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Phytochemical Screening, Determination of Total Phenolic Contents and Total Flavonoid Contents of Pod Purified Extract from *Caesalpinia pulcherrima* (L.) Sw.

Febriyanti Br Surbakti[1], Sovia Lenny[2]*, Juliati Br Tarigan[2]
[1]*Postgraduate School of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia*
*Corresponding Author: sofia1@usu.ac.id

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ABSTRACT

Caesalpinia pulcherrima (L.) Sw. is traditionally used in medicine and is thought to be influenced by the presence of secondary metabolites, especially phenolic compounds and flavonoids. However, identifying the phytochemical compounds of *Caesalpinia pulcherrima* (L.) Sw. plants, especially on the pods, are less discussed. Here, we propose research on the identification of secondary metabolite compounds of pod-purified extract from *Caesalpinia pulcherrima* (L.) Sw. using phytochemical screening and also the determination of total phenolic and total flavonoid content. The extraction of *Caesalpinia pulcherrima* (L.) Sw. pods were processed by maceration and fractionation methods to obtain the purified extract. Total phenolic contents were determined using the *Folin-Ciocalteu* method. Meanwhile, total flavonoid contents were determined using the $AlCl_3$ method. The result showed that the purified extract of *Caesalpinia pulcherrima* (L.) Sw. pods contained phenolics, flavonoids, alkaloids, and saponins. The total phenolic and total flavonoid content in the pod purified extract were 326.038 mg GAE/g extract and 290.026 mg QE/g extract, respectively.

Keywords: *Caesalpinia pulcherrima (L.)* Sw, Total Phenolic Content, Total Flavonoid Content, UV-Vis Spectrophotometry, *Folin-ciocalteu*.

ABSTRAK

Caesalpinia pulcherrima (L.) Sw. sering digunakan dalam pengobatan tradisional dan diduga dipengaruhi oleh adanya senyawa metabolit sekunder di dalamnya, terutama senyawa fenolik dan flavonoid. Namun, identifikasi senyawa tanaman *C. pulcherrima* (L.) Sw. terutama pada polongnya masih sedikit dibahas. Disini kami mengusulkan penelitian tentang identifikasi senyawa metabolit sekunder dari ekstrak terpurifikasi dari *Caesalpinia pulcherrima* (L.) Sw. menggunakan skrining fitokimia dan juga penentuan kandungan fenolik total dan flavonoid totalnya. Ekstraksi polong *Caesalpinia pulcherrima* (L.) Sw. dilakukan melalui proses maserasi dan fraksinasi untuk mendapatkan ekstrak terpurifikasi. Kandungan fenolik total ditentukan dengan metode *Folin-Ciocalteu.* Sementara itu, kadar flavonoid total ditentukan dengan menggunakan metode $AlCl_3$. Hasil penelitian menunjukkan bahwa ekstrak terpurifikasi dari polong *Caesalpinia pulcherrima* (L.) Sw. mengandung senyawwa fenolik, flavonoid, alkaloid, dan saponin. Total kadar fenolik dalam ekstrak terpurifikasi dari polong adalah 326.038 mg GAE/g ekstrak dan total kadar flavonoid adalah 290,026mg QE/g ekstrak..

Kata Kunci : *Caesalpinia pulcherrima* (L.) Sw., Kadar Fenolik Total, Kadar Flavonoid Total, UV-Vis Spectrophotometry, *Folin-Ciocalteu*.

1. Introduction

Numerous herbal treatments have been utilized in medical systems to treat ailments. One significant application is their use as cytotoxic agents for cancer and tumour treatment. Numerous studies have examined various species of the *Caesalpinia* genus, assessing their antimicrobial, anticancer, anti-inflammatory, anti-psoriatic, antidiabetic, antioxidant, antibacterial, immunomodulatory, and hypoglycemic properties. [1].

One of the plants included in the *Caesalpinia* genus is *Caesalpinia pulcherrima* (L.) Sw. *Caesalpinia pulcherrima* (L.) Sw, a member of the Fabaceae family, is an evergreen shrub widely referred to as the peacock flower, primarily found in tropical and subtropical parts of Asia and Africa [2, 3]. This plant is known by various regional names. The peacock flower is usually planted in gardens as an ornamental plant but is sometimes found growing wild. The peacock flower plant is a type of upright shrub with a height of 2-4 m, many branches with twigs, sometimes with sticky thorns, and a white stem, and solid and rigid [4].

Parts of this plant have been widely used as an essential ingredient for traditional medicine because it has antidiabetic, anticancer, anti-derma-topic [5], antimalarial and antioxidant [1,5,6], cytotoxic, anti-inflammatory, and leishmanicidal potential [1,7,8]. This pharmacology activity can be associated with the presence of secondary metabolite compounds it contains [5]. Based on the literature approach, the secondary metabolites contained in the *Caesalpinia* genus which has been successfully isolated are polyphenols, glycosides, saponins, diterpenoids, phenolics, flavonoids, triterpenoids, benzoic acid, and lactones [6,9,10,11,12,13,14].

Phenolic chemicals are the predominant category of secondary plant metabolites, with more than 8000 identified structures exhibiting significant structural diversity. They are mostly composed of polymerized or monomeric components and can exist as matrix or free-bound compounds, glycosides, or aglycones [15]. These chemicals have attained a notable status owing to their extensive presence in plant-based diets and substantial data indicating a negative association between their consumption and cancer, diabetes, and cardiovascular illnesses. Epidemiological and clinical research indicates that diets rich in polyphenols may lower the incidence of several age-related chronic illnesses [16].

Flavonoids are a group of polyphenols that come from vegetables, fruit and other plants [17]. Flavonoids have antidiabetic and antitoxic potential in diabetes mellitus sufferers. In addition, flavonoids also have antioxidant effects [18], bronchodilator effects, anti-asthma [19], anticancer [20], therapeutic agents [21], antibacterial, antiglycation [22], hypoglycemic and hypolipidemic effects [23].

From the description above, we propose research on pod-purified extract from *Caesalpinia pulcherrima* (L.) Sw. as one of the uses of *Caesalpinia pulcherrima* (L.) Sw. waste, which still has other uses to become something that we can use and is related to the benefits and uses of this plant in pharmacology. We also conducted a study to test the secondary metabolite content, especially the total phenolic and flavonoid contents from pod-purified extract from *Caesalpinia pulcherrima* (L.) Sw. to see the content of purified extracts compared to crude extracts that have been done previously. Where research on purified extracts from *Caesalpinia pulcherrima* (L.) Sw. is still very limited. Therefore, we develop innovations in this study.

2. Materials and Methdos

2.1. Equipments

The instruments used in this research were vacuum rotary evaporator Buchi R-300, waterbath, UV mini-1240 (UV-Vis Spectrophotometer Shimadzu), UV lamp.

2.2. Materials

Caesalpinia Pulcherrima (L.) Sw was obtained from Telagah, Sei Bingei, Langkat Regency, Indonesia. Chemicals used in this research are methanol, HCl 1 M, ethyl acetate, H_2SO_4, *n*-hexane (Merck), HCl 37%, Mg powder, $FeCl_3$ 5%, Mayer's reagent, Dragendorff's reagent, Bouchardart's reagent, Salkowski's reagent, Liebermann-Burchard's reagent, ammonia 25%, distilled water.

2.3. Preparation and Extraction Procedure

3 kg of peeled and chopped pods of *Caesalpinia Pulcherrima* are macerated with 30 L of methanol (sample: solvent = 1:1) and stirred several times for 48 hours at room temperature. The maceration procedure for the sample residue was conducted three times and subsequently concentrated to produce a methanol extract using a rotary evaporator set at 65°C and 80 rpm. Ethyl acetate was used as a solvent to extract the methanol, resulting in the ethyl acetate extract. After dissolving this extract in methanol, it was fractionated with n-hexane for 15 minutes using a separating funnel, and the process was repeated ten times.

Flavonoids: Flavonoids were detected by dissolving 1mg of pods' purified extract with ethyl acetate. The filtrate was mixed with 2-3 mg of Mg powder and concentrated with 2-3 ml of HCl. The formation of yellow, pink, blue, orange, or red colour indicates the presence of flavonoids.

Alkaloids: Alkaloids were detected by dissolving 4 mg of pods in purified extract ethanol, and the filtrate was tested in four test tubes. Tube 1 was tested with Mayer's reagent; tube 2 was tested with Bouchardat's reagent; and tube 3 was tested with Dragendorff's reagent, five drops each. The formation of yellowish-white, brown, orange, or reddish-brown precipitates indicates the presence of alkaloids.

Phenolics: Phenolics were detected by dissolving the pods' purified extract with methanol, and the filtrate was mixed with 3 drops of $FeCl_3$ 5%. Black colloid indicates the presence of tannins.

Saponins: Saponins were detected by dissolving 1 mg of pods' purified extract with methanol. The filtrate was combined with 10 mL of distilled water and briskly shaken. Saponins can be detected by stable foam production within 10 minutes.

Steroids: Steroids were detected by dissolving 2 mg of pods' purified extract with *n*-hexane, and the filtrate was tested in two test tubes. Tube 1 was tested with Salkowski's reagent and tube 2 was tested with Liebermann-Burchard's reagent, five drops each. The formation of brown, reddish brown, or bluish-green indicates the presence of Steroids.

Triterpenoids: Triterpenoids were detected by dissolving 2 mg of pods' purified extract with chloroform, and the filtrate was tested in two test tubes. Tube 1 was tested with Salkowski's reagent and tube 2 was tested with Liebermann-Burchard's reagent, five drops each. The formation of brown or bluish-green indicates the presence of triterpenoids.

2.4. Determination of Total Phenolic Contents(TPC)

The TPC of *Caesalpinia Pulcherrima* pod purified extracts was estimated by using the *Folin-Ciocalteu* method. Briefly, 5 mg plant extract was diluted with 4.5 mL distilled water and 0.5 mL methanol pa to make 5 mL. 0.2 mL of the mixture was mixed with 15.8 mL of distilled water and 1 mL of *Folin-Ciocalteau* reagent (10 %v/v). Following an interval of 8 minutes, the mixture that has been made is put into a 10% w/v dilute sodium carbonate solution and continued with an incubation process for 2 hours at room temperature. After 2 hours of incubation, the reaction mixture obtained was analyzed using a UV spectrophotometer at 765 nm, with a blank without extract as a reference. Gallic acid was used as a standard with concentration variations of 1, 1.25, 1.5, 1.75, and 2 ppm to quantify the total phenolic content, so TPC in the purified extract was calculated as mg equivalents of gallic acid per g of extract (mg GAE/g extract).

2.5. Determination of Total Flavonoid Contents (TFC)

The TFC of *Caesalpinia Pulcherrima* (L.) Sw pod extracts were estimated by using the spectrophotometric method. Briefly, 5 mg plant extract was diluted with 5 ml methanol pa to obtain a sample solution of 1000 ppm. Following the addition of 0.2 mL of 1 M potassium acetate, which was thoroughly mixed and incubated for 5 minutes, 0.3 mL of $AlCl_3$ 10%, which was also thoroughly mixed and incubated for 6 minutes, and 3 mL of methanol pa, the mixture's volume was immediately increased to 10 mL by adding 5.6 mL of distilled water, which was thoroughly mixed, and absorbance measurements were performed using a UV spectrophotometer against a blank containing no extract at 440 nm. The TFC of the purified extract was measured in mg QE/g extract.

3. Results and Discussion

3.1. Extraction and fractination

The sample residue was macerated three times before being concentrated in a rotary evaporator. The resulting concentrated methanol extract was 15.63% w/w. Ethyl acetate was used as a solvent to extract the methanol, resulting in the ethyl acetate extract to produce ethyl acetate extract with 10.9 % w/w yield, which was then dissolved in methanol, followed by fractionation with n-hexane utilizing a separating funnel for 15 minutes, and repeated 10 times. The process of fractionation was conducted iteratively until the n-hexane layer exhibited clarity, which means there were no more non-polar compounds in the extract. The methanol extract was concentrated in a rotary evaporator to obtain purified extract with a 2.17 % w/w yield. The purified extract obtained was visually greenish black in colour (Table 1).

Table 1. % Yield of *Caesalpinia Pulcherrima* (L.) Sw pod purified extract

Sample	Colour	Sample Weight (g)	Weight of Purified Extract (g)	% Yield of Purified Extract
Caesalpinia Pulcherrima (L.) Sw pod	Greenish black	3000	65.2422	2.17

3.2. Phytochemical Screening Test

Testing for the content of secondary metabolite compounds contained in samples is carried out using qualitative phytochemical tests. The test is carried out by adding a reagent appropriate to the compound to be identified. Colour changes indicated the presence of these compounds upon reaction with specific standard reagents, a common method for determining such metabolites. Saponin identification was done by shaking the substance vigorously to produce stable foam for up to 10 minutes in this study from 1 cm to 10 cm height. The foam persisted even after 2 N hydrochloric acid is added in a single drop [26,27]. The foam produced as a positive reaction in this test is due to the glycoside content in saponins, which will be hydrolyzed into glucose and other compounds to produce stable foam [26]. Understanding the phytochemical components present in the extracts is crucial for elucidating the biological and pharmacological effects of the plan [28]. The purified extract from *Caesalpinia pulcherrima* pods contains alkaloids, flavonoids, saponins, and phenolics (Table 2).

Table 2. Identification of secondary metabolite compounds results

Secondary Metabolites	Indicator	Result
Alkaloid	- Mayer	+
	- Dragendorff	+
	- Bouchardart	+
Flavonoid	Mg- HCl	+
Steroid	Lieberman-Burchard	-
	Salkowski	-
Triterpenoid	Lieberman-Burchard	-
	Salkowski	-
Saponin	Aquadest	+
Phenolic	$FeCl_3$ 10%	+

Description:
(+) = indicates presence of compound
(-) = indicates absence of compound

3.3. Determination of Total Phenolic Contents (TPC)

The total phenolic contents of the purified extract of *Caesalpinia Pulcherrima* (L.) Sw collected from Telagah, Sei Bingei, Langkat Regency, Indonesia, was analyzed using the *Folin-Ciocalteu* technique, and the standard used was gallic acid [29]. The *Folin-Ciocalteu* method is the most prevalent technique employed in laboratories globally for quantifying total polyphenol content due to its efficiency and cost-effectiveness. *Folin-Ciocalteu* reagent is composed of lithium sulfate, water, two sodium salts, namely tungstate and molybdate, and two inorganic acids, namely phosphate and chloride. Gallic Acid is the predominant chemical standard employed in the *Folin-Ciocalteu* method because of its good solubility and in an anhydrous state is relatively stable. Moreover, it is comparatively economical [30]. The assessment of total phenolic content relies on the interaction between the *Folin–Ciocalteu* reagent and phosphomolybdic acid with the sample's phenolic compounds, yielding a variety of blue oxides (Figure 1) [31].

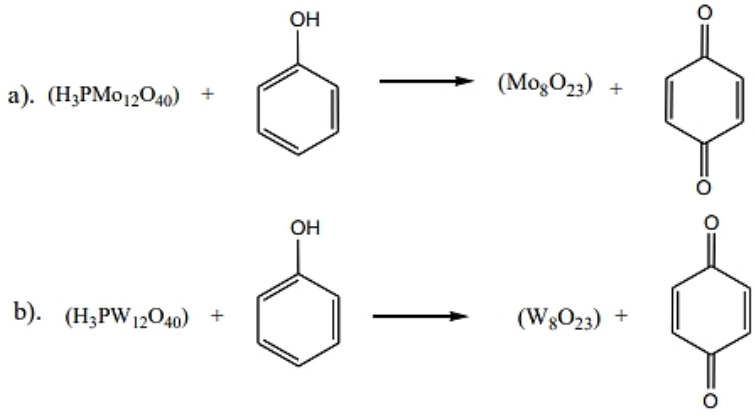

Figure 1. The interaction of the *Folin-Ciocalteu* reagent with phenolic compounds can be delineated as follows: a). the reaction of phosphomolybdic acid with phenolic compounds; b). the reaction of phosphotungstic acid with phenolic compounds.

The complex of phosphomolybdic/phosphotungstic acid exhibits a yellow hue in its oxidized state, whereas it transforms into a vivid blue colour upon reduction by phenolate ions. The development of colour is fundamentally rooted in the transfer of electrons, which facilitates the reduction of the acid complex, leading to the formation of chromogens characterized by metals exhibiting lower valence [30]. The measurement of this compound's absorption was carried out at a wavelength of 765 nm using a spectrophotometer.

Gallic acid was used as a standard with concentration variations of 1, 1.25, 1.5, 1.75, and 2 ppm to quantify the total phenolic content. Gallic acid is used as a standard because it is found in almost all plants and widely found in food matrices, which have three hydroxyl groups. Gallic acid is also a simple phenolic derivate [32,33]. The findings regarding the absorbance, as depicted in the standard curve of gallic acid, are illustrated in Figure 2.

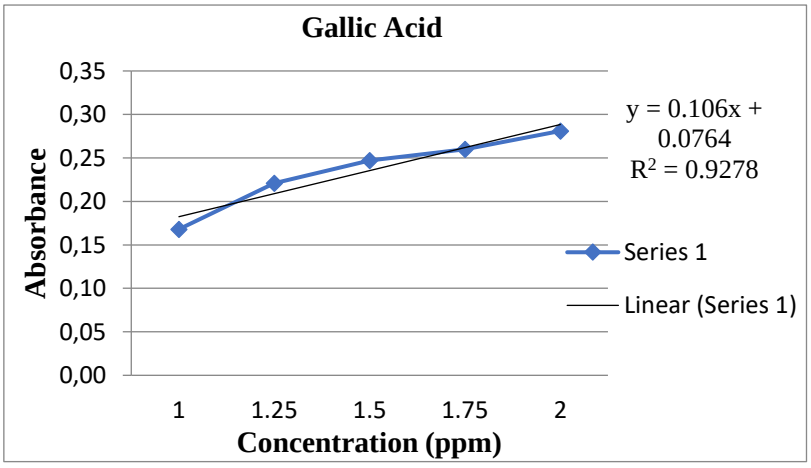

Figure 2. Gallic Acid standard solution curve

From these measurements, the absorbance value increases with increasing solution concentration. In line with Lambert-Beer's law, which asserts that absorbance and sample solution concentration have a linear relationship, where the absorbance value obtained also met the good absorbance range [34]. The standard curve of gallic acid obtained was plotted between the concentration and absorbance. From the curve above, y = 0.106x + 0.0764 and R^2 = 0.9278 are obtained, which can be used to compare the concentration of total phenolic compounds in the sample. The linear relationship between absorbance value and gallic acid content is best described by R^2. The correlation coefficient (R^2) shows the strength and direction of the association between two variables. A number of +1 indicates positive correlation, while a value of -1 indicates negative correlation. The connection is quite strong when the R^2 value falls between 0.80 and 1.0 [35].

There was a significant positive correlation between the two calibration curve variables when taking into account the results for R^2 = 0.9278, indicating their linearity within the tested standard range. Next, the

sample was determined by repeating the treatment three times to minimise errors and improve the validity of the results for data reliability. Measurement of the absorbance value can be seen in Table 3.

Table 3. Determination of total phenolic content of sample

Sample	Replication	Absorbance	Absorbance average	Total Phenolic Content (mgGAE/g extract)
CP	1	0.425		
Purified	2	0.418	0.422	326.038
Extract	3	0.424		

TPC of purified extract obtained 326.038 mgGAE/g extract. In this study, TPC of purified extract from *Caesalpinia Pulcherrima* pods was lower than the total phenolic content reported for *Caesalpinia Pulcherrima* fruit by Lukita *et al.* [36]. The total phenolic content obtained in a previous study was 341.71 mgGAE/ g extract. In this study, 326.038 mgGAE/ g extract was used. This is due to the study's differences in plant parts and solvents. In the previous study, ethanol solvent was used, which has a higher polarity than the solvent used in this study, so the total phenolic content obtained was lower than in previous studies. TPC is influenced by the variety of plants, the environment, processing, solvents used for extraction, climatic conditions and farming of the plant, the storage of the plants after harvest, and analytical methods [37].

3.4. Determination of Total Flavonoid Contents (TFC)

$AlCl_3$ was used to create complexes, causing a wavelength shift towards the visible spectrum, as evidenced by the solution exhibiting a yellow hue. Wavelengths in the visible region are stabilized by adding potassium acetate [38]. In this method, flavonoids that have *o*-dihydroxy or hydroxyl ketone groups will create complex compounds with aluminium metal, resulting in a bathochromic shift. In the $AlCl_3$ colorimetric method, the ketone group at C-4 and the hydroxyl group on the flavonoid will react with $AlCl_3$ to form a stable acid complex. In addition, $AlCl_3$ forms a persistent acid combination with the *o*-dihydroxy group located on the A or B ring of the flavonoid. (Figure 3) [39,40].

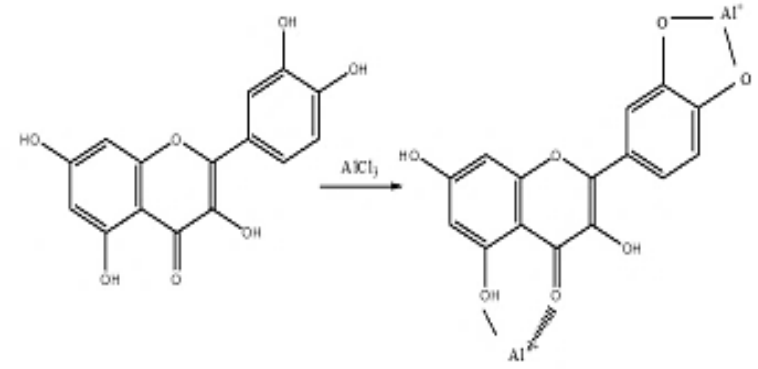

Figure 3. The reaction between flavonoid groups with $AlCl_3$ forms a stable acid complex [34]

Meanwhile, potassium acetate is used to stabilize the structure by ionizing the 3 and 4'-OH groups, which are not complex with Al^{3+} and the 7-OH group, so that the structure still provides absorption in the visible area (Figure 4). The total flavonoid content was assessed using UV-Vis spectrophotometry due to the presence of conjugated aromatic systems in flavonoids, which exhibit pronounced absorption bands in both the ultraviolet and visible light spectra. Quercetin, a prevalent flavonoid, is frequently employed as a standard for quantifying flavonoid levels [41]. Quercetin is used as a comparison because it has all the OH groups which can react with Al^{3+} and potassium acetate [42].

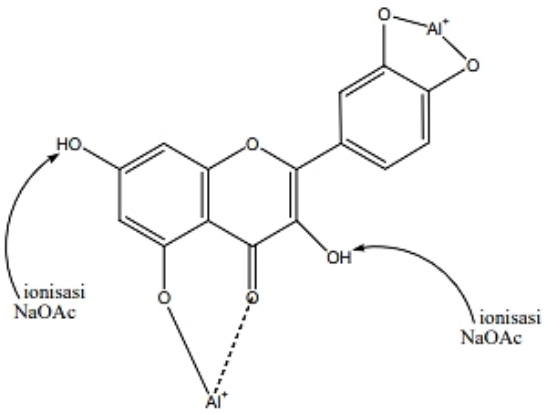

Figure 4. The reaction between flavonoid groups with $AlCl_3$ forms a stable acid complex[34].

Standard quercetin absorbance measurements were carried out at λ 440 nm. The regression equation of the calibration curve (y = 0.0378x + 0.0237) is expressed as milligrams of quercetin equivalents (QE) per gram of dry weight sample (mg QE/g). The method's linearity was assessed within the range of 2 to 10 ppm, yielding a regression coefficient of R^2 = 0.9789, as depicted in Figure 5. The correlation coefficient was close to 1 indicating that the regression equation is linear.

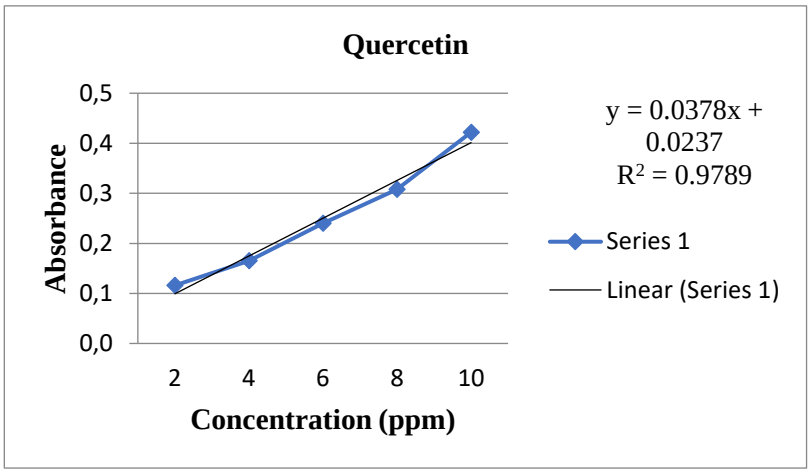

Figure 5. Quercetin standard solution curve

The standard curve of quercetin obtained was plotted between the concentration and its absorbance. From the curve above, y = 0.0378x + 0.0237 and R^2 = 0.9789 is obtained, which can be used to compare the concentration of TFC in the sample. TFC of purified extract obtained was 290.026 mg QE/g (Table 4). The total flavonoid content of purified extract from *Caesalpinia Pulcherrima* (L.) Sw pods in the present study were higher than the value reported for *Caesalpinia Pulcherrima* (L.) fruit by Lukita *et al.* [36]. In a previous study, TFC obtained was 2.26 mg QE/g extract, whereas it was 290.026 mg QE/g extract in this study. This is due to differences in plant parts and solvents used in the study. Where in the previous study, ethanol solvent was used, which has a higher polarity than ethyl acetate, so flavonoid compounds will be more attracted to ethyl acetate because flavonoid compounds are semi-polar. So, the total flavonoid content obtained was higher than in previous studies.

Table 4. Determination of Total Flavonoid Content of sample

Sample	Replication	Absorbance	Absorbance average	Total Flavonoid Content (mg QE/g)
CPP purified extract	1	1.120	1.120	290.026
	2	1.119		
	3	1.121		

A previous study conducted by Purnama *et al.* examined the effect of several solvents on chemical compounds. The results showed that the total flavonoid content of the n-hexane fraction (6.27 mg QE/g sample) was higher than the water fraction (3.09 mg QE/g sample) because flavonoid compounds had low polarity (semi-polar to non-polar). This was caused by the difference in the polarity of the compounds in each solvent [43]

4. Conclusion

The yield of purified extract of *Caesalpinia Pulcherrima* (L.) Sw pods were obtained at 2.17%, and the qualitative result of phytochemical screening was positive for alkaloids, saponins, phenolics, and flavonoids. The purified extract of *Caesalpinia Pulcherrima* (L.) Sw pods had a total phenolic content of 326.038 mg GAE/g extract and a total flavonoid of 290.026 mgQE/g extract.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

References

[1] Pournaghi N, Sigaroodi FK, Safari E, Hajiaghaee R. A review of the genus Caesalpinia L.: emphasis on the cassane and norcassane compounds and cytotoxicity effects. J. Med. Plants. 2020; 19(76): 1 -20.

[2] Qiao Y, Xu Q, Hu Z, Li XN, Xiang M, Liu J, Huang J, Zhu H, Wang J, Luo Z, Xue Y, Zhang Y. Diterpenoids of the Cassane Type from Caesalpinia decapetala. J. Nat. Prod, 2016; 79(12): 3134-3142

[3] Wang DS, Nie W, Jiang TT, Ding LF, Song LD, Wu XD, Zhao QS. Caesalpanins A-C, Three Dimeric Cassane Diterpenoids from the Seeds of Caesalpinia sappan L. Chem. Biodiversity. 2020: 1-10

[4] Sihotang ISA. Uji Aktivitas Antibakteri Ekstrak Metanol, Etil Asetat dan NHeksana dari Daun Kembang Merak Bunga Kuning (Caesalpinia pulcherrima [L.]Sw.). [skripsi]: Universitas Sumatera Utara; 2018.

[5] Deepika S, Selvaraj CI, Roopan SM. Screening bioactivities of Caesalpinia pulcherrima L. swartz and cytotoxicity of extract synthesized silver nanoparticles on HCT116 cell line. Materials Science & Engineering C. 2020; 106: 110279-88.

[6] Ogbeide O, Okhomina OK, Omoregie IG, Unuigbe CA, Augustine I, Isacc A, Iheanacho M, Akubuiro P, Solomon A, Irabor EEI, Owolabi JB, Falodun A. Antimalarial, ferric reducing antioxidant power and elemental analysis of Caesalpinia pulcherrima leaf extract. J Chem Soc Nigeria 2020; 45(4):704 -711.

[7] Erharuyi O, Adhikari A, Falodun A, Aimas J, Ahmmad M, Rehan I, Choudhary MI. Cytotoxic, antiinflmmatory, and leishmanicidal activities of diterpenes isolated from the roots of Caesalpinia pulcherrima. Planta Med. 2017; 83(1- 2):100-110.

[8] Erharuyi O, Adhikari A, Falodun A, Imad R, Choudhary MI. Derivatization of cassane diterpenoids from Caesalpinia pulcherrima (L.) Sw. and evaluation of their cytotoxic and leishmanicidal activities. Tetrahedron Lett. 2016; 57(20):2201-2206.

[9] Sadaf S, Erharuyi O, Falodun A, Choudhary MI, Sammer Y. Crystal structure of (3S*,4S*,4aS*,5R*,6R*,6aS*,7R*,11aS*,11bR*)-5,6-bis-(benzoyloxy)3,4adihydroxy -4,7,11btrimethy l,1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydrophenanthro[3,2-b]-furan -4-carboxylic acid methanol monosolvate. Acta Crystallogr. 2015; E71:739- 740.

[10] Yun X, Chen XM, Wang JY, Lu W, Zhang ZH. Cassane diterpenoids from Caesalpinia pulcherrima and their anti-inflammatory and a-glycosidase inhibitory activities. Nat. Prod. Res 2022; 36:4636–4644. [11] Faloye K, Famuyiwa S, Akinwunmi K, Tata C, Ayoola M, Fakola E, Akinyele O, Ndinteh D. Antioxidant Potentials of the Pod Extract of Caesalpinia Pulcherrima Swarta (Fabaece) and the Theoretical Evaluation of the Antioxidant Property of the Isolated Compounds. 2021; 1-14

[12] Ogbeide OK, Akhigbe IU, Unuigbe CA, Erharuyi O, Oseghale I, Imieje V, Iheanacho C, Ikeke K, Ayeni B, Irabor E, Owolabi JB, Falodun A. Isolation, characterisation and in vivo anti-malarial investigation of pulcherrimin A from Caesalpinia pulcherrima stem bark. GSC Biological and Pharmaceutical Sciences. 2020; 12(02):056-063.

[13] Khan F, Nida D, Mehreen L, Muhammad Y, Zulfiqar AM, Ahmed M, shaheen F, Shahana U. Immunomodulatory activities of extracts of Caesalpinia pulcherrima. Journal of Herbs, Spices & Medicinal Plants. 2018.

[14] Oladoja FA, Irokosu ES, Ayoola MD, Elijah OO, Akanji MA, Beatrice OT, Samuel OA. Evaluation of the Antidiabetic Activity and Toxicological Properties of Hippocratea Velutina (Afzel.). Clinical Complementary Medicine and Pharmacology. 2023; 3(2):100080.
[15] Alara OR, Abdurahman NH, Ukaegbu CI. Extraction of phenolic compounds: A review. Current Research in Food Science. 2021: 2000-2014.
[16] Debelo H, Li M, Ferruzzi MG. Processing influences on food polyphenol profiles and biological activity. Curr. Opin. Food Sci. 2020; 32: 90– 102
[17] Serra D, Almeida LM, Dinis TCP. Dietary polyphenols: A novel strategy to modulate microbiota-gutbrain axis, Trends Food Sci. Technol. 2018; 78: 224–233. https://doi.org/10.1016/j.tifs.2018.06.007
[18] Uddin J, Ali Shah SW, Zahoor M, Ullah R, Alotaibi A. Chalcones: The flavonoid derivatives synthesis, characterization, their antioxidant and in vitro/in vivo antidiabetic potentials. Heliyon, 2023; 9(11). https://doi.org/10.1016/j.heliyon.2023.e22546
[19] Abdel-Kader MS, Saeedan AS, Rehman NU, Faqihi HM, Solimin GA. New flavonoids with multiple bronchodilator activity pathways from Tephrosia purpurea L. (Pers.) growing in Saudi Arabia. Saudi Pharmaceutical Journal. 2024; 32(2024) 101992. https://doi.org/10.1016/j.jsps.2024.101992
[20] Seo JI, Yu JS, Zhang Y, Yeo HH. Evaluating flavonoids as potential aromatase inhibitors for breast cancer treatment: In vitro studies and in silico predictions. Chemico-Biological Interactions. 2024; 2797(24)00073-5. https://doi.org/10.1016/j.cbi.2024.110927
[21] Ren Z, Yu Y, Ji Z, Li H, Li X, Lin H, Ge R, Zhu Q. Structure-activity relationship and docking analysis of nature flavonoids as inhibitors of human and rat gonadal 3βhydroxysteroid dehydrogenases for therapeutic purposes. Journal of Steroid Biochemistry and Molecular Biology. 2024; 238:106450-61. https://doi.org/10.1016/j.jsbmb.2023.106450
[22] Mohsin A, Hussain MH, Zaman WQ, Mohsin MZ, Zhang J, Liu Z, Tian X, Rehman SU, Khan IM, Niazi S, Zhuang Y, Guo M. Advances in sustainable approaches utilizing orange peel waste to produce highly value-added bioproducts. Crit. Rev. Biotechnol. 2021; 42(8): 1284–1303. https://doi.org/10.1080/07388551.2021.2002805
[23] Arifah FH, Nugroho AE, Rohman A, Sujarwo W. A review of medical plants for the treatment of diabetes mellitus: The case of Indonesia. South African Journal of Botany. 2022; 149: 537-558. https://doi.org/10.1016/j.sajb.2022.06.042
[24] Lumbantoruan MS, Lenny S, Sembiring H. Antioxidant Activity Assay of Eucalyptus pellita Leaf Extract. International Journal of Ecophysiology. 2023; 5(02): 26-33.
[25] Harborne JB. Phytochemistry. London. Academic Press; 1993. 89-131 p.
[26] Kumalasari MLF dan Andiarna F. Uji Fitokimia Ekstrak Etanol Daun Kemangi (Ocimum basilicum L). Indonesian Journal fot Health Sciences. 2020; 4(1): 39. https://doi.org/10.24269/ijhs.v4il.2279
[27] Inarah F, Hariyanto IH, Irfan RS, Monica S. Skrining fitokimia dan analisis kromatografi lapis tipis dari ekstrak etanol buah lerak (Sapindus rarak). Jurnal Pendidikan Informatika dan Sains. 2017; 6(2): 243-256.
[28] Safitri A, Fatchiyah F, Sari DRT, Roosdiana A. Phytochemical screening, in vitro anti-oxidant activity, and in silico anti-diabetic activity of aqueous extracts of Ruellia tuberosa L. J. Appl. Pharm. Sci. 2020; 10(3): 101–108.
[29] Hagos M, Chandravanshi BS, Abshiro MR, Yaya EE. Determination of total phenolic, total flavonoid, ascorbic acid contents and antioxidant activity of pumpkin flesh, peel and seeds. Bull. Chem. Soc. Ethiop. 2023; 37(5): 1093-1108.
[30] Raposo F, Borja R, Gonzales JAG. A comprehensive and critical review of the unstandardized FolinCiocalteu assay to determine the total content of polyphenols: The conundrum of the experimental factors and method validation. Talanta. 2024; 272(125771): 1-10.
[31] Bancuta OR, Chilian A, Bancuta I, Ion RM, Setnescu R, Setnescu T, Gheboianu. Improvement of spectrophotometric method for determination of phenolic compounds by statistical investigations. Romanian Journal of Physics. 2016; 1-11
[32] Fachriyah E, Kusrini D, Haryanto IB, Wulandari SMB, Lestari WI, Sumariyah. Phytochemical Test, Determination of Total Phenol, Total Flavonoids and Antioxidant Activity of Ethanol Extract of Moringa Leaves (Moringa oleifera Lam). Journal of Scientific and Applied Chemistry. 2020; 23(8): 290–294.
[33] Sam S, Malik A, Farmasi F, Indonesia UM. Penetapan kadar fenolik total dari ekstrak etanol bunga rosella berwarna merah (Hibiscus Sabdariffa L.). 2020; 3(2): 182-187
[34] Zealviani S. Analisis nilai absorbansi untuk menentukan kadar flavonoid daun jarak merah (Jatropha Gossypyfolia L.) menggunakan spektrofotometer UV-Vis. Jurnal Fisika dan terapannya. 2021; 8:56-64

https://doi.org/10.24253/jft.v8i2.23379
[35] Fogarasi M, Socaiu MI, Salagean CD, Ranga F, Farcas AC, Socaci SA, Socaciu C, Ibulca D, Fogarasi S, Semeniuc CA. Comparison of different extraction solvents for characterization of antioxidant potential and polyphenolic composition in Boletus edulis and Cantharellus cibarius mushrooms from Romania. Molecules. 2021; 26:7508.
[36] Lukita BL, Budiardjo SB, Elya B. 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. Iranian Journal of Pharmaceutical Sciences. 2019; 15(4): 1-10.
[37] Ayele DT, Akele ML, Melese AT. Analysis of total phenolic contents, favonoids, antioxidant and antibacterial activities of Croton macrostachyus root extracts. BMC Chemistry. 2022; 16(30): 1-9.
[38] Ahmad AR, Juwita, Ratulangi SAD, Malik A. Penetapan Kadar Fenolik dan Flavonoid Total Ekstrak Metanol Buah dan Daun Patikala (Etlingera elatior (Jack) R.M.SM). Pharm Sci Res. 2025; 2(1): 1-10
[39] Markham KR. Cara Mengidentifikasi Senyawa Flavonoida. Terjemahan Kosasi ed. Bandung. ITB Press; 1998.
[40] Cornard, J. P. & Merlin, J. C., Complexes of aluminium (III) with isoquercitrin: spectroscopic characterization and quantum chemical calculations. Polyhedron, 21: 2801–2810 (2002).
[41] Nurlinda, Handayani V, Rasyid FA. Spectrophotometric Determination of Total Flavonoid Content in Biancaea Sappan (Caesalpinia sappan L.) Leaves. Jurnal Fitofarmaka Indonesia. 2021; 8(3): 1-4. https://doi.10.33096/jffi.v8i3.712
[42] Sjahid LR, Aqshari A, Sediarso. Penetapan Kadar Fenolik dan Flavonoid Hasil Ultrasonic Assisted Extraction Daun Binahong (Anredera cordifolia [Ten] Steenis). Jurnal Riset Kimia. 2020; 11(1): 1-8. https://doi.org/10.25077/jrk.v11i1.34
[43] Purnama RA, Sunarta S, and Ismail H. Effect of Several Solvents on the Chemical Compound, Antioxidant Activity, and Cytotoxicity Levels of Acacia decurrens Willd. Gum Extract. Jurnal Ilmu Kehutanan. 2022; 16(2): 171-183. https://doi.org/10.22146/jik.v16i2.2254.

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