Pharmacogn J. 2018; 10(1): 123-127
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Phytochemical Screening, Total Flavonoid and Total Phenolic Content and Antioxidant Activity of Different Parts of *Caesalpinia bonduc* (L.) Roxb

Elin Novia Sembiring, Berna Elya, Rani Sauriasari

ABSTRACT

Background: *Caesalpinia bonduc* (L.) Roxb are traditionally used in Indonesia to treat various diseases, but still limited study about different part of this plant. **Objective:** The aim of this study was to screen the phytochemicals, to evaluate the total flavonoid and total phenolic contents as well as antioxidant activity of ethanol extract of root, stem, leaves, and seed kernel of *C. bonduc*. **Methods:** Each part of plant were extracted by reflux using 70% ethanol as the solvent for 2 h and repeated 3 times. Total flavonoid content was determined by aluminium chloride colorimetric assay on 415 nm. Total phenolic content was determined with Folin-Ciocalteu 1:4 on 765 nm using microplate reader. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenger methods. **Results:** Phytochemical screening showed that all of samples positively contain flavonoid and saponin. Total flavonoid content was the highest in leaf and the lowest in root whereas total phenols content was highest in leaf and the lowest in seed kernel. The crude extracts displayed DPPH free radical scavenging activity with highest value in leaf extract followed by root, stem, and seed kernel. **Conclusion:** The 70% ethanol leaf extract of *C. bonduc* showed the highest yield, total flavonoid content and total phenolic content among other parts investigated. Moreover, leaf extract has highest DPPH free radical scavenging activity (79.802 μg/ml) which could be related to its higher phenolic content.

Key words: *Caesalpinia bonduc*, Phytochemical screening, DPPH, Total flavonoid, Total phenolic content.

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History

- Submission Date: 02-10-2017;
- Review completed: 27-10-2017;
- Accepted Date: 20-10-2017

DOI : 10.5530/pj.2018.1.22

Article Available online
http://www.phcogj.com/v10/i1

INTRODUCTION

Genus of Caesalpinia comprise more than 500 species, which have many beneficial effects for humans health based on their pharmacological activity.[1] One of medicinal plant from this genus was *Caesalpinia bonduc* (L.) Roxb (syn. *Caesalpinia bonducella* (L.) Flemming, *Caesalpinia crista* (Linn)), from Fabaceae family.[2,3] This plant was grown in Indonesia, which is one of country in the world with rich source of medicinal plants.[4] *C. bonduc* also distributed in other tropical and subtropical part of Asia, such as India, Shrilanka, Vietnam, China, Myanmar and Bangladesh.[5] In Indonesia, *C. bonduc* known as Bagore, Kalici, Tinglur, Areuy Matahiang.[6,7]

C. bonduc is thorny perennial shrub with length reach of 15 m, stems are covered in curved spines. It's about 2 cm gray seeds known as nicker nuts. The kernel is about 1.23- 1.75 cm in diameter with hard, pale yellowish – white, circular to oval, furrowed and ridged surface. Branches are armed with hooked and straight hard yellow prickles. Leaves are bipinnate, 25-40 cm long and petioles prickly. Leaflets 7-9 pairs, ovate-elliptic, 1.5-3.5 x 1-2 cm, glabrous above, and pubescent below. Stipules are foliaceous. Peduncle is long, 3 to 4 cm, prickled, bracts long, linear-lanceolate and deciduous. Pedicel is 0.5-1.5 cm and very short in bud.[2,7,8] Each part of the plant as seen in Figure 1.

Whole plant of the *C. bonduc* contain steroidal saponins, fatty acids, hydrocarbons, phytosterols, isoflavone, amino acids and phenolics.[2] Caesalpinins H—P and norcaesalpinin F were isolated from the CH_2Cl_2 extract of the seed kernels of *Caesalpinia crista*, together with 13 known diterpenes.[9]

In Indonesia, seed kernel of this plant traditionally used for cough, malaria, and as anthelmintic.[6,10] Leaves for inflammation and liver disease, and the root was used for gastric and blood disorder.[6,7] Part of *C. bonduc* possesses several activities such as anthelmintic, antimalaria, antidiabetic, antioxidant, antibacterial, antimalarial and antidiarrhoeal.[2,11,12]

Antioxidants could be used for management of some pathophysiological conditions, which involve free radical, such as cardiovascular and neurodegenerative disease.[13] One established *in vitro* method to evaluate antioxidant activity was DPPH scavenging activity. This method is furthermore rapid, not involved with many steps and reagents, and

Cite this article: Sembiring EN, Elya B, Sauriasari R. Phytochemical Screening, Total Flavonoid and Total Phenolic Content, and Antioxidant Activity of Different Parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacog J. 2018;10(1):123-7.

inexpensive compare to other method.[14] Flavonoid and phenolic compounds may be useful as antioxidant from natural sources. Distribution of phenolic compounds varies between different parts of plant.[15]

According to the literature search, phytochemical and antioxidant activity of different parts of *C. bonduc* have never been comparatively evaluated. The study was carried out to screen the phytochemicals as well as to evaluate total flavonoid contents, total phenolic contents and antioxidant activity of root, stem, leaf and seed kernel ethanolic extract.

MATERIAL AND METHODS

Chemicals

Gallic acid, Foline-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, quercetin, aluminium chloride, sodium acetate, sodium carbonate was purchased from Sigma Aldrich (Singapore). Ethanol were purchased from Merck (Germany).

Plant Materials

Parts of *C. bonduc* were collected in January 2017 from Bulukumba, South Sulawesi, Indonesia. All plants were identified by Indonesia Science Institution, Center for Plant Conservation-Bogor Botanical Garden, Indonesia.

Preparation of Samples

The plants materials were collected and cleaned, dried at room temperature, crushed into powder and stored in an air tight glass container. Fifty g of powdered samples was extracted by reflux using 70% ethanol as the solvent for 2 h and repeated 3 times. The extracts then evaporated using a vacuum rotatory evaporator, and then dried in water bath on 50°C.

Determination of Percentage Yield (%)

The percentage yield of the extract was determined using the dry weight of extract (a) and soaked samples material (b) using the formula:

$$\text{Percentage yield (\%)} = a/b \times 100$$

The extraction yield was calculated for each extract.

Phytochemical Screening

The qualitative phytochemistry test were conducted according to Indonesian Materia Medica[16] and Harborne.[17] Alkaloid test with Mayer, Dragendorff, and Bouchardat reagents; flavonoid test with Shinoda test; tannin test with gelatin test, gelatin-salt test, and test with ferrous (III) chloride; saponin test with froth test; quinone with Borntrager test; terpenoid/steroid test with Liebermann-Burchard reagent.

Determination of Total Flavonoid Content

Total flavonoid content was determined by aluminium chloride colorimetric assay adapted from Chatatikun *et at*[18] and Roy *et al*[19] with slight modification. Standard solution of quercetin in concentration 30,40,50,60,70,80,90,100 μg/ml were prepared in 96% ethanol. 50 µl of extracts (1 mg/ml) or standard solution was added to 10 μl of 10% the aluminium chloride solution and followed by 150 μl of 96% ethanol. 10 μl of 1 M sodium acetate was added to the mixture in a 96 well plate. 96% ethanol was used as reagent blank. All reagents were mixed and incubated for 40 min at room temperature protected from light. The absorbance was measured at 415 nm with a microplate reader (Versamax Microplate Reader, USA). Total flavonoid contents were expressed as mg Quercetin Equivalents (QE) per g of plant extract.

Determination of Total Phenolic Content

The microplate total phenolic content method was based on the 96-well microplate Folin–Ciocalteu method adapted from Ahmad *et al* [20] with some modifications. A total of 25 µL of the diluted extract of each part of *C. bonduc* were mixed with 100 µL of 1:4 diluted Folin–Ciocalteu reagent and shaken for 60 sec in a flat-bottom 96-well microplate. The mixture was left for 240 secs and then 75 µL of sodium carbonate solution (100 g/L) were added and the mixture was shaken at medium-continuous speed for 1 min. After 2 h at room temperature, the absorbance was measured at 765 nm using the microplate reader (Versamax Absorbance Microplate Reader, USA). The absorbance of the same reaction with ethanol instead of the extract or standard was subtracted from the absorbance of the reaction with the sample. Gallic acid dilutions (10–200 mg/L) were used as standards for calibration. Total phenolic contets were expressed as mg Gallic Acid Equivalents (GAE) per g of plant extract.

Antioxidant activity

DPPH scavenging ability assay was used to evaluate the antioxidant activity of each extract. Test was conducted in a 96-well plate according to Zahratunnisa *et al*.[21] with slight modification. 20 μL stock solution of extracts in different concentrations (100, 500, 1000, 1500, 2000 ppm) and 180 μL of DPPH solution 0.147 mM were added to each well. After 30 min incubation at room temperature in dark room, absorbance was read at 517 nm using micro-plate reader of Versamax Microplate Reader (USA). Methanol was used as blank. The scavenging ability (%) was calculated as follows:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of standard} - \text{Absorbance of crude extract}}{\text{Absorbance of standard}} \times 100$$

Ascorbic acid was used as positive standard. All tests were performed in triplicate. Concentration of samples resulting in 50% inhibition on DPPH (IC_{50} value) were calculated.

RESULTS

Percentage Yield of Extract

The results showed the highest yield was associated with leaves (33.8%), followed by seed kernel (28.26%), root (18.52%) and then stem (10.48%) as seen on Figure 2.

Phytochemical screening

The extracts were further investigated to determine phytochemical compound in the extract. The common phytochemistry content from plant such as flavonoid, alkaloid, terpenoid, steroid, tannin, and saponin have identified (Table 1).

Total Flavonoid Content

The result of total flavonoid contents of the four crude extracts is given in Table 2. Equation of calibration curve of quercetin standard was $y = 0.0291x - 0.0397$, $R^2 = 0.9904$.

Among the four crude extracts, leaf contained the highest (31.05 ± 0.35 mgQE/g) amount of total flavonoid content compounds followed by stem (21.82 ± 0.46), seed kernel (13.21 ± 1.35) and then root (12.55 ± 0.08 mgQE/g).

Total Phenolic Content

The result of total phenolic content determination from 70% ethanolic extracts of different parts of *C. bonduc* are shown in Table 3. Calibration curve from gallic acid showed maximum absorbances at 765 nm wavelength (equation $y = 0.0531x + 0.0003$, $R^2 = 0.9951$).

The total phenol contents of four crude extracts determined by Folin-Ciocalteu method were reported as gallic acid equivalents. Among the four crude extracts, leaf contained the highest (146.64 ± 3.94 mgGAE/g) amount of total phenolic content compounds followed by stem (144.42

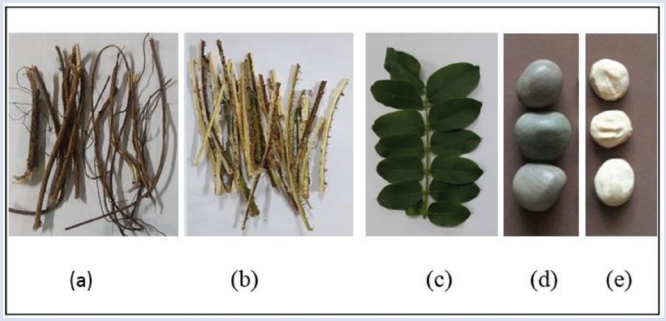

Figure 1: *Caesalpinia bonduc* (L.) Roxb (a) root; (b) stem; (c) leaf; (d) seed; (e) seed kernel.

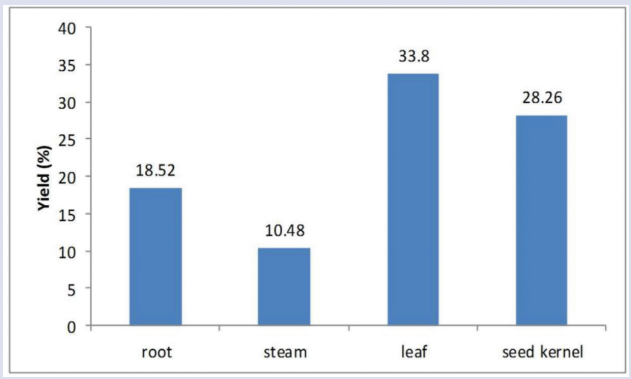

Figure 2: Percentage of yield.

± 16.05), root (89.81 ± 3.00) and then seed kernel (70.34 ± 10.59 mgGAE/g).

Antioxidant Activity

The ability of extract in scavenging DPPH radical are shown in Table 4. The leaf has the highest ability to scavenge free radicals than other parts.

DISCUSSION

Extraction was done using 70% ethanol as solvent. According to Jing *et al*,[22] this solvent is preferred to extract phenolic compounds from plants. Reflux method was chosen as extraction method to optimize phenolic and flavonoid content. Temperature was maintained not exceeding 70°C to avoid any degradation of phenolic and flavonoid as targeted compound.[23]

Different parts of plant could have different phytochemical compound, which may contribute to different pharmacological effect of each part. Phytochemical screening showed that all of extracts from root, stem, leaf and seed kernel of *C. bonduc* contain flavonoid and saponin, and absent in alkaloid and quinone. Among other part, only leaf contained tannin, one of polyphenol that can donate hydrogen and act as antioxidant.[15,24]

Total flavonoid content was determined using aluminium chloride method. Aluminium chloride will form stable complex with carbonyl group at C4 and hydroxyls at C3 (flavonols) and C5 in flavonols and flavones. It could also form labile acid complexes with hydroxyls in the ortho position in B rings of flavonoids.[25]

Table 1: **Phytochemical screening of the extracts.**

Phytochemical Constituents	Root	Stem	Leaf	Seed kernel
Alkaloids	-	-	-	-
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Triterpenoids	+	-	-	+
Steroid	-	-	+	+
Tannin	-	-	+	-
Quinone	-	-	-	-

Note: - = absent, + = present

Table 2: **Total Flavonoid Content.**

Sample	Total Flavonoid Content (mgQE/gram)
Root	12.55 ± 0.08
Stem	21.82 ± 0.46
Leaf	31.05 ± 0.35
Seed kernel	13.21 ± 1.35

Data are mean ± SEM for triplicate measurements.

Table 3: **Total Phenolic Content.**

Sample	Total Phenolic Content (mgGAE/gram)
Root	89.81 ± 3.00
Stem	144.42 ± 16.05
Leaf	146.64 ± 3.94
Seed kernel	70.34 ± 10.59

Data are mean ± SEM for triplicate measurements.

Table 4: **Antioxidant DPPH Scavenging Activity.**

Sample	IC_{50} (µg/mL)
Root	135,778
Stem	169.92
Leaf	79,802
Seed kernel	> 200
Ascorbic acid	2.24

The total phenols contents in four different crude extracts were evaluated in the present study. The highest amount of phenolics compounds was present in leaf (146.64 mg of GAE/g of crude extract) and the lowest was in seed kernel extract (70.34 mg of GAE/g). Leaf also have highest total flavonoid content (31.05 ± 0.35 mgQE/g) while the lowest was in root (12.55 ± 0.08 mgQE/g).

The molecule DPPH is a free radical by the delocalisation of the spare electron over the molecule. The delocalization of electron rises to the deep violet colour. If a solution of DPPH is mixed with samples that can donate a hydrogen atom, DPPH will be converted into colourless purple. The amount of reduced DPPH was measured in absorbance at 517 nm.[26] In present study, ascorbic acid as a well known potent antioxidant, was used as positive control for DPPH scavenging activity. Leaf part showed highest ability in DPPH scavenging activity compare to other plant part which measured by the lowest IC_{50} value, but it has lower antioxidant capacity compared to ascorbic acid. The phenolic content in the leaf may contribute to the antioxidative action by hydrogen donating ability. Antioxidant activity of leaf and seed kernel of *C. bonduc* in this study were found different from study in other country.[27,28] The variation might be

caused by different phytogeographic region and plant nutrition, which could modify the secondary metabolites of the plant[29] and due to the different method of extraction and solvents polarities.

CONCLUSION

The 70% ethanol leaf extract of *C. bonduc* showed the highest yield, total flavonoid content and total phenolic content compared to other part investigated. Moreover, leaf extract has highest DPPH free radical scavenging activity (79.802 μg/mL) which could be related to its higher phenolic content.

ACKNOWLEDGEMENT

This study was supported by Universitas Indonesia via a grant "Hibah PITTA 2017".

CONFLICT OF INTEREST

No conflict of interest

ABBREVIATION USED

***C. bonduc*:** *Caesalpinia bonduc* L. Roxb; **GAE:** Gallic acid equivalent; **QE:** Quercetin equivalent, **DPPH:** 1,1-diphenyl-2-picrylhydrazyl; **IC_{50}:** Concentration of samples resulting in 50% inhibition.

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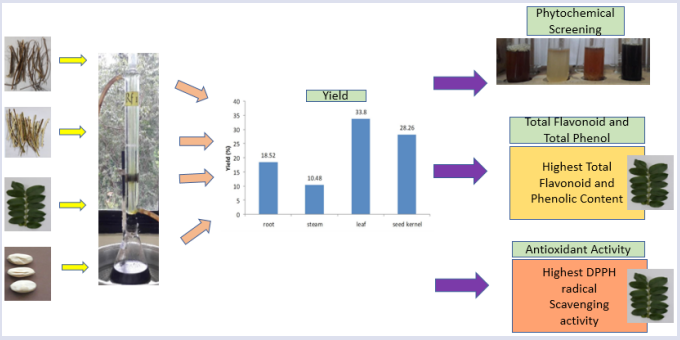
GRAPHICAL ABSTRACT



SUMMARY

- Each part of *C. bonduc* (root, stem, leaf, seed kernel) were extracted by reflux using 70% ethanol as the solvent for 2 hours and repeated 3 times.
- The highest yield was leaf (33.8%). Phytochemical screening showed that all of samples positively contain flavonoid and saponin.
- Total flavonoid content was the highest in leaf (31.05 ± 0.35 mgQE/g) and the lowest in root whereas total phenols content was highest in leaf (146.64 ± 3.94 mgGAE/g) and the lowest in seed kernel.
- The highest DPPH free radical scavenging activity was in leaf extract (79.802 µg/mL) followed by root, stem, and seed kernel.

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Cite this article: Sembiring EN, Elya B, Sauriasari R. Phytochemical Screening, Total Flavonoid and Total Phenolic Content, and Antioxidant Activity of Different Parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacog J. 2018;10(1):123-7.