Biochemical Systematics and Ecology 64 (2016) 70–73

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Chemical constituents from *Ilex urceolatus*

De-Qing Wang [a], Cheng-Nan Wu [b], Yang Song [b], Hai-Qiang Jiang [c], Hong-Lei Zhou [c, **], Chao Zhang [c], Hui-Fen Li [c], Xue-Lan Zhang [c], Yi Wu [b, *]

[a] College of Life and Environment Science, Huangshan University, Huangshan 245041, PR China
[b] Institute of Traditional Chinese Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, #1 Weigang, Nanjing 210095, Jiangsu Province, PR China
[c] College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250355, Shandong Province, PR China

ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form 21 November 2015
Accepted 28 November 2015
Available online 17 December 2015

Keywords:
Ilex urceolatus
Triterpenoids
Flavonoids
Phenylpropanoids
Chemotaxonomy

ABSTRACT

Twenty-four compounds were obtained from the extract of the leaves of *I. urceolatus*, which were divided into saturated fatty alcohols (**1** and **2**), triterpenoids (**3**–**8** and **14**–**16**), lignanoids (**9**, **20** and **22**), coumarins (**10** and **19**), flavonoids (**11**–**13**, **21**, **23** and **24**) and others (**17** and **18**). Among them, compounds **1**, **2**, **17** and **18** were firstly obtained from the genus *Ilex*, others were isolated from this species for the first time. The chemotaxonomic relationships between *I. urceolatus* and other species of genus *Ilex* were also discussed. As a result, the isolated compounds closely matched the ones obtained in other species of the genus.

1. Subject and source

The genus *Ilex* (Aquifoliaceae) contains approximately 500–600 species, most of which distribute in tropical and subtropical regions of the world (Fan et al., 2015). *Ilex urceolatus* C. B. Shang, K. S. Tang et D. Q. Du, as a native evergreen arbor tree, is found in Hunan. The dried leaves of this plant were collected in Lianyuan, Hunan Province of China in July 2012, and were authenticated by Dr. Deyun Wang (Institute of Traditional Chinese Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University), where a voucher specimen (No. IU20120702) was deposited.

2. Previous work

Up to now, phytochemical investigations on genus *Ilex* have exhibited the presence of triterpene saponins (Tang et al., 2005), lignanoid glycosides (Wang et al., 2014), phenolic glycosides (Zhang et al., 2005), flavonoids (Del Pero Martinez et al., 1997), hemiterpene glycosides (Fuchino et al., 1997), alkaloids (Xu et al., 2009) and sterols (Wu et al., 2014).

3. Present work

The air dried leaves of *I. urceolatus* (10 kg) were extracted with aqueous EtOH (85%, v/v) under refluxed three times. The combined solvent was concentrated under reduced pressure to yield a brown crude extract (1.02 kg). The extract was then

* Corresponding author.
** Corresponding author.
E-mail addresses: zhouhongleitcm@163.com (H.-L. Zhou), wuyi2001cn@163.com (Y. Wu).

http://dx.doi.org/10.1016/j.bse.2015.11.018
0305-1978/

dissolved in water and partitioned successively with petroleum ether (PE, boiling point 60–90 °C), ethyl acetate (EtOAc) and *n*-BuOH, then evaporated to dryness under reduced pressure to give PE (160 g), EtOAc (225 g) and *n*-BuOH (360 g) extracts, respectively.

The EtOAc fraction was subjected to a silica gel (100–200 mesh) column chromatography (CC) and eluted with a step gradient of CH_2Cl_2–MeOH (from 29:1 to 0:1, v/v) to yield four fractions (Fr. I–IV). Fr. I (31 g) was subjected to a silica gel CC (200–300 mesh) eluting with PE–EtOAc (from 19:1 to 1:1, v/v) to obtain four sub-fractions (sub-fraction Ia–Id). Sub-fraction Ia was separated on repeated silica gel CC with PE–acetone (gradient, 30:1 to 10:1, v/v) and PE–EtOAc (gradient, 20:1 to 8:1, v/v) to yield **1** (36 mg) and **2** (20 mg). Fr. III (26 g) was performed on a silica gel CC (200–300 mesh) eluting with PE–acetone (from 15:1 to 1:1, v/v) to obtain five sub-fractions (sub-fraction IIIa–IIIe). Sub-fraction IIIc was purified by repeated silica gel H CC with *n*-hexane–EtOAc–MeOH (gradient, 10:1:1 to 0:4:1, v/v) to give **3** (12 mg), **4** (16 mg) and **5** (23 mg). Sub-fraction IIId was further separated using repeated silica gel CC (200–300 mesh) with *n*-hexane–acetone (gradient, 10:1 to 1:1, v/v) to obtain **6** (57 mg), **7** (13 mg) and **8** (11 mg). Fr. IV (13 g) was subjected to a silica gel CC (200–300 mesh) with CH_2Cl_2–MeOH (from 25:1 to 1:1, v/v) to give four sub-fractions (sub-fraction IVa–IVd). Compounds **9** (18 mg) and **10** (10 mg) were furnished from sub-fraction IVb after being re-chromatographed over silica gel H CC with PE–acetone–MeOH (gradient, 4:4:1 to 0:3:1, v/v). Sub-fraction IVc was applied to silica gel CC eluted with cyclohexane–acetone–MeOH (gradient, 9:3:1 to 0:2:1, v/v) to give **11** (25 mg), **12** (17 mg) and **13** (32 mg).

The *n*-BuOH fraction was chromatographed over a macroporous resin D101 column eluted by aqueous alcohol (0:100, 30:70, 50:50, 70:30 and 100:0, v/v) to yield five fractions (Fr. A–E). The Fr. B (49 g, v/v 30% part) was subjected to a silica gel (100–200 mesh, inactivated) CC with a step gradient of CH_2Cl_2–MeOH–H_2O (from 19:1:0.1 to 1:1:0.1, v/v) to give six groups (Fr. B1–B6). Fr. B3 was applied to ODS column eluted with aqueous MeOH (gradient, from 20% to 80%, v/v) to give five fractions (Fr. B3a–B3e). Compounds **14** (34 mg), **15** (11 mg), **16** (22 mg), **17** (10 mg) and **18** (8 mg) were isolated by repeated preparative TLC with $CHCl_3$–MeOH–H_2O (gradient, 90:10:10 to 55:45:10, v/v) from Fr. B3a–B3e, respectively. Fr. C (35 g, v/v 50% part) was performed on silica gel (100–200 mesh, inactivated) CC with CH_2Cl_2–MeOH–H_2O (from 15:1:0.1 to 1:1:0.1, v/v) to give four sub-fractions (sub-fraction C1–C4). Fr. C4 was further applied to ODS column eluted with MeOH–H_2O (gradient, 1:9–9:1, v/v) and then isolated by Sephadex LH-20 CC with MeOH–H_2O (gradient, 3:1–1:1, v/v) to obtain **19** (9 mg), **20** (16 mg) and **21** (19 mg). The Fr. D (27 g, v/v 70% part) was fractioned by ODS CC eluting with MeOH–H_2O (gradient, 1:9–9:1, v/v) to afford six sub-fractions (sub-fraction D1–D6). Sub-fraction D3 was further separated by preparative TLC with *n*-BuOH–HAc–H_2O (gradient, 6:1:1–3:1:1, v/v) and $CHCl_3$–MeOH–H_2O (gradient, 90:10:10 to 55:45:10, v/v) repeatedly, then purified by Sephadex LH–20 CC with MeOH–H_2O (1:1, v/v) to yield **22** (14 mg), **23** (37 mg) and **24** (26 mg).

The structures of the isolated compounds were determined on the basis of the MS, ^{1}H NMR, ^{13}C NMR and by comparison with reported data in the related literature. They are identified as eicosanol (**1**) (Wang et al., 2009), docosanol (**2**) (Liu et al., 2011), hederagenin (**3**) (Wei et al., 2000), uvaol (**4**) (Xie et al., 2007), 2α-hydroxyursolic acid (**5**) (Jiang et al., 2013), lupeol (**6**) (Lin et al., 1996), lup-20(29)-ene-3β-ol-24-methyl ester (**7**) (Ouyang et al., 1997), lup-20(29)-ene-3β,24-diol (**8**), (Xie et al., 2007) (Liu et al., 2003) syringaresinol (**9**) (Li et al., 1997), esculetin (**10**) (Yang and Yan, 2002), quercetin (**11**) (Xu et al., 2009), kaempferol (**12**) (Xu et al., 2009), isorhamnetin (**13**) (Yang and Yan, 2002), ilexoside XXXVIII (**14**) (Amimoto et al., 1993), ilexoside XXX (**15**) (Amimoto et al., 1992), ilexoside XXXVI (**16**) (Amimoto et al., 1993), betulalbuside A (**17**) (Pan, 2011), roseoside (**18**) (Okamura et al., 1981), esculin (**19**) (Zhang et al., 2000), syringaresinol O-β-D-glucopyranoside (Li et al., 1997), (**20**), quercetin-3-O-β-glucopyranoside (**21**) (Zhou, 2007), liriodendrin (**22**) (Yang et al., 2006; Wang et al., 2014), rutin (**23**) (Xu et al., 2009), kaempferol-3-O-β-rutinoside (**24**) (Xu et al., 2009) (Fig. 1).

4. Chemotaxonomic significance

In this study, 24 compounds were isolated by chromatographic methods from the aqueous EtOH extract of the leaves of *I. urceolatus*. This is the first report of compounds **1**, **2**, **17** and **18** from the genus Ilex, whereas others (**3**–**16** and **19**–**24**) have been isolated from the genus but not previously reported to occur in *I. urceolatus*.

Previous investigation of *I. urceolatus* revealed the existence of triterpenoid saponins and sapogenins (Wu et al., 2014). In this study, triterpenoid sapogenins (**3**–**8**) and triterpenoid saponins (**14**–**16**) were also obtained. These compounds can be divided into oleanane (**3**), ursane (**4**, **5**, **14**–**16**) and lupine (**6**–**8**) type triterpenoids. These compounds were reported from *Ilex kwangtungensis* Merr. (Wei et al., 2000) (**3**), *Ilex pernyi* Franch. (Xie et al., 2007) (**4**), *Ilex pubescens* Hook et Arn (Jiang et al., 2013) (**5**), *Ilex centrochinensis* S. Y. Hu (Lin et al., 1996) (**6**), *Ilex kudingcha* C. J. Tsing (Ouyang et al., 1997) (**7**), *I. pernyi* Franch. (Xie et al., 2007) (**8**) and *Ilex rotunda* Thunb. (**14**–**16**) (Amimoto et al., 1992, 1993, 15 and 16, 14), respectively. This suggests the close chemotaxonomic relationships among these species of *Ilex*. Additionally, other ursane and oleanane triterpenoids have been reported from various species of *Ilex* such as *I. pernyi* (Xie et al., 2007), *Ilex purpurea* Hassk (Liao et al., 2005), *Ilex hainanensis* Merr. (Peng et al., 2012), *Ilex amara* (Vellozo) Loes. (Pezzuto de Andrade et al., 2002), *Ilex crenata* cv. Convexa Makino (Kakuno et al., 1991), *I. pubescens* Hook. et Arn. var. glabra Chang (Lin et al., 2011), *I. centrochinensis* (Li et al., 2013) and *I. pubescens* (Lin et al., 2015). Therefore, ursane and oleanane triterpenoids can be recognized as biomarkers for *Ilex*.

Among the five phenylpropanoids obtained in this study, two coumarins (**10** and **19**) were reported from *Ilex cornuta* Lindl. (Yang and Yan, 2002, **10**) and Erigeron breviscapus (Vant.) Hand.-Mazz. (Asteraceae) (Zhang et al., 2000) (**19**), while the lignans were previously reported from *Ilex asprella* (Hook. Et Arn.) Champ. ex Benth. (Li et al., 1997, **9** and **20**), *I. pubescens* (Yang et al., 2006, **22**) and *I. rotunda* Thunb. (Wang et al., 2014, **22**). Three flavonoid aglycones (**11**–**13**) and three flavonoid glycosides (**21**, **23** and **24**) were isolated from *Ilex paraguariensis* St. Hil. (Xu et al., 2009, **11**, **12**, **23** and **24**), *I. cornuta* (Yang and