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Antiproliferative effect of phenolic glucosides isolated from *Philodendron bipinnatifidum* on HepG2 cells

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Abstract

Cytotoxic activity of methanol extract of the leaves of *Philodendron bipinnatifidum* and its three phenolic compounds was determined using the SRB Assay on human tumor cell line (Hepatocyte generation 2, HepG2). Chromatographic fractionation of the methanol extract led to isolation of three new phenolic compounds named *p*-hydroxyphenyl ethyl *β*-D-glucopyranosyl (1→2),*β*-D-glucopyranosyl (1→3),4-*O-trans*caffeoyl-*O*-*α*-L-rhamnopyranoside (**1**), 2-phenyl ethyl 4-*O-trans*-caffeoyl-*O*-*α*-L-rhamnopyranoside (**2**) and 2,3dimethoxy-phenylethyl-4-*O-trans*caffeoyl-*β*-D-glucopyranosyl (**3**). The structure of these compounds was established on the basis of NMR and mass spectral data. This preliminary study indicates promising potential of *P. bipinnatifidum* as a potential source of new anticancer compounds.

Keywords: *Philodendron bipinnatifidum;* phenolic, glucosides; HepG2

Introduction

Araceae is a family of monocotyledonous flowering plants in which flowers are borne on a type of inflorescence called a spadix. This family contains of 110 genera and over 2000 species. Saponins, flavonoids, polyphenols, cyanogenic compounds and alkaloids are secondary metabolites characteristic of this family (Williams et al 1981; Pangi, 1982; Kite et al 1997). *Philodendron* is a large genus of flowering plants in the Araceae family; it consists of close to 900 species. *Philodendron* species can be found in many diverse habitats in the tropical Americas and west India. *Philodendron bipinnatifidum* is widely cultivated in Egypt gardens as ornamental plants. There is little information on its chemical constituents in literature; β–sitosterol, polyprenoid hexaprenol and 6-β–hydroxy stigmast-4-en-3-one were isolated from *Philodendron imbe* (Feitosa and Bezerra 2007). Plant–derived phenolic compounds have received considerable attention in treatment of many health problems as antioxidant and anticancer agents (Cai et al 2004).

In this study cytotoxic investigations were carried on Methanol extract of *Philodendron bipinnatifidum* and its phenolic constituents. Chromatographic fractionation was carried on its methanol extract; three phenolic compounds were isolated and identified on the basis of spectroscopic analysis.

Material and Methods

Plant material

The leaves of *Philodendron bipinnatifidum* Schott ex Endlicher (*P. selloum*) were collected from El-Giza gardens, Egypt. The plant was authenticated by Mrs. Terase Labib, General Manager and Head of Plant Taxonomy in El-Orman Botanical Garden. Voucher specimens [Ph-s I] are kept in the herbarium of Medicinal Chemistry Laboratory, Theodor Bilharze Research Institute, El-Giza, Egypt.

Equipment

^{1}H-NMR (δ [ppm], *J*[Hz]) and ^{13}C-NMR spectra were recorded in CD_3OD on Varian Mercury 300 and Bruker APX-400 instrument, operating at 300 MHz for proton and 75 MHz for carbon 13. Chemical shifts (δ) are reported using TMS as internal standard. Mass spectra were recorded on a Finnigan TSQ 700 GC/MS equipped with a Finnigan electrospray source (ESI-MS). Paper chromatography sheet (Whattman No 1), using 15 % acetic acid as solvent system, the chromatograms were visualized under UV light (at 254 and 366 nm). Column chrzomatography was performed using a glass column (120 × 7 cm) and using polyamide as stationary phase.

Extraction and isolation

The air dried powdered leaves (1.1 Kg) were exhaustively extracted with 85 % methanol (7 L × 3) under reflux (70 ^{o}C). After evaporation of the solvent, the concentrated residue was defatted with light petroleum ether (60-80^{o}C) to give the crude extract which was further extracted with chloroform and ethyl acetate successively to give crude methanol extract (144 g). The crude extract dissolved in water and the water insoluble residue was removed by filtration, the water soluble portion was desalted by precipitation with excess methanol to give a dry brownish residue (63.3g) that was suspended in water and fractionated on polyamide column (Ø 7.0 × 120 cm). Elution was started with water followed by gradual increase of methanol. On the basis of comp-TLC and PC with the use ofUVlight, the individual 120 fraction (250 ml each) were collected into seven collective fractions (I-VII). Fraction I was found to be dark sugar material of no phenolic characters. Fraction II was subjected to cellu-lose column with elution system methanol:water (8:2) to give one pure compound **1**.Fraction III subjected to Sephadex LH- 20 column with methanol : water (7:3) as an eluent, resulting in pure compound **2**. Compound **3** was isolated from fraction IV and purified on Sephadex LH-20 column with methanol:water (5:5).

Measurement of Potential Cytotoxicity (SRB Assay)

The sulforhodamine B (SRB) assay which was developed in 1990, remains one of the widely used methods for in vitro cytotoxicty screening (Skehan *et al.,* 1996). The assay relies

on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloro acetic acid. SRB is a bright–pink amino oxanthene dye with two sulfonic groups that bind to basic amino acid residues under mild acidic conditions, and dissociate under basic conditions (Lillie 1977). The SRB method has proven to be sensitive, practical and suited to large scale screening applications as well as research.

Human tumor cell lines

They were obtained frozen in liquid nitrogen (-180 ^{0}C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

Chemicals

All chemicals were purchased from Sigma Chemicals Co (St. Louis, Mo U.S.A.). Dimethylsulphoxide (DMSO), RPMI-1640 medium, Sodium bicarbonate, Trypan blue, Fetal Bovine Serum (FBS), Penicillin/Streptomycin, Acetic acid, Sulphorhodamine-B (SRB), Trichloroacetic acid (TCA), 100 % isopropanol and 70 % ethanol.

Buffers

Tris base 10 mM (PH 10.5): It was used for SRB dye solubilization. 121.1 gm of tris base was dissolved in 1000 ml of distilled water and PH was adjusted by HCl acid (2 M).

Sulphorhodamine-B (SRB) assay of cytotoxic activity

This method was carried out according to that of Skehan et al. (1996). Cells were used when 90 % confluence was reached in T25 flasks. Adherent, cell lines were harvested with 0.025% trypsin. Viability was determined by trypan blue exclusion using the inverted microscope (Olympus 1x70, Tokyo, Japan). Cells were seeded in 96-well microtiter plates at a concentration of $5x10^4$-10^5 cell/well in a fresh medium and left to attach to the plates for 24 hrs. After 24 hrs, cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200 µl volume/well using fresh medium and incubation was continued for 24, 48 and 72 hrs. Control cells were treated with vehicle alone. For each drug concentration, 4 wells were used. Following 24, 48 and 72 hrs treatment, the cells were fixed with 50 µl cold 50 % trichloroacetic acid for 1 hr at 4 ^{0}C. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid. The wells were then washed 4 times with 1 % acetic acid. The plates were air-dried and the dye was solubilized with 100 µl/well of 10 mM tris base (ph 10.5) for 5 min on a shaker (Orbital shaker OS 20, Boeco, Germany) at 1600rpm. The optical density (O.D.) of each well was measured spectrophotometrically at 564nm with an ELIZA microplate reader (Meter tech. Σ 960, U.S.A.). The mean background absorbance was automatically subtracted and mean values of each drug concentration was calculated. The percentage of cell survival was calculated as follows:

Survival fraction = O.D. (treated cells)/ O.D. (control cells).

The IC_{50} values (the concentrations of thymoquinone required to produce 50 % inhibition of cell growth). The experiment was repeated 3 times for each cell line.

Results

Compound 1

p-hydroxyphenylethyl-*β*-D-glucopyranosyl(1→2), *β*-D-glucopyranosyl(1→3)4-*O*-*trans*caffeoyl-*O*-*α*-L-rhamnopyranoside**.** ^{1}H NMR:2.05 (2H, m,H-7), 3.53, 4.53 (2H, m, H_2-8), 6.6 (1H,d, *J*=2.0 Hz, H-2""), 6.74 (1H, dd, *J*=8.0, 2.0Hz, H-6""), 6.84 (1H,d, J=8.0 Hz, H-5""), 6.83 (2H, d, *J* = 8.5 Hz, H-3, H-5), 7.29 (2H, d, *J*=8.5 Hz, H-2,H-6), 7.52, 6.74 (1H, f, *J*=16.0 Hz, H-7"", H-8"").4.69 (1H, d, *J*=7 Hz, H-1"), 4.99 (1H, d, *J*= 7.0 Hz, H-1"') 5.9 (1H, br s, H-1Rha). ESI-MS: m/z 770.90 $[M]^-$, m/z, 607.75, 455.46, 325.66 [M-2 × 162 – $C_8H_9O_2$]. ^{13}C NMR (Table 1).

Compound 2

2-phenyl ethyl 4-*O*-*trans*caffeoyl-*O*-*α*-L-rhamnopyranoside.^{1}H NMR: δ 2.4 (2H, m, H-7), δ 3.7, 4.5 (1H each, m, H-8), δ 7.78 – 7.80 (5 H, m, H-2, H-3, H-4, H-5, H-6), δ 6.82 (1H, d, *J*=2.0 Hz, H-2""), δ 6.85 (1H, dd*J*= 9.2, 1.5 Hz, H-6""), 6.7 (1H, br, s, H-5""). δ 5.3 (1H, br, s, H-1 Rha), 1.11 (3H, d, *J*=7.0 Hz, Me Rha).ESI-MS: m/z 445.07 [M], m/z 282.23 [M-163($C_9H_7O_3$)]$^-$,m/z 325.03 (base beak) [M-(C_8H_9O)].^{13}C NMR Table 1.

Compound 3

2,3-dimethoxy-phenyl ethyl4-*O*-*trans*caffeoyl-*β*-D-glucopyranosyl. ^{1}H NMR: δ 2.4 [2H, m, H-7], δ 3.56, 4.59 [1H each, m, H-8], 3.69, 3.46, δ 6.4 [1H, d, *J*=2.0 Hz, H-2], 5.85 [1H, d, *J*=2.0 Hz, H-2""], δ 6.65[1H, dd*J*= 8.0, 2.0 Hz, H-6], 7.8[1H, dd*J*= 8.0, 2.0 Hz, H-6""], 7.7[1H, d, *J*=8.0 Hz, H-5], 7.6 [1H, d, *J*=8.0 Hz, H-5""], 5.2. ESI-MS: m/z 504.98 $[M]^-$ ($C_{25}H_{38}O_{11}$).^{13}C NMR Table 1.

Discussion

The search for anticancer agents from natural sources has been successful worldwide; active constituents have been isolated and are nowadays used to treat human tumors. The methanol extract of *Philodendron bipinnatifidum* showed significant cytotoxic effects (LC_{50} 15.9 µg/ml) using HEPG2 cell line (Table 2). The methanol extract was subjected to chromatographic fractionation, it afforded three compounds which according to (Deciga- Campos *et al.,* 2007) criteria showed significant cytotoxic activity.

Compound **1** (Figure 1), oily brown substance, showed phenolic properties, it gives dark color changed to yellow under UV lamp, its R_f value0.21[15 % acetic acid]. It showed complicated eoyl protons at 7.52 and 6.74 both (2H, d, *J*=16.0 Hz, H-7"", H-8"") (Zhao et al 2008, Kanc hanapoom et al 2001), ^{1}H NMR spectra showed signals assignable to ortho and meta coupled ABC-type aromatic protons at 6.6 (1H,d, *J*=2.0 Hz, H-2""), 6.74 (1H, dd, *J*=8.0, 2.0Hz, H-6""), 6.84 (1H,d, *J*=8.0 Hz, H-5"") corresponding to caffeoyl moiety (Morikawa et al 2010). Two terminal β-D-glucopyranosyl moieties at 4.69 (1H, d, *J*=7 Hz, H-1"), 4.99 (1H, d, *J*= 7.0 Hz, H-1"') and core α-L-rhamnopyranosyl moiety at 5.9 (1H, br s, H-1"").

Negative ESI-MS spectrum showed a molecular ion at m/z 770.90 $[M]^-$, followed by loss of two terminal glucose moieties at m/z, 607.75 and 455.46. M/z 325.66 [M- 2 x 162 –

$C_8H_9O_2$] which suggest the removal of *p*-hydroxy phenylethanoid unit. On the basis of above analysis data, the structure of compound 1 was elucidated as p-hydroxy phenyl ethyl *β*-D-glucopyranosyl(1-2),*β*-D-glucopyranosyl(1-3 4-*O*-trans caffeoyl-*O*-*α*-L-rhamnopyranoside. According to the literature the compound was isolated for the first time.

Compound **2**, yellow powder substance, m.p. 147°C, showed phenolic properties, it give dark color changed to yellow under UV lamp, its $R_f$value0.48 [15 % acetic acid]. The ^1H

Table 1: ^{13}C NMR of Compound 1, 2 and 3.

Carbon No	Compound 1	Compound 2	Compound 3
1	149.8	140.2	145.6
2	118.6	130	115.1
3	118.1	129.9	150.3
4	153.5	127.8	148.7
5	117.8	129.9	117.1
6	119.9	130.2	122
7	40.7	39.6	40.2
8	66.2	70.4	70.6
	Rhamnose	Rhamnose	Glucose
1'	102.4	101.3	102.6
2'	73.9	71.6	77.2
3'	75.4	72.4	75.7
4'	77.8	79.5	70.7
5'	71.3	70.4	75.9
6'	17.6	18.9	60.8
	Glucose		OMe-3 56.7
1"	101.9		OMe-4 56.8
2"	75.4		
3"	77.3		
4"	71.6		
5"	76.5		
6"	61.8		
	Glucose		
1'"	101.4		
2'"	76.1		
3'"	77.9		
4'"	77.4		
5'"	70.7		
6'"	62		
	Caffeoyl	Caffeoyl	Caffeoyl
1""	126.8	127.7	127.5
2""	117.3	117.8	117.2
3""	145	146	146.4
4""	147.1	150.8	150.5
5""	116.5	118	117.4
6""	122	123.9	123.7
7""	141	143	143.1
8""	115.3	114	114.6
9""	166.3	167	166.8

Table 2. LC_{50} of the methanol extract of *P. bipinnatifidum* and the isolated compounds.

Test sample	LC_{50} (µg/ml)
Methanol extract	15.9
Compound 1	14.9
Compound 2	23.7
Compound 3	18.2
Doxorobsin*	0.6

NMR spectrum in the aromatic region exhibited five aromatic protonsappeared at δ 7.78 – 7.80 [5 H, m, H-2, H-3, H-4, H-5, H-6] indicating that no substituted group in the phenylethyl moiety in addition to two methylene signals appeared at δ 2.4 [2H, m, H_2-7] and δ 3.7, 4.5 [2H each, m, H_2-8], It also showed ortho and meta coupled ABC- type aromatic protons at δ 6.82 [1H, d, *J*=2.0 Hz, H-2""], δ 6.85[1H, dd, *J*= 9.2, 1.5 Hz, H-6""] and 6.7 [1H, br s, H-5""] corresponding to caffeoyl moiety. It showed signal of one anomeric protons at δ 5.3 [1H, br, s, H-1 Rhamnose] and its methyl protons at 1.11[3H, d, *J*=7.0 Hz, Me Rhamnose].^{13}C NMR spectrum showed presence of two aromatic nucleuses, it gives 23. carbon resonance signals, 6 signal for rhamnose sugar moiety and 17 signals corresponding to two phetnolic acids. The anomeric carbon of rhamnose moiety appears at δ 101.3 and its methyl car-

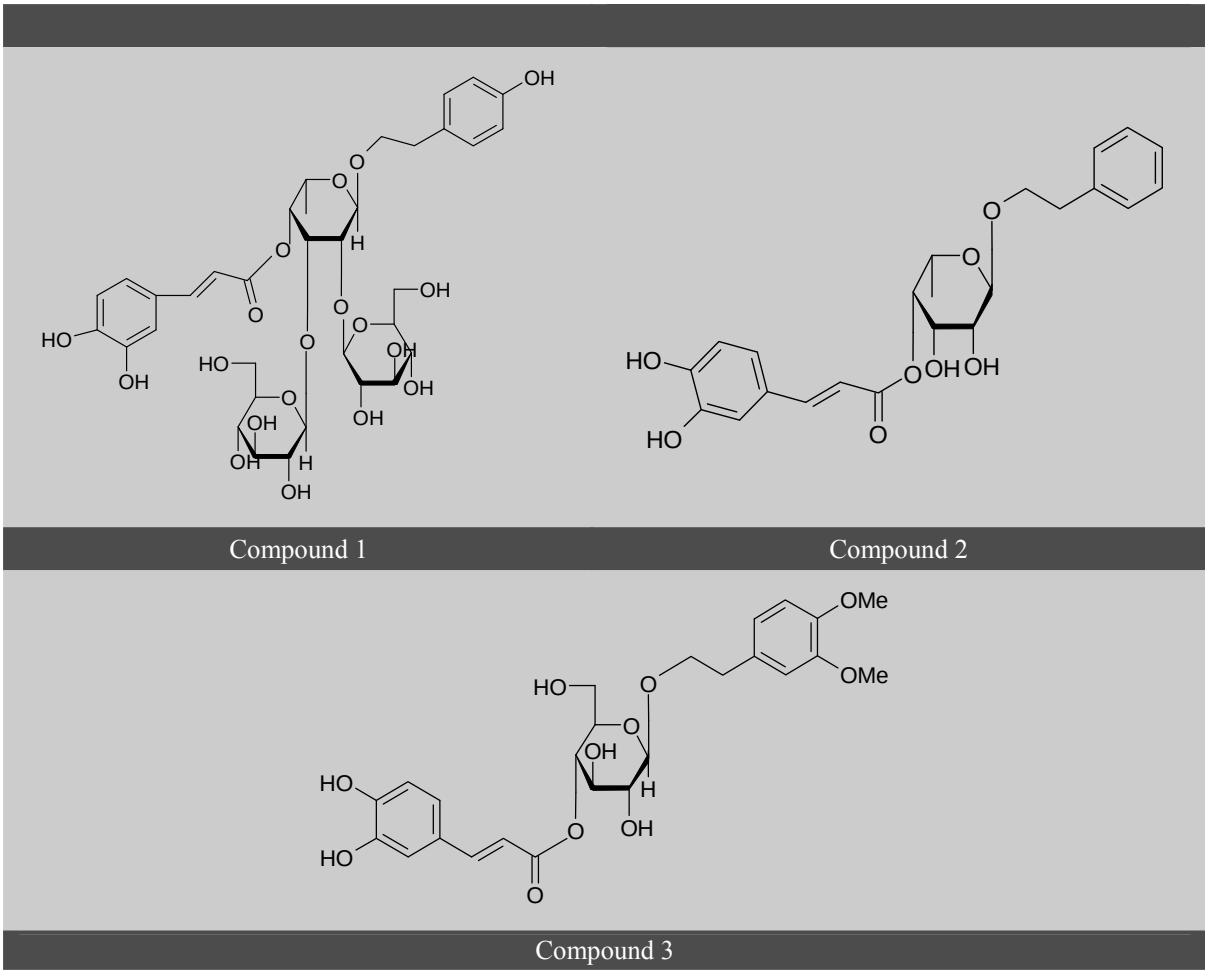

Figure 1. Chemical structures of isolated compounds (**1-3**).

bon at 18.9. The downfield shift value at 79.5 at C-4 means that the point of attachment of caffeoyl moiety is at carbon 4 of rhamnose. Negative ESI-MS spectrum showed a molecular ion at m/z 445.07 $[M]^-$, lose of caffeoyl moiety give peak at m/z 282.23 $[M- 163(C_9H_7O_3)]^-$, m/z 325.03 (base beak) $[M-(C_8H_9O)]$). The above data suggest that the structure of compound **2** was elucidated as 2-phenyl ethyl 4-*O*-*trans*caffeoyl-*O*-*α*-L- rhamn-opyranoside and the comp-ound was isolated for the first time.

Compound 3, brown powder compound, m.p. 95°C showed phenolic properties, it gives dark color changed to yellow under UV lamp, its R_f values 0.55 [15 % acetic acid]. ^{1}H NMR spectrum showed a characteristic feature similar to compound **2**, it showed two methylene signals at δ 2.4 [2H, m, H-7] and δ 3.56 (1H, m, H-8), 4.59 [1H m, H-8], two strong methoxyl singlet at 3.69, 3.46, two units of ortho and meta coupled ABC- type aromatic protons at δ 6.4[1H, d, *J*=2.0 Hz, H-2],δ5.85[1H, d, *J*=2.0 Hz, H-2""] δ 6.65[1H, dd, *J*= 8.0, 2.0 Hz, H-6], δ7.8[1H, dd, *J*= 8.0, 2.0 Hz, H-6""] and δ7.7[1H, d, *J*=8.0 Hz, H-5], δ7.6 [1H, d, *J*=8.0 Hz, H-5""], anomeric proton of β-D glucopyranosyl moiety appeared at δ5.2. ^{13}C NMR spectrum indicates presence of two aromatic acids substituted on glucose moiety unit at C-1 and C-4. It also showed presence of two methoxy groups at 56.7 and 56.8. Negative ESI-MS spectrum showed a molecular ion at m/z 504.98 $[M]^-(C_{25}H_{38}O_{11})$. The above data suggest that the structure of a new compound determined as 2, 3 dimethoxy-phenyl ethyl 4-*O*-*trans*caffeoyl-*β*-D-glucopyranosyl.

Comparison of the structures of the compounds 1,2 and 3bear close resemblance to each other. Comparison of the cytotoxic activity of the three compounds [**1**(14.9 µg/ml), 2 (23.7 µg/ml) and 3 (18.2 µg/ml)] showed that compound **1**is the most active compound, it may be due more number of sugar units which enhance the cytotoxic activity, compound **3** slightly active than compound 2 which may be due to presence of glucose sugar moiety instead of rhamnose and/or presence of two methoxy groups which may enhance the cytotoxic activity.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

References

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 tradional Chinese medicinal plants associated with anticancer. *Life Sciences*, 74, 2157-2184.

Déciga-Campos M, Rivero-Cruz I, Arriaga-Alba M, Castañeda-Corral G, Angeles-López GE, Navarrete A, Mata R (2007). Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. *J Ethnopharmacol,* 110, 334–342.

Feitosa CM, Benzerra MZB (2007). Constituents qimicos de *Philodendron* imbes chott. *QuímicaNova* 30, 41-44.

Kanchanapoom T, Kasai R, Yamasaki K (2001). Lignan and phenylethanoid glycosides from *Fernando adenophylla*. *Phytochemistry* 57, 1245-1248.

Kite HI, Sharp HJ, Hill PS Boyce PC (1997). Polyhydroxy alkaloids in the *Aroid tribes Nephthytideae* and *Agloanemateae*: Phytochemical support for internal relationship. *Biochemical Systematics and Ecology*, 25, 757-766.

Lillie RHJ (1977). Conn's Biological stains 9th edn (Williams& Wilkins), Baltimore.

Morikawa T, Pan Y, Ninomiya K, Imura K, Matsuda H, Yoshikawa M, Yuan D, Muraoka O (2010). Acylated phenylethanoid oligoglycosides with hepatoprotective activity from the desert plant *Cistanchetubulosa*. *Bioorganic*& Medicinal *Chemistry*, 18, 1882-1890.

Pagani FI (1982). Phytoconstituents of Arisarum vulgare O. Targ-Tozz. Araceae. *Bollettino chimico farmaceutico* 121, 460-464.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT,

Bokesch H, Kenney S, Boyd MR (1996). New colorimetric cytotoxicity assay for anticancer-drug screening.*Food and Chemical Toxicology,*34, 449-456.

Williams CA, Harbone JB, Mayo SJ (1981). Anthocyanin pigment and leaf flavonoids in the family *Araceae. Phytochemistry,*20, 217-234.

Zhao P, Tanaka T, Hirabayashi K, Zhang Y, Yang C, Kouno I (2008). Caffeoylarbutin and related compounds from the buds of *Vaccinium dunalianum*. *Phytochemistry*, 69, 3087-3094.

