



## Evaluation of the Antimicrobial Activity of *Caesalpinia pulcherrima* (L) Swartz Extract against Microbes that Cause Dental and Oral Infections and Determination of the Total Flavonoid and Total Phenolic Contents of the Plant

Bertha Lolo Lukita<sup>a</sup>, Sarworini Bagio Budiardjo<sup>b</sup>, Berna Elya<sup>a\*</sup>

<sup>a</sup>Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia, <sup>b</sup>Faculty of Pediatric Dentistry Dental Health, Universitas Indonesia, Depok, Indonesia

### Abstract

In Indonesia, *Caesalpinia pulcherrima* (L) Swartz is widely grown as an ornamental, whereas it is used as a medicinal herb in Mexico as an alternative treatment for dental and oral infections. At present, only fruit and root parts of the plant are used. The aim of this study was to evaluate the anti-microbial activity of ethanol extract of fruit, leaves, flowers and stems against microbes that cause dental and oral infections and determination of the total flavonoid and total phenolic contents of *C. pulcherrima* (L) Swartz. *Porphyromonas gingivalis*, *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans* are microbes that cause dental and oral infections. All parts of the plant were extracted by maceration using 70% ethanol for 24 hours at 15<sup>o</sup>-30<sup>o</sup>C, and extracts were concentrated using a reflux method and dried over a water bath. The total flavonoid contents were determined using the aluminium chloride colorimetric assay at 415 nm. The total phenolic content was determined using Folin-Ciocalteu reagent in a 1:4 ratio at a wavelength of 750 nm using a microplate reader. Antimicrobial activity was determined by the diffusion method. The total flavonoid content was expressed as milligram quercetin equivalent (QE) per gram of plant extract. The highest total flavonoid content per gram of plant extract was found in the flowers (10.06 ± 0.08 mg QE/g), and the lowest total content was found in the leaves (0.34 ± 0.02 mg QE/g). The highest phenolic content was also found in the flowers (498.52 ± 1.96 mg gallic acid equivalent [GAE]/g), and the lowest total phenolic content was detected in the leaves (216.76 ± 1.00 mg GAE/g). A 100% crude ethanol extract of the flower and fruit parts exhibited antimicrobial activity against bacteria *P. gingivalis*, *S. mutans*, *E. faecalis* and *C. albicans* that cause dental and oral infections. A 2% crude flower extract showed the highest antimicrobial activity against *P. gingivalis* (14.49 ± 0.465 mm), *S. mutans* (9.02 ± 0.607 mm), *E. faecalis* (9.67 ± 0.297 mm) and *C. albicans* (19.44 ± 0.207 mm). The ethanol extract of the flowers had the highest contents of total flavonoids and total phenols and the most antimicrobial activity against the studied compared to the other parts. This results of the present study revealed valuable information and also support the continued sustainable use of *C. pulcherrima* (L) Swartz in a traditional system of medicine.

**Keywords:** antimicrobial activity, *Caesalpinia pulcherrima* (L) Swartz, ethanol extract of flowers, total flavonoids, total phenol

Corresponding Authors: Berna Elya, Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia

Tel: (+62)813-1416-1497

E-Mail: [berna.elya@farmasi.ui.ac.id](mailto:berna.elya@farmasi.ui.ac.id)

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## 1. Introduction

Products from plants, animals and minerals are used as sources for treating diseases in humans. Awareness of plants used as food and medicine and various applications of these plants have been achieved through trial and error [1]. The field of dentistry has begun to exploit herbs for the relief of dental pain, as well as for their antiseptic, analgesic, anti-fungal, anti-oxidant, anti-viral and antibacterial properties [2, 3]. The *Caesalpinia* genus contains more than 500 species, which have many health benefits based on the pharmacological activities of these species [4]. *Caesalpinia pulcherrima* (L) Swartz, which belongs to the *Caesalpiniceae* family and common names for this species peacock flower, originated in America and then spread to Africa and Asia. In Indonesia, the plant is widely grown as an ornamental [5]. It is also commonly used in traditional medicine in Indonesia. In Mexico, the fruit and root parts of *C. pulcherrima* (L) are used as alternative medicines in oral and dental treatment [6]. According to the researches, methanol and water extracts from aerial parts of *C. pulcherrima* (L) Swartz have antimicrobial activities [7]. More than 700 bacterial taxa have been identified in samples taken from

oral cavities [8]. The accumulation of these bacteria in the oral cavity are considered the primary cause of dental caries, gingivitis, periodontitis, peri-implant infections, and stomatitis [9]. Taxa isolated in the oral cavity include the bacteria *Porphyromonas gingivalis*, *Streptococcus mutans* and *Enterococcus faecalis* and the yeast *Candida*. *Porphyromonas gingivalis*, a gram-negative oral anaerobe, is considered the main aetiological factor in periodontal inflammatory diseases [8]. *Streptococcus mutans*, a gram-positive anaerobe, is found in the oral cavity and considered a major pathogen in the initiation of dental caries [9]. *Enterococcus faecalis* is aerob gram-negative bacterium. In the oral cavity, *E. faecalis* is found in carious lesions in periodontal diseases [10, 11]. *Candida* species are commensal yeasts and opportunistic pathogens [12]. They reside on mucosal surfaces and cause oropharyngeal infections [12]. We examined the antimicrobial activity of ethanol extract of fruit, leaves, flowers, and stems against microbes that cause dental and oral infections and to determine the total flavonoid and total phenolic contents of ethanol extract from fruit, leaves, flowers and stems of *C. pulcherrima* (L) Swartz.

## 2. Materials and Methods

### 2.1. Plant Materials

*Caesalpinia pulcherrima* (L) Swartz which were obtained from Depok, West Java Region on January 2018 and Identified at The Center for Plant Conservation Botanical Gardens-

LIPi, Jl. Ir. H. Juanda No. 13 Bogor, Indonesia.

## 2.2. Chemicals and Culture Media

Folin–Ciocalteu reagent, gallic acid, sodium carbonat (Sigma, St.Louis, America), ethanol (Merck, Darmstadt, German), dimethyl sulfoxide/DMSO (Merck, Darmstadt, German), triphenyl tetrazolium chloride (Merck, Darmstadt, German), nutrient broth (Oxoid, Cheshire, England), nutrient agar (Oxoid, Cheshire, England), Brucella Agar (Difco, Franklin Lakes, USA) were used.

## 2.3. Microorganisms

The test microbes consisted of gram-positive bacteria, a gram-negative bacterium and a fungus. The gram-positive bacteria were *S. mutans* type C and *E. faecalis* American Type Culture Collection (ATCC) 29212. The gram-negative bacterium was *P. gingivalis* ATCC 33277. The fungus was *Candida albicans* ATCC 10231. The microbes were obtained from the Microbiology Laboratory of the National Center of Food and Drug Testing

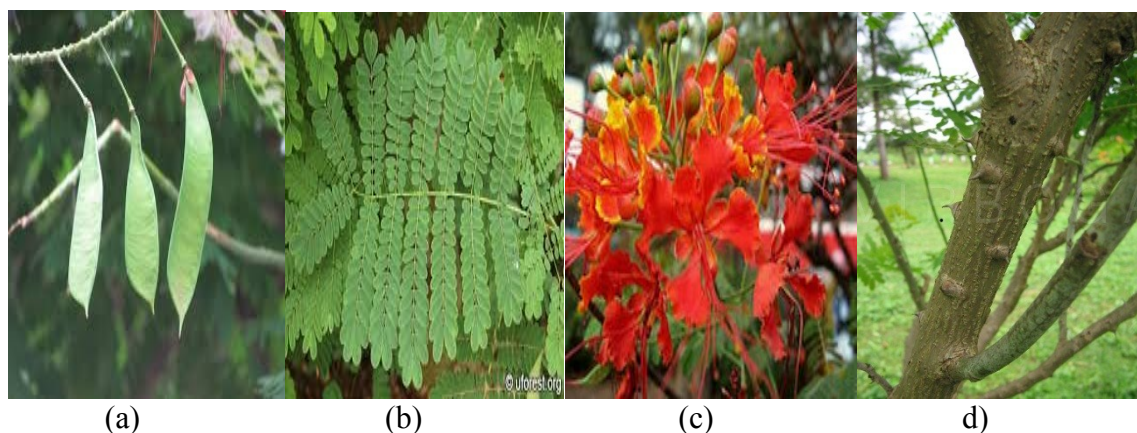
of Indonesia, Jakarta. Medical Microbiology Departement, FKUI-RSCM Clinic, Jakarta, Indonesia.

## 2.4. Sample Collection

Samples of fruits, leaves, flowers and stems were collected. Some of the fruits used were completely green, while others were partly dark brown. Only intact healthy dark green leaves and intact healthy orange flowers were used. The stems were obtained from the branch of the tree. After collection, all parts of the plants were cleaned and then dried at room temperature, as described in Section 2.5.

## 2.5. Extract Preparation

Fresh plant materials that were not contaminated by bacteria or fungi were removed from healthy plants. Plant organs were washed to remove dirt and then sliced into small segments, after which they were left to dry naturally at 15<sup>o</sup>-30<sup>o</sup> °C, air-cooled until the plant material were totally dry. The plant parts were dried to prevent microbial fermentation and degradation of metabolites,



**Figure 1.** *Caesalpinia pulcherrima* (L) Swartz (a) Fruit; (b) Leaf; (c) Flower; (d) Stem.

in addition to minimizing chemical reactions that occur due to ultraviolet light from direct sunlight. The dried plant materials were stored in a tightly closed container in a dry, cold place until use. The individual plant materials were later milled using a blender or grinding machine to produce dried plant powder to increase the surface area and to increase solvent penetration in cells, thereby increasing the extract yield [13]. The dried part of the stems, flowers, fruits and leaves were extracted by maceration using 70% ethanol for 24 hours at 15<sup>o</sup> -30<sup>o</sup>C. The solvent used was of technical grade. It was purchased from PT Duta Pratama Chemika, Bogor, Indonesia and was refined before use. The resulting extracts from each part of the plant were separated from the solvent by filtering using No. 1 Whatman paper. The filtrate was then evaporated using reflux method and dried over a water bath. Subsequently, the extract was collected and stored at 4° C before use.

#### 2.6. Determination of the Extract Yield

The yield of each extract was determined based on the dry weight of the extract (a) and a soaked sample (b) using the following formula:

$$\text{Yield (\%)} = a/b \times 100.$$

The yield value of each extract was then calculated.

#### 2.7. Determination of the Total Flavonoid Content

The determination of total flavonoids was done using the aluminium chloride colorimetric test, adopted from the methods of

Chatatikun *et al.* [14] and Sandip *et al.* [15], with little modification. The standard solution used was quercetin at concentrations of 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL in 96% ethanol. Fifty microlitres of extract (1 mg/mL or standard solution) were added to 150 µl of 96% ethanol. Then, 10 µl of 10% aluminium chloride solution were added, followed by the addition of 10 µl of 1M sodium acetate and mixing in 96 wells on a microplate. Ethanol 96% was used as a blank. All the reagents were mixed and incubated for 40 min at 15<sup>o</sup>-30<sup>o</sup> C and protected from light. Absorption was measured at a wavelength of 415 nm using a microplate reader (Versamax Microplate Reader, Carolina-USA). The total flavonoid content was expressed as milligram quercetin equivalent (QE) per gram of plant extract.

#### 2.8. Determination of the Total Phenolic Content

The total phenolic content of each extract was determined using a 96-microplate well, in accordance with the method of Ahmad *et al.*[16] with some slight modification. Extract solution (25 µl) from each part of the *C. pulcherrima* (L) Swartz plant was reacted with 200 µl of a 1:8 Folin–Ciocalteu reagent mixture, homogenized using a shaker for 60 sec and left for 240 sec and then 75 µl sodium carbonat solution (100 g/L) was shaken for 60 sec and incubated for 2 h at 15<sup>o</sup>-30<sup>o</sup>C and protected from light. Absorption was measured at a wavelength of 750 nm using a microplate reader (Versamax Absorbance Microplate Reader, Carolina-USA). The

absorbance of the same reaction with ethanol instead of the extract or standard was deduced from the absorbance of the reaction with the sample. Gallic acid (6.25–200 mg/L) was used as a standard for calibration. The total phenolic concentration was expressed as milligrams of gallic acid equivalent (GAE) per gram of plant extract.

### 2.9. Antimicrobial Activity: Agar Diffusion

The microbial stocks used were 24-h-old microbes that were cultured in nutrient agar, which had been incubated at 37° C for 24 h. Microbial stock cultures were sub-cultured in nutrient broth and was compared as judged by eyes, to reach estimated 0.5 Mc Farland (  $10^9$  bacteria/mL) [17]. Discs (6 mm) were prepared using Whatman filter paper. The discs were placed in a petri dish, which was then sterilized using an autoclave at a temperature of 121° C, 1 atm, for 15–20 min. Sterile discs were prepared and impregnated with each extract. The antimicrobial activity was carried out in two steps, the first step, the discs were infused/impregnated with 100% extract. In the second step, inhibition zone test only use the positive results from the first step, used 2% crude extracts in DMSO. They were then aseptically inoculated on the surface of the agar media. Subsequently, they were incubated at 37° C for 24 h, with petri dish

placed upside down [17]. The zone of inhibition against the selected pathogens after incubation were determined and recorded. Antimicrobial activity was evaluated by measuring the diameter of the inhibitory zone around the disc using a calliper. Chlorhexidine commercially available standard antibiotic disc was used as a positive control. Each disc was infused with 20 µl of extract, and each experiment was carried out with three replications.

### 3. Results and Discussion

The highest extract yield was obtained from the leaves (23.8%) followed by flowers (17.6%), fruits (15.6%) and stems (11.6%). Table 1 shows the total flavonoid contents of the four crude extracts and the total phenolic contents of the extracts obtained from the different parts of the plant. The equation of the calibration curve of the quercetin standard was  $y = 0.0758x + 0.1327$ ,  $R^2 = 0,9981$ . The calibration curve from gallic acid showed maximum absorbances at a 765 nm wavelength (equation  $y = 0.0315x + 0.0006$ ,  $R^2 = 0.9999$ ). The total phenolic contents of the four crude extracts as determined by the Folin–Ciocalteu method are reported as GAEs. Among the four crude extracts, the flowers contained the highest amount of total phenols ( $498.52 \pm 1.96$  mg GAE/g), and the lowest

**Table 1.** Total flavonoids and total phenol contents.

Part	Total Flavonoid Content (mgQE/gram)	Total Phenolic Content (mgGAE/gram)
Fruit	2,26 ± 0,01	341,71 ± 0,54
Leaf	0,34 ± 0,02	216,76 ± 1,00
Flower	10,06 ± 0,08	498,52 ± 1,96
Stem	0,70 ± 0,04	318,59 ± 0,34

was in leaves ( $216.76 \pm 1.00$  mg GAE/g). Flowers also have highest total flavonoid content ( $10.06 \pm 0.08$  mg QE/g) and the lowest was in leaves ( $0.34 \pm 0.02$  mg GAE/g). The antimicrobial activity test results of each extract of 100% and extract of 2% in DMSO of the different parts of the plant against the four studied microbes are presented in tables 2 and 3. All the extracts showed inhibitory activity against the selected pathogens. The ethanol 100% extracts of flowers and fruits showed to inhibit the activity of microbes *P. gingivalis*, *S. mutans*, *E. faecalis* and *C. albicans* and the ethanol 100% extracts of *C. pulcherrima* (L) Swartz leaves showed inhibit the activity of the microbes *E. faecalis* and *C. albicans*. The 100% extracts of *C. pulcherrima* (L) Swartz stems inhibit the activity of the microbes *P. gingivalis* and *C. albicans*. The zone of inhibition of the 2% extracts of the *C. pulcherrima* (L) Swartz plant parts was compared with that of commercially available

standard antibiotic disc. The standard antibiotic (chlorhexidine) showed to inhibit the activity of the microbes *P. gingivalis* ( $20.24 \pm 0.246$  mm), *S. mutans* ( $20.18 \pm 0.490$  mm), *E. faecalis* ( $19.73 \pm 0.533$  mm) and *C. albicans* ( $28.24 \pm 1.686$  mm). The result of antimicrobial activity of the four crude extract 2% in DMSO different part, extracts of flowers the highest to inhibit the activity of four studied microbes *P. gingivalis* ( $14.49 \pm 0.465$  mm), *S. mutans* ( $9.02 \pm 0.607$  mm), *E. faecalis* ( $9.67 \pm 0.297$  mm) and *C. albicans* ( $19.44 \pm 0.207$  mm). In the present study, all parts of the plant were extracted by maceration with 70% ethanol. The maceration is carried out at  $15^{\circ}$ - $30^{\circ}$  C, at this temperature some compounds are difficult to dissolve and cannot be efficiently extracted. Maceration also tends to cause degradation of thermolabile metabolites [13]. The choice of solvent is determined by its selectivity towards the substance to be extracted. The use of 70%

**Tabel 2.** Antimicrobial activity of 100% various extract part of *Caesalpinia pulcherrima* (L) Swartz.

Extract	Microorganisms			
	<i>P. gingivalis</i>	<i>S. mutan</i>	<i>E. faecalis</i>	<i>C. albicans</i>
Fruit	+	+	+	+
Leaf	-	-	+	+
Flower	+	+	+	+
Stem	+	-	-	+

Antimicrobial activity: -, No inhibition; +, Zone of inhibition

**Tabel 3.** Antimicrobial activity of 2% various extract part of *Caesalpinia pulcherrima* (L) Swartz.

Extract	Microorganisms			
	<i>P. gingivalis</i>	<i>S. mutan</i>	<i>E. faecalis</i>	<i>C. albicans</i>
	Diameter of inhibition zone in mm (mean)			
Fruit	$8,55 \pm 0,469$	$8,56 \pm 0,125$	$8,00 \pm 0,101$	$14,61 \pm 0,270$
Leaf	0	0	0	$17,17 \pm 0,522$
Flower	$14,49 \pm 0,465$	$9,02 \pm 0,607$	$9,67 \pm 0,297$	$19,44 \pm 0,207$
Stem	$10,50 \pm 0,153$	0	0	$11,20 \pm 0,507$
K+/klorheksidin	$20,24 \pm 0,246$	$20,18 \pm 0,490$	$19,73 \pm 0,533$	$28,24 \pm 1,686$

Mean of three replicate determination  $\pm$  SD; Standard antibiotic Chlorhexidine

ethanol as a solvent for extraction is preferred because it can extract phenol compounds from plants [18]. In this study, the extract was concentrated using the reflux method. The reflux method can optimize phenol and flavonoid compounds [19]. When using this method, the temperature should be maintained at no more than 70° C to avoid degradation of phenolic and flavonoid compounds [19]. This study was conducted to prove ethno medicinal value of the plant, investigating antimicrobial activity and determination of the total Flavonoid and total phenolic contents of flowers of *C.pulcherrima*. This study showed extracts of flowers the highest to inhibit the activity of four studied microbes. According to previous research, methanol extracts from flower parts have antimicrobial activities [20]. The antimicrobial activity found in plant extracts has been linked to several secondary metabolites [21]. Extraction with various types of solvents affected the inhibitory zones of antimicrobial activity [22]. In this study, the antimicrobial activity was using the agar diffusion method. Methods for detecting antimicrobial activity can be classified into three groups: diffusion, dilution and bioautographic [23]. One advantage of the diffusion method is its suitability for screening pure substances [24]. Different parts of the plant may have different secondary metabolites, which can have an impact on their pharmacological. In the present study, the flowers of *C. pulcherrima* (L) Swartz had the highest flavonoid and phenolic content as compared with that in the other parts (i.e. fruits, stems and leaves). This condition

provides results that are relevant to the antimicrobial activity shown by the flowers, which have a high total flavonoid and phenolic content. Antimicrobial activity of part of *C.pulcherrima* in this study were found different from study in other countries [7]. The variation might be caused by different phytogeographic region and plant nutrition, which could modify the secondary metabolites of the plant [25]

#### **4. Conclusion**

The results provide data to support the continued use of *C. pulcherrima* (L) Swartz as a traditional remedy. Further research is needed to determine the type of compound responsible for the antimicrobial effects of *C. pulcherrima* (L) Swartz. In this study, the 70% ethanol extract of the flowers showed inhibit the activity of microbial in vitro againsts microbial that cause dental and oral infection. This result sheds light on the efficacy of *C. pulcherrima* (L) Swartz as an alternative remedy for the treatment of dental and oral complaints.

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