DETERMINATION OF SCIENTIFIC NAME AND ISOLATION OF FOUR SAPONIN TRITERPEN FROM *POLYSCIAS BALFOURIANA* GROWING IN CAN THO, VIETNAM

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ABSTRACT

Can Tho city is a city directly under the Central Government in the Mekong Delta region with many valuable medicinal species including *Polyscias balfouriana*, but there have not been many in-depth studies on the chemical composition grown in this area. Therefore, this issue needs to be studied in order to develop and preserve the precious medicinal resources of Can Tho City, Vietnam. Leaves of *Polyscias balfouriana* grown in Can Tho were sampled, identified the correct species of the study, then extracted and fractionated by the technique of exhaust extraction and liquid-liquid extraction, obtained fractions. The fractions were further isolated by chromatographic techniques and their chemical structures determined by nuclear magnetic resonance spectroscopy. The results isolated 4 saponin triterpen compounds including Ladyginosid A (1), 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucuronopyranosyl oleanolic 28-*O*-*β*-D-glucopyranosyl ester (2), acid 3-*O*-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-(6-*O*-methyl) glucuronopyranosyloleanolic (3), 3-*O*-*β*-*D*-glucuronopyranosyloleanolic 28-*O*-*β*-D-glucopyranosyl ester (4). The structure was determined by 1-dimensional 2D NMR nuclear magnetic resonance and compared with reference material. The above four compounds found in the leaves of *Polyscias balfouriana* provide basic data for further studies on pharmacological effects later.

Keywords: *Polyscias balfouriana, Araliaceae, saponin triterpen.*

INTRODUCTION

In recent years, the world has tended to return to natural sources of medicinal herbs due to the discovery of many undesirable effects of many drugs produced by chemical synthesis. Especially, medicinal herbs with tonic, energy-boosting and anti-stress effects are noticed because people's lives are increasingly improved, so the need to protect and improve health is concerned by society. Ginseng is a tonic that has been trusted by people for a long time. Moreover, in recent years, Ginseng's hepato- and kidney-protective effects against the effects of chemotherapy drugs have been interested and proven by researchers [5], [7], [14]. However, Ginseng is difficult to grow, rare and expensive, so for about 50 years, domestic and foreign scientists have always been looking for other medicinal species of the Ginseng family to replace some of the effects brought from Ginseng. ginseng, including plants of the genus *Polyscias*.

The plant belongs to the genus *Polyscias*, there are about 150 species in the world, mainly distributed in Africa, tropical Asia, New Guinea, and the Pacific Ocean. The plant is native to the Pacific Ocean, first discovered on the island of Polynesia. According to an investigation by the Vietnam Ginseng Center in the southern provinces, there are 6 species of Dinglingopods: *Polyscias fruticosa* (L) Harm, *Polyscias balfouriana* Bailey, *Polyscias filicifolia* (Merr et Fourn) Bailey, *Polyscias guilfeyleivar* lacinita Bailey, *Polyscias guilfeylei* (Cogh). et March) Bailey), *Polyscias scutellarie* (N.L.Burn) Fosberg [11]. Previous studies only paid much attention to the species *Polyscias fruticosa* (L.) Harms and showed that Dinguncle contains 2 main and important groups of compounds, saponins [1], [13] and polyacetylene [8]. However, with the species *Polyscias balfouriana*, there have not been many studies, so the study was carried out to determine the scientific name of a tree species, possibly through analysis of morphological characteristics, analysis of DNA genetic code and comparison with

the original DNA gene in the gene bank. In order to confirm the exact scientific name of the round-leaf clover collected in Can Tho City, it is helpful to investigate the chemical composition with the hope that it will contribute to clarifying the chemical composition of this species to contribute scientific evidences to Vietnam's precious medicinal herbs storehouse, thereby contributing to the rational exploitation and use of plant resources.

MATERIALS AND METHODS

Materials

Leaves of *Polyscias balfouriana* were collected in Le Binh ward, Cai Rang district, Can Tho city, Vietnam to extract, isolate and analyze DNA for gene sequencing, compared with the gene sequence samples of species of the genus *Polyscias*.

Chemicals and equipment for DNA analysis: CTAB Buffer (2% CTAB, 100 mM Tris pH 8.0, 20 mMEDTApH8.0,1.4MNaCl), β-mercaptoethanol, Chloroform: Isoamylalcohol (24:1), Enzyme RNase, Isopropanol, ethanol (70%). All chemicals used in this experiment were sourced from Merck, Germany. PCRMix (NEXpro, Korea), PCR 2X MasterMix, purified agarose, GelRed dye, TAE 1X, Loading dye 6x, Ladder 1 kb plus (Thermo Scientific, USA), TE, purified water (2 times distilled and purified water) pasteurized at 121oC for 20 minutes). ATTO CORPORATION AE 7344 electrophoresis, Polyacrylamide ATTA Compact PAGE-Twin gel electrophoresis machine (ATTA, Japan), GeneAmp PCR System 2700 PCR machine (Amplied Biosystems – Malaysia), UV gel reader (BioBlockScientific, France).

Chemicals and equipment for isolation: Methanol, diethyl ether, ethyl acetate, *n*-butanol (Merck, Germany)….Nuclear magnetic resonance spectra: ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HSQC, HMBC recorded on a BRUCKER AVANCE (500 MHz) chemical shift in δ (ppm), interaction constant (J) in Hz. Mass spectra were measured on an AGILENT TECHNOLOGIES 6120 (Quadrupole LC/MS). Thin layer chromatography (TLC) was performed on a pre-coated Merck-GF60 F254 aluminum silica gel plate, size 20 × 20 cm, adsorbent layer thickness 0.2 mm of Merck, Germany. Medium-pressure column chromatography using silica gel 60, Merck, particle diameter 0.040-0.063 mm; diaion HP-20; reverse phase silica gel RP - 18 (particle size 30 - 50 m).

Methods

Identify the scientific name

* Morphological description method

Observation and description of the external morphology of Dinguncle. Based on the improved method of [9], the parts described include: Stems, leaves...

* Total extraction and purification

Whole DNA was isolated from fresh leaves according to an improved CTAB extraction procedure [2].

First, 100 mg of leaf samples were weighed in a mortar and finely ground in 1 mL of CTAB 2X solution incubated at 65 ^{o}C for 15 min. Place the sample that has been ground in CTAB into the tube and add the CTAB, titrate to the 1.5 mL mark. Mix well and centrifuge at 13000 rpm for 10 min. After centrifugation, withdraw 1000 µL of the supernatant from each tube in turn and place in a new tube. Then add 10 µL of β-mercaptoethanol/tube. Carry out incubation at 65 ^{o}C for 60 minutes (every 10 minutes mix the samples well). Next, add 500 µL of chloroform to each tube, mix well and centrifuge at 13000 rpm for 10 minutes. Pipette 750 µL of the above solution into a new tube, then continue to add 500 µL of chloroform, mix well and centrifuge at 13000 rpm for 10 minutes. Transfer 550 µL of the above solution to a new tube, then add 500 µL of chloroform to each tube and centrifuge at 13000 rpm for 10 min. Withdraw 350 µL of the supernatant into a new tube, then add 5 µL of RNase to each tube, shake well and incubate the sample at 37 °C for 2 h. After 2 h of incubation, add 300 µL CTAB 2X and 500 µL chloroform to each tube. The sample was centrifuged at 13000 rpm for 10 min. Next, withdraw each tube 400 µL of the supernatant and put it in a new tube, and at the same time add

400 µL isopropanol (1:1 ratio), mix well and incubate at -20 ^{o}C for 30 minutes. The sample is centrifuged at 13000 rpm for min, carefully discarding the upper solution, leaving the precipitate deposited below. Add 500 µL 70% ethanol to each tube and centrifuge at 13000 rpm for 5 min to rinse the sample, then discard the alcohol and leave the precipitate. Add 500 µL of 70% ethanol further to each tube to rinse the sample a second time and centrifuge at 13000 rpm for 5 min. Then discard the alcohol and leave the precipitate. Use a micropipette to suck up the remaining alcohol in each tube and let the sample dry (under a ceiling fan) for 1 hour. Finally, 30 µL TE were added to each tube (pH = 8.0) to dissolve the DNA and refrigerated at -20 °C.

* DNA amplification by PCR reaction

The DNA sequence was amplified using primers rbcLa-F: 5'-ATGTCACCACAAACAGAGACTAAAGC-3' and rbcLa-R: 5'-GTAAAATCAAGTCCACCRCG-3' [6].

Thermal cycling for a CPR reaction: Performed in 35 heating cycles, including 5 min at 95 ^{o}C, 30 s at 95 ^{o}C, 30 s at 60 ^{o}C, 30 s at 72 ^{o}C, stretching the series for 5 min at 72 °C and the product was stored at 10 °C for 20 min.

* DNA electrophoresis on agarose gel

DNA after being extracted and purified will be checked by electrophoresis on 1% agarose gel. After electrophoresis, the gel was stained with redsafe dye (Biobasic, UK), and the results were recorded

* PCR product electrophoresis and sequencing

PCR products were electrophoresed and purified using the Wizard SV Gel kit and PCR Clean-up System (Promega). Based on the Sanger method [12]. Each dideoxynucleotide is labeled with a different colored fluorescent agent. Thus, all oligonucleotides terminating at the same dideoxynucleotide will have the same color. DNA sequences were sequenced by Phu Sa Biochem company (Vinh Long city) on an automatic sequence reader.

* Analyze data and compare DNA sequences

Molecular weight was calculated using Gel Analyzer software. Sequencing results were stored in FASTA format and analyzed using the latest BioEdit software version 7.0.5 [4]. Then by BLAST method on the NCBI gene bank system (National Center for Biotechnology Information) used for species identification.

Extraction and isolation

Polyscias balfouriana leaf powder (1000 g) was extracted continuously with methanol (MeOH), filtered off the residue, the extract was concentrated by solvent under low pressure to obtain crude MeOH (200 g). Then, crude MeOH extract was added with little liquid-liquid extract with diethyl ether (Et_2O), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) respectively, to obtain the respective concentrations Et_2O (50 g), EtOAc. (10 g), *n*-BuOH (40.0 g) and aqueous solution.

The *n*-BuOH fraction (40 g) was isolated by column chromatography (SKC) with the stationary phase HP-20. The solvent system was water, 50% MeOH, 80% MeOH, MeOH and Me2CO, respectively. The results obtained 5 main segments are PBN01-PBN05 respectively.

+ Segment PBN02 (9 g) carried out chromatography of normal phase silica gel column eluted with $CHCl_3$-MeOH solvent system (100:0 → 0:100) to obtain 7 segments PBN02.1-PBN02.7. Segment PBN02.2 (2.0 g) chromatography of normal phase silica gel column with solvent system $CHCl_3$-MeOH-H_2O (90:10:0→75:25:1) obtained 5 fractions PBN02.2.1- PBN02 .2.5. PBN02.2.4 fractionation (0.50 g) multiple times normal phase silica gel column chromatography with solvent $CHCl_3$-MeOH-H_2O (80:20:0.1) and reverse phase column chromatography of MeOH- H_2O system (50: 50) obtained compound PB1 (25 mg). Segment PBN02.4 (2.2 g) chromatography of normal phase silica gel column with solvent system $CHCl_3$-MeOH-H_2O (90:10:0→75:25:1) obtained 5 fractions PBN02.4.1-

PBN02 .4.5. Fraction PBN02.4.3 (0.4 g) silica gel column chromatography with repeated mixing with $CHCl_3$-MeOH-H_2O solvent (85:15:0.5) and multiple purification to obtain PB2 compound (30 mg).

+ Segment PBN03 (12 g) carried out chromatography of normal phase silica gel column with $CHCl_3$-MeOH solvent system (100:0→0:100) to obtain 5 segments PBN03.1-PBN03.5. Fraction PBN03.1 (4.0 g) multiple times normal-phase silica gel column chromatography with solvent $CHCl_3$-MeOH-H_2O (95:5:0→80:20:1) and reverse phase column chromatography of MeOH- system. H_2O (60:40) yielded compound PB3 (33 mg).

+ Segment PBN03.2 (8.5 g) column chromatography of silica gel normal phase with solvent system $CHCl_3$-MeOH (90:10→80:20:1) obtained 5 segments PBN03.2.1- PBN03.2.5. Segmentation PBN03.2.2 (1.6 g) chromatography of normal phase silica gel column with multiple times of $CHCl_3$-MeOH-H_2O solvent (90:10:0.05) and reverse phase column chromatography of MeOH- H_2O system (60: 40) obtained compound PB4 (28 mg).

RESULTS AND DISCUSSION

Determination of scientific name by *rbc*L gene sequencing

* Morphological characteristics of plant *Polyscias balfouriana*

Tall, fragrant shrub. Body bronze color speckled with gray spots. Compound leaves usually have 3 leaflets on a long petiole, the first leaflets sometimes appear larger than the rest of the leaves; leaf blade round, heart-shaped at the base, dark green, hairless, edge with sharp teeth, slit ears, margin edges or white veins; leaf veins propeller-shaped, petioles 1 cm; peduncle has a sheathed bottom, hugging the body. The canopy has a canopy with a large canopy of 1 - 1.5 cm.

Figure 1. Morphology of *Polyscias balfouriana*

Samples of fresh leaves of *Polyscias balfouriana* were extracted, DNA separated, gene sequenced, compared with the published gene sequence samples of species of the genus Polyscias, giving the following results:

* Extract the total DNA and perform the PCR reaction:

The total DNA after separation was electrophoresis on 1% agarose gel for clear DNA lines, clean electrophoresis tape, no RNA mixed. The total DNA after performing the gene multiplication reaction with the rbcL fragment was electrophoresed and compared with the standard 1000 bp ladder, showing that the obtained sequence size was about 600 bp. The product line on the electrophoresis tape is bold, clear, intact, and unbroken, so it is eligible for further purification to perform the sequencing reaction.

Figure 2. Total DNA electrophoresis

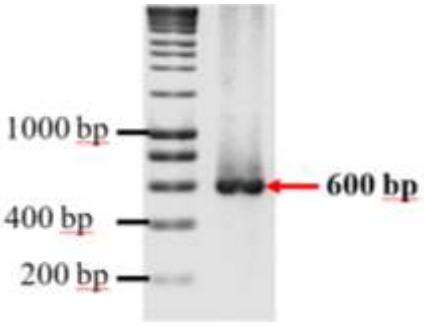

Figure 3. PCR product electrophoresis

* *rbc*L gene sequence

The obtained DNA sequence after sequencing consists of 545 bp, of which 542 bp is evident, and is included to compare with the published sequence, in which the G-C ratio is 42 %, the A-T ratio is 58 %. The NCBI/Blast tool was used to compare with the sequence published on the world gene bank (gene bank code: KX783977.1), showing that the obtained gene sequence is similar to the *Polyscias balfouriana* species sequence (Andre) L.H. Bailey published with 541/542 homologous nucleotides (corresponding to 99% similarity rate).

Sequence ID: KX783977.1 **Length:** 552 **Number of Matches:** 1

Range 1: 1 to 541 GenBank Graphics ▼ Next Match ▲ F

Score	Expect	Identities	Gaps	Strand
994 bits(538)	0.0	541/542(99%)	1/542(0%)	Plus/Minus

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Sbjct  541  AAACCGCTCTACCGTAGTTTTTAGCAGATAACCCCAATTTAGGTTTAATAGTACATCCCA  482

Query  61   ATAGGGGACGACCATACTTGTTCAATTTATCTCTCTCAACTTGGATGCCATGAGGCGGTC  120
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Query  121  CTTGGAAAGTTTTAATATAAGCAACAGGGATTCGCAGATCTTCCAGACGTAGAGCACGCA  180
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Sbjct  421  CTTGGAAAGTTTTAATATAAGCAACAGGGATTCGCAGATCTTCCAGACGTAGAGCACGCA  362

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Query  541  CT  542
            ||
Sbjct  2    CT  1
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Figure 4. Comparison of the *rbc*L gene sequence of the study sample with the species sequence published in the world

In which: Query is the sequence of the research sample and Sbjct is the sequence of the species *P. balfouriana* (Andre) L.H. Bailey published on the world gene bank (gene bank code: KX783977.1) [3]. The results of gene sequencing and comparison of the gene sequence of *Polyscias balfouriana* with the gene sequence of *P. balfouriana* (Andre) L.H. Bailey is a reliable basis for confirming the scientific name of the round-leaf clover, collected in Le Binh ward, Cai Rang district, Can Tho city, which is *Polyscias balfouriana* (Andre) L.H. Bailey belongs to the Ginseng family (Araliaceae).

Determine the structure of 4 isolated compounds

Extraction and isolation results from *n*-BuOH fraction obtained 4 triterpene saponins PB01 (25 mg), PB02 (30 mg), PB3 (33 mg) and PB4 (28 mg) all in powder form, color white, readily soluble in methanol.

Compound PB01

The ^{13}C-NMR spectrum (125 MHz, pyridine-d5, δ ppm) combined with the DEPT 90, DEPT 135 spectrum shows that compound PB01 has 42 carbons: 2 >C=O, 1 >C=, 1 -CH= type carbon, 2 –O–CHO– type carbon, 1 –CH_2–O– type carbon, 9 –CHO– type carbon, 6 >C< type carbon, 3 >CHO– type carbon, 10 –CH_2– type carbon, 7 –CH_3 type carbon. The presence of: 7 tertiary methyl carbons, 1 carbonyl carbon at δ_C 180.5, together with 1 >C= type carbon at δ_C 144.6, 1 -CH= type carbon at δ_C 122.4 characterizes 2 carbons olefin C13; C12 of the olean-12-en-28-oic acid framework. In the presence of 2 acetal carbons at δ_C 106.2; 104.5; 8 carbon oxymethin and 1 carbon oxymethylene at δ_C 62.0, indicating that compound PB01 is an oleanolic framework triterpene saponin with 2 sugar units, D-glucuronopyranoside and D-glucopyranoside, respectively. The ^{1}H-NMR spectrum (500 MHz, pyridine - d_5, δ ppm) also proves that the aglycon framework is oleanolic acid in the presence of signals: 1 olefin proton at δ_H 5.41 (1H, br s, H12); 1 oxymethin proton at δ_H 3.23 (1H, dd, J = 4.0 and 11.5 Hz, H3); 1 methine proton at δ_H 3.21 (1H, dd, J = 4.0 and 13.5 Hz, H18) and 7 tertiary methyl groups at δ_H 0.68 - 1.25. In addition, the presence of 2 proton anomers at δ_H 5.09 (1H, d, J = 7.0 Hz, H1') and 4.82 (1H, d, J = 7.5 Hz, H1′′) combining HSQC spectra, confirming compound PB01 has 2 sugar units, β-D-glucuronic and β-D-glucose. HMBC spectra show oxymethin proton at δ_H 3.23 (1H, dd, J = 4.0 and 11.5 Hz, H3) with 2 methyl carbons at δ_C 28.0 (C-23) and 16.8 (C- 24); proton methine at δ_H 3.21 (1H, dd, J = 4.0 and 13.5 Hz, H18) with 2 olefin carbons at δ_C 144.6 (C-13) and 122.4 (C-12); protons at δ_H 2.07 (1H, m, H-16a) with carbons at δ_C 180.5 (C-28), which reconfirmed the key sites of the framework. On the other hand, the proton anomer at δ_H 5.09 (1H, d, J = 7.0 Hz, H1') interacts with the oxymethin carbon at δ_C 89.1 (C3); besides, the proton anomer at δ_H 4.82 (1H, d, J = 7.5 Hz, H1′′) interacts with the oxymethin carbon at δ_C 83.6 (C4′); showed that the D-glucuronic sugar unit attached to the aglycon skeleton at the C3 position and the D-glucose sugar unit attached to the D-glucuronic sugar unit at the C4′ position.

From ^{1}H-NMR, ^{13}C-NMR spectral data, combined with DEPT, HSQC, HMBC spectrum; The physical characteristics and comparison with the published documents [13], we identified the compound PB01 as: acid 3-O-[β-D-glucopyranosyl-(1→4)]-β-D -glucuronopyranosyloleanolic also known as Ladyginoside A.

Compound PB02

^{1}H-NMR and ^{13}C-NMR spectral data of compound PB02 show that there are 48 carbon atoms, including the aglycon part is oleanolic acid and the glycoside part is 3 sugar units. Compound PB02 is an oleanolic acid triterpene saponin with 3 sugar units β-D-GlcA and 2 sugars β-D-Glc.

HMBC spectra show a correlation between oxymethin proton at δ_H 3.28 (1H, dd, J = 4.0 and 11.5 Hz, H-3) with 2 methyl carbons at δ_C 28.0 (C-23) and 16.8 (C-24); methine protons at δ_H 3.23 (1H, dd, J = 4.0 and 13.5 Hz, H-18) with 2 olefin carbons at δ_C 144.6 (C-13) and 122.4 (C-12); protons at δ_H 2.07-2.10 (1H, m, H-16^a) with carbons at δ_C 180.1 (C-28), which reconfirmed the critical positions of

the framework. On the other hand, the proton anomer at δ_H 4.94 (1H, d, J = 8.0 Hz, H-1') interacts with the oxymethin carbon at δ_C 89.3 (C-3); besides, proton anomer at δ_H 4.96 (1H, H-1'') interacts with carbon oxymethin at δ_C 82.6 (C-4'); showed that the D-glucuronic sugar unit attached to the aglycon skeleton at the C-3 position and the D-glucose sugar unit attached to the D-glucuronic sugar unit at the C-4' position. In addition, HMBC also showed the interaction between proton anomer at δ_H 6.28 (1H, d, J = 8.0 Hz, H-1''') with carbonyl carbon δ_C 176.5 (C-28). Shows that the sugar unit D-glucose is attached to the aglycon skeleton at position C-28.

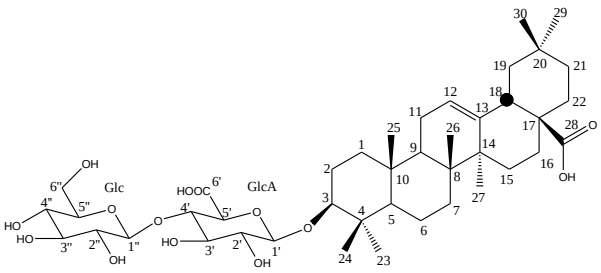
From the analysis of ^{1}H, ^{13}C-NMR spectral data, combined with DEPT, HMBC, HSQC, COSY spectra and compared with documents [13], [10], there are similarities. Therefore, compound structure PB02 was determined as 3-O-β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl oleanolic 28-O-β-D-glucopyranosyl ester. This compound was isolated by Vo Duy Huan in 1998 from *P. fruticosa* [13].

Compound PB03

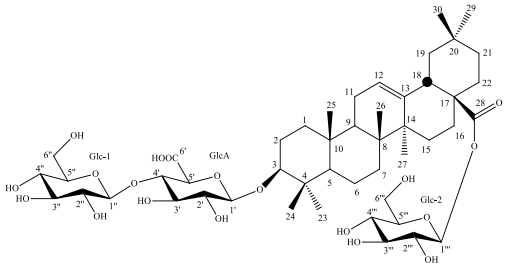
The ^{13}C and ^{1}H-NMR spectra of compound PB03 are similar to those of PB01. However, there is an extra oxymethyl group at δ_C 52.4 which is correlated with protons at δ_H 3.84 (3H, s, OMe), indicating that the compound PB03 is an oleanolic triterpene saponin with 2 sugar units, β-D-GlcA, β-D-Glc and an oxymethyl group. HMBC spectra showed that the oxymethyl proton at δ_H 3.84 (3H, s, OMe) interacts with the C 170.0 carbonyl carbon, which identified methylated β-D-GlcA at the C-6 position. Also, 2 proton anomers at δ_H 4.93 (1H, d, J = 7.5 Hz, H-1′) and 4.96 (1H, d, J = 7.5 Hz, H-1′′) interacting with 2 carbons at δ_C 89.2 (C-3) and 82.3 (C-4′), respectively, like compound PB01. From NMR spectral data and comparison with parent documents [13], we identify the compound PB03 as: acid 3-O-[β-D-glucopyranosyl-(1→4)]-β-D-(6-O-methyl) glucuronopyranosyloleanolic.

Compound PB04

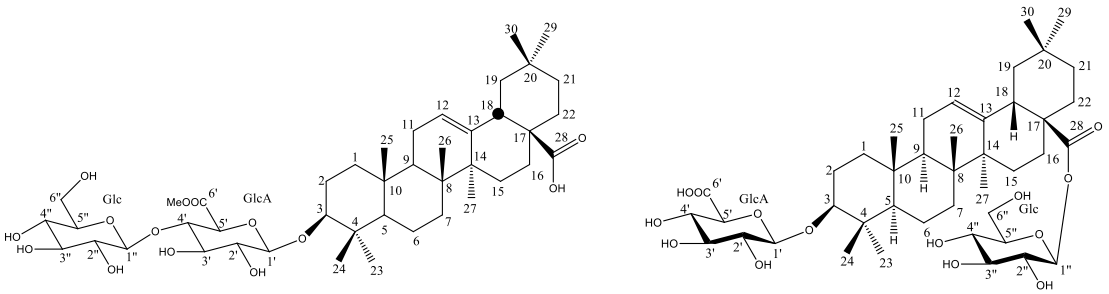
The ^{13}C and ^{1}H-NMR spectra of compound PB04 are similar to those of compound PB01, showing that compound PB04 is also an oleanolic triterpene saponin with 2 sugar units, β-D-GlcA, β-D-Glc. HMBC spectra show that the proton anomer at δ_H 4.79 (1H, d, J = 8.0 Hz, H1′) interacts with the oxymethin carbon at δ_C 88.8 (C3); showed that β-D-GlcA binds to the aglycon at C3. In addition, the interaction between proton anomer at δ_H 6.14 (1H, d, J = 8.0 Hz, H1′′) with carbonyl carbon δ_C 176.6 (C28), proves that β-D-Glc binds to the aglycon at C-28. From NMR spectral data and comparison with documents [13], we identified the compound PB04 as: 3-O-β-D-glucuronopyranosyloleanolic 28-O-β-D-glucopyranosyl ester.



PB01



PB02



PB03 PB04

Figure 5. Structure of 4 isolated triterpene saponins

Table 1: ^{13}C and ^{1}H-NMR spectral data of compounds PB (01), (02), (03) và (04)

Location C	^{13}C NMR (C_5D_5N; δ_c, 125 MHz)				^{1}H, NMR (C_5D_5N; δ_H, 500 MHz)			
	(PB01)	(PB02)	(PB03)	(PB04)	(PB01)	(PB02)	(PB03)	(PB04)
1	38,3	38,5	38,4	38,4				
2	26,1	26,3	26,3	26,0				
3	89,1	89,1	89,2	88,8	3,23 (1H, *dd*, *J* = 4,0 và 11,5)	3,24 (1H, *dd*, *J* = 4,0 và 11,5)	3,23 (1H, *dd*, *J* = 4,0 và 11,5)	3,36 (1H, *dd, J* = 4,5 và 12,0)
4	39,2	39,3	39,3	39,1				
5	55,5	55,6	55,6	55,5				
6	18,2	18,3	18,3	18,2				
7	33,0	32,3	33,0	32,8				
8	39,5	39,7	39,6	39,5				
9	47,7	47,8	47,8	47,6				
10	36,7	36,7	36,8	36,6				
11	23,5	23,6	23,5	23,4				
12	122,4	123,3	122,4	122,6	5,41 (1H, *br s*)	5,39 (1H, *br s*)	5,41 (1H, *br s*)	5,40 (1H, *brs*)
13	144,6	144,0	144,6	143,8				
14	41,9	42,0	42,0	41,8				
15	28,1	28,0	28,1	27,9				
16	23,5	23,6	23,6	23,0				
17	46,5	47,8	46,5	46,8				
18	41,8	41,6	41,8	41,4	3,21 (1H, *dd*, *J* = 4,0 và 13,5)		3,21 (1H, *dd*, *J* = 4,0 và 13,5)	3,18 (1H, *dd*, *J* = 3,5 và 13,5)
19	46,3	46,1	46,3	45,9				
20	30,7	30,6	30,8	30,4				
21	34,0	32,8	34,1	33,6				
22	33,0	32,4	33,1	32,2				
23	28,0	28,0	28,0	27,9	1,21 (3H, *s*)	1,24 (3H, *s*)	1,21 (3H, *s*)	1,29 (3H, *s*)
24	16,8	16,8	16,8	16,7	0,90 (3H, *s*)	0,93 (3H, *s*)	0,90 (3H, *s*)	097 (3H, *s*)
25	15,2	15,4	15,3	15,2	0,71 (3H, *s*)	0,75 (3H, *s*)	0,71 (3H, *s*)	0,81 (3H, *s*)

26	17,2	17,3	17,2	17,1	0,89 (3H, *s*)	1,06 (3H, *s*)	0,89 (3H, *s*)	1,07 (3H, *s*)
27	26,0	26,0	26,0	25,8	1,25 (3H, *s*)	1,24 (3H, *s*)	1,25 (3H, *s*)	1,26 (3H, *s*)
28	180,5	176,5	180,1	176,6				
29	33,0	32,9	33,0	32,8	0,90 (3H, *s*)	0,87 (3H, *s*)	0,90 (3H, *s*)	0,89 (3H, *s*)
30	23,6	23,5	23,6	23,3	0,95 (3H, *s*)	0,84 (3H, *s*)	0,95 (3H, *s*)	0,87 (3H, *s*)
3-*O*-GlcA								
1'	106,2	106,4	106,6	106,3	5,09 (1H, *d*, *J* = 7,0)	4,83 (1H, *d*, *J* = 8,0)	5,09 (1H, *d*, *J* = 7,0)	5,01 (1H, *d*, *J* = 8,0)
2'	74,6	74,9	74,5	74,8				
3'	76,5	76,5	75,8	78,0				
4'	83,6	83,8	82,3	73,3				
5'	74,7	76,5	74,9	77,6				
6'	*****	175,2	170,0	*				
Ome			52,4				3,84 (3H, *s*)	
Glc (1→4) GlcA								
1''	104,5	104,6	104,8		4,82 (1H, *d*, *J* = 7,5)	4,85*	4,82 (1H, *d*, *J* = 7,5)	6,30 (1H, *d*, *J* = 8,5)
2''	74,6	74,9	74,2					
3''	77,2	78,1	77,8					
4''	71,1	71,7	71,3					
5''	78,0	78,5	78,3					
6''	62,0	62,0	62,3					
28-*O*-Glc						6,24 (1H, *d*, *J* = 8,0)		
1'''		95,6		95,3				
2'''		73,8		73,4				
3'''		79,0		78,7				
4'''		71,2		70,6				
5'''		78,5		78,7				
6'''		61,8		61,7				

* *Weak signal*

CONCLUSION

By method of barcoded DNA combined with morphological characteristics, *Polyscias balfouriana* was collected in Le Binh ward, Cai Rang district, Can Tho city, Vietnam with scientific name *Polyscias balfouriana* (Andre) L.H. Bailey belonging to the Ginseng family. (Araliaceae). This result helps to accurately identify the scientific names of the research subjects by the rbcL gene sequencing method.

From the leaves of *Polyscias balfouriana* grown in Can Tho City, Vietnam for the first time, we isolated and identified the structure of 4 triterpene saponin compounds: Ladyginoside A (1), 3-O-β-D-glucopyranosyl-(1→4)-β-D-glucuronopyranosyl oleanolic 28-O-β-D-glucopyranosyl ester (2), acid 3-O-[β-D-glucopyranosyl-(1→4)]-β-D-(6- O-methyl)glucuronopyranosyloleanolic (3), 3-O-β-D-glucuronopyranosyloleanolic 28-O-β-D-glucopyranosyl ester (4). These compounds help guide further studies in terms of biological effects as well as deeper chemistry.

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