Journal of Functional Foods 23 (2016) 378–388

Available online at www.sciencedirect.com

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

Chemical constituent and antioxidant activity of the husk of Chinese hickory

Limin Xiang [a,b], *Yihai Wang* [a], *Xiaomin Yi* [a], *Xinluan Wang* [c], *Xiangjiu He* [a,*]

[a] *School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China*
[b] *School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, China*
[c] *Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China*

ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 26 February 2016
Accepted 1 March 2016
Available online 31 March 2016

Keywords:
Chinese hickory husks
Carya cathayensis Sarg
Bioactive constituents
Antioxidant
Health benefit

ABSTRACT

Chinese hickory (*Carya cathayensis* Sarg.) is a common and nutritious nut. Twenty-nine phenolic compounds, together with three quinones and three triterpenoids, were isolated from Chinese hickory husks. Their structures were determined by comprehensive spectroscopic methods. The total methanolic extract, chloroform and ethyl acetate fractions and all the isolated compounds were evaluated for antioxidant activity and neuroprotective effects on SH-SY5Y cells, as well as the effects on the expression of PPAR-γ in 3T3-L1 cells. Quercetin (**10**) and protocatechuic acid methyl ester (**24**) showed pronounced antioxidant activities in all of the different antioxidant assays and neuroprotective effect against H_2O_2-induced oxidative stress. Some of the flavonoids could significantly decrease the mRNA expression level of PPAR-γ by approximately 70%. The present study provided supplement information for the evaluation of antioxidant activity and health benefit of Chinese hickory.

1. Introduction

Evidence from epidemiological studies has shown that regular consumption of nut can protect against the risk of chronic degenerative diseases such as heart disease (Ros et al., 2004), neurodegenerative disease (Alzheimer disease, Parkinson disease, Huntington disease) (Poulose, Miller, & Shukitt-Hale, 2014) and type 2 diabetes (Kendall, Josse, Esfahani, & Jenkins, 2010). Since oxidative stress is common in chronic

Abbreviations: SH-SY5Y, human neuroblastoma cell; 3T3-L1, mouse embryonic fibroblast-adipose like cell line; CCM, methanol extract of Chinese hickory husk; CCC, chloroform fraction of Chinese hickory husk methanol extract; CCE, ethyl acetate fraction of Chinese hickory husk methanol extract; NMR, nuclear magnetic resonance; TMS, tetramethylsilane; HPLC, high-performance liquid chromatography; ODS, octadecylsilyl; DMSO, dimethyl sulphoxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; SARS, superoxide anion radical-scavenging; PMS, phenazine methosulphate; NADH, β-nicotinamide adenine dinucleotide; NBT, nitroblue tetrazolium chloride; FRAP, ferric reducing antioxidant potential; TPTZ, 2,4,6-Tris-(2-pyridyl)-S-triazine; VC, ascorbic acid; GA, gallic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMEM, Dulbecco's modified Eagle's medium; FBS, foetal bovine serum; PBS, phosphate buffer solution; IBMX, 3-isobutyl-1-methylanxthine; PPAR-γ, peroxidase proliferation content activated receptor-γ; SD, standard deviation

* *Corresponding author.* School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China. Tel.: +86 20 3935 2132; fax: +86 20 3935 2132.
E-mail address: hexiangjiu@163.com (X. He).

Chemical compounds: Quercetin (PubChem CID: 5280343); Quercetin-3-α-O-L-rhamnoside (PubChem CID: 5280459); 5-Hydroxy-7-methoxyflavone (PubChem CID: 5281954); Wogonin (PubChem CID: 5281703); Isosclerone (PubChem CID: 44576009); Protocatechuic acid (PubChem CID: 72); Protocatechuic acid methyl ester (PubChem CID: 287064); (-)-Lyoniresinol (PubChem CID: 9888378); Evofolin-B (PubChem CID: 5317306); (-)-Syringaresinol (PubChem CID: 11604108).

http://dx.doi.org/10.1016/j.jff.2016.03.001
1756-4646/

degenerative diseases, dietary antioxidants in plant sources may provide a beneficial effect. Nuts have been identified as being a rich source of antioxidants. Phytochemicals, including phenolic acids, flavonoids, quinones, lignans, dicarboxylic acid derivatives and α-tetralonyl glucosides are present in different nuts (Wu, Bi, Cao, Zhang, & Zhao, 2012; Yu, Zhang, Li, Zeng, & Ruan, 2013). As antioxidants, phenolic compounds prevent or control the formation of free radicals with deleterious health effects and are therefore important in disease risk reduction (Shahidi, 2000).

Chinese hickory (*Carya cathayensis* Sarg.) is a common and nutritious nut which is produced by a deciduous tree with pinnately compound leaves and big nuts in the family Juglandaceae that grows throughout Anhui and Zhejiang provinces of China. The kernel of Chinese hickory is a special local nut and is widely used as a kind of healthy food which is famous for its daintiness and nutritional content. The kernel of Chinese hickory consists of 50% lipid, 20% protein and a substantial amount of vitamins (He, Fu, & Mao, 2012; Ye, Ren, Wu, Wang, & Liu, 2013; Zhu, Deng, & Shi, 2008). The husks of Chinese hickory are by-products of cracking and shelling process. The extracts of immature walnuts and husk from Chinese hickory have been used in folk medicine more than four hundred years for treatment of rheumatic arthritis, gynaecological disorders, constipation, and tumours, etc. In modern pharmacological studies, it has been demonstrated that they also possess anti-cancer, free radical scavenging, cardiotonic, anti-inflammation and antinociception actions (Zhang, Chen, & Li, 2012).

However, more attention is being on its eatable nuts. Every year, a huge amount of the epicarp is produced, with nearly 1:1 between the weights of its fruit and its skin. It would be a great waste and also brings environmental problems in developing countries if thrown away (Liu, Lv, Yuan, & Yang, 2011). Converting by-product materials into value-added functional ingredients by applying biotechnology is a desirable approach (Guo, Qi, Zhu, & Wang, 2015). However, only a few studies have been conducted on the constituent and bioactivity on the husk.

The aim of this study has been to investigate the chemical composition, as well as the antioxidant, neuroprotection and related bioactivities, of the total methanolic extract, chloroform and ethyl acetate fractions and of some secondary metabolites isolated from the husk of Chinese hickory in order to evaluate its antioxidant activity and health benefit. The study sheds light on the potential use of the husk of Chinese hickory in food ingredients.

2. Materials and methods

2.1. Chemicals

HPLC grade methanol was purchased from Hanbon Sci. & Tech. (Jiangsu, China). Silica gel (200–300 mesh, Anhui Liangchen Silicon Material Co. Ltd. Anhui, China), Sephadex LH-20 (Pharmacia Biothech AB, Uppsala, Sweden), and ODS (40–60 μm, Merck KGaA, Darmstadt, Germany) were used for column chromatography. Standard chemicals of gallic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) in free radical form were purchased from Aladdin Chemical Co. Ltd. (Shanghai, China). Phenazine methosulphate (PMS) was obtained from J&K Chemical Ltd. (Shanghai, China). 2,4,6-Tris-(2-pyridyl)-S-triazine (TPTZ) and 3-isobutyl-1-methylanxthine (IBMX) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). β-Nicotinamide adenine dinucleotide (NADH) was purchased from Roche Ltd. (Basel, Switzerland). Ascorbic acid, nitroblue tetrazolium chloride (NBT), ethyl acetate and all other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Foetal bovine serum (FBS) and DMEM (Dulbecco's modified Eagle's medium) were purchased from HyClone Laboratories (Logan, UT, USA).

2.2. General experimental procedure

All NMR spectra were obtained on a Bruker DPX-400 instrument using standard Bruker pulse programmes (Bruker Biospin GmbH, Rheinstetten, Germany). Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard and coupling constants were reported in Hz. Semi-preparative HPLC was performed on a Rainin pump equipped with a Dynamax absorbance detector model UV-D II with the observing wavelength set at 210 nm (Rainin Instrument Co. Inc., Woburn, MA, USA) and a Cosmosil packed column (5C_{18}-AR-II, 10 ID × 250 mm, Nacalai Tesque, Kyoto, Japan). The qualitative and quantitative analyses were carried out on a Waters 600 instrument equipped with a photodiode array detector (PDA) and empower software (Waters Corporation, Milford, MA, USA). Microplate reader (Kehua Technologies, Inc., Shanghai, China) was used for antioxidant assay.

2.3. Plant material

The husk of Chinese hickory was collected from Ningguo, Anhui province, China, in September 2010 and was identified as *C. cathayensis* Sarg. by Prof. Xiangjiu He, the School of Pharmacy, Guangdong Pharmaceutical University. A voucher specimen (No. GDPU-NPR-2010001) was deposited in the Department of Medicinal Chemistry, Guangdong Pharmaceutical University, Guangzhou, China.

2.4. Extraction, fractionation and isolation

The dry husk (9.5 kg) of Chinese hickory was crushed and then exhaustively extracted with methanol (3 × 50 L). The methanolic extract was filtered and then evaporated under vacuum at 45 °C to yield methanol extract (CCM, 400 g). CCM was dissolved in the mixture of chloroform and methanol, and absorbed with 800 g silica gel (100~200 mesh). The silica gel was directly eluted with 34 L chloroform, then with 26 L ethyl acetate, respectively. The organic solvents were evaporated under vacuum to yield chloroform fraction (CCC, 40 g) and ethyl acetate fraction (CCE, 35.9 g).

CCC was further purified by silica gel chromatography (200~300 mesh, 600 × 80 mm) and eluted with a cyclohexane/ethyl acetate gradient elution (the ratios of cyclohexane/ethyl acetate were from 100:0 to 1:1, v/v). The cyclohexane/ethyl acetate (50:1, v/v) eluent (5.9 g) was recrystallized to yield compound **1** (900 mg) as colourless cubic crystals. Orange needle crystals of compound **18** (700 mg) were collected from the cyclohexane/ethyl acetate (20:1, v/v) fraction (8.55 g) of CCC