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ISOLATED COMPOUNDS FROM THE STERM OF *ROUREA MINOR*

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Abstract: *Rourea minor subsp. microphyllais a species which belongs to the family of Connaraceae. According to traditional medicine, R.minor is used to treat wounds, prevent infection and hemostatic effect. In this report, three compounds were isolated from the ethyl acetate extract of Rourea minor stem including protocatechuic aldehyde (**1**), procyanidin A1 (**2**) and isoquercetrin (**3**). The isolated compounds were structurally elucidated by modern spectroscopic methods and compared with previously published information on these compounds.*

Keywords: *Rourea minor, isolation, protocatechuic aldehyde, procyanidin A1, isoquercetrin.*

1. Introduction

Rourea minor subsp. *microphylla* Vidal. (*R. minor*) belongs to the *Rourea* genus, Connaraceae family [1]. The genus *Rourea* consists of climbing shrubs or small trees, often with prominent dermal pores, with unbranched inflorescences bearing five-petaled flowers in the calyx, and curved glabrous fruits [2]. Species of the genus *Rourea* are widely distributed in the Amazon, Pacific, Africa and Asia such as Angola, Benin, Kenya, Mali, Nigeria, Papua New Guinea, Niue, Samoa, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, China, and Vietnam [3]. R. minor is a woody vine or climbing shrub, 1-4 m tall. The plant is harvested almost year-round and is used both fresh and dried [4]. According to tradition medicine, this plant is used as a tonic for women after giving birth to regain strength, has diuretic effects, is used to treat yellow urine, frequent urination, boils, crushed leaves are used to drink to regulate menstruation, and is used as a medicine to prevent miscarriage. The chemical composition and biological activity of *R. minor* have been few studies. The study of Zhen-Dan He et al. showed that the $CHCl_3$ extract of *R. minor* has antimalarial properties. In this study, five compounds were isolated including rourinoside, rouremin, 1-(26-hydroxyhexacosanoyl)-glycerol (3), 1-*O*-*β*-*D*-glucopyranosyl-(2*S*,3*R*,4*E*-8*Z*)-2-N-(20-hydroxypalmitoyl)-octadecasphinga-4,8-dienine, 9S,12S, 13S-trihydroxy-10E-octadecenoic acid, dihydrovomifoliol-9-*β*-*D*-glucopyranoside, β-sitosterol glucoside [5]. Several studies

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have shown that ethanol and methanol extracts of *R. minor* collected in India have hypoglycemic, serum insulin-increasing, antidiabetic and antidiabetic effects in diabetic mice [6][7]. In Vietnam, a study by Hieu Nguyen Ngoc et al. showed that from *R. minor* plant samples from Ba Vi Mountain, Hanoi, the authors isolated compounds belonging to the phenolic group, alkaloids, fatty acids and glucosides [8]. Recent research on the plant collected from Ben En National Park, Thanh Hoa province, has isolated seven compounds including: β-sitosterol, vanilic acid, sitoindoside I, vanillin, protocatechuic acid, daucosterol and quercitrin [9][10]. To continue to improve the research on compounds of the *R. minor*, in this study, we report our isolation of three compounds isolated from *R. minor* samples from Ben En National Park, Thanh Hoa Province.

2. Materials and Methods

2.1. Plant materials

The plant samples were collected from Ben En National Park in May 2025 and identified by Dr. Do Ngoc Dai, Vinh University of Economics with the scientific name as *Rourea minor* subsp. *Microphylla* Vidal., belonging to the Connariaceae family. A voucher specimen (MK-302) was deposited at the Faculty of Natural Sciences, Hong Duc University.

2.2. General experimental procedures

The ^{1}H-NMR (500MHz), ^{13}C-NMR (125MHz), DEPT and two-dimensional HMBC, HSQC spectra were measured on a Bruker Avance 500 MHz machine at the Institute of Chemistry - Vietnam Academy of Science and Technology. Polar rotation was measured on a JASCO P-2000 Polarimeter at the Institute of Marine Biochemistry. Mass spectra (ESI-MS) were measured on an Agilent 1260 LC/MS, using the electron spray method, melting temperature and polar rotation were measured on a MELT-TEMP 3.0 at the Institute of Marine Biochemistry.

Thin layer chromatography is performed on silica gel 60F_{254} (Merck) coated plates, developed under UV light at 254 nm or sprayed with 10% sulfuric acid. Column chromatography is usually performed on silica gel (Merck) with a particle size of 40-63 μm or Sephadex LH-20.

The solvents used are ethyl acetate ($CH_3COOC_2H_5$), acetone (CH_3COCH_3), n-hexane (n-C_6H_{14}), methanol (CH_3OH), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), and distilled water. The solvents used for plant sample extraction are all pure (Pure), when used for thin layer chromatography and column chromatography, analytical grade (PA) is used.

2.3. Extraction and isolation

The plant sample from the stem of *R. minor* was dried in the shade, then ground into powder. The dry powder (4.5 kg) was extracted with methanol at room temperature for 24 hours, then the extract was taken out and the stem residue was further extracted with methanol, this process was repeated 3 times to separate all the active ingredients in

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the residue. The obtained extracts were combined and evaporated under reduced pressure to obtain methanol residue. This residue was mixed with 1 liter of distilled water, then partitioned with ethyl acetate solvent. The extract after removing the solvent yielded 14.8 g of ethyl acetate residue. The ethyl acetate residue (14.8 g) was applied to a silica gel column to separate substances of different polarity, eluted with a gradient with the solvent system n-hexane/EtOAc (from 0-100% ethyl acetate). Results were tested by thin layer chromatography, combine solutions of the same composition and distill the solvent to obtain 9 fractions (E1-E9).

The fraction E5 (0.48 g) was purified through a silica gel column with the solvent system n-hexane/EtOAc (8/2) to obtain five sub-fractions E5.1 to E5.5. The sub-fraction E5.2 (23 mg) was purified through a normal phase silica gel column, eluted with the solvent system n-hexane/EtOAc (6/4) to obtain pure compound **1** (5.1 mg).

Fraction E6 (0.28 g) was purified through a reversed-phase silica gel column with MeOH/water (1/3) solvent system to obtain five sub-fractions E6.1 to E6.5. The sub-fraction E6.1 (90 mg) was purified through a reversed-phase silica gel column, eluted with MeOH/water (1/3) solvent system to obtain pure compound **2** (4.5 mg).

The fraction E8 (2.1 g) was purified through a Sephadex column, eluted with methanol solvent to obtain four sub-fractions E8.1 to E8.4. The sub-fraction E8.3 (0.64 g) was purified through a normal phase silica gel column, eluted with the solvent system CH_2Cl_2/EtOAc (19/1) to obtain pure compound **3** (4.2 mg).

2.4. Properties and spectral values of the isolated compounds

Protocatechuic aldehyde (1): Compound **1** was obtained as a brown solid. ESI-MS *m/z* 139,1 $[M+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H (ppm): 9.71 (1H, s, CHO), 7.33 (1H, dd, *J* = 8.0 Hz, 1.5 Hz, H-6), 7.31 (1H, d, *J* = 1.5 Hz, H-2), 6.93 (1H, d, *J* = 7.5 Hz, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): δ 193.2 (C-7), 154.8 (C-3), 147.3 (C-4), 130.9 (C-1), 126.5 (C-2), 116.3 (C-6), 115.5 (C-5).

Proanthocyanidin A1 (2): Compound **2** was obtained as a white solid. ESI-MS *m/z* 577 $[M+H]^+$.^{1}H-NMR (500 MHz, DMSO) δ (ppm): 7,15 (d, *J* = 2,5 Hz, H-10), 6,83 (d, *J* = 8,0 Hz, H-13), 7,07 (dd, *J* = 2,0; 8,0 Hz, H-14), 6,08 (d, *J* = 2,5 Hz, H-8), 5,98 (d, *J* = 2,5 Hz, H-6), 4,09 (d, *J* = 3,5 Hz, H-3) và 4,25 (d, *J* = 3,5 Hz, H-4). 6,93 (s, H-10’), 6,83 (d, *J* = 8,0 Hz, H-13’); 6,83 (dd, *J* = 2,5; 8,0 Hz, H-14’); 6,11 (s, H-6’); 4,17 (m, H-3’), 2,58 (dd, 8,5, 16,5, H-4α), 2,95 (dd, 5,5, 16,5 H-4β). ^{13}C NMR (125 MHz, DMSO) δ (ppm): 156.53 (C-7), 155.70 (C-5), 152.67 (C-8a), 145.11 (C-11) 144.29 (C-12), 130.58 (C-9), 117.83 (C-14), 115.22 (C-13), 114.68 (C10), 102.66 (C-4a), 101.41 (C-2), 98.76 (C-6), 96.76 (C-8), 65.68 (C-3), 28.34 (C-4). 154.35 (C-5’), 150.53 (C-7’), 149.97 (C-8a), 145.42 (C-11’), 144.85 (C-12’), 129.90 (C-9’), 118.66 (C-14’), 115.00 (C-10’), 114.72 (C-13’), 105.84 (C-8’), 102.30 (C-4a’), 94.58 (C-6’), 81.73 (C-2’), 66.09 (C-3’), 27.74 (C-4’).

Isoquercitrin (3): Compound **3** was obtained as a brown solid. ESI MS *m/z* 465 $[M+H]^+$. ^{1}H NMR (500 MHz, CD_3OD) δ (ppm): 7.72 (1H, d, *J* = 2.5 Hz, H-2'), 7.60 (1H, dd, *J* = 2.5, 8.5 Hz, H-6'), 6.88 (1H, d, *J* = 8.5 Hz, H-5'), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.22 (1H, d, *J* = 2.5 Hz, H-6), 5.26 (1H, d, *J* = 7.5 Hz, H-1''), 3.73 (1H, dd, *J* = 2.5, 12.0 Hz, H-6''a), 3.59 (1H, dd, *J* = 5.0, 12.0 Hz, H-6''b). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm): 179.5

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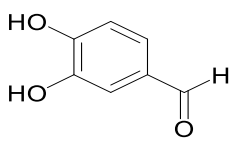
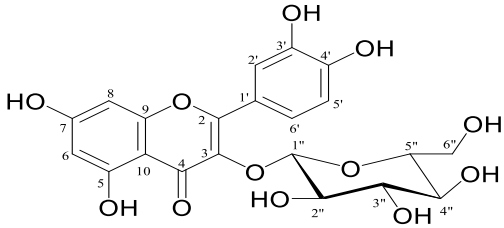
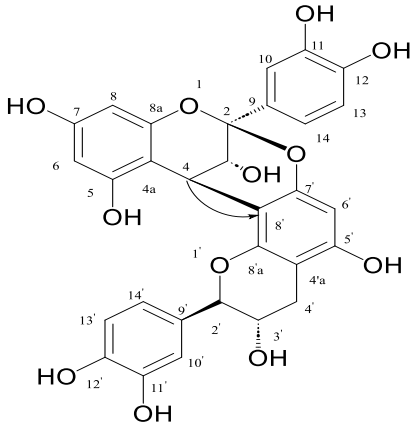
(C-4), 166.0 (C-7), 163.0 (C-5), 159.2 (C-2), 158.5 (C-9), 149.9 (C-4'), 145.9 (C-3'), 135.6 (C-3), 123.1 (C-1'), 123.2 (C-6'), 117.6 (C-2'), 116.0 (C-5'), 105.7 (C-10), 104.4 (C-1''), 99.9 (C-6), 94.7 (C-8), 78.3 (C-5''), 78.2 (C-3''), 75.7 (C-2''), 71.2 (C-4''), 62.6 (C-6'').



Protocatechuic aldehyde (**1**)



Isoquercetrin (**3**)



Procyanidin A1 (**2**)

Figure 1*. Chemical structures of the isolated compounds **1-3***

3. Results and Discussion

3.1. Determination of the structure of Protocatechuic aldehyde (1)

Compound **1** was obtained as a brown solid. The ESI-MS mass spectrum of compound **1** showed a protonated molecular ion peak at *m/z*139,1 $[M+H]^+$ corresponding to the compound with molecular formula $C_7H_6O_3$ molecular mass is 138. On the 1H-NMR spectrum, characteristic signals of the ABX system appeared with proton signals at δ_H 7.33 (1H, dd, *J* = 8.0 Hz, 1.5 Hz, H-6), 7.31 (1H, d, *J* = 1.5 Hz, H-2), 6.93 (1H, d, *J* = 7.5 Hz, H-5). There is also an aldehyde group signal at δ_H 9.71 (1H, s, CHO). Compound **1** was identified as protocatechuic aldehyde. The ^{13}C-NMR spectrum showed an aldehyde group signal at 193.2 (C-7) and 6 aromatic carbon signals at 154.8 (C-3), 147.3 (C-4), 130.9 (C-1), 126.5 (C-2), 116.3 (C-6), 115.5 (C-5). Therefore, based on the mass spectrometry and nuclear magnetic resonance spectral data, and comparison with published literature, compound **1** was identified as protocatechuic aldehyde [11]. The results show the similarities in the following table:

Table 1. *Comparison of ^{1}H-NMR and ^{13}C-NMR spectral values of compound **1** with reference*

C position	**^{1}H-NMR** δ_H (ppm)		**^{13}C-NMR** δ_C(ppm)	
	Compound 1	**Reference [11]^a**	**Compound 1**	**Reference [11]^a**
1	-	-	130.9	130.8
2	7.31 (1H, d, *J* = 1.5 Hz, H-2)	7.30 (1H, d, *J*=1.8 Hz, H-2)	126.5	126.3
3	-	-	154.8	153.7

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4	-	-	147.3	147.2
5	6.93 (1H, d, *J* = 7.5 Hz, H-5)	6.90 (1H, d, *J*=7.8 Hz, H-5)	115.5	115.7
6	7.33 (1H, dd, *J* = 8.0 Hz, 1.5 Hz, H-6)	7.29 (1H, dd, *J*=8.0, 1.8 Hz, H-6)	116.3	116.2
7 CHO	9.71 (1H, s, CHO)	9,68 (1H, s, CHO)	193.2	193.0

a) Measurement in CD_3OD solvent, 600 MHz

3.2. Determination of the structure of Proanthocyanidin A1 (2)

Compound **2** was isolated as a white solid. ESI-MS mass spectrum gave a pseudomolecular ion peak *m/z* 577 $[M+H]^+$ allowing to predict compound **2** with the molecular formula $C_{30}H_{24}O_{12}$ (M = 576).

The ^{1}H-NMR spectrum of 2 showed characteristic signals for two 5,7,11,12-tetrahydroxy-flavan-3-ol moieties. The ABX proton signals of the B ring in the flavanol skeleton appeared at δ_H 7.15 (d, *J* = 2.5 Hz, H-10), 6.83 (d, *J* = 8.0 Hz, H-13) and 7.07 (dd, J = 2.0; 8.0 Hz, H-14). The two meta-interacting protons were at δ_H 5.98 (d, *J* = 2.5 Hz, H-6) and 6.08 (d, *J* = 2.5 Hz, H-8). On the pyran ring, only 2 proton signals appeared at δ_H 4.09 (d, *J* = 3.5 Hz, H-3) and 4.25 (d, *J* = 3.5 Hz, H-4). In addition to the proton signals of the ABC ring characteristic of an epicatechin unit, protons with chemical shifts at δ_H 6.93 (s, H-10'), 6.83 (d, *J* = 8.0 Hz, H-13'); 6.83 (dd, *J* = 2.5; 8.0 Hz, H-14'); 6.11 (s, H-6'); 4.17 (m, H-3') and 2.58 (dd, 8.5, 16.5, H-4a), 2.95 (dd, 5.5, 16.5) correspond to the A'B'C' ring of a catechin unit. The units were identified as catechin and epicatechin based on the spin interaction constant between the H-3 and H-4 proton pair with *J* = 3.5 Hz corresponding to epicatechin and *J* = 8.5 Hz corresponding to catechin.

In the epicatechin unit, only 1 pair of spin interactions between protons H-3 and H-4 appeared, the carbon at position C-2 shifted up to δ_C 100.35 and the proton signal at position C-8' of the catechin unit did not appear, allowing to predict that compound 2 is an oligomeric flavonoid of catechin and epicatechin through the bridge between C-4 and C-8' and the ether bond between C-2 and C-7'. This bond was also proven through the HMBC spectrum showing the interaction between proton H-4 and carbon C-8'. The configuration at position C-4 was determined to be β because the interaction constant between H-3 and H-4 was 3.5 Hz. Through the combined spectral data and comparison with published paper [12], compound **2** was identified as Procyanidin A1. The results show the similarities in the following table:

Table 2. *Comparison of ^{1}H-NMR and ^{13}C-NMR spectral values of compound **2** with reference*

C position	**^{1}H-NMR δ_H (ppm)**		**^{13}C-NMR δ_C(ppm)**	
	Compound 2	**Reference [12]^b**	**Compound 2**	**Reference [12]^b**
2	-	-	100.34	101.41

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3	4.09 (d, *J* = 3.5 Hz, H-3)	4,07 (d, *J* = 3.5 Hz, H-3)	97.79	65.68
4	4.25 (d, *J* = 3.5 Hz, H-4)	4.24 (d, *J* = 3.5 Hz, H-4)	29.23	28.34
4a	-	-	104.06	102.66
5	-	-	156.76	155.70
6	5.98 (d, *J* = 2.5 Hz, H-6)	5.90 (d, *J* = 2.4 Hz, H-6)	98.22	98.76
7	-	-	158.14	156.53
8	6.08 (d, *J* = 2.5 Hz, H-8)	6.05 (d, *J* = 2.4 Hz, H-8)	96.78	96.76
8a	-	-	154.24	152.67
9	-	-	132.30	130.58
10	7.15 (d, *J* = 2.5 Hz, H-10)	7.13 (d, *J* = 2.4 Hz, H-10)	115.72	114.68
11	-	-	146.79	145.11
12	-	-	145.65	144.29
13	6.83 (d, *J* = 8.0 Hz, H-13)	6.79 (d, *J* = 8.4 Hz, H-13)	116.36	115.22
14	7.07 (dd, *J* = 2.0; 8.0 Hz, H-14),	7.01 (dd, *J* = 2.4; 8.4 Hz, H-14)	119.87	117.83
2’	4.60 (d, *J* = 6.5 Hz; H-2’)	4.74 (d, *J* = 7.8; Hz, H-2’)	84.49	81.73
3’	4.17 (m, H-3’)	4.16 (m, H-3’)	68.12	66.09
4’	2.58 (dd, 8.5, 16.5, H-4α) 2.95 (dd, 5,5, 16.5 H-4*β*)	2.58 (dd, 8.1, 16.5, H-4α) 2.81 (dd, 5.4, 16.5 H-4*β*)	28.96	27.74
4’a	-	-	103.16	102.30
5’	-	-	156.15	154.35
6’	6.11 (s, H-6’)	6.08 (s, H-6’)	96.58	94.58
7’	-		152.19	150.53
8’	-	-	106.80	105.84
8’a	-	-	151.42	149.97
9’	-	-	130.58	129.90
10’	6.93 (s, H-10’)	6.91 (s, H-10’)	115.77	115.00
11’	-	-	146.79	145.42
12’	-	-	146.34	144.85
13’	6.83 (d, *J* = 8.0 Hz, H-13’)	6.80 (d, *J* = 8.0 Hz, H-13’)	115.72	114.72
14’	6.83 (dd, *J* = 2.5; 8,0	6.80 (dd, *J* = 2,0; 7.8	120.73	118.66

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	Hz, H-14')	Hz, H-14')		

b) Measurement in CD_3OD solvent, 270 MHz

3.3. Determination of the structure of Isoquercitrin (3)

Compound **3** was isolated as a white solid. ESI-MS mass spectrum gave a pseudomolecular ion peak *m/z* 465 $[M+H]^+$ allowing to predict compound **3** with the molecular formula $C_{21}H_{20}O_{12}$ (M=464). The ^{1}H-NMR spectrum of compound 3, characteristic signals of flavonoid glycoside compounds appeared with signals of 2 protons at the meta position at δ_H 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.22 (1H, d, *J* = 2.5 Hz, H-6); 3 aromatic protons of the ABX system at δ_H 7.72 (1H, d, J = 2.5 Hz, H-2'), 7.60 (1H, dd, *J* = 2.5, 8.5 Hz, H-6'), 6.88 (1H, d, *J* = 8.5 Hz, H-5'). The sugar moiety signals were found with the proton anomer signal at 5.26 (1H, d, *J* = 7.5 Hz, H-1") and the oxymethylene and oxymethine group signals at 3.73 (1H, dd, *J* = 2.5, 12.0 Hz, H-6"α), 3.59 (1H, dd, *J* = 5.0, 12.0 Hz, H-6"β). ^{13}C-NMR spectrum showed 21 carbon signals, including 15 signals of flavonoid skeleton at 179.5 (C-4), 166.0 (C-7), 163.0 (C-5), 159.2 (C-2), 158.5 (C-9), 149.9 (C-4'), 145.9 (C-3'), 135.6 (C-3), 123.1 (C-1'), 123.2 (C-6'), 117.6 (C-2'), 116.0 (C-5'), 105.7 (C-10), 99.9 (C-6), 94.7 (C-8), and 6 carbon signals of sugar at 104.4 (C-1"), 78.3 (C-5"), 78.2 (C-3"), 75.7 (C-2"), 71.2 (C-4"), 62.6 (C-6"). Based on the coupling coefficient *J* = 7.5 Hz and the carbon signals, it was determined that it was β-D-glucose. Through the combined spectral data compared with reference documents, compound **3** was identified as quercetin 3-O-β-D-glucopyranose or Isoquercitrin [13][14][15].The results show the similarities in the following table:

Table 3. *Comparison of ^{1}H-NMR and ^{13}C-NMR spectral values of compound **3** with reference*

C position	**^{1}H-NMR** δ_H (ppm)		**^{13}C-NMR** δ_C(ppm)	
	Compound 3	**Reference [14][c]**	**Compound 3**	**Reference [14][c]**
2	-	-	159.2	159.2
3	-	-	135.6	135.8
4	-	-	179.5	179.6
5	-	-	163.0	163.2
6	6.22 (1H, d, *J* = 2.5 Hz, H-6)	6.20 (1H, s, H-6)	99.9	100.1
7	-	-	166.0	166.3
8	6.40 (1H, d, *J* = 2.0 Hz, H-8)	6.39 (1H, s, H-8)	94.7	94.9
9	-	-	158.5	158.6
10	-	-	105.7	105.8
1'	-	-	123.1	123.2

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2’	7.72 (1H, d, *J* = 2.5 Hz, H-2')	7.70 (1H,s, H-2')	117.6	117.7
3’	-	-	145.9	146.1
4’	-	-	149.9	150.0
5’	6.88 (1H, d, *J* = 8.5 Hz, H-5')	6.87 (1H, d, *J* = 8.3 Hz, H-5')	116.0	116.1
6’	7.60 (1H, dd, *J* = 2.5, 8.5 Hz, H-6')	7.58 (1H, d, *J* = 8.3 Hz, H-6')	123.2	123.3
1’’	5.26 (1H, d, *J* = 7.5 Hz, H-1")	5.24 (1H, d, *J* = 7.6 Hz, H-1")	104.4	104.5
2’’	3.48 (m, 1H, H-2’’)	3.48t (8.4)	75.7	75.9
3’’	3.43 (m, 1H, H-3’’)	3.43t (8.9)	78.2	78.3
4’’	3.37 (m, 1H, H-4’’)	3.35t (9.2)	71.2	71.4
5’’	3.35 (m, 1H, H-5’’)	3.22m	78.3	78.5
6’’	3.73 (1H, dd, *J* = 2.5, 12.0 Hz, H-6"α) 3.59 (1H, dd, *J* = 5.0, 12.0 Hz, H-6"β)	3.70 (1H, d, *J* = 11.8 Hz, H-6"α) 3.57 (1H, dd, *J* = 5.3, 11.8 Hz, H-6"β)	62.6	62.7

c) Measurement in CD_3OD solvent, 600 MHz

4. Conclusion

From the ethyl acetate extract of the *Rourea minor* plant collected from Ben En National Park, Thanh Hoa Province, the structure of three compounds including Protocatechuic aldehyde, Proanthocyanidin A1 and Isoquercitrin was isolated and determined. The results of the study on the chemical composition of the *Rourea minor* species are the basis for further research on other species of the *Rourea* genus in the future.

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