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Flavonoid from the Sao Pedro Petro of tubers of cassava (*Manihot esculenta* Crantz)

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Abstract

*The flavonoid compound, quarcetin-3-O-rutinoside (**1**) has been isolated from n-BuOH extract of the fresh of Sao Pedro Petro of Tubers of Cassava (Manihot esculenta Crantz). The chemical structure of compound (**1**) was identified by spectroscopic data including UV, IR, NMR (^1H, ^{13}C, DEPT 135°, HMQC, HMBC, ^1H-^1H COSY) and MS and by comparing with previously reported spectral data.*

Keywords: Cassava, *Manihot esculenta* Crantz, Sao Pedro Petro, flavonoid, quarcetin-3-*O*-rutinoside.

Introduction

Cassava (*Manihot esculenta* Crantz; Euphorbiaceae) is the only species in its genus as a food crop in South America where it originated. Cassava is the third largest sources of carbohydrates for human food in the world produced for industrial purpose in Brazil[1], In the late 18th century, some of locations for the early arrival of cassava were in India, Java and the Philippines[2]. The *Manihot esculenta* Crantz plant is rich in various macro and micronutrients[3,4]. It also contains various antioxidant like α-carotene[5].

Additionally, plant also contains vitamin C, vitamin A, anthocyanins (flavonoids), saponins, steroids and glycosides. Popularly known as cassava is also one of these plants with various medicinal properties is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy and tuberous root which is a major source of carbohydrates.[6]

Subramanian et al[7] identified flavonoids (quercetin 3-*O*-rhamnosylglucoside) from leaves of Cassava *M. esculenta* Pohl (= *M.esculenta* Crantz). Flavonoid is found in many plants widely distributed in edible fruits and vegetables. Quercetin is a potent anticancer agent in human. Recently quercetin was found to inhibit the cell growth of leukemia cells[8].

The Sao Pedro Petro of Tubers of *Cassava (Manihot esculenta* Crantz*)* from Cisarua, Bogor Indonesia is a cassava variety locally used as sample in this research. The cytotoxic effect is inhibiting growth of P-388 murine leukemia cancer cells with inhibition concentration (IC_{50}) value of 50.24µg/mL in butanolic extract[9].

Material and Methods

General Experimental Procedure: UV spectra were measured by using a TECAN Infinite M200 pro with MeOH. The IR spectra were recorded on a Shimadzu IR Prestige-21 in KBr. The mass spectra were recorded with a Waters Xevo QTOF MS. NMR data were recorded on a JEOL spectrometer at 500 MHz for ^1H and 125 MHz for ^{13}C using TMS as internal standard. Column chromatography was conducted on silica gel 60. TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in EtOH followed by heating.

Plant Material: The Cassava (*Manihot esculenta* Crantz) was planted in Cisarua Village, Bogor Discrict, West Java Province, Indonesia in April 2018. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia.

Plant Extraction: Fresh cassava root cortex (5 Kg) of Cassava (*Manihot esculenta* Crantz) was extracted with EtOH exhaustively (49 L) at room temperature for 7 days. After removal of the solvent under vacuum, the viscous concentrate of EtOH extract (340.01 g) was first suspended in H_2O and then partitioned with *n*-hexane, EtOAc and *n*-butanol, successively. Evaporation resulted in the crude extracts of *n*-hexane (10.90 g), EtOAc (25.18 g) and *n*-BuOH (228.63 g) respectively. The *n*-BuOH soluble fraction (228.63 g) was fractionated by column chromatography on silica gel using a gradient *n*-hexane, EtOAc and MeOH to give fractions A–H combined according to TLC results.

Fraction B (1.73 g) was subjected to column chromatography over silica gel using a gradient mixture of CH_2Cl_2-EtOAc (10:0-1:1) as eluting solvents to afford 6 subfractions (B1-B6). Subfraction B3 (460 mg) was chromatographed on a column of silica gel, eluted with CH_2Cl_2:EtOAc (7:3) to give five subfractions (B3A–B3E). Subfraction B3B was chromatographed on a column of silica gel eluted with CH_2Cl_2:EtOAc:MeOH (7:2:1), to give (**1**) (6.5 mg). Compound (**1**) is yellow solid which crystallized from MeOH as yellow granules, m.p. 192-94 °C, $[\alpha]^{25}_D$ + 5.8^o (*c* 0.27, EtOH). IR, UV, NMR and MS data were consistent with structure.

Results and Discussion

Fresh cassava root cortex of *Manihot esculenta* Crantz were ground and successively extracted with EtOH and partition

with *n*-hexane, EtOAc and *n*-BuOH. The *n*-BuOH extract was chromatograph over a column chromatographed packed with silica gel 60 by gradient elution. The fractions were repeatedly subjected to normal-phase column chromatography to afford flavonoid glycosides compounds (**1**) (Figure 1).

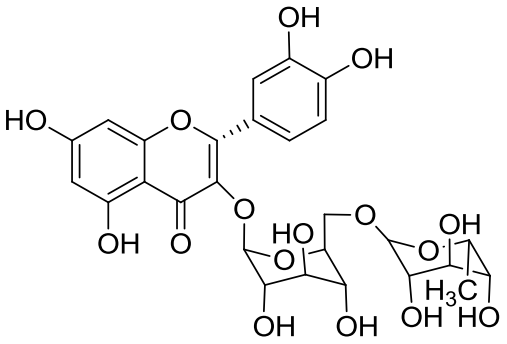

Figure 1: Structure of compound 1

Table 1
Date NMR of Compounds (1) in MeOH.

Position	^{13}C NMR δc (mult.)	^{1}H NMR δ_H (Int., mult, *J*=Hz)
2	156.4 (s)	-
3	135.1 (s)	-
4	178.2 (s)	-
5	161.8 (s)	-
6	98.3 (d)	5.94 (1H, d, 2.1)
7	166.4 (s)	-
8	94.0 (d)	6.25 (1H, d, 2.1)
9	158.8(s)	-
10	104.5 (s)	-
1′	122.8 (s)	-
2′	115.3 (d)	6.72 (1H, d, 1.7)
3′	145.9 (s)	-
4′	146.5 (s)	-
5′	117.2 (d)	6.93 (1H, d, 8.6)
6′	121.8 (d)	7.15 (1H, dd, 1.7, 8.6)
Glucose		
1″	109.6 (d)	5.68 (1H, dd, 1.8, 7.2)
2″	75.1 (d)	3.71 (1H, dd, 7.2, 8.1)
3″	76.6 (d)	3.49 (1H, dd, 1.8, 8,1)
4″	71.8 (d)	3.40 (1H, dd, 1.8, 3)
5″	80.8 (d)	4.00 (1H, dd, 1.9, 3)
6″	68.6 (t)	3.63 (1H, ddd, 3; 4.9, 11.8) 3.38(1H, ddd, 3, 4.9, 11.8)
Rhamnose		
1‴	112.0 (d)	5.03 (1H, dd, 1.8, 7.2)
2‴	73.8 (d)	3.73 (1H, dd, 7.2, 8.1)
3‴	72.4 (d)	3.49 (1H, dd, 1.8, 8,1)
4‴	73.7 (d)	3.40 (1H, dd, 1.8, 3)
5‴	74.2 (d)	3.85 (1H, dd, 1.9, 3)
6‴	17.0 (q)	1.18 (3H, d, 3)

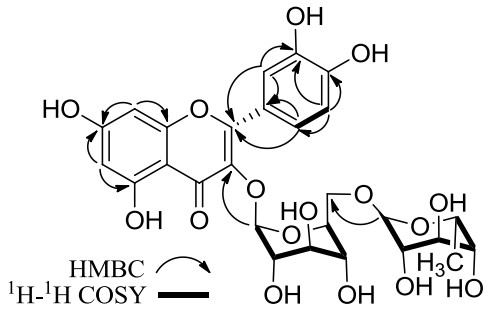

Figure 2: Selected HMBC and ^{1}H-^{1}H COSY correlations for 1

Compound (**1**) was obtained as a yellow solid. HR-TOFMS spectrum of **1** showed a $[M+H]^+$ ion at *m/z* 611.1578 (calcd *m/z* 611.1612), which corresponds to a molecular formula $C_{27}H_{30}O_{16}$ and thus required thirteen degrees of unsaturation, originating from fourteen C sp^2, one carbonyl, the remaining tricyclic flavonoid and two substituted glucose rings. The UV spectrum in MeOH showed λ_{max} at 275 nm (log ε 3.93) indicating the presence of benzene chromophore. IR spectra showed absorption peaks at 3327, 1570, 1156, 1051 and 827 cm^{-1} suggesting the presence of hydroxyl groups, C=C olefin rings, symmetric and asymmetric C-O-C and substituted benzene ring respectively.

The ^{13}C NMR spectrum of **1** (Table 1) exhibited 27 signals divided into 15 C-sp^2 and 12 C-sp^3 signals, suggesting that the compound is a glycoside derivative of flavonoid. The presence of carbon signals at δ_C 178.2 (conjugated C=O) and 135.1 (=C-O) is indicating a flavonol structure (DBE = 11). The 12 C-sp^3 signals consisted of 11 oxygenated C-sp^3 and methyl signals and thus the compound is a flavonol glycoside containing a glucoside and a rhamnoside moiety.

From HMQC spectrum the anomeric proton and carbon signals of the glucoside and rhamnoside moieties were at δ_H 5.68/δ_C 109.6 and δ_H 5.03/δ_C 112.0 respectively.

In the ^{1}H NMR spectrum of **1** (Table 1), a pair of *meta*-coupled (J = 2.1 Hz) aromatic signals (δ_H 6.25 and 5.94) and ABC signals (δ_H 7.15, 6.93 and 6.72) were observed indicating that the flavonol has a structure of quercetin. The presence of HMBC correlations from proton to carbon signals of δ_H 5.03 → δ_C 109.6 and δ_H 5.68 → δ_C 135.1 indicated that the glycoside is a rutinoside and is attached to C-3. Thus, compound **1** was determined as quercetin 3-*O*-rutinoside.

Comparison of the NMR data of 1 with those reported for quercetin 3-*O*-rutinoside[7] showed close agreement in their NMR parameters[10].

The ^{1}H-NMR spectrum of compound **1** showed signals of a *meta*-couple and the presence of five olefinic methine groups, resonating at δ_H 5.94 and 6.25 (each 1H, d, J = 2.1 Hz, H-6, H-8), 6.72 (1H, d, J = 1.7 Hz, H-2′), 6.93 (1H, d, J = 8.6 Hz, H-5′) and 7.15 (1H, dd, J = 1.7, 8.6 Hz, H-6′), two

glucose or rutinoside at δ_H 5.68 (1H, dd, *J*=1.8, 7.2 Hz, H-1″), 3.71 (1H, dd, *J*=7.2, 8.1 Hz, H-2″), 3.49 (1H, dd, *J*=1.8, 8,1 Hz, H-3″), 3.40 (1H, dd, *J*=1.8, 3.0 Hz, H-4″), 4.00 (1H, dd, *J*=1.9, 3.0 Hz, H-5″), one oxymetylene at δ_H 3.63 (1H, ddd, *J*=3.0, 4.9, 11.8 Hz, H-6″), 3.38 (1H, ddd, *J*=3.0, 4.9, 11.8 Hz, H-6″) and δ_H 5.03 (1H, dd, *J*=1.8, 7.2 Hz, H-1‴), 3.73 (1H, dd, *J*=7.2, 8.1 Hz, H-2‴), 3.49 (1H, dd, *J*=1.8, 8,1 Hz, H-3‴), 3.40 (1H, dd, *J*=1.8, 3.0 Hz, H-4‴), 3.85 (1H, dd, *J*=1.9, 3.0 Hz, H-5‴), one metil at δ_H 1.18 (3H, d, *J*=3.0 Hz, H-6‴). Two *meta*-protons at ring (a) were evidenced by *J* constant coupling of H-6 and H-8 (2.1 Hz) and HMBC correlations between H-6 to C-5, C-7 and H-8 to C-7, C-9 (Figure 2).

Benzene tri-substitution in ring (b) was observed at δ_H 6.72 (1H, d, *J* = 1.7 Hz, H-2′), 6.93 (1H, d, *J* = 8.6 Hz, H-5′) and 7.15 (1H, dd, *J* = 1.7, 8.6 Hz, H-6′) and ^{1}H-^{1}H COSY cross peak H-5′/H-6′ (Figure 2). The ^{13}C NMR of compound (**1**) (MeOH 125 MHz) and DEPT 135^0 spectra showed the presence of five olefinic methines and nine quaternary olefinic carbon (fourteen sp^2 carbons), one carbonyl and rutinoside. These functionalities accounted for seven and one carbonyl of the total thirteen degrees of unsaturation, two degrees of unsaturation from rutinoside and the remaining three degrees of unsaturation were consistent with the flavone structure. Cytotoxic evaluation of **1** against P-388 cells showed its IC_{50} was 50.24 ug/mL[9].

Conclusion

A known flavonoid, quarcetin-3-*O*-rutinoside (**1)** was isolated from *n*-BuOH extract of the fresh of the Sao Pedro Petro tubers of Cassava. *Manihot esculenta* Crantz.

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