

# Analysis of total phenolics and flavonoids content from methanol extract of *Caesalpinia bonduc* (L.) Roxb. seeds and antioxidant activity assay

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**ABSTRACT:** *Caesalpinia bonduc* (L.) Roxb. seed is known as gorek seed contain secondary metabolites such as alkaloids, tannins, flavonoids, and phenolics, due to the content of these secondary metabolites so that this plant has antioxidant activity. This study aimed to determine total phenolic and flavonoid contents of methanol extract of gorek *C. bonduc* seeds as well as antioxidant activity. Extraction of gorek *C. bonduc* seeds was carried out with methanol, then quantitative determination of total phenolic by the Folin-Ciocalteu method as Gallic Acid Equivalent (GAE)/gram of extract, flavonoid content by AlCl<sub>3</sub> method as Quercetin Equivalent (QE)/gram of extract, and in vitro antioxidant activity with DPPH (2,2-diphenyl-1-picrylhydrazyl) method was in IC<sub>50</sub> (inhibition concentration). Based on the results of research, the phytochemical screening of methanol extract of gorek *C. bonduc* seeds showed alkaloids, flavonoids, saponins, tannins, and phenolics. The determination of the total phenolic and flavonoid content were obtained  $0.55 \pm 0.02$  mgGAE/g and  $0.50 \pm 0.02$  mgQE/g, respectively. The antioxidant activity of methanol extracts and ascorbic acid showed IC<sub>50</sub> values were 3770.77 ppm and 3.36 ppm respectively.

## INTRODUCTION

Free radicals are intermediate products formed in various chemical reactions in the body and also from polluted environments such as cigarette smoke, vehicle fumes, pollutants and radiation [1]. Free radicals are highly reactive compounds and can bind or attack electrons in surrounding molecules such as carbohydrates, lipids, proteins, and DNA [2]. Imbalance occurs if the free radicals produced exceed the limit of their cellular antioxidant protection capability, the free radicals will affect the process of disease emergence [3-4]. Free radicals are one of the causes of several diseases, such as Parkinson disease, Alzheimer type dementia etc. Free radical production is effectively combated by the use of antioxidants [5].

Plants containing flavonoids and phenolics are known to possess strong antioxidant properties [6] and potent antioxidant activity leading therefore to various defensive and disease fighting properties. Phenolic compounds are plants secondary metabolites considered as very important plant constituents due to the presence of one or more hydroxyl groups on their aromatic ring. Those phenolic compounds being non harmful to human's health [7].

*Caesalpinia bonduc* (L.) Roxb. is a medicinal plant belonging to the family Caesalpiniaceae. It is a prickly shrub widely distributed all over the world especially in Indian tropical regions such as Kerala, Andaman and Nicobar Islands and Sri Lanka. It is popular in indigenous system of medicine in India. It has been considered as an important remedy for the treatment of several diseases. In Indonesia known as gorek plant. All parts of the plant have medicinal properties so it is a very valuable medicinal plant which is utilized in

traditional system of medicine. The plant has been reported to possess antidiabetic, antiinflammatory, antimalarial, antimicrobial, antifungal, antitumor, and antioxidant [9-10]. The antioxidant activity of *Caesalpinia bonduc* (L.) Roxb. may be it is caused by the presence of flavonoid dan fenolic compound in it. Hence, This study aimed to determine total phenolic and flavonoid contents of methanol extract of gorek *Caesalpinia bonduc* (L.) Roxb. seeds as well as antioxidant activity as a basic information to develop the research about this plant in health, pharmacology, and medic.

## RESULT AND DISCUSSION

### Extraction

The extraction result of *C.bonduc* seeds with methanol as solvent can be seen in **Table 1**.

**Table 1.** Extraction Results of *C.bonduc* Seeds

Material	Weight of sample	Weight of extract	Yield (%b/b)
<i>C.bonduc</i> seeds	300 g	61,67 g	20,6%

### Phytochemical Screening

The methanol extract of *C. bonduc* seeds were obtained are then tested to determine the phytochemical components contained there in. Common phytochemical content of plants such as flavonoids, alkaloids, terpenoids, steroids, tannins, and saponins can be identified, can be seen in **Table 2**. Phytochemical analysis conducted by Manikandaselvi, et al. [18] that the seed powder of *C. bonduc* had alkaloids, phenols, flavonoids, tannins, lignins, carbohydrates, protein, lipids, and fiber.

**Table 2.** Phytochemicals Screening of *C. bonduc* Seeds

Secondary metabolite	Test result
alkaloid	+
phenol	+
flavonoid	+
tannin	+
Terpenoid/steroid	-
saponin	+

### Total Phenolic Content

The total phenolic content of *C. bonduc* seeds was determined by Folin-Ciocalteu method were reported as gallic acid equivalents (GAE) mg/g of extract. The gallic acid calibration curve can be seen in Figure 1 which shows the maximum absorbance at a wavelength 750 nm with the equation  $y = 0.055x + 0.006$ ,  $R = 0.999$ . It is known that the total phenolic content in the methanol extract of *C.bonduc* seeds was  $0.55 \pm 0.02$  mgGAE/g. As compared with other study from several references, the total phenolic content obtained in this study was lower.

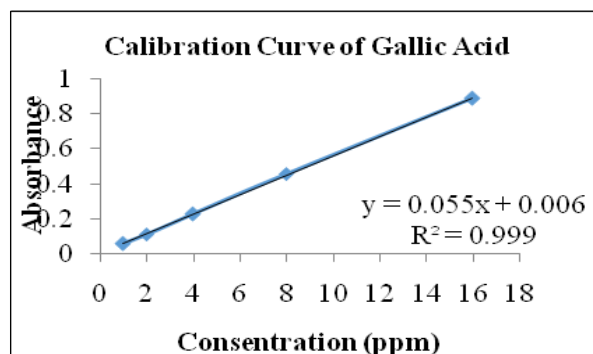


Figure 1. Calibration Curve of Gallic Acid

Sembiring, et al. (2018)[19] reported the total phenolic content of the ethanolic extract of *C.bonduc* seeds were  $146.64 \pm 3.94$  mgGAE/g (leaves),  $144.42 \pm 16.05$  mgGAE/g (stems),  $89.81 \pm 3.00$  mgGAE/g (roots), and  $70.34 \pm 10.59$  mgGAE/g (seeds). Shukla, et al. [4] reported the total phenolic content of the ethanolic extract of *C.bonduc* seeds was 62.50 mg/g, and  $21.96 \pm 2.12$  g/mL in 1000 g/mL chloroform extract [20].

### Flavonoid Content

The flavonoid content of *C.bonduc* seed was determined by  $AlCl_3$  method were reported as Quercetin equivalent (QE) mg/g of extract.  $AlCl_3$  will cause a complex reaction with flavonoid compounds [21]. Quercetin calibration curve can be seen in Figure 2 which shows the maximum absorbance at a wavelength 428 nm with  $y = 0.009x - 0.001$ ,  $R = 0.997$ . It is known that the flavonoid content in the methanol extract of *C.bonduc* seeds was  $0.50 \pm 0.02$  mgQE/g. As compared with other study from several references, the flavonoid content obtained in this study was lower. Sembiring, et al. [19] reported the flavonoid content of the ethanolic extract of *C. bonduc* seeds were  $31.05 \pm 0.35$  mgQE/g (leaf),  $21.82 \pm 0.46$  mgQE/g (stem),  $12.55 \pm 0.08$  mgQE/g (root), and  $13.21 \pm 1.35$  mgQE/g (seed).

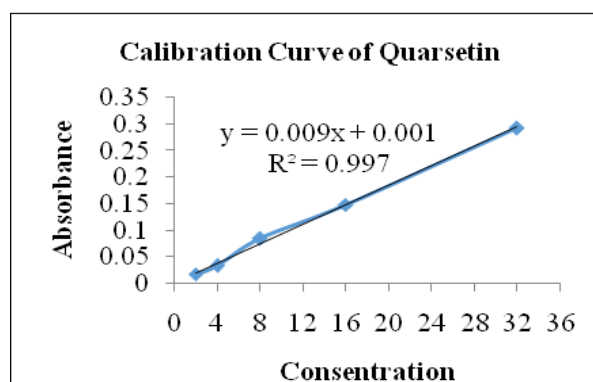


Figure 2. Calibration Curve of Quercetin

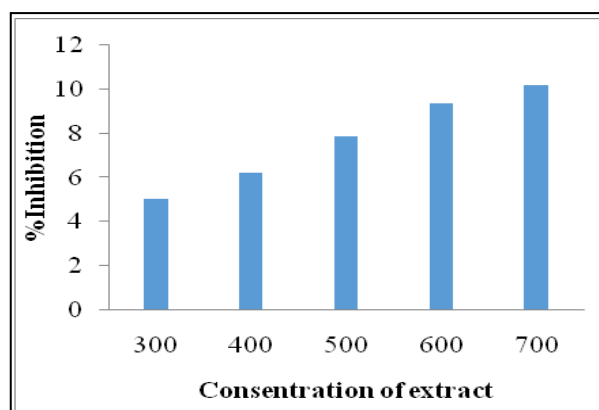
### Antioxidant Activity

The antioxidant capacity of plants can be tested using a wide variety of methods. In the present study, the antioxidant activity of the fresh produce was evaluated in terms of their free radical scavenging capacity by DPPH assay. DPPH method is a simple antioxidant measurement method that does not require a lot of reagents like other methods. DPPH

reagents are compounds that can counteract or reduce the negative impact of oxidants by donating one electron to compounds that are oxidants so that the activity of these oxidant compounds can be inhibited [1].

DPPH is a free radical that is stable at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH radical is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals is initiated by lipid autooxidants [22]. Radical inhibitory activity is determined from the reduction reaction that occurs. The radical scavenging activity of sample was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical by antioxidants. A positive DPPH test indicates that the sample is a free radical scavenger [23].

In this study, antioxidant activity of *C. bonduc* seeds was measured by DPPH at wavelength 515 nm. Ascorbic acid with known antioxidant potential was used as a positive control. The antioxidant activity of the extract and positive control was shown in **Figure 3** and **4**. The antioxidant activity of the extract and positive control was determined according to the regression equation,  $y = 0.013x + 0.980$ ,  $R^2 = 0.989$  for the extract and  $y = 15.50x - 2.019$ ,  $R^2 = 0.998$  for the control positive, so that the  $IC_{50}$  value can be calculated, it was 3770.77 ppm and 3.36 ppm, respectively. The ability of the methanol extract of *C. bonduc* seeds inhibiting DPPH radicals was lower than ascorbic acid. According to Matheos, et al. [24], vitamin C or ascorbic acid has two sites for the hydrogen release process which are connected internally so that there is a further release process after the first release. Ascorbic acid has high antioxidant activity because ascorbic acid has 2 hydroxyl groups which makes it easier to donate hydrogen.



**Figure 3.** Inhibition ability of methanol extract of *C. bonduc* seed

Antioxidant activity of *C. bonduc* seeds obtained in this study is relatively low. This is consistent with the low total phenolic and flavonoid content obtained. As it is known that the content of phenolic compounds may contribute to antioxidant activity through the ability of these compounds to donate hydrogen so that their levels in the extract can determine high or low antioxidant activity. Antioxidant properties of *C. bonduc* seeds had been reported by Sachan, et al. [20] that the chloroform extract of *C. bonduc* seed had an  $IC_{50}$   $170 \pm 4.08$  g/mL and a total phenolic content of  $21.96 \pm 2.12$  g/mL in 1000 g/mL, the water extract of shell *C. bonduc* had a value of  $IC_{50}$   $350.638$   $\mu$ g/ml [25], and the ethanol extract of *C. bonduc* seed had  $IC_{50}$   $74.73$   $\mu$ g/ml [4]. The variation might be caused by

different phytogeographic region and plant nutrition, which could modify the secondary metabolites of the plant and due to the different method of extraction and solvents polarities [19].

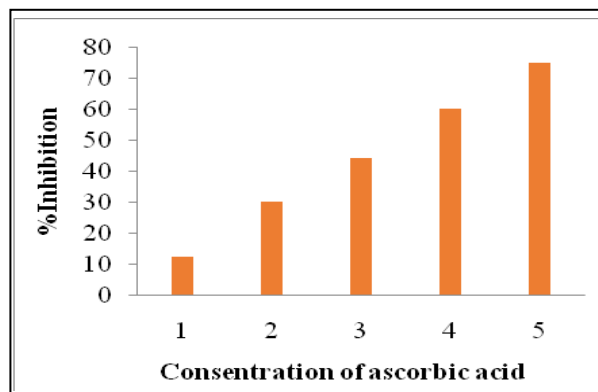


Figure 4. Inhibition ability of ascorbic acid

## CONCLUSION

The result showed that methanol extract of *C. bonduc* seeds contains alkaloids, phenols, flavonoids, saponins, and tannins. The total phenolic and flavonoid contents respectively were  $0.55 \pm 0.02$  mgGAE/g and  $0.50 \pm 0.02$  mgQE/g, as well as antioxidant activity with IC<sub>50</sub> 3770.77 ppm, and ascorbic acid as positive control has a value of IC<sub>50</sub> 3.36 ppm.

## MATERIAL AND METHOD

### Materials

Materials used in this research were Gorek *Caesalpinia bonduc* (L.) Roxb. seeds, dragendrof reagen, wagner reagen, NaOH 10%, H<sub>2</sub>SO<sub>4</sub>, anhidrat asetic acid, Liberman buchard, NaCl, FeCl<sub>3</sub>, ascorbic acid, Folin-Ciocalteu, AlCl<sub>3</sub>10%, CH<sub>3</sub>COOK 1 M, Na<sub>2</sub>CO<sub>3</sub>, gallic acid, quersetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, ethanol, and aquadest.

### Procedures

#### Sample Preparation

*C. bonduc* seeds used in this research were obtained from Gusung Timur, Selayar, South Sulawesi, Indonesia. *C. bonduc* seeds were cut into small sizes. Then dried in an oven for 2 days at 40°C, then crushed in an electric grinder into powder [11].

#### Extraction

The extraction process was carried out based on Devi, et al. (2020) [12]. which has been modified. Powdered of *C. bonduc* seeds was weighed as much as 300 grams was extracted by maceration method using methanol as the solvent for 24 hours and repeated 3 times. The crude extract was filtered. The filtrate obtained was concentrated under vacuum in a rotary evaporator at 30-40°C for further use.

#### Phytochemical Screening

The extract that obtained was continued to the phytochemical test. Phytochemical screening for alkaloid, phenolic, flavonoid, tannin, saponin, and terpenoid/steroid was performed according to the standard procedure described by Harbone, et al. [13].

#### *Determination of Total Phenolic Content*

The total phenolic content was determined by Folin–Ciocalteu method adapted from Chanwitheesuk, et al. (2004) [14] with slight modification.

#### *Sample Preparation*

The sample was weighed as much as 0.05 g, then added 10 mL of hot distilled water (80 °C). After that, it was allowed to stand for 10 minutes, then filtered to obtain a sample filtrate. Samples are diluted if necessary.

#### *Determination of Total Phenolic Content*

A total of 5 mL of sample was added 0.25 mL of 50% Follin reagent, then 0.5 mL of saturated  $\text{Na}_2\text{CO}_3$  was added, then the mixture was left for 30 minutes. The absorbance of the mixture was measured at the maximum wavelength 750 nm using a UV-Vis spectrophotometer. Gallic acid was used as standard in various concentration 1, 2, 4, 8, 16 ppm were prepared in ethanol and distilled water was used as blank. Using a standard curve, the total phenolic compound content can be calculated and expressed as gallic acid equivalents (GAE) in mg/g of extract.

#### *Determination of Flavonoid Content*

Total flavonoid content was determined by aluminium chloride method adapted from Singleton and Rossi (1965)[15] with slight modification.

#### *Sample Preparation*

The sample was weighed as much as 0.05 g, then dissolved in 10 mL of ethanol. Then filtered if necessary to obtain a sample filtrate. Samples are diluted if necessary.

#### *Determination of Total Flavonoid Content*

Samples of 0.5 mL were added 3 mL of methanol, was added 0.2 mL of  $\text{AlCl}_3$  and 0.2 mL of 10%  $\text{CH}_3\text{COOK}$  1 M. Then volume was made up to 10 mL by adding 6.2 mL aquadest. The absorbance of the mixture was measured at the maximum wavelength 428 nm using a UV-Vis spectrophotometer. Quercetin was used as a standard in various concentration 2, 4, 8, 16, 32 ppm were prepared in methanol and methanol was used as a blank. By using a standard curve, the content of flavonoid can be calculated and expressed as quarcetin equivalents (QE) in mg/g extract.

#### *Determination of Antioxidant Activity*

DPPH scavenging ability assay was used to evaluate the antioxidant activity of extract. Test was conducted in a a UV-Vis spectrophotometer according to Molyneux (2004) [16] with slight modification. The extract of *C. bonduc* seeds were made in concentrations of 300, 400, 500, 600 and 700 ppm. Ascorbic acid was used as positive control in different concentrations 1, 2, 3, 4, and 5 ppm. After that, the measuring series solution was added with 1 mL of 0.4 mM DPPH each solution and added methanol until a total volume of 5 mL was obtained. After 30 minutes incubation at room temperature in dark room, absorbance



was read at 515 nm using a UV-Vis spectrophotometer [17]. The scavenging ability (%inhibition) was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{control abs.} - \text{sample abs.}}{\text{control abs.}} \times 100\%$$

Concentration of samples resulting in 50% inhibition on DPPH (IC<sub>50</sub> value) were calculated.

## DECLARATION

There is no conflict of interest from authors for this research.

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## REFERENCES

- [1] Sayuti, K.; Yenrina, R., (2015) *Antioksidan Alami dan Sintesis*, Andalas University Press, Padang.
- [2] Jang, H. D.; Chang, K. S.; Huang, Y.; Hsu, C. L.; Lee, S. H.; Suet, M.S., (2007) *Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants*, Food Chemistry, **103**, 749-56.
- [3] Halliwell, B.; Gutteridge, J. M.; Aruoma, O. I., (1987) *The deoxyribose method: a simple "test tube" assay for determination of rate constants for reactions of hydroxyl radicals*, Analytical Biochemistry, **165**, 215-219.
- [4] Shukla, S.; Mehta, A.; John, J.; Singh, S.; Mehta, P.; Vyas, S. P., (2009) *Antioxidant activity and total phenolic content of ethanolic extract of Caesalpinia bonducella seeds*, Food and Chemical Toxicology, **47**, 1848-1851.
- [5] Sivasankari, K. V.; Janaky, S.; Sekar, T., (2011) *Antioxidant Status of Leaves of Caesalpinia Bonduc*, International Journal of Pharmaceutical Applications, **4**(2), 262-266.

- [6] Badami, S.; Moorkoth, S.; Rai, S. R.; Kannan, E.; Hojraj, S. B., **(2003)** *Antioxidant Activity of Caesalpinia sappan Heartwood*, Biol. Pharm. Bull, **26**(11), 1534-1537.
- [7] Kahkonen, M. P.; Hopia, A. I.; Vuorela, H. J.; et al., **(1999)** *Antioxidant activity of plant extracts containing phenolic compounds*, J Agric Food Chem, **47**, 3954-3962.
- [8] Billah, M. M.; Islam, R.; Khatun, H.; Parvin, S.; Islam, S. M. A.; Mia, A. A., **(2013)** *Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of Caesalpinia bonducella (L.) Roxb leaves*, BMC Complementary and Alternative Medicine, **101**(13), 1-7.
- [9] Vadlakonda, V.P. dan Chaudhari, S.G., 2019, *The Pharmacological Potential Of Caesalpinia Bonduc*, Indo American Journal of Pharmaceutical Research, **9**(12), 584-594.
- [10] Gadakh, M. J.; Jadhav, R. S.; Vikhe, D. N., **(2020)** *Biological Potential of Caesalpinia bonducella Seeds: A Review*, Journal of Drug Delivery & Therapeutics, **10**(3-s): 308-310.
- [11] Jana, K.; Chatterjee, K.; Ali, K. M.; De, D.; Bera, T. K.; Ghosh, D., **(2012)** *Antihyperglycemic and antioxidative effects of the hydro-methanolic extract of the seeds of Caesalpinia bonduc on streptozotocin-induced diabetes in male albino rats*, pharmacognosy Research, **4**(1), 57-82.
- [12] Devi, S.; Ropiqa, M.; Murti, Y. B.; Nugroho, A. K.; **(2020)** *Screening of Extraction Process and The Estimation of Total Alkaloids in Carica papaya Linn. Leaf*, Traditional Medicine Journal, **25**(2), 90-95.
- [13] Harborne, J. B., **(1987)** *Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan Edisi ke-2*, ITB, Bandung.
- [14] Chanwitheesuk, A.; Teerawutgulrag A.; Rakariyatham N., **(2004)** *Screening of Antioxidant Activity and Antioxidant Compounds of Some Edible Plants of Thailand*, Food Chemistry, **92**, 491-497.
- [15] Singleton, V. and Rossi, J., **(1965)** *Colorimetry of Total Phenolic Compounds with Phosphomolybdic-Phosphotungstic Acid Reagents*, American Journal of Enology and Viticulture, **16**, 144-158.
- [16] Molyneux, P., 2004, *The Use of The Stable Free Radikal Diphenyl Picrylhydrazyl (DPPH) For Estimating Antioxidant Activity*, Journal Science of Technology, **26**(2), 211-219.
- [17] Junopia, A. C.; Natsir, H.; Dali, S., (2020) *Effectiveness of Brown Algae (Padina australis) Extract as Antioxidant Agent*, Journal of Physics, **1463**, 1-5.
- [18] Manikandaselvi, S.; Vadivel, V.; Brindha, P., **(2016)** *Studies on Nutraceutical Properties of Caesalpinia bonducella L.: An Indian Traditional Medicinal Plant*, Research Journal of Medicinal Plants, **10**, 127-139.
- [19] Sembiring, E. N.; Elya, B.; Sauriasari, R., **(2018)** *Phytochemical Screening, Total Flavonoid and Total Phenolic Content and Antioxidant Activity of Different Parts of Caesalpinia bonduc (L.) Roxb*, Pharmacognosy Journal, **10**(1), 123-127.
- [20] Sachan, N. K.; Verma, S.; Sachan, A. K.; Arshad, H., **(2010)** *An Investigation to antioxidant activity of Caesalpinia Bonducella seeds*, Annals of Pharmacy and Pharmaceutical Science, **2**(10), 88-91.
- [21] Aryal, S.; Baniya, M. K.; Danekhu, K.; Kunwar, P.; Gurung, R.; Koirala, N., **(2019)** *Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal*, Plants, **8**(96), 1-12.
- [22] Kumar, R. S.; Sivakumar, T.; Sunderam, R. S.; Gupta, M.; Mazumdar, U. K.; Gomathi, P.; Rajeshwar, Y.; Saravanan, S.; Kumar, M. S.; Muruges, K.; Kumar, K. A., **(2005)** *Antioxidant and antimicrobial activities of Bauhinia racemosa L. stem bark*, Brazilian Journal of Medical and Biological Research, **38**, 1015-1024



- [23] Rajeshwar, Y.; Kumar, G. P. S.; Gupta, M.; Mazumder, U. K., **(2005)** *Studies On In Vitro Antioxidant Activities Of Methanol Extract Of Mucuna Pruriens (Fabaceae) Seeds*, European Bulletin of Drug Research, **1**(13), 31-39.
- [24] Matheos, H.; Runtuwene, M. R. J.; Sudewi, S., **(2014)** *Antioxydant Activity of Kayu Bulan leaf Extract (Pisonia alba)*, Jurnal Ilmiah Farmasi, **3**(3), 235-246.
- [25] Subbiah, V.; Nagaraja, P.; Narayan, P.; Nagendra, H. G. R., **(2019)** *Evaluation of Pharmacological Properties of Caesalpinia bonducella Seed and Shell Extract*, Pharmacogn J., **11**(1), 150-154.