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Antibacterial Activity of *Pennisetum purpureum* CV Extracts from Microwave-Assisted Extraction and Maceration Methods

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ABSTRACT

Pennisetum purpureum cv. Mott contains secondary metabolites such as flavonoids, tannins, saponins, and alkaloids, which are suspected to have antibacterial potential. This study was aim to test the antibacterial activity of *P. purpureum* ethanol extract against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion and dilution methods. To obtain a valid data, this study employed both qualitative and quantitative methods. The sample used was a thick extract of *P. purpureum* extracted using maceration and Microwave-Assisted Extraction (MAE) methods. The obtained Inhibitory Zone Width data were analyzed using One-Way ANOVA and Duncan's follow-up test. The results of the qualitative phytochemical test showed that the *P. purpureum* extract with the maceration and MAE extraction methods positively contained secondary metabolite compounds such as flavonoids, tannins, saponins, and alkaloids. The antibacterial test showed that the extract with the MAE method at a concentration of 40 % provided the highest inhibition zone width against *S. aureus* (15.36 mm) and *E. coli* (16.68 mm), both of which were in the strong category. As conclusion, the MAE extraction method is the most effective method in producing *P. purpureum* extract that has active compounds as antibacterials.

Keywords: antibacterial; inhibitory zone; maceration; MAE; *Pennisetum purpureum*

INTRODUCTION

Diarrhea is one of the most common infections in adults and children (Manetu et al., 2021). In Indonesia, diarrhea is classified as an endemic disease. One of the bacteria that causes diarrhea is *Staphylococcus aureus* and *Escherichia coli* (Anggraini & Kumala, 2022). Antibiotics are drugs frequently used to treat this problem. Common antibiotic therapies include ampicillin, cortimoxazole, trimethoprim, azithromycin, and gentamicin (Sartika et al.,2020).

P. Purpureum, known locally as "rumput odot" is a green plant that commonlu used used for animal feed and grows in tropical environments (Qohar et al., 2021). *P. purpureum* contains quite high levels of primary metabolites, such as protein, fat and carbohydrates. *P. purpureum* also contains secondary metabolites.

Maceration is an extraction technique by soaking (Septian et al., 2022). The principle of the maceration method is the extraction of active substances which is carried out by soaking in a suitable solvent for several days at room temperature and protected from exposure to light (Saepudin et al., 2024). The Microwave Assisted Extraction (MAE) method is an extraction method that uses microwave radiation to accelerate selective extraction by heating the solvent quickly and efficiently (Utami et al., 2020). The principle of the MAE method is based on the direct effect of microwaves on the molecules of a material. Ionic conduction and dipole rotation convert electromagnetic energy into heat in the solvent and sample (Sulistiyono et al., 2018).

The purpose of using/comparing maceration with MAE is to determine which extraction method produces the greatest antibacterial power. The maceration extraction method (cold method) and MAE (hot extraction) will produce different extracted

secondary metabolites. *S. aureus* and *E. coli* were used as objects in this study because they easily stick to the skin, although other bacteria are also dangerous and endemic. The solvent used in extraction is 96 % Ethanol, because 96 % ethanol plays an important role in dissolving chemical compounds that are difficult to dissolve in water.

METHODS

Tools and Materials

The tools used in this study were glassware (Pyrex®), aluminum foil, autoclave (Clinoclave®), 40 mesh sieve (CBN), blender (Philip®), Bunsen burner, suction bulb (Bulp), brown bottle, steam dish, petri dish (Pyrex®), Erlenmeyer flask (Pyrex®), hot plate, incubator (Nuve®), cotton, batis cloth, filter paper (Whatman®), porcelain crucible, volumetric flask (Pyrex®), laminar air flow (LAF), microwave (Samsung®), micropipette (Gilson®), oven (Memmert®), round loop, dropper pipette, volume pipette (Iwaki®), water bath, tweezers, ratory evaporator (IKA®), test tube (Pyrex®), furnace, analytical balance.

The materials used in this study were Sulfuric Acid (H_2SO_4), Aquadest, *S. aureus*, *E. coli* Bacteria, Dimethyl Sulfoxide (DMSO), Ciprofloxacin 500 mg, Ethanol 96 %, $FeCl_3$, HCl, $BaCl_2$ Solution 1 %, NaCl Solution 0.9 %, Nutrient Agar Medium (NA), Mayer Reagent, Wagner Reagent, Dragendorff Reagent, *P. purpureum*, Mg Powder. All material obtained from marketplace accompanied with certificate of analysis.

Preparation of *P. purpureum* Dry Powder

The collected *P. purpureum* leaves were wet sorted and then washed under running water. They were then chopped to facilitate the drying process, oven-dried at 50 °C until dry. After drying, dry sorting was performed to separate the samples from remaining impurities and burnt samples. The dried leaves were then powdered using a grinding machine, sieved through a 40-mesh sieve, weighed, and packaged.

Extraction process of *P. purpureum* Extract

Maceration Extraction

About 100 g of *P. purpureum* powder was placed in a closed container and soaked in 1000 mL of 96 % ethanol, then left for 3 days at room temperature with occasional stirring. The filtrate and residue were separated with filter paper, then the filtrate was collected and evaporated using a rotary evaporator at 40 °C (Sukasih et al., 2020).

Microwave Assisted Extraction (MAE)

The *P. purpureum* plant powder was weighed as much as 100 g and 1000 mL of 96 % ethanol solvent. Then the simplicia powder and 96 % ethanol were put into 2 500 mL Erlenmeyer flasks, each containing 50 g of plant powder and 500 mL of 96 % ethanol with a sample: solvent ratio of 1:10. Then put the Erlenmeyer into a 600 watt microwave for 4 minutes 30 seconds alternately. The solution was irradiated in the microwave periodically (irradiation for 30 seconds and 2 minutes off) to prevent the temperature from rising to 80 ºC. The filtrate was then filtered using filter paper and evaporated using a rotary evaporator at 40 ºC (Kemenkes RI., 2017).

Quality Test of *P. purpureum* Extract and Powder

Organoleptic Test

Organoleptic testing is carried out visually using the five senses, including examining the shape, color, smell and taste of the plant powder and extract (Octavia et al., 2023).

Determination of Water Content

The water content of the powdered medicinal plants and thick extract was determined using the gravimetric method. Accurately weigh 2 g of the powdered medicinal plants and thick extract of *P. purpureum*. Place them in a tared steam dish for 10 minutes in an oven at 105 °C. Evaporate the mixture in the oven at 105 °C for 5 hours, and then weigh until constant weight is achieved (Sukasih et al., 2020).

Determination of Ash Content

Determination of the ash content of the plant powder and thick extract was carried out by weighing 2 g of each sample, then placed in a crucible and incinerated using a furnace at a temperature of 600ºC for 5 hours until the charcoal was used up, then placed in a desiccator and weighed. The incineration was carried out until a constant weight was achieved (Kumar & Rani, 2024).

Phytochemical Screening

Flavonoids

About 100 mg thick extract of odot grass was placed in a test tube, 1 mL of distilled water was added. After that, it was heated for 5 minutes, then a few drops of concentrated HCl were added. Then, 0.2 g of Mg powder was added. A positive result was indicated by the appearance of a reddish-purple color (Suhendar & Fathurrahman, 2019).

Tannin

About 100 mg of thick *P. purpureum* extract was placed in a test tube, then water was added until dissolved. The solution was then separated into test tubes and 2 to 3 drops of 1 % FeCl3 solution were added (Anggraini & Kumala, 2022).

Saponin

About 100 mg of thick odot grass extract was placed in a test tube, 10 mL of distilled water was added

to completely submerge the sample, and the mixture was boiled for 2-3 minutes, cooled, and then shaken vigorously for several minutes. The tube was kept upright for 30 minutes. A positive result was indicated by the formation of a stable foam (Kemenkes RI, 2017).

Alkaloids

About 100 mg of thick *P. purpureum* extract was placed in a test tube, then H_2SO_4 was added and stirred until thoroughly mixed. The mixture was filtered and then Meyer's, Bouchardart's, and Dragendorff's reagents were added, respectively (Pehino, et al., 2021).

Antibacterial Activity

Test Rejuvenation of Test Bacteria

The test bacteria were taken with a loop and then suspended in a tube containing 2 mL of 0.9% NaCl solution until the same turbidity was obtained as the standard turbidity solution of 0.5 Mc Farland solution.

Preparation of Mc. Farland Standard Solution

The determination of the 0.5 Mc turbidity standard. Farland is made by mixing 9.95 mL of 1% H_2SO_4 solution and 0.05 mL of 1% $BaCl_2$ solution with half a test tube of distilled water, then shaking until a cloudy solution is formed. This turbidity is used as the standard turbidity of the bacterial suspension being tested and is equivalent to a bacterial density of 10^8 CFU/mL (Alouw et al., 2022).

Minimum Inhibitory Concentration (MIC) Test

The minimum inhibitory concentration (MIC) was determined using the dilution method on nutrient agar (NA) media. 10 ml of sterile NA media were prepared in 10 cm diameter petri dishes containing 1 mL of extract with various concentration ratios. As a positive control, NA media without the addition of bacteria was used. 0.2 mL of *S. aureus* and *E. coli* bacteria with a concentration of 0.5 Mc. Farland were inoculated into each NA media, homogenized, and incubated at 37°C for 24 hours (Pehino et al., 2021).

Determination Of Inhibitory Zone Width

Inhibitory power was determined using the disc diffusion method. A 0.2 mL bacterial suspension was poured into 15 mL of NA medium and then homogenized. Paper discs containing ethanol extract of *P. purpureum*, followed by positive and negative controls, were placed into the medium. The medium was then incubated at 37°C for 24 hours. The resulting clear zone was then measured with a caliper (Anggraini & Kumala, 2022).

Data Analysis

The statistical data analysis used in this study used the SPSS® (Statistical Package For the Social Sciences) version 26 program with One Way ANOVA using the Completely Randomized Design (CRD) method with a factorial pattern. The parameters analyzed included data on the width of inhibition power of each extract concentration based on the type of Maceration and MAE extraction methods against *S. aureus* and *E. coli* bacteria with 3 repetitions. If there is a significant difference in the method of each treatment with increasing concentration, it be analyzed using the Duncan's follow-up test.

RESULTS AND DISCUSSIONS

Quality of Extract and Dry Powder of *P. Purpureum*

Dry Powder and Extract Yield

The *P. purpureum* herbaceous plant was obtained in the amount of 4,500 g with a yield of 11.82 % of the dry powder. The powder yield is influenced by various processes such as drying at high temperatures that causes water migration from the material to the environment, sieving that causes some particles to be trapped in the filter media, and various other processes. The dried herbal extract was then extracted using maceration and MAE methods. The yield of the *P. purpureum* extract can be seen in Table 1 below:

Table 1. Results of *P. purpureum* Extract Yield

Methods	Yield (%)
Maceration	15.09
MAE	12.49

Based on the results of the yield percentage (Table 1), the extract yield with the maceration method was greater than the MAE method, this was because the time used in the maceration extraction was 3 days, then re-maceration was carried out 2 times so that more polar compounds were attracted. Meanwhile, in the MAE method, the power used is large so it will damage the bioactive compounds contained in the plant extract and show lower yields.

Water content

The water content of the medicinal plants and extracts plays a crucial role in ensuring their quality and stability. The method for determining the water content of the medicinal plants and extracts uses the gravimetric method, using an oven at 105°C to evaporate the physically bound water in the sample, achieving a constant weight. The results of the water content calculations for the medicinal plants and extracts of *P. purpureum* are shown in Table 2.

Table 2. Water content of *P. purpureum* powder and extract

Sample	Water content ± SD (%)
Maceration	7.3347 ± 0.4306
Maceration	7.3151 ± 0.5397
MAE	7.3899 ± 0.2451

Ash Content

The principle of determining ash content is that organic substances can be burned at high temperatures 500–600 °C in a furnace, until all organic components decompose and disappear as gas, while the remaining solids (ash) represent the inorganic components. The results of the ash content calculations for the crude drug and *P. purpureum* extract are shown in Table 3.

Table 3. Ash content of *P. purpureum* powder and extract

Sample	Water content ± SD (%)
Maceration	5.6731 ± 0.5714
Maceration	6.8653 ± 0.3764
MAE	6.3443 ± 0.2507

Phytochemical Screening

Phytochemical testing was conducted qualitatively by observing the color changes that occurred after the addition of reagents. The results of the qualitative phytochemical test of *P. purpureum* extract can be seen in Table 4.

Table 4. Phytochemical test of P. purpureum extract

Test Type	Resul ts	
	Maceration extract	MAE extract
Flavonoid	+	+
Saponin	+	+
Tannin	+	+
Alkaloid	+	+

The (+) symbol indicates compounds detected in the extract and dry powder. From Table 4 it can be concluded that the extract and dry powder of P. purpureum contain compounds from the polar secondary metabolite group or phenol and polyphenol derivatives.

Antibacterial Activity

Minimum Inhibitory Concentration (MIC)

The results of the MIC test show that the higher the concentration of the extract used, the greater the inhibitory power. This is indicated by the clear media (no bacterial growth) which can be used as a basis for determining the concentration for the Width of Inhibitory Zone test. The minimum inhibitory concentration is determined to find out what concentration starts to be meaningful as an antibacterial. The extract concentrations used to determine the MIC were 10 %, 15%, 20 %, and 25 % for *S. aureus* and *E. coli* bacteria. The results of the MIC testing of *P. purpureum* extract using the maceration and MAE methods showed that the 25 % concentration could inhibit bacterial growth.

Width of Inhibition Zone

The paper disc diffusion method was chosen because it is quick and easy to perform, requiring no special equipment. The formation of a clear zone around the disc indicates there is antibacterial activity. The width of the resulting zone of inhibition can be measured using a vernier caliper. The concentrations of *P. purpureum* extract used in this test were 30 %, 35 %, and 40 %. A bacterial turbidity of 0.5 McFarland was used as a reference within the test microbial range. The positive control (K+) used was the antibiotic ciprofloxacin, because ciprofloxacin has a broad spectrum against both gram-negative and gram-positive bacteria, with strong bactericidal activity against *S. aureus* and *E. coli*. The negative control (K-) used was dimethyl sulfoxide (DMSO 10 %) because DMSO 10 % has been shown not to provide inhibition to the test bacteria. The results of the calculation of the width of the inhibition zone test of *P. purpureum* extract against *S. aureus* and *E. coli* bacteria can be seen in Table 5.

The results of antibacterial testing of *P. purpureum* extract against *S. aureus* and *E. coli* bacteria have a wide inhibitory zone so *P. purpureum* extract have antibacterial activity (Table 5.). In *S. aureus* bacteria, using the maceration method extract at a concentration of 30 % to 40 % produced inhibition zone with a strong category. The positive control (K+) with an average inhibition zone of 21.21 mm was categorized as very strong, and the negative control (K-) with an average inhibition zone of 0 mm was categorized as non-inhibitory. Using the MAE method at a concentration of 30% to 40% produced inhibition zone with a strong category. The positive control (K+) with an average inhibition zone of 22.48 mm was categorized as very strong, and the negative control (K-) with an average inhibition zone of 0 mm was categorized as non- inhibitory.

In testing on *E. coli* bacteria, the results obtained using the maceration method at a concentration of 30% to 40% produced inhibition zone with a strong category. The positive control (K+) with an average inhibition zone of 21.23 mm was in the very strong category, and the negative control (K-) with an average inhibition zone of 0 mm was in the non-inhibitory category. Meanwhile, using the MAE method at a concentration of 30% to 40% produced inhibition zone with a strong category. The positive control (K+) with an average inhibition zone of 22.26 mm was categorized as very strong, and the negative control (K-) with an inhibition zone inhibitory value of 0 mm was categorized as non-inhibitory.

Table 5. Inhibitory zone width of *P. purpureum* extract against *S. aureus* and *E. coli*

Bacteria	Method	C (%)	IZW Average ± SD (mm)	Description
S.aureus	Maceration	C⁻	0.00 ± 0.00	does not hinder
		30	11.21 ± 0.10	strong
		35	13.13 ± 0.12	strong
		40	14.25 ± 0.13	strong
		C⁺	21.21 ± 0.16	very strong
	MAE	C⁻	0.00 ± 0.00	does not hinder
		30	13.26 ± 0.16	strong
		35	14.11 ± 0.10	strong
		40	15.36 ± 0.18	strong
		C⁺	22.48 ± 0.12	very strong
E. *coli*	Maceration	C⁻	0.00 ± 0.00	does not hinder
		30	11.23 ± 0.10	strong
		35	13.61 ± 0.17	strong
		40	14.31 ± 0.18	strong
		C⁺	21.23 ± 0.15	very strong
	MAE	C⁻	0.00 ± 0.00	does not hinder
		30	14.21 ± 0.10	strong
		35	15.31 ± 0.17	strong
		40	16.68 ± 0.12	strong
		C⁺	22.26 ± 0.15	very strong

Description: Inhibition area ≥ 20 mm is stated as Very Strong, 10-20 mm as Strong, 5-10 mm as Medium, and ≤ 5 mm as Weak. (Simanjuntak et al., 2022)

The best concentration in inhibiting the growth of *S. aureus* and *E. coli* bacteria was a concentration of 40% (Figure 3). In general, the MAE methods have numerous benefits compared to the conventional ones, such as quicker extraction, greater output, and reduced environmental impact (Lopez-Salazar et al., 2023). Active substances that have antibacterial power are alkaloids, flavonoids, saponins, and tannins. This is due to the ability of this flavonoid compound in the methylation process which can increase the role of flavonoids in the field of medicine flavonoids possess antibacterial, antiviral, antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties (Roy et al., 2022).

The mechanism of alkaloids as antibacterials is through inhibition of cell wall synthesis which will cause lysis of the cell wall of a bacteria so that bacterial growth is inhibited. The mechanism of saponin as an antibacterial is by denaturing proteins and used to kill a variety of microorganisms (Thawabteh et al., 2019). In the studies of bioactive saponins, some found that their antimicrobial properties may be related to their interaction with the cell membranes. the interaction of saponins with microbial membranes can change the membrane surface morphology and even destroy its integrity (Li, 2023). Tannin has antibacterial activity with its ability to inactivate enzymes and interfere with protein transport in the inner layer of cells. The amount of hydroxyl groups in tannins and the release of hydrogen peroxide are important indicators as antibacterial properties (Tong et al., 2021).

MAE extract is better than maceration extract in inhibiting bacteria from this study, because at a concentration of 40 % MAE extract can inhibit 15.36 ± 0.18 mm against *S. aureus* while maceration inhibits 14.25 ± 0.13 mm, and against *E. coli* MAE extract 16.68 ± 0.12 mm while maceration extract 14.31 ± 0.18 mm

The results of measuring the width of the inhibition zone of *P purpureum* extract against *S. aureus* and *E. coli* bacteria were analyzed using the SPSS computer statistics program. The data were tested using the One Way ANOVA test to find

significant differences in the average at different concentrations. In *S. aureus* and *E. coli* bacteria, the p value (0.000) < α (0.05) was obtained, which means that there was a significant difference in each concentration of *P. purpureum* ethanol extract in inhibiting the growth of *S. aureus* and *E. coli* bacteria, because the significant requirement of One Way ANOVA is p < 0.05. Duncan's further test on *S. aureus* and

E. coli bacteria showed that the Significance (Sig.) value was 1.000, indicating that there was no significant difference between the average groups in the subset. This indicates that each treatment can be considered homogeneous or can be interpreted as there is no significant difference in each treatment. From this test, it can be seen that the MAE method appears to be more effective than Maceration in producing extracts with higher inhibitory power. The results of the statistical analysis showed no significant difference between the two treatments, but the data from the different MAE extracts, although slightly different, had an effect on inhibiting bacteria that affect health.

CONCLUSION

MAE extract is better at inhibiting bacteria than maceration extract. *P. purpureum* cv. Mott extract has the potential as an antibacterial. *P. purpureum* extract both in the maceration and MAE extraction methods contain secondary metabolite compounds such as flavonoids, tannins, saponins, and alkaloids. In the antibacterial activity test, *P. purpureum* extract with the MAE extraction method inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria more widely than maceration. The Minimum Inhibitory Concentration (MIC) value in both extraction methods on the growth of *S. aureus* and *E. coli* bacteria was obtained at a concentration of 25%. The MAE method extract with a concentration of 40% both on *S. aureus* bacteria (average LDH 15.36) and on *E. coli* bacteria (average LDH 16.68) showed a strong category.

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CONFLICT OF INTEREST

The authors declared no conflict of interest in the manuscript.

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