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Three new oleanane-type triterpenoid saponins from the seeds of *Celosia cristata* L.

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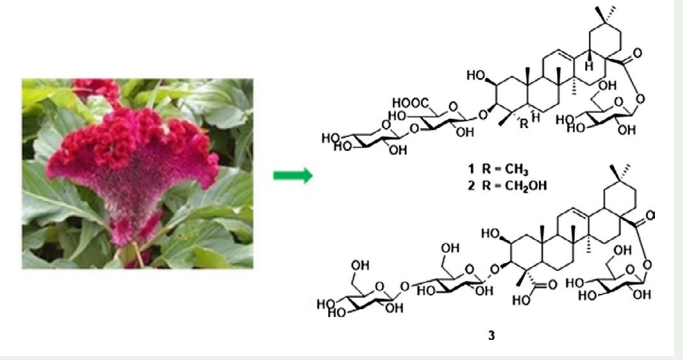
ABSTRACT

Phytochemical investigation of the 1-butanol soluble fraction of 60% ethanol extract of the seeds of *Celosia cristata* L. led to the identification of three new oleanane-type triterpenoid saponins. Using ^{1}D and ^{2}D NMR experiment methods, ESI-MS analysis and acid hydrolysis, their structures were identified as 3-*O*-[*β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucuronopyranosyl]-2*β*-hydroxy-oleanolic acid-28-*O*-β-D-glucopy ranoside (**1**), 3-*O*-[*β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucuronopyranosyl]-2*β*, 23-dihydroxy-oleanolic acid-28-O-*β*-D-glucopyranoside (**2**) and 3-*O*-[*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranosyl]-2-hydroxyl-medicagenic acid-28-O-*β*-D-glucopyranosyide (**3**), respectively.



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Celosia cristata; chemical constituent; triterpenoid saponin

1. Introduction

Celosia cristata is a member of the genus *Celosia*, and is commonly known as cockscomb because the flower looks like the head on a rooster. It belongs to the class Mangoliopsida, order Caryophyllales and family Amaranthaceae. Other than its ornamental value due to vibrant coloured inflorescence, *C. cristata* is also known for its activities for the treatment of

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haematemesis, abnormal uterine bleeding, haematochezia, haemorrhoidal bleeding, leucorrhea, chronic dysentery with persistent diarrhoea, redness of the eye and dizziness due to excessive heat of fire in the liver (Cai et al. 2003). Semen *C. cristata* is the seeds of *C. cristata* L. named 'Ji Guan Hua Zi' in Chinese, and used as a traditional Chinese medicine for removing 'liver-heat', improving eyesight, clearing wind-heat and as an anti-inflammatory agent (Wang et al. 2010). It has long been used as a folk medicine for the treatment of hypertension, palsy, cataract, keratitis, diabetes, fatigue, atherosclerosis, leucorrhoea and osteoporosis (Wang et al. 2010; Sun et al. 2011). Recent phytochemical studies reported the chemical constituents of flavonoids, phenolic glycosides, coumaronochromones and saponins in Semen *C. cristata* (Wen et al. 2006; Wang et al. 2010; Sun et al. 2011; Yan and Zhang 2016). Modern pharmacological research has demonstrated that *C. cristata* possesses antiviral, antioxidant, anthelmintic and hepatoprotective activities (Balasubrahmanyam et al. 2000; Gholizadeh et al. 2004; Rubini et al. 2012). Therefore, its health benefits have aroused increasing attention interest.

Triterpenoid saponins, as an important class of natural products, are distributed widely in the plant kingdom. Their pharmacological effects, such as anticancer, antibacterial, antimutagenesis, anti-inflammatory and inhibition of cardio-cerebral vascular diseases, have been reported recently (Soetan et al. 2014). Triterpenoid saponins are also known to be important bioactive constituents in *Celosia* plants. Previous phytochemical and pharmacological studies on *Celosia* genus plants have resulted in the isolation of several triterpenoid saponins which have been reported to possess anti-inflammatory, antitumour and hepatoprotective activities (Wen et al. 2006; Wang et al. 2010; Sun et al. 2011; Wu et al. 2013). Therefore, the present study focused on the isolation and structure elucidation of three new triterpenoid saponins from the 1-butanol soluble fraction of 60% ethanol extract of Semen *C. cristata*. Their structures were identified by extensive use of ^{1}D and ^{2}D NMR experiments along with HR-ESIMS analysis and acid hydrolysis.

2. Results and discussion

Three new triterpenoid saponins were isolated and identified from the 1-butanol fraction by chromatographic methods and spectrometric data. The optical rotation, ^{1}H and ^{13}C NMR and mass spectra analysis were used to identify these compounds (Figure 1).

Compound **1**, obtained as a white amorphous powder, m.p., 280 ~ 282 °C; $[\alpha]_D^{25}$, +20.8 (*c* 0.25, MeOH), positive to Liebermann–Burchard reaction. The IR spectrum showed the presence of hydroxyl group (3418 cm^{-1}) and carbonyl group (1735 cm^{-1}). The HR-ESI-MS of **1**

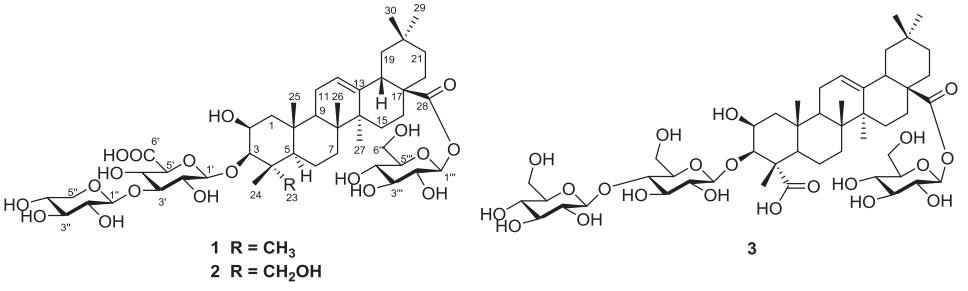

Figure 1. Chemical structures of compounds **1**–**3** isolated from the seeds of *C. cristata*.

exhibited a $[M + Na]^+$ ion at *m/z* 965.4713, indicating a molecular formula of $C_{47}H_{74}O_{19}$. There were 47 carbon signals in the ^{13}C NMR spectrum, among which 30 carbons were assigned to the aglycone, with another 17 carbons belonging to the sugar moieties. The ^1H NMR of **1** showed that aglycone contained seven methyl protons at δ_H 0.85, 0.88, 1.11, 1.26, 1.30, 1.36 and 1.45 (each 3H, *s*), one olefinic proton at δ_H 5.40. Correspondingly, seven methyl carbons at δ_C 23.6, 33.1, 17.5, 26.1, 29.5, 18.5 and 16.5, two olefinic carbons at δ_C 123.0 and 144.0, and one carbonyl carbon at δ_C 176.4 exhibited in the ^{13}C NMR spectrum, respectively. A feature of the ^1H NMR spectrum was the signal at δ_H 5.40 typical of H-12 of Δ^{12} skeleton, which was also indicated by the carbon signals at δ_C 123.0 and 144.0, due to C-12 and C-13 in the ^{13}C NMR spectrum. The presence of two oxygenated methines was deduced from the signals at δ_H 3.21 (1H, *m*) and 4.63 (1H, *m*), which correlated in the HSQC spectrum with the carbon resonances at δ_C 89.7 and 70.4, respectively. These signals are typical of ring *A* of a 2*ß*-hydroxy substituted oleanolic acid. The configuration of C-2 and C-3 in the triterpene structure was confirmed by NOESY experiments, in which a cross peak between H-2 and H-3 was observed, confirming the presence of 2*ß*,3*ß*-dihydroxyoleanane skeleton. Furthermore, in the HMBC spectrum, the methine proton (H-3, δ_H 3.36) was correlated with an anomeric carbon (δ_C 106.1) of the sugar moiety. Three anomeric proton signals at δ_H 5.31 (1H, *d*, *J* = 7.4 Hz, H-1′), 5.02 (1H, *d*, *J* = 7.1 Hz, H-1″), 6.29 (1H, *d*, *J* = 8.1 Hz, H-1‴) and three corresponding anomeric carbon signals at δ_C 106.1, 106.4 and 95.7, were also observed revealing that compound **1** contained three sugar units (Figure S1). Comparison of ^1H and ^{13}C NMR data of **1** with those of celosin *H*, isolated from the seeds of *C. argentea*, showed that **1** was almost identical to celosin *H*, except for the absence of an aldehyde group and the presence of a methyl group (Pang et al. 2014).

Acid hydrolysis of **1** followed by GC-MS analysis of its derivative enabled further identification of the existence of D-glucuronic acid, D-xylose and D-glucose. The *β*-configurations of D-glucuronic acid, D-xylose and D-glucose were determined by the $J_{1,2}$ values of 7.4 Hz, 7.1 Hz and 8.1 Hz, respectively. *A* ^1H-^1H COSY experiment allowed analysis of their spin systems and assignments of their spin systems and proton resonances. The assignment of their corresponding carbons, made by a HSQC spectrum, indicated that xylose was a terminal unit. The Xyl-(1 → 3)-GlcA structure of the disaccharide moiety at C-3 of the oleanolic acid residue was deduced from the HMBC correlations from H-3 (δ_H 3.36) to C-1′ (δ_C 106.1) of the GlcA unit and from H-3′ (δ_H 4.33) of the GlcA unit to C-1″ (δ_C 106.4) of the Xyl unit. The glycosylation position of glucose was confirmed due to the long-range HMBC correlations from H-1‴ at *δ* 6.29 to C-28 at *δ* 176.4 (Figure S1). Therefore, we speculated that celosin *H* was a product of methyl (C-23) oxidation in **1** (Pang et al. 2014). This speculation was confirmed by the HMBC correlations between H-23 (δ_H 1.30) and C-2 (δ_C 70.4), C-3 (δ_C 89.7). In addition, the NOESY correlation of H-24 (δ_H 1.36) and H-25 (δ_H 1.45) revealed that the *β*-configuration of 24-CH_3 and *α*-configuration of 23-CH_3, the correlations of 23-CH_3 with H-2 (δ_H 4.63) and H-3 (δ_H 4.15) revealed the *α*-configurations of H-2 and H-3, thus, the *β*-configurations of 2-OH and 3-OH were determined. Consequently, the structure of **1** was elucidated as 3-*O*-[*β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucuronopyranosyl]-2*β*-hydroxy-oleanolic acid-28-*O*-*β*-D-glucopyranoside, named celosin *K*.

Compound **2**, white amorphous powder, m.p., 281 ~ 282 °C; α_D^{25}, +22.6 (*c* 0.25, MeOH), and the Molish and Liebermann–Burchard reactions were positive. The IR spectrum showed the presence of hydroxyl group (3413 cm^{-1}) and carbonyl group (1728 cm^{-1}). The molecular formula of **2** was determined as $C_{47}H_{74}O_{20}$ from its positive-ion HR-ESI-MS (*m/z* 981.4667

$[M + Na]^+$, calcd. 981.4671). The ^1H and ^{13}C NMR data of **2** indicated a pentacyclic triterpenoid saponin containing one triterpene aglycone and three monosaccharides. The ^1H NMR data exhibited the characteristic signals for six tertiary methyl groups at δ_H 1.32 (H-24), 1.54 (H-25), 1.13 (H-26), 1.21 (H-27), 0.86 (H-29) and 0.84 (H-30), the corresponding carbon signals at δ_C 17.2, 14.9, 17.6, 26.2, 33.1 and 23.6, respectively. Two characteristic oxygenated methine protons were also observed at δ_H 4.34 (1H, *m*) and 4.79 (1H, *m*), which correlated in the HSQC spectrum with the carbon resonances at δ_C 82.7 and 70.9, respectively. The anomeric proton signals at δ_H 5.26 (1H, *d*, *J* = 7.7 Hz, H-1′), 5.24 (1H, *d*, *J* = 7.6 Hz, H-1″) and 6.28 (1H, *d*, *J* = 8.1 Hz, H-1‴) showed HSQC correlations with anomeric carbon signals at δ_C 105.7 (C-1′), 106.1 (C-1″) and 95.7 (C-1‴). In addition, one trisubstituted olefinic proton at δ_H 5.38 (H-12) with two typical olefinic carbon signals at δ_C 123.0 and 144.1, one oxydic-methine at δ_C 82.7 and one carbony signal at *δc* 176.4, indicating the aglycone of **2** was a derivative of oleanolic acid. The NMR data of **2** were very similar to those of **1**, except for the absence of a methyl group and appearance of $–CH_2–OH$ group.

The presence of D-glucuronic acid, D-xylose and D-glucose in a 1:1:1 ratio was established by acid hydrolysis followed by GC-FID analysis of the corresponding derivatives. Comparison of the ^1H and ^{13}C NMR data of the oligosaccharide part with those of **1** indicated that **2** contained the same sugar units as **1**. The linkage points of the sugar units to each other and to the aglycone were determined by following HMBC correlations: δ_H 5.26 (H-1′) with δ_C 82.7 (C-3), δ_H 5.24 (H-1″) with δ_C 86.3 (C-3′) and δ_H 6.28 (H-1‴) with δ_C 176.2 (C-28) (Figure S1). Therefore, we speculated that compound **2** was a product of methyl (C-23) oxidation in **1.** This speculation was confirmed by the HMBC correlations between H-23 (δ_H 3.64) and δ_C 82.7 (C-3), 42.9 (C-4), 47.5 (C-5) and 14.9 (C-24) (Figure S1). Hence, compound **2** was identified as 3-*O*-[*β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucuronopyranosyl]-2*β*, 23-dihydroxy-oleanolic acid-28-*O*-*β*-D-glucopyranside, named celosin *L*.

Compound **3** was obtained as a white amorphous powder, m.p., 281 ~ 283 °C; α_D^{25}, +20.3 (*c* 0.18, MeOH), and the Molish and Liebermann–Burchard reactions were positive. The IR spectrum showed the presence of hydroxyl group (3395 cm^{-1}) and carbonyl group (1682 cm^{-1}) Its molecular formula, $C_{48}H_{76}O_{21}$, was deduced from HR-ESI-MS: *m/z* 1011.4771 $[M + Na]^+$ (calcd., 1011.4777), indicating the presence of 11 unsaturation degrees. There were 48 carbon signals in ^{13}C NMR spectrum, among which 30 carbon signals were assigned to the aglycone, and 18 carbon signals to the sugar moiety. The NMR data of compound **3** showed the characteristic signals of a triterpenoid saponin. Six tertiary methyl groups at δ_H 1.95 (H-24), 1.52 (H-25), 1.12 (H-26), 1.20 (H-27), 0.86 (H-29) and 0.84 (H-30), and the corresponding six sp^3 carbons sp^3 carbons at δ_C 14.1, 16.8, 17.4, 26.1, 33.0 and 23.6 were observed, as well as one olefinic proton at δ_H 5.38 (1H, *t*, *J* = 3.7 Hz) with two typical olefinic carbon signals at δ_C 122.8 and 144.1. The presence of 2-OH group was indicated by the signal at δ_H 4.72 in the ^1H NMR and 70.0 in the downfield region of ^{13}C NMR. The ^1H NMR spectrum showed that three anomeric proton signals at δ_H 5.03 (1H, *d*, *J* = 7.8 Hz, H-1′), 5.15 (1H, *d*, *J* = 7.9 Hz, H-1″) and 6.29 (1H, *d*, *J* = 8.1 Hz, H-1‴), and three corresponding anomeric carbon signals at δ_C 105.1 (C-1′), 104.8 (C-2′), 95.7 (C-1‴) were observed using HSQC spectrum, revealing that **3** contained three sugar units. Acid hydrolysis of **3** followed by GC-FID analysis identified the sugar units as D-glucose. The *β*-configurations of D-glucose were determined by the $J_{1,2}$ values of 7.8, 7.9 and 8.1 Hz, respectively. The HMBC spectrum showed that the cross peaks from H-1′ (δ_H 5.03) to C-3 (*δc* 86.3), from H-1″ (δ_H 5.15) to C-4′ (*δc* 80.7) and from H-1‴ (δ_H 6.28) to C-28 (*δc* 176.4), which revealed the glycosylation positions and sugar

sequence of **3** (Figure S1). Compound **3** had the almost same aglycone structure as **1**, except for the absence of a methyl group and appearance of carboxylic group by comparing their NMR data. Therefore, we speculated that compound **3** is a product of methyl (C-23) oxidation in **1.** This speculation was confirmed by the HMBC correlations between δ_H 4.64 (H-3) and δc 180.6 (C-23), δ_H 1.95 (H-24) and δc 180.6 (C-23) (Figure S1). Hence, compound **3** was identified as 3-*O*-[*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranosyl]-2-hydroxyl-medicagenic acid-28-*O*-*β*-D-glucopyranosyide, named celosin *M*.

3. Experimental

3.1. Materials and chemicals

The melting point was measured on a Yanaco micromelting point apparatus without correction. ^{1}H (600 MHz), ^{13}C (150 MHz) and ^{2}D NMR spectra were obtained on a Bruker AVANCE DRX-600 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. HR-ESI-MS was performed on a Waters Acquity UPLC system coupled to a Waters Micromass LCT Premier time-of-flight mass spectrometer (Milford, MA, USA), equipped with electrospray interface. Optical rotations were measured on a Rudolph Autopol VI90079 polarimeter. UV spectra were recorded on a Beckman Coulter DU800 spectrophotometer. IR spectra were recorded from KBr disc on the FT-IR (Bruker Tensor 27). Gas chromatography analysis was operated on an HP-5892 II with FID detector, and an HP-20 M (Carbowx 20 M) capillary column (25 m × 0.32 mm × 0.3 μm) was used. Silica gel (100–200 mesh, and 200–300 mesh, Qindao Haiyang Chemical Co. Ltd. Qingdao, China), Sephadex LH-20 (Amersham Pharmcia Biotech. Ltd. Shanghai, China) column chromatography, ODS (50 mesh, AA12550, YMC) and semiprep high-performance liquid chromatography (HPLC) were used for separation. L-xylose, D-glucose and D-glucuronic acid were purchased from J&K Scientific Co. Ltd (Beijing, China).

3.2. Plant material

The seeds of *C. cristata* were purchased from Suqian city, Jiangsu Province, China, in September 2013, and identified by Prof. Xiujia Zhou from Shanghai University of Traditional Chinese Medicine (Shanghai, China). A voucher specimen (2013072105) was originally deposited in the State Key Laboratory of New Drugs in Shanghai Institute of Pharmaceutical Industry (Shanghai, China).

3.3. Extraction and isolation

The air-dried seeds of *C. cristata* (5 kg) were powdered and extracted with 60% ethanol under reflux for two times (each for 3 h). After filtration, combination and evaporation, the residues were suspended in water, and partitioned successively with ethyl acetate and 1-butanol. The 1-butanol soluble fraction (40.0 g) was subjected to a MCI gel (CHP 20P) column chromatograph eluted with MeOH/H_2O (0:100, 10:90, 20:80, 40:60, 60:40, 80:20 and 100:0), yielding seven sub-fractions (Fr.A–Fr.G). The sub-fraction Fr.E (5.0 g) was subjected to reversed phase silica gel column employing MeCN/H_2O (20:80–100:0), yielding three portions (Fr. E1–Fr.E3). Fr.E2 was repeatedly chromatographed on a reverse-phase RP-C8 column eluting with MeCN/H_2O (33:67) to yield six fractions (Fr.E2a–Fr.E2f). Purification of sub-fraction Fr.E2b

by semi-prep HPLC eluting with MeOH/H_2O (45:55) yielded compound **1** (18 mg). Fr.E2d was purified by semi-prep HPLC eluting with MeOH/H_2O (50:50) yielded compounds **2** (15 mg) and **3** (10 mg).

3.3.1. Celosin K (1)

White amorphous powder, m.p., 280 ~ 282 °C; α_D^{25}, +20.8 (*c* 0.25, MeOH). UV (MeOH), λ_{max} (log ε): 218.4 (4.50); IR ν_{max} 3418, 2936, 1735, 1632, 1462, 1371, 1258, 1159, 1070 cm^{-1}; HR-ESI-MS *m/z*: $[M + Na]^+$ 965.4713. ^1H NMR (pyridine-d_6, 600 MHz): δ_H 2.15 (1H, *d*, *J* = 13.8 Hz, H-1), 1.07 (1H, *m*, H-1), 4.63 (1H, *m*, H-2), 3.36 (1H, *m*, H-3), 0.91 (1H, *d*, *J* = 10.8 Hz, H-5), 1.53 (2H, *m*, H-6), 1.64 (1H, *m*, H-7), 1.33 (1H, *m*, H-7), 1.58 (1H, *m*, H-9), 2.16 (1H, *m*, H-11), 1.94 (1H, *m*, H-11), 5.40 (1H, *t*, *J* = 3.7 Hz, H-12), 2.33 (1H, *t*, *J* = 13.0 Hz, H-15), 1.17 (1H, *d*, *J* = 13.6 Hz, H-15), 2.06 (1H, *m*, H-16), 1.89 (1H, *m*, H-16), 3.16 (1H, *dd*, *J* = 13.6, 4.4 Hz, H-18), 1.74 (1H, *m*, H-19), 1.22 (1H, *dd*, *J* = 14.3, 3.6 Hz, H-19), 1.32 (1H, *m*, H-21), 1.05 (1H, *m*, H-21), 1.80 (1H, *m*, H-22), 1.71 (1H, *m*, H-22), 1.30 (3H, *s*, H-23), 1.36 (3H, *s*, H-24), 1.45 (3H, *s*, H-25), 1.11 (3H, *s*, H-26), 1.26 (3H, *s*, H-27), 0.88 (3H, *s*, H-29), 0.85 (3H, *s*, H-30), 5.02 (1H, *d*, *J* = 7.1 Hz, H-1′), 4.06 (1H, *t*, *J* = 8.4 Hz, H-2′), 4.33 (1H, *m*, H-3′), 4.43 (1H, *d*, *J* = 3.9 Hz, H-4′), 4.61 (1H, *d*, *J* = 9.6 Hz, H-5′), 5.31 (1H, *d*, *J* = 7.4 Hz, H-1″), 4.01 (1H, *m*, H-2″), 4.12 (1H, *m*, H-3″), 4.32 (1H, *m*, H-4″), 4.31 (1H, *m*, H-5″), 3.68 (1H, *t*, *J* = 10.2 Hz, H-5″), 6.29 (1H, *J* = 8.1 Hz, H-1‴), 4.17 (1H, *d*, *J* = 8.5 Hz, H-2‴), 4.25 (1H, *d*, *J* = 8.9 Hz, H-3‴), 4.32 (1H, *m*, H-4‴), 3.99 (1H, *m*, H-5‴), 4.42 (1H, *dd*, *J* = 12.0, 2.5 Hz, H-6‴), 4.36 (1H, *dd*, *J* = 12.0, 4.4 Hz, H-6‴); ^{13}C NMR (pyridine-d_6, 150 MHz): δ_C 44.2 (C-1), 70.4 (C-2), 89.7 (C-3), 39.0 (C-4), 56.0 (C-5), 18.3 (C-6), 33.1 (C-7), 40.0 (C-8), 48.4 (C-9), 37.0 (C-10), 23.4 (C-11), 123.0 (C-12), 144.0 (C-13), 42.2 (C-14), 28.1 (C-15), 23.9 (C-16), 46.9 (C-17), 41.7 (C-18), 46.1 (C-19), 30.7 (C-20), 34.0 (C-21), 32.5 (C-22), 29.5 (C-23), 18.5 (C-24), 16.5 (C-25), 17.5 (C-26), 26.1 (C-27), 176.4 (C-28), 33.1 (C-29), 23.6 (C-30), 106.1 (C-1′), 74.2 (C-2′), 86.1 (C-3′), 71.4 (C-4′), 77.1 (C-5′), 172.4 (C-6′), 106.4 (C-1″), 75.2 (C-2″), 78.0 (C-3″), 70.8 (C-4″), 67.3 (C-5″), 95.7 (C-1‴), 74.1 (C-2‴), 78.8 (C-3‴), 71.3 (C-4‴), 79.2 (C-5‴), 62.2 (C-6‴) (Table S1 and S2).

3.3.2. Celosin L (2)

White amorphous powder, m.p., 281 ~ 282 °C; α_D^{25}, +22.6 (*c* 0.25, MeOH). UV (MeOH), λ_{max} (log ε): 218.4 (4.46); IR ν_{max} 3413, 2926, 1728, 1680, 1432, 1205, 1141, 1074, 1034 cm^{-1}; HR-ESI-MS *m/z*: $[M + Na]^+$ 981.4667. ^1H NMR (pyridine-d_6, 600 MHz): δ_H 2.18 (1H, *d*, *J* = 13.8 Hz, H-1), 1.14 (1H, *m*, H-1), 4.79 (1H, *m*, H-2), 4.34 (1H, *m*, H-3), 1.78 (1H, *m*, H-5), 1.46 (2H, *m*, H-6), 1.64 (1H, *m*, H-7), 1.33 (1H, *m*, H-7), 1.69 (1H, *m*, H-9), 2.07 (1H, *m*, H-11), 1.95 (1H, *m*, H-11), 5.38 (1H, *t*, *J* = 3.7 Hz, H-12), 2.31 (1H, *t*, *J* = 13.1 Hz, H-15), 1.09 (1H, *m*, H-15), 2.02 (1H, *m*, H-16), 1.91 (1H, *m*, H-16), 3.14 (1H, *dd*, *J* = 13.7, 4.0 Hz, H-18), 1.71 (1H, *m*, H-19), 1.19 (1H, *m*, H-19), 1.30 (1H, *m*, H-21), 1.04 (1H, *d*, *J* = 11.2 Hz, H-21), 1.80 (1H, *m*, H-22), 1.70 (1H, *m*, H-22), 4.32 (1H, *m*, H-23), 3.65 (1H, *m*, H-23), 1.32 (3H, *s*, H-24), 1.54 (3H, *s*, H-25), 1.13 (3H, *s*, H-26), 1.21 (3H, *s*, H-27), 0.86 (3H, *s*, H-29), 0.84 (3H, *s*, H-30), 5.26 (1H, *d*, *J* = 7.7 Hz, H-1′), 4.08 (1H, *t*, *J* = 8.6 Hz, H-2′), 4.18 (1H, *m*, H-3′), 4.42 (1H, *d*, *J* = 3.9 Hz, H-4′), 4.52 (1H, *d*, *J* = 9.6 Hz, H-5′), 5.24 (1H, *d*, *J* = 7.4 Hz, H-1″), 4.00 (1H, *m*, H-2″), 4.14 (1H, *m*, H-3″), 4.31 (1H, *m*, H-4″), 4.32 (1H, *m*, H-5″), 3.68 (1H, *m*, H-5″), 6.28 (1H, *J* = 8.1 Hz, H-1‴), 4.16 (1H, *m*, H-2‴), 4.25 (1H, *d*, *J* = 8.9 Hz, H-3‴), 4.33 (1H, *m*, H-4‴), 3.99 (1H, *m*, H-5‴), 4.43 (1H, *dd*, *J* = 9.4, 2.8 Hz, H-6‴), 4.37 (1H, *m*, H-6‴); ^{13}C NMR (pyridine-d_6, 150 MHz): δ_C 44.3 (C-1), 70.9 (C-2), 82.7 (C-3), 42.9 (C-4), 47.5 (C-5), 17.9 (C-6), 32.8 (C-7), 40.0 (C-8), 48.5 (C-9), 36.9 (C-10), 23.9 (C-11), 123.0 (C-12), 144.1 (C-13), 42.2 (C-14), 28.2 (C-15), 23.3 (C-16), 47.0 (C-17), 41.7 (C-18), 46.1 (C-19), 30.7 (C-20), 33.9 (C-21),

32.5 (C-22), 64.7 (C-23), 14.9 (C-24), 17.2 (C-25), 17.6 (C-26), 26.2 (C-27), 176.4 (C-28), 33.1 (C-29), 23.6 (C-30), 105.7 (C-1′), 74.2 (C-2′), 86.3 (C-3′), 71.4 (C-4′), 77.1 (C-5′), 172.5 (C-6′), 106.1 (C-1″), 75.2 (C-2″), 78.0 (C-3″), 71.0 (C-4″), 67.3 (C-5″), 95.7 (C-1‴), 74.1 (C-2‴), 78.8 (C-3‴), 71.1 (C-4‴), 79.2 (C-5‴), 62.2 (C-6‴) (Table S1 and S2).

3.3.2. *Celosin M* (3)

White amorphous powder, m.p., 281 ~ 283 °C; α_D^{25}, +20.3 (*c* 0.18, MeOH). UV (MeOH), λ_{max} (log ε): 218.4 (4.33); IR ν_{max} 3395, 2945, 1682, 1444, 1204, 1137, 1072 cm^{-1}; HR-ESI-MS *m/z*: $[M + Na]^+$ 1011.4777. ^1H NMR (pyridine-d_6, 600 MHz): δ_H 2.27 (1H, *m*, H-1), 1.27 (1H, *m*, H-1), 4.72 (1H, *m*, H-2), 4.64 (1H, *d*, *J* = 3.7 Hz, H-3), 1.73 (1H, *m*, H-5), 1.62 (2H, *m*, H-6), 1.62 (1H, *m*, H-7), 1.35 (1H, *m*, H-7), 1.73 (1H, *m*, H-9), 2.12 (1H, *m*, H-11), 1.98 (1H, *m*, H-11), 5.38 (1H, *t*, *J* = 3.7 Hz, H-12), 2.31 (1H, *m*, H-15), 1.09 (1H, *m*, H-15), 2.06 (1H, *m*, H-16), 1.89 (1H, *m*, H-16), 3.16 (1H, *dd*, *J* = 13.7, 4.8 Hz, H-18), 1.71 (1H, *m*, H-19), 1.21 (1H, *m*, H-19), 1.30 (1H, *m*, H-21), 1.04 (1H, *m*, H-21), 1.80 (1H, *m*, H-22), 1.69 (1H, *m*, H-22), 1.95 (3H, *s*, H-24), 1.52 (3H, *s*, H-25), 1.12 (3H, *s*, H-26), 1.20 (3H, *s*, H-27), 0.86 (3H, *s*, H-29), 0.84 (3H, *s*, H-30), 5.03 (1H, *d*, *J* = 7.8 Hz, H-1′), 3.90 (1H, *t*, *J* = 8.6 Hz, H-2′), 3.84 (1H, *m*, H-3′), 4.26 (1H, *m*, H-4′), 4.15 (1H, *m*, H-5′), 4.44 (1H, *m*, H-6′), 4.39 (1H, *m*, H-6′), 5.15 (1H, *d*, *J* = 7.9 Hz, H-1″), 4.03 (1H, *m*, H-2″), 4.16 (1H, *m*, H-3″), 4.32 (1H, *m*, H-4″), 4.15 (1H, *m*, H-5″), 4.44 (1H, *m*, H-6″), 4.35 (1H, *m*, H-6″), 6.29 (1H, *d*, *J* = 8.1 Hz, H-1‴), 4.16 (1H, *m*, H-2‴), 4.25 (1H, *d*, *J* = 8.9 Hz, H-3‴), 4.33 (1H, *m*, H-4‴), 3.94 (1H, *m*, H-5″), 4.47 (1H, *m*, H-6‴), 4.23 (1H, *m*, H-6‴); ^{13}C NMR (pyridine-d_6, 150 MHz): δ_C 44.2 (C-1), 70.0 (C-2), 86.0 (C-3), 52.8 (C-4), 48.7 (C-5), 21.1 (C-6), 32.9 (C-7), 40.3 (C-8), 48.7 (C-9), 36.8 (C-10), 23.9 (C-11), 122.8 (C-12), 144.1 (C-13), 42.2 (C-14), 28.1 (C-15), 23.3 (C-16), 46.9 (C-17), 41.7 (C-18), 46.1 (C-19), 30.7 (C-20), 33.9 (C-21), 32.5 (C-22), 180.6 (C-23), 14.1 (C-24), 16.8 (C-25), 17.4 (C-26), 26.1 (C-27), 176.4 (C-28), 33.0 (C-29), 23.6 (C-30), 105.1 (C-1′), 74.6 (C-2′), 76.4 (C-3′), 80.7 (C-4′), 76.5 (C-5′), 62.2 (C-6′), 104.8 (C-1″), 74.7 (C-2″), 79.2 (C-3″), 71.0 (C-4″), 78.1 (C-5″), 61.9 (C-6″), 95.7 (C-1‴), 74.1 (C-2‴), 78.8 (C-3‴), 71.1 (C-4‴), 78.3 (C-5‴), 62.2 (C-6‴) (Table S1 and S2).

3.4. *Acid hydrolysis and sugar analysis of compounds* 1–3

The acid hydrolysis and sugar analysis of the isolated three new compounds were performed as previously described in the literature (Zong et al. 2015). Each compound (3 mg) was dissolved in 2 M trifluoroacetic acid for 2 h at 120 °C. The reaction mixture was extracted with dichloromethane, and the supernatant was evaporated to dryness under N_2 flow. The residue was dissolved in 0.2 mL pyridine containing L-cysteine methyl ester hydrochloride (10 mg/mL) and reacted at 70 °C for 1 h. This reaction was evaporated under N_2 flow, after which 0.2 mL trimethysilyimidazole was added. The mixture was heated at 70 °C for another 1 h, and then partitioned between *n*-hexane and water. The organic layer was analysed by GC-FID. Temperature conditions were as follows: injector temperature at 280 °C, the initial oven temperature was 160 °C for 1 min, then linearly increased to 280 °C and held for 5 min. The standard sugar samples were subjected to the same reaction and GC-FID conditions. The sugar unit of compounds **1**–**3** were identified by comparision with authentic samples: L-xylose (t_R = 10.67 min), D-glucose (t_R = 13.84 min), D-glucuronic acid methyl ester (t_R = 15.08 min), respectively.

4. Conclusion

Phytochemical investigation of Semen *C. cristata* allowed the isolation and characterisation of three new oleanane-type saponins. Previous investigation revealed the existence of triterpenoid saponin in *C. cristata*, this results could have a significant chemotaxonomical meaning in the further studies for classification of the *Celosia* species. Moreover, the biological activities of these new compounds should be evaluated in the next work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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