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*Research Article*

# β-Sitosterol: The Isolated Compound from n-Hexane Fraction of *Baccaurea racemose* (Reinw. Ex Blume) Mŭll. Arg. Pulp and Its Antioxidant Activity

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**Abstract:** *Baccaurea racemosa* is one of the most widespread plants in Indonesia. The constituent compound at *Baccaurea racemosa* influenced its biological activity. However, the isolation of compounds from the n-hexane fraction of *B. racemosa* pulp has never been reported. This study aimed to isolate and spectroscopic analysis of compound from n-hexane fraction of *Baccaurea racemosa* pulp and its antioxidant activity. The fraction of n-hexane was isolated using the preparative thin-layer chromatography (PLC) method. The purified compound was identified its structure compound using the spectroscopic method. Then the antioxidant activity was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. UV-Vis spectrum of isolated compounds revealed the absence of conjugated double bonds. Furthermore, the infrared spectrum showed OH, C-H aliphatic, C-O, C-H vanillic, and C=C groups. The molecular ion of the isolated compound was 414 g/mol. The NMR spectrum showed several peaks at various chemical shifts. Based on the spectral analysis, the isolated compound was indicated as β-sitosterol. This compound has antioxidant activity with $IC_{50}$ > 2.4155 mM.

**Keywords:** n-Hexane fraction; Antioxidant activity; *Baccaurea racemosa* pulp; β-sitosterol; Isolation

## 1. INTRODUCTION

Genus Baccaurea was one of the genus of plants that are utilizing to improve human health. As traditional, genus Baccaurea was used as anti-pain [1]. The secondary metabolites at the plant belonging to genus Baccaurea contributed to its biological activity. Genus Baccaurea contained various secondary metabolites such as phenolic, flavonoid, organic acid, alkaloids, fatty acids, phytosterols, and terpenoids [2–5]. Many compounds were successfully isolated from genus Baccaurea. These compounds were blumenol B, β-sitosterol, daucosterol, phytol, epihydrotutin, epicatechin, and picrotoximaesin[6–10]. Those compounds exhibited many biological activities, such as anti-bacterial [11,12], antifungal, antioxidant and cytotoxic activity [13].

Kepundung (*Baccaure racemosa*) is one of the species from the genus Baccaurea. *B. racemosa* pulp contains protein, carbohydrates, and high calcium to improve human health. The secondary metabolites were reported in the ethyl acetate fraction of *B. racemosa,* such as gallic acid belonging to the phenolic compound [14]. *B. racemosa* has various biological activities such as antioxidant, anti-bacteria, and antidiabetic [15–17]. These compounds influenced the biological activities in *B. racemosa*. However, the isolation of compounds from the n-hexane fraction of *B. racemosa* has not been reported.

This study aimed to isolate, and spectroscopic analyze the compound from the n-hexane fraction of *B. racemosa* pulp and its antioxidant activity using the DPPH free radical scavenging activity method. The results of the present study help explore the compounds from *B. racemosa* pulp which are beneficial for health.

## 2. MATERIALS AND METHODS

### *2.1. General Experiments Procedures*

The infrared spectrum was analyzed by Fourier-transform infrared spectroscopy (FTIR) using FTIR 100 PERKIN ELMER with KBr pellet. The ultraviolet spectrum was obtained using UV-Vis spectrophotometry (Hitachi UH5300). Mass spectrometry (MS) spectrum was identified by gas chromatography-mass spectrometry (GC-MS) with electron impact using GCMS-QP2010S SHIMADZU. The spectra of $^{1}$H-NMR (400 MHz), $^{13}$C-NMR (100 MHz), and 2-D NMR were recorded by JEOL ECS-400 with $CDCl_3$ using TMS as internal standard. Silica gel 60 $GF_{254}$ was used for PLC. The antioxidant activity was measured using a microplate reader (SH-1000).

### *2.2. Plant materials*

The *B. racemosa* pulps were obtained from East Java, Indonesia. *B. racemosa* was authenticated by I Gde Mertha (senior lecturer in Plant Taxonomy) in the Laboratory of Biology, Mathematics and Science Faculty, Mataram University, West Nusa Tenggara, Indonesia. The voucher number of the sample was 01-LP.

### *2.3. Extraction and isolation*

The *Baccaurea racemosa* pulp was separated from its seed and dried using the oven. The extraction and fractionation process of the *Baccaurea racemosa* pulp was modified from the previous study by Permatasari et.al.[18] Fraction of n-hexane was isolated using preparative thin-layer chromatography (PLC) techniques. N-hexane fraction was eluted by n-hexane: ethyl acetate (7:3). The left and right sides of the PLC plate, about 1 cm, were sprayed with cerium sulfate and then were heated. The spot that showed the purplish-red color at $R_f$ 0.625 was scraped off and extracted using methanol: chloroform (1:1). The filtrate was concentrated using a vacuum rotary evaporator to get an isolated compound.

### *2.4.Thin-layer chromatography*

The crystals of the isolated compound were observed with a plate of silica gel 60 $F_{254}$ using three different developing systems to ensure the purity of crystals. The developing systems used chloroform: acetone (7:3); *n*-hexane: ethyl acetate (1:1); dichloromethane: Petroleum ether (3:1). To visualization of spot, the plate was sprayed with cerium sulfate.

### *2.5. DPPH antioxidant assay*

An amount of 6.4 mg DPPH was dissolved with ethanol until 100 ml. The DPPH solution (100 µl) was mixed with of 100 µl various concentration of isolated compound at ethanol (0.4831; 0.9662; 1.4493; 1.9324; and 2.4155 mM). For the control, 100 µl DPPH was mixed with 100 µL ethanol. All mixtures were shaken and kept at room temperature for 30 mins. The absorbance of all mixtures was recorded at 515 nm using a microplate reader. Quercetin was used as control positive. The antioxidant activity of the isolated compound was measured with $IC_{50}$ values and calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \text{ x } 100\%$$

*2.6. Statistical analysis*

The results of the DPPH free radical scavenging activity of β-sitosterol and quercetin were analyzed using the Statistical Package for the Social Sciences (SPSS) version 16. The statistical analysis method was used an independent T-test test with significances $p<0.05$.

## 3. RESULTS AND DISCUSSION

The Phytochemical constituent isolated from ethyl acetate fraction of *Baccaurea racemosa* pulp has been investigated, including 5-hydroxymethylfurfural and tartaric acid[18]. In this study, we will report the isolation and identification of compounds from the n-hexane fraction of *Baccaurea racemosa* pulp.

*3.1. Thin-layer chromatography*

The crystals obtained one spot at the TLC plate that was using three developing systems. The TLC sprayed with cerium sulfate showed one spot at Rf 0.20; 0.50; and 0.82 (Figure 1). The results indicated that the crystal of the isolated compound was pure.

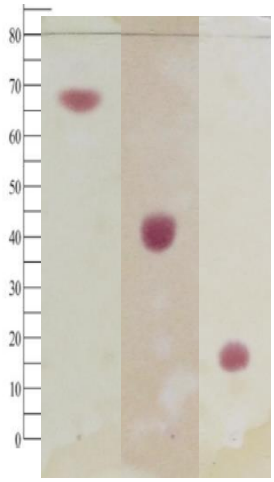


**Figure 1.** TLC profile of an isolated compound. Mobile phase: (a) chloroform: acetone= 7:3; (b) n-hexane: ethyl acetate= 1:1; (c) dichloromethane: Petroleum ether= 3:1

*3.2. Spectroscopis Analysis*

The isolated compound was the white crystal (25 mg). Based on the infrared spectrum (Figure 2), the wavenumber at 3274.96 $cm^{-1}$ reported the presence of hydroxyl group. Estimated the hydroxyl group was the hydroxyl alcohol. In addition, the sharp peak at wave number 2936.60 $cm^{-1}$ indicated the stretching vibration of C-H aliphatic. The infrared spectrum showed the C-H bending at 1376.18 $cm^{-1}$. The peak at 1064.06 $cm^{-1}$ indicated the C-O group.

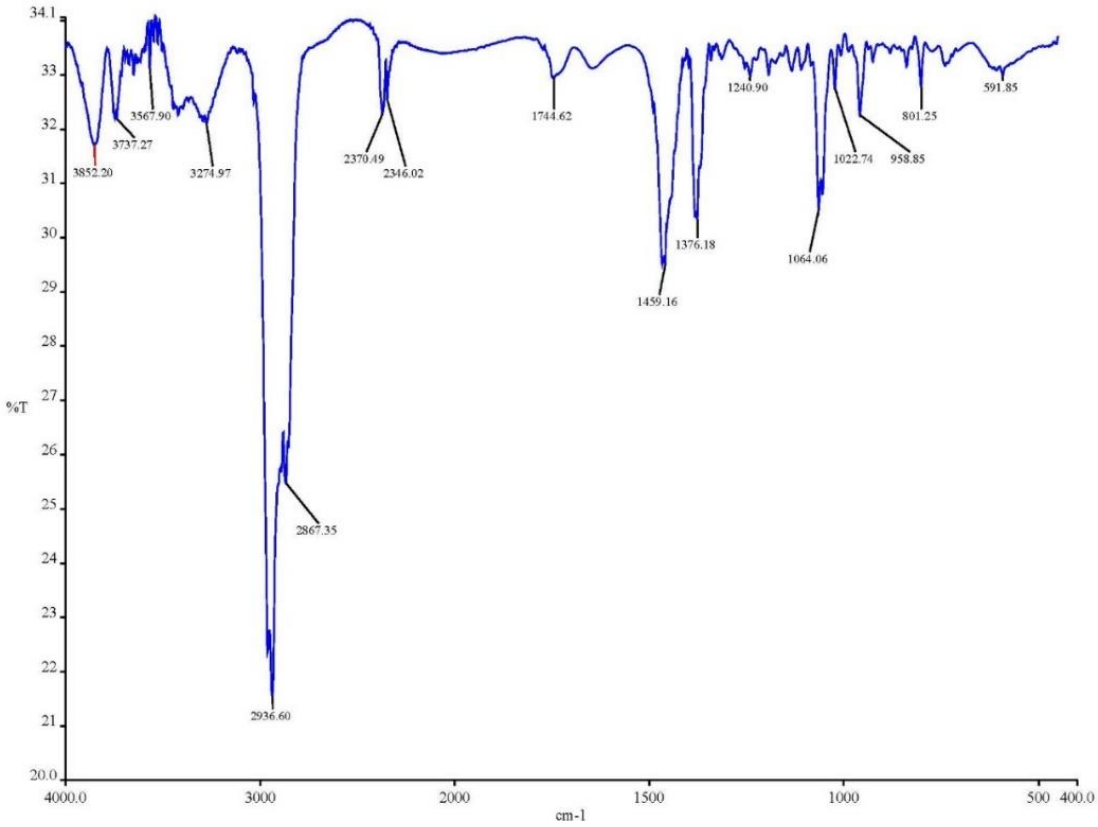


**Figure 2.** Infrared Spectrum

The spectrum of $^{1}$H-NMR showed the multiple signals at δ 3.52 ppm (1H, H-3, m) that indicated the proton was coupling with many protons. Meanwhile, δ 5.34 (1H, H-6) appeared as a doublet of doublet signals. H-6 was coupling with 2 diastreotropic proton bonded to carbon number 7 (C-7). The proton at C-7 was the diastreotropic proton because there was the chiral carbon in C-8. Two specific methyl groups at δ 0.69 ppm (3H, H-18, s) dan 1.07 ppm (3H, H-19, s) appeared as a single signal. The other protons of the isolated compound appeared at chemical shift 0.6-2.0 ppm. The other chemical shift of proton can be seen in Table 1.

The $^{13}$C-NMR spectrum showed the isolated compound containing 29 carbon (Table 1). The signal at δ 121.8 ppm (C-6) dan δ 140.8 ppm (C-5) indicated the double bond of carbon. Meanwhile, the chemical shift at δ 71.9 ppm (C-3) indicated tertiary carbon attaching to the hydroxyl group. The DEPT 135 spectrum showed the presence of 26 carbons. The results showed that there were six primary carbons, 11 secondary carbons, nine tertiary carbons. Meanwhile, there were three quaternary carbons. The HSQC spectrum supported the data on the DEPT 135 spectrum, where there were only 26 carbon atoms directly bonded to proton atoms. There were many cross-peaks in the COSY spectrum. One of them was between H-6 and H-7. The HMBC spectrum showed many cross-peaks correlated between proton and carbon separated by 2-4 bonds (Figure 3). Full COSY and HMBC correlation data can be seen in Table 1.

**Table 1.** $^{13}$C-NMR, $^{1}$H-NMR, and 2D NMR spectrum of β-sitosterol from n-hexane fraction of *Baccaurea racemosa* pulp.

| **Position** | **δ$^{13}$C (100 MHz) $CDCl_3$** | **δ$^{1}$H (400 MHz) $CDCl_3$** | **COSY** | **HMBC** |
|---|---|---|---|---|
| 1 | 37,3 | 1,81 | H-19 | - |
| 2 | 31,9 | 1,83 | H-3 | C-3, C-5, C-10 |
| 3 | 71,9 | 3,52 (m) | H-4, H-2 | - |
| 4 | 42,4 | 2,27 | H-3 | C-2, C-3, C-5, C-6, C-10 |
| 5 | 140,8 | - | - | - |
| 6 | 121,8 | 5,345 (dd) | H-7 | C-4, C-8, C-10 |
| 7 | 31,7 | 1,99 | H-6 | C-9 |
| 8 | 32 | 1,45 | - | C-5, C-6, C-7, C-9, C-14 |
| 9 | 50,2 | 0,92 | - | C-1, C-5, C-10 |
| 10 | 36,6 | - | - | - |
| 11 | 21,2 | 1,47 | H-12 | - |
| 12 | 39,8 | 1,99 | H-11 | - |
| 13 | 42,3 | - | - | - |
| 14 | 56,8 | 1,08 | - | - |
| 15 | 24,4 | 1,54 | H-16 | C-16 |
| 16 | 28,3 | 1,82 | H-15 | C-15 |
| 17 | 56,1 | 1,06 | - | C-12, C-13, C-17, C-18, C-20 |
| 18 | 11,9 | 0,69 (s) | - | C-12, C-13, C-17 |
| 19 | 19,5 | 1,07 (s) | H-1 | C-1, C-5, C-9 |
| 20 | 36,2 | 1,34 | - | - |
| 21 | 18,9 | 0,79 | - | - |
| 22 | 34,0 | 1,34 | - | - |
| 23 | 26,1 | 1,14 | - | - |
| 24 | 45,9 | 0,91 | - | - |
| 25 | 29,2 | 1,63 | H-26 | C-23, C-28 |
| 26 | 19,9 | 0,82 | H-25 | C-24, C-25 |
| 27 | 19,1 | 0,79 | - | C-26 |
| 28 | 23,1 | 1,24 | - | C-23, C-24, C-25, C-29 |
| 29 | 12,0 | 0,83 | - | C-28 |

The mass spectra showed that the molecular ion of the isolated compound was 414 g/mol. The ion peak of the isolated compound at m/z 396 indicating the release of $H_2O$ groups. Meanwhile, there was releasing of $C_6H_{13}$ indicated by an ion peak at m/z 329. Furthermore, fragmentation occurred again with the release of $C_4H_8$ and $H_2O$ indicated by an ion peak at m/z 255.

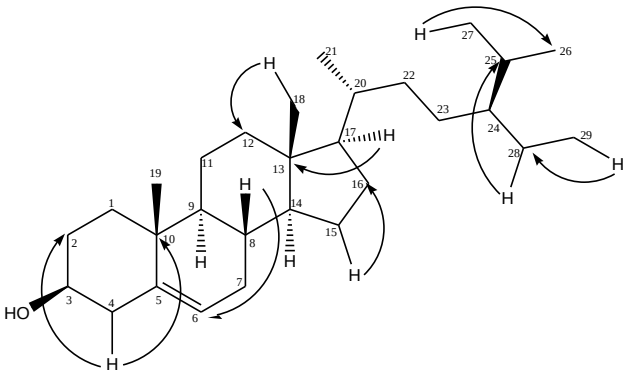

**Figure 3.** HMBC Correlation

*3.3. Antioxidant Activity*

The antioxidant activity of β-sitosterol was classified as weak with $IC_{50}$ >2.4155 mM against DPPH radical (Table 2). Meanwhile, quercetin as a positive control had high antioxidant activity. β-sitosterol has a hydroxyl group as an electron donor but no conjugated double bond. Therefore, β-sitosterol was unable to stabilize DPPH radicals. However, β -sitosterol was reported to be able to increase the level of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)[15]. These three enzymes converted superoxide radicals ($O_2^{-}$) and hydrogen peroxide ($H_2O_2$) into water molecules. β-sitosterol can be acted as a non-enzymatic antioxidant [19]. Baskar et al.[20] reported, β-sitosterol decreased the peroxidation lipid in the liver. So that, β-sitosterol could be used as a candidate for the antioxidant agent.

**Table 2.** DPPH Free Radical Scavenging Activity of β-sitosterol

| **Sample** | **Concentration (mM)** | **% radical scavenging** | | | **rata-rata $IC_{50}$ ± SD (mM)** |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| β-sitosterol | 0.4831 | 9.1904 | 9.1904 | 2.4070 | > 2.4155[a] |
| | 0.9662 | 7.6586 | 6.3457 | 9.1904 | |
| | 1.4493 | 11.3786 | 15.0985 | 10.5033 | |
| | 1.9324 | 12.2538 | 10.9409 | 9.8468 | |
| | 2.4155 | 10.9409 | 10.9409 | 8.7527 | |
| quercetin | 0.0033 | 25.3669 | 27.4633 | 24.9476 | 0.0075 ±0.0002[b] |
| | 0.0066 | 48.0084 | 48.6373 | 45.0734 | |
| | 0.0099 | 64.9895 | 61.4256 | 66.8763 | |
| | 0.0132 | 79.6646 | 80.7128 | 78.6164 | |
| | 0.0166 | 88.0503 | 85.7442 | 85.7442 | |

The statistical analysis is measured by the T-test method. The values of $IC_{50}$ are represented as mean ± standard deviation (SD), n=3. Samples with the same letter at the same column show no significant difference ($p>0.05$).

## 4. CONCLUSION

The phytochemical studies of *Baccaurea racemosa* succeeded in isolating β-sitosterol from the n-hexane fraction from the pulp. β-sitosterol was the first time isolated from *B. racemosa* pulp. This research can be a guide to find other compounds from *B.racemosa* pulp. The antioxidant activity of β-sitosterol was with $IC_{50}$ value >2.4155 mM. β-sitosterol could be developed to be an antioxidant compound.

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**Conflicts of interest:** The authors declare no conflict of interest.

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