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New sesquiterpene lactones from *Inula oculus-christi* L.

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ABSTRACT

Investigation of the aerial parts of *Inula oculus-christi* L. led to the identification of four new sesquiterpene lactones: an eudesmanolide (4α,15α-epoxypulchellin E) and three guaianolides (2α-acetoxy-4α,9β-dihydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide, 9β,10β-epoxygaillardin, 9α,10α-epoxy-2-*epi*-gaillardin), in addition to the known gaillardin, pulchellin E, pulchellin C and 3-*O*-palmitates of 16β-hydroxylupeol, 16β-hydroxy-β-amyrin, and faradiol. The structures of all compounds have been elucidated on the basis of their spectral data.

1. Introduction

Inula is a large genus in Asteraceae family containing more than one hundred species widespread mainly in Africa, Asia and Europe. Of them 19 are identified in Europe and they are distributed predominantly in the Mediterranean area. It is reported that 16 *Inula* species are known as plants used in traditional medicine mainly in some Asian countries. Moreover, some of them (such as *I. helenium* L., *I. japonica* Thunb) are constituents of commercial herbal preparations or supplements with medicinal applications. *Inula* species are target of scientific investigation to determine their active principles, to elucidate the mechanisms of action, to find new pharmacological applications, or to isolate new bioactive compounds. Numerous chemical compounds are reported so far, mostly terpenoids, flavonoids, essential oils, etc. (Seca et al., 2014, 2015; Wang et al., 2014). Some *Inula* species showed to contain significant amount of sesquiterpene lactones, which belong to 14 different structural types, the major of which were eudesmane, guaiane, pseudoguaiane, and germacrane. These secondary metabolites from *Inula* species have attracted the attention of scientists because of their diverse biological activity – antiproliferative, bactericidal, cytotoxic against tumor cell lines, antineoplastic and anti-inflammatory, etc. (Cheng et al., 2011; Harvala et al., 2002; Hu et al., 2012; Konishi et al., 2002; Qin et al., 2012).

Inula oculus-christi L. is widespread in eastern Central Europe, the Balkan Peninsula, Turkey, Iran, Iraq and the Caucasus (GBIF, 2016). It is not known as a plant used in ethnopharmacology, but cytotoxic, antioxidant, acetylcholinesterase, antimicrobial and amoebicidal activities as well as DNA damage protection potential (Berk et al., 2011; Degerli et al., 2012; Hajimehdipoor et al., 2014; Mosaddegh et al., 2006, 2010) have been found. Phytochemical study of *I. oculus-christi* from Iran (Mosaddegh et al., 2010) and Montenegro (Vajs et al., 2003) revealed the presence of sesquiterpene lactones of two biogenetically related skeletal types—guaianolides (gaillardin and ergolide) and eudesmanolides (pulchellin C and pulchellin E). Gaillardin has been firstly isolated from the species originating from Azerbaidzhan SSR (Kiseleva et al., 1969). It was found that this compound exhibited acetylcholinesterase activity and could be a good agent for Alzheimer treatment (Hajimehdipoor et al., 2014). On the other hand, based on the results from the investigation of cytotoxic activity gaillardin could be regarded as a promising candidate for studies in cancer therapy (Mosaddegh et al., 2010, Moghadam et al., 2013). The obtained amoebicidal activity of extract from *I. oculus-christi* (Degerli et al., 2012) is also a reason for further more detailed phytochemical research.

In continuation of our interest on *Inula* species growing in Bulgaria this work represents the results from phytochemical study of *Inula oculus-christi*.

2. Results and discussion

The chloroform extract of the aerial parts of *I. oculus-christi* was subjected to Sephadex LH-20 and Silica gel column chromatography as well as to procedures for purification to give seven sesquiterpene lactones (Fig. 1): the known gaillardin (**1**) (Fischedick et al., 2013), pulchellin E (**2**) (González-Romero et al., 2000), pulchellin C (**3**) (González-Romero et al., 2000) and four new sesquiterpene lactones (**4** – **7**).

Compound **4** possessed a molecular formula $C_{17}H_{22}O_6$ according to quasi-molecular ion $[M + H]^+$ at *m/z* 323.14872 (calcd for $C_{17}H_{23}O_6$ 323.14891), observed in HR-ESI mass spectrum. ^1H and ^{13}C NMR data (Tables 1 and 2) of **4** showed the presence of 3 methyl, 2 methylene

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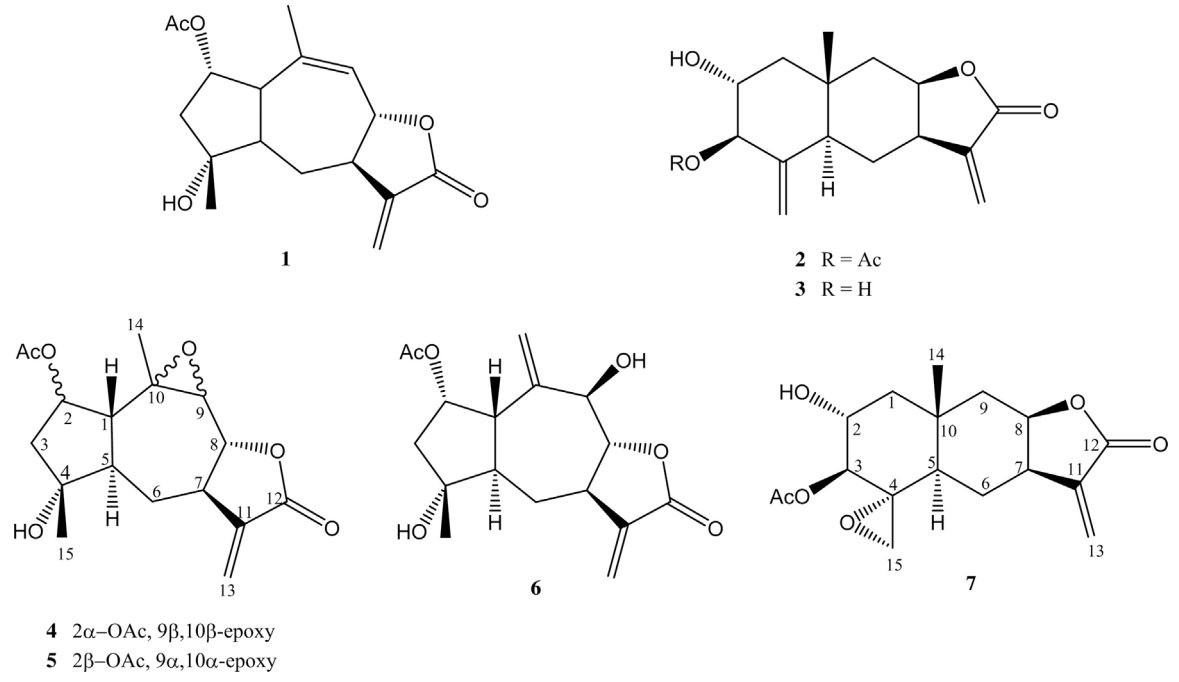

Fig. 1. Chemical structures of compounds **1–7**.

and 1 exomethylene groups, 3 methine and 3 oxo methine protons, 2 carbonyl functions as well as 2 oxygenated and 1 olefinic quaternary carbons. Further, the spectral data showed that **4** has α-methylene-γ-lactone ring (δ_H 6.28 d and 5.58 d, δ_C 121.1, 137.3 (exomethylene), 169.0 (C═O)), acetyl (δ_H 2.12 s (CH_3), δ_C 169.6 (C═O)), tertiary hydroxyl (δ_C 76.9) and trisubstituted epoxide (δ_H 3.02 d, δ_C 64.8 and 58.9) moieties. The sequence of bonding of C-3–C-2–C-1–C-5–C-6 – C-7–C-8 was demonstrated by cross peaks in COSY spectrum between δ_H 2.03 (2H-3)/5.30 (H-2), 5.30/1.81 (H-1), 1.81/2.19 (H-5), 2.19/1.20 (H-6′) and 2.45 (H-6), 1.20 and 2.45/2.85 (H-7), 2.85/4.02 (H-8), 2.85/6.28 (H-13) and 5.58 (H-13′). The only position for the tertiary hydroxyl group was at C-4 and for the epoxide ring was C-9/C-10. All these data and additional COSY, HSQC, HMBC revealed that **4** is a derivative of gaillardin (**1**) differing at C-9 and C-10 positions, i.e. a replacement of the trisubstituted $\Delta^{9,10}$ double bond with an epoxide group. Further, NOESY interactions H-7/H-5, H-7/H-9, H-14/H-9 and H-14/H-5 (Fig. 2) showed their *syn*-α-orientation, assuming biogenetical α-position of H-7. These correlations confirmed *cis*-β-epoxide. *Trans*-connection of C-1/C-5 was proven by cross peak between βH-8 (4.02 ppm) and H-1 (1.81 ppm). NOE interaction of H-1 with H-2, H-8, H-15 and H-6′ (1.20 ppm) showed their β-disposition. Thus, compound **4** was identified as 9β, 10β-epoxygaillardin.

Compound **5** was isolated as an oil with a molecular formula of $C_{17}H_{22}O_6$ as determined by the HRESIMS with quasimolecular ion at *m/z* 323.14871 $[M + H]^+$ (calcd for $C_{17}H_{23}O_6$ 323.14891). Comparison of ^1H and ^{13}C NMR data (Tables 1 and 2) of **5** with those of lactone **4** revealed that they are isomers differing in the stereochemistry at C-2, C-9 and C-10. The large vicinal coupling constant $J_{7,8}$ = 10.6 Hz revealed *trans*-connection of the lactone ring, i.e. H-8 was assumed to be β-orientated. NOESY correlations between αH-7/H-5 and βH-8/H-1 (Fig. 2) confirmed *trans*-fusion of 5- and 7-membered rings. Further, NOESY interactions H-8/H-6′ (δ_H 0.94), H-6′/H-1 and H-1/H-15 (δ_H 1.19)

Table 1
^1H NMR (600 MHz) data of compounds **4-7** in $CDCl_3$ (*J* in Hz).

H	4	5	6	7
1	1.81 (dd, 11.7, 7.6)	2.04 (dd, 12.7, 4.7)	2.61 (dd, 12.4, 6.8)	2.07 (dd, 13.2, 5.0)
1′				1.36 (dd, 13.2, 11.7)
2	5.30 (brq, 7.6)	5.26 (brt, 4.7)	5.12 (brq, 6.8)	3.90 (ddd, 11.7, 9.5, 5.0)
3	2.03 (d, 7.6)[a]	1.98 (d, 15.6)	2.20 (dd, 13.5, 6.8)	5.02 (d, 9.5)
3′	2.03 (d, 7.6)[a]	2.16 (dd, 15.6, 4.7)	2.10 (m)[b]	
5	2.19 (td, 11.7, 11.7, 3.0)	2.31 (td, 12.7, 12.7, 3.1)	2.10 (m)[b]	1.82 (dd, 12.9, 2.7)
6	2.45 (dt, 13.0, 3.0, 3.0)	2.26 (dt, 13.0, 3.1, 3.1)	2.34 (m)	1.66 (ddd, 14.0, 6.5, 2.7)
6′	1.20 (ddd, 13.0, 11.7, 10.7)	0.94 (ddd, 13.0, 12.7, 10.6)	1.33 (m)	0.98 (ddd, 14.0, 12.9, 11.8)
7	2.85 (tq, 11.7, 10.7, 3.5, 3.2, 3.0)	2.97 (tq, 10.6, 10.6, 3.3, 3.1, 3.1)	2.70 (m)	2.91 (ddd, 11.8, 6.5, 4.6)
8	4.02 (dd, 10.7, 6.0)	4.19 (d, 10.6)	4.14 (dd, 9.8, 6.8)	4.49 (brt, 4.6)
9	3.02 (d, 6.0)	3.30 (s)	4.31 (d, 6.8)	2.29 (dd, 15.4, 1.4)
9′				1.52 (dd, 15.4, 4.6)
13	6.28 (d, 3.5)	6.25 (d, 3.3)	6.28 (d, 3.5)	6.14 (s)
13′	5.58 (d,3.2)	5.56 (d, 3.1)	5.56 (d, 3.3)	5.60 (s)
14	1.54 (s)	1.38 (s)	5.30 (s)	1.02 (s)
14′			5.35 (s)	
15	1.24 (s)	1.19 (s)	1.23 (s)	2.55 (d, 4.7)
15′				2.88 (d, 4.7)
Ac	2.12 (s)	2.15 (s)	2.06 (s)	2.10 (s)

[a] ABX system.
[b] Overlapped signals.

Table 2
^{13}C NMR (150 MHz) data of compounds **4-7** in $CDCl_3$.

C	4	5	6[a]	7
1	52.2	49.5	45.3	48.1
2	73.2	75.1	73.9	68.7
3	47.4	48.3	48.3	76.0
4	76.9	79.1	77.2	58.1
5	51.4	50.1	53.7	42.7
6	30.0	26.7	29.8	23.1
7	43.8	41.2	41.8	40.1
8	83.0	82.3	88.6	76.3
9	64.8	60.3	80.0	41.4
10	58.9	57.8	147.5	33.0
11	137.3	138.9	138.6	141.3
12	169.0	169.6	169.8	170.8
13	121.1	120.2	121.3	121.3
14	20.3	22.8	118.9	19.8
15	24.1	26.0	23.7	44.9
CH_3 (Ac)	21.6	21.6	21.6	21.0
C=O (Ac)	169.6	170.9	171.1	170.2

[a] Data were extracted from ^{13}C NMR, HSQC and HMBC spectra of a mixture of pulchellin C (**3**) and **6**.

indicated their β-disposition. It should be also noted the significant difference which was observed in the shape of H-9 signals in ^{1}H NMR spectra of **4** and **5**.Thus, H-9 appeared as a singlet in the spectrum of **5** and as a doublet in that of **4.** Inspection of the Dreiding molecular model showed that this is possible when H-9 is β-oriented i.e. the dihedral angle between H-8 and H-9 in this case was around 90°. NOE correlations between H-9/H-14 and H-2/H-14 showed α-orientation of the epoxide group. The Dreiding model showed that H-2/H-14 cross peak is possible only when H-2 is α-disposed, i.e. C-2 acetoxy group was β. Based on the above evidence, the structure of **5** was established as 9α, 10α-epoxy-2-*epi*-gaillardin.

Compound **6** was obtained as yellowish oil. Its HRESIMS exhibited a quasimolecular ion peak $[M + H]^+$ at *m/z* 323.14878 (calcd for 323.14891), consistent with the molecular formula $C_{17}H_{23}O_6$. ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) of **6** showed that this component contained one acetyl (δ_H 2.06 s (CH_3), δ_C 171.1 (C=O)), one secondary hydroxyl (δ_H 4.31 d and δ_C 80.0), one tertiary hydroxyl group (δ_C 77.2), and α-methylene-γ-lactone ring (δ_H 6.28 d and 5.56 d, δ_C 121.3 and 138.6 (exomethylene), 169.8 (C=O)). The location of the functional groups and the connectivity of C-3–C-2–C-1–C-5–C-6–C-7–C-8–C-9 were assigned using ^{1}H–^{1}H COSY interactions. Further, a pair of singlets at δ_H 5.30 and 5.35 for two olefinic protons as well as the corresponding carbon at δ_C 118.9 and a signal for a quaternary carbon at δ_C 147.5 revealed the presence of an exomethylene group. HMBC correlations between these carbons and H-9 (δ_H 4.31 d) demonstrated its position at C-10/C-14. The relative configuration at the chiral centers of **6** was elucidated by coupling constants of protons and NOESY spectrum. Thus, NOESY correlations H-7/H-5 and H-7/H-9 (Fig. 2) indicated their *syn*-α-orientation. The vicinal coupling constant $J_{7,8}$ = 9.8 Hz showed *trans*-fusion of the lactone ring, i.e. H-8 was β. The observed NOE correlation between H-8 and H-1 revealed *trans*-connectivity of C-1/C-5. α-Disposition of the acetyl ester and β-orientation of C-4 methyl group were established from H-1/H-2 and H-1/H-15 NOESY interactions, respectively. Thus, compound **6** was identified as 9β-hydroxy derivative of 2α-acetoxy-4α-hydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide. The latter one was isolated previously from *I. lineariifolia* (Nie et al., 2010).

The HRESIMS of compound **7** indicated a quasimolecular ion peak at *m/z* 323.14864 $[M + H]^+$, corresponding to a molecular formula $C_{17}H_{23}O_6$ (calcd for 323.14891), which required 7degrees of unsaturation. ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **7** showed two downfield shifted signals at δ_H 3.90 and 5.02 each for one proton, and corresponding carbons at δ_C 68.7 and 76.0, which revealed the presence of two oxygen containing functional groups. The first one should be hydroxyl group, while the second one was acetyl (δ_H 2.10, CH_3 and δ_C 170.2, C=O). Other two oxygen atoms in the molecule belonged to a lactone ring, the evidence for which were IR absorption band at 1767 cm^{-1}, broad triplet for oxymethine at δ_H 4.49 (δ_C 76.3) as well as characteristic multiplet for H-7 at 2.91 ppm. The observed two singlets at δ_H 5.60 and 6.14, a carbon signal at δ_C 121.3 and a signal for a quaternary carbon at δ_C 141.3 corresponded to an exomethylene group. Further, two doublets at δ_H 2.55 and 2.88 with J_{gem} 4.7 Hz for a

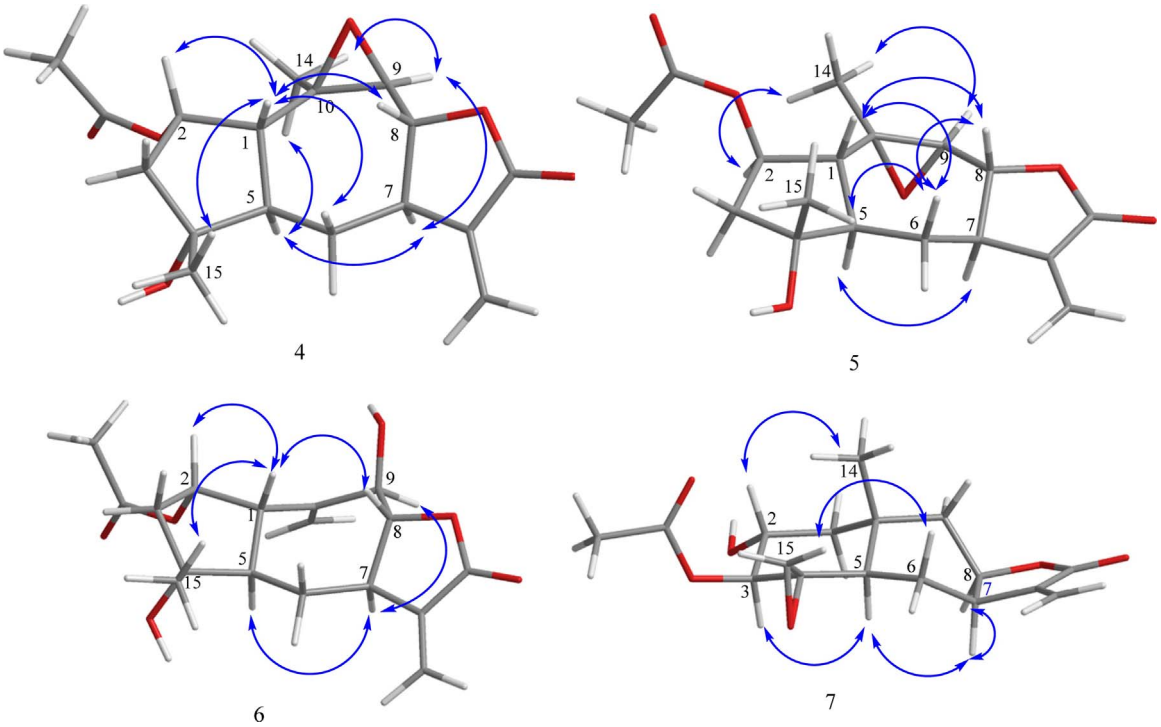

Fig. 2. Selected NOESY correlations (blue solid arrows) for compounds **4–7**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

methylene group, its carbon signal at δ_C 44.9 and a signal for quaternary carbon at δ_C 58.1 confirmed the presence of spirocyclic epoxide ring positioned at C-4. Thus, the proposed epoxide ring, acetyl moiety, and unsaturated lactone ring corresponded to 5degrees of unsaturation. The other two degrees required bicyclic carbon skeleton of the compound. COSY and HSQC experiments allowed determination of the position of the functional groups in an eudesmane structure, while the proposed relative stereochemistry was based on the coupling patterns of protons and NOESY spectrum. The observed interaction between H-3/H-5, H-5/H-7 and H-7/H-8 (Fig. 2) revealed their α-orientation, assuming biogenetical α-position of H-7. The coupling constant $J_{2,3}$ = 9.5 Hz, confirmed *trans*-disposition of H-2 and α-H-3. β-Orientation of C-10 methyl group was supported from NOESY cross peak between H-2/H-14. The large coupling constants $J_{5,6'}$ = 12.9 Hz and $J_{6',7}$ = 11.8 Hz showed β-H-6′, which signal was at 0.98 ppm. NOE correlation between the latter and H-15 exhibited β-orientation of C-4/C-15 bond. Thus, compound **7** was identified as 4α,15α-epoxypulchellin E.

IR spectrum of less polar fraction contained absorption band at 1735 cm^{-1}, which showed presence of compounds bearing some ester groups. Three triterpenoids were isolated by prep. TLC and their structures were proved to be 3-*O*-palmitates of 16β-hydroxylupeol (Kundakovic et al., 2004), 16β-hydroxy-β-amyrin (Kundakovic et al., 2004), and faradiol (Ragasa et al., 2005) using ^{1}H NMR data. All these compounds were earlier found in *I. britannica* (Ivanova et al., 2017).

The isolated sesquiterpene lactones are closely related compounds with guaiane (**1**, **4-6**) and eudesmane (**2**, **3** and **7**) carbon skeleton. It was found that guaianolides were dominating and gaillardin (**1**) was the principal one like in *I. oculus-christi* of other origins (Vajs et al., 2003; Mosaddegh et al., 2010). Pulchellin E (**2**) was detected in *I. oculus-christi* from Montenegro (Vajs et al., 2003), while pulchellin C (**3**) – in the plant from Iran (Mosaddegh et al., 2010). Our results are consistent with the previous reports and enrich the knowledge on the lactone profile of *I. oculus-christi* with the newly identified components. The obtained results could be of chemotaxonomic interest.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a Perkin Elmer Model 341 Polarimeter. IR spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer (software OMNIC, Version 7.0). The samples were prepared as KBr pellets. Spectral data were collected in the mid-IR range (4000–400 cm^{-1}) with 64 scans and 2 cm^{-1} resolution. A background spectrum (32 scans) was recorded before every sample spectrum. The 1D and 2D NMR spectra were recorded on a Bruker Avance II+ 600 spectrometer with operating frequency 600 MHz (^{1}H) and 150 MHz (^{13}C), using the residual solvent signal (δ_H 7.26 in ^{1}H and δ_C 77.16 in ^{13}C for $CDCl_3$, as a reference. The chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hz. The 1D and 2D NMR (^{1}H and ^{13}C NMR, COSY, HSQC and HMBC) spectra were recorded using the standard Bruker pulse sequence. HRESIMS analyses were conducted on a Thermo Scientific Q Exactive Plus (Bremen, Germany) mass spectrometer. Column chromatography (CC) was performed with Silica gel 60 (230–400 mesh, Merck, Germany) and Sephadex LH-20 (Pharmacia, Fine Chemicals AB, Fluka). Prep. TLC was carried out on TLC plates (Silica gel 60 F_{254}, Merck). Fractions were monitored by TLC detection which was achieved by spraying the TLC plates with conc. H_2SO_4 followed by heating. All solvents used were of analytical grade.

3.2. Plant material

The aerial parts of *I. oculus-christi* were collected in August 2016 in full flowering stage. The species was identified by Dr. Ina Aneva from the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences. A voucher specimen (SOM – 1360) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.

3.3. Extraction and isolation

Air-dried and finely powdered aerial parts of *I. oculus-christi* (95 g) were successively extracted with $CHCl_3$ (3 × 500 mL) at room temperature. After filtration, the solvent was evaporated under vacuum at low temperature (40 °C) to give 4.5 g of crude extract. The extract was dissolved in $CHCl_3$ and subjected to CC on Silica gel. Elution of the column with $CHCl_3$/Acetone mixtures with increasing polarity (from 20:1 to 0:1) afforded nine fractions. Prep. TLC (Hexane/Et_2O, 5:1, x3) of a portion of Fr. 2 (250 mg) afforded 16β-hydroxylupeol 3-*O*-palmitate (3.2 mg), 16β-hydroxy-β-amyrin 3-*O*-palmitate (3 mg), faradiol 3-*O*-palmitate (2.8 mg), and β-sitosterol (5 mg). Fr. 6 (170 mg) after CC (Silica gel, $CHCl_3$/Acetone, 8:1) gave 4 subfractions. Fr. 6/3 gave one spot on TLC and after recrystallization afforded 40 mg mixture of gaillardin (**1**) and pulchellin E (**2**) in ratio 3:1 (calculated from ^{1}H NMR). CC ($CHCl_3$/MeOH, 30:1) of Fr. 7 (320 mg) afforded 4 subfractions. Prep. TLC of subfr. 7/1 (Cyclohexane/EtOAc, 1:1, x 4) gave compounds **4** (2 mg) and **5** (3 mg). Prep. TLC of subfr. 7/2 ($CHCl_3$/MeOH, 30:1, x 3) afforded **7** (2 mg) and prep. TLC ($CHCl_3$/EtOAc, 1:1, x3) of subfr. 7/3 yielded **3** (3 mg) and **6** (0.8 mg).

9β,10β-Epoxygaillardin (**4**): Colorless oil. $[\alpha]_D^{20}$ −2 (c 0.1, MeOH); IR (KBr) ν_{max} 3435, 2925, 2854, 1770, 1735, 1666 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 323.14872 $[M + H]^+$ (calcd for $C_{17}H_{23}O_6$ 323.14891)

9α,10α-Epoxy-2-*epi*-gaillardin (**5**): Colorless oil. $[\alpha]_D^{20}$ +15 (c 0.1, MeOH); IR (KBr) ν_{max} 3422, 2925, 2853, 1769, 1732, 1667, 1644 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 323.14871 $[M + H]^+$ (calcd for $C_{17}H_{23}O_6$ 323.14891)

2α-Acetoxy-4α,9β-dihydroxy-1β-guaia-11(13),10(14)-dien-12,8α-olide (**6**): Colorless oil. $[\alpha]_D^{20}$ +18 (c 0.05, MeOH); IR (KBr) ν_{max} 3423, 2957, 2924, 2852, 1774, 1737, 1666, 1651 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 323.14878 $[M + H]^+$ (calcd for $C_{17}H_{23}O_6$ 323.14891)

4α,15α-Epoxypulchellin E (**7**): Colorless oil. $[\alpha]_D^{20}$ +12 (c 0.05, MeOH); IR (KBr) ν_{max} 3440, 2952, 2924, 2852, 1767, 1737, 1636 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 323.14864 $[M + H]^+$ (calcd for $C_{17}H_{23}O_6$ 323.14891)

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2017.07.008.

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