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ISOLATION OF ANTIOXIDANT COMPOUNDS FROM KEBIUL *(CAESALPINIA BONDUC L)* SEED SHELLS

Moch Abdussalam [1)], Indah Permata Yuda [2)], Juniarti [3)*]

[1,2] Herbal Research Centre, YARSI University, Jl Letjed Soeprapto Cempaka Putih, Jakarta
[3] Medicine Faculty, YARSI University, Jl Letjed Soeprapto Cempaka Putih, Jakarta

[*]Email : juniarti@yarsi.ac.id

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Antioxidant
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Penulis Korespondensi

Name : Juniarti
Affiliation : YARSI University
E-mail : juniarti@yarsi.ac.id

ABSTRACT

Caesalpinia bonduc L. is a medicinal plant widely distributed in tropical and subtropical regions and has long been utilized in traditional medicine for the treatment of various ailments. Its seeds are recognized as a rich source of bioactive secondary metabolites, particularly flavonoids, phenolic compounds, terpenoids, and diterpenes, which have been associated with antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities. However, studies focusing on the isolation, purification, and characterization of individual bioactive compounds from C. bonduc seeds remain limited. Therefore, this study aimed to isolate, purify, identify, and evaluate the antioxidant activity of bioactive compounds obtained from C. bonduc L. seeds. Bioactive compounds were isolated from the seed extract through successive extraction, chromatographic separation, and purification procedures. The purified compound was subsequently characterized by phytochemical identification and evaluated for its free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The isolated compound was identified as a flavonoid based on its chemical characteristics. Antioxidant evaluation demonstrated that the isolated flavonoid exhibited potent free radical scavenging activity, with a half-maximal inhibitory concentration (IC_{50}) of 3.31 ± 0.12 µg/mL, indicating a very strong antioxidant capacity. The findings demonstrate that C.

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bonduc seeds constitute a valuable source of naturally occurring antioxidant compounds and support their traditional medicinal use.

INTRODUCTION

Indonesia possesses vast and diverse natural resources, notably its abundance of tropical flora traditionally employed in ethnomedicine. For generations, herbal plants have been extensively utilized by traditional communities across the archipelago for their therapeutic and curative properties. This long-standing practice is fundamentally underpinned by the presence of specific bioactive molecules within these plants, known as secondary metabolites. These secondary metabolites—which include chemical groups such as alkaloids, terpenes, and phenolics—are synthesized by the plants as defense mechanisms but are precisely the compounds responsible for the observed pharmacological effects, thereby validating the traditional uses of these botanical resources in healthcare (Rice & Glutinosa, 2019), (Rintelen et al., 2017). The immense chemical diversity inherent in Indonesia's medicinal flora offers fertile ground for bioprospecting and the discovery of novel drug candidates.

In the realm of biochemistry, metabolic products are broadly categorized into primary and secondary metabolites, each serving distinct roles in organismal survival and interaction. Primary metabolites are essential compounds integral to basic physiological processes, directly supporting the life and growth of an organism. These include fundamental biomolecules such as carbohydrates, proteins, lipids, and nucleic acids (Pagare et al., 2015). In contrast, secondary metabolites are typically specialized, non-essential chemical compounds that possess valuable biological activities (bioactivities). While not directly involved in growth or reproduction, these compounds often play crucial ecological roles, such as defense mechanisms against predators or pathogens, or signaling molecules. These specialized chemicals are predominantly generated as the end products of metabolic pathways in higher plants (Forrister et al., 2023), though they are also found in microorganisms and some animals. Their diverse structures and potent bioactivities make them the focus of intense research for pharmaceutical and agricultural applications.

Secondary metabolites are recognized as essential non-primary compounds, crucial for the survival and ecological adaptation of plants. Their primary function lies in mediating plant defense mechanisms against a wide spectrum of biological and environmental threats. Flavonoids, a prominent class of polyphenolic compounds, exemplify this multifunctionality: they are vital for attracting specific insect species to facilitate pollination while also acting as powerful defensive agents. Specifically, flavonoids provide protection against herbivory and exhibit significant antimicrobial activity against disease-causing pathogens, including bacteria, fungi, and viruses (Panche, Diwan, 2016). Given the intense biological pressures in the tropics, it is not surprising that tropical plants are rich sources of diverse secondary metabolites. These compounds encompass several key structural classes, notably alkaloids, flavonoids, steroids, terpenoids, and tannins, all of which possess high functional significance within the producing organisms and represent a vast resource for chemical investigation.

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Flavonoids are a phenolic group isolated from many tropical plants. Based on previous research, groups of flavonoid compounds function actively in plants as photoreceptors, visual attractants, antioxidants, antimicrobials, and antifungals (Panche, Diwan, 2016). Many studies have suggested that flavonoids exhibit biological activity effects, including anti-allergic, antiviral, anti-inflammatory, and vasodilator activity. Currently, flavonoid research has focused on antioxidant activity, because of its ability to reduce the free radical formation and fight free radicals in the human body. Research related to flavonoid antioxidants is related to the development of degenerative diseases in modern times. The ability of flavonoids to act as antioxidants in vitro has been the subject of several studies in recent years, and their structural activity is remarkable (Hassanpour & Doroudi, 2023). The relationship between antioxidant activity has been established (Abdussalam & Yuda, 2020). The antioxidant properties of flavonoids in vivo are rarely documented, perhaps due to limited knowledge about their absorption in humans (Tang et al., 2025). Flavonoid compounds that are ingested are extensively degraded into various carboxylic acids.

This major group of phenolic compounds that are widely isolated from numerous tropical plant species. Extensive prior research indicates that these compounds perform multiple active functions in plants, including acting as photoreceptors, visual attractants, and providing protection through their properties as antioxidants, antimicrobials, and antifungals (Bakri et al., 2025). Furthermore, numerous human studies suggest that flavonoids exhibit a broad spectrum of biological activities, such as anti-allergic, antiviral, anti-inflammatory, and vasodilatory effects. Contemporary research has increasingly focused on their antioxidant activity due to their crucial ability to reduce and scavenge free radicals in the human body. The link between flavonoid antioxidants and the development of degenerative diseases in modern society has spurred significant interest in this area. The capacity of flavonoids to act as antioxidants in vitro has been the subject of intensive investigation, often highlighting the remarkable relationship between their structure and activity (Hassanpour & Doroudi, 2023). Conversely, documentation of their antioxidant properties in vivo remains relatively sparse (Tang et al., 2025). This scarcity is likely attributable to limited knowledge regarding their absorption and bioavailability in humans, as ingested flavonoid compounds are known to undergo extensive degradation into various carboxylic acids.

Caesalpinia bonduc L. (commonly known as Kebiul) is a plant belonging to the Magnoliopsida class and thrives predominantly as a shrub in tropical regions (Sopianti et al., 2026). Its natural habitats are frequently observed in marginal areas, such as the outskirts of communal forests, plantation fields, cliff regions, and along riverbanks (Ramachandran, 2025). Previous research has demonstrated that extracts derived from Kebiul seeds possess significant antidiabetic activity and the ability to reduce elevated triglyceride levels back to a normal state (Ramachandran, 2025). Furthermore, the administration of the ethanol extract from C. bonduc seeds also exhibits potent inhibitory properties against the alphaglucosidase enzyme (Febriza et al., 2025).

The ethanol extract derived from *C. bonduc* was characterized by significant levels of both total flavonoids and total phenolics. Crucially, the extract exhibited remarkably high DPPH free radical scavenging activity, a finding that can be directly attributed to its elevated

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phenolic content (Lobo et al., 2010). This correlation supports the established role of phenolics as key natural antioxidants.

METHOD

General Experiment Procedure

Ultra-violet spectra were analyzed in methanol on the Shimadzu UV-1575 spectrophotometer. NMR spectral data were performed on a Bruker spectrometer 500 MHz) with acetone-d6 as a solvent, chemical shifts were given on a δ (ppm) scale and tetramethylsilane (TMS) internal standard. Column chromatography was conducted on silica gel 60 (Merck, Germany)). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in EtOH, followed by heating and analyzed under UV light at wavelength 254 and 365 nm.

Plant material

The Seeds of *Caesalpinia Bonduc L* was collected in Manna, South Bengkulu, Indonesia on November 2020. The plant was identified by the staff of the Herbarium in Lembaga Ilmu pengetahuan Indonesia (LIPI) Cibinong.

Extraction and isolation

The dried seeds of *Caesalpinia Bonduc* (1,1 kg) was successively extracted with n-hexane, Ethyl acetate, and Ethanol. Each extract was evaporated under a vacuum. After evaporation three crude were obtained: n-hexane (1,7 g), ethyl acetate (6,6 g), and Ethanol (67,5 g). The ethyl acetate extract was separated by vacuum liquid chromatography using 100 g of silica gel as the stationary phase. The mobile phase used for this separation is a mixture of h-hexane: ethyl acetate the polarity is increased gradually from the solvent with an increase in the polarity of the solvent by 10%. Ethyl acetate extract as much as 6.5 grams was separated to obtain 6 fractions namely fractions A (460 mg), B (232 mg), C (240 mg), D (365 mg), E (460 mg), and F (2.9 g). The resulting fraction was then analyzed by thin-layer chromatography (TLC) to obtain the target compound. The TLC plate is sprayed with DPPH solution to select the spot for the antioxidant active compound, namely the spot that changes the DPPH color to yellow. Fraction 3 (240 mg) had yellow spots and was well separated. Therefore, fraction 3 was further purified using radial chromatography. The eluent used is a mixture of n-hexane-ethyl acetate (4: 6) as much so that the desired spot can be separated. Thin-layer chromatography (TLC) analysis showed a single spot indicating the presence of pure isolation.

DPPH assay

In vitro testing of DPPH radical inhibitor activity was carried out based on research by Sharma & Bhat (Samer Salem Alhamami, 2025) which has been modified. A total of 4 mL of the extract solution in methanol with the concentration of the extract at the measurement: 1; 2; 3; 4 and 5 g/mL were put into each test tube and then 1 mL of DPPH solution with a concentration of 160 g/L in methanol was added. Inhibition activity was measured based on the reading of the maximum wave absorption of DPPH at 517 nm. Each blank used methanol.

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The percentage inhibition of DPPH radical inhibition activity can be calculated using the formula:

% inhibition : $[A_0-A_1)/A_0] \times 100\%$

Description: A_0: absorbance of blank; A_1: absorbance of extract.

Calculation of IC_{50} value The five concentrations (1; 2; 3; 4 and 5 ppm) were analyzed respectively. their respective inhibition of the enzyme. The IC_{50} value is calculated through a linear regression equation, y = a + bx, where the x-axis is the sample concentration and the y-axis is the % inhibition, then the IC_{50} value can be calculated using the formula: IC_{50}= (50-a)/b.

RESULT

The ethyl acetate extract of the seeds *C. Bonduc* was chromatographed over vacuum-liquid chromatography (VLC) and silica gel column chromatography to afford compounds. The isolated compound was obtained as a pale yellow amorphous solid. The UV spectrum (**Fig.1**) showed absorption peaks at 211 and 280 nm

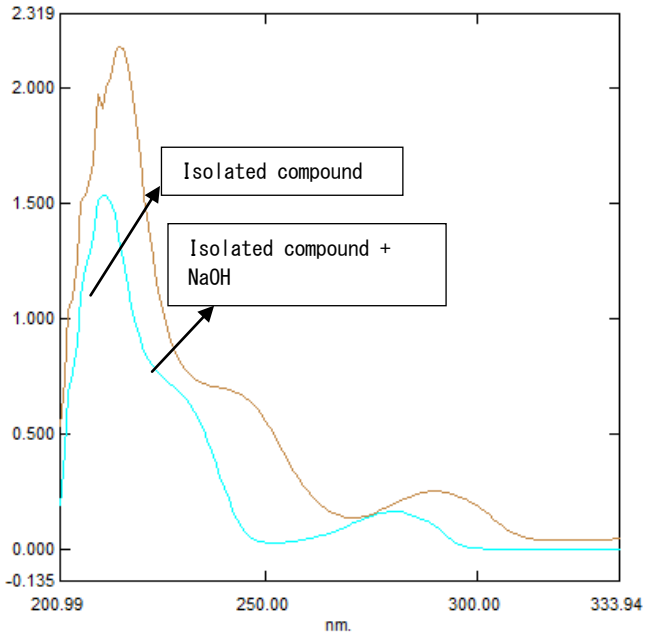

Fig.1: Spectrum UV-Visible of Isolated Compound

The spectrum is consistent with conjugated π-π* transitions arising from the aromatic rings. A maximum wavelength of 211 and 280 nm indicates the presence of a chromophore (Karakaya, 2026). The addition of 1% NaOH to the isolated compound methanol solution will give a bathochromic shift in the spectrum. The bathochromic shift indicates the presence of an auxochromic group attached to the chromophore. These results were similar to those reported in the literature (Parwata et al., 2018).

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Isolated compounds were identified using a 500 MHz NMR spectrometer to determine the type and chemical environment of the carbon and hydrogen atoms. This will make it easier to determine the exact molecular structure.

The ^{1}H-NMR spectrum of the isolated compound confirmed that the proton H-3 became split by H-2 and H-4, ensuing in multiples at 4.21 ppm. The proton at H-4, damaged down by H-3, offers a double-double δ_H 2.86 ppm with J=4.6 &16.6 Hz and δ_H 2.76 with J = 3.3&16.6 Hz. The H-2 position, the chemical shift (δ_H 4.89) shows that the remoted compound has a flavan shape framework with cis-2, three stereochemistry. This description is supported via way of means of small values for coupling (<1 Hz) between the protons H-2 and H-3, which seems as singlet on H-2 (δ_H 4.89)

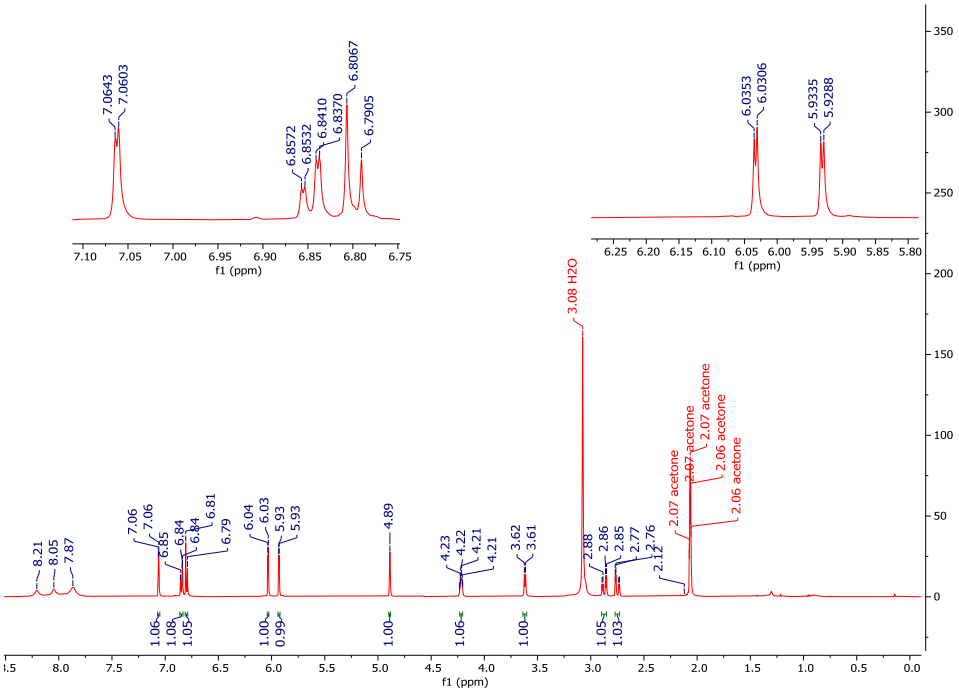

Fig. 2 Spectrum 1H-NMR of Isolated Compound

The standard sign of a B-ring aromatic proton is of kind ABX. The proton methin H-2` is broken down via way of means of H-6' withinside the meta position results in a doublet multiplicity at δ_H 7.06 (d, J = 2 Hz), and H-5' divided by H-6' in the ortho position offers a doublet at δ_H 6.79 (d, J = 8.1 Hz) and H-6' divided by H-2' and H-5' given a doublet doublet at δ_H 6.84 (dd J=1.7 & 8.2 Hz). The A ring of aromatic protons additionally shows H-6 and H-8 coupling, with ensuing doublets at δ_H 5.93 and 5.96 (d, 2.3 Hz, respectively). The fundamental structure of this compound is consequently concluded as 3,3',4',5,7-penta-hydroxyflavan.

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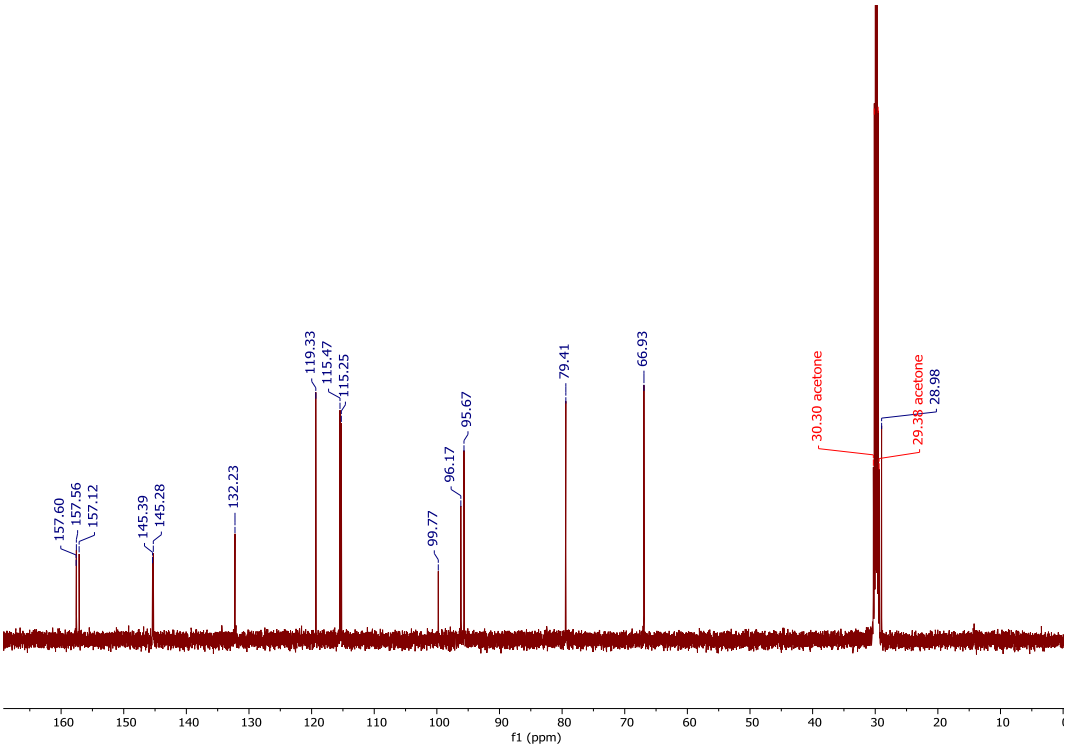

Fig. 3 Spectrum ^{13}C-NMR flavonoid of Isolated Compound

The thirteen C-NMR spectrum (**Fig.3**) confirmed fifteen carbon signals consisting of one methylene, seven methines, and seven quaternary carbon. These signals are as follows: 79.41 (C-2), 66.93 (C-3), 28,98 (C-4), 157.12 (C-5), 96.17 (C -6), 157.60 (C-7), 95.67 (C-8), 157.56 (C-9), 99.77 (C-10), 132.23 (C-1'), 115.25 (C-2'), 145.28 (C-3'), 145.39 (C-4'), 115.47 (C-5'), 119.33 (C-6`).

NMR spectrum (**Table 1**) was assigned by comparing with the literature data (Usman et al., 2016). By observing spectral data isolates with compounds that have been reported by previous researchers. obtained the value of chemical shift (ppm) which is almost the same. Chemical shear value compounds other than those caused by the environment atomic chemistry is also affected by external factors such as solvent and conditions NMR tool. In the 13C-NMR spectrum, the measurement of identical compounds can be given a maximum difference of 2 ppm (Gerothanassis, 2026).

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Position	Isolated compound		Epicatechin (literatur data)	
	δc (ppm)	δ_H (ppm)	δc (ppm)	δ_H (ppm)
2	79.41	4,89 1 H (s)	79.9	4.83 1 H (s)
3	66.93	4,21 1 H (m)	67.5	4.19 1 H (m)
4	28,98	2,86 1H (dd) J = 4,6 &16.6 Hz	29.3	2.86 1H (dd) J = 4,8 &16.8 Hz
		2,76 1H (dd) J = 3.3 &16.6 Hz		2.73 1H (dd) J = 2.7 &16.8 Hz
5	157.12		157.4	
6	96.17	5,93 5.93 1H (d) J = 2.3 Hz	96.4	5.93 1H (d) J = 2.3 Hz
7	157.60		157.9	
8	95.67	6.03 1H (d) J = 2.3 Hz	95.9	5.96 1H (d) J = 2.3 Hz
9	157.56		157.7	
10	99.77		100.1	
1`	132.23		132.3	
2`	115.25	7,06 1H (J = 2 Hz)	115.3	6.99 1H (J = 1.7 Hz)
3`	145.28		145.9	
4`	145.39		145.8	
5`	115.47	6,79 1H (J = 8.1 Hz)	115.9	6.77 1H (J = 8.2 Hz)
6`	119.33	6,84 1H (J = 2 & 8.1Hz)	119.4	6.81 1H (J = 1.7 & 8.2Hz)

Tabel 1. Comparison NMR spectrum between isolated compound and literature data

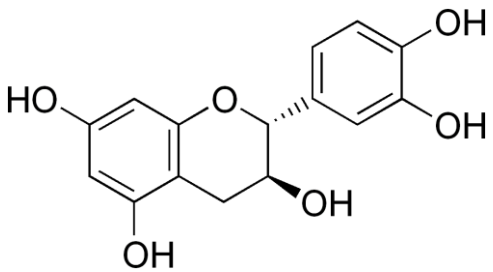

Fig. 4. Flavonoid structure from isolated compound

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The measurement results of the DPPH assay inhibition test show results in **Table 2** below.

Table 2. Antioxidant DPPH assay

Repeat	IC_{50} (µg/mL)	R^2	ST DV
1	3.42	0.9637	0.12
2	3.32	0.9938	
3	3.18	0.9996	
mean	3.31		

The isolated compound has an IC_{50} value of the DPPH assay 3.31±0.12 µg/mL. Specifically a compound is said to be a very strong antioxidant if the value of IC_{50} less than 50 p µg/mL (IC_{50}< 50 µg/mL), strong (50 µg/mL < IC_{50}< 100 µg/mL), medium (100 µg/mL < IC_{50}< 150 µg/mL), weak (150 µg/mL < IC_{50} < 200 µg/mL), and very weak (IC_{50}>200 µg/mL) (20).

CONCLUSION

Flavonoid isolated compounds are polyphenols with high antioxidant bioactivity and the isolation of these metabolites from Caesalpinia Bonduc L seems to support several uses of herbal plants in the development of medicinal products. Isolation of natural products was carried out using chromatographic separation and their chemical structures were elucidated by spectroscopic techniques.

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