

SOME OF URS-AND OLEAN-SKELETON PENTACYCLIC TRITERPENES FROM DICHLOROMEHANE EXTRACT OF LEAVES OF *CHRYSOPHYLLUM ROXBURGHII*

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TÓM TẮT

MỘT SỐ HỢP CHẤT TRITERPENE NĂM VÒNG KHUNG URSANE AND OLEANANE TỪ DỊCH CHIẾT DICHLOROMETHANE CỦA LÁ LOÀI *CHRYSOPHYLLUM ROXBURGHII*

Một số các hợp chất triterpene năm vòng khung ursane và oleanane đã được phân lập từ dịch chiết dichloromethane của lá loài Sảng sáp (*Chrysophyllum roxburghii*) gồm tarexarol (1), ursolic acid (2), barbenevic acid (3), diospyric acid B (4), và serratogenic acid (5). Cấu trúc của chúng đã được xác định bởi các phương pháp phổ và sự so sánh dữ liệu phổ của chúng với các giá trị đã được công bố trong tài liệu. Đây là lần đầu tiên các hợp chất 3, 4, 5 đã được phân lập từ loài Sảng sáp cũng như chi Vú sữa (*Chrysophyllum*).

Từ khóa: *Chrysophyllum roxburghii*, triterpene, ursolic acid, barbenevic acid, diospyric acid B, serratogenic acid.

1. INTRODUCTION

Chrysophyllum roxburghii is one of the two species belonging to a Genus *Chrysophyllum* growth in Viet Nam. The genus *Chrysophyllum* comprises 40 species distributed throughout the world's tropical zones, most found in the northern south America [1]. Some of the species of the genus were used in traditional medicine and reported on the chemical constituents or biological activity. In Africa, *Chrysophyllum albidum* (*C. albidum*) was utilized in the treatment of malaria and yellow fever, skin eruptions, stomachache and diarrhea [2]. From this species, flavonoids, phenols, glycosides, terpenoids, saponins, steroids, alkaloids, tanin, anthraquinones were found [3-5].

Two alkaloids, namely eleagnine (1,2,3,4-tetrahydro-1-methyl-β-carboline) and skatole (3-methylindole) exhibited potential anti-inflammatory and antioxidant activities [2, 6]. From *C. roxburghii* fruits, glutamic acid, aspartic acid, lysine, and proline were found [7]. Recently, from *C. lacourtianum* [8] and *C. cainito* [9], pentacyclic triterpenes were reported. In our project, from ethylacetate extract of *C. roxburghii* leaves, five new oleanane triterpene glycosides chryroxosides A-E and five known compounds were reported [10]. To continue, some of urs- and olean-skeleton pentacyclic triterpenes were isolated from dichloromethane extract of the leaves of *Chrysophyllum roxburghii* including

tarexarol (**1**), ursolic acid (**2**), barbivevic acid (**3**), diospyric acid B (**4**), and serratagenic acid (**5**).

2. MATERIALS AND METHODS

2.1. Plants materials

The sample (leaves) of *Chrysophyllum roxburghii* G.Don. were gathered in Dak Lak province, Viet Nam. The taxonomic identification of the sample was identified by Dr Do Van Hai, Institute of Ecology and Biological Resources, VAST. Voucher specimen (HSB2019CL) was preserved at the Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

ESI-MS was performed using an Agilent 1100 Series LC/MSD Trap SL. NMR spectra, ¹H-NMR (500 MHz or 600 MHz), ¹³C (100 or 150 MHz), HSQC and HMBC were measured on a Bruker Avance NEO spectrometer (500MHz or 600 MHz). Column chromatography (CC) was implemented on silica gel (Kieselgel 60, Merck), or RP-18 resins (30-50 μ m), or sephadex LH-20 (Merck). Thin layer chromatography was conducted on silica gel 60 F₂₅₄ or RP-18 F_{254S} plates (Merck).

2.3. Extraction and isolation

The dry leaves of *C. roxburghii* (1.5 kg) were pulverized then sonicated in MeOH at 50 °C for 30 minutes (4.5 L \times 4). The extracts were collected and concentrated to dryness *in vacuo* by a rotary evaporator. The residue (MeOH extract, 125 g) was suspended in 500 mL of water, then thoroughly extracted with *n*-hexane, dichloromethane and ethyl acetate (EtOAc), resulting in respectively residues, *n*-hexane (CH, 57 g), dichloromethane (CD, 23 g) and EtOAc (CE, 25 g) and a water layer (MW).

The CD residue was separated by silica gel CC, eluted with gradient solvent systems of *n*-hexane/EtOAc (5/1, v/v), *n*-

Hexane/EtOAc (3/1, v/v) CH₂Cl₂/EtOAc (5/1, v/v), CH₂Cl₂/EtOAc (1/1, v/v) and EtOAc (100%) to give five fractions (fr. CD1-CD5), respectively.

Subfraction CD2 (2.7 g) was subjected to silica gel CC and eluted with *n*-hexane/EtOAc (5/1, v/v) to give four fractions (CD2A-CD2D). Compound **1** (9 mg) was obtained from fraction CD2B (300 mg) by a column chromatography on silica gel eluting with *n*-hexane/EtOAc (6/1, v/v).

Subfraction CD3 (7.5 g) was subjected to silica gel CC and eluted with CH₂Cl₂/EtOAc (5/1, v/v) to obtain five fractions (CD3A-CD3E). Fraction CD3A (1.6 g) was separated on a silica gel column using CH₂Cl₂/EtOAc (8/1, v/v) as eluent to yield four fractions (CD3A1-CD3A4). Fraction CD3A2 (57 mg) was chromatographed on an RP-18 column using an isocratic elution of MeOH/H₂O (1/3, v/v), then was further purified by CC on a sephadex LH-20 using MeOH as an eluted solvent to give compound **2** (6.0 mg). Compound **3** (6.0 mg) was yielded from fraction CD3A3 (35 mg) by separation on an RP-18 column using MeOH/H₂O (2/5, v/v) as an eluted solvent, then purified on a sephadex LH-20 column eluting with MeOH.

Subfraction CD5 (8.5 g) was separated on a silica gel column eluting with CH₂Cl₂/EOAc /MeOH (20/10/1, v/v/v) to generate five fractions CD5A-CD5E). Fraction CD5B (1.2 g) was subjected on a RP-18 silica gel column eluting with MeOH/H₂O (1/1, v/v) to obtain five fractions (CD5B1- CD5B5). From fraction CD5B3 (65 mg), compound **4** (6.0 mg) was afforded by a sephadex LH-20 column chromatography eluting with MeOH. Similaly, compound **5** (5.0 mg) was yielded from fraction CD5B4 (57 mg) by a sephadex LH-20 column chromatography eluting with MeOH.

Compound **1** (Taxterol): White powder. ESI-MS: m/z 427.1 [M+H]⁺. ¹H-NMR (CD₃OD, 500 MHz): δ_H 0.80 (3H, *s*, H-25), 0.82 (3H, *s*, H₃-30), 0.90 (3H, *s*, H₃-28), 0.91 (3H, *s*, H₃-27), 0.93 (3H, *s*, H₃-24), 0.95 (3H, *s*, H₃-29), 0.98 (3H, *s*, H₃-23), 0.98 (3H, *s*, H₃-23), 1.09 (3H, *s*, H₃-26), 1.92 (1H, *dd*, *J* = 2.5, 14.5 Hz, H-1a), 2.03 (1H, *dt*, *J* = 3.0, 12.5 Hz, H-7), 3.19 (1H, *dd*, *J* = 4.5, 11.0 Hz, H-3), 5.34 (1H, *dd*, *J* = 3.0, 8.0 Hz, H-15); and ¹³C-NMR (CD₃OD, 125 MHz) see Table 1.

Compound **2** (Ursolic acid): ESI-MS: m/z 457.3 [M+H]⁺; ¹H NMR (CD₃OD, 600 MHz): δ_H 0.77 (1H, *brd*, *J* = 11.0 Hz, H-5), 0.87 (3H, *s*, H₃-26), 0.80 (3H, *s*, H₃-24), 0.79 (3H, *d*, *J* = 6.5 Hz, H₃-29), 0.98 (3H, *s*, H₃-25), 0.99 (3H, *d*, *J* = 6.5 Hz, H₃-30), 1.00 (3H, *s*, H₃-23), 2.05 (1H, *td*, *J* = 4.5, 13.5 Hz, H-16), 1.14 (3H, *s*, H₃-27), 2.22 (*brd*, *J* = 11.0 Hz, H-18), 3.18 (1H, *dd*, *J* = 4.5, 11.0 Hz, H-3), 5.25 (1H, *t*, *J* = 3.5 Hz, H-12), and ¹³C NMR (CDCl₃, 125 MHz) see Table 1.

Compound **3** (Barbnevic acid): White powder. ESI-MS: m/z 489.2 [M+H]⁺. ¹H-NMR (CD₃OD, 500 MHz): δ_H 0.76 (3H, *s*, H₃-26), 0.94 (3H, *s*, H₃-25), 0.95 (3H, *d*, *J* = 8.5 Hz, H₃-30), 0.95 (3H, *d*, *J* = 8.5 Hz, H₃-30), 1.06 (3H, *s*, H₃-23), 1.21 (3H, *s*, H₃-29), 1.38 (3H, *s*, H₃-27), 2.52 (1H, *brs*, H-18), 2.61 (1H, *td*, *J* = 4.5, 13.0 Hz, H-16a), 3.70 and 3.42 (2H, 2 \times *d*, each *J* = 11.0 Hz, H₂-H₂-24), 3.79 (1H, *brs*, H-3), 5.31 (1H, *brs*, H-12); and ¹³C-NMR (CD₃OD, 125 MHz) see Table 1.

Compound **4** (Diospyric acid B): White powder. ESI-MS: m/z 503.2 [M+H]⁺. ¹H-NMR (CD₃OD, 500 MHz): δ_H 0.84 (3H, *s*, H₃-26), 0.91 (3H, *s*, H₃-25), 0.95 (3H, *d*, *J* = 7.0 Hz, H₃-30), 1.2 (3H, *s*, H₃-29), 1.28 (3H, *s*, H₃-23), 1.39 (3H, *s*, H₃-27), 2.53 (1H, *brs*, H-18), 2.53 (1H, *brs*, H-18), 2.59 (1H, *td*, *J* = 4.5, 8.5 Hz, H-16a), 4.00 (1H, *t*, *J* = 2.5 Hz, H-3), 5.31 (1H, *t*, *J* = 3.5 Hz, H-12); and ¹³C-NMR (CD₃OD, 125 MHz) see Table 1.

Compound **5** (serratagenic acid): White powder. ESI-MS: m/z 487.3 [M+H]⁺. ¹H-NMR (CDCl₃, 500 MHz): δ_H (ppm) 0.80 (3H, *s*, H-24), 0.85 (3H, *s*, H-26), 0.97 (3H, *s*, H-25), 1.00 (3H, *s*, H-23), 1.19 (3H, *s*, H-27), 1.27 (3H, *s*, H-30), 2.05 (1H, *td*, *J* = 3.5, 13.0 Hz, H-16), 2.18 (1H, *dd*, *J* = 13.5, 14.0 Hz, H-19), 2.91 (1H, *dd*, *J* = 4.0, 14.0 Hz, H-18), 3.18 (1H, *dd*, *J* = 5.0 Hz, 11.5 Hz, H-3), 5.31 (1H, *t*, *J* = 3.5 Hz, H-12). ¹³C-NMR (125 MHz, CDCl₃): Table 1.

3. RESULTS AND DISCUSSIONS

Compound **1** was a white amorphous powder. On the ESI-MS spectrum of **1**, the pseudomolecular ion was at m/z 426 [M]⁺ combining with ¹³C NMR of **1** (Table 1), which suggested a molecular formula C₃₀H₄₈O₃ (M = 427) for **1**. On the ¹H NMR of **1**, signals of an olean-skeleton pentacyclic triterpene were observed including eight methyl groups at 1.09 (3H, *s*, H₃-26), 0.98 (3H, *s*, H₃-23), 0.95 (3H, *s*, H₃-29), 0.93 (3H, *s*, H₃-24), 0.91 (3H, *s*, H₃-27), 0.90 (3H, *s*, H₃-28), 0.82 (3H, *s*, H₃-30) and 0.80 (3H, *s*, H-25); an olefinic proton at δ_H 5.34 (1H, *dd*, *J* = 3.0, 8.0 Hz, H-15), 3.19 (1H, *dd*, *J* = 4.5, 11.0 Hz, H-3ax), 2.03 (1H, *dt*, *J* = 3.0, 12.5 Hz, H-7), 1.92 (1H, *dd*, *J* = 2.5, 14.5 Hz, H-1a). The coupling constant of proton H-3 (*J* = 11.0 Hz) was assigned to a 3 β -OH group. On the ¹³C NMR spectrum of **1**, signals of 30 carbon were observed, including eight methyls at δ_C 28.0 (C-23), 15.4 (C-24, C-25), 25.9 (C-26), 21.3 (C-27), 29.8 (C-28), 33.2 (C-29), and 29.9 (C-30); one oxygenated methine at δ_C 79.1 (C-3); one olefinic methine at δ_C 116.9 (C-15), and three saturated methines at δ_C 55.6 (C-5), 49.3 (C-9) and 48.8 (C-18); ten methylenes at δ_C 37.8 (C-1), 27.1 (C-2), 18.8 (C-6), 41.3 (C-7), 17.5 (C-11), 33.7 (C-12), 37.7 (C-16), 36.7 (C-19), 33.1 (C-21) and 35.1 (C-22); and seven quaternary carbons at δ_C 39.8 (C-4),

39.0 (C-8), 37.6 (C-10), 38.0 (C-13), 158.1 (C-14), 35.8 (C-17) and 28.8 (C-20). The HMBC correlations (Figure 2) between H-15 (δ_H 5.34) and C-13/C-16/C-8/C-17 revealed the positions of the olefinic group 14-C=CH-15 and this was also confirmed by the HMBC correlations from the methyl protons (26-CH₃ and 27-CH₃) to C-14. The HMBC correlations between H-3 α and C-2/C-23, H₃-23/H₃-24 and C-4 also suggested the positions of the groups 3 β -OH. From the above analysis and comparison of the ESI-MS and NMR spectral data of **1** with those of taraxerol in literature (Table 1) [11], compound **1** was identified as taraxerol (3 β -D-Friedoolean-14-en-3-ol). Taraxerol was isolated from some plants as *Barringtonia maunwongyathiae* [11], *Kydia glabrescens* [12], *Lumnitzera racemosa* [13]....

Compound **2** was obtained as a white amorphous powder. The ¹H and ¹³C-NMR (Table 1) of **2** indicated signals very similar to the signals of ursolic acid,

which was isolated from *Sarcosperma kontumense* [14]. Seven methyl groups including two doublet methyls at δ_H 0.90 and δ_H 0.99 [2 \times (d, J = 6.5 Hz), 29-CH₃ and 30-CH₃], one oxymethine group at δ_H 3.06 (1H, dd, J = 4.5, 11.0 Hz, H-3ax) and one olefinic proton at δ_H 5.25 (1H, t, J = 3.5 Hz, H-12) were observed on the ¹H NMR spectrum of **2**. On the ¹³C-NMR of **2**, signals of 30 carbons including one carboxylic group, seven methyls, seven methines, nine methylenes and six quaternary carbons were indicated. The carboxylic group was identified by the HMBC (Figure 2) correlation between H-16 at δ_H 2.05 (1H, td, J = 4.5, 13.5 Hz) and the carboxylic group 28-COOH at δ_C 182.3. The ESI-MS, ¹H and ¹³C-NMR spectral data closely matched those of ursolic acid as reported in the literature [14]. Thus, the compound **2** was determined to be ursolic acid (3 β -hydroxyurs-12-ene-28-oic acid).

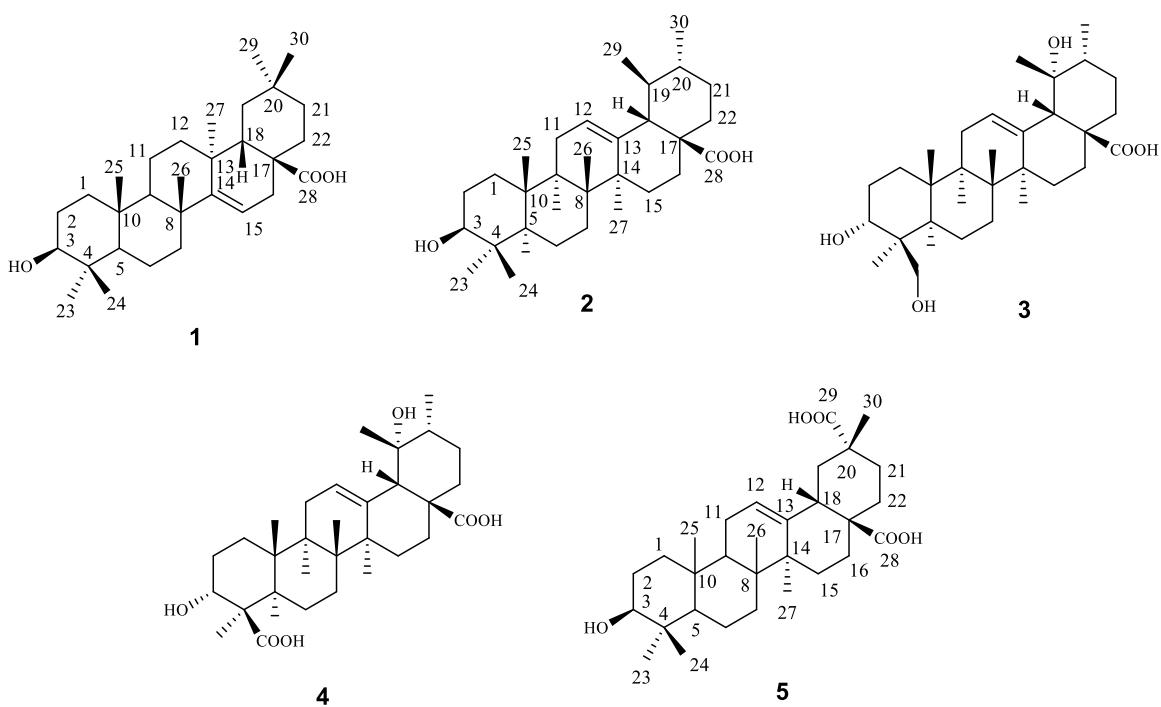


Figure 1: Chemical structure of compounds **1-5**

Table 1: ^{13}C -NMR spectral data of compounds **1-5** and references

C	1		2		3		4		5	
	$\delta_{\text{C}}^{a,b}$ [11]	$\delta_{\text{C}}^{a,c}$	$\delta_{\text{C}}^{d,e}$ [14]	$\delta_{\text{C}}^{c,d}$	$\delta_{\text{C}}^{g,h}$ [15]	$\delta_{\text{C}}^{c,d}$	$\delta_{\text{C}}^{g,c}$ [16]	$\delta_{\text{C}}^{c,d}$	$\delta_{\text{C}}^{c,d}$ [17]	$\delta_{\text{C}}^{c,d}$
1	37.7	37.8	40.0	40.0	34.0	34.2	35.1	35.0	38.5	38.5
2	27.2	27.1	27.9	27.8	26.5	26.1	28.0	27.2	26.5	26.5
3	79.1	79.1	79.7	79.7	69.9	71.3	71.0	71.7	78.3	78.4
4	38.8	39.8	39.8	39.8	44.0	44.0	48.7	48.8	38.5	38.5
5	55.5	55.6	56.8	56.7	50.2	50.7	50.0	50.2	55.3	55.4
6	18.8	18.8	19.5	19.5	19.2	19.6	21.2	21.2	18.1	18.1
7	41.3	41.3	34.4	34.3	34.1	34.5	34.5	34.5	32.6	32.6
8	39.0	39.0	40.8	40.8	40.6	39.0	40.9	41.1	39.2	39.3
9	49.3	49.3	49.0	49.0	47.8	48.7	47.5	47.6	47.7	47.4
10	38.0	37.6	38.2	38.3	37.5	38.0	38.7	38.7	36.8	36.8
11	17.5	17.5	24.4	24.4	24.3	24.8	24.6	24.7	23.2	23.2
12	33.7	33.7	126.9	126.9	128.1	129.5	128.7	129.7	123.0	123.1
13	37.6	38.0	139.7	139.6	140.0	139.9	140.3	139.9	143.1	143.1
14	158.1	158.1	43.3	43.2	42.1	42.7	42.7	42.8	41.5	41.5
15	116.9	116.9	29.3	29.2	29.3	29.6	29.6	29.6	27.3	27.4
16	37.7	37.7	25.4	25.3	26.4	26.7	26.8	26.7	22.8	22.8
17	35.8	35.8	48.9	48.8	48.3	48.8	48.7	49.2	46.0	46.1
18	48.8	48.8	54.4	54.4	54.6	55.1	55.1	55.2	40.2	40.2
19	36.7	36.7	40.5	40.4	72.7	73.6	73.1	73.6	39.8	39.8
20	28.8	28.8	40.4	40.4	42.4	43.1	42.8	43.1	41.7	41.8
21	33.1	33.1	31.8	31.8	27.0	27.3	27.3	27.3	28.2	28.2
22	35.1	35.1	38.1	38.1	38.5	41.2	38.8	39.0	31.3	31.3
23	28.0	28.0	28.8	28.8	23.7	22.8	25.7	24.9	27.4	27.4
24	15.5	15.4	16.4	16.5	65.7	66.3	181.1	181.4	15.0	15.0
25	15.4	15.4	16.0	16.0	16.0	16.1	14.1	13.7	14.6	14.7
26	25.9	25.9	17.9	17.8	17.2	17.4	17.7	17.4	16.4	16.4
27	21.3	21.3	24.1	24.1	24.6	24.8	24.9	24.7	25.1	25.0
28	29.8	29.8	#181.5	#182.3	180.7	182.3	181.1	182.3	180.0	180.0
29	33.4	33.2	17.7	17.6	27.1	27.1	27.5	27.1	181.0	181.1
30	29.9	29.9	21.6	21.5	16.8	16.6	17.1	16.6	18.5	18.5

Recorded in ^a CDCl_3 , ^b100MHz, ^c125 MHz, ^d CD_3OD , ^e 150 MHz, ^g pyridine-*d*₅, ^h90MHz [#]Signal identified by HMBC spectrum.

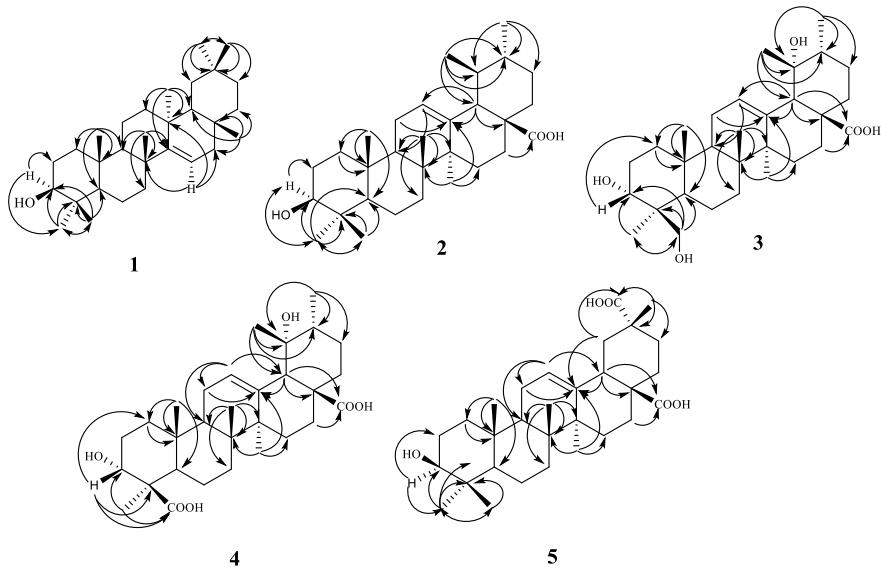


Figure 2: Key HMBC interactions of compounds **1-5**

Compound **3** was a white amorphous powder. The ^1H and ^{13}C -NMR spectra of **3** exhibited similar signals to those of **2**, a ursane skeleton pentacyclic triterpene, except for the addition of a new hydroxymethylene (24- $\text{CH}_2\text{-OH}$) at δ_{H} 3.70 and 3.41 ($2 \times d$, each $J = 11.0$ Hz, $\text{H}_{2-24}/\delta_{\text{C}}$ 66.3 (C-24), and a hydroxy group (19-OH) at δ_{C} 73.6 (C-19). A characteristic broad singlet of H-18 (δ_{H} 2.52) suggested the presence of the 19-O substituted urs-12-ene skeleton. Due to the anisotropic effect of a 19-OH group, signals of H-18 and Ha-16 (td) were downfield shifted at δ_{H} 2.52 and δ_{H} 2.61 respectively. The NMR spectra also showed a trisubstituted olefinic double bond observed at δ_{H} 5.31 (brs, H-12)/ δ_{C} 129.5 (C-12)/ δ_{C} 139.9 (C-13) and an oxymethine at δ_{H} 3.79 (brs, H-3 β)/ δ_{C} 71.3 (C-3). The small proton coupling constant ($J_{\text{H-2/H-3}}$ is *brs*) suggested the 3 α -OH group. The ^{13}C NMR, DEPT and HSQC spectra of **3** revealed 30 carbons, including one carboxylic group, six methines, ten methylenes, six methyls, seven non-proton quaternary carbons. In HMBC spectrum of **3**, the HMBC correlations [Figure 2] between Ha-24 and C-3/C-23; H₂-24 and

C-3/C-4/C-5/C-23; between H-18 and C-28/C-12/C-13/C-17/C-19/C-16/C-29 showed the locations of group 24-methylene and group 19-hydroxyl, respectively. The ESI-MS spectrum of **3** showed a pseudomolecular ion peak at m/z 488 $[\text{M}]^+$, suggesting the molecular formula of **3** as $\text{C}_{30}\text{H}_{48}\text{O}_5$. All NMR spectral data of **3** were in good agreement with those of barbnevic acid in literature (Table 1) [15]. Thus, the compound **3** was deduced to be barbnevic acid ($3\alpha,19\alpha$ -24-trihydroxyurs-12-ene-28-oic acid).

The ^1H and ^{13}C -NMR spectra of **4** showed very similar signals to those of **3** except for the exchange of 24- CH_2OH group to 24-COOH. The ^{13}C NMR spectra of **3** showed the signals of 30 carbons, including two carboxylic groups at δ_{C} 182.3 (C-28) and δ_{C} 181.4 (C-23); one oxymethine at δ_{C} 71.7 (C-3); six methyls at 27.1 (C-29), 24.9 (C-23), 24.7 (C-27), 17.4 (C-26), 16.6 (C-30) and 13.7 (C-25); six methines at δ_{C} 129.7 (C-12), 71.7 (C-3), 55.2 (C-18), 50.2 (C-5), 47.6 (C-9) and 43.1 (C-20); nine methylenes at δ_{C} 35.0 (C-1), 27.2 (C-2), 21.2 (C-6), 34.5 (C-7), 24.7 (C-11), 29.6 (C-15), 26.7 (C-16), 27.3 (C-21), and 39.0

(C-22); 48.8 (C-4), 41.1 (C-8), 38.7 (C-10), and six non-proton carbons at δ_C 48.8 (C-4), 41.1 (C-8), 38.7 (C-10), 139.9 (C-13), 42.8 (C-14), 49.2 (C-17), and 73.6 (C-19). The 1H NMR spectra of **4** showed characteristic signals of the 19-O substituted urs-12-ene skeleton including an oxymethine proton at 4.00 (*t*, 2.5 Hz, H-3 β), an olefinic proton at 5.31 (*t*, 3.5 Hz, H-12 β), a methylene proton at 2.59 (*td*, *J* = 4.5, 8.5 Hz, H-16a), a methin proton at 2.53 (*brs*, H-18), and six methyl at 1.39 (*s*, H₃-27), 1.28 (*s*, H₃-23), 1.21 (*s*, H₃-29), 0.95 (*d*, *J* = 7.0 Hz, H₃-30), 0.91 (*s*, H₃-25), 0.84 (*s*, H₃-26). The HMBC correlations of **4** [Figure 2] between methyl protons 23-CH₃ and 24-COOH, H-16/H-18 and 28-COOH, and between H-12/H-18/H-27 and 13-C=CH, H-18 and 12-CH=C-13 suggested positions of 23-COOH, 28-COOH and 12-CH=C-13, respectively. The ESI-MS of **4** showed a pseudomolecular ion peak at *m/z* 503 [M+H]⁺, suggesting the molecular formula of **4** C₃₀H₄₆O₆ (M = 502). The MS, 1H and ^{13}C -NMR spectral data closely matched those of diospyric acid B as reported in the literature [16]. Thus, compound **4** was determined to be diospyric acid B (3 α ,19 α -dihydroxyurs-12-ene-24,28-dioic acid).

The 1H and ^{13}C -NMR (Table 1) of **5** showed the signals of olean-12-ene-28-oic skeleton with the addition of a carboxylic acid. The 1H -NMR spectrum revealed six methyl singlets, an olefinic proton, an oxymethine proton. The coupling constant of proton H-3 (*J* = 11.5 Hz) confirmed configuration of 3 β -OH group. The ^{13}C -NMR spectrum gave the signals of 30 carbons including two carboxylic groups at δ_C 181.1 (C-29) and δ_C 180.0 (C-28), an trisubstituted double at δ_C 123.1 (C-12) and δ_C 143.1 (C-13), an carbinol carbon at δ_C 78.4. The HMBC spectrum of **5** [Figure 2] showed the correlations between protons of 30-CH₃ to C-29, C-20, C-19, and C-21. The

1H and ^{13}C -NMR data of compound **5** was close to those of serratagenic acid, a compound isolated from flowers of *Macropanax membranifolius*, which was reported in literature [17]. Thus, compound **5** was deduced to be serratagenic acid (3 α -hydroxyolean-12-ene-28,29-dioic acid).

4. CONCLUSION

From the dichloromethane extract of the leaves of *C. roxburghii*, collected in Dak Lak province, two olean-skeleton pentacyclic triterpenes including tarexarol (**1**) and serratagenic acid (**5**) and three urs-skeleton pentacyclic triterpenes including ursolic acid (**2**), barbnevic acid (**3**), diospyric acid B (**4**), were isolated. Their structures were elucidated by spectroscopic methods and compared to their spectral data with reported values. This was the first time that compounds (**3**, **4**, **5**) were isolated from the *Chrysophyllum roxburghii* as well as from genus *Chrysophyllum*.

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REFERENCES

1. <http://www.worldfloraonline.org/taxon/wfo-4000008232>.
2. Idowu T.O., Iwalewa E.O., Aderogba, M.A., Akinpelu B.A. and Ogundaini A.O.. Antinociceptive, (2006). Anti -inflammatory and Antioxidant activities of Eleagnine: an alkaloid isolated from seed cotyledon of *C. albidum*. *J. Biol. Sci.*, **6** (6), 1029 - 1034.
3. Ushie, O. A.; Adamu, H. M.; Abayeh, O. J.; Chindo, I. Y.; Lawal, U., (2014). Phytochemical screening of (*Chrysophyllum albidum*) leaf extracts. *J. Appl. Chem*, **2**(2), 40-46.

4. Ogbuagu, M. N.; Chikezie, O., (2010). The phytochemical screening and anti-microbial activities of the aqueous extracts of the seed kernels of *Chrysophyllum albidum* and *Carica papaya*. *J. Chem. Soc. Nigeria*, **35(1)**, 5-7.
5. Moses S Owolabi, AA Omowonuola, Oladipupo A Lawal, Lajide, Labunmi, Noura S Dosoky, Jasmine T Collins, Ifedayo Victor Ogungbe and William N Setzer, (2017). Phytochemical and bioactivity screening of six Nigerian medicinal plants, *J Pharmacogn Phytochem*, **6(6)**, 1430-1437.
6. Idowu T. O.; Onawunmi, G. O.; Ogundaini, A. O.; Adesanya, S. A., (2003). Antimicrobial constituents of *Chrysophyllum albidum* seed cotyledons. *Nigerian J. Nat. Prod. Med*, **7**, 33-36.
7. Barthakur NN, Arnold NP., (1991). A chemical analysis of the Indian star apple (*Chrysophyllum roxburghii*) fruit. *J Food Compos Anal.*, **4(4)**, 354-359.
8. Talla RM, Jouda JB, Mawabo IK, Tegasne C, Happi GM, Kapche GDWF, Lenta BN, Sewald N, Wandji J., (2023). One new constituent from the stem bark of *Chrysophyllum lacourtianum* De Wild. (Sapotaceae). *Nat Prod Res.*, **37(7)**, 1067-1073.
9. Herrera-España AD, Aguiar-Pech JA, Alvarez-Sánchez ME, Arana-Argáez VE, Palomar-Gómez CK, Jiménez-Ross AG, Cáceres-Castillo D, Carballo RM, Torres-Romero JC., (2022). Lupeol acetate isolated from *Chrysophyllum cainito* L. fruit as a template for the synthesis of N-alkylarylsulfonamide derivatives and their synergistic effects with metronidazole against *Trichomonas vaginalis*. *Nat. Prod. Res.*, **36(21)**, 5508-5516.
10. Pham Thi Hang, Nguyen Thi Hue, Nguyen Hoang Nam, Nguyen Le Tuan, Pham Van Cuong, Nguyen Quoc Vuong, Nguyen Huy Hoang, Bui Huu Tai & Phan Van Kiem, (2024). Chryroxosides A-E: five new triterpene saponins from the leaves of *Chrysophyllum roxburghii* G. Don. and their cytotoxic activity. *Nat. prod. Res.*, **38(5)**, 735-743.
11. Aranya Jutiviboonsuk, Hong-Jie Zhang, Tamara P. Kondratyuk, Angkana Herunsalee, Wongsatit Chaukul, John M. Pezzuto, Harry H.S. Fong & Nuntavan Bunyapraphatsara, (2007). Isolation and Characterization of Cancer Chemopreventive Compounds from Barringtonia maunwongyathiae. *Pharmaceutical Biology*, **45(3)**, 185-194.
12. Nguyễn Quyết Chiến, Nguyễn Văn Hùng, Trần Văn Sung, (2004). Nghiên cứu thành phần hóa học cây *Kydia glabrescens*. *Vietnam J. Chem.*, **42 (1)**, 71 - 75.
13. Châu Ngọc Địệp, Nguyễn Hoài Nam, Lê Đức Đạt, Vũ Anh Tú, Ninh Thị Ngọc, Nguyễn Phương Thảo, Phan Thị Thanh Hương, Nguyễn Xuân Cường, Ninh Khắc Bản, Phan Văn Kiệm và Châu Văn Minh, (2004). Các hợp chất tritecpen và tritecpen glycosit phân lập từ cây Cóc trắng (*Luminitzera racemosa*). *Vietnam J. Chem.*, **50(5A)** 219-223.
14. Nguyen Quoc Vuong, Nguyen Thi Hue, Pham Thi Hang, Nguyen Hoang Nam, Nguyen Le Tuan, Le Nguyen Thanh, Pham Van Cuong, Duong Thi Hai Yen, Nguyen Xuan Nghiem, Bui Huu Tai, Phan Van Kiem, (2023). Secondary metabolites from *Sarcosperma kontumense*. *VJST*, **61 (6)**, 943-952.

15. Bao-Ning Su, Young-Hwa Kang, Rosa Elena Pinos, Bernard D. Santarsiero, Andrew D. Mesecar, D. Doel Soejarto, Harry H.S. Fong, John M. Pezzuto, A., (2003). Douglas Kinghorn. Isolation and absolute stereochemistry of coussaric acid, a new bioactive triterpenoid from the stems of *Coussarea brevicaulis*. *Phytochemistry*, **64**, 293–302.
16. Phuong Thien Thuong, Chul Ho Lee, Trong Tuan Dao, Phi Hung Nguyen, Wan Gi Kim, Sang Jun Lee and Won Keun Oh, (2008). Triterpenoids from the Leaves of *Diospyros kaki* (Persimmon) and Their Inhibitory Effects on Protein Tyrosine Phosphatase 1B. *J. Nat. Prod.*, **71**, 1775–1778.
17. Pham Thi Hang, Vu Van Chien, Nguyen Thi Hue, Nguyen Hoang Nam, Vu Thi Hue, Pham Van Cuong, Le Nguyen Thanh, Nguyen Quoc Vuong, Diep Thi Lan Phuong, (2023). Chemical constituents of *Macropanax membranifolius*. *Tạp chí phân tích Hóa, Lý và Sinh học*, **29 (1)**, 6-12.