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

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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 aluminum 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. 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±5.82%). Key words: Caesalpinia bonduc, Phytochemical screening, DPPH, Total flavonoid, Total phenolic content.

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 bonduella (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.9 Caesalpins H—P and norcaesalpin F were isolated from the CHCl3 extract of the seed kernels of Caesalpinia crista, together with 13 known diterpenes.10 In Indonesia, seed kernel of this plant traditionally used for cough, malaria, and as anthelmintic.5,10 Leaves for inflammation and liver disease, and the root was used for gastric and blood disorder.5,9 Part of C. bonduc possesses several activities such as anthelmintic, antimalaria, antiadibetic, antioxidant, antibacterial, antimalarial and antiarrrheoidal.2,11 Antioxidants could be used for management of some pathophysiological conditions, which involve free radical, such as cardiovascular and neurodegenerative disease.11 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
inexpensive compare to other methods. Flavonoid and phenolic compounds may be useful as antioxidant from natural sources. Distribution of phenolic compounds varies between different parts of plant. 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 rotary 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:

$$\text{Percentage yield} = \frac{a}{b} \times 100$$

The extraction yield was calculated for each extract.

**Phytochemical Screening**

The qualitative phytochemistry test were conducted according to Indonesian Materia Medica and Harborne. Alkaloidal 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; tannin-test with gelatin-test, gelatin-salt test, and test with ferrous (III) chloride; saponin test with froth test; quinone with Borntrager test; tannin-test with Liebermann-Burchard reagent.

**Determination of Total Flavonoid Content**

Total flavonoid content was determined by aluminium chloride colorimetric assay adapted from Chatatikun and Sandip et al. 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 (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. 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 sec 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 contents 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. 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:

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\% \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 compounds followed by seed kernel (28.26%), root (18.52%) and then stem (10.48%) as seen on Figure 2.

**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...
± 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., this solvent is preferred to extract phenolic compounds from plants. Reflush 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.

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.

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.

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. 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<sub>50</sub> value, but it has lower antioxidant capacity compared to ascorbic acid.

The variation might be
caused by different phytophysiographic region and plant nutrition, which could modify the secondary metabolites of the plant and due to the different method of extraction and solvents polarities.

CONCLUSION

The 70% ethanolic 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.

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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*<sub>50</sub>: Concentration of samples resulting in 50% inhibition.

REFERENCES


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.
- The 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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