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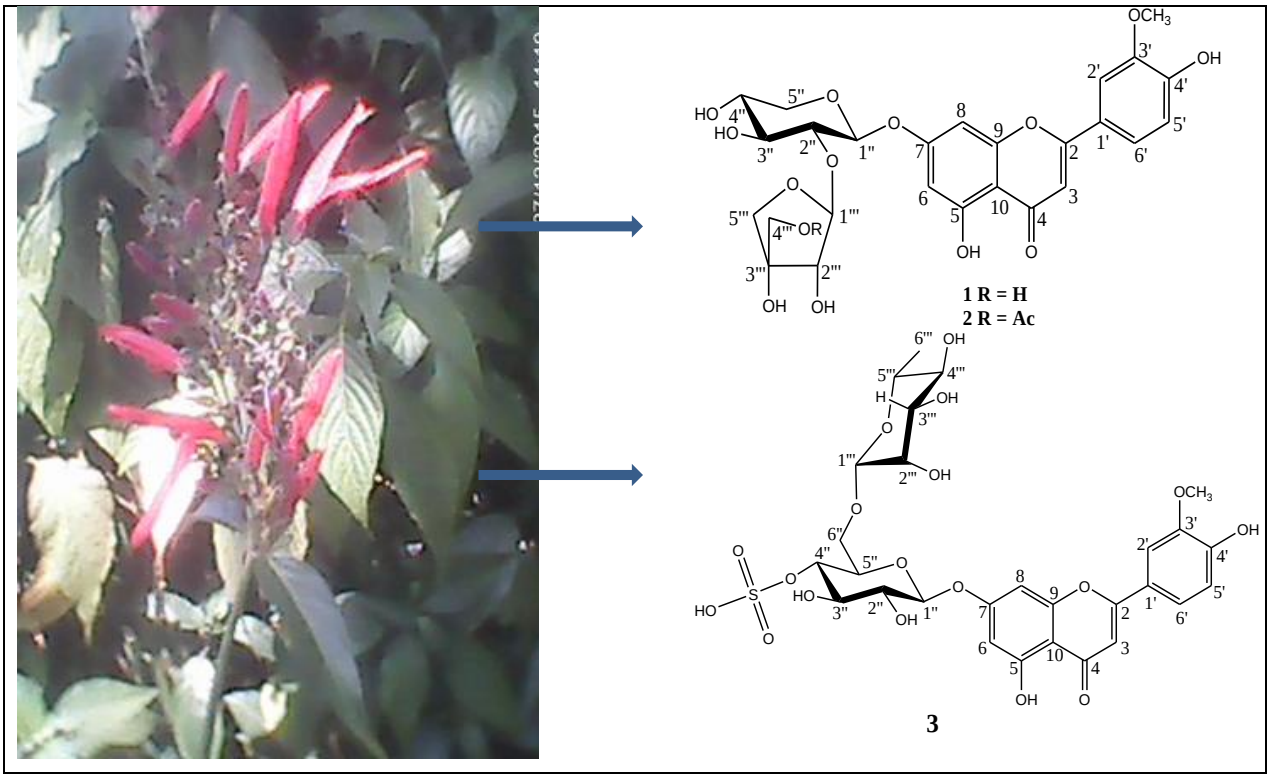
***Graphical Abstract**



Highlights

- Granduloside A-C, three new flavonoids glycosides were isolated from the aerial parts of *Graptophyllum grandulosum* Turill.
- The structure was characterized by extensive 2D-NMR studies
- Four known compounds were also isolated from this plant

Three new flavonoid glycosides from the aerial parts of *Graptophyllum grandulosum* Turril (Acanthaceae)

Cyrille NGOUFACK TAGOUSOP [a], David NGNOKAM[a*], Dominique HARAKAT[b] and Laurence VOUTQUENNE-NAZABADIOKO[c]

[a]Faculty of Science, Department of Chemistry, University of Dschang, P.O. Box 67. Dschang, Cameroon

[b]Service Commun d'Analyses, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 7312, Bat. 18 B.P. 1039, 51687 Reims Cedex 2, France

[c]Groupe Isolement et Structure, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 7312, Bat. 18 B.P. 1039, 51687 Reims Cedex 2, France

Corresponding authors:

* Professor David Ngnokam, Laboratory of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, PO. Box 67, Dschang, Cameroon, Tel: +237 696 710 992. E-mail: dngnokam@yahoo.fr/ngnokam@univ-dschang.org

Abstract

Grandulosides A-C, three new flavonoid glycosides, were isolated from the aerial parts of *Graptophyllum grandulosum* Turill and identified as chrysoeriol-7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-xylopyranoside **(1),** chrysoeriol-7-*O*-[4’’’-*O*-acetyl-*β*-D-apiofuranosyl-(1→2)]-*β*-D-xylopyranoside **(2)** and 7-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-(4’’-hydrogeno sulfate) glucopyranoside **(3).** Four known compounds, chrysoeriol-7-*O*-*β*-D-xyloside **(4)**, isorhamnetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside **(5)**, luteolin-7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-xylopyranoside **(6)** and sucrose **(7)** were also obtained. The structures of these compounds were established by interpretation of their spectral data, mainly HR-TOFESIMS, 1D-NMR (^{1}H, ^{13}C) and 2D-NMR (COSY, NOESY, HSQC and HMBC) and by comparison with the literature data.

Keywords: Acanthaceae, *Graptophyllum grandulosum*, flavonoid glycosides, granduloside.

1. Introduction

The genus *Graptophyllum* is a member of the Acanthaceae family and occurs mainly in tropical and some temperate environments. Globally, there are about 15 species of *Graptophyllum*, comprising shrubs or small trees that predominantly grow in the Pacific region and West and Central Africa (Barker 1986). Many species of this genus, such as *Graptophyllum pictum*, are used as medicinal plants in folk medicine (Perry, 1980; Kasahara and Mangunkawatjia, 1986). Previous studies of this genus revealed the presence of alkaloids, triterpenes, coumarins and flavonoids (Corrêa and Alcântara, 2012). In the course of our continuing search for secondary metabolites of biological importance from medicinal plants, we investigated the *n*-BuOH extract of the aerial parts of *Graptophyllum grandulosum* and isolated three new flavonoid glycosides whose structures are discussed in the present report.

2. Results and discussion

Purification of the *n*-BuOH soluble fraction of the crude MeOH extract afforded three new compounds, grandulosides A, B and C (**1-3**), and four known compounds (**4-7**). The known compounds were identified as chrysoeriol-7-*O*-*β*-D-xyloside (**4**) (Markham and Chari,1982 ; Markham *et al*., 1978), isorhamnetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside **(5)** (Mona-antonia and Hanns, 1999)**,** luteolin-7-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-xylopyranoside (**6**) (Koffi *et al*., 2013) and sucrose **(7) (**Hee-Jeong *et al*., 2014) by comparison of their spectroscopic data with those reported in the literature.

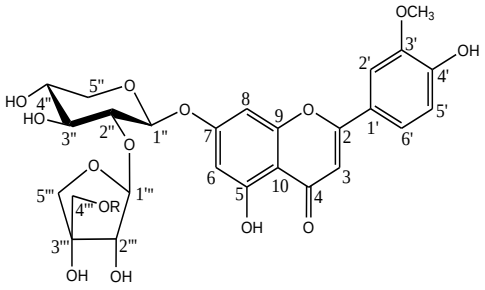
Compound **1** was obtained as a yellow powder that reacted positively with Shinoda and Molisch reagents, thus revealing the presence of a glycosylated flavonoid. Its molecular formula $C_{26}H_{28}O_{14}$ was determined on the basis of a pseudo-molecular ion peak at *m/z* 587.1370 $[M+Na]^+$ (calcd. for $C_{26}H_{28}O_{14}Na$ 587.1377) in its HR-TOFESIMS spectrum. The ^{1}H-NMR (Table 1) spectrum of **1**, showed signals at $\delta_{H:}$ 6.70 (H-3, s), 6.45 (H-6, d, J = 2.2 Hz), 6.77 (H-8, d, J = 2.2 Hz), 7.52 (H-2', d, J = 2.1 Hz), 6.95 (H-5', d, J = 8.2 Hz) and 7.56 (H-6', dd, J = 8.2, 2.1 Hz). These protons showed correlations in the HSQC spectrum with the carbons at $\delta_{C:}$ 104.5 (C-3), 100.9 (C-6), 95.9 (C-8), 110.4 (C-2'), 116.7 (C-5') and 121.9 (C-6'), respectively, indicating a 3',4',5,7-tetrasubstituted flavone skeleton. The HMBC correlation of a methyl signal at δ_H 3.98 (s) with the carbon signal at δ_C 149.5 indicated the presence of a methoxyl group at C-3'. Thus the aglycone is 5,7,4'-trihydroxy-3'-methoxyflavone (chrysoeriol) (Facundo *et al*., 2012). The ^{1}H-NMR spectrum also exhibited the anomeric protons of two sugar units at δ_H 5.16 (H-1'', d, J = 7.2 Hz) and δ_H 5.46 (H-1''', d, J = 1.3 Hz). Correlations in the HSQC spectrum readily identified the corresponding

anomeric carbons at δ_C 100.6 (C-1'') and δ_C 110.0 (C-1'''), respectively. Complete assignment of the protons and carbons of the sugar units was achieved by analysis of COSY, ^{1}H-^{1}H, NOESY, HSQC and HMBC spectra. The sugars were identified as a xylopyranose (from δ_H 5.16) and an apiofuranose (from δ_H 5.46) characterized by its quaternary carbon C-3'''. Acid hydrolysis of **1** afforded xylose and apiose which were detected by TLC. The D-configuration of these sugars was confirmed by GC-MS after derivatization. Their anomeric configuration was determined to be β from the $^3J_{H1-H2}$ values of the anomeric proton signals and the chemical shifts of the anomeric carbons (Agrawal, 1992). The HMBC spectrum showed a correlation between xylose H-1 and C-7 of the aglycone, confirming its direct attachment to the aglycone. The deshielded nature of C-2'' (δ_C 78.6) suggested a substitution at this position. This was readily confirmed by the HMBC correlation from apiose H-1 to carbon C-2'' (δ_C 78.6) of xylose. The NMR spectral data of compound **1**, without the signal of the methoxyl group are in good agreement with those reported (Koffi *et al*., 2013). Thus the structure of compound **1,** granduloside A, is chrysoeriol-7-*O*-β-D-apiofuranosyl-(1→2)-β-D-xylopyranoside.

Compound **2** was obtained as a yellow amorphous powder that reacted positively with Shinoda and Molisch reagents. Its molecular formula $C_{28}H_{30}O_{15}$ was determined on the basis of a pseudo-molecular ion peak at *m/z* 629.1471 $[M+Na]^+$ (calcd. for $C_{28}H_{30}O_{15}Na$ 629.1482) in its positive HR-TOFESIMS spectrum. The ^{1}H-NMR and ^{13}C-NMR spectra of **2** (Table 1) were very similar to those of compound **1**, except for the presence of an acetate group [δ_C 172.5 (CO), 20.5 (CH_3)] in the ^{13}C-NMR spectrum. The deshielding of C-4''' (δ_C 68.3) of apiose and the HMBC correlations between protons 2H-4''' (δ_H 4.12, 4.07) and the ester carbonyl carbon at δ_C 172.5 confirmed the presence of an apiose C-4''' acetate. Acetylation at this position has already been reported in acacetin-7-*O*-[4'''-*O*-acetyl-β-D-apiofuranosyl-(1→3)]-β-D-xylopyranoside by Zhang *et al*. 2014. Thus compound **2**, granduloside B. is chrysoeriol-7-*O*-[4'''-*O*-acetyl-β-D-apiofuranosyl-(1→2)]-β-D-xylopyranoside.

Compound **3** was obtained as a yellow amorphous powder that reacted positively with Shinoda and Molisch reagents. Its molecular formula $C_{28}H_{31}O_{18}S$ was determined on the basis of a pseudo-molecular ion peak at *m/z* 687.1226 $[M-H]^-$ (calcd. for $C_{28}H_{31}O_{18}S$ 687.1231) in its negative HR-TOFESIMS spectrum. The ^{1}H-NMR and ^{13}C-NMR spectra of **3** (Table 1), revealed the presence of a 5,7,4'-trihydroxy-3'-methoxyflavone (chrysoeriol) (Facundo *et a*l., 2012) as in compound **1**. In addition, its ^{1}H-NMR spectrum showed the anomeric protons of two sugar units at δ_H 5.15 (H-1'', d, J = 7.7 Hz) and δ_H 4.74 (H-1''', *d, J*

= 1.3 Hz) which had HSQC correlations with the corresponding anomeric carbons at δ_C 104.0 (C-1'') and 102.6 (C-1'''), respectively. The complete assignment of the protons and carbons of a glucopyranose (from δ_H 5.15) and a rhamnopyranose (from δ_H 4.75), with its methyl signal at δ_H 1.21, was achieved as for compound **1**. The sugar composition was confirmed by TLC, after acid hydrolysis, to be glucose and rhamnose. Their respective D and L absolute configurations were established by GC-MS after derivatization. The anomeric proton configurations of glucose and rhamnose were assigned as β and α, respectively from the $^3J_{H1\text{-}H2}$ values of the anomeric protons and the chemical shifts of the anomeric carbons (Agrawal, 1992). An HMBC correlation between H-1'' of glucose and C-7 of the aglycone confirmed the direct attachment of glucose to the aglycone. The correlation between H-1''' of rhamnose and C-6'' of glucose established the connectivity of the two sugar units. The deshielding of C-4'' (δ_C 77.5) indicated that it was linked to an electron withdrawing group, identified as a hydrogen sulfate (HSO_4-) group by mass spectroscopy. Thus compound **3**, granduloside C, is chrysoeriol-7-*O*-α-L-rhamnopyranosyl (1→6)-β-D-(4''-hydrogeno sulfate) glucopyranoside.



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2 R = Ac

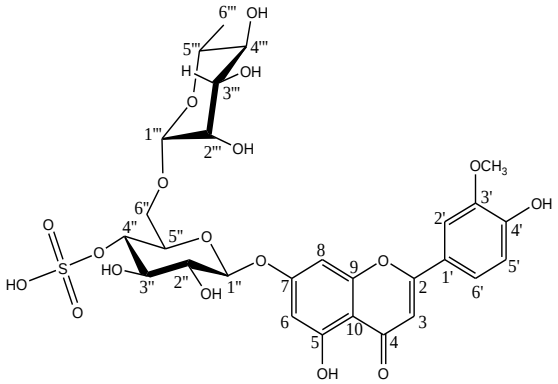

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Fig.1. Structures of compounds **1**-**3** isolated from the *n*-BuOH soluble extract of *Graptophyllum grandulosum*

Table 1: ^{1}H-NMR (600MHz) and ^{13}C-NMR (150MHz) data of compounds **1-3** in CD_3OD

N^{O}	**1**		**2**		**3**	
	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
	Aglycon		Aglycon		Aglycon	
1	-	-	-	-	-	-
2	166.6	-	166.8	-	166.8	-
3	104.5	6.70 (1H, s)	104.6	6.73 (1H, s)	104.5	6.73 (1H, s)
4	184.0	-	184.1	-	184.1	-
5	162.9	-	163.0	-	163.1	-
6	100.9	6.45 (1H, d, 2.1)	100.9	6.44 (1H, d, 2.0)	101.1	6.56 (1H, d, 2.1)
7	164.4	-	164.4	-	164.0	-
8	95.9	6.77 (1H, d, 2.1)	95.7	6.78 (1H, d, 2.0)	96.2	6.84 (1H, d, 2.1)
9	158.9	-	159.0	-	159.0	-
10	107.0	-	107.1	-	107.2	-
1’	123.4	-	123.5	-	123.6	-
2’	110.4	7.52 (1H, d, 2.1)	110.8	7.56 (1H, d, 2.0)	110.8	7.55 (1H, d, 2.1)
3’	149.5	-	149.6	-	149.6	-
4’	152.3	-	152.3	-	152.3	-
5’	116.7	6.95 (1H, d, 8.4)	116.8	6.97 (1H, d, 8.4)	116.9	6.98 (1H, d, 8.4)
6’	121.9	7.56 (1H, dd, 8.4, 2.1)	122.0	7.59 (1H, dd, 8.4, 2.0)	122.0	7.59 (1H, d, 8.4, 2.1)
OCH_3	56.6	3.98 (3H, s)	56.7	4.00 (3H, s)	56.7	3.99 (3H, s)
	Xyl		Xyl		Glc	
1’’	100.6	5.16 (1H, d, 7.1)	100.5	5.17 (1H, d, 7.3)	100.9	5.15 (1H, d, 7.8)
2’’	78.6	3.68 (1H, dd, 9.0, 7.1)	77.8	3.71 (1H, dd, 9.1, 7.3)	74.5	3.61 (1H, dd, 9.1, 7.8)
3’’	77.9	3.63 (1H, m)	78.2	3.61 (1H, m)	76.8	3.84 (1H, t, 9.1)
4’’	70.9	3.62 (1H, m)	71.0	3.60 (1H, m)	77.5	4.32 (1H, dd, 9.9, 9.1)
5’’	66.9	3.97 (1H, m) 3.48 (1H, t, 9.6)	67.0	3.97 (1H, dd, 10.9, 4.3) 3.48 (1H, t, 10.9)	75.3	3.89 (1H, m)
6’’					67.1	4.10 (1H, m) 3.68 (1H, m)
	Api		Api		Rha	
1’’’	110.0	5.46 (1H, d, 1.7)	110.3	5.49 (1H, d, sl)	102.4	4.75 (1H, d, 1.3)
2’’’	78.1	3.98 (1H, m)	78.5	3.87 (1H, d, 3.6)	71.9	3.95 (1H, dd, 3.4, 1.3)
3’’’	80.7	-	79.0	-	72.3	3.72 (1H, dd, 9.5, 3.4)
4’’’	65.8	3.56 (2H, sl)	68.3	4.12 (1H, d, 11.3) 4.07 (1H, d, 11.3)	74.2	3.32 (1H, t, 9.5)
5’’’	75.4	4.05 (1H, d, 9.5) 3.84 (1H, d, 9.5)	75.4	4.14 (1H, d, 9.6) 3.86 (1H, d, 9.6)	69.8	3.42 (1H, m)
6’’’					17.9	1.21 (3H, d, 6.2)
Ac			172.5 20.5	- 1.93 (3H, s)		

3. Experimental

3.1. General experimental procedures

Melting points were recorded with a Schorpp Gerätetechnik apparatus. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoprobe (^{1}H at 600 MHz and ^{13}C at 150 MHz). 2D NMR experiments were recorded by means of standard Bruker microprograms (Xwin-NMR version 2.1 software TopSpin 3). Chemical shifts (δ) are reported in parts per million (ppm) using the residual solvent signals

as secondary reference relatively to TMS (δ = 0), while the coupling constants (*J* values) are given in Hertz (Hz). TOF-ESIMS and HR-TOFESIMS spectra were recorded using a Micromass Q-TOF micro instrument (Manchester, UK) equipped with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 μL min-1. Column chromatography was run on Merck silica gel 60 (70-230 mesh) and gel permeation on Sephadex LH-20 while TLC was carried out on silica gel GF254 pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 ^oC, or by visual inspection under UV lamp at 254 and 365 nm.

3.2. Plant material

The aerial parts of *G. grandulosum* were collected at FOTO Village (Menoua Division, Western region of Cameroon) in November 2015. Authentication was done by Mr. Fulbert Tadjouteu, a Botanist of the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N^o 65631/HNC) has been deposited.

3.3. Extraction and isolation.

The air-dried plant material (4 kg) was powdered and extracted at room temperature with methanol (3 x 20 L, 72 h). The solvent was evaporated under reduced pressure, leaving an extract (240 g). Part of this extract (235 g) was suspended in water (300 mL) and successively extracted with equal volumes (500 mL) of ethyl acetate (EtOAc) and *n*-BuOH yielding respectively 37 g and 13 g fractions after evaporation to dryness. A part of the *n*-BuOH fraction (10 g) was subjected to silica gel column chromatography gradient using EtOAc-MeOH (100:0 →0:100) graduated elution. 33 fractions of 150 mL each were collected and combined on the basis of their TLC profiles to give 6 fractions: A (1-2), B (4-9), C (10-18), E (19-24) and F (25-33). Fraction B (1.5 g) was purified on sephadex LH-20 column eluted with methanol to give compound **4** (15.3 mg). Fraction C (2.1 g) and D (3.2 g) were respectively purified on sephadex LH-20. Fractions of 10 mL were collected and combined on the basis of TLC profiles to give 4 sub-fractions C_1, C_2, D_1 and D_2. Sub-fractions C_2 (1.2 g) and D_2 (500 mg) were combined and subjected to silica gel column chromatography using EtOAc-MeOH (95:5) as eluent to give compounds **1** (45.5 mg), **2** (25.2 mg) and **6** (35.2 mg). Fraction E (1.3 g) yielded compounds **3** (35 mg) and **5** (20.5 mg) after multiple chromatographic steps over silica gel using EtOAc-MeOH-H_2O (9-1-0.5) and sephadex LH-20 using MeOH, while compound **7** (40.1 mg) was obtained after purification of fraction F (0,9 g) by gel column chromatography on silica gel eluted with EtOAc-MeOH-H_2O (8-2-1).

3.4. New compounds

Granduloside A: yellow powder; m.p. = 181.8 °C; ^{1}H and ^{13}C-NMR data, see Tables 1; $[\alpha]_D$ -3.4 (MeOH, *c* 0.29); IR (KBr) ν_{max} (cm^{-1}) 3350-3300, 1070, 1040 (OH), 1643 (C=O), 1605, 1580, 1520 (C=C aromatic), 1650 (C-O); HR-TOFESIMS (positive ion mode) *m/z*: 587.1370 $[M+Na]^+$ (calcd. for $C_{26}H_{28}O_{14}Na$ 587.1377).

Granduloside B: yellow amorphous powder; ^{1}H and ^{13}C-NMR data, see Tables 1; $[\alpha]_D$ -30.1 (MeOH, *c* 0.08); IR (KBr) ν_{max} (cm^{-1}) 3350-3300, 1070, 1045 (OH), 1648 (C=O), 1610, 1580, 1525 (C=C aromatic), 1648 (C-O); HR-TOFESIMS (positive ion mode) *m/z*: 629.1471 $[M+Na]^+$ (calcd. for $C_{28}H_{30}O_{15}Na$ 629.1482).

Granduloside C: yellow amorphous powder; ^{1}H and ^{13}C-NMR data, see Tables 1; $[\alpha]_D$ -37.7 (MeOH, *c* 0.58); IR (KBr) ν_{max} (cm^{-1}) 3350-3300, 1070, 1040 (OH), 1643 (C=O), 1605, 1580, 1520 (C=C aromatic), 1650 (C-O); HR-TOFESIMS (negative ion mode) m/z: 687.1226 $[M-H]^-$ (calcd. for $C_{28}H_{31}O_{18}S$ 687.1231).

*3.5. Acid hydrolysis and determination of absolute configuration of monosaccharide*s

Compounds **1**-**3** (15 mg) were respectively dissolved in MeOH-2N HCl (1:4) (10 mL) and refluxed at 80 °C for 3 h. After removal of MeOH under reduced pressure, the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). The combined CH_2Cl_2 extracts were washed with H_2O and evaporated to dryness to afford the aglycones (3.5 and 4 mg respectively). The aqueous layer was neutralized with dilute NaOH and concentrated to dryness. The resultant residue was dissolved in pyridine (1 mL), then $(CH_3)_3SiNHSi(CH_3)_3$ (1 mL) was added. After 10 min at room temperature, the solution was blown to dryness under a stream of nitrogen. The residue was dissolved in diethyl ether and then subjected to chiral GC-MS analysis. The monosaccharides in compounds **1** and **2** were found to be D-xylose and D-Apiose, while those of compound **3** were identified as D-glucose and L-rhamnose by comparison of their retention times with literature values (Hara *et al.*, 1987).

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References

Agrawal, P. K., 1992. NMR spectroscopy in structural elucidation of oligosaccharides and glycosides. *Phytochemistry* **31**, 3307-3330.

Barker, R.M., 1986. *Graptophyllum Nees*. *Journal of the Adelaide Botanic Gardens* **9**,156-166.

Corrêa, G. M. Alcântara A. F. C., 2012. Chemical constituents and biological activities of species of *Justicia* - a review. *Brazilian Journal of Pharmacognosy* **22**, 220-238.

Facundo, V. A., Azevedo, M. S., Rodrigues, R. V., Do Nascimento, L. F., Militão, J. S. L. T., Da Silva, G. V. J., Braz-Filho, R., 2012. Chemical constituents from three medicinal plants: *Piper renitens, Siparuna guianensis* and *Alternanthera brasiliana. Brazilian Journal of Pharmacognosy* **22**, 1134-1139.

Hara, S., Okabe, H., Mihashi, K., 1987. Gas-liquid chromatographic separation of aldose enantiomers as trimethylsilyl ethers of methyl 2 (polyhydroxyalkyl) thiazolidine-4(*R*)-carboxylates. *Chemical and Pharmaceutical Bulletin* **35**, 501-506.

Hee-Jeong, M., Tae-Seong, L., Young-Soo, B., 2014. Structure Determination of Sucrose by Acetylation and Acid Hydrolysis. *Journal of the Korean Wood Science and Technology* **42** 183-192.

Hiroshi, N., Naoyuki, K., Mitsuru, Y., Hiroshi, O., Rika, I., Takuji, T., 2011. Isolation and Identification of Flavonoids Accumulated in Proanthocyanidin-free Barley. *Journal of Agricultural and Food Chemistry* **59**, 9581-9587

Kasahara, Y. S., Mangunkawatjia, S., 1986. Medicinal Herb index in Indonesia, Eisai Indonesia, p. 318.

Koffi, E. N., Le Guernevéc, C., Lozanoa, P. R., Meudecc, Adjéd, F. A., Bekrob,Y. A., Lozanoa, Y. F., 2013. Polyphenol extraction and characterization of *Justicia secunda* Vahl. leaves for traditional medicinal uses. *Industrial Crops and Products* **49**, 682-689.

Markham, K. R., Chari, V. M., 1982. Carbon-13 NMR spectroscopy of flavonoids. In: The Flavonoids: Advances in Research (Harborne J.B. and Mabry T.J., eds.). Chapman and Hall, London.

Markham, K. R., Ternai, B., Stanley, R., Geiger, H., Mabry, T. J., 1978. Carbon-13 NMR studies of flavonoids. III. Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron* **34**, 1389-1397.

Mona-Antonia, B., Hanns H., 1999. Flavonol glycosides from *Eschscholtzia californica*. *Phytochemistry* **50**, 329-332.

Perry, L. M., 1980. Medicinal plants of East and South-East Asia; Attributed Properties and Uses MIT press, Cambridge, United States and London, p. 2.

Zhang, X.L., Guo, Y.S., Wang, C.H., Li, G.Q., Xu, J.J., Chung, H.Y., Ye, W.C., Li. Y.L., Wang, G.C., 2014. Phenolic compounds from *Origanum vulgare* and their antioxidant and antiviral activities. *Food Chemistry***. 152**, 300-306.