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FLAVONOID GLYCOSIDE COMPOUND FROM TOMBILI SEED (*Caesalpinia bonducella*) AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

Caesalpinia bonducella, known as 'tombili' belongs to the family of *Fabaceae.* Tombili has been empirically used as traditional medicine. In a previous study, phytochemical screening showed that tombili seed contained alkaloid, flavonoid, terpenoid, and tannin compounds. This research aimed to isolate an antioxidant compound from tombili seed extract. Fractionated using *n*-hexane, ethyl acetate, and methanol as a solvent. All fractions were tested for their antioxidant potential. The ethyl acetate fraction gave a better antioxidant potential (IC_{50} 86.153±4.22 ppm) than it has purified. Characterization of an isolated compound using various spectroscopies data, including UV, FTIR, 1D-NMR, 2D-NMR, and LCMS/MS. The structure of the isolated compound was suggested as 7- (β-D-Glucopyranosyloxy) 5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (homoplantaginin). The IC_{50} value of the isolated compound was 40.53 ±3.13 ppm, indicating the isolated compound has potent antioxidant activity.

Keywords: *Caesalpinia Bonducella,* Flavonoid, Homoplantaginin, and Tombili.

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INTRODUCTION

Caesalpinia bonducella known as tombili belongs to the family of *Fabaceae.*[1,2] Tombili can be found in Gorontalo province, Indonesia.[3] In Indonesia, *C. bonducella* is known as tombili and areuy.[4,5] Previous studies showed that seed extract of tombili contained alkaloids, flavonoids, terpenoids, and tannin compounds.[6-9] The seed kernel of tombili has traditionally been used by the community as a medicinal plant to treat cough, malaria, and anthelmintic diseases. The seed extract of tombili has several antioxidant, antidiabetic, and antibacterial activities. According to Esmaeili *et al*., 2015 tombili extract has a pharmacological activity that can decrease fasting glucose levels. In addition, tombili seed extract has an activity to reduce glucose levels.[10] Tombili seed methanol extract was also reported to have antihyperglycemic and hypolipidemic activity in induced diabetic rats.[11-12] The flavonoid compounds have the potential as natural antioxidants. In this study, isolated compounds using the chromatography method. Antioxidant content analysis from an isolated compound of tombili seed was evaluated.[13] Antioxidant activity *in vitro* method was used DPPH scavenging. Based on the previous study, this research aimed to isolate flavonoid glycoside compound from tombili seed extract as a natural antioxidant.[13]

EXPERIMENTAL

The materials used in this research are tombili seeds, collected from Bobohu villages, Gorontalo province, Indonesia. Tombili was identified at the Laboratory of Biology Department (plant taxonomy), Universitas Negeri Gorontalo, and Indonesia. The seed powder of tombili (1.0 kg) was macerated in methanol for 2 x 24 h. The methanol extract (210 g) was partitioned to obtain *n*-hexane, ethyl acetate, and methanol fractions. The total solvent used was 2.5 L, respectively. The fractions were obtained from *n*-hexane (29.5

g) and ethyl acetate (50.2 g), and methanol (70.2 g) extracts. All extracts test for antioxidant activity according to inhibition percentage. The higher antioxidant activity of ethyl acetate fraction then purification to give pure isolated antioxidant compound.

Antioxidant Activity Test

DPPH Solution Preparation Crystalline DPPH was weighed and dissolved in ethanol at a concentration of 0,004 %. This solution was freshly prepared every time and kept under low temperature and light. The antioxidant test was performed following Gurning *et al.* (2021).[13] Extract (1.2 mL) was added to 0.3 m DPPH solution to obtain a 1.5 mL mixture, then incubated the mixture of sample, methanol, and DPPH for 30 minutes. The amount of unreacted DPPH was determined using UV-Vis at 515 nm. A similar procedure was applied to the blank solution (DPPH without sample). The following formula calculated the percentage of free radical inhibition by the sample:

$$\%\ \text{Inhibition} = \frac{absorbance\ control - absorbance\ sample}{absorbance\ control}\ x\ 100\%$$

The antioxidant activity data were analyzed using probit analysis to obtain the IC_{50}. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Antioxidant, Isolation, and Determination of Isolated Compound

The highest antioxidant activity of all fractions was the ethyl acetate fraction (Tabel-1). Bioactivity-guided chromatography isolated the flavonoid glycoside compound from ethyl acetate fraction of tombili seed. TLC checked the purification of the isolated compound on Silica G60 F254 with observed on UV at λ 254 and 365 nm. The isolated compound reacted with the coloring reagent of $FeCl_3$ to give amorphous yellow spots. Then the structure of the isolated compound was elucidated by analysis of various spectroscopies data (UV, FT-IR, NMR, and LCMS).

Table-1: Antioxidant Activity Test of Fractions of Tombili Seed

fractions	IC_{50} value replicated			IC_{50} average ± SD
	1	2	3	
n-Hexane	100.798	99.505	102.495	100.933± 1.50
Ethyl acetate	88.499	81.286	88.675	86.153±4.22
Methanol	93.551	95.890	92.718	94.053±1.64

The isolated compound from ethyl acetate fraction (20 mg) was obtained in yellow amorphous form. The isolated compound was soluble in methanol solvent. The IR spectra of the isolated compounds showed an absorptions peak at 3360 $cm^{-1}$for hydroxyl group (-OH). Absorptions peaks at 1658.33 and 1326.42 cm^{-1} for carbonyl (C=O) and olefinic (C=C) functional groups. Absorptions peaks at 2941.33 $cm^{-1}$for (C-H) group. [14-16] LCMS spectra data showed that isolated compounds were $C_{22}H_{22}O_{11}$ (m/z 463.1222) $[M+H]^+$. The ^{1}H-NMR spectrum (500 MHz, methanol D-4): showed proton signal at δH 11.91ppm (1H, s, 5-OH), δH 7.46 ppm (2H, d, H-2', 6'), δH 6.92 ppm (1H, s, H-8), δH 6.94 ppm (2H, d, H-3', 5'), δH 6.83 ppm (1H, s, H-3), δH 5.11ppm (1H, d, H-1''), δH 3.77 ppm (3H, s, 6-OCH3), δH 3.35 ppm (1H, brd, H-6''a), δH 3.19-3.42 ppm (5H, H-2'', 3'', 4'', 5'', 6b''). The ^{13}C-NMR spectra (125 MHz, methanol D-4): δC 178.31 ppm (C-4) belongs to carbonyl group (C=O), δC 163.25 (C-2) belongs to olefinic compound, δC 160.45 (C-4'), δC 149.21 (C-7), δC 147.76 (C-9), δC 145.12 (C-5), δC 131.49 (C-6), δC 127.56 (C-2', 6'), δC 118.89 (C-1'), 116.08 (C-3', 5'), 105.73 (C-10) belongs to methin hydroxy group (CH-OH), 102.64 (C-3), 100.22 (C-1''), 92.42 (C-8), 77.26 (C-3''), 76.29 (C-5''), δC 73.12 (C-2''), δC 69.32 (C-4''), δC 59.49 (C-6''), δC 60.59 (6-OCH_3) belong to methoxy group (Table-2).

Table-2: The NMR Data of Isolated Compound (Homoplantaginin)

Carbon position	δC (ppm)	δH (ppm)
C4	178.31	11.91 (1H, s, 5-OH)
C2	163.25	7.46 (2H, d, H-2', 6')
C4'	160.45	6.92 (1H, s, H-8)
C7	149.21	6.94 (2H, d, H-3', 5')

C9	147.76	6.83 (1H, s, H-3)
C5	145.12	5.11 (1H, d, H-1")
C6	131.49	3.77 (3H, s, 6-OCH3)
C2', C6'	127.56	3.35 (1H, H-6")
C1	118.89	3.19-3.42 (5H, H-2", 3", 4", 5", 6")
C3', C5'	116.08	6.65 (1H, s, H-3)
C10	105.73	
C3	102.64	
C1"	100.22	
C8	92.42	
C3"	77.26	
C5"	76.29	
C2"	73.12	
C4"	69.32	
C6"	59.49	
C6	60.59 OCH_3	

The ^{13}C-NMR, DEPT 135°, and ^{1}H-NMR showed that the isolated compound contained 22 signals of carbons (Table-2). The ^{13}C-NMR spectra (Fig.-1) of the isolated compound indicated signals for twenty-two carbons, including one methoxy signal (-OCH_3), one methylene (CH_2), and eleven sp^2 methynes (-CH), and nine quaternary carbons. Chemical sift at δc 60.59 ppm indicated the isolated compound contained methoxy group (-OCH_3).[17] The chemical sift at δc 59.49-77.26 ppm means the isolated compound had glucose groups.[18] The glucose group was confirmed by the HMBC spectra data by correlation of H-1" connected with δC C-7 (149.21 ppm). Therefore the isolated compound was suggested as 7- (β-D-Glucopyranosyloxy) 5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (homoplantaginin).[19-20] According to a previous study from various literature, the isolation of homoplantaginin compound from ethyl acetate fraction of tombili seed was first reported.

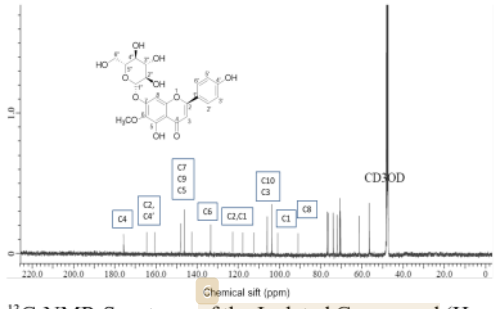

Fig.-1: The ^{13}C-NMR Spectrum of the Isolated Compound (Homoplantaginin)

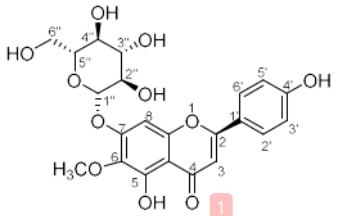

Fig.-2: The Chemical Structure of Isolated Compound 7- (β-D-Glucopyranosyloxy) 5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (Homoplantaginin)

The LCMS/MS showed the molecular formula of the isolated compound was $C_{22}H_{22}O_{11}$ with LCMS-MS: m/z 463.1222 [M+H]+ (Fig.-3). The retention time of the isolated compound was 7.16 min (Fig.-4). Homoplantaginin compounds have been isolated by Kil et al., 2020 from *Salvia plebeia* using HPLC.

Jang et al., 2017 and lee et al., 2010 have isolated homoplantaginin compounds from *Salvia plebeian*. According to Meng et al., 2022 homoplantaginin has an activity to protect VECs by activating Nrf2 and thus inhibited atherosclerosis in apoE-/- mice.[18-21]

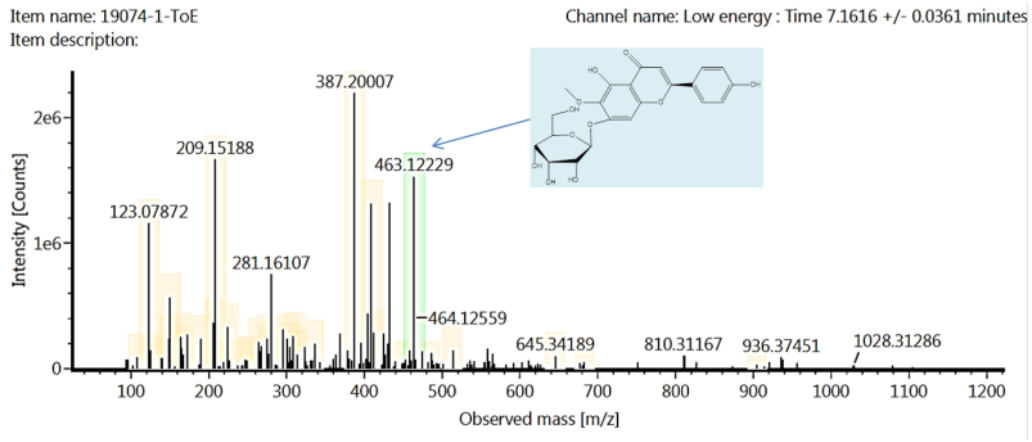

Fig.-3: The LCMS/MS Mass Spectra of the Isolated Compound

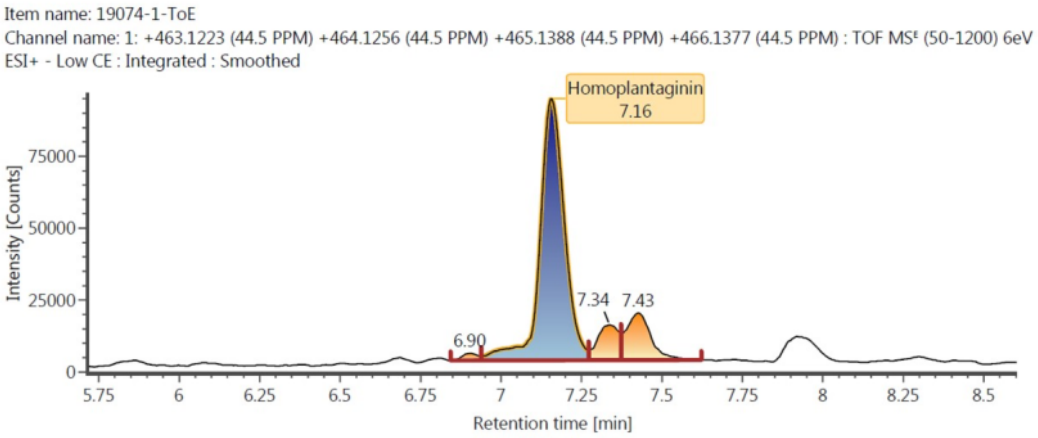

Fig.-4: The Retention Time (Min) of the Isolated Compound

Antioxidant Activity of 7- (β-D-Glucopyranosyloxy) 5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (homoplantaginin)

Antioxidant activity of isolated compound using DPPH method shown in Table-3. The concentration and inhibition percentage curve were constructed, and the linear regression equation obtained from the curve was used to calculate the IC_{50}. The IC_{50} value of the isolated compound was 40.53 ±3.13 ppm. This result indicated that the isolated compound has potent antioxidant activity.

Table-3: Absorbance and Inhibition Percentage of the Isolated Compound

Concentration (ppm)	Absorbance replicated		% Inhibition replicated	
	1	2	1	2
0	0.601	0.612	0	0
20	0.397	0.411	33.94	32.84
40	0.258	0.244	57.07	60.13
60	0.126	0.133	79.03	78.27
80	0.068	0.073	88.68	88.07

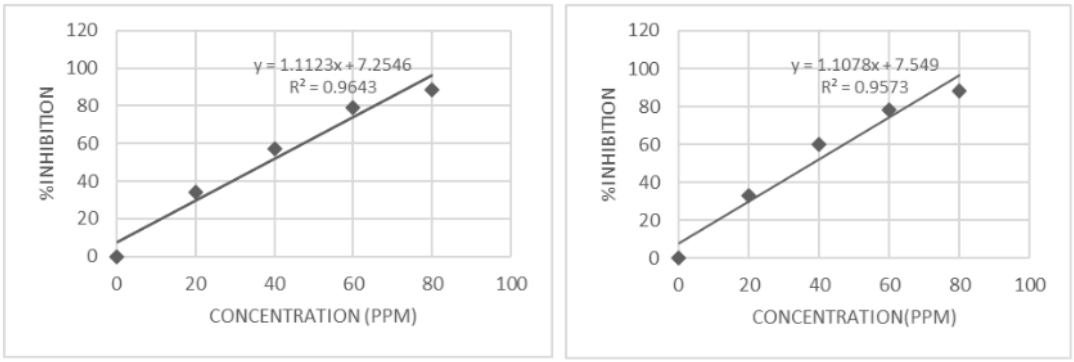

Fig.-5: Linear Regression of an Antioxidant of the Isolated Compound

CONCLUSION

The isolated compound was suggested as 7- (β-D-Glucopyranosyloxy) 5-hydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-1-benzopyran-4-one (homoplantaginin). The IC_{50} value of the isolated compound was 40.53 ±3.13 ppm, indicating that the isolated compound has potent antioxidant activity.

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