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FLAVONOID GLYCOSIDE FROM THE ETHYL ACETATE EXTRACT OF KELADI TIKUS *TYPHONIUM FLAGELLIFORME* (LODD) BLUME LEAVES

Yunahara Farida[1], P.S. Wahyudi[2], S. Wahono [3,1], M. Hanafi[4]

[1] Faculty of Pharmacy, Pancasila University, Jakarta,
[2] Department of Chemistry, FMIPA, University of Indonesia, Depok,
[3] Agency for the Assessment and Apllication of Technology, (BPPT)
[4] Research Centre for Cheμg/mL mistry, Indonesian Institute of Sciences,
INDONESIA.
[1] yunahara_farida@yahoo.com

ABSTRACT

A flavonoid glycoside has been isolated from keladi tikus (Typhonium flagelliforme (Lodd) Blume) leaves. Keladi tikus is a plant traditionally uses as anticancer. The ethyl acetate extract was isolated using Vacuum Liquid Chromatography, then fractionated with column chromatography and identified by UV-VIS, FTIR, LCMS-MS and ^{1}H-NMR, ^{13}C-NMR, 2D-NMR (HMQC, HMBC, DEPT and COSY). The result from the isolation of the ethyl acetate fraction T. flagelliforme (Lodd) Blume obtained 6-glucosyl apigenine namely isovitexin. Isovitexin has antioxidant activity (DPPH free radical scavenging) with IC_{50} 34.39 μg/mL and cytotoxic activity using BSLT (LC_{50} 15.84 μg/mL).

Keywords: Flavonoid glycoside, ethyl acetate, Typhonium flagelliforme.

INTRODUCTION

Indonesia is a tropical country that has the second largest biodiversity in the world after Brazil, has the natural resources that have not been fully utilized. Therefore, research of natural resources as source of medicines has developed especially discovery of active compound in order to find new drugs, one of them as anticancer.

Keladi Tikus (*T. flagelliforme* (Lodd) Blume), familia Araceae, commonly known as the 'rodent tuber', is often included as an essential ingredient in various herbal remedies recommended for cancer therapies in Malaysia (Choo *et al*., 2001). This plant is widely used in traditional medicine in Southeast Asia to treat various diseases. This plant is used to soothe swelling, coughing and more predominantly for the treatment of cancer (Lee and Wong, 2004), as anti-inflammatory, analgesic and sedative (Zhong *et al*., 2001); as antibacterial and antioxidant activities (Mohan, *et al.,* 2008).

A number of plant secondary metabolites including flavonoids, saponins, alkaloids and terpenoids have previously been reported from this plant.

Several chemical constituents had been identified from *T. flagelliforme*. The hexane extract was reported to contain saturated hydrocarbons and aliphatic acids (Choo *et al*., 2001a), while the ethyl acetate extract was found to contain aromatic fatty acids (Chen *et al*., 1997). The aim of this research is to determine the flavonoid compound of T. *flagelliforme* leaves and their activities.

MATERIALS AND METHODS

Plant Material

The Leaves of T. flagelliforme were collected in Balittro, Bogor, Indonesia. A voucher specimen has been deposited at Research Centre of Biology, LIPI, and Cibinong Bogor.

Extraction and Isolation

The dried powdered leaves (3.4 kg) of T. *flagelliforme* were macerated with methanol at room temperature for 24 hours (three times), and the crude methanolic solution was subsequently concentrated using rotary evaporator. The methanol extract was partitioned by the following solvents with increasing polarity: *n*-hexane, ethyl acetate, and *n*-buthanol. The extracts were concentrated to dryness by rotary evaporator. The ethyl acetate extract was fractionated by Vacuum Liquid Chromatography (dichloromethane–isopropanol gradient (100:0 ~ 50:50) followed by methanol to give 9 fractions. Fraction 7 (7.27 g) was further chromatographed using Sephadex LH20 with methanol as the eluent, obtained 8 subfractions. Flavonoid compound was obtained from subfraction 7.6 (108.8 mg) by purification with the preparative reversed phase HPLC with methanol-water (100:0 ~0:100) and then identified by UV, IR, LCMS and 1D, 2D NMR spectroscopy data.

Antioxidant Activity (DPPH Free Radical Scavenging Activity)

The method was carried out according to the method described by Brand-Williams *et al*. (1995) and Blois (1958) with slight modification.

Briefly, samples of various concentration (5, 10, 25, 50, 100 ppm) was added to 1mL of 0.1 mM methanolic DPPH solution. After 30 minutes incubation period at room temperature, the absorbance was recorded at 515 nm using UV-Vis spectrophotometer. The results are expressed as IC_{50} (µg/mL), the compound concentration providing 50% scavenging of the DPPH radical present in solution. Vitamin C was used as standard in concentration 2-10 µg/mL). The reduction of the absorbance (inhibition, %) for DPPH reagen was calculated according to the following equation.

DPPH radical scavenging (%) = [($Abs_{control}$ - Abssample) / $Abs_{control}$] x 100, where $Abs_{control}$ is the absorbance of the control reaction (DPPH reagent) and Abs_{sample} is the absorbance of the test compound. The IC_{50} value was calculated from the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate.

Brine Shrimp Lethality Test (BSLT).

The method was carried out using the method described by Meyer, *et al*. (1982). Briefly, *Artemia salina* Leach was allowed to hatch and mature as nauplii (larvae) in seawater for 48h at 25^{o}C. Serially diluted sample solution was added to the seawater (5 mL) containing 10 nauplii. After incubation for 24h at 25^{o}C, the number of survivors was counted. The LC_{50} (50% lethal concentration, µg/mL) was determined from triplicate experiments using Probit analysis as described by Finney (1971).

RESULT AND DISCUSSION

The compound was isolated as a yellow powder, mp 201-204^{o}C. LCMS m/z 432,11 $[M]^{+}$ with a combination of ^{1}H NMR and ^{13}C NMR data having molecular formula $C_{21}H_{20}O_{10}$.

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UV λmax (MeOH) at 273, 333 nm. The IR (KBr) spectra gives 3243 cm^{-1} is the stretching vibration of the hydroxyl group (-OH), 1711 cm^{-1} is the C=O stretching vibration, 1660 cm^{-1} is the stretching vibration of C=C bonds of aromatic ring, 1450-1356 cm^{-1} is the bending vibration of CH bond, 1182-1081 cm^{-1} is the C-O-C bond of ether, 832 cm^{-1} is the C-H bond outside the field.

The ^{1}H NMR (500 MHz, CD_3OD) indicates the compound is a group of flavonoid glycosides. The presence of aromatic protons at δ_H 6.91 (2H, d, 8.6Hz, H-2´, H-6´) and 7.82 (2H, d, 8.6 Hz, H-3´, H-5´). Besides, the two unpaired aromatic proton as singlets at δH 6.49 (1H, s, H-8) and 6.58 (1H, s, H-3). This compound has a hydroxy group of a glucoside at δ_H 3.43 (d, 1H), 3.48 (m, 1H), 3.73 (dd, 1H; 5.5; 11.2 Hz).

^{13}C NMR (125 MHz, CD_3OD) showed that the compound containing 21 carbon atoms consisting of one methylene group, eleven methyne group consist of five methyne for typical area of sugar at δ_C 75.4 (C-1″); 72.6 (C-2″); 80.2 (C-3″), 71.9 (C-4″) and 82.7 (C-5″), nine quarternary carbons group at δ_C 166.2 (C-2), 184.1 (C-4), 162.1 (C-5), 109.3 (C-6), 165.2 (C-7), 158.8 (C-9), 105.2 (C-10), 123.1 (C-1´), and 162.9 (C-4´) with the formula $C_{21}H_{20}O_{10}$.

The HMQC and HMBC spectra showed an aromatic protons at δ_H 6.49 correlated to C-signals at 95.3 (C-6), correlated to the carbon at C-10, C-6, C-9 and C-7. The correlation between proton at δ_H 6.58 to C-signals at 103.9 (C-3), correlated to the carbon at C-10, C-1´, C-2 and C-4. The aromatic proton at δ_H 7.82 correlated to C-signals at 129.5 (C-2′, C-6′), correlated to the carbon at C-4´ and C-2. The aromatic proton at δ_H 6.91 correlated to C-signals at 117.1 (C-3′, C- 5′), correlated to the carbon at C-1´ and C-4´. Glucoside protons at δH 4.89 correlated to C-signals at 75.4 (C-1″), correlated to the carbon at C-2″, C-3″, C-6, C-5 and C-7. Proton at δ_H 4,18 correlated to C-signals at 72.6 (C-2″), correlated to the carbon at C-3˝). The correlation between δH 3.47 to C-signals at 80.2 (C-3″), correlated to the carbon at C-4″ and δ_H 3.48 correlated to C-signals at 71.9 (C-4″), correlated to the carbon at C-3˝ and δ_H 3.43 correlation with 82.7 (C-5″), correlated to the carbon at C-4˝ and C-3˝.

Based on the HMBC spectra showed a correlation between the anomeric proton with δ_C 109.3; 162.1 and 165.2 ppm that indicate the group is located in the 6C-position. Analysis ^{1}H NMR, ^{13}C NMR and HMBC of the Compound as seen as figure 1 and table 1.

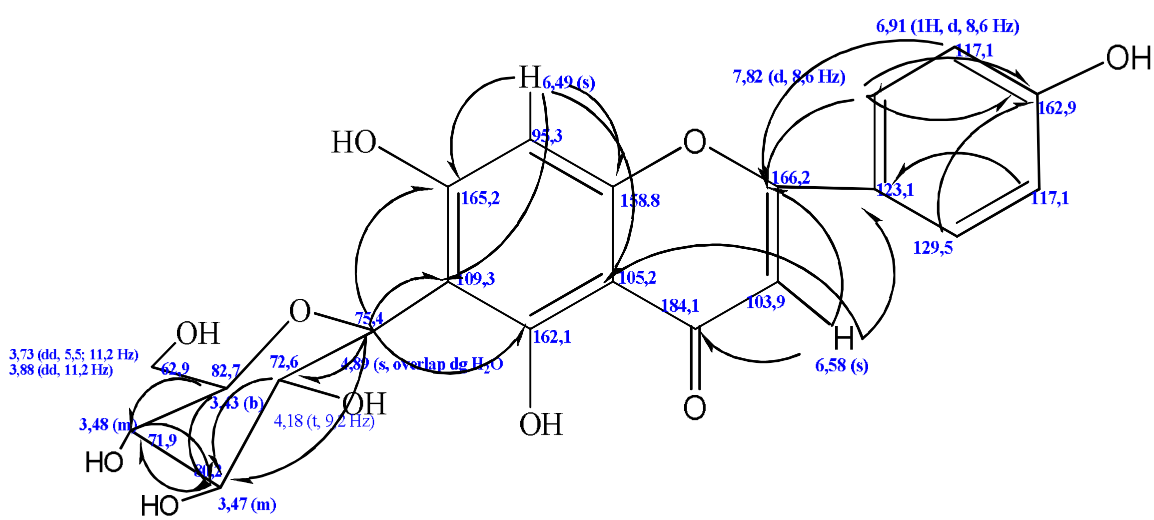

Figure 1. HMBC Correlation of the Compound

Table 1. ^{1}H NMR, ^{13}C NMR and HMBC of the Compound

No	^{13}C NMR (δ_C, ppm)	^{1}H NMR (δ_H, ppm)	HMBC (δ_C, ppm)	No	^{13}C NMR (δ_C, ppm)	^{1}H NMR (δ_H, ppm)	HMBC (δ_C, ppm)
2	166.2	-	-	3′	117.1	6.91 (d, 8.6Hz)	123.1; 162.9
3	103.9	6,58 (1H, s)	105.2; 123.1; 166.2; 184.1	4′	162.9	-	-
4	184.1	-	-	5′	117.1	6.91 (d, 8.6Hz)	123.1; 162.9
5	162.1	-	-	6′	129.5	7.82 (d, 8.6Hz)	162.9; 166.2
6	109.3	-	-				
7	165.2	-	-	1″	75.4	4.89 (s, overlap with H_2O)	72.6; 80.2; 109.3; 162.1; 165.2
8	95.3	6,49 (1H, s)	105.2; 109.3; 158.8; 165.2	2″	72.6	4.18 (t, 9.2Hz)	80.2
9	158.8	-	-	3″	80.2	3.47 (m)	71.9
10	105.2	-	-	4″	71.9	3.48 (m)	80.2
1′	123.1			5″	82.7	3.43 (b)	71.9; 80.2
2′	129.5	7.82 (d, 8.6Hz)	162.9; 166.2	6″	62.9	3.73(dd,5.5,11.2Hz) 3.88 (dd;11.2Hz)	-

Comparing with the reported data, by ^{1}H NMR and ^{13}C NMR and comparison with its literature data (Wen, P. *et al*., 2007), the compound identified as isovitexin (6-glucosyl apigenine) as seen in Figure 2.

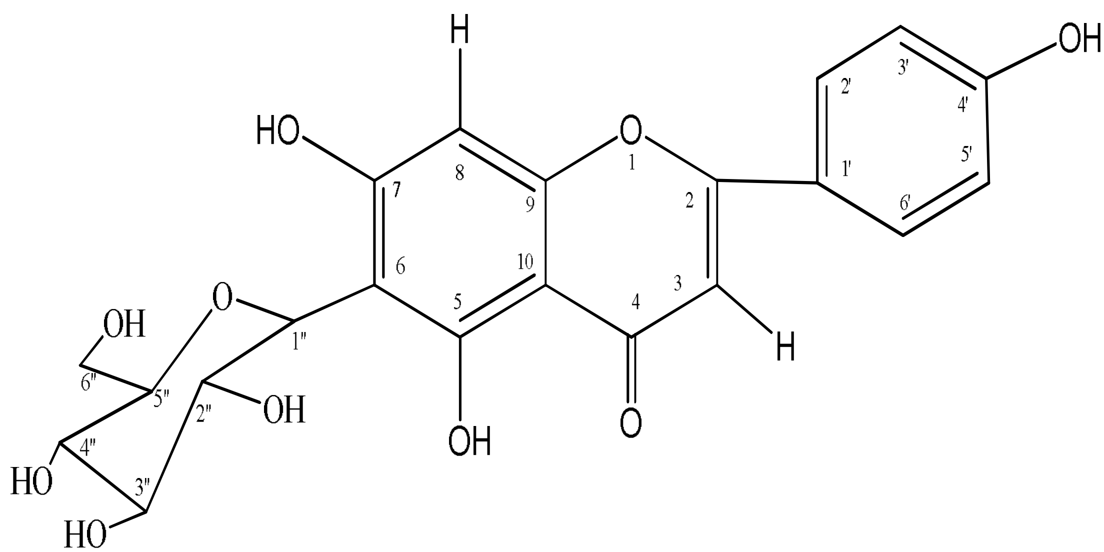

Figure 2. The structure of 6-glucosyl apigenine (isovitexin)

The antioxidant activity of the compound showed potent antioxidant with IC_{50} 34.39 µg/mL (<200 µg/mL) as seen in Figure 3. The flavonoid like flavonols and flavones containing a catechol group in ring B are highly active as antioxidant, with flavonols more potent than corresponding flavones because of the presence of 3-hydroxyl group. On the contrary, the presence of only one hydroxyl in ring B diminishes the activity (Pietta, PG, 2000)

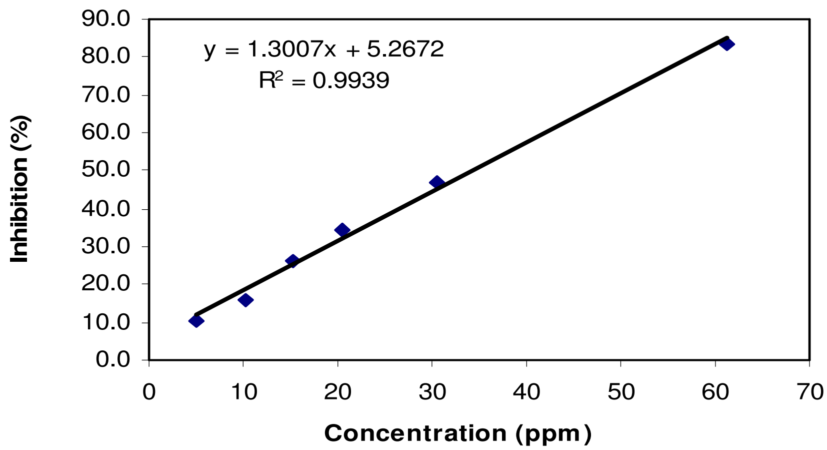

Figure 3. Antioxidant activity of the 6-glucosyl apigenine

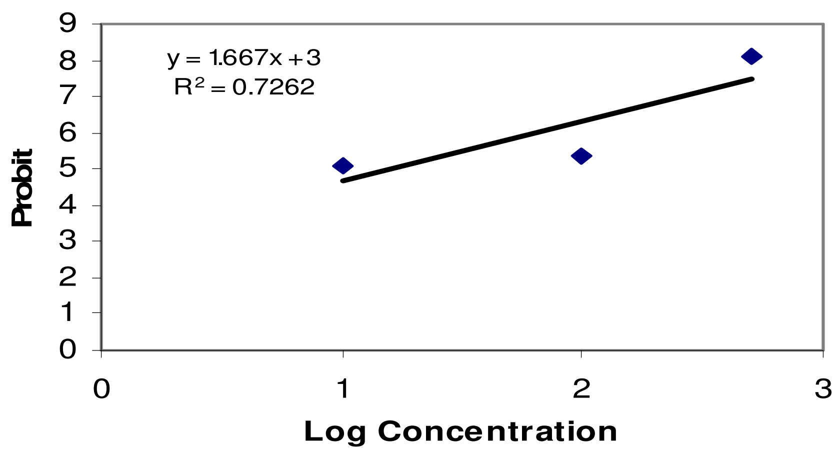

Figure 4. Relationship between log concentration versus probit (BSLT)

The brine shrimp lethality test (BST) was used to predict trends of toxicity and possible presence of potential anticancer compounds (Moshi *et al.*, 2004). The results for cytotoxic activity on brine shrimp nauplii are interpreted as follows: LC_{50} < 1.0 µg/ml – highly toxic; LC_{50} 1.0-10.0 µg/ml – toxic; LC_{50} 10.0-30.0 µg/ml – moderately toxic; LC_{50} > 30 < 100 µg/ml–mildly toxic, and > 100µg/ml as non-toxic. The results showed the compound was exhibited moderately toxic with LC_{50} 15.84 µg/mL (Figure 4.)

CONCLUSION

1. The ethyl acetate fraction has cytotoxic activity against brine shrimp nauplii (*A.salina*), has antioxidant activity
2. Flavonoid from ethyl acetate fraction of methanol extract *T.flagelliforme* leaves was isolated and identified as 6C-glucosyl apigenine, namely isovitexin

Acknowledgements

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REFERENCES

Blois, M.S., (1958). Antioxidant determinations by the use of a stable free radical. *Nature* 26 pp. 1199-1200

Brand-Williams, W., Cuvelier,M.E., & Berset, C.(1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft and Technologie , 28*, 25-30.

Choo,C.Y., Chan, K.L, Takeya,K, Itokawa, H., (2001). Cytotoxic activity of *Typhonium flagelliforme* (Araceae). *Phytotherapy Research* 15(3): 260–262.

Choo,C.Y., Chan, K.L., Sam, T.W., Hitotsuyanagi, Y., Takeya,K. (2001a). The cytotoxicity and chemical constituents of the hexane fraction of Typhonium flagelliforme (Araceae). *J. of Ethnopharmacology*, 15(1), 129–131.

Chen, S.X., Goh, C.J., Kon, O.L., (1997,Dec). Fatty acids from Typhonium flagelliforme. *Planta Med.* 63(6): 580.

McLaughlin and Anderson JE. (1991). A blind comparation of single benzsch-top bioassay and human tumor cell. Cytotoxicities studies as Anti tumor prescreens. *Phytochemical Analysis*. Vol. 2; p.107-111.

Meyer BN, Ferrigni,NR., Putnam JE., Jacobsen, LB., Nichols, DE., MCLaughlin, JL. (1982). Brine shrimp: a convenient general bioassay for active plant constituent. *Planta Medica*, Vol. 45 : 31-34.

Mohan, S., Abdul, AB., Wahab, SIA., Al-Zubairi,AS., Elhassan, MM., Yousif,M., (2008). Antibacterial and antioxidant activities of *Typhonium*

Moshi, MJ., Cosam, JC., Mbwambo,ZH., Kapingu,M. and Nkunya MHH., (2004). Testing beyond ethnomedical claims:brine shrimp lethality of some Tanzanian plants. *Pharmaceutical Biology,* 42, 547-551.

Pietta, PG., (2000). Flavonoids as antioxidants. *J.Nat.Prod,* 63 (7), 1035-1042.

Reiser, M.J., Zhe-Ming,G., Xin-Ping,F., Zeng,L., Wood, KV., McLaughlin, JL., (1996). Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*. *J. Nat. Prod.* 59, 100-108.

Silverstein, R.M., Webster, F.X. dan Kiemle, D.J. (2005). Spectrometric Identification of Organic Compounds. 7th edition, John Wiley &Sons,Inc.USA.

Wen, P., Han, H., Wang,R., Wang,N., Yao,X., (2007). C-glycosylfavones and aromatic glycosides from Campylotropis hirtella (Franch.) Schindl. *Asian Journal of Traditional Medicines,* 2007, 2 (4) http://www.asianjtm.com/qikan/manage/wenzhang/AJTM07-2(4)-4.pdf

Zhong, Z., Zhou, G., Chen, X., Huang, P.(2001). Pharmacological study on the extracts from *T. flagelliforme* Blume. Zhongyaocai 24 (10), 735-738.