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Chemical Constituents of *Excoecaria agallocha* L. (Euphorbiaceae) Growing in Vietnam

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Three known triterpenoids, taraxer-14-ene-3-one (**1**), taraxer-14-ene-3α-ol (**2**), taraxer-14-ene-3β-ol (**3**) and one known acylated steroid glucoside, stigmast-5-en-3β-yl (6'-*O*-heptadecanoyl-*β*-D-glucopyranoside) (**4**) were isolated from the *n*-hexane fraction of methanol extract of the leaves of *Excoecaria agallocha* L. collected in the Thai Binh province,Vietnam. Their structures were elucidated on the basis of spectroscopic data and by comparison with their spectral data reported in literature. This is the first isolation of compound **4** from this species.

Key words: *Excoecaria agallocha* L.; triterpenoid; taraxer-14-ene-3-one; taraxer-14-ene-3α-ol; taraxer-14-ene-3β-ol; acylated steroid glucoside; stigmast-5-en-3β-yl (6'-*O*-heptadecanoyl-β-D-glucopyranoside); methanol; *n*-hexane fraction

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Excoecaria agallocha L. (*E. agallocha*) (Euphorbiaceae) is a small mangrove tree found extensively on seashores and edge-mangroves throughout tropical Africa, Asia, and northwest Australia. The plant has been used in traditional medicine in many countries as Thailand, China, India etc. against rheumatism, leprosy, epilepsy, paralysis, conjunctivitis, dermatitis and as a dart and fish poison [1]. The plant has been reported to have various biological activities such as anti HIV, antimicrobial, antioxidant, antiulcer, and antifilarial properties [2].

In Vietnam, this tree grows widely in the tidal forests and swamps in the coastal areas. It is traditionally used by the local people as folk medicine to treat epilepsy, ulcers etc. [3]. There is no report on the phytochemicals of this plant collected in Vietnam. Hence, the present phytochemical study of the *n*-hexane fraction of methanol extract of the leaves of this plant led to the isolation and structure characterization of taraxer-14-ene-3-one (**1**), taraxer-14-ene-3*α*-ol (**2**), taraxer-14-ene-3*β*-ol (**3**), and stigmast-5-en-3*β*-yl (6'-*O*-heptadecanoyl-*β*-D-glucopyranoside) (**4**). Compound **4** was isolated for the first time from this species.

EXPERIMENTAL SECTION

General Procedure

Melting points (m.p.) were determined on a Botius melting point apparatus (Germany), ESI-LC/MS/MS-Xevo TQMS. The NMR (^{1}H-NMR, ^{13}C-NMR, and DEPT) spectra were recorded on a Bruker Avance 500 MHz spectrometer with tetramethylsilane (TMS) as a zero internal standard. Silica gel (0.040–0.063 mm, Merck, Germany) was used for column chromatography (CC). The TLC was performed on Merck pre-coated TLC DC-Alufolien silica gel 60F_{254} (Merck). The plates were dipped in vanillin-H_2SO_4 and heated (110°C for 5 min.) for visualization.

Plant Material

The leaves of *E. agallocha* (Euphorbiaceae) were collected in the Thai Binh province, Vietnam, in 2013. The species was identified by Prof Tran Huy Thai, Institute of Ecology and Biological

Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (No. BK-B03) has been deposited at the Hanoi University of Science and Technology, Vietnam. The leaves were air-dried and powdered after collection.

Extraction and Isolation

The dried and powdered leaves of *E.agallocha* (5.7 kg) were extracted with 95% aqueous MeOH at room temperature. The MeOH extract was evaporated *in vacuo*, and the residue was partitioned with *n*-hexane followed by ethyl acetate (EtOAc). The *n*-hexane extract (50 g) was subjected to silica gel CC with solvents of increasing polarity ranging from (0–100% EtOAc in *n*-hexane) to give 7 fractions. Fraction I [6.6 g, 5% EtOAc in *n*-hexane] was subjected to silica gel CC with a mixture of *n*-hexane/EtOAc (65:1, v/v) to afford 3 subfractions: I.1, I.2 and I.3. The fractions I.1 and I.3 were further purified by crystallization in *n*-hexane to give taraxer-14-en-3-one (**1,** 150 mg) and 14-taraxerene-3*a*-ol (**2,** 96 mg). Fraction II [12.4 g, 10% EtOAc in *n*-hexane] was subjected to silica gel CC with a mixture of *n*-hexane/EtOAc (50:1, v/v) followed by crystallization in CH_2Cl_2 to yield taraxer-14-ene-3*β*-ol [(**3**), 580 mg)]. Fraction VII [2.2 g, 50% EtOAc in *n*-hexane] was subjected to silica gel CC with a mixture of CH_2Cl_2/MeOH (50:1, v/v). It resulted in stigmast-5-en-3*β*-yl (6'-*O*-heptadecanoyl-*β*-D-glucopyranoside) [(**4**), 34.6 mg].

Taraxer-14-ene-3-one (**1**). These are white crystals: m.p. 241–243°C (lit.[7] m.p. 242–245°C); R_f 0.52 on silica gel (*n*-hexane/EtOAc, 20:1, v/v); ESI-MS: *m/z* 425 $[M + H]^+$ ($C_{30}H_{49}O$); ^1H NMR ($CDCl_3$, 500 MHz) *δ* ppm: 5.55 (1H, *dd*, *J* = 8.0, 3.5 Hz, H-15), 2.56 (1H, *ddd*, *J* = 16.0, 7.6, 3.5 Hz, H_a-2), 2.32 (1H, *ddd*, *J* = 16.0, 6.5, 3.5 Hz, H_b-2), 1.08 (3H, *s*, H-23), 1.06 (3H, *s*, H-24), 1.09 (3H, *s*, H-25), 1.14 (3H, *s*, H-26), 0.92 (3H, *s*, H-27), 0.83 (3H, *s*, H-28), 0.91 (3H, *s*, H-29), 0.95 (3H, *s*, H-30); ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* ppm given in Table 1.

Taraxer-14-ene-3α-ol (**2**). This white needle-shaped crystals, m.p. 268–269°C (lit.[8] m.p. 267–269°C), R_f 0.33 (*n*-hexane/EtOAc, 20:1, v/v); ESI-MS: *m/z* 427 $[M + H]^+$ ($C_{30}H_{51}O$); ^1H-NMR (500 MHz, $CDCl_3$) *δ* ppm: 5.52 (1H, *dd*, *J* = 8.0, 3.5 Hz, H-15), 3.40 (1H, *br s*, H-3), 0.94 (3H, *s*, H-23), 0.82 (3H, *s*, H-24), 0.94 (3H, *s*, H-25), 1.09 (3H, *s*, H-26), 0.91 (3H, *s*, H-27), 0.86 (3H, *s*, H-28), 0.91 (3H, *s*, H-29), 0.93 (3H, *s*, H-30); ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* ppm are given in Table 1.

Taraxer-14-ene-3β-ol (**3**). This colourless plates, m.p. 279–280°C (lit.[9] m.p. 278–279°C); R_f 0.61 on silica gel (*n*-hexane/EtOAc, 10:1, v/v); ESI-MS: *m/z* 427 $[M + H]^+$ ($C_{30}H_{51}O$); ^1H-NMR (500 MHz, $CDCl_3$) *δ* ppm: 5.54 (1H, *dd*, *J* = 8.0, 3.5 Hz, H-15), 3.19 (1H, *br s*, H-3), 0.93 (3H, *s*, H-23), 0.80 (3H, *s*, H-24), 0.91 (3H, *s*, H-25), 0.97 (3H, *s*, H-26), 1.09 (3H, *s*, H-27), 0.82 (3H, *s*, H-28), 0.91 (3H, *s*, H-29), 0.95 (3H, *s*, H-30); ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* ppm are given in Table 1.

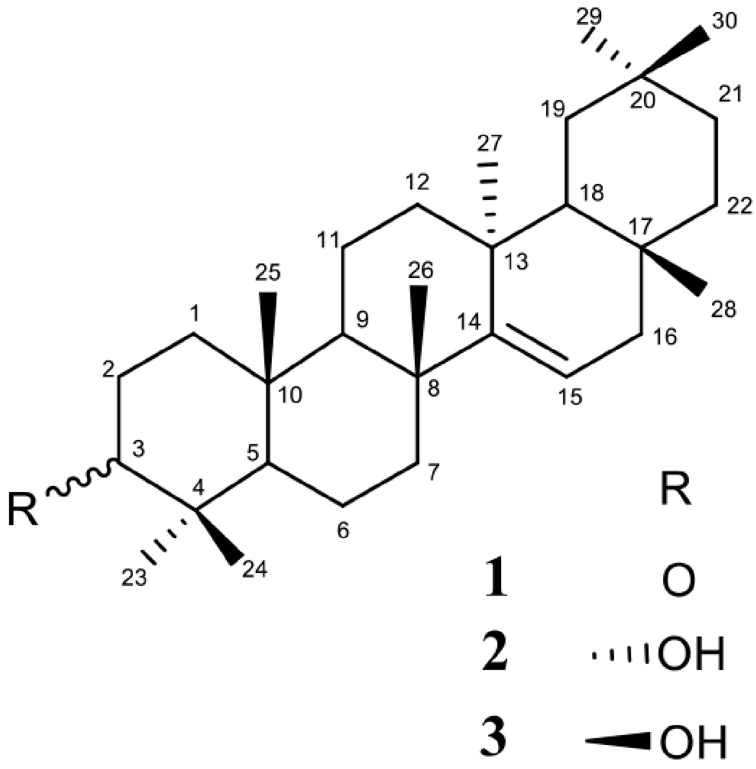
Stigmast-5-en-3β-yl (6'-O-heptadecanoyl-β-D-glucopyranoside) (**4**). This is a colourless oil, *Rf* 0.57 on silica gel (CH_2Cl_2/MeOH, 15:1, v/v); ESI-MS: *m/z* 850 $[M+Na-H]^-$ ($C_{52}H_{91}O_7Na$); ^1H NMR ($CDCl_3$, 500 MHz) *δ* ppm: 5.35 (1H, *br s*, H-6), 4.37 (1H, *d*, *J* = 7.5 Hz, H-1'), 4.33 (2H, *br s*, H-6'), 3.56-3.34 (4H, *m*, H-2', H-3', H-4' and H-5'), 3.54 (1H, *m*, H-3), 1.25 (*br s*, CH_2 chain), 0.68 (3H, *s*, H-18), 0.99 (3H, *s*, H-19), 0.91 (3H, *d*, *J* = 6.5 Hz, H-21), 0.82 (6H, *d*, *J* = 6.5 Hz, H-26 and H-27), 0.84 (3H, *t*, *J* = 6.5 Hz, H-29), 0.88 (3H, *t*, *J* = 6.6 Hz, H-17"); ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* ppm given in Table 1.

RESULTS AND DISCUSSION

The separation of *n*-hexane extract (50g) using chromatography and crystallization methods yielded four compounds **1-4**. The structure of all four compounds were identified based on comparison with published spectral data mentioned below (Figure 1). The ^1H- and ^{13}C-NMR spectra of compounds **1** to **3** were very similar and suggested that compound **4** was a acylated glycoside. Compounds **1** to **3** have been previously isolated from *E.agallocha* [4] and compound **4** from *Gynura divaricata* and *Ficus glumosa* Del. [5, 6].

Table 1. ^{13}C-NMR spectral data of compounds 1-4 ($CDCl_3$, 125 MHz).

Position	1	2	3	4
1	38.3	37.7	37.6	37.3
2	34.1	25.0	26.7	29.4
3	217.5	76.2	78.8	79.7
4	47.5	39.1	38.8	38.9
5	55.7	49.2	55.4	140.3
6	19.9	18.7	18.6	122.1
7	35.1	33.7	35.0	32.0
8	38.8	38.0	38.6	31.9
9	48.7	48.9	48.6	50.2
10	35.7	37.5	37.4	36.7
11	17.4	17.4	17.3	21.1
12	37.6	35.1	37.5	39.8
13	37.7	37.3	35.6	42.3
14	157.6	158.2	158.0	56.8
15	117.1	116.7	116.7	24.3
16	36.6	36.7	36.5	28.2
17	37.5	35.8	37.8	56.2
18	48.8	48.7	49.5	11.9
19	40.6	41.2	41.2	19.4
20	28.7	28.8	28.6	36.2
21	33.5	33.1	33.5	18.8
22	33.1	32.2	32.9	33.9
23	26.1	33.3	27.7	26.2
24	21.3	22.1	15.3	45.8
25	14.8	29.9	15.2	29.2
26	29.8	26.0	29.6	19.8
27	25.5	29.8	25.7	19.0
28	29.9	21.2	29.7	23.1
29	33.3	28.2	33.1	11.8
30	21.4	15.2	21.1	–
1’	–	–	–	101.3
2’	–	–	–	73.4
3’	–	–	–	76.2
4’	–	–	–	70.4
5’	–	–	–	73.7
6’	–	–	–	63.5
1”	–	–	–	174.3
2”	–	–	–	34.3
3”	–	–	–	25.0
4”–14”	–	–	–	29.3–29.9
15”	–	–	–	31.9
16”	–	–	–	22.7
17”	–	–	–	14.1



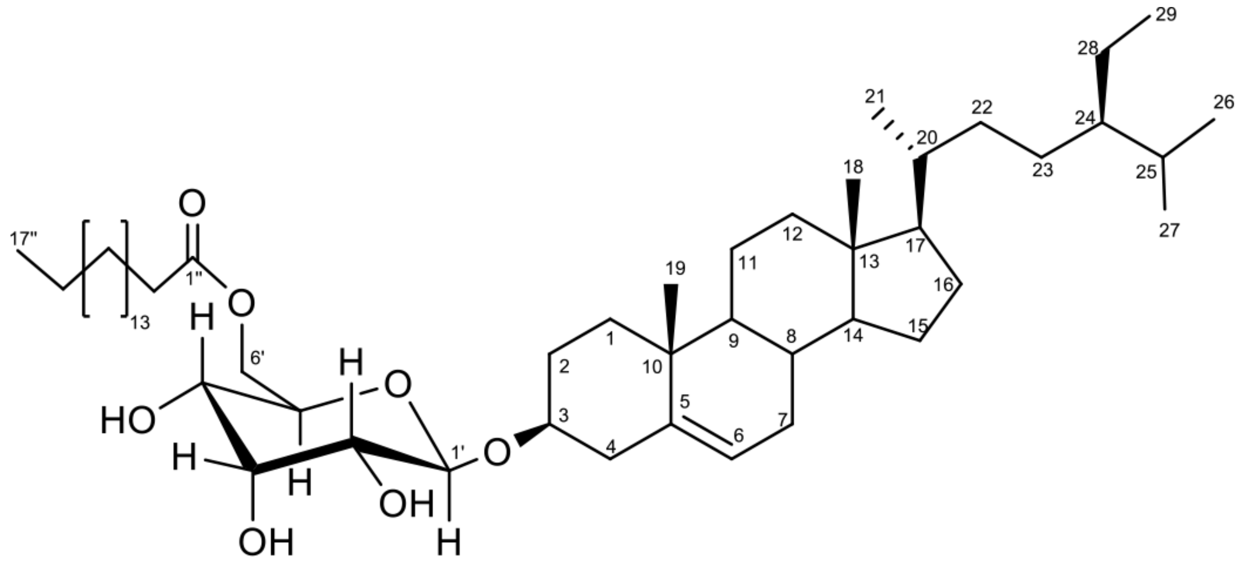


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Figure 1. Structures of compounds **1–4**.

Compound **1** was obtained as white crystals. Its molecular formula $C_{30}H_{48}O$ was deduced from comparison of the ESI-MS ($m/z = 425$ $[M+H]^+$) and ^{13}C-NMR data with published values suggesting that this compound is the triterpenoid taraxerone (taraxer-14-ene-3-one) [7].

Compound **2** was obtained as white needle-shaped crystals. The ESI-MS spectrum of **2** indicated a molecular ion peak at $m/z = 427$ $[M + H]^+$, corresponding to $C_{30}H_{48}O$ based on MS and NMR data. The structure of **2** was identified as epitaraxerol (14-taraxeren-3α-ol) when compared with published data [8].

Compound **3** was isolated as colorless plates. It gave the same molecular ion peak ($m/z = 427$ $[M + H]^+$) and molecular formula ($C_{30}H_{50}O$) as compound **2** from ESI-MS and ^{13}C-NMR data. Compound **3** was identified as 3β-OH, taraxerol when compared with published spectral data [9]. Taraxerol, a member of the family of triterpenoids, has been isolated from a variety of plants including *E. agallocha*. It has been reported to exhibit anti-diabetic potential and potent antiinflammatory effects [10].

Compound **4** was isolated as colourless oil. It revealed the molecular ion peak at m/z 850 $[M+Na-H]^-$ in the negative ESI-MS. The MS and NMR data suggested the molecular formula $C_{52}H_{92}O_7$. Compound **4** was identified as stigmast-5-en-3β-yl (6'-*O*-heptadecanoyl-β-D-glucopyranoside) after comparison of the ^1H and ^{13}C NMR spectra with reported data [6].

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APPENDIX. EA4−$CDCl_3$−HMBC.