

IDENTIFICATION OF RBCL GENE FRAGMENT SEQUENCE AND ISOLATION OF SOME COMPOUNDS FROM *POLYSCIAS SERRATA*

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ABSTRACT

Taxonomic identification of plant species is crucial for various scientific and practical applications. The use of molecular techniques, such as the Ribulose-1,5-bisphosphate Carboxylase/Oxygenase large subunit (rbcL) gene sequencing, has emerged as a reliable method for determining scientific names accurately. *Polyscias serrata*, a member of the Araliaceae family, poses taxonomic challenges due to morphological variations. Investigating its taxonomic identity using the rbcL gene sequence can provide valuable insights. This study aims to determine the scientific name of *Polyscias serrata* through rbcL gene sequencing and to isolate compounds from this species for further analysis. They include β -sitosterol (1), acid caffeic (2), acid ferulic (3), acid betulenic (4), daucosterol (5), quercetin (6), quercitrin (7), myricitrin (8). Genomic DNA extraction, PCR amplification of the rbcL gene, sequencing, and phylogenetic analysis were conducted to determine the taxonomic identity of *Polyscias serrata*. Additionally, compounds were isolated from the plant material using chromatographic techniques. The rbcL gene sequencing revealed the taxonomic identity of *Polyscias serrata*, providing valuable information for its classification. Furthermore, the isolation of compounds from the plant material yielded promising results for further investigation of its phytochemical profile. The study successfully determined the scientific name of *Polyscias serrata* using the rbcL gene sequence method and identified compounds from the species, laying a foundation for future research on its taxonomy and pharmacological potential.

Keywords: Taxonomic identification, rbcL gene sequence, *Polyscias serrata*, phytochemical analysis, compound isolation.

1. INTRODUCTION

Polyscias is the second largest genus in the family Araliaceae. The Araliaceae family includes 55 genera and more than 1500 species, distributed mostly in tropical and subtropical regions, of which many species are used as oriental medicine and ornamental plants [9]. The genus *Polyscias* is derived from two Greek words: 'poly' meaning many and 'skia' meaning shade indicating thick foliage, which is characteristic of the genus *Polyscias*. Species of the *Polyscias* genus are usually perennial shrubs, rarely flowering, and are usually small or medium-sized trees with slender shapes and beautiful foliage. Pinnately compound leaves or simple leaves with pinnate lobes or pinnately compound leaves with leaflets of variable shape; Stipules absent or fused at the base into a small appendage. Native to Southeast Asia and Polynesian islands in the Pacific, tropical island areas. In addition, some species also grow and are cultivated in Indonesia, Malaysia, Lao and Vietnam [1].

Currently, in the world according to the plant monograph page of "The Plant list" [2], there are a total of 655 names, of which 176 species are accepted, there are 473 species. meaning, 6 unresolved species. Meanwhile, the plant classification system according to "Catalogue of Life" [7] has 174 species, 4 subspecies and 6 subspecies of the genus *Polyscias*. Previous studies only paid much attention to the species *Polyscias fruticosa* (L.) Harms. As for *Polyscias serrata*, it is rarely studied, *Polyscias serrata*, the shield aralia or plum aralia, is a tropical shrub or small tree reaching 2–6 meters in height. So the project was carried out to determine the scientific name of a tree species, possibly through analyzing morphological characteristics, analyzing the rbcL gene segment and comparing it with the gene segment original rbcL in Genbank. To confirm the exact scientific name of the round-leaved *Polyscias serrata* plant collected in (10°14'58.0"N latitude, 105°34'47.3"E

longitude) Tan Loc ward, That Not District, Can Tho City, Vietnam we help survey the chemical composition with the hope that it will contribute to clarifying the chemical composition of the *Polyscias serrata* species. This type of leafy lentil aims to contribute scientific evidence to Vietnam's precious medicinal herb warehouse, thereby contributing to the exploitation and rational use of plant resources.

2. MATERIALS AND METHODS

2.1. Materials

Leaves of *Polyscias serrata* were collected in (10°14'58.0"N latitude, 105°34'47.3"E longitude) Tan Loc ward, That Not 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 mM EDTA pH 8.0, 1.4M NaCl), 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 121°C for 20 minutes). ATTO CORPORATION AE 7344 electrophoresis, polyacrylamide ATTA Compact PAGE-Twin gel electrophoresis machine (ATTA, Japan), GeneAmp PCR System 2700 PCR machine (Applied 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: ¹H-NMR, ¹³C-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; Sephadex LH-20.

2.2. Methods

2.2.1. Identify the scientific name

* Morphological description method

Observation and description of the external morphology of Dinguncle. Based on the improved method of [13], the parts described include: Roots, stems, leaves...

* Total extraction and purification

Whole DNA was isolated from fresh leaves according to an improved CTAB extraction procedure [5].

Initially, 100 mg of leaf samples were weighed in a mortar and finely ground in 1 mL of CTAB 2X solution incubated at 65 °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 μ L of the supernatant from each tube in turn and place in a new tube. Then add 10 μ L of β -mercaptoethanol/tube. Carry out incubation at 65 °C for 60 minutes (every 10 minutes mix the samples well). Next, add 500 μ L of chloroform to each tube, mix well and centrifuge at 13000 rpm for 10 minutes. Pipette 750 μ L of the above solution into a new tube, then continue to add 500 μ L of chloroform, mix well and centrifuge at 13000 rpm for 10 minutes. Transfer 550 μ L of the above solution to a new tube, then add 500 μ L of chloroform to each tube and centrifuge at 13000 rpm for 10 min. Withdraw 350 μ L of the supernatant into a new tube, then add 5 μ L of RNase to each tube, shake well and incubate the sample at 37 °C for 2 h. After 2 h of incubation, add 300 μ L CTAB 2X and 500 μ L chloroform to each tube. The sample was centrifuged at 13000 rpm for 10 min. Next, withdraw each tube 400 μ L 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 $-20\text{ }^{\circ}\text{C}$ 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 $-20\text{ }^{\circ}\text{C}$.

* DNA amplification by PCR reaction

The DNA sequence was amplified using primers rbcLa-F: 5'-ATGTCACCACAAACAGAGACTAAAGC-3' and rbcLa-R: 5'-GTAAAATCAAGTCCACCRCG-3' [10].

Thermal cycling for a CPR reaction: Performed in 35 heating cycles, including 5 min at $95\text{ }^{\circ}\text{C}$, 30 s at $95\text{ }^{\circ}\text{C}$, 30 s at $60\text{ }^{\circ}\text{C}$, 30 s at $72\text{ }^{\circ}\text{C}$, stretching the series for 5 min at $72\text{ }^{\circ}\text{C}$ and the product was stored at $10\text{ }^{\circ}\text{C}$ 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 [16]. 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 [6]. Then by BLAST method on the NCBI gene bank system (National Center for Biotechnology Information) used for species identification.

Extraction and isolation

From 2,0 kg of *Polyscias serrata* leaves were extracted with methanol to obtain the extract, then vacuum evaporated to obtain 400 g of total extract. This high amount was dissolved in a minimum amount of water, then the resulting solution was shaken with the following solvents: Diethyl ether, ethyl acetate, *n*-buthanol. The obtained fractions were concentrated under reduced pressure to yield 50 g diethyl ether, 20 g ethyl acetate, 40 g *n*-buthanol. The diethyl ether fraction (30 g) was purified by silica gel column chromatography (CC) using *n*-hexane–EtOAc–acetone (7:2:1) as eluent to afford five major fractions, labeled R1–R9. Fractions R4 and R5 were selected for further isolation. The major fraction R4 (4.2 g) was loaded onto a Sephadex LH-20 column using MeOH, yielding subfractions R4.1–R4.5. Subfraction R4.3 (90 mg) was separated by silica gel CC using CHCl_3 –EtOAc–acetone (8:8:2) to afford 1 (15 mg) and 2 (12 mg). Subfraction R4.4 (120 mg) was separated in the same way to give 3 (16 mg) and 4 (22 mg). Fraction R5 (5,2 g) was subjected to Sephadex LH-20 and eluted with MeOH to give subfractions R5.1–R5.5. Subfraction R5.3 (90 mg) was further purified by silica gel CC using *n*-hexane– CHCl_3 –EtOAc–MeOH (10:2:1:1) to yield 5 (12 mg), 6 (14 mg), 7 (15 mg), and 8 (10 mg). Determination of the structure of the isolated and purified compounds was based on spectroscopic methods tests such as $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, COSY, HSQC and HMBC. At the same time, it is based on references previously published by scientists.

3. RESULTS AND DISCUSSION

3.1. Determination of scientific name by *rbcL* gene sequencing

* Morphological characteristics of plant *Polyscias serrata*

Shrub shape 50 – 150 cm tall; body grey-white, hairless; green young branches. Leaves are fragrant, feathered twice; lobed; leaf shape changes, broad, dark green, smooth, with sharp teeth, pointed or not.



Figure 1. Morphology of *Polyscias serrata*

Samples of fresh leaves of *Polyscias serrata* 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

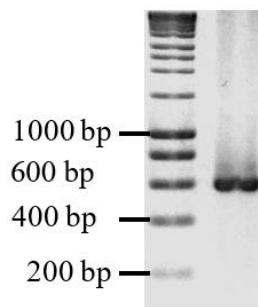


Figure 3. PCR product electrophoresis

* RBCL gene sequence

The obtained DNA sequence after sequencing consists of 551 bp, of which 551 bp is evident, and is included to compare with the published sequence, in which the G-C ratio is 40%, the A-T ratio is 60%. The NCBI/Blast tool was used to compare with the sequence published on the world gene bank (gene bank code: MN117992.2) [12], showing that the obtained gene sequence is similar to the *Polyscias serrata* Balf. published with 550/551 homologous nucleotides (corresponding to 99% similarity rate):

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AAGTGTGGATTCAAAGCTGGTGTAAAGATTACAAATTGACTTATTATACTCCTGACTATGAAA
CCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAG
AAGCAGGGGCTGCGGTAGCTGCCGAATCTTCTACTGGTACATGGACAACCTGTGTGGACCGAT
GGACTTACCAGCCTTGATCGTTACAAAGGGCGATGCTACGGAATCGAGCCGTTACTGGAGA
AGAAAATCAATATATTGCTTATGTAGCTTACCCATTAGACCTTTTTGAAGAGGGTTCTGTTACTA
ATATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTGCGTGCTCTACGTCTGGA
AGATCTGCGAATCCCTGTTGCTTATATTAACCTTTCCAAGGACCGCCTCATGGCATCCAAGTT
GAGAGAGATAAATTGAACAAGTATGGTCGTCCCTATTGGGATGTACTATTAACCTAAATTGG
GGTTATCTGCTAAAACTACGGTAGAGCGGTTT
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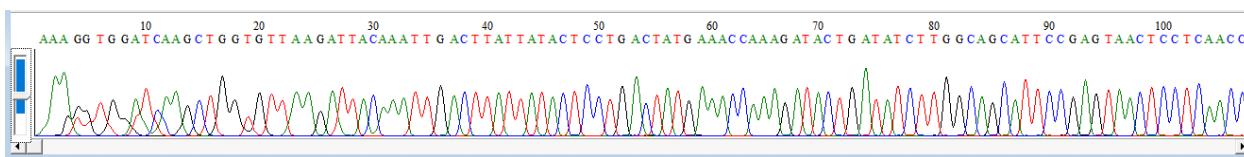


Figure 4. Sequencing results of *rbcL* gene segment of *Polyscias serrata* sample using ABI 3100 machine (Applied Biosystem, USA) reading with Bioedit software

In which: Query is the sequence of the research sample and Sbjct is the sequence of the species *Polyscias serrata* (Burm.f.) Fosberg published on the world gene bank (gene bank code: MN117992.2) [12]. The results of gene sequencing and comparison of the gene sequence of *Polyscias serrata* with the gene sequence of *Polyscias serrata* is a reliable basis for confirming the scientific name of the Dinh lang rang, collected in Tan Loc ward, Thot Not district, Can Tho city, Vietnam which is *Polyscias serrata* belongs to the Ginseng family (Araliaceae).

3.2. Determine the structure of 8 isolated compounds

From 2,0 kg of *Polyscias serrata* leaves were extracted with methanol to obtain the extract, then vacuum evaporated to obtain 400 g of total extract. This high amount was dissolved in a minimum amount of water, then the resulting solution was shaken with the following solvents: Diethyl ether, ethyl acetate, *n*-buthanol. The diethyl ether fraction (30 g) was purified by silica gel column chromatography (CC) eight pure compounds were obtained. They include β -sitosterol (1), acid caffeic (2), acid ferulic (3), acid betulenic (4), daucosterol (5), quercetin (6), quercitrin (7), myricitrin

(8). Determination of the structure of the isolated and purified compounds was based on spectroscopic methods tests such as $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, COSY, HSQC and HMBC. At the same time, it is based on references previously published by scientists.

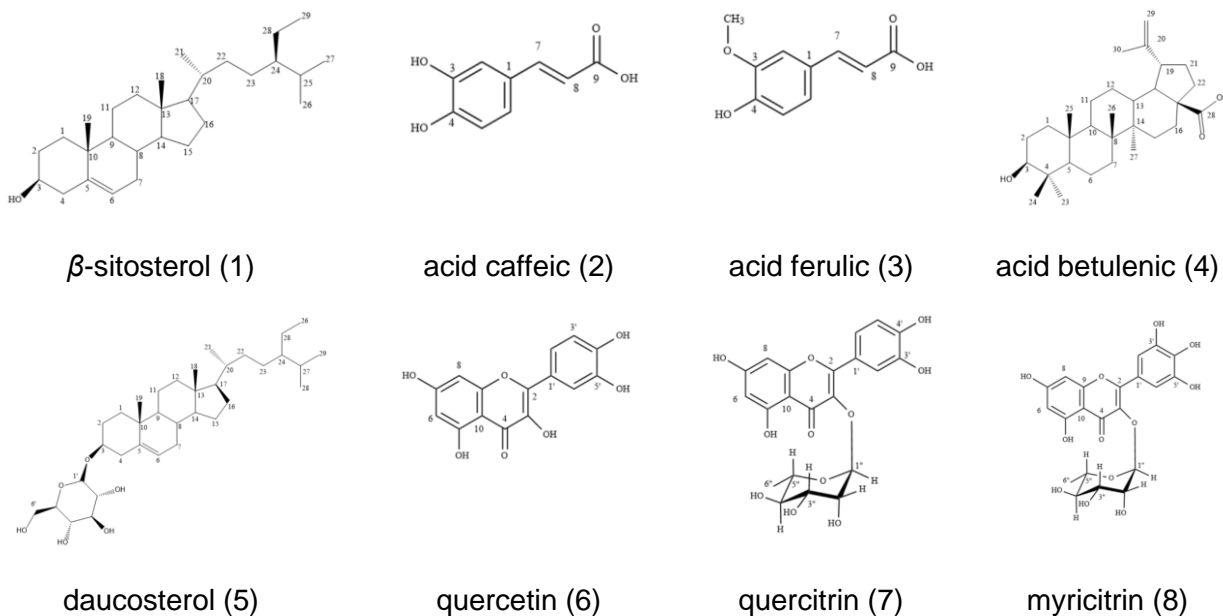


Figure 5. Structure of the isolated compounds

(1) β -sitosterol: β -sitosterol was obtained as a white amorphous powder [14].

Property	Value
Appearance	White amorphous powder
$^1\text{H-NMR}$ Spectrum (CDCl_3)	
δ (ppm)	Multiplicity
5.38	Multiplet, H-6
3.53	Multiplet, H-3
1.03	Singlet, CH_3 -19
0.95	Doublet, CH_3 -21 ($J = 6.5$ Hz)
0.87	Triplet, CH_3 -29 ($J = 7.4$ Hz)
0.86	Doublet, CH_3 -26 ($J = 6.7$ Hz)
0.84	Doublet, CH_3 -27 ($J = 6.7$ Hz)
0.71	Singlet, CH_3 -18
$^{13}\text{C-NMR}$ Spectrum (CDCl_3)	
δ (ppm)	Chemical Shift
140.79	C-5
121.72	C-6
71.83	C-3
56.80	C-14
56.10	C-17
50.18	C-9
45.88	C-24

Property	Value
42.35	C-4
42.34	C-13
39.81	C-12
37.29	C-1
36.53	C-10
36.16	C-20
33.99	C-22
31.94	C-7, C-8
31.70	C-2
29.20	C-25
28.26	C-16
26.14	C-23
24.32	C-15
23.11	C-28
21.11	C-11
19.83	C-19
19.41	C-27
19.06	C-26
18.80	C-21
12.00	C-29
11.88	C-18

(2) **Acid caffeic:** Acid caffeic was isolated as a white solid [15].

Property	Value
¹ H-NMR Spectrum (CD ₃ OD)	
δ_{H} (ppm)	Multiplicity
7.05	Doublet, H-2 (J = 2.0 Hz)
6.80	Doublet, H-5 (J = 8.0 Hz)
6.95	Doublet of doublets, H-6 (J = 2.0 and 8.0 Hz)
7.55	Doublet, H-7 (J = 16.0 Hz)
6.23	Doublet, H-8 (J = 16.0 Hz)
¹³ C-NMR Spectrum (CD ₃ OD)	
δ_{C} (ppm)	Chemical Shift
127.8	C-1
115.1	C-2
146.8	C-3
149.5	C-4
116.5	C-5
122.8	C-6
147.0	C-7
115.6	C-8

Property	Value
171.0	C-9

(3) Acid ferulic: Acid ferulic was obtained as white needle-shaped crystals [8].

Property	Value
ESI-MS Spectrum	
Peaks	m/z 191.0 [M+H] ⁺ , 193.0 [M-H] ⁻
¹ H-NMR Spectrum (CD ₃ OD)	
δ_{H} (ppm)	Multiplicity
7.19	Doublet, H-2 (J = 1.0 Hz)
6.83	Doublet, H-5 (J = 8.0 Hz)
7.08	Doublet of doublets, H-6 (J = 1.0 and 8.0 Hz)
7.62	Doublet, H-7 (J = 16.0 Hz)
6.33	Doublet, H-8 (J = 16.0 Hz)
3.91	Singlet, 3-OCH ₃
¹³ C-NMR Spectrum (CD ₃ OD)	
δ_{C} (ppm)	Chemical Shift
127.8	C-1
111.8	C-2
150.5	C-3
149.4	C-4
116.5	C-5
124.0	C-6
146.9	C-7
115.9	C-8
170.9	C-9
56.5	3-OCH ₃

(4) Acid betulenilic: Acid betulenilic was obtained as white needle-shaped crystals [17].

Property	Value
¹ H-NMR Spectrum (500 MHz, CD ₃ OD)	
δ_{H} (ppm)	Multiplicity
4.74	Singlet, H-29 α
4.61	Singlet, H-29 β
3.18	Doublet of doublets, H-3 (J = 11.0 and 5.0 Hz)
3.00	Multiplet, H-19
1.69	Singlet, H-30
0.98	Singlet, H-27
0.97	Singlet, H-23
0.94	Singlet, H-26
0.83	Singlet, H-25
0.76	Singlet, H-24
¹³ C-NMR Spectrum	

Property	Value
(125 MHz, CD ₃ OD)	
δ_c (ppm)	Chemical Shift
14.7	C-27
15.4	C-24
16.0	C-26
16.1	C-25
18.3	C-6
19.4	C-30
20.9	C-11
25.5	C-12
27.4	C-2
28.0	C-23
29.7	C-21
30.6	C-15
32.2	C-16
34.4	C-7
37.0	C-22
37.2	C-10
38.4	C-13
38.7	C-1
38.9	C-4
40.7	C-8
42.5	C-14
46.9	C-19
49.3	C-18
50.6	C-9
55.4	C-5
56.3	C-17
79.0	C-3
109.7	C-29
150.4	C-20
179.5	C-28

(5) Daucosterol: Daucosterol was obtained as a white solid [11].

Property	Value
ESI-MS Spectrum	
Peaks	m/z 599.3 [M+Na] ⁺ , 575.4 [M-H] ⁻
¹ H-NMR Spectrum (500 MHz)	
Solvent	CDCl ₃ & CD ₃ OD
δ_H (ppm)	Multiplicity
3.59	Multiplet, H-3
5.37	Triplet, H-6 (J = 3.5 Hz)

Property	Value
0.68	Singlet, H-18
1.00	Singlet, H-19
0.92	Doublet, H-21 (J = 6.5 Hz)
0.86	Triplet, H-26 (J = 7.5 Hz)
0.81	Doublet, H-28 (J = 7.0 Hz)
0.81	Doublet, H-29 (J = 7.0 Hz)
Glucose Signals	
δ_H (ppm)	Multiplicity
4.41	Doublet, H-1' (J = 8.0 Hz)
3.24	Multiplet, H-2'
3.44	Multiplet, H-3', H-4'
3.29	Multiplet, H-5'
3.84	Doublet of doublets, H-6'a (J = 2.0 and 12.0 Hz)
3.75	Doublet of doublets, H-6'b (J = 4.5 and 12.0 Hz)
¹³ C-NMR Spectrum (125 MHz)	
Solvent	CDCl ₃ & CD ₃ OD
δ_C (ppm)	Chemical Shift
37.4	C-1
29.7	C-2
79.3	C-3
38.8	C-4
140.4	C-5
122.3	C-6
32.0	C-7
31.9	C-8
50.3	C-9
36.8	C-10
21.2	C-11
39.9	C-12
42.4	C-13
56.9	C-14
24.4	C-15
28.3	C-16
56.2	C-17
11.9	C-18
19.4	C-19
36.3	C-20
18.8	C-21
34.1	C-22
26.2	C-23
45.9	C-24
29.3	C-25

Property	Value
19.9	C-26
19.1	C-27
23.1	C-28
12.0	C-29
Glucose Signals	
δ_c (ppm)	Chemical Shift
101.2	C-1'
73.6	C-2'
76.5	C-3'
70.1	C-4'
75.8	C-5'
61.9	C-6'

(6) **Quercetin:** Quercetin was isolated as a yellow solid [3].

Property	Value
ESI-MS Spectrum	
Peaks	m/z 303.0 [M+H] ⁺ , 301.0 [M-H] ⁻
¹ H-NMR Spectrum (500 MHz)	
Solvent	acetone-d6
δ_H (ppm)	Multiplicity
6.26	Doublet, H-6 (J = 2.0 Hz)
6.51	Doublet, H-8 (J = 2.0 Hz)
7.82	Doublet, H-2' (J = 2.0 Hz)
6.99	Doublet, H-5' (J = 8.5 Hz)
7.69	Doublet of doublets, H-6' (J = 2.0 and 8.5 Hz)
¹³ C-NMR Spectrum (125 MHz)	
Solvent	acetone-d6
δ_c (ppm)	Chemical Shift
146.9	C-2
136.7	C-3
176.5	C-4
162.3	C-5
99.1	C-6
165.0	C-7
94.4	C-8
157.7	C-9
104.1	C-10
123.8	C-1'
115.7	C-2'
145.8	C-3'
148.3	C-4'
116.2	C-5'

Property	Value
121.4	C-6'

(7) **Quercitrin:** Quercitrin was obtained as a yellow solid [4].

Property	Value
ESI-MS Spectrum	
Peak	m/z 471.0 [M+Na] ⁺
¹ H-NMR Spectrum (500 MHz)	
Solvent	CD ₃ OD
δ _H (ppm)	Multiplicity
6.22	Doublet, H-6 (J = 1.5 Hz)
6.39	Doublet, H-8 (J = 1.5 Hz)
7.36	Doublet, H-2' (J = 2.0 Hz)
6.93	Doublet, H-5' (J = 8.0 Hz)
7.33	Doublet of doublets, H-6' (J = 2.0 and 8.0 Hz)
5.37	Broad singlet, H-1''
4.24	Broad doublet, H-2'' (J = 1.0 Hz)
3.78	Doublet of doublets, H-3'' (J = 3.0 and 9.0 Hz)
3.36	Multiplet, H-4''
3.45	Multiplet, H-5''
0.95	Doublet, H-6'' (J = 6.0 Hz)
¹³ C-NMR Spectrum (125 MHz)	
Solvent	CD ₃ OD
δ _C (ppm)	Chemical Shift
158.5	C-2
136.3	C-3
179.7	C-4
163.2	C-5
99.8	C-6
165.9	C-7
94.7	C-8
159.3	C-9
105.9	C-10
123.0	C-1'
116.4	C-2'
146.4	C-3'
149.8	C-4'
117.0	C-5'
122.9	C-6'
103.6	C-1''
71.9	C-2''
72.2	C-3''
73.3	C-4''

Property	Value
72.0	C-5"
17.7	C-6"

(8) Myricitrin: Myricitrin was obtained as a yellow powder [18].

Property	Value
ESI-MS Spectrum	
Peak	m/z 487 [M+ Na] ⁺
¹ H-NMR Spectrum (500 MHz)	
Solvent	CD ₃ OD
δ_{H} (ppm)	Multiplicity
6.22	Doublet, H-6 (J = 2.0 Hz)
6.38	Doublet, H-8 (J = 2.0 Hz)
6.97	Singlet, H-2', H-6'
5.34	Doublet, H-1" (J = 1.5 Hz)
4.24	Doublet of doublets, H-2" (J = 1.5 and 3.0 Hz)
3.81	Doublet of doublets, H-3" (J = 3.0 and 9.0 Hz)
3.36	Multiplet, H-4"
3.54	Multiplet, H-5"
0.99	Doublet, H-6" (J = 6.0 Hz)
¹³ C-NMR Spectrum (500 MHz)	
Solvent	CD ₃ OD
δ_{C} (ppm)	Chemical Shift
159.5	C-2
136.3	C-3
179.7	C-4
163.2	C-5
99.8	C-6
165.9	C-7
94.7	C-8
158.5	C-9
105.9	C-10
122.0	C-1'
109.6	C-2', C-6'
146.9	C-3', C-5'
137.9	C-4'
103.6	C-1"
71.9	C-2"
72.1	C-3"
73.4	C-4"
72.0	C-5"
17.7	C-6"

4. CONCLUSION

By method of barcoded DNA combined with morphological characteristics, *Polyscias serrata* was collected in Tan Loc ward, Thot Not district, Can Tho city, Vietnam with scientific name *Polyscias serrata* Balf. 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 serrata* grown in Thot Not district, Can Tho City, Vietnam for the first time, we isolated and identified the structure of 8 compounds. They include: β -sitosterol (1), acid caffeic (2), acid ferulic (3), acid betulenic (4), daucosterol (5), quercetin (6), quercitrin (7), myricitrin (8). The chemical structures of these phytochemicals were characterized and isolated by various chromatographic methods. Their structures were elucidated by NMR (1D and 2D-NMR) in reference to the literature. To the best of our knowledge, compounds 1–8 were isolated from this plant for the first time, while compounds (2), (3), (4) and (8) were first isolated from the genus *Polyscias*. These compounds help guide further studies in terms of biological effects as well as deeper chemistry.

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REFERENCES

1. Ashmawy N.S., Gad H.A., Ashour M.L., El-ahmady S.H., Singab A.N.B. (2020). The genus *Polyscias* (Araliaceae): A phytochemical and biological review. *Journal of herbal Medicine*, 23, pp. 1-16.
2. Bernal R., Gradstein S.R., Celis M. (eds.) (2015). *Catálogo de plantas y líquenes de Colombia*. Instituto de Ciencias Naturales. Universidad Nacional de Colombia, Bogota. <http://www.plantsoftheworldonline.org/taxon/> [Cited 2023 12-October].
3. Bui T. T., Nguyen H. T., Duong T. L. H., Le T. T. H., Vu D. L., Nguyen H. T. (2015). "Flavonoids from leaves of *Tetracera scandens* L.". *Journal of Chemical and Pharmaceutical Research*, 7(3), pp.2123-2126.
4. Cota B. B., Siqueira E. P., Oliveira D. M. D., Alves T., Sobral M. E., Rabello A., Zani C. L. (2012). "Chemical constituents and leishmanicidal activity from leaves of *Kielmeyera variabilis*". *Revista Brasileira de Farmacognosia*, 22(6), pp.1253-1258.
5. Doyle J.J., Doyle J.L., (1990). Isolation of Plant DNA from fresh tissue. *Focus*, 12(6), pp. 13 – 15.
6. Hall T.A., (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41, pp. 95-98.
7. <http://www.theplantlist.org/1.1/browse/A/Araliaceae/Polyscias/> [Cited 2023 23-October].
8. Khalil H. E., Kamel M. S. (2015). "Phytochemical and biological studies of *Cichorium endivia* L. leaves". *Journal of Pharmaceutical Sciences and Research*, 7(8), pp.509.
9. Kim K., Nguyen V.B., Dong J., Wang Y., Park J.Y., Lee S.C., Yang T.J. (2017). Evolution of the Araliaceae family inferred from complete chloroplast genomes and 45S nrDNAs of 10 Panax-related species. *Scientific Reports*, 7 (1), pp.1-9.
10. Kress W.J and Erickson D.L. (2007). A two-locus global DNA barcode for land plants: the coding rbcLa gene complements the non-coding trnH-psbA spacer region. *PLoS One*. 6, pp. 1-10.
11. Lendl A., Werner I., Glasl S., Kletter C., Mucaji P., Presser A., Reznicek G., Jurenitsch J., Taylor D. W. (2005). "Phenolic and terpenoid compounds from *Chione venosa* (SW.) Urban var. *venosa* (Bois Bande´)". *Phytochemistry*, 66(19), pp.2381-2387.
12. Mai D.V., Luan T.C. and Ky H. (2021). *Polyscias* sp. 'serrata' isolate 4 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. GenBank: MN117992.2.
13. Nguyen Nghia Thin (2006). Plant research methods. Education publisher.

14. Parvin S., Kader M. A., Muhit M. A., Haque M. E., Mosaddik M. A., & Wahed, M. I. I. (2011). "Triterpenoids and phytosteroids from stem bark of *Crataeva nurvala* buch ham". *Journal of Applied Pharmaceutical Science*, 1(9), pp. 47-50.
15. Paul B., Mitra P., Ghosh T., Salhan R., Singh T. A., Chakrabarti A., Gupta S., Basu B., Mitra, P. K. (2013). "Isolation and structural determination of an anti bacterial constituent from the leaves of *Cassia alata* Linn". *Journal of Pharmacognosy and Phytochemistry*, 2 (1), pp. 326-333.
16. Sanger S., Nicklen S., and Coulson A.R., (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 74 (12), pp. 5463–5467.
17. Yili A., Aisa H. A., Isaev M. I. (2009). Betulinic acid and sterols from *Astragalus altaicus*. *Chemistry of natural compounds*, 45(4), pp. 592-594.
18. Zhang Z., ElSohly H. N., Li X. C., Khan S. I., Broedel S. E., Raulli R. E., ... & Walker L. A. (2003). Phenolic Compounds from *Nymphaea odorata*. *Journal of natural products*, 66(4), pp. 548-550.