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Antibacterial activity of *Paederia foetida* leaves using two different extraction procedures against pathogenic bacteria

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Abstract. *Yunita M, Ohiwal M, Elfitrasya MZ, Rahawarin H. 2023. Antibacterial activity of* Paederia foetida *leaves using two different extraction procedures against pathogenic bacteria. Biodiversitas 24: 5920-5927.* The objective of this study was to compare the effectiveness of the extraction methods between maceration and infundation of *Paederia foetida* (Linn.) leaves in suppressing the growth of *Staphylococcus aureus* and *Escherichia coli* with concentrations of 10, 25, 50, 75, and 100% using the disc-diffusion method. While phytochemical testing was performed by decoction method. The results of this study revealed that the macerated extract was able to inhibit both *S. aureus* and *E. coli* at all concentrations with the inhibition zones varied ranging from 5.8 ± 0.85 - 20.43 ± 0.06 mm and 4.9 ± 0.57 - 18.18 ± 0.67 mm, respectively. While the leaf infusion obtained from infundation method was found to be less able to inhibit both pathogenic bacteria (<1 mm). Results of phytochemical testing confirmed the result of inhibitory testing where macerated extract contained alkaloids, saponins, steroids, terpenoids, and tannins, while the leaf infusion only contained alkaloids and terpenoids. It can be concluded that macerated extracts of *P. foetida* leaves were far more effective in inhibiting the growth of *S. aureus* and *E. coli*. The study implies that the leaf extract of *P. foetida* can be considered and developed into a strong antibacterial in the future through good and appropriate bioprospecting.

Keywords: Decoction, disc-diffusion, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

Infectious diseases caused by *Staphylococcus aureus* and *Escherichia coli* are a growing crisis due to the increasing bacterial resistance to various types of antibiotics (Assis et al. 2017). In 2017, the World Health Organization (WHO) released a priority list of pathogenic bacteria that are resistant to certain antibiotics in an effort to conduct research and development of novel antibiotics. The priority list is divided into three groups based on the order of priority for the exploration of novel antibiotics, namely critical, high and medium. Pathogenic *S. aureus* is included in the high category (Tacconelli and Magrini 2013) due to its resistance to methicillin (Lee et al. 2018), tetracycline (Velhner and Milanov 2016), gentamicin (Rahimi 2016), and vancomycin (Cong et al. 2020). While *E. coli* is included in the critical category because it has been found to be resistant to carbapenem and third-generation cephalosporins (Tacconelli and Magrini 2013). Furthermore, 5 strains of *E. coli* were reported to have toxins that cause diarrhea with different mechanisms, namely enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), and Enteroinvasive *E. coli* (EIEC) (Murray et al. 2016).

Given the risk of *S. aureus* and *E. coli* resistance, it encourages the exploration of alternative antibiotics that have antibacterial properties, for example, using plant-based natural products that are endemic to a region (Nji et al. 2021). One of the native medicinal plants from Indonesia is *Paederia foetida* (Linn.), commonly known by local people as *sembukan* leaves. This plant has a variety of uses. It is used to treat herpes, diarrhea, and dysentery, and to relieve the discomfort caused by flatulence. Additionally, it is used to treat cramps, colic, dysentery, and other gastrointestinal issues. The results of phytochemical test on *P. foetida* leaves previously revealed that this plant contains alkaloids, tannins, flavonoids, phenol, terpenoid, saponin, and cardiac glycosides (Upadhyaya 2013), thus could be able to inhibit *S. aureus* and *Bacillus subtilis.* Another study conducted by (Silaban 2021) revealed that the ethanol extract of *P. foetida* leaves successfully inhibited the growth of *E. coli*.

The extraction method may affect the antibacterial properties of *P. foetida* leaf extract in inhibiting the growth of pathogenic bacteria. Hence, the choice of extraction method is very important because it can affect the amount and type of secondary metabolite substances produced (Balakrishna et al. 2016). Cecille and Vicencio (2021) used the bioautography method with maceration technique on *P. foetida* leaves and found that *P. foetida* leaf extract was able to inhibit the growth of *S. aureus.* Maceration is a simple extraction method that is usually used for the extraction of natural materials. Plant leaves are mashed and soaked with alcohol as a solvent, then allowed to stand for several hours to several days. However, the maceration method has disadvantages such as long extraction time and the use of quite a lot of solvents (Balakrishna et al. 2016).

Another more conventional extraction method that can be carried out is infundation, where the extraction process is carried out by grinding natural ingredients into a fine powder, then a hot extraction solvent is poured over the medicinal material, soaked, and stored for a short time. This method uses water as a solvent because it is considered a universal solvent (Zhang et al. 2019). However, research on the extraction of *P. foetida* leaves by the infundation method has never been conducted previously. Therefore, in the current investigation, we compared the two extraction methods in extracting *P. foetida* leaves. This study aimed to determine and measure the effectiveness of extraction methods between maceration and infundation methods on *P. foetida* leaves in inhibiting the growth of pathogenic gram-positive and negative pathogenic bacteria, namely *S. aureus* and *E. coli*, as well as determining the optimum concentration in inhibiting both pathogens. The findings of this study are expected to contribute to the exploration and development of novel antibacterials based on natural ingredients in the future as an alternative to antibiotics.

MATERIALS AND METHODS

Study area

This study was conducted during June - July 2022 at the Microbiology Laboratory of the Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia. Leaf samples were collected in Halong Village, Baguala Sub-district, Ambon City, Maluku, Indonesia.

Sample preparation

The fresh and dark green leaves of *P. foetida* were used in this study for sample preparation. The leaves of *P. foetida* were washed properly under running water to remove impurities on the leaves and then air-dried. Furthermore, the leaves are dried using an oven at 50°C until the leaves are very dry and can be kneaded. Subsequently, the leaves were pulverized with a blender and then filtered to obtain fine powder (Cecille and Vicencio 2021).

Preparation of leaf extract using maceration method

A total of 1 kg leaves powder of *P. foetida* was weighed and then soaked in 96% ethanol solution with 1:10 ratio for 24 hours while stirring. Furthermore, the yield was filtered using filter paper of Whatman 42 to separate the extract from the residue. The filtrate was then evaporated using a rotatory evaporator (RE100-Pro D-Lab) at a temperature of 40°C until no solvent dripped or a concentrated extract was obtained (Cecille and Vicencio 2021). Yield percentage of leaf extract was calculated by the following formula (Dhanani et al. 2017):

% Yield Extract: Simplicia weight / Concentrated extract x 100

Preparation of leaf extract using infundation method

Leaf powder of *P. foetida* was weighed and then soaked in distilled water with a ratio of 1:10 for 24 hours. Leaf infusion with a concentration of 100% was prepared by dissolving 1 g leaf simplicia in 10 mL distilled water in an Erlenmeyer, in other words, the ratio between simplicia and solvent was 1:10. Erlenmeyer was placed in a beaker filled with water and then heated on a hot plate for 15 minutes when the water temperature reached 90°C while occasionally stirring. After 15 minutes, the extract was filtered using a sterile flannel cloth into the Erlenmeyer. If the volume of *infusa* had not reached the desired volume, heated distilled water was added and filtered again using the remaining leaf pulp until the appropriate volume of leaf infusion was obtained (Balakrishna et al. 2016).

Preparation of extract concentrations

In this study, the results of extraction using maceration and infundation techniques were made in concentrations of 10, 25, 50, 75, and 100%. Stock concentrations were prepared using the following formula (Ernilasari et al. 2021):

Vs x Cs = Vn x Cn

Where:
Vs: Initial volume
Cs: Initial concentration, i.e. 100%
Vn: Expected volume, i.e. 5 mL
Cn: Expected concentration
(C1=10%, C2=25%, C3=50%, C4=75%)

Preparation of bacterial growth media

Media preparation was carried out by dissolving and heating 18g Nutrient Agar (Merck) in 1 L of distilled water on a hot plate (Thermo Scientific Cimarec II) and stirring until homogeneous. The media was sterilized using an autoclave for 15 minutes at 121°C. Before pouring into petri dishes, the media was allowed to stand until the temperature reached approximately 40°C. The media that was poured into Petri dishes was stored for 24 hours at room temperature so that the medium solidifies perfectly (Yunita et al. 2022).

Antibacterial activity test of leaf extract against pathogenic bacteria

Paederia foetida leaf extract and infusion were prepared at 10, 25, 50, 75, and 100% concentrations using maceration and infundation procedures, respectively. Antibacterial activity was measured using Kirby-Bauer disc paper diffusion method. Ciprofloxacin was used as positive control and distilled water as negative control. The medium used was Nutrient Agar (Merck), which was pre-streaked with *S. aureus* and *E. coli*. The disc paper was soaked for 15 minutes in the three treatments namely ciprofloxacin, distilled water, and each leaf extract with serial concentrations. The disc paper was then placed on the surface of the media, then incubated at 37°C for 24 hours. The clear zone formed around the disc paper was measured using a ruler. The clear zone around the disc paper indicates the presence of antibacterial activity against pathogenic bacteria. The result is valid if no clear zone is seen on the negative control and a clear zone is seen on the positive control. Antibacterial activity is calculated and

categorized (Table 1) using the following formula (Davis and Stout 1971):

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

Where:
DV: Diameter of vertical zone of inhibition
DH: Diameter of horizontal zone of inhibition
DC: Diameter of disc paper

Phytochemical test of *P. foetida* leaves

To determine the bioactive compounds contained in the leaf extract and infusion, phytochemical analysis was performed including alkaloids, tannins and flavonoids, saponin, steroids, and terpenoids (Elboughdiri 2018). As an important piece of information, phytochemical tests on the infusion of *P. foetida* leaves have never been studied previously.

Alkaloids content

Leaf extract of *P. foetida* was put into a test tube and then added with 10 mL chloroform (Merck) and 0.5 mL of H_2SO_4 (Merck) and shaken. The solution was then allowed to stand until two layers formed in the test tube. The top layer formed was transferred to a new test tube and then added 1-2 drops of Dragendorff reagent. A pink or orange precipitate indicates the presence of alkaloid content (Elboughdiri 2018).

Flavonoids content

A total of 3 mL of aqueous solution of the leaf extract was put into a test tube and 1 mL of magnesium and 1 mL of concentrated HCl (Merck) were added. Discoloration of the solution to yellow indicates the presence of flavonoids (Elboughdiri 2018).

Tannins content

A total of 2 mL of aqueous solution of the leaf extract was put into a test tube and then 10 mL of distilled water and heated. The solution was cooled and filtered, and then 1-2 drops of $FeCL_3$ 1% (Merck) were added. The color change of the solution to dark blue or black reflects the presence of tannins (Elboughdiri 2018).

Saponins content

A total of 3 mL of the aqueous solution of the leaf extract were mixed with 10 mL distilled water in a test tube. The test tube was stoppered and shaken vigorously using a vortex for 5 minutes Thirty minutes later, foam was observed as a positive reaction to saponin presence (Elboughdiri 2018).

Table 1. Inhibitory response according to Davis and Stout (1971)

Clear zone diameter (mm)	Inhibitory response
<5	Weak
5-10	Moderate
10-20	Strong
>20	Very strong

Steroids content

The amount of 1 mL of the aqueous solution of the leaf extract was dissolved in 10 mL chloroform and filtered using filter paper. For the following tests, the solution was separated into two equal parts. The solution was first mixed with 1 mL of acetic anhydride, then 1 mL of concentrated H_2SO_4 (Merck) was added down the side of the test tube to produce a layer underneath. The test tube was observed for greenish coloration as indicative of steroids (Elboughdiri 2018).

Terpenoids content

The second portion of the solution resulting from steroid testing was mixed with concentrated H_2SO_4 (Merck) carefully. The appearance of a reddish-brown color indicated a positive result for terpenoids (Elboughdiri 2018).

Data analysis

To assess the significant differences between the antibacterial effects of the extract and infusion of *P. foetida* leaves resulting from maceration and infundation methods, the data were statistically analyzed by the Mann-Whitney U test using SPSS 16. Antibacterial activities were examined qualitatively by measuring the clear zone around the paper disk.

Ethical clearance and plant determination

Ethical approval was issued by the Ethics Committee of the Faculty of Medicine, Universitas Pattimura with Number: 102/FK-KOM.ETIK/VIII/2022. While *P. foetida* plant was determined in the Department of Biology, Faculty of Mathematics and Natural Science, Universitas Pattimura. This study meets generally recognized scientific requirements and is based on adequate and relevant up-to-date references. The methods used are in accordance with the objectives and are supported by adequate facilities and infrastructure.

Data analysis

The collected data was provided in the form of tabulations and figures, and it was descriptively assessed. The difference in the results of the inhibition test between macerated extract and leaf infusion on the growth of pathogenic bacteria was analyzed statistically using the Mann-Whitney U Test.

RESULTS AND DISCUSSION

Extraction of *P. foetida* leaves was carried out using two methods, i.e., maceration and infundation. The extraction yield resulting from the two methods is presented in Table 2. While According to the appearance, there was no significant difference in color. However, the leaf extract using the maceration method appears to have a more concentrated color than the infundation extract. A comparison of the two leaf extracts is presented in Figure 1.

The greater the yield produced, the more efficient the treatment applied in the extraction procedure without compromising other properties. According to the findings of this study, it is reasonable to assume that the leaf extract contains more bioactive components than the leaf infusion. This is consistent with the findings of Dhanani et al. (2017), who revealed that a high yield value indicated a high concentration of bioactive components. Several factors can influence yield results, including the extraction method employed, the stirring process during maceration, the kind of solvent, time and temperature used during the extraction process (Zlotek 2016).

Extraction by maceration is one of the main methods used to extract natural materials using universal solvents such as ethanol and methanol. Several important factors influencing *P. foetida* L. leaf extract to have antibacterial activity are the choice of solvent type, the ratio of solvent to simpicilia, extraction temperature, and extraction time (Balakrishna et al. 2016; Luliana et al. 2019). Moreover, Cecille and Vicencio (2021) compared *P. foetida* leaf extract using two different solvents and found that leaf extract using ethanol had greater antibacterial activity (21.00-22.70 mm) compared to ethyl acetate (13.95-14.20 mm). Meanwhile, ciprofloxacin as positive control showed strong inhibition against pathogenic S. *aureus* and *E. coli* with the antibacterial zone of 19.38 and 21.17 mm, respectively. Ciprofloxacin works by inhibiting gyrase (topoisomerase II), which plays a role in negative supercoiling of bacterial DNA to prevent excessive DNA twisting during replication and transcription processes. Inhibition of the gyrase enzyme may cause the lysis of bacterial cells so ciprofloxacin is bactericidal and has a broad spectrum that can inhibit the growth of gram-positive and negative bacteria (Diniatik and Anggraeni 2021). In accordance with this study, Hanum et al. (2022) found a similar result where ciprofloxacin could inhibit the growth of *E. coli* and *S. aureus* with the antibacterial zone of 58.12 mm and 44.92 mm, respectively.

Macerated extract of *P. foetida* was prepared by dissolving 100 g of simpicilia with 1000 mL of 96% ethanol to obtain 20 mL of leaf extract. While the leaf infusion using the infundation methods was obtained by dissolving 25 g of simpicilia in 250 mL of sterile distilled water so that 250 mL of infusion was obtained. The leaf extracts obtained from each of these methods were then diluted again until the concentrations reached 10, 25, 50, 75, and 100%. Each concentration was tested for its inhibitory effect on *E. coli* and *S. aureus*. Antibacterial activity is presented in Tables 3 and 4.

In this study, we found that antibacterial activity was present in macerated leaf extracts of *Paederia foetida* L. in all concentrations tested. This is evidenced by the presence of inhibition zones around the disc papers treated in the leaf extract. Otherwise, we found that *P. foetida* leaf infusion obtained by infundation method had weak antibacterial activity and was only present at concentrations of 75 and 100%. This is confirmed by the inhibition zone <1 mm, which is included in the weak category.

Table 2. Comparison of yield percentage of *Paederia foetida* leaf extract

Extraction method	Simplicia weight (g)	Concentrated extract weight (g)	Yield percentage (%)
Infundation	1000 g	93.2	9.32
Maceration	1000 g	120.7	12.07

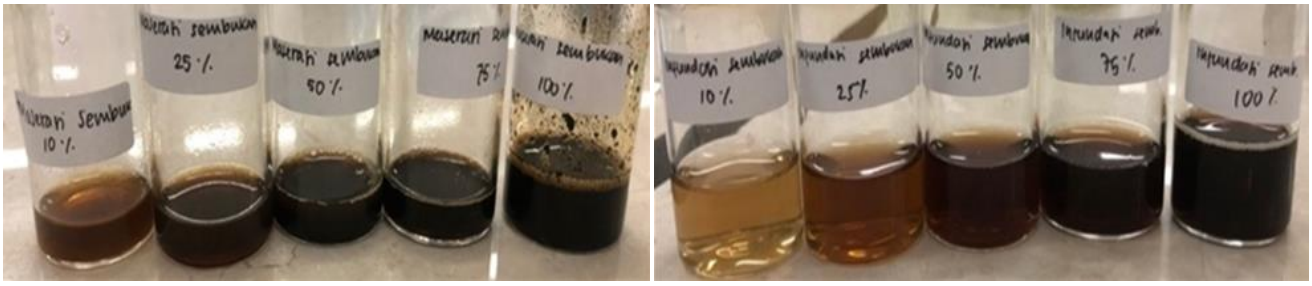

Figure 1. Appearance of extract (*left*) and infusion (*right*) of *P. foetida* leaves

Table 3. Antibacterial activity of macerated extract and infusion on the growth of *S. aureus*

Concentration	Macerated extract		Leaf infusion	
	Diameter of inhibition zone (mm)	Inhibitory response	Diameter of inhibition zone (mm)	Inhibitory response
10%	5.8 ± 0.85	Moderate	0.00 ± 0.00*	-
25%	9.48 ± 1.17	Moderate	0.00 ± 0.00*	-
50%	12.12 ± 1.41	Strong	0.00 ± 0.00*	-
75%	14.43 ± 0.67	Strong	0.19 ± 0.04*	Weak
100%	20.43 ± 0.06	Very strong	0.26 ± 0.675*	Weak
K (+)	22.88 ± 1.80	Very strong	22.63 ± 4.42	Very strong
K (-)	0.00 ± 0.00	-	0.00 ± 0.00	-

Note: Values followed by (*) are not significantly different from positive control at $p<0.05$

Table 4. Antibacterial activity of macerated extract and infusion on the growth of *E. coli*

Concentration	Macerated extract		Leaf infusion	
	Diameter of inhibition zone (mm)	Inhibitory response	Diameter of inhibition zone (mm)	Inhibitory response
10%	4.9 ± 0.57	Weak	0.00 ± 0.00*	-
25%	7.6 ± 1.06	Moderate	0.00 ± 0.00*	-
50%	10.8 ± 1.27	Strong	0.15 ± 0.04*	Weak
75%	11.15 ± 0.92	Strong	0.39 ± 0.06*	Weak
100%	18.18 ± 0.67	Very strong	0.68 ± 0.12*	Weak
K (+)	22.18 ± 3.71	Strong	22.55 ± 1.56	Very Strong
K (-)	0.00 ± 0.00	-	0.00 ± 0.00	-

Note: Values followed by (*) are not significantly different from positive control at $p<0.05$

Tables 2 and 3 showed that the macerated extract of *P. foetida* leaves at all concentrations could be able to suppress the growth of *S. aureus* and *E. coli*. Meanwhile, *P. foetida* infusion had a very small inhibition with a weak category at concentrations of 75 and 100%. Visualization of the antibacterial activity of *P. foetida* leaf extract and infusion in vitro is presented in Figures 2 and 3. Meanwhile, a comparison graph of the antibacterial activity of *P. foetida* leaf extract against *S. aureus* and *E. coli* is presented in Figure 4. There was a high correlation between the macerated extract of *P. foetida* leaves against *S. aureus* and *E. coli* with R-value of $R^2 = 0.9666$.

According to the results of the Mann-Whitney U test (Table 5), there was a significant difference between the extract and infusion of *P. foetida* leaves with p-values of 0.034 and 0.048, respectively, which indicated that there was a significant difference between the two extract methods in inhibiting the growth of *S. aureus* and *E. coli*. However, based on the results of statistical analysis and measurement of the inhibition zone, *P. foetida* leaf extract has more optimum effectiveness in inhibiting *S. aureus* and *E. coli* compared to its infusion. Meanwhile, a phytochemical test as further testing were carried out to confirm and determine the bioactive compound contained in *P. foetida* leaf extract and infusion of. The results of the phytochemical testing can be presented in Table 6.

In accordance with the findings of this study, Borges et al. (2020) found that the ethanol extracts of olive and mimosa leaves had greater inhibition against *S. aureus* and *E. coli* than the methanol extracts. The maceration methods used on plant extract (*Etlingera elatior*) using ethanol solution 70% at a concentration of 0.5, 1, 1.5, and 2% showed antibacterial activity only at a concentration of 2% with inhibition zones of 8.4 and 2.4 mm against *E. coli* and *S. aureus*, respectively (Ernilasari et al. 2021). Furthermore, Jeyaseelan and Jashothan (2012) revealed that both hot and cold ethanol extracts were also known to significantly and effectively inhibit the growth of *S. aureus* and *E. coli* with a minimum inhibitory concentration and bactericidal concentration of 5 and 10 mg/mL, respectively. Ethanol is commonly preferred in the extraction process as a solvent over other solutions because it is less expensive, polar, easier to get, environmentally friendly, less poisonous, relatively more soluble, and has a lower boiling point (Khotimah et al. 2020). A low boiling point can be helpful in the process of separating ethanol with the extract using the right temperature so as to prevent damage to the bioactive compounds contained in the extract (Jeyaseelan and Jashothan 2012).

The presence of inhibition zones in the macerated extract of *P. foetida* leaves against the two pathogenic bacteria is supported by the results of phytochemical testing, which confirm that it contains bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids and steroids. Research conducted by Upadhyaya (2013) obtained similar results where this leaf extract contains various bioactive compounds such as saponins, tannins, phenols, flavonoids, cardiac glycosides, alkaloids, and reducing sugars. Furthermore, Jeyaseelan and Jashothan (2012) also found alkaloids, saponins, cardiac glycosides, tannins, resins, flavonoids, and terpenoids in *Ricinus communis* leaf extract which enabled it to suppress the growth of *S. aureus* and *E. coli*.

Table 5. The results of statistical analysis using the Mann-Whitney U test

Pathogenic bacteria	Sample	N	Mean rank	*P*-value
S. aureus	Macerated extract	7	9.71	0.034
	Leaf infusion	7	5.29	
E. coli	Macerated extract	7	9.57	0.048
	Leaf infusion	7	5.43	

Table 6. Results of phytochemical testing of macerated extract and infusion of *P. foetida* leaves

Bioactive compound	Indicator	Results	
		Macerated extract	Leaf infusion
Alkaloids	Orange precipitate	+	-
Flavonoids	Yellow	+	-
Saponins	Foam	+	+
Tannins	Dark blue	+	-
Terpenoids	Brown ring	+	+
Steroids	Red ring	+	-

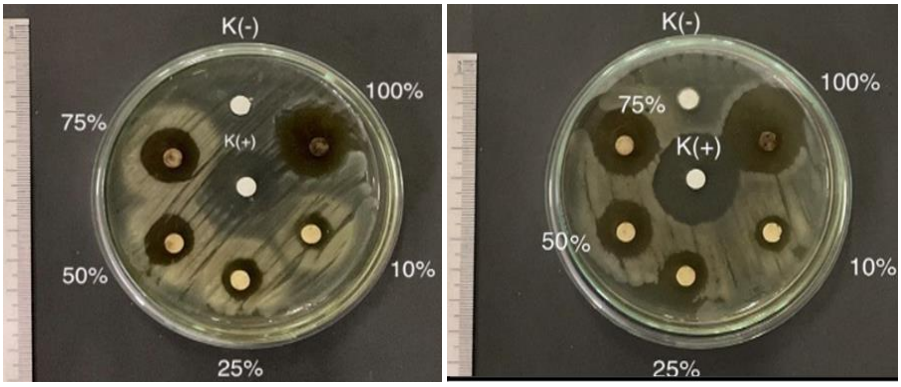

Figure 2. Visualization of the antibacterial activity of *P. foetida* leaf extract *in vitro* against *S. aureus* (*left*) and *E. coli* (*right*)

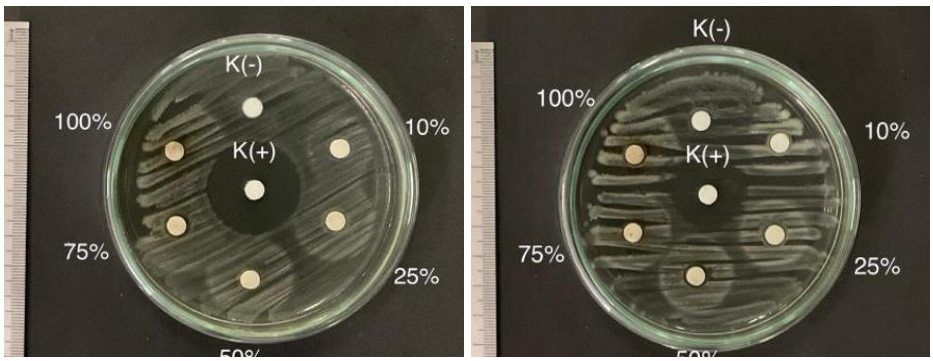

Figure 3. Visualization of the antibacterial activity of *P. foetida* leaf infusion *in vitro* against *S. aureus* (*left*) and *E. coli* (*right*)

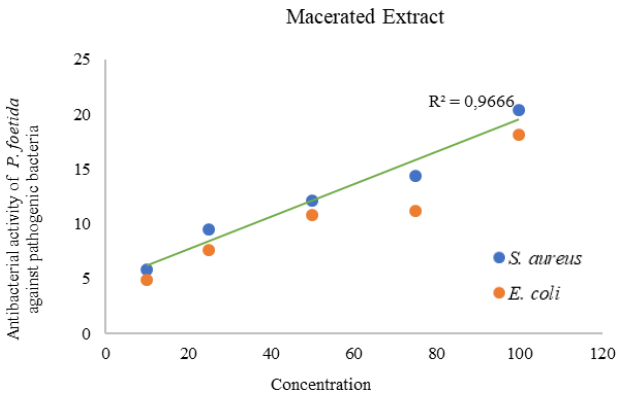

Figure 4. Comparison of the antibacterial activity of *P. foetida* leaf extract against *S. aureus* and *E. coli*

Alkaloids can inhibit bacterial growth by inhibiting protein and nucleic acid synthesis, disrupting the function of bacterial cell membranes, inhibiting drug efflux pumps on bacterial membranes, and inhibiting ATP synthesis (Yan et al. 2021). Flavonoids act as antibacterial through three mechanisms, i.e. interfere with metabolism for bacterial cell energy synthesis, inhibit nucleic acid synthesis, and inhibit the function of the cytoplasmic membrane by inhibiting ATPase and phospholipase enzyme binding thereby interfering with the permeability of bacterial cell membranes (Xie et al. 2014). Saponins, terpenoids and steroids have antibacterial activity by lowering the permeability of cell membranes by different mechanisms. In detail, saponins are natural detergents that have antibacterial activity by affecting the permeability of the outer membrane or by interacting with proteus in the lipopolysaccharide layer. This causes wall rupture which in turn will lyse the cell walls of pathogenic bacteria (Cankaya and Somuncuoglu 2021; Xue et al. 2020).

Otherwise, extraction using the infundation method is carried out by soaking the simpicilia in sterile distilled water that is heated to 90°C for 15 minutes. The results of the phytochemical test of *P. foetida* leaf infusion confirmed the absence of antibacterial activity due to the negative results for alkaloids, flavonoids, tannins, terpenoids and steroids. The main factor that most likely causes the formation of very small inhibition zones and even no inhibition zones at almost all concentrations is the temperature that is too high during the boiling process of the simplicia causing damage and breakdown in the structure of the bioactive compounds. The contrasting results between macerated extract and leaf infusion of *P. foetida* may occur due to several factors. A study conducted by (Chaaban et al. 2017) revealed that the use of high temperatures could damage or change the core structure of the active compounds so that they also affect the action mechanism of the flavonoids. In addition,

Wardatun et al. (2017) revealed that the technique and type of solvent used greatly affected the inhibitory activity and content of bioactive compounds, whereas extraction by maceration methods using ethanol solution 96 and 70% had a far greater effect on the content of bioactive compounds in plant extracts than using infundation and soxhletation and methods. Unfortunately, we cannot compare our findings with other results on the same leaf infusion as in the present study due to the paucity of accessible literature on this subject.

In conclusion, the current study indicated that maceration, rather than infundation, is the superior method or procedure for extracting *Paederia foetida* leaves. When compared to its leaf infusion, macerated extract of *P. foetida* leaves was more effective in inhibiting the growth of pathogenic bacteria at all concentrations of 10, 25, 50, 75, and 100%. Furthermore, due to their high inhibition indexes of 20.43 ± 0.06 and 18.18 ± 0.67 mm, respectively, all concentrations were found to have the potential to be developed as antibacterials against gram-positive bacterium (*S. aureus*) and gram-negative bacterium (*E. coli*). Although this work successfully explored the antibacterial capabilities of leaf extract, more research into their bioactive components in quantitative and antibacterial formulations is required in order to develop this extract as a natural source of alternative antibiotics in the future.

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