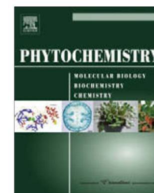
Phytochemistry 70 (2009) 1173–1181

Contents lists available at ScienceDirect

Phytochemistry

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



Neural cell protective compounds isolated from *Phoenix hanceana* var. *formosana*

Yi-Pei Lin [a], Tai-Yuan Chen [b], Hsiang-Wen Tseng [c], Mei-Hsien Lee [d,e,*], Shui-Tein Chen [f,g]

[a] School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei 110, Taiwan
[b] Clinical Genomics and Proteomics Core Laboratory, Department of Medical Research, Chang Gung Memorial Hospital at Kaohsiung, Kaohsiung 833, Taiwan
[c] Biomedical Engineering Research Laboratories, Industrial Technology Research Institute, Hsinchu 30011, Taiwan
[d] Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei 110, Taiwan
[e] Center for Reproductive Medicine and Sciences, Taipei Medical University Hospital, Taipei 110, Taiwan
[f] Institute of Biochemical Sciences, National Taiwan University, Taipei 10617, Taiwan
[g] Institute of Biological Chemistry and Genomic Research Center, Academia Sinica, Taipei 11529, Taiwan

ARTICLE INFO

Article history:
Received 14 September 2008
Received in revised form 27 April 2009
Available online 21 July 2009

Keywords:
Phoenix hanceana var. *formosana*
Palmae
6-Hydroxydopamine
Neuroprotective activity
Isovitexin
Luteolin-7-*O*-β-D-glucopyranoside
(+)-Catechin
Antiapoptosis
Cellular ROS scavenging assay

ABSTRACT

A platform for screening drugs for their ability to protect neuronal cells against cytotoxicity was developed. Nerve growth factor (NGF) differentiates PC12 cells into nerves, and these differentiated PC12 cells enter apoptosis when challenged with 6-hydroxydopamine (6-OHDA). A screening spectrophotometer was used to assay cytotoxicity in these cells; pretreatment with test samples allowed identification of compounds that protected against this neuronal cytotoxicity. The 95% ethanol extract of *Phoenix hanceana* Naudin var. *formosana* Beccari. (PH) showed potential neuroprotective activity in these assays. The PH ethanol extract was further fractionated by sequential partitioning with *n*-hexane, ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), and water. Subsequent rounds of assaying resulted in the isolation of ten constituents, and their structures were characterized by various spectroscopic techniques and identified by comparison with previous data as: isoorientin (**1**), isovitexin (**2**), veronicastroside (**3**), luteolin-7-*O*-β-D-glucopyranoside (**4**), isoquercitrin (**5**), tricin-7-neohesperidoside (**6**), tricin-7-*O*-β-d-gluco-pyranoside (**7**), (+)-catechin (**8**), (−)-epicatechin (**9**), and orientin 7-*O*-β-D-glucopyranoside (**10**). Among these compounds, isovitexin (**2**), luteolin-7-*O*-β-D-glucopyranoside (**4**) and (+)-catechin (**8**) showed significant neuroprotective activity in cell viability (WST-8 reduction), anti-apoptosis (Annexin V-FITC/propidium iodide double-labeled flow cytometry), and cellular ROS scavenging assays (besides isovitexin (**2**)), as well as a decreased caspase-8 activity in 6-OHDA-induced PC12 cells. Hence, isovitexin (**2**), luteolin-7-*O*-β-D-glucopyranoside (**4**), and (+)-catechin (**8**) protected PC12 cells from 6-OHDA-induced apoptotic neurotoxicity.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Neurons are post-mitotic cells that cannot proliferate or regenerate, except under a few special conditions (Pizarro et al., 2004). Accumulated evidence has shown that either neuronal apoptosis or cell death can be induced by a wide range of stimuli, such as oxidative stress (Steckley et al., 2007), inadequate trophic factors (Brewster et al., 2006), and by neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (Ryu et al., 2002). Excessive neuronal apoptosis may lead to extensive neuronal tissue damage, as seen in human neurodegenerative diseases like Parkinson's and Alzheimer's diseases. Thus, identification of anti-apoptotic agents conferring neuroprotection could potentially lead to therapies that slow or ameliorate the progression of such neurodegenerative diseases.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Blum et al., 2001). Although the mechanism of neuronal degeneration has yet to be established, the data indicate that mitochondrial dysfunction results in excessive production of reactive oxygen species (ROS) (Castello et al., 2007; Hanrott et al., 2006; Inden et al., 2005; Shackelford, 2006; Song et al., 2007; Sultana et al., 2006), which can trigger apoptosis of dopaminergic neurons. The PC12 cell line, which is derived from a rat pheochromocytoma, has many properties similar to dopaminergic neurons, and is widely used as a neuronal cell model for probing the principal mechanisms of PD (Meng et al., 2007; Takano et al., 2007).

Phoenix hanceana Naudin var. *formosana* Beccari. (Palmae) is a plant indigenous to Taiwan. It is usually displayed as an ornamental plant, though its fruits are edible, its growing tips (shoots) are eaten as a vegetable, and its leaves are used for brooms (Huang,

* Corresponding author. Address: Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei 110, Taiwan. Tel.: +886 (2) 2736 1661x6151; fax: +886 (2) 27357983.
E-mail address: lmh@tmu.edu.tw (M.-H. Lee).

0031-9422/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2009.06.006

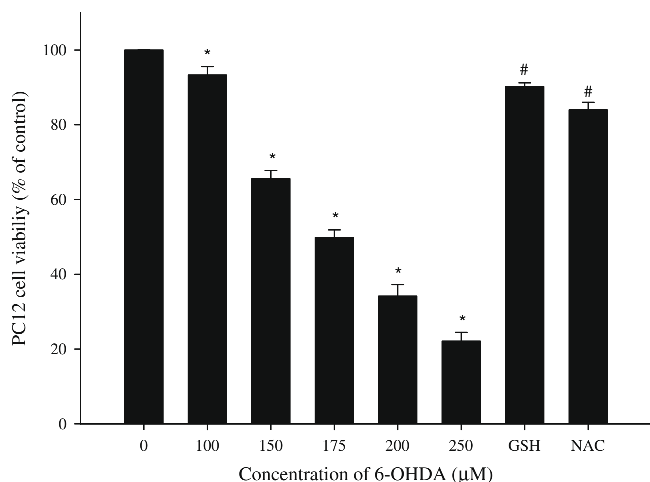


Fig. 1. Effect of various concentrations of 6-OHDA on viability of PC12 cells. mNGF-differentiated PC12 cells were treated with various amounts of 6-OHDA (100, 150, 175, 200, or 250 μM). GSH (1 mM) and NAC (1 mM) were used as positive controls and applied prior to treatment with 6-OHDA (175 μM). Results are expressed as the mean ± S.D. ($N = 4$). $^{*}P < 0.05$ when compared with 6-OHDA (0 μM). $^{\#}P < 0.05$ when compared with 6-OHDA (175 μM).

2000; Lai, 1995; Liu et al., 1978). Other *Phoenix* species display antioxidant (Al-Farsi et al., 2005; Hong et al., 2006; Mansouri et al., 2004), anti-cancer (Ishurd and Kennedy, 2005), anti-mutagenic (Vayalil, 2002), gastrointestinal transit (Al-Qarawi et al., 2003), anti-bacterial, anti-fungal, dose-dependent CNS depressive (Al-Yahya, 1986), and anti-platelet aggregation (Hussein et al., 1998) activities. Free radical scavenging effects have also been observed, we found that the 95% ethanol extract of *P. hanceana* var. *formosana* (PH) exhibited DPPH scavenging activity (data not shown); however, pure compounds derived from PH that display neuroprotective bioactivity have not been explored. Our preliminary results showed that 95% ethanol extracts of PH could prevent PC12 cells from undergoing 6-OHDA-induced apoptosis. In the present study, the protective effect of PH ethanol extracts on the 6-OHDA-induced apoptosis of PC12 cells was investigated further. Several isolation methods, coupled with a cell-protection screening assay, were used to purify compounds for large-scale isolation, structural identification, and characterization of their biological activities on cell viability (WST-8 reduction), apoptosis (Annexin V-FITC/propidium iodide double-labeled flow cytometry), scavenging of ROS, and caspase-8 activity in 6-OHDA-induced, mouse nerve growth factor (mNGF)-differentiated PC12 cells.

2. Results and discussion

2.1. mNGF-differentiated PC12 cells in 6-OHDA-induced neurotoxicity

There have been several reports on searches for neuroprotective components from natural products (Stevenson and Hurst, 2007; Yu et al., 2007), and it is important to seek a variety of compounds that might contribute to the development of medical drugs for treating various neurodegenerative diseases. In this paper, we established a rapid screening platform for assessing the neuroprotection activities of active compounds isolated from PH.

To determine the cytotoxicity of 6-OHDA, PC12 cell viability was measured using a WST-8 mitochondrial function assay. When PC12 cells were cultured with variable amounts of 6-OHDA for 24 h, the cell viability decreased in a concentration-dependent manner. The cell viability was significantly decreased at concentrations of 6-OHDA exceeding 100 μM. Based on this result, we used 6-OHDA (175 μM) as the 50% lethal dose (LD_{50}) in all subsequent analyses (Fig. 1). The positive controls, GSH and NAC, greatly inhibited 6-OHDA-induced PC12 cell death (Fig. 1).

As shown in Fig. 1 6-OHDA neurotoxicity was inhibited by presence of thiol antioxidants GSH and NAC. It has previously been reported that these thiol antioxidants effectively attenuate the insults induced by 6-OHDA (Shimizu et al., 2002; Soto-Otero et al., 2000). Both positive controls, GSH (1 mM) and NAC (1 mM), prevented 6-OHDA autoxidation. It appears that the inhibitory effect of GSH against 6-OHDA-induced cytotoxicity is mediated via suppression of 6-OHDA autoxidation in the extracellular fluid (Hanrott et al., 2006).

2.2. Purification of neuroprotective constituents from *P. hanceana* var. *formosana*

As shown in Fig. 2, an ethanolic extract of the leaves of PH was concentrated and then partitioned with a sequence of *n*-hexane, EtOAc, and *n*-BuOH. The *n*-BuOH layer showed the highest PC12-cell protective activity (69.2 ± 0.6%, Fig. 2) when the concentration was 100 μg/ml. Chromatographic analysis was then performed on the *n*-BuOH fraction using a Diaion HP 20 column, and the PH-1-3 (61.5 ± 4.5%, Fig. 3) and PH-1-4 fractions (72.8 ± 1.0%, Fig. 3) were obtained. PH-1-3 and PH-1-4 were subjected to size exclusion chromatography, and PH-2-4 and PH-3-4 were obtained based on results of the protective assay (data not shown). PH-2-4 and PH-3-4 were further purified by reversed-phase HPLC, and 10 pure compounds were obtained.

Structural identification was performed by comparing the ^{1}H and ^{13}C NMR spectra with those reported in the literature. The chemical structures are presented in Fig. 4. These 10 compounds were identified as the flavonoids isoorientin (**1**) (Krafczyk and Glomb, 2008), isovitexin (**2**) (Krafczyk and Glomb, 2008), veronicastroside (**3**) (Kikuchi and Matsuda, 1996), luteolin-7-*O*-β-D-glucopyranoside (**4**) (Wang et al., 1998; Ono et al., 2001), isoquercitrin (**5**) (Marzouk et al., 2006), tricin-7-neohesperidoside (**6**) (Asami et al., 1991), tricin-7-*O*-β-D-glucopyranoside (**7**) (Li et al., 2005), (+)-catechin (**8**) (Hirose et al., 1990), (−)-epicatechin (**9**) (Ishizu et al., 2008), and orientin 7-*O*-β-D-glucopyranoside (**10**) (S. Rayyan et al., 2005), respectively.

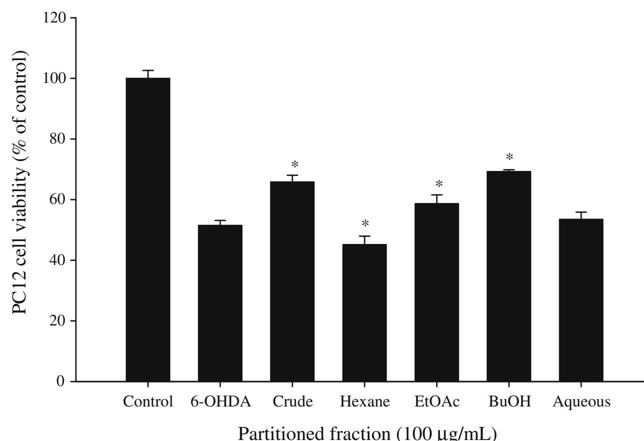


Fig. 2. Protective effect of partitioned fractions of an extract of *Phoenix hancena* var. *formosana* against 6-OHDA toxicity. mNGF-differentiated PC12 cells were treated with 6-OHDA (175 μM) in the presence of partitioned fractions (100 μg/ml). All methods are described in Section 4.5. Cell viability was determined by the WST-8 assay. 6-OHDA: 6-OHDA (175 μM); Crude: PH ethanolic extract; Hexane: partitioned *n*-hexane layer; EtOAc: partitioned EtOAc layer; BuOH: partitioned *n*-BuOH layer; Aqueous: partitioned aqueous layer. Results are expressed as the mean ± S.D. ($N = 3$). $^{*}P < 0.05$ when compared with 6-OHDA.

Download English Version:

https://daneshyari.com/en/article/5166493

Download Persian Version:

https://daneshyari.com/article/5166493

Daneshyari.com