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GLYCOSYLATED FLAVONOID COMPOUND FROM COCOA PARASITE LEAVES (*Dendrophthoe pentandra* (L.) Mig.)

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ABSTRACT

Dendrophthoe pentandra (L.) Mig) is a parasitic plant that grows on cacao trees (*Theobroma cacao* L). Methanol extract of *D. pentandra* (L.) Mig) leaves had an IC_{50} value of 30.31 µg/mL as antioxidant activity and the results of phytochemical screening were contained flavonoid compounds. The aim of this research is to isolate and identification of a glycosylated flavonoid compound from *D. pentandra* (L.) Mig) leaves. Extraction of *D. pentandra* (L.) Mig) leaves powder using methanol was performed by maceration. The crude extract was partitioned with ethyl acetate and the flavonoid compound in the ethyl acetate extract was separated by column chromatography [(SiO_2, i). $CHCl_3$; ii). $CHCl_3$-MeOH = 10: 1 ~ 1: 1 iii). Preparative TLC ($CHCl_3$-ethyl acetate = 1: 4)] yielded a compound A. Based on the interpretation of ultraviolet (UV), infrared (FT-IR), Nuclear Magnetic Resonances spectra [NMR 1 D (^{1}H- & ^{13}C-, DEPT), 2 D (COSY and HMBC)], compound A was determined as quercitrin (quercetin 3-O-ramnosida).

Keywords: *Dendrophthoe pentandra* (L.) Mig.), Glycosylated Flavonoid, Quercitrin

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INTRODUCTION

Indonesia is a mega biodiversity country that has great potential in finding unique plant species to be developed as a source of antioxidants and one of them is parasitic plants.[1-3] Parasitic plants can be found in various ecosystems, which uptake carbon, water and other nutrients from host plants by attaching and penetrating their host xylem through specialized organs called haustoria. Mistletoes or parasite in Indonesia is semi-parasitic plant[4], a plant that is harmful because it damages the host plant.[5] However, the parasites have been utilized as a mixture in traditional or ethnomedicines.[6,7] Traditionally a number of parasitic species since ancient times have been used to prevent and treat various diseases including cough[8], diuretics, wounds or fungal infection, anti-inflammatory[9] and antibacterial.[10] The chemical compounds in the parasites are mainly phenolic compounds, tannins, alkaloids, saponins[11] and flavonoids.[12] Flavonoid compounds play an active role as antioxidants. Flavonoid compounds have a chemical structure that can easily contribute hydrogen or electrons to hydrogen acceptors such as reactive oxygen species or peroxy groups from fat, so they can reduce the activity of oxygen and peroxy radicals.[13]

Dendrophthoe pentandra (L.) Miq. is a parasitic plant species that grow on cacao trees commonly found on cocoa plantations[14] in Karo District, North Sumatera, Indonesia. This parasite is very detrimental to cocoa farmers because it damages their commercial crops.[15] Some people have harvested this species as a medicinal herb and traditionally used it for anticancer treatment. This parasitic plant can grow on several different host plants[16,17], and each host plant has a different chemical content.[18]

A previous study has reported that the methanol extract of cacao parasite leaves had an IC_{50} of 30.31 µg/mL as antioxidant activity and the result of phytochemical screening were contained flavonoid compounds.[10,12,19] The purpose of this study was to isolate and identify glycosylated flavonoid compounds

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from the leaves of *D. pentandra* (L.) Mig originating from cocoa plantations, Kabanjahe, Karo District, North Sumatera, Indonesia.

EXPERIMENTAL

Isolation and Purification of Compound A

The leaves of *D. pentandra* (L.) Mig) collected from cocoa plantations, Kabanjahe, Karo District, North Sumatera, Indonesia. Methanol, chloroform, ethylacetate and *n*-hexane used were of analytical grade, silica gel 60 HF_{254} (Merck KGaA) for column chromatography, and TLC Silica gel 60 F_{254} for preparative TLC. The ^{1}H-NMR and ^{13}C-NMR spectrum was recorded on a Agilent-2 NMR 500 MHz spectrometer instrument with CD_3OD as a solvent and TMS as an internal standard and chemical shifts are given in δ (ppm). IR spectrum was recorded on FT-IR (Shimadzu), UV spectrum was recorded on Spectrophotometer UV-Vis (Hewlett Packard Agilent), solvent evaporation with a rotary evaporator (Heidolph), monitoring sample spots with UV lights (254 nm/366 nm, UVGL 58). *D. pentandra* (L.) Mig leaves are blended[20] and the powder (2000 g) macerated with methanol.[16, 22] Procedure for isolation and purification of compound A from parasites plant (*D. pentandra* (L.) Mig) can be seen in Fig.-1.

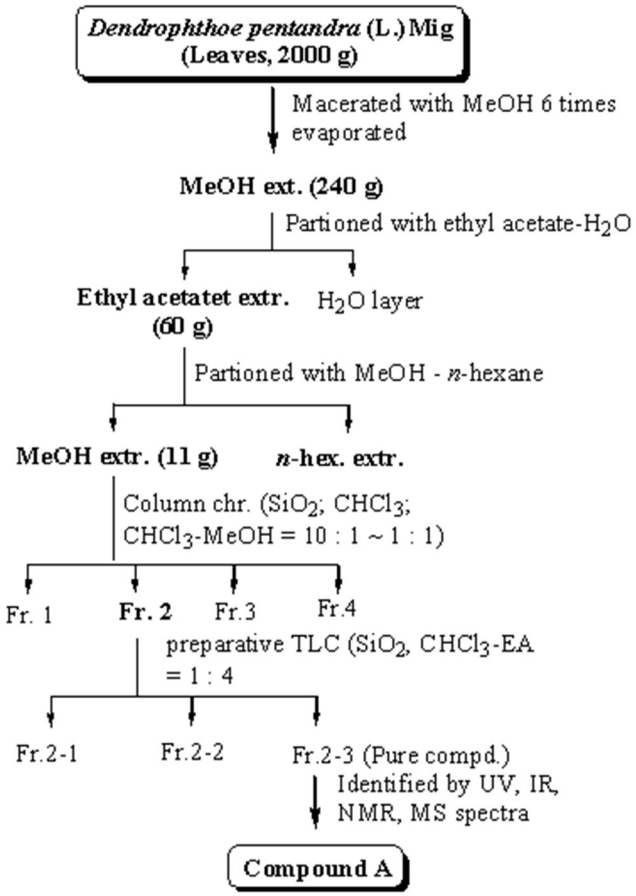

Fig.-1: Scheme of Isolation and Purification of Flavonoid Compound from Parasites Plant (*D. pentandra* (L.) Miq).

RESULTS AND DISCUSSION

Interpretation of Compound A

The sample used in this research was the leaves of *D. pentandra* (L.) Miq. (Fig.-2c) is the Loranthaceae family, with the local name "coklat “chocolate". *D. pentandra* (L.) Miq. is a cocoa parasitic plant (Fig.-2b) that grows on the branches of a cocoa plant (Fig.-2a). In this study, column chromatography was performed for fractions two[12,19] and fraction 2-3 was collected as a yellow powder (compound A, 6.5 mg). There were several other fractions, but in small amounts (below 5 mg). compound A was purified by Preparative Thin

Layer Chromatography using chloroform-ethyl acetate (2:8). Identification of compound A was determined by UV-Vis, FT-IR and 1H-NMR spectroscopic analysis.

Ultra-violet (UV-vis.) spectra for compound A provide a λ_{max} (MeOH) at 350 nm which is characteristic of flavones, chalcones, flavonols (3-OH free) and flavonols (3-OH substituted). Infrared (FT-IR) spectra (KBr, υ_{max}, cm^{-1}) gave wave numbers at 3433.2 (O-H overlaps with sp^2 C-H stretching), 2924.09 and 2854.65 (sp^3 C-H stretching), 1627.92 (C = O); 1527.62 (C = C), 1381.03 (sp^3 C-H bending), 1273.02 (C-O), indicated that compound A has an aromatic ring commonly found in flavonoid compound. Compound A has a glycoside bond with sugar, this is indicated by the presence of the sp^3 C-H stretching and bending at υ 2924.09 cm^{-1} and 1381.03 cm^{-1} of methyl moiety derived from sugar.

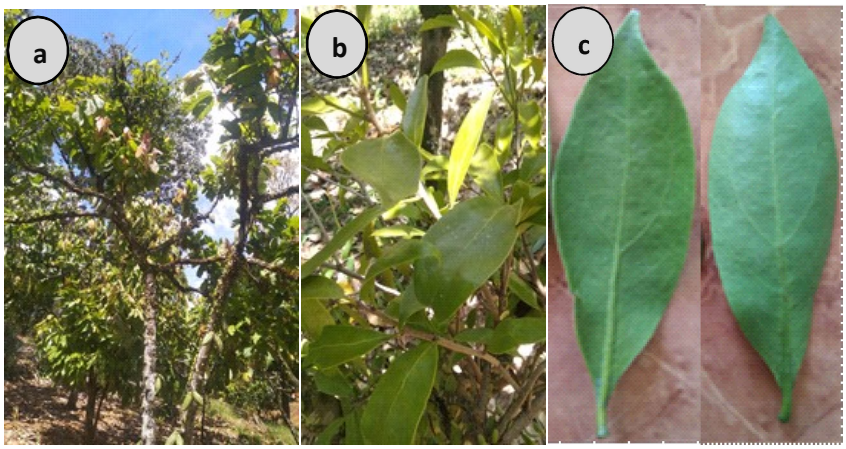

Fig.-2: Cocoa Tree was overgrown with parasites plants (a); The parasitic plants of cocoa (*D. pentandra* (L.) Miq (b); the leaves of *D. pentandra* (L.) Miq (c).

Mass spectra analysis (LC-MS) gave two peaks with *m/z* 287 and *m/z* 449. This spectrum showed that compound A had molecular ion peak $[M+H]^+$ at *m/z* 449, indicating that compound A had molecular weight 448 with molecular formula $C_{21}H_{20}O_{11}$. Peak with *m/z* 287 $[(M+H)^+ - 162]$ suggesting the loss of a sugar.[4] Investigation of ^{1}H-NMR spectra showed the presence of five aromatic protons and eleven sugar protons. Spectra of ^{13}C-NMR showed the presence of twenty one carbon atoms including six carbon atoms of sugar and fifteen carbon atoms of aglycon flavonoids. The chemical shifts of each proton and carbon in compound A are listed in Table-1.

Table-1: ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data for Compound A compared to the isolated Compound of quersetin3-O-rhamnoside.

No	Position C/H	Compound A		Reference[4]	
		δ_C (ppm), methanol-D_4,	δ_H (ppm) *J* (Hz), methanol-D_4	δ_C (ppm), Various Solvents,	δ_H (ppm, Hz)
1	2	146.48	-	149.8	-
2	3	136.55	-	136.2	-
3	4	179.64	-	179.6	-
4	5	158.61	-	159.3	-
5	6	100.14	6.18 (s)	99.8	6.19 , d 2.2
6	7	166.50	-	165.9	
7	8	95.02	6.34 (s)	94.7	6.39, d 2.1
8	9	159.33	-	163.2	
9	10	105.82	-	105.9	
10	1’	123.05	-	123.0	
11	2’	117.08	7.34 (s)	116.9	7.64
12	3’	146.48	-	146.4	
13	4’	149.90	-	149.8	

14	5’	116.55	6.90 (d, 8)	116.4	6.97 dd 8.2, 1.8
15	6’	123.05	7.29 (d, 8)	122.9	7.32, d 2.1
	Rham				
16	1’’	103.61	5.35 (s)	103.5	5.44 d 1.5
17	2’’	72.13	4.24 (s)	72.1	4.35 dd 1.5, 3.3
18	3’’	72.13	3.76 (dd, 2.5, 2.0))	71.9	3.92 dd 3.3,9.5
19	4’’	73.37	3.35 (t 7)	73.3	3.47 t 9.5
20	5’’	72.13	3.76 (dd, 2.5, 2.0)	72.0	3.64 dd 9.5, 6.2
21	6’’	17.78	0,94 (d, 6,5)	17.7	1.08 d 6.3

Two-dimensional ^{1}H-^{1}H COSY spectrum shows coupling between neighboring protons reveaeled as cross peaks in the spectrum. ^{1}H-^{1}H COSY analysis for compound A showed that proton H-6 (δ_H 6.18 ppm) was coupled with proton H-8 (δ_H 6.34 ppm); H-6’ (δ_H 7.29 ppm) was coupled with H-5’ (δ_H 6.90 ppm). The ability to see long-range interaction between ^{13}C and ^{1}H nuclei ($^{2,3}J_{CH}$) using Spectrum 2-D ^{1}H-^{13}C HMBC (Hetero Multiple Bond Connectivity). The 2D HMBC spectrum (Fig.-3) shows that the proton H-6 (δ_H 6.18 ppm) was correlated with carbon C-5 (δ_C 158.61 ppm) and C-10 (δ_C 105.82 ppm). Proton H-2’ (δ_H 7.34 ppm) was correlated with C-3’ (δ_C 146.48 ppm) and C-4’ (δ_C 149.90 ppm). Rhamnoside sugar is attached to the flavonoid aglycone at the C-3 position, this can be proven to have a correlation of proton H-1” (anomeric proton) at δ_H 5.35 ppm with carbon C-3 δ_C 136.55 ppm. These data indicate that compound A is a flavonoid compound of the flavonol group (3-OH substituted). The -OH group at the C-3 position of flavonols is substituted with rhamnoside sugar a glycosylated flavonoid. Analysis of 2 D NMR (COSY and HMBC experiment) for Compound A can be seen in Fig.-3. Based on the interpretation of spectra (UV-Vis, FT-IR, 1D & 2D ^{1}H NMR, ^{13}C NMR and LC-MS) and comparison of chemical shifts with isolated compound by Katrin *et al* (2011), compound A was determined as a compound of quercitrin (quercetin 3-O-rhamnoside) (Fig. -4).

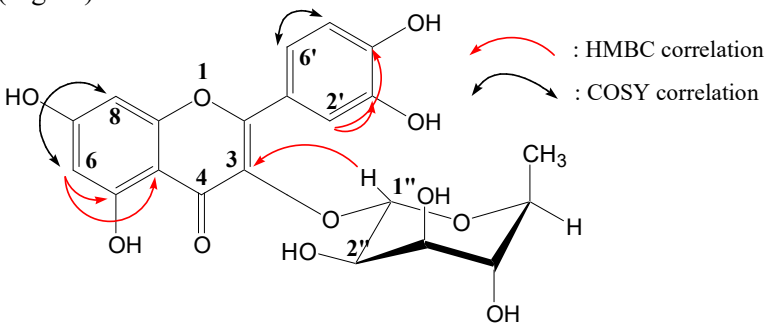

Fig.-3: Analysis of 2D-NMR (COSY and HMBC experiment) for Compound A.

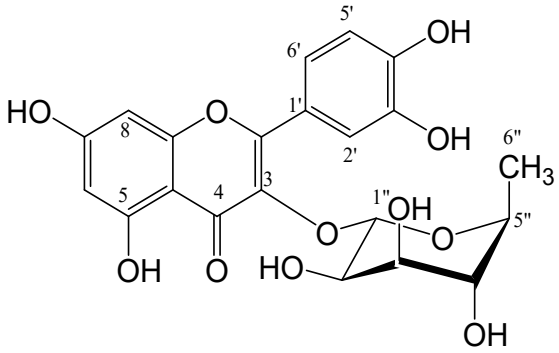

Fig.-4 : Chemical Structure of Quercitrin (quercetin 3-O-rhamnoside) isolated Compound from Parasitic Plants (*D.pentandra* (L.) Miq)

CONCLUSION

A glycosylated flavonoid compound had been isolated and identified from parasite leaves (*Dendrophthoe pentandra* (L.) Mig) collected in Karo District, North Sumatera, Indonesia as quercitrin (quercetin 3-*O*-rhamnoside).

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