Journal of Applied Science, Engineering, Technology, and Education *ISSN: 2685-0591 (online)*
Vol. 4 No. 2 (2022)
https://doi.org/10.35877/454RI.asci772

Inhibitory Test of Bay Leaf (*Eugenia polyantha*) Extract Against *Pseudomonas aeruginosa* and *Escherichia coli*

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Abstract

Bay leaf has traditionally been used by Indonesians as a cooking spice and for disease treatment. Bay leaves contain secondary metabolites that are known to have antibacterial properties. The secondary metabolites produced are influenced by several factors, including the growth area and the extraction solvent. The main causative bacteria in nosocomial infections are *Pseudomonas aeruginosa* and *Escherichia coli*. This study aimed to evaluate the antibacterial activity of bay leaf extract against the two gram-negative bacteria. The maceration method and 70% ethanol were used to extract bay leaves. The antibacterial activity was evaluated using the disc diffusion method. The extract exhibits the widest zone of inhibition (11.91±0,84 mm) at 100% of concentration against *Pseudomonas aeruginosa*. The extract showed antibacterial activity against *Pseudomonas aeruginosa* but not against *Escherichia coli*.

Keywords: antibacterial activity; bay leaf; *Escherichia coli*; *Pseudomonas aeruginosa*; Salam (*Eugenia polyantha*)

Received: 15 September 2020 Revised: 29 November 2021 Accepted: 2 December 2021

1. Introduction

Bay leaf (Indonesian synonym = Salam) was a plant that was deeply associated with the daily lives of Indonesians. Bay leaves are frequently utilized in medicine and as a food ingredient in the community (Hidayati et al., 2017; Kusuma et al., 2011; Sholikhah, 2016). Several studies have found that bay leaves contain antifungal, antidiarrheal, antidiabetic, antibacterial, antioxidant, anticancer, and antihypertensive properties (Aini et al., 2016; Hidayati et al., 2017; Ismail & Wan Ahmad, 2019). Secondary metabolites found in bay leaves are thought to be responsible for their pharmacological properties. Bay leaves have been shown to contain secondary metabolites of flavonoids, phenolics, alkaloids, tannins (Dewi & Arlita, 2021), steroids, sesquiterpenes, triterpenoids, steroids, and essential oils (Dewijanti et al., 2018; Ismail & Wan Ahmad, 2019). Bay leaves have also been shown to contain chemicals such as myricetin, eugenol, orientin (Kusuma et al., 2011), quercetin, quercitrin, α-pinene (Hamad et al., 2017), squalene, and phytol (Rahim et al., 2018).

Differences in a growth area and extraction solvent affect the content of secondary metabolites and the pharmacological activity provided (Dewijanti et al., 2020; Verdiana et al., 2018). Dewijanti et al. (2020) discovered that the flavonoid and alkaloid content of bay leaf extract from West Java was higher than that of East Java. Furthermore, total phenol and flavonoid levels were found to be greater in bay leaves from West Java (Dewijanti et al., 2020). West Java bay leaf extract contains retusine, but East Java bay leaf extract contains coniferin. Another study found that the yield of a bay leaf extract from the Sukabumi area was higher than that of Bogor (Sembiring et al., 2015).

The extract's rich phenol and flavonoid content could enhance its antibacterial properties. Previous studies show that using 70% ethanol in the extraction of bay leaf extract leads to higher total phenol and flavonoid levels than using 96% ethanol (Dewijanti et al., 2018). Furthermore, Mamay et al. (2018) observed antibacterial activity against *Salmonella sp.* so the yield of bay leaves grown in the lowlands was significantly higher than bay leaves grown in the highlands. In the lowlands, an ethanolic extract of 96% bay leaf produced an inhibition zone of 27,55±3,85 mm,

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whereas the highland area was 21,06±7,12 mm. This difference in pharmacological activity was due to synergistic or antagonistic interactions with the extract's different components (Syahrir et al., 2016).

Antibacterial testing on plants was required as an alternative therapy in the treatment of infections. Long-term antibiotic treatment for illnesses carries the potential of resistance (Subramani et al., 2017). Nosocomial infections were infections that had a high morbidity and mortality rate. According to Laverty et al., 2014), the most common causes of nosocomial infections were *Pseudomonas aeruginosa* and *Escherichia coli*. Ramli et al. (2017) observed that a 96% ethanol extract of bay leaves from Bandung had antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* of 7.00±0.28 and 7.00±0.31 mm, respectively. Meanwhile, Utami & Ramadhani, 2020) reported that the inhibitory zone in the test against *Escherichia coli* was 20 mm. The antibacterial activity of a 70% ethanol extract of Sukabumi bay leaves against *Pseudomonas aeruginosa* and *Escherichia coli* bacteria was investigated in this study.

2. Materials and Methods

2.1. Materials

The main ingredient was bay leaf obtained from Sukabumi, West Java. Bacteria used were *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. The instruments used in this research include analytical balance (Thermo scientific®), vacuum rotary evaporator (Buchi R-100®), water bath (CAPP CRWB-30®), autoclave (Hirayama®), oven (Memmert®), laminar airflow (Thermo Scientific®) and incubator (Memmert®).

2.2. Sample preparation

Eugenia polyantha (Wight) Walp was identified as the sample at the School of Biological Sciences (SITH), Bandung Institute of Technology. Bay leaves were washed, sorted for the dark-green old leaf, then air-dried. The powdered sample was then extracted in a 1:10 maceration ratio to 70% ethanol. Immerse at room temperature for 24 hours, stirring occasionally, and repeated till substantial color changes occur. The crude extract was obtained after the solvent had been evaporated and concentrated using a vacuum rotary evaporator and subsequently a water bath.

2.3. Phytochemical screening

The extract compounds were qualitatively identified. According to Maigoda et al. (2022) procedures, the substances evaluated included flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids.

2.4. Inhibitory test

The disc diffusion method was applied to quantify inhibitory activity. Oven or autoclave sterilized materials and glassware were used and done in laminar airflow. The bacterial suspension was created in 0.9% physiological NaCl at turbidity comparable to the McFarland standard 0.5 (equivalent to $1.5x10^8$ CFU/mL). The suspended bacteria were added to solidified Mueller Hinton Agar (MHA) media using the spread-plate technique. Fifty µl of the suspended bacteria were dripped onto the media surface. The suspension was distributed with an L-rod and rotated until thoroughly distributed. Six-mm discs were immersed for ten minutes in different sample concentrations (25, 50, 75, and 100%) with quadruple repetitions. A DMSO solvent was utilized as a negative control, streptomycin as a positive control, and a bacterial suspension without discs as a normal/growth control. Tweezers were used to attach the disc containing the sample, negative control, and positive control to the surface of the bacterial suspension-treated media. Following that, the petri dishes were inverted for 24 hours at a 37°C temperature. With a calliper, the inhibitory zone was measured diagonally, vertically, and horizontally across the clear area, minus the disc's diameter (Haerussana et al., 2021).

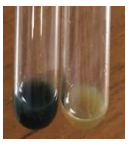
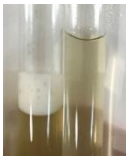
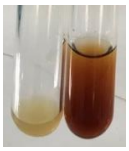
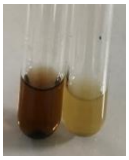
2.5. Statistical analysis

The results were averaged by four repeats on each plate (SD). The data was processed with IBM SPSS Statistic 25. Analysis of Variance (ANOVA) was performed in the statistical analysis, which was followed by Post-Hoc analysis. A significant difference was given as a p value<0.05 (*) or p<0.01 (**).

3. Results and Discussion

Phytochemical screening was assessed to identify the presence of secondary metabolites that play a role in the pharmacological activity of bay leaf extract. Table 1 shows that the extract contains tannin, saponin, flavonoid, terpenoid, and alkaloid metabolites. The antibacterial activity of bay leaves was formerly related to phytol (terpenoid), quercetin (flavonoid), eugenol (phenol), citral (terpenoid), and tannin components (Dewijanti et al., 2019; Irdawati et al., 2017; Murbarani et al., 2021). Phytol, from the terpenoid class, was also mentioned as the major compound in the maceration of bay leaves utilizing ethanol as a solvent (Rahim et al., 2018).

Table 1. Phytochemical screening of bay leaf (*Eugenia polyantha*) extract

Secondary Metabolites Class	Methods	+/-	Results	Result Description
Tannin	$FeCl_3$	+++		Thick blue-black colour was formed
Saponin	Foam test	++		The stable foam was formed
Flavonoid	Shinoda test	++		The solution is brownish-orange in hue
Terpenoid and steroid	Liebermann-Burchard	++		Reddish-brown colour resulted
Alkaloids	Mayer and Wagner	+		The colour slightly changed, but no precipitate or hard turbidity was formed, and the colour of the blank was lighter than the test results.

*(+++) high, (++) medium, and (+) low presence

The antibacterial activity was determined by measuring the diameter of the inhibitory zone produced around the disc. Bacterial growth on the test medium (turbid) was inhibited, resulting in clear media surrounding the disc. The extract had the largest inhibition zone against *Pseudomonas aeruginosa* at a 100% concentration of 11.91±0.84 mm but did not provide an inhibitory zone against *Escherichia coli*. Table 2 shows that the positive control inhibited both bacteria. The diameter of the positive control zone against *Pseudomonas aeruginosa* was smaller than that of the extract concentration of 100 percent. As a negative control, DMSO, which was used as a solvent in extract dilution, did not affect on either bacteria. The statistical test results revealed that the data distribution was normal and homogeneous. The test was then repeated with one-way ANOVA, which revealed significant differences in each group.

Table 2. Inhibition zone of bay leaf extract (*Eugenia polyantha*) against *Pseudomonas aeruginosa* and *Escherichia coli*

Sample		Inhibition zone (mm)	
%	mg/mL	*Pseudomonas aeruginosa*	*Escherichia coli*
25	250	7.05±0.35**	-
50	500	8.05±0.70**	-
75	750	9.28±0.75**	-
100	1000	11.91±0.84**	-
Positive control (streptomycin)		10.49±0.67**	12,25±0,55
Negative control (DMSO 10%)		-	-

**significantly different from negative control ($p<0.01$)

The higher the concentration of bay leaf extract, the wider the inhibitory zone. The ease of damage to bacterial cells increased with increasing concentrations of the supplied extract due to an increase in the number of metabolites (Dewi & Arlita, 2021). The mechanism of quercetin's bacterial growth inhibition was comparable to that of streptomycin, both of which interfere with bacterial protein synthesis, affect protein expression in cells, and cause cell damage/lysis (Wang et al., 2018; Waters & Tadi, 2021). Ramadhania et al. (2018) discovered that a Probolinggo bay leaf methanol extract has antibacterial efficacy against *Pseudomonas aeruginosa*. Meanwhile, the ethanol extract of Tangerang bay leaves tested negative for antibacterial action against *Pseudomonas aeruginosa* DSM 19882 (Dewijanti et al., 2018). Hidayati et al. (2016) reported no inhibitory zone in the test against *Escherichia coli* using a 70% methanol extract of bay leaves from Banda Aceh and Pariaman. Similarly, (Hamad et al., 2017) discovered that bay leaf extract extracted using the water distillation method had no antibacterial efficacy against *Escherichia coli* (Ismail & Wan Ahmad, 2019). Interestingly, (Evendi, 2017) achieved the best inhibitory zone of 12.00 mm in the same test utilizing Kalimantan bay leaves. Variances in growing locations, differences in the use of solvents and extraction procedures, and differences in test bacteria can all lead to discrepancies in the results of antibacterial activity testing with bay leaf extract.

4. Conclusion

The 70% ethanolic extract of Sukabumi bay leaves demonstrated antibacterial activity against *Pseudomonas aeruginosa* with an inhibition zone of 11.91±0.84 mm, while there was no inhibition zone in *Escherichia coli*.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

Contribution: Ayu Nala El Muna Haerussana: concept and design, data analysis/ interpretation, drafting manuscript, technical or material support. Wulan Putri Dwiastuti: data acquisition. Cindi Arwan Sukowati: concept and design, data acquisition. Widyastiwi: statistical analysis.

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