Industrial Crops & Products 125 (2018) 114–122

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Novel flavonoids from *Lonicera japonica* flower buds and validation of their anti-hepatoma and hepatoprotective activity *in vitro* studies

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ARTICLE INFO

Keywords:
Lonicera japonica
Flavonoids
Anti-hepatoma activity
Hepatoprotective activity

ABSTRACT

Lonicera japonica, a widely used traditional Chinese medicine, possessed antiviral, hepatoprotective, anti-tumor and other activities. Meanwhile, *L. japonica* could be used as healthy food, cosmetics, and so on. Herein, four novel flavonoids, japoflavone A–D (**2**-**3**, **5**, **12**), together with ten known flavonoids, were isolated and identified on the basis of spectroscopic evidence from *L. japonica*. In addition, all isolates were assayed for their anti-hepatoma and hepatoprotective activities *in vitro*. **5** and **8** showed significant anti-hepatoma activity in SMCC 7721 cell with IC_{50} values of 13.01 ± 2.62 and 16.69 ± 0.35 μg/ml; Compound **12** showed significant hepatoprotective activity against H_2O_2-induced injury in SMCC 7721 and HepG 2 cells ($p < 0.001$). Drop in the levels of catalase and superoxide dismutase caused by H_2O_2 were remarkably reversed in a dose-dependent manner after treatment with **12**. This study provided a phytochemical evidence for further development and utilization of *Lonicera japonica* in health products.

1. Introduction

The flower buds of *Lonicera japonica* Thunb. (Caprifoliaceae), known as "JinYin Hua" in Chinese, provide some of the most common ingredients for formulations used in traditional Chinese medicine for treating influenza, cold, fever, and infections (Chinese Pharmacopoeia Commission, 2015; Teng et al., 2000). Moreover, they have also been used in indigenous beverages in Korea and China for many years, such as tea (Lee et al., 2010; Wang et al., 2008). Pharmacological studies have shown that extracts of the flower buds of *L. japonica* have a broad spectrum of biological activity, including antibacterial, anti-inflammatory, antioxidant, antiangiogenic, anticancer, antiviral, and hepatoprotective effects (Lee et al., 2005; Shang et al., 2011; Tae et al., 2003; Xiong et al., 2013; Yoo et al., 2008). In anti-hepatoma and hepatoprotective study, the extract of *L. japonica* could trigger HepG2 cell death in a JNK-dependent manner (Yip et al., 2006) and showed significantly hepatoprotective effect by pathological analysis in the dimethylnitrosamine induced liver fibrosis rats (Sun et al., 2010). A number of chemical constituents with diverse structures, including flavonoids, organic acids, iridoids, and saponins, have been isolated from this high plant (Lee et al., 2010; Kuroda et al., 2014; Kumar et al., 2005; Yu et al., 2013, 2015). Modern pharmacological researches thought that these effects may be related to the active compositions volatile oils, chlorogenic acid and flavones. Furthermore, the extract of *L. japonica*, which decreases the raised liver and spleen indexes, and improves the aggravated liver histopathologic changes, was attributed to its flavone constituents (Shang et al., 2011).

As part of the ongoing search of promising new anti-hepatoma and hepatoprotective compounds from traditional medicinal plants, bioactivity-guided isolation and fractionation of the flower buds of *L. japonica* were carried out and four novel flavonoids, japoflavone A–D (**2**-**3**, **5**, **12**), together with ten known flavonoids (**1**, **4**, **6**-**10**, **11**, **13**-**14**), were isolated and identified from the EtOAc fraction of *L. japonica* flower buds extracts. The chemical structures of all identified compounds are shown in Fig. 1. Their potential anti-hepatoma effects on SMCC 7721 cell were evaluated. In addition, the hepatoprotective activities against H_2O_2-induced oxidative damage of several compounds were determined.

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https://doi.org/10.1016/j.indcrop.2018.08.073
Received 9 May 2018; Received in revised form 27 August 2018; Accepted 28 August 2018

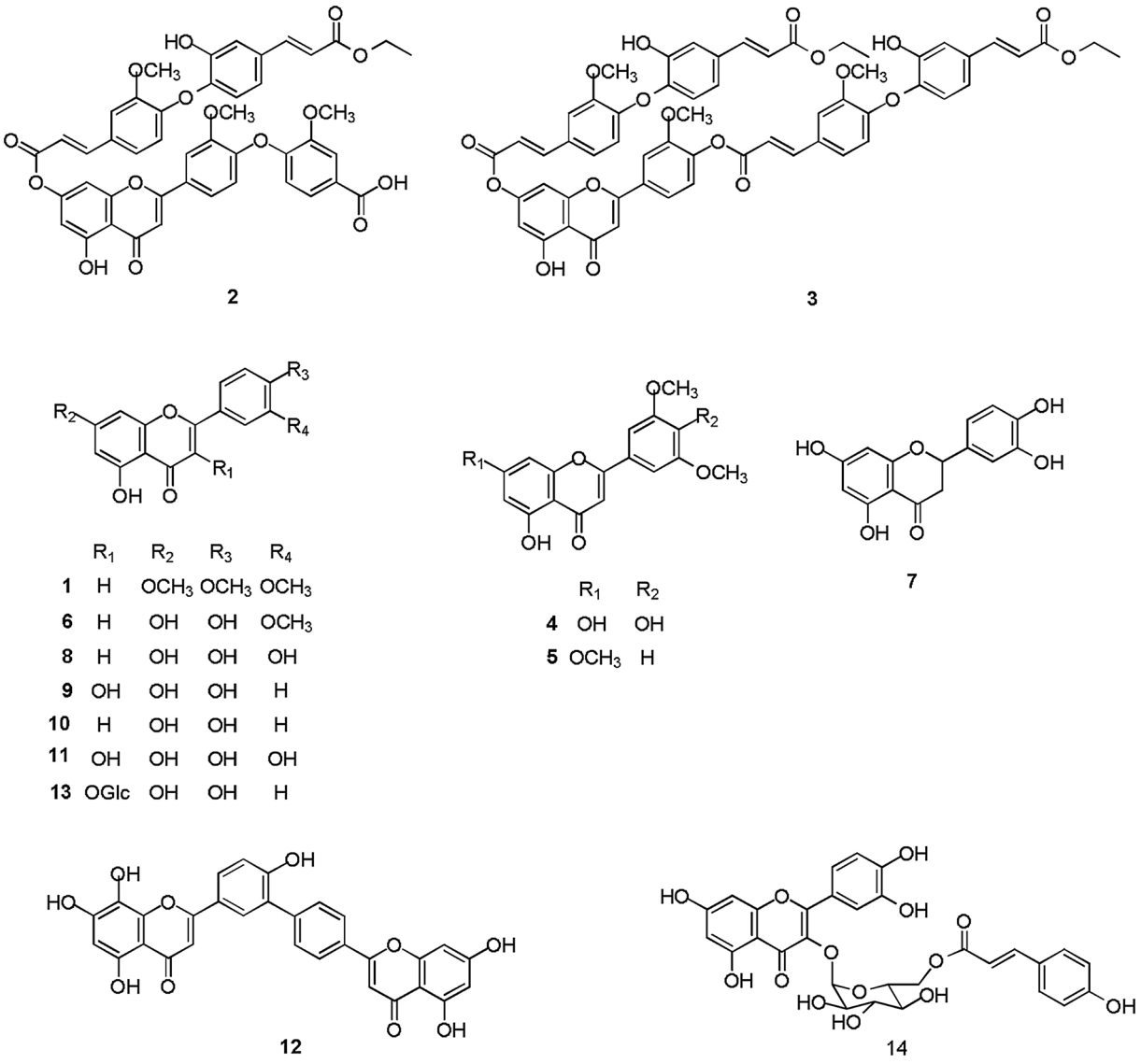

Fig. 1. Chemical structure of all flavonoids in *Lonicera japonica* flower buds.

2. Materials and methods

2.1. General experimental procedures

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX-400 spectrometer using standard Bruker pulse programs (Bruker, Karlsruhe, Germany). Chemical shifts were shown as δ-values with reference to tetramethylsilane (TMS) as an internal standard. Electrospray ionization mass spectrometry (ESI-MS) data of positive ions were obtained on a Bruker Esquire LC 200-Ion trap mass spectrometer (Bruker, Karlsruhe, Germany), and high-resolution ESI-MS (HR-ESIMS) data of positive ions were measured on a Bruker microTOF-QIImass spectrometer (Bruker, Karlsruhe, Germany). Sephadex LH-20 (GE, Beijing, China), silica gel (Qingdao Ocean Chemical Co., Ltd, Qingdao, China), and ODS (40–63 μm, Merck, Darmstadt, Germany) were used for column chromatography. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} (Qingdao Ocean Chemical Co., Ltd, Qingdao, China), and spots were visualized by spraying the plates with 15% H_2SO_4, and heating them at 105 °C. Preparative high-performance liquid chromatography (HPLC) was performed using an octadecyl silica (ODS) column (Cosmosil $5C_{18}$-MS-II, 20 × 250 mm, Nacalai Tesque, Inc., Nijo Karasuma, Japan). Each chromatographic run was carried out at a flow rate of 8 mL/min with a binary mobile phase consisting of methanol (A) and ultrapure water (B) using isocratic elution. All other chemicals were analytical or HPLC grade and were obtained from Shanghai Chemical Reagents Co., Ltd (Shanghai, China).

2.2. Plant material

Flower buds of *L. japonic*a were purchased from Fukangwanjia Pharmaceuticals (Haozhou, Anhui Province, China) in September 2017, and identified by Dr. XB Zeng of Shenzhen People's Hospital. A voucher specimen (No. 20170930) was deposited at Center Lab of Longhua Branch, Shenzhen People's Hospital, Second Clinical Medical College of Jinan University, Shenzhen, China.

2.3. Extraction and isolation

The air-dried flower buds of *L. japonic*a (6.5 kg) were powdered and extracted twice at room temperature with 75% EtOH. After filtration and removal of solvent by evaporation *in vacuo*, a dry alcohol extract (1500 g) was obtained. The alcohol extract was suspended in distilled water and then successively partitioned three times with cyclohexane, EtOAc, and *n*-BuOH, which yielded the cyclohexane fraction (130.3 g),