Journal of Ethnopharmacology 147 (2013) 341–348

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Anti-inflammatory compounds of "Qin-Jiao", the roots of *Gentiana dahurica* (Gentianaceae)

Yan-Ming Wang [a,b], Min Xu [a,*], Dong Wang [a], Chong-Ren Yang [a,c], Yang Zeng [d], Ying-Jun Zhang [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Weihe Biotech Research and Development Center, Yuxi 653101, China
[d] School of Life and Geographical Sciences, Qinghai Normal University, Xining 81008, China

ARTICLE INFO

Article history:
Received 28 November 2012
Received in revised form
5 February 2013
Accepted 7 March 2013
Available online 15 March 2013

Keywords:
Gentiana dahurica
Anti-inflammatory
Nitric oxide (NO)
Cyclooxygenase (COX)
Zebrafish model

ABSTRACT

Ethnopharmacological relevance: "Qin-Jiao" is a well-known traditional Chinese medicinal (TCM) herb having been used generally for fighting rheumatoid arthritis (RA) since ancient times. The root of *Gentiana dahurica* Fisch (Gentianaceae) is one of the four officially validated "Qin-Jiao" as listed in the Chinese Pharmacopoeia. In addition, it is a common Tibetan medicinal herb used for the treatment of tonsillitis, urticaria, and RA, while the flowers have been used as a Mongolian herb for curing cough sore throat and eliminating the phlegm due to its anti-inflammatory effect.

Aim of the study: The aim of the study was to characterize the anti-inflammatory compounds in "Qin-Jiao", on the basis of detailed investigation on not only the phytochemical study of *Gentiana dahurica*, but also the bioactive evaluation on compounds obtained presently and previously from different "Qin-Jiao" origins and *Gentiana* species.

Materials and methods: The ethanol extract of air-dried roots of *Gentiana dahurica* was suspended into H_2O and extracted with EtOAc and *n*-BuOH, successively. Repeated column chromatography (CC) and semi-preparative HPLC were carried out on each of the fractions. The isolated compounds were determined by detailed spectroscopic analysis and acidic hydrolysis. Anti-inflammatory activities of 18 isolates, together with 12 typical compounds obtained previously by our group from the other "Qin-Jiao" origins (*Gentiana crassicaulis*, *Gentiana straminea*) and *Gentiana rigescens*, were tested by inhibitory effects on LPS-induced NO production in macrophage RAW264.7 cells and TPA-induced cyclooxygenases-2 and -1 (COXs-2/1) production on zebrafish model.

Results: A new lignan glycoside (**1**) was identified, together with 20 known compounds, including 10 iridoid glycosides (**2**–**11**), three steroids (**12**–**14**), four lignans (**15**–**18**), one phenylpropanoid (**19**) and two triterpenes (**20**–**21**). Anti-inflammatory bioassay showed that only compound **21** displayed potential inhibitory effect on NO production (IC_{50} = 16.85 μM), while 20 tested compounds had inhibitory activities on COXs-2/1. Among them, the triterpenoid **21** was the most active compound with an inhibitory value of 78% at a concentration of 30 μM. All the tested compounds showed no cytotoxicity on five human cancer cell lines (40 μM) and zebrafish (30 μM), except for **21** displaying weak cytotoxicity on human myeloid leukemia HL-60 (IC_{50} = 16.43 μM).

Conclusion: Most of compounds particularly iridoid glycosides from "Qin-Jiao" display potential inhibitory effect on COXs-2/1. The results support the historical importance of the well-known TCM herb, "Qin-Jiao", having been commonly used for fighting RA. As major components, the bioactive iridoid glycosides should play important role in the anti-inflammatory effect of "Qin-Jiao". Although further research will be required to evaluate the selective activities of the COXs-2/1 inhibitors, this work validates the medicinal use of "Qin-Jiao" and provides information for different "Qin-Jiao" origins having different treating effects on RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune, and inflammatory disease that primarily attacks the joints, but also the skin, cardiovascular system, lungs, and muscles of human beings. RA is a major cause of disability and affects up to 0.5–1.0%

* Corresponding author. Tel.: +86 871 65223235.
E-mail addresses:
xumin@mail.kib.ac.cn (M. Xu),
zhangyj@mail.kib.ac.cn (Y.-J. Zhang).

0378-8741/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.03.016

of the adult population worldwide (Ernest and Gabriel, 2001; Gary, 2003; Gerard et al., 2000). Autoimmune targeting of normal joint proteins results in inflammation, with resultant local release of cytokines, TNF, growth factor, and interleukins, all of which induce cyclooxygenase (COX) expression. In addition, induction of nitric oxide (NO) synthesis has been identified as one of the major responses to inflammatory stimuli in macrophages (Nathan and Hibbs, 1991). COX pathway inhibitors are some of the most frequently prescribed drugs in medicine. The non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used agents in this class (Park et al., 2011).

"Qin-Jiao", being used for the treatment of rheumatism, arthralgia, stroke, hemiplegia, pains, jaundice, and infantile malnutrition, has been an important traditional Chinese medicinal (TCM) herb for fighting rheumatoid arthritis (RA) since ancient times in China (Cai et al., 2010). In Chinese Pharmacopoeia, the roots of four plants from the genus *Gentiana* (Gentianaceae) including *Gentiana macrophylla* Pall., *Gentiana crassicaulis* Duthie., *Gentiana straminea* Maxim., and *Gentiana dahurica* Fisch. are recorded as the original materials of "Qin-Jiao" (Chinese Pharmacopoeia, 2010). To date, the former three, *Gentiana macrophylla*, *Gentiana crassicaulis*, and *Gentiana straminea* have been chemically and biologically investigated by several groups (Lv et al., 2012; Singh, 2008; Tan et al., 1996; Xu et al., 2009a; Liu et al., 1994). The results suggested that "Qin-Jiao" contained mainly iridoid glycosides and the water extracts from roots of *Gentiana macrophylla* displayed a significant inhibitory effect on acute treatment of rheumatoid rats (Yu et al., 2004). Loganic acid (**10**), an iridoid glucoside widely existed in *Gentiana* plants, could inhibit the carrageenan-induced mouse paw edema (María del et al., 1994). Gentiopicroside (**2**), another major iridoid glucoside, also showed inhibitory effect on inflammatory mediators NO and COX-2 (An and Jin, 2007).

Gentiana dahurica is distributed in most areas of China except for Yunnan province and Northeast area (Li et al., 2006). In addition to be one of the officially validated "Qin-Jiao", it is a common Tibetan medicine used for fighting RA and the flowers have been used as a Mongolian medicine for curing sore throat, cough and cleaning away lung-heat. Previously, five iridoid glycosides and seven triterpenoids with moderate cytotoxicities were reported from the roots of *Gentiana dahurica* (Fan et al., 2010). However, its anti-inflammatory constituents have so far not been known. As a part of an ongoing effort to search for new bioactive compounds from *Gentiana* medicinal plants and to characterize the anti-inflammatory compounds in "Qin-Jiao" (Liu et al., 1994; Xu et al., 2007, 2009a, 2011, 2008, 2009b; Lv, et al., 2012), the phytochemical investigation on the roots of *Gentiana dahurica* was carried on. This led to the isolation of one new lignan glycoside together with 20 known compounds. The anti-inflammatory activities of most of the isolates and 12 typical compounds obtained previously by our group from the other "Qin-Jiao" origins (*Gentiana crassicaulis*, *Gentiana straminea*) and *Gentiana rigescens*, were tested by inhibitory effects on LPS-induced NO production in macrophage RAW264.7 cells and TPA-induced cyclooxygenases-2 and 1 (COXs-2/1) production on zebrafish model, one of the prominent iso-enzyme at sites of inflammation. The structure-activity relationship (SAR) of tested compounds was discussed.

2. Material and methods

2.1. Plant material and compounds tested for anti-inflammatory activities

The roots of *Gentiana dahurica* were collected in Xining, Qinghai province, PR China and identified by Professor Yang Zeng (School of Life and Geographical Sciences, Qinghai Normal University). A voucher (KUN_551024) specimen has been deposited in Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (CAS).

Thirty compounds were tested for anti-inflammatory activities. These include 18 isolates (**1**–**6**, **8**–**10**, **12**–**17,** and **19**–**21**) reported in the present study. Since compounds **8** and **9** were isolated a**s** a mixture from the title plant, the purified ones for bioassay were obtained previously from *Gentiana rhodantha* by our group (Xu et al., 2008). In addition, 11 typical iridoid glycosides (**22**–**32**) and one chromene glycoside (**33**) were obtained previously by our group from the other "Qin-Jiao" and related *Gentiana* species, referring to 6′–*O*-β-D-glucosylloganic acid (**22**), qingjiaosides A–C (**23**–**25**), and 4′–*O*-β-D-glucosylgentiopicroside (**26**) from *Gentiana crassicaulis* (Lv et al., 2012), macrophyllosides A, E–F (**27**–**29**), loganic acid 11-*O*-β-D-glucopyranosyl ester (**30**), and macrophylloside D (**33**) from *Gentiana straminea* (Xu et al., 2009a), and 2-(*o*,*m*-di-hydroxybenzoyl)-sweroside (**31**) and sweroside (**32**) from *Gentiana rigescens* (Xu et al., 2006).

2.2. General experimental techniques

Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR spectra were measured on a Bio-Rad FTS-135 series spectrometer in dry film. UV spectra were recorded on a Shimadzu UV2401A ultraviolet–visible spectrophotometer. ESIMS and HRESI-MS were run on an API QSTAR Pular-1 spectrometer. NMR spectra measured in DMSO or CD_3OD solution and recorded on a Bruker AV400, DRX-500 or Avance III-600 spectrometer at 25 °C, using TMS as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (J) were expressed in Hz. Column chromatography (CC) were carried out over silica gel (200–300 mesh, Qingdao Marine Chemical Factory), Diaion HP20SS (Mitsubishi Chemical Industry, Ltd.), MCI-gel CHP-20P (75–150 μm, Mitsubishi Chemical Industry, Ltd), Rp-18 (40–63 μm, Merck), and Rp-8 (40–63 μm, Merck). Pre-coated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done under UV light (254 nm and 365 nm) and by spraying the plates with 10% sulfuric acid followed by heating. An Agilent series 1100 (Agilent Technologies) were used for HPLC. An Agilent ZORBAX SB-C_{18} column 5 μm 143 Å column (250 mm × 9.4 mm) were used for semi-preparative HPLC separations. GC analysis was run on Agilent Technologies HP5890 gas chromatography equipped with an H_2 flame ionization detector. The column was 30QC2/AC-5 quartz capillary column (30 m × 0.32 mm) with the following conditions: column temperature: 180 °C/280 °C; programmed increase, 3 °C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4 μl, split ratio: 1/50.

2.3. Extraction and isolation of compounds

As previously reported on the extraction and isolation of secondary metabolites from Gentianaceous medicinal plants (Geng, et al., 2009; Xu et al., 2006), the air-dried roots (2.57 kg) of *Gentiana dahurica* were extracted with 95% ethanol two times, following with 50% ethanol two times, each time 3 h under reflux at 80 °C. After concentrated in vacuum, the combined extract (147.4 g) was suspended into H_2O and partitioned with EtOAc and *n*-BuOH, successively.

The H_2O layer (dry weight 68.9 g) was loaded on a Diaion HP20SS column, eluting with MeOH/H_2O (0:1–1:0), to give eight fractions (H1–H8). H1 (13.7 g) was applied to a silica gel column chromatograph (CC), eluting with $CHCl_3$/MeOH/H_2O (8:2:0.2–7:3:0.5) to afford compound **10** (35 mg). H2 (3.4 g) was almost the pure compound **10**. H3 (1.5 g) was subjected to CC over