



Antibacterial Activity and Chemical Composition of Red Peacock Flower (*Caesalpinia pulcherrima* L.) Leaf Essential Oil

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Abstract

Peacock flower (*Caesalpinia pulcherrima* L.) leaves contain essential oils which can be used as an ingredient in cosmetics, perfume, aromatherapy, medicine, and supplements. The study was conducted to obtain essential oils from peacock flower leaves and determine the antibacterial activity against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*). Antibacterial activity test was carried out by the agar diffusion method, using paper discs. Measurements were made for the inhibition zone diameter (IZD) that appeared, while the essential oil component was analyzed using GC-MS. The results showed that the peacock flower leaves (*C. pulcherrima*) had a moderate to strong antibacterial effect at a concentration of 7.5%–20% against gram-positive bacteria (*B. subtilis* and *S. aureus*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*). Gram-negative *E. coli* bacteria are relatively more sensitive to peacock flower leaf essential oil compared to other test bacteria. Peacock flower (*C. pulcherrima*) leaf essential oil is composed of 7 main components namely β -Cubebene 33.87%; Caryophyllene 23.00%; γ -Elemene 13.18%; α -Pinene 10.96%; Cadina-1(10),4-diene 10.20%; Copaene; 7.09%; β -Pinene 1.70%.

1. Introduction

Peacock flower (*Caesalpinia pulcherrima* L.) is one of the ornamental plants of the Fabaceae family that grows in Indonesia. Guno et al. (2009) reported that peacock flower leaves contain essential oils, flavonoids, glycosides, and sterols. Essential oils can be used as ingredients in the fields of cosmetics, perfume, aromatherapy, medicine, and supplements [1].

Usman et al. [2] reported on the acquisition of red and yellow varieties of peacock flowers (*C. pulcherrima*) leaves in Nigeria using the distillation method. The essential oil compositions of the peacock flower (*C. pulcherrima*) leaves of the red and yellow varieties obtained were 0.50% and 0.52% v/w [2]. In the study of Vivek et al. [3], it is reported that the methanol extracts of leaves and flowers of the peacock flower (*C. pulcherrima*) plant show antimicrobial activity against pathogenic bacteria. The results show that the flower extract inhibited more

bacterial growth than leaf extract from the peacock flower plant. At a dose of 100 μ L leaf or flower extract (20 mg/mL), each yielded IZD of 1.9 cm and 1.4 cm, respectively [3]. Antibacterial test with diffusion method was carried out on pathogenic bacteria composing of *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans*, and *Cryptococcus neoformans*. Peacock flower plant contains secondary metabolites such as diterpenoids, phenolics, flavonoids, triterpenoids, and lactones that cause peacock flowers to have biological activity [4].

Njoku Isaac et al. [5] reported that the attainment of peacock flower (*C. pulcherrima*) leaf essential oil of the red flower variety in Nigeria using the distillation method with differing drying condition, namely fresh, air-dried, sun-dried, and oven-dried leaves, each yielded 0, 63%, 0.90%, 0.20%, and 0.58%. The analysis of chemical composition contained in the essential oil of peacock

flower leaves using GC-MS detected the presence of 26 chemical compounds in the fresh leaf essential oil [5].

Leaf and stem bark extracts from the Peacock Flower (*C. pulcherrima*) plant displayed antibacterial activity. With the agar diffusion method, antibacterial activity tests were carried out on six bacteria, i.e., *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*, which were then observed by calculating the inhibition zone diameter. Peacock flower (*C. pulcherrima*) bark extract showed higher antibacterial activity compared to the leaf extract, especially against *S. typhi* bacteria. At a concentration of 100 mg/mL, the bark extract of peacock flower (*C. pulcherrima*) produced IZD of 15 mm, while the leaf extract produced IZD of 12 mm. The extracts have inhibitory activity against organisms due to the presence of bioactive compounds such as alkaloids, flavonoids, and tannins [6].

In general, essential oils have an antibacterial effect, and, therefore, red peacock flower leaves were selected as a source of essential oil. The red variety of the plant was chosen because it is more often found than the yellow variety. Additionally, the constituent components of the red flower plant kind are more abundant (55 components with nine significant components each having levels of more than 4%) than the yellow flower plant (53 components with three significant components, each having levels of more than 4%) [2].

Research reports on peacock flowers in Indonesia generally use crude extracts from methanol solvent. However, there are no data for essential oils. Meanwhile, research reports on the existing peacock leaf essential oil originate from Africa, meaning that the novelty of this study is the use of peacock leaf essential oil from Indonesia as an antibacterial agent. The primary purpose of this study is to obtain red peacock flower leaf essential oil, determine its antibacterial activity against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), as well as identifying the chemical composition of essential oil of red peacock flower plant using GC-MS.

2. Methodology

2.1. Materials and Equipment

The materials used in this study include the leaves of the red peacock flower (*C. pulcherrima*) plant obtained from the Salatiga and its surrounding areas (Central Java, Indonesia). This plant has been determined by Mr. Soenarto Notosudarmo M.Sc (Jelami taxonomist), and the voucher is stored at the FSM Natural Product Chemistry Laboratory. The research was also carried out at the Satya Wacana Christian University's FSM Natural Product Chemistry Laboratory in October-December 2018. The chemicals used were pro-analysis standards from E-Merck or Smart Lab.

2.2. Sample Preparation

The leaves of peacock flower (*C. pulcherrima*) leaves with red-colored flowers was cleaned and separated from the stalks.

2.2.1. Essential Oil Extraction

A total of 1 kg of fresh peacock flower leaves was distilled (steam distillation) for 8 hours (until no essential oil was released). The water and essential oil were separated using a Clevenger apparatus. Na_2SO_4 anhydrous was added so that the essential oil was wholly separated from the water. The essential oil obtained was stored in a sample bottle and placed in a refrigerator until a series of analysis was carried out.

2.2.2. Antibacterial Activity Test with Agar Diffusion Method (Kirby –Bauer in American Society for Microbiology, 2016) [7]

Testing of the antibacterial activity of the essential oil of peacock flower (*C. pulcherrima*) against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, was carried out by diffusion method using Schleicher and Schuell 2668 Ref. No. 10321260, D6022274-1 with a diameter of 6 mm and Tween 80 as an emulsifier. A paper disc that had been dripped with essential oils with variation in the concentrations of 7.5%, 10%, 12.5%, 15%, and 20% (w/v), as much as 20 μL , with the same relative distance from one to another, was put onto the solidified agar medium. The disk was then incubated at 37°C for 24 hours. As a negative control, Tween 80 was used whereas for the positive control, tetracycline 30 μg was used. The measurements were done by looking at the Inhibition Zone Diameter (IZD) of the resulting inhibition areas.

2.3. Essential Oil Chemical Components Analysis using GC-MS

Analysis of the chemical composition of peacock flower (*C. pulcherrima*) essential oil was done using Gas Chromatography-Mass Spectrometry (GC-MS) of the Perkin Elmer Clarus 680 brand. Operating conditions used include the use of the Perkin Elmer Elite 5 MS column with a length of 30 meters, diameter 250 μm , and a temperature of 70°C. The sample was injected as much as 1 μL with an oven temperature of 70°C increased to 200 °C in 2 minutes. The split injector was at 250°C in temperature with a pressure of 13.7 kPa, and a split ratio of 70: 1. Whereas the helium carrier gas was streamed at 1 mL/min with EI⁺ ionization.

2.4. Data Analysis

The resulting research data were analyzed statistically using the Variance Analysis method for sample observation (Randomized Design of Sub Sampling Group) with 5 treatments in the form of a dose of essential oil which are 7.5%, 10%, 12.5%, 15%, and 20% (w/v), 5 replications, and 4 subsampling (2 Gram-Positive bacteria and 2 Gram-Negative bacteria). Inter-average testing used the Honest Significant Difference (HSD) test with a significance level of 5%.

3. Results and Discussion

3.1. Yield and physical properties of red peacock flower (*C. pulcherrima*) leaf essential oil

The average yield and physical properties of the red peacock (*C. pulcherrima*) essential oil obtained through the steam distillation method for 8 hours are presented in Table 1. When compared with other two similar studies, the yield of the red peacock flower (*C. pulcherrima*) fresh leaf essential oil obtained in this study was 0.32%, relatively lower than that of [2] at 0.50% and Njoku Isaac *et al.* [5] at 0.63%. The difference in the yields of essential oils from the same species is possible because of the differing conditions of the place of growth and climates, which are very influential on the essential oil products [8].

Table 1. The yields and physicochemical properties of the distillation of peacock flower leaf essential oil

Parameter	The results of peacock flower (<i>C. pulcherrima</i>) leaves essential oil			Description
	Current study	Usman <i>et al.</i> [2]	Njoku Isaac <i>et al.</i> [5]	
Yield	0.32%	0.50%	0.63%	Fresh leaves
Color	Yellow	-	-	-
Aroma	Unique peacock flower aroma	-	-	-
Density	0,9320 g/mL	-	-	-
Essential oil components	12	57	26	Fresh leaves
Most abundant component	β -Cubebene 33.87%	γ -Terpinene 44,4%	Caryophyllene 15.5%	Fresh leaves

The density of peacock flower (*C. pulcherrima*) essential oil obtained is 0.9320 g/mL. This result is supported by the finding of Nugraheni *et al.* [9], which states that the density of essential oils at 25°C is between 0.696-1.188 g/mL. The product of the distillation of peacock flower (*C. pulcherrima*) leaf essential oil can be seen in **Figure 1**.



Figure 1. Peacock flower (*C. pulcherrima*) leaf essential oil

3.2. Antibacterial Activity Test Results of Peacock Flower (*C. pulcherrima*) Essential Oil on Gram-Positive and Gram-Negative Bacteria

Measurements of the inhibition zone diameter that appeared as a mean of testing of antibacterial effect are presented in Table 2.

Table 2. The average diameter of the inhibition zone ($\bar{x} \pm S$ mm) of the essential oil of peacock flower (*C. pulcherrima*) leaves on gram-positive and gram-negative bacteria

Bacteria	W	Essential oil concentration ($\bar{x} \pm S$ mm)					
		7.5%	10%	12.5%	15%	20%	
Gram-positive	<i>B. subtilis</i>	0.5542	8.62 ± 0.79	9.74 ± 1.58	11.21 ± 0.63	12.18 ± 0.68	13.43 ± 1.36
			(a)	(b)	(c)	(d)	(e)
Gram-positive	<i>S. aureus</i>	0.6012	11.56 ± 1.03	12.21 ± 1.45	13.86 ± 1.07	16.47 ± 0.96	19.45 ± 0.84
			(a)	(b)	(c)	(d)	(e)
Gram-negative	<i>E. coli</i>	0.6937	12.65 ± 1.57	13.91 ± 1.74	15.45 ± 1.68	16.49 ± 2.44	16.42 ± 2.88
			(a)	(b)	(c)	(d)	(d)
Gram-negative	<i>P. aeruginosa</i>	1.0033	10.63 ± 0.83	11.10 ± 0.87	12.33 ± 0.97	11.15 ± 1.78	10.67 ± 0.48
			(a)	(a)	(b)	(a)	(a)
Positive Control	Tetracycline				± 35.00		
Negative Control	Tween 80			0.00			

Description:

- ✓ W = HSD 5%
- ✓ The numbers followed by the same letters indicate that the treatments did not differ significantly, while the numbers followed by different letters showed that the treatments were significantly different.

Table 2 shows that the essential oil of peacock flower (*C. pulcherrima*) leaves has positive antibacterial results against both gram-positive and gram-negative bacteria. The antibacterial reacts with the test bacteria by damaging the cell wall, damaging the plasma membrane, inhibiting protein synthesis, inhibiting the synthesis of nucleic acids, or inhibiting the synthesis of important metabolites in bacteria [10].

Elgayyar *et al.* [11] reported that the antibacterial effect of the material is shown by IZD, which is grouped into three categories: strong (IZD > 11 mm), moderate (IZD between 6-11 mm) and low (IZD < 6 mm) [11]. The results indicate that the essential oil of peacock flower (*C. pulcherrima*) leaves have a moderate to strong antibacterial effect at a concentration of 7.5%–20%.

Gram-negative bacteria (*E. coli*) turned out to be relatively more sensitive to the essential oil produced

compared to *P. aeruginosa* and gram-positive bacteria (*B. subtilis* and *S. aureus*). Table 2 shows that the antibacterial effect on *E. coli* bacteria has higher IZD than that of other test bacteria. According to Pelezar and Chan (2008), gram-negative bacteria have a thick cell wall structure (outer membrane and lipopolysaccharide), high lipid levels, and single peptidoglycan. The peptidoglycan layer of gram-negative bacteria is thinner compared to gram-positive bacteria, which implies that if the cell wall is damaged, it is easier to penetrate compounds from the outside [12]. Essential oils that penetrate the membrane can damage the cytoplasm and protein [13]. In addition, bacterial phospholipids are also soluble in essential oils due to the presence of phenol or alcohol groups [14].

In this study, tetracycline was used as a standard antibiotic or positive control since tetracycline is an antibiotic that can inhibit the growth of almost all types of bacteria, both gram-negative and gram-positive. Tetracycline works to inhibit protein synthesis by blocking the addition of amino acids of the peptide chain in bacterial growth [15]. The antibacterial ability of the peacock flower (*C. pulcherrima*) leaf essential oil is lower than the positive control (Tetracycline), shown in the diameter of the tetracycline inhibition with an (IZD) value of ± 35.00 mm. Meanwhile, an emulsifier (Tween 80) with a 10% concentration was used as a negative control. The result obtained did not show any antibacterial effect on gram-positive or gram-negative bacteria shown by the diameter of the inhibition area (IZD) of the 10% Tween 80 at 0.00 mm. The essential oil solution with concentrations of 7.5%; 10%; 12.5%; 15%; and 20%, each contains Tween 80 with concentrations of 3%; 4%; 5%; 6%; and 8%. Concentrations of Tween 80 contained in the essential oil are lower than 10%; thus, it would not affect the IZD produced in the antibacterial testing of peacock flower leaf essential oil. The use of Tween 80 is very effective as an emulsifier for essential oils because it has the ability to increase solubility in water [16].

3.3. Analysis of Composition of Peacock Flower (*C. pulcherrima*) Essential Oil

The results of the GC-MS analysis show that there are seven main components making up the essential oil, each of which appears as a peak on the chromatogram (Figure 2).

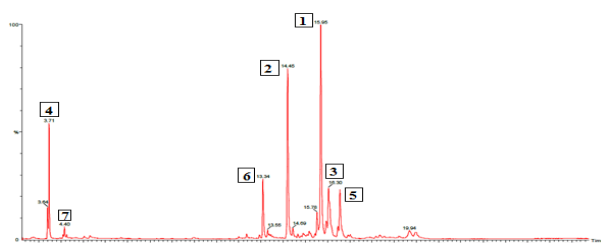


Figure 2. Chromatogram of Peacock Flower (*C. pulcherrima*) Essential Oil

Chromatogram of peacock flower (*C. pulcherrima*) leaf extract shows seven peaks of dominant components: peaks 1, 2, 3, 4, 5, 6 and 7 (Figure 2) with successive retention times of 15.95; 14.45; 16.30; 3.71; 16.81; 13.34; and 4.40. Identification analysis was done by comparing

each peak that appears with the Wiley database. Peak number 1, with a retention time of 15.95, has fragmentation similar to the β -Cubebene compound (Wiley's database). Thus, the peak is believed to be a β -Cubebene compound. β -Cubebene appears at the first peak with a level of 33.87%, it has a molecular formula of $C_{15}H_{24}$ with a molecular weight of 204,357 g/mol, and it is a class of organic compounds known as sesquiterpenoids or terpene [17].

The identification of peak number 2 with the Wiley database was conducted in the same way. Peak number 2 with a retention time of 14.45 has fragmentation similar to Caryophyllene compounds (Wiley's database). Peak number 2 is, therefore, designated as a Caryophyllene compound with a level of 23.00%. Caryophyllene is the most common sesquiterpenoid compound contained in many essential oils that have a role as non-steroidal anti-inflammatory drugs, metabolites from plants and fungi, medicinal/pharmaceutical ingredients, or source health benefits [18]. Caryophyllene has the molecular formula $C_{15}H_{24}$ with a molecular weight of 204.357 g/mol. In the same way, the identification of the rest of the seven peaks detected implies that all peaks have fragmentation similar to the Wiley database shown in Table 3.

Table 3. Main Chemical Components of Peacock Flower (*Caesalpinia pulcherrima* L.) Essential Oil

No Peak	Retention Time	Chemical Component	Content (%)
1	15.949	β -Cubebene	33.87%
2	14.453	Caryophyllene	23.00%
3	16.299	γ -Elemene	13.18%
4	3.714	α -Pinene	10.96%
5	16.809	Cadina-1(10), 4-diene	10.20%
6	13.338	Copaene	7.09%
7	4.404	β -Pinene	1.70%

Usman *et al.* [2] reported that peacock leaf essential oil is composed of 57 components, including 28.6% sesquiterpenoid. Its most dominant compounds are γ -Terpinene 44.4%; Germacrene B 14.3%; β -Pinene 7.4%; Alloocimene 5.9%; Myrcene 5.6%; Farnescene 5.3%; β -Caryophyllene 5.1%, and α -Pinene 4.2% [2]. Whereas Njoku Isaac *et al.* [5] reported that the peacock leaf essential oil is composed of 26 components with 54.20–70.0% are sesquiterpenoids and its most dominant compounds are Caryophyllene 15.51%; α -Cadinol 14.36%; γ -Muurolene 13.28%; Nerolidol 8.32%; Candiene 4.78%; γ -unsure 4.70%; Copaene 2.31% and β -Cubebene 0.32% [5]. When compared with the two studies mentioned, there are differences found, both in the composition and level of content of the essential oil components of peacock flower (*C. pulcherrima*) essential oil, such as the Caryophyllene compounds. Caryophyllene is a sesquiterpenoid compound that is found in all essential oils of peacock flower leaves studied despite having different levels. The percentage content of the compound Caryophyllene in this study is 23.00%; in the study of Usman *et al.* [2] is 5.1%, while Njoku Isaac *et al.* [5] is 15.51%. For the β -Cubebene compound which is one of the main components found in this study was only found in a relatively small amount in the research of Njoku Isaac *et*

al. [5] (0.32) and did not make an appearance in the study of Usman *et al.* [2]. The α -Pinene compound at 10.96% was also found in the study of Usman *et al.* [2] (4.2%); however, it did not appear in the study of [2, 5, 8]. Differences in the composition and level of compounds in the essential oil of peacock flower (*C. pulcherrima*) leaves are possible due to the differences in environmental factors where plants grow, such as soil and climate conditions that determine the quality of essential oils produced [8].

The components in the essential oil with a relatively high level of content such as β -Cubebene 33.87%, Caryophyllene 23.00%, and so forth are influential on the bioactivity of the essential oil as anti-bacteria. In essential oils, the bioactivity effect that appears can be caused by the synergistic effects of several components. This research can be used as primary data to enrich the scientific knowledge of plant essential oils in Indonesia. In general, the antibacterial bioactivity possessed by essential oils has the potential to be developed into natural antibiotics. This study also opens knowledge of the opportunities present to increase the benefits of peacock flower plants not only as ornamental plants but also as sources of essential oils and medicinal plants.

4. Conclusion

The peacock flower (*C. pulcherrima*. L) essential oil yielded was 0.32%. This essential oil has a strong antibacterial effect against gram-negative bacteria, especially against *E. coli* and *P. aeruginosa*, at a concentration of 7.5%. This is also true for the gram-positive bacteria, especially *S. aureus*, at a concentration of 7.5% and *B. subtilis* at a concentration of 12.5%. The seven main constituent components of peacock flower (*C. pulcherrima*. L) essential oil are β -Cubebene 33.87%; Caryophyllene 23.00%; γ -Elemene 13.18%; α -Pinene 10.96%; Cadina-1 (10), 4-diene 10.20%; Copaene 7.09%; and β -Pinene 1.70%.

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