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A New Triterpene from Ludwigia hyssopifolia (G.Don) Exell

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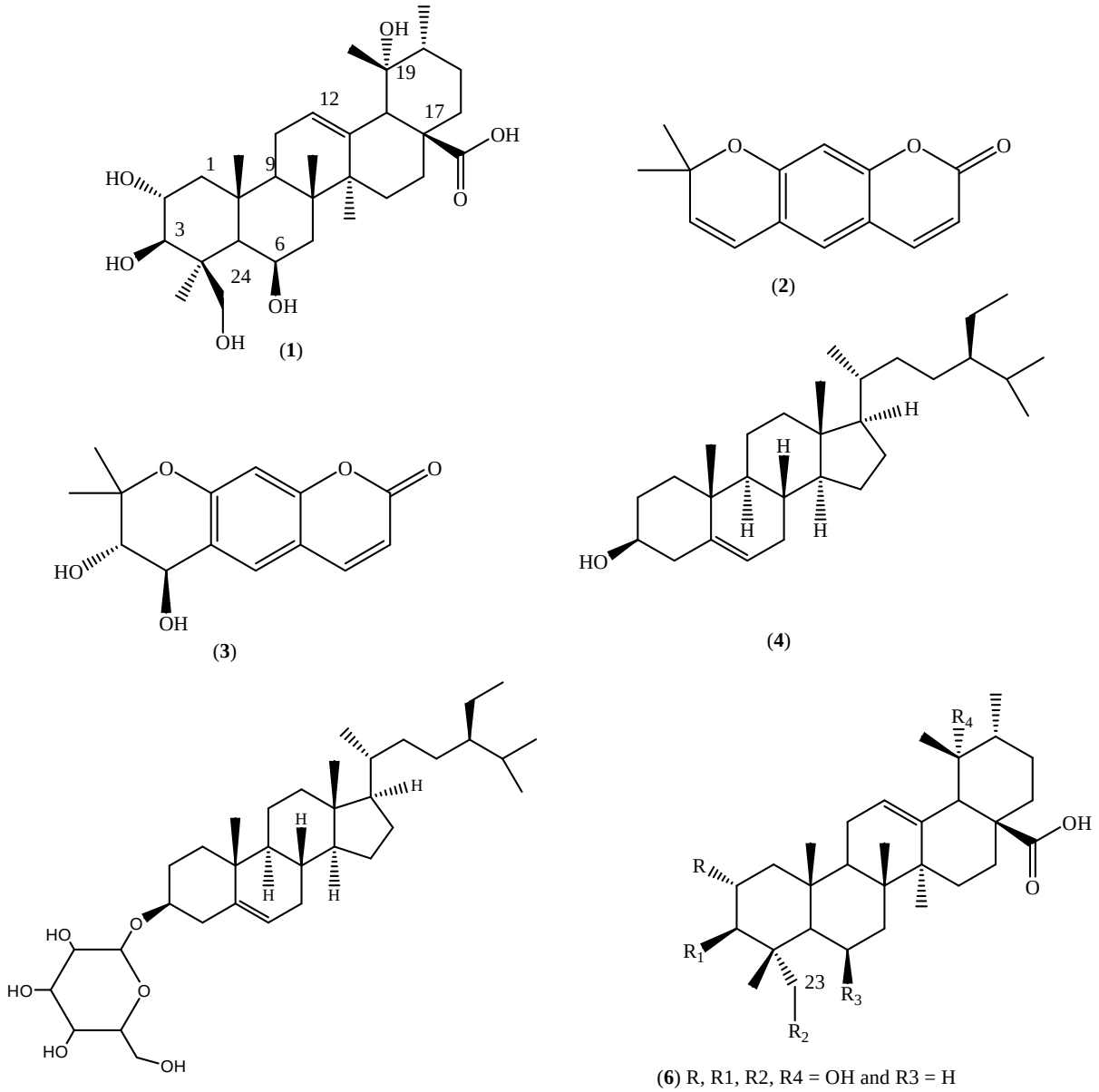
Abstract: Chemical studies of the ethanol extract of *Ludwigia hyssopifolia* whole plant led to isolation of one new compound and seven known compounds. The new compound was elucidated as 6*β*, 24 hydroxy tormnetic acid [2*α*, 3*β*, 19*α*, 6*β*, 24- penta hydroxylurs-12-en-18-oic acid (**1**)] by spectroscopic data. The known compounds were identified as xanthyletin (**2**), (+) *trans*-decursidinol (**3**), *β*-sitosterol (**4**), *β*-sitosterol-*β*-D-glucopyranoside (**5**), 6*β*, 23-hydroxy tormentic acid (**6**), 23-hydroxy tormentic acid (**7**) and 6*β*, 23-hydroxy tormentic acid (**8**) by using comparison with available data and spectroscopic studies.
Key Words: *Ludwigia hyssopifolia*, Onagraceae, Coumarins and triterpenes.

Introduction:

Ludwigia hyssopifolia Linn. (Synonym: *Jussiaea hyssopifolia* G. Don, *Jussiaea linifolia* Vahlnon and *Ludwigia linifolia* Poir) belongs to Onagraceae Family, is extensively grown in Bangladesh, in all parts of India and Ceylon. This plant is considered as astringent, anthelmintic, carminative diuretic and decoction is used in diarrhea and dysentery, flatulence, leucorrhoea, spitting of blood, extensive use for the vermifuge and purgative leaves were useful for poulticing in orchitis and glands in the neck[1]. Previous phytochemical investigation of *L. hyssopifolia* yielded piperine, vitexin, isovitexin, orientin and isoorientin[2].

As a part of our research program to identify chemical biomarkers from medicinal plants[3,4], we herein report the isolation (Fig. 1) of a new pentacyclic triterpenoid, 6β, 24 hydroxy tormnetic acid [2*α*, 3*β*, 19*α*, 6*β*, 24- penta hydroxylurs-12-en-18-oic acid (**1**)]. Seven known compounds which were isolated were identified as xanthyletin (**2**)[5], (+) *trans*- decursidinol (**3**)[6] , *β*-sitosterol (**4**)[7] , *β*-sitosterol-*β*-D-glucopyranoside (**5**) [8], 6*β*-hydroxy tormentic acid (**8**)[9] , 23-hydroxy tormentic acid (**7**)[10] and 6*β*, 23-hydroxy tormentic acid (**6**) [11].

Fig. 1: Compounds isolated from *Ludwigia hyssopifolia* (1-8)



(**1**)

(**2**)

(**3**)

(**4**)

(**5**)

(**6**) R, R1, R2, R4 = OH and R3 = H

(**7**) R, R1, R4 = OH and R2, R3 = H

(**8**) R, R1, R2, R3, R4 = OH

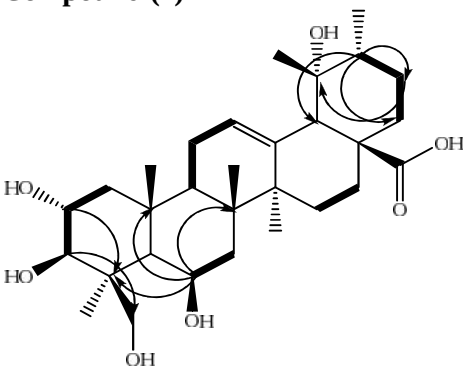
Compound **1** was obtained as a white solid. The molecular formula was established as $C_{30}H_{48}O_7$ on the basis of HRMS. ^{13}C NMR and DEPT experiments showed the presence of thirty carbons of which of six methyls, eight methylenes, eight methines and eight quaternary carbons. 1H & 13C NMR spectrum suggested a triterpenoid with basic structure similar to 24-hydroxy tormentic acid[7]. 1H & 13C NMR spectra (Table 1) of **1** displayed similarities to compound **8** (6β, 23-hydroxy tormentic acid)**.** The major difference is the substitution of hydroxyl group at C-24 position instead of position C-23. The 13C NMR spectrum showed two major differences as compared to the reference compound 6β, 23-hydroxy tormentic acid[9] at C-3 appeared at δ 84.2, whereas in compound **1** showed at δ 78.4, C-5 position at δ 54.3 which were shifted to higher fields and C-4 showed at δ 44.5 shifted to down fields compared to reference compound[9]. With the above observation, we have concluded a hydroxyl group substituted at C-24 but not C-23.

Table 1: ^{13}C NMR (Pyridine-d_5, 100 MHz) spectral data of compound 1 and 8

S. No	Compound 1	Compound 8
1	48.3	50.2
2	67.8	67.9
3	78.4	84.2
4	44.5	40.2
5	48.9	54.3
6	69.0	69.2
7	41.4	42.0
8	39.9	43.5
9	48.4	48.5
10	38.5	39.2
11	24.3	23.2
12	128.4	129.4
13	139.4	139.5
14	42.7	41.0
15	29.2	27.8
16	26.9	25.6
17	49.3	48.2
18	54.6	56.4
19	72.1	73.2
20	42.4	43.0
21	26.4	26.5
22	38.2	38.4
23	15.9	14.5
24	66.2	68.5
25	18.9	18.5
26	18.2	18.3
27	24.7	23.8
28	180.7	180.5
29	27.1	26.5
30	16.8	14.3

The position of all the functional groups including hydroxyls, construction of methylenes is established on the basis of COSY and HMBC (fig. 2) analysis and literature. Based on the literature value and 1-D & 2-D NMR data **1** was elucidated as a new penta cyclic triterpinoid, named as 6β, 24-hydroxy tormentic acid.

Fig. 2: Key HMBC and COSY correlations for Compound (1)



^{1}H (400 MHz), ^{13}C (100 MHz) and 2D-NMR spectra were recorded using the residual solvent signal as internal standard on a Varian AS 400 spectrometer. IR spectra were measured on a Bruker Tensor 27 FTIR spectrometer. UV spectra were obtained on a Varian Cary 50 Bio UV-visible spectrophotometer. Optical rotations were obtained at the sodium D line at ambient temperature on a Rudolph Research Analytical Autopol IV automatic polarimeter. HRIMS were obtained on an Agilent Series 1100 SL mass spectrometer. TLC was carried out on aluminum-backed plates precoated with silica gel F254 (20 × 20 cm, 200 µm, 60 Å, Merck). Visualization was accomplished by spraying with *p*-anisaldehyde [0.5 mL in glacial acetic acid (50 mL) and sulfuric acid (97%, 1 mL)] spray reagent followed by heating. Flash silica gel (60-120 µm, 60 Å, SiliCycle), SiliaBond C18 silica gel (40-63 µm, 60 Å, 17% carbon loading, SiliCycle) and Sephadex LH-20 (25-100 µm, lipophilic, Sigma-Aldrich) were used for column chromatography.

Whole plant of *Ludwigia hyssopifolia* were obtained from the University of Mississippi and authenticated by Dr. V.C. Joshi at the National Center for Natural Products Research, University of Mississippi, where a voucher specimen (No. 3287) was deposited.

Powdered whole plant of *L. hyssopifolia* (400.0 g) was extracted with EtOH through sonication at room temperature. The combined extracts were filtered and the solvent evaporated to afford a brown powder (13.0 g), followed by vacuum liquid chromatography over C18 silica (200.0 g) [H_2O (100%), H_2O/MeOH (2:8, 4:6, 6:4, 8:2), MeOH (100%)] yielding seven fractions (F1-F7). Fractions F1 [H_2O (100%)], F7 [MeOH (100%)] primarily contained sugars, and aliphatic acids and esters, respectively, based on GC-MS and TLC analysis, and were not further investigated. Fractions F2 (1.8 g) followed by fractionation over silica (250 g) [$CHCl_3$/MeOH (8:2)] provided **2** and **3** (136.4 mg and 11.1 mg), respectively. Fraction F4 (6.0 g) was fractionated over Sephadex LH-20 (100 g) [MeOH (100%)], yielding F4(1)-F4(20). Subsequent fractionation of F4(1) (200.0 mg) by Sephadex LH-20 (100 g) [MeOH (100%)] and final purification over C18 silica (250 g) [MeCN/H_2O (4:6)] provided **1** (15.0 mg), and F4(20) (1.5 g) by C18 silica (250 g) [MeCN/H_2O (3:7)] provided **6** (36.0 mg), **7** (14.2 mg)and **8** (12.8 mg). Fraction F5 (4.2 g) was fractionated by C18 silica (250 g) [MeCN/H_2O (1:1)], yielding F5 (1), (1.64 g), F5 (2) (0.3 g) and F5 (3) (2.18 g), which gave **4** (19.0 mg) and **5** (4.0 mg), respectively, after final purification over C18 silica [MeCN/H_2O (1:1)].

2*α*, 3*β*, 19*α*, 6*β*, 24- penta hydroxylurs-12-en-18-oic acid (1):

White solid (10.2 mg). Mp 268-269^{O}C. $[\alpha]_D^{25}$ +17.2 (MeOH, c 1.10). UV (MeOH)max (log ε) 281 (4-01) and 355 (4.30) nm**;** HRIMS *m/z* 521.3233 $[M+1]^+$, calculated 520.3400 ($C_{30}H_{48}O_7$); IR cm^{-1}; 3438, 2920, 1682, 1153, 1016. ^{1}H NMR (400MHz, C_5D_5): 5.30 (1H, m, H-12), 5.10 (1H, s, 23H), 4.35 (1H, m, H-2), 3.85 (1H, d, *J* = 9.0 Hz; H-3), 2.85 (1H, m, H-16a), 2.80 (1H, s, H-18),1.90-1.20 (12H, H-1, 4, 15, 19, 20, 21), (0.94, 3H, 30-Me), 0.72, 0.74, 0.92, 1.12, 1.32 (each 3H, s). ^{13}C NMR: 48.3 (C-1), 67.8 (C-2), 78.4 (C-3), 44.5 (C-4), 48.9 (C-5), 69.0 (C-6), 41.4 (C-7), 39.9 (C-8), 48.4 (C-8), 38.5 (C-10), 24.3 (C-11), 128.4 (C-12), 139.4 (C-13), 42.7 (C-14), 29.2 (C-15), 26.9 (C-16), 49.3 (C-17), 54.6 (C-18), 72.1 (C-19), 42.4 (C-20), 26.4 (C-21), 38.2 (C-22), 15.9 (C-23), 66.2 (C-24), 18.9 (C-25), 18.2 (C-26), 24.7 (C-27), 180.7 (C-28), 27.1 (C-29) and 16.8 (C-30)

Compound (**8**): 50.2 (C-1), 67.9 (C-2), 84.2 (C-3), 40.2 (C-4), 54.3 (C-5), 69.2 (C-6), 42.0 (C-7), 43.5 (C-8), 48.5 (C-8), 39.2 (C-10), 23.2 (C-11), 129.4 (C-12), 139.5 (C-13), 41.0 (C-14), 27.8 (C-15), 25.6 (C-16), 48.2 (C-17), 56.4 (C-18), 73.2 (C-19), 43.0 (C-20), 26.5 (C-21), 38.4 (C-22), 68.5 (C-23), 14.5 (C-24), 18.5 (C-25), 18.3 (C-26), 23.8 (C-27), 180.5 (C-28), 26.5 (C-29) and 14.3 (C-30)

The new compound isolated from ethanol extract of *Ludwigia hyssopifolia*, 6β, 24 hydroxy tormnetic acid [2*α*, 3*β*, 19*α*, 6*β*, 24- penta hydroxylurs-12-en-18-oic acid (**1**)] could possibly used as a chemical biomarker for identifying *Ludwigia hyssopifolia* plant.

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