterial activity test of the ethanol extract *A. thyrsiflora* leaves against *S. epidermidis* did not have a minimum bacterial killing capacity. The ethanol extract *A. thyrsiflora* leaves is known to be bacteriostatic against bacterial growth and does not have bactericidal properties (Mogana et al., 2020). The minimum killing concentration is the lowest level or concentration of antimicrobials that can kill 99.9% of microorganisms after incubation for 24 hours under appropriate conditions (Deradjat et al., 2019). Based on this, it is known that the ethanol extract of tiger gagatan leaves is only bacteriostatic against bacterial growth and does not have bactericidal properties or kill bacteria as a whole.

5. Conclusions

The increase in the power used and a longer extraction time resulted in a higher ethanol yield. The optimum power and watt were found to be high total phenol (300 W) and 7 min (232.0088±4.54 mg GAE/g). The optimization results of ethanol extract of *A. thyrsiflora* leaves have MIC activity against Staphylococcus epidermidis bacteria with concentration 6.25 mg/mL and diameter of inhibitory zone 7.93 ± 0.81 mm and does not have MBC.

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Ethical considerations

Not applicable.

Conflict of Interest

The authors declare there is no conflict of interest.

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