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ARTIKEL

ISOLATION AND IDENTIFICATION OF APIGENIN, A FLAVONOID COMPOUND FROM *Macaranga hypoleuca* (REICHB.F. & ZOLL.)

[*Isolasi dan identifikasi apigenin, senyawa golongan flavonoid dari Macaranga hypoleuca (Reichb.f. & Zoll.)]*

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ABSTRACT

The study on *Macarang hypolue*ca (Reichb.f. & Zoll.), which was collected from secondary forests around Samarinda City, East Kalimantan, involved phytochemical investigations that led to the isolation of a flavone-type compound of flavonoid from the ethyl acetate fraction. To separate the compounds, silica gel column chromatography was utilized with a gradient solvent system of n-hexane and ethyl acetate, along with the addition of 5%. Infrared analysis (FTIR), mass spectrum (LC-ESI-MS), and nuclear magnetic resonance (1D- and 2D-NMR) were used to identify and elucidate the structure. Based on spectroscopic data and comparison with appropriate references, the isolated compound was identified as apigenin.

Keywords: *Macaranga hypoleuca* (Reichb.f. & Zoll.), flavonoid, flavone, apigenin.

ABSTRAK

Kajian terhadap tumbuhan Macarang hypolueca (Reichb.f. & Zoll.) dari hutan sekunder sekitar Kota Samarinda, Kalimantan Timur, termasuk studi fitokimia telah berhasil mengisolasi senyawa flavonoid jenis flavon dari fraksi etil asetat daun Macaranga hypoleuca. Proses pemisahan dilakukan dengan metoda kromatografi kolom dengan eluen n-heksana dan etil asetat yang dinaikkan tingkat kepolarannya secara gradien sebesar 5%. Identifikasi dan elusidasi struktur kimia dilakukan menggunakan data hasil analisis inframerah (FTIR), massa (LC-ESI-MS), dan resonansi magnetik inti (1D- dan 2D-NMR). Berdasarkan data-data hasil analisis spektroskopi serta hasil komparasi data spektroskopi dengan literatur dapat diidentifikasi bahwa isolat murni yang berhasil diisolasi adalah senyawa apigenin.

Kata kunci: *Macaranga hypoleuca (Reichb.f. & Zoll.), flavonoid, flavon, apigenin.*

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INTRODUCTION

The *Macaranga* genus, which belongs to the Euphorbiaceae family, is a well-known source of flavonoid compounds. Over 91 flavonoid compounds have been identified from 26 *Macaranga* species, which is more than the number of stilbenes (16), tannins (45), terpenes (12), coumarin (1), steroids (3), and other compounds (29) found in the same genus (Joseph J. Magadula, 2014). Macaranga plants are native to tropical regions of Africa, Asia, Australia, and the Pacific region, including Indonesia, where around 125 of the 300 *Macaranga* species can be found (Ilimu and Syah, 2019). Even though *Macaranga hypoleuca* is one of the *Macaranga* species, published data regarding the results of phytochemical studies on this species is still limited.

Apigenin, an active antioxidant compound (Poureini *et al.* 2022; Tian *et al.* 2021; Sianturi *et al.* 2023), is a flavonoid compound that is commonly found and isolated in plants of the *Macaranga* genus, such as *M. gigantea*, *M. gigantifolia*, and *M. magna* (Tanjung *et al.* 2009; Aminah *et al.* 2014; Johari *et al.* 2019), *M. gigantifolia* (Fajriah *et al.* 2016), *M. magna* (Minarti *et al.* 2021). However, information regarding research activities related to flavone compounds (flavonoids) from *M. hypoleuca* is still very lacking. Therefore, the aim of this research is to carry out a phytochemical study of *M. hypoleuca* as a source of active compounds from the genus of *Macaranga*.

MATERIALS AND METHODS

Plant Material

Macaranga hypoleuca leaves were collected from a secondary forest around Samarinda City, East Kalimantan, Indonesia. Specimen (coll. No. AH 5471) were identified and deposited at Herbarium Bogoriense, National Research and Innovation Agency of Indonesia (BRIN).

Instrumentations

All chemicals used for this research are pro-analytical grade obtained from Merck. Extraction and gravitation column chromatography used technical grade solvent and redistilled before use. 1D- and 2D-NMR spectra were recorded on JEOL ECZR 500 spectrometer. The IR spectrum was recorded on Prestige-21 Shimadzu. LC-MS were measured with Mariner Biospectrometry. Column chromatography was carried out with silica gel 60 (0.063 - 0.200 mm, Merck 1.07734.1000). Compounds detection was used TLC plate (silica gel 60 F254, Merck 1.05554.0001) with 5% H_2SO_4 in ethanol as the compound detection reagent.

Isolation

The secondary metabolites were isolated using a general method that started with a maceration process using methanol solvent for 24 hours and repeated three times. The methanol extract was obtained by evaporating the extract with a rotary evaporator. The methanol extract was then fractionated using n-hexane and ethyl acetate solvents. Three fractions (n-hexane, ethyl acetate, and methanol fractions) were obtained. The next isolation step was carried out using the silica gel column chromatography method, and the number of compounds was qualitatively identified using a TLC plate. The next isolation step was carried out using the silica gel column chromatography method and the number of compounds was qualitatively identified using a TLC plate.

RESULTS

Isolation

About 900 grams of dried *Macaranga hypoleuca* leaf powder underwent a process of maceration with methanol (MeOH) for 24 hours, and it was repeated three times to obtain 157.9 grams (17.55% w/w) of methanol extract. 100 grams of methanol extract were then fractionated using *n*-hexane and ethyl acetate (EtOAc). 18.8 grams, 31.17 grams, and 27.75 grams of *n*-hexane, EtOAc, and MeOH extracts were obtained, respectively. Afterward, around 7 grams of the EtOAc fraction was subjected to a silica gel chromatography column where a solvent system was utilized starting from *n*-hexane, EtOAc to MeOH was utilized with a gradient solvent system of *n*-hexane and ethyl acetate, along with the addition of 5%. Based on the spot pattern on TLC, 18 sub-fractions (SF-1 to

SF-18) were obtained. Compound **1** (6.2 mg) is obtained from the purification and recrystallization process of the SF-12 fraction.

Compound **1**. UV-Vis (Agilent Cary 60) ⌊max 273 nm dan 338 nm. FT-IR (Shimadzu Prestige-21) 3,246; 1,654; 1,570 cm^{-1}, LC-ESI-MS (*m/z*) 270 [M^+], ^{1}H-NMR (500 MHz, in DMSO-*d6*) d_H 6.78 (1H, s, H-3), 6.17 (1H, d, *J* 1.95 Hz, H-6), 6.46 (1H, d, *J* 1.95 Hz, H-8), 7.92 (2H, d, *J* 1.95 & 7.14 Hz, H-2'/6'), 6.92 (2H, d, *J* 1.95 & 7.14 Hz, H-3'/5'). ^{13}C-NMR (125 MHz in DMSO-*d6*) d_C 163.7 (C-2), 102.8 (C-3), 181.7 (C-4), 161.3 (C-5), 98.9 (C-6), 164.6 (C-7), 94.1 (C-8), 157.4 (C-9), 103.6 (C-10), 121.2 (C-1), 128.5 (C-2'/6'), 116.0 (C-3'/5'), 161.5 (C-4')

DISCUSSION

To the best of our knowledge, no information or publication exists regarding the isolation of flavonoid compounds from the *Macaranga hypoleuca* species. However, the content of secondary metabolite compounds from the *Macaranga* genus is mainly composed of flavonoid compounds, with approximately 91 flavonoid compounds having been isolated and identified from around 26 *Macaranga* species (Magadula, J.J. 2014).

The methanol extract of the *Macaranga hypoleuca* leaves extract was partitioned with *n*-hexane and EtOAc. EtOAc fraction was further subjected to column chromatography on silica gel to obtain 18 sub-fractions (SF-1 to SF-18). Purification and re-crystallization of SF-12 afforded compound **1**, yellow crystalline powder. A UV-Vis spectrum of compound **1** showed 2 maximum wavelength bands (λ_{max}) at 273 dan 338 nm indicating that compound **1** flavonoid compound from the flavone type. The FTIR wavelength number (cm^{-1}) data of compound **1** shows the presence of various groups. The wavelength number of 3,246 cm^{-1} indicates the presence of a hydroxyl group (-OH), 1,654 cm^{-1} is a typical wavelength number for a carbonyl group (-C=O), and the wavelength number of 1,570 cm^{-1} indicates the presence of aromatic groups (Figure 1). The LC-ESI-MS analysis revealed that compound **1** had a molecular weight (m/z) of 271 [M+H] or 270 [M+] (Figure 2).

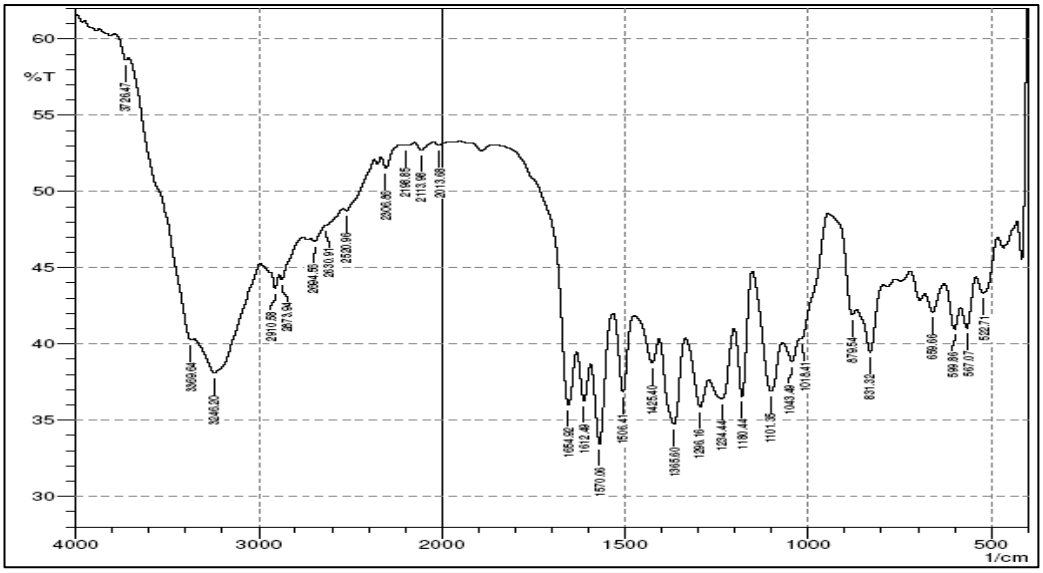

Figure. 1. FTIR spectrum of compound **1** (*spektrum FTIR dari senyawa **1***).

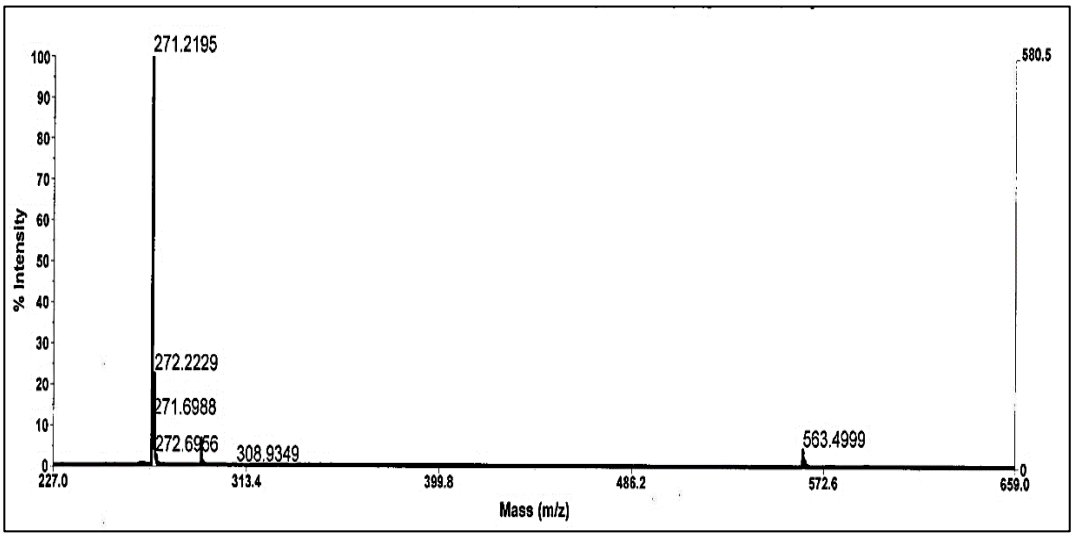

Figure. 2. LC-ESI-MS spectrum of compound **1** (*spektrum LC-ESI-MS dari senyawa **1***).

^{1}H-NMR spectrum of compound **1**, showed AA'XX' proton system with 4 aromatic proton signals at ^{1}H 6.17 (1H, *d*, *J* 1.95 Hz, H-6), 6.46 (1H, *d*, *J* 1.95 Hz, H-8), 6.92 (2H, dd, *J* 1.95 & 7.14 Hz, H-3'/H-5'), and 7.92 (2H, d, *J* 1.95 & 7.14 Hz, H-2'/6'), a singlet signal of carbon double bond atom at ^{1}H 6.78 (1H, *s*, H-3), and a broad singlet signal at ^{1}H 12.94 (Figure 3). Signal proton at ^{1}H 6.92 (H-3'/H-5') and 7.92 (H-2'/H-6') showed four symmetric proton atoms which correlated each other at meta (*J* 1.95 Hz) and ortho (*J* 7.14 Hz) positions. 4 other proton signals at ^{1}H 6.17 (H-6) and 6.46 (H-8) have a *J* coupling value 1.95 Hz, indicating that both protons were correlated at the meta position. Meanwhile, a broad singlet signal at ^{1}H 12.94 indicates that compound **1** also has a hydrogen bridge (-OH). Based on ^{1}H-NMR can be identified that compound **1** at least has 2 aromatic rings.

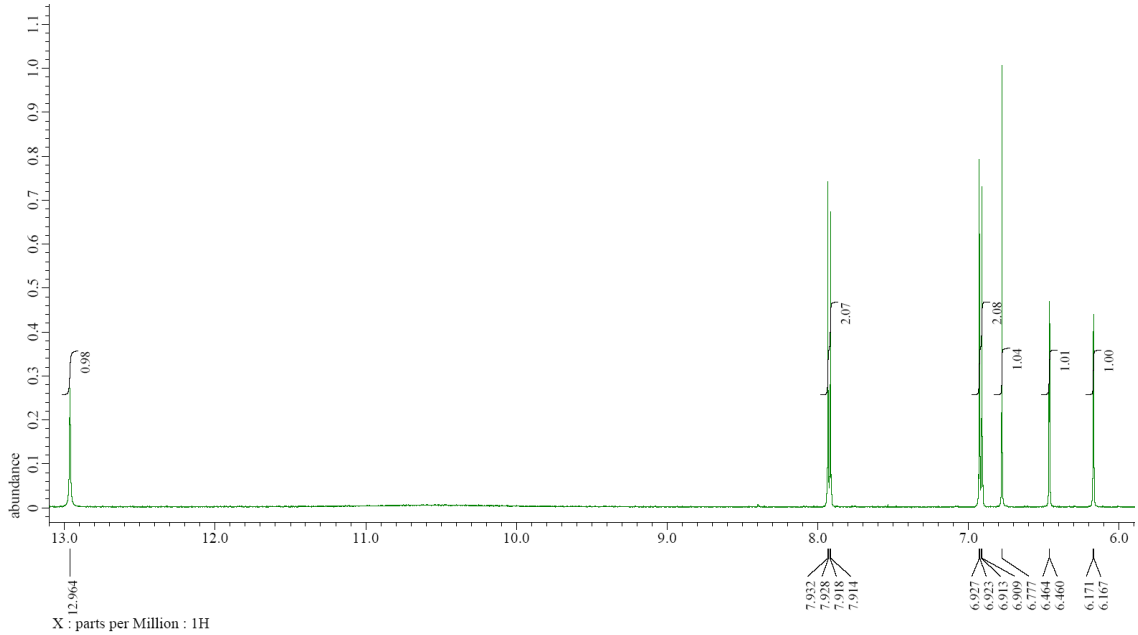

Figure. 3. ^{1}H-NMR spectrum of compound **1** (*spektrum ^{1}H-NMR dari senyawa **1**)*

^{13}C-NMR spectrum showed that compound **1** has 13 carbon signals at ™$_{C}$ 94.1 (C-8), 99.0 (C-6), 102.8 (C-3), 103.6 (C-10), 116.0 (C-3'/C-5'), 121.2 (C-1'), 128.5 (C-2'/C-6'), 157.4 (C-9), 161.2 (C-5), 161.5 (C-4'), 163.7 (C-2), 164.6 (C-7), and 181.7 (C-4/C=O). Carbon atom type was confirmed with 135 DEPT-NMR, where C-2, C-4 (C=O), C-5, C-7, C-9, C-10, C-1', C-4' are

quaternary carbon atoms (Figure 4). Based on HMQC (Figure 5) and HMBC spectrums (Figure 6) showed that compound **1** is a flavonoid from the flavone class indicated by the carbon-carbon double bond between C-2 and C-3, and the proton atom attached to C-3 in ring C (Tabel 1, Figure 7.a). Correlation between proton at ^{1}H 6.75 (H-3) with a carbon atom at ^{13}C 121.2 (C-1') indicated that signal carbon at ^{13}C 121.2 (C-1') located on B ring. Correlation between proton signal at ^{1}H 6.90 (C-3'/H-5') with C-1', correlation between proton signal ^{1}H 7.92 (H-2'/H-6') and ^{13}C 161.5 (C-4'), and correlation based on ortho/meta position between proton signal at ^{1}H 7.92 (H-2'/H-6') and 6.92 (H-3'/H-5') indicates that proton signals at ^{1}H 7.92 (H-2'/H-6'), 6.92 (H-3'/H-5') and carbon signal at ^{13}C 121.2 (C-1') and ^{13}C 161.5 (C-4') located on B ring (Figure 7.b). The correlation between the proton signal at ^{1}H 6.17 and the quaternary carbon signal at ^{13}C 103.6, supported by the correlation between the proton signal at ^{1}H 6.75 (H-3) and the carbon signal at ^{13}C 103.6, indicates that the proton signal at ^{1}H 6.17 located on A ring at H-6 position. Based on this, all correlations involving the H-6 proton signal can be confirmed that all proton signals and carbon signals are in ring A (Figure 7.b).

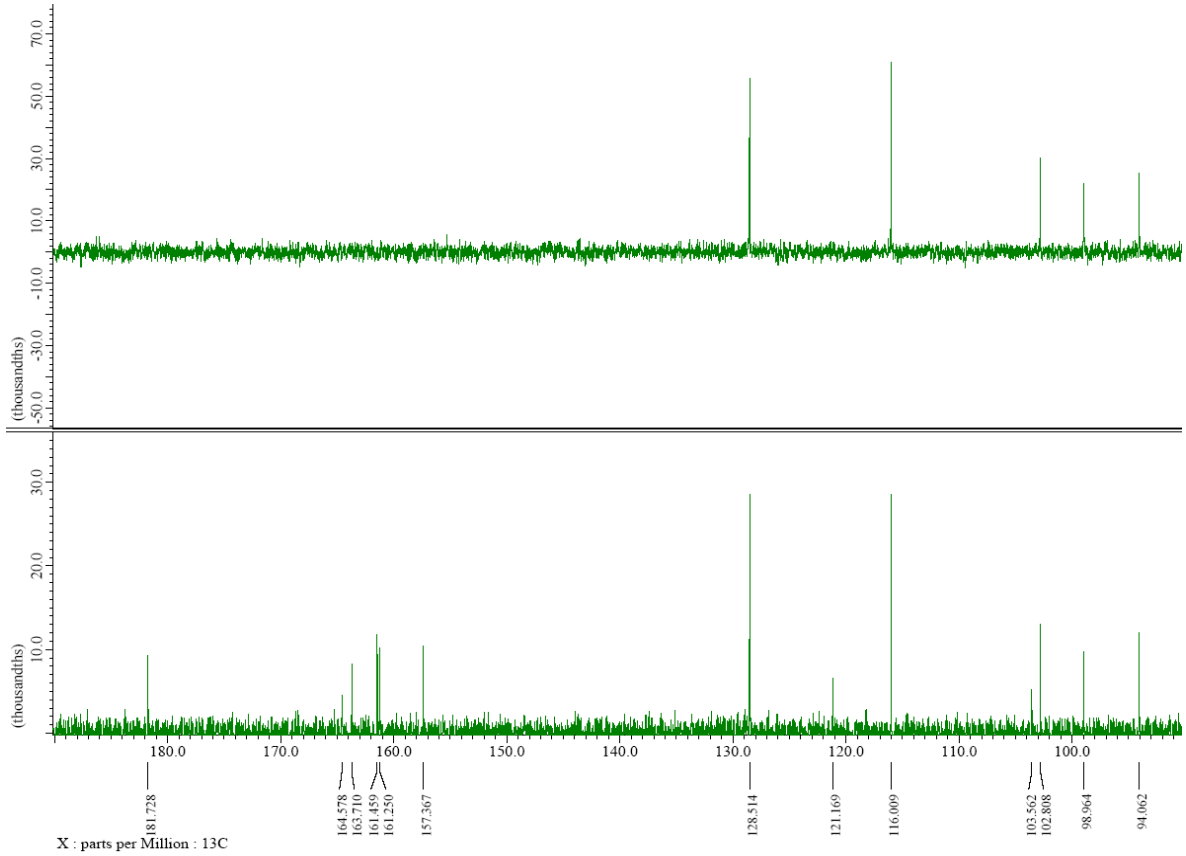

Figure. 4. ^{13}C- and DEPT 135-NMR spectrum of compound **1** (*spektrum ^{13}C- dan DEPT 135-NMR dari senyawa **1**)*

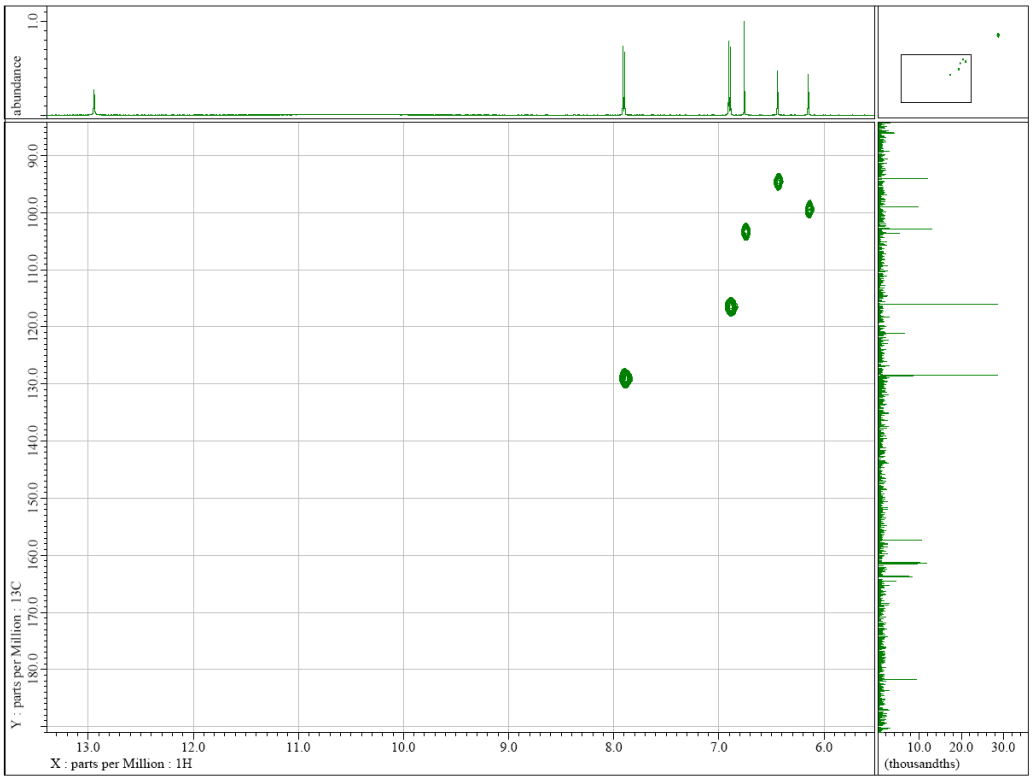

Figure. 5. HMQC-NMR spectrum of compound **1** (*spektrum HMQC-NMR dari senyawa **1**)*

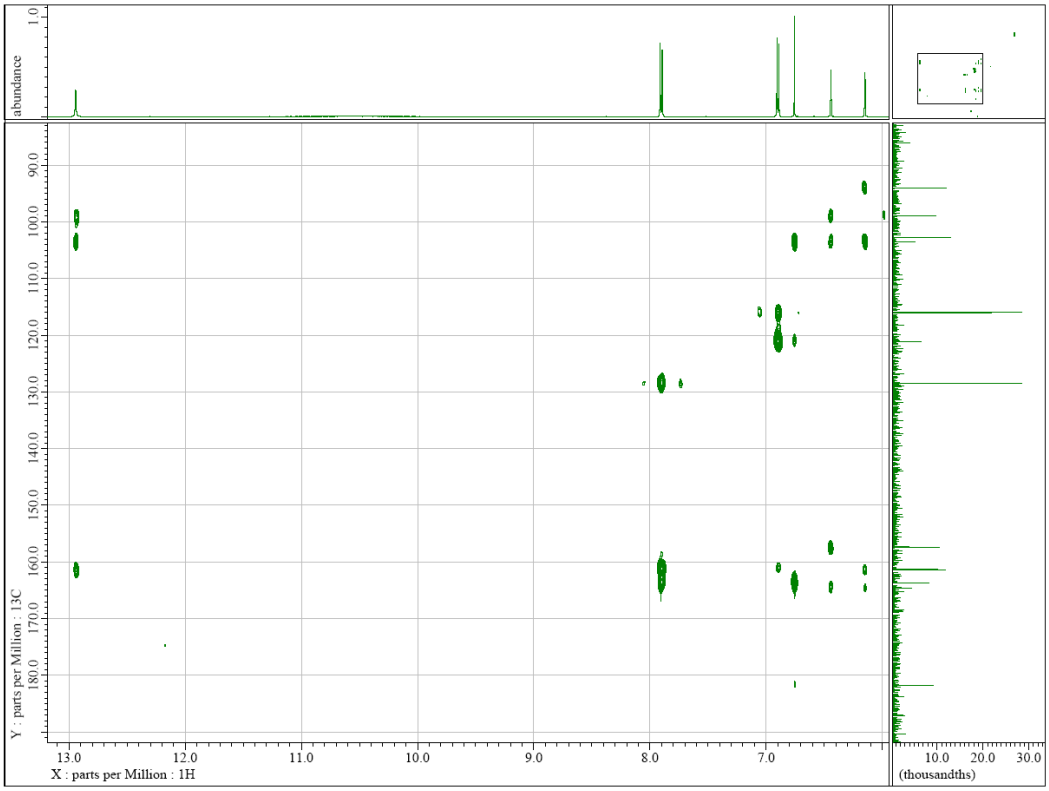

Figure. 6. HMBC-NMR spectrum of compound **1** (*spektrum HMBC-NMR dari senyawa **1**)*

Tabel 1. 1D- and 2D-NMR data of compound **1** (*Data 1D- dan 2D-NMR dari senyawa **1***)

No.	Apigenin (Tavakoli *et al.* 2022)		Compound 1 (*Senyawa 1*)					
	δ_H (ppm) (ΣH, mult., *J* Hz)	δ_C (ppm)	δ_H (ppm) (ΣH, mult., *J* Hz)	δ_C (ppm)	HMBC 1	2	3	4
1		-		-				
2		164.17		163.7				
3	6.78 (1H, *s*)	103.73	6.78 (1H, *s*)	102.8	103.6	121.2	163.7	
4		181.80		181.7				
5		161.49		161.3				
6	6.47 (1H, *d*, *J* 2)	98.86	6.17 (1H, d, *J* 1.95)	98.9	94.1	103.6	161.3	164.6
7		163.76		164.6				
8	6.18 (1H, *d*, *J* 2)	94.00	6.46 (d; 1H; 1,95)	94.1	99.0	103.6	164.6	157.4
9		157.34		157.4				
10		105.83		103.6				
1’		121.63		121.2				
2’ & 6’	7.92 (2H, *d*, *J* 8.8)	128.52	7.92 (2H, *d*, *J* 1.95 & 7.14)	128.5	161.5	163.7		
3’ & 5’	6.91 (2H, *d*, *J* 8.8)	115.99	6.92 (2H, *d*, *J* 1.95 & 7.14)	116.0	121.2			
4’		161,21		161.5				
			12.94 (1H, *sb*)	5-OH				

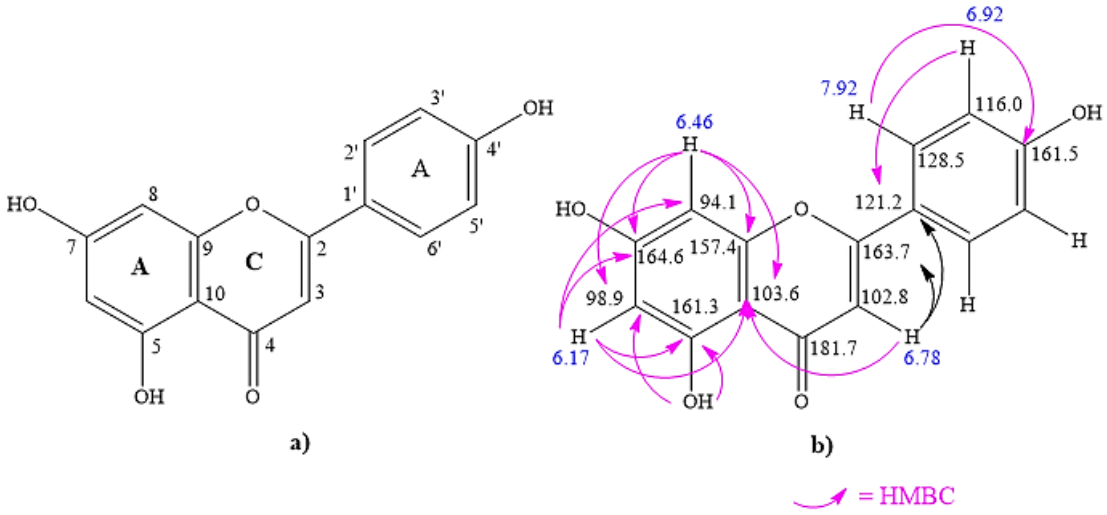

Figure 7. a) Chemical structure of Apigenin (*struktur kimia apigenin*), b) HMBC correlation of compound **1** (*korelasi HMBC dari senyawa **1***)

Based on a series of spectroscopic analysis data and supported by the comparison of NMR data results from Nawal and Atta (2013), the chemical structure of compound **1** has been elucidated. It has been confirmed that compound **1** is apigenin, a flavonoid compound from the flavone type.

CONCLUSION

According to the results of phytochemical studies on the leaves of *Macaranga hypoleuca*, a flavonoid compound from the flavone group called apigenin (compound **1**) has been successfully separated and purified. As far as we know, there has been no previous documentation of flavonoid compounds in the *M. hypoleuca* species.

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AUTHORSHIP CONTRIBUTION

All authors have contributed equally to this paper. The specific roles of each author are as follows: SA drafts the manuscript and is also responsible related to the isolation and purification process. MG, MN, and NA were responsible for screening, fractionation, isolation, and purification processes. SK, AH, and MH were responsible for sample collection, determination, preparation, and extraction process. HHK, GP, and AD were responsible for purification, characterizing, and elucidating the chemical structure of the isolate. in addition, all authors contributed to the writing process, reviewing and editing the paper per their respective roles. They are also responsible for proofreading each other's work to ensure the final paper is complete.

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