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Antioxidant and heme oxygenase-1 (HO-1)-induced effects of selected Taiwanese plants

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Abstract

Recent studies have shown biological effects of heme oxygenase-1 (HO-1) induction and antioxidation in cardiovascular disorders. The ethanol extracts of leaves of 12 selected indigenous Taiwanese plants were investigated for their antioxidant activities, evaluated using assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, and superoxide radicals scavenging and reducing power activities as well as the induction of heme oxygenase-1 (HO-1). *Acer albopurpurascens*, *Cinnamomum kanehirai*, *Diospyros discolor*, *Excoecaria kawakamii*, *Koelreuteria henryi*, and *Syzygium formosanum* showed better DPPH-scavenging activities than the other plants. IC_{50} values ranged from 1.7 to 8.7 μg/mL. Excepting *Millettia pulchra* var. *microphylla* and *Pittosporum moluccanum*, the extracts displayed hydroxyl-scavenging activities (IC_{50} of 0.16–0.67 μg/mL). *A. albopurpurascens*, *D. discolor*, *K. henryi*, and *S. formosanum* also showed good superoxide anion radical scavenging activities and IC_{50} values ranged from 12.9 to 28.5 μg/mL. *D. discolor*, *K. henryi*, and *S. formosanum* showed potent reducing power and *M. pulchra* var. *microphylla* and *S. formosanum* exhibited potent HO-1 induced activity. These active plant extracts also contained abundant phenolic constituents. The present results provide candidates to isolate the active constituents and develop natural antioxidants.

Keywords: Indigenous Taiwanese plants; Superoxide; Reducing power; Heme oxygenase-1

1. Introduction

Oxidative stress, which is a common prelude to increased amounts of reactive oxygen species (ROS), is a causative factor in several human chronic degenerative diseases including inflammatory and neurodegenerative disorders and cardiovascular diseases [1]. ROS are highly reactive molecules. Examples include the hydroxyl radical ($OH^{\bullet}$), superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and peroxyl ($ROO^{\bullet}$).

The reactivity of ROS species generates metabolic products that attack deoxyribonucleic acid and the lipid component in cell membranes. Eukaryotic cells are equipped with endogenous scavenging systems or other substances that act to protect the cells from ROS activity.

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Cells are impaired by an imbalance between ROS-generating and -scavenging systems. Recently, heme oxygenase-1 (HO-1) was shown to be another protective antioxidant. Its increased expression reduces oxidative damage of tissues [2].

HO is a rate-limiting catalyst in the degradation of heme to produce biliverdin, which is further converted by biliverdin reductase to yield free iron, carbon monoxide (CO) and the antioxidant bilirubin [3]. Three HO isozymes have been identified (HO-1, HO-2 and HO-3), which are encoded by separate genes [1]. HO-1 is a stress–response protein that can be induced by various oxidative-inducing agents including heme, heavy metals, UV radiation, cytokines and endotoxin [4,5]. Induction of HO-1 is an important cellular protective mechanism against oxidative injury both in vitro and in vivo [4,5]. HO-1 is also a heat shock protein that has been implicated in a cytoprotective mechanism to prevent tissues from oxidative damage [6,7].

Given these protective effects, the therapeutic potential of HO-1 has been explored. In animal models, the adenovirus-mediated gene transfer of HO-1 protects against hyperoxia-induced lung injury [8], reperfusion-induced injury of transplanted liver [9] and atherosclerosis [10].

These notable effects have prompted us to search for an alternative medicine that would function as a potent inducer of HO-1 production and activity. Plant extracts or secondary metabolites may serve as antioxidants in phytotherapeutic medicines to protect against various diseases [11]. Examples include phenolics, flavonoids, tannins, proanthocyanidins and, in particular, phenolics. Taiwan contains an abundance of plant species and so represents a suitable region for an evaluation of phenolic phytotherapeutics. Using the scavenging activities of 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide and hydroxyl ($OH^{\bullet}$) radicals, as well as assays of reducing power and HO-1 induction, a systematic survey of selected plant material extracts from plants indigenous to Taiwan was undertaken to identify potential new sources of natural antioxidants.

2. Experimental

2.1. Plant materials

All indigenous plant materials were selected and collected from the Taiwan Endemic Species Research Institute (TESRI) garden in Nantou County, central Taiwan. These plants were identified by Chih-Hui Chen at TESRI. Herbarium voucher specimens were deposited in the Graduate Institute of Pharmacognosy, Taipei Medical University, Taiwan.

2.2. Preparation of crude extracts

Dried leaves of plants were pulverized and extracted with two applications of twice the volume of 95% EtOH. The extracts were concentrated in vacuo, freeze-dried and stored at −20 °C until used (Table 1).

Table 1
Yields of 12 Taiwanese plants leaves ethanolic extracts

Plants	Family	Voucher specimen	% Yield*
A. albopurpurascens Hayata	Aceraceae	M-51	8.9
A. formosana (Burk.) Makino	Betulaceae	M-54	10.2
C. kanehirai Hayata	Lauraceae	M-43	29.6
D. discolor Willd.	Ebenaceae	M-47	13.7
E. kawakamii Hayata	Euphorbiaceae	M-41	11.2
F. formosana Hayata	Oleaceae	M-44	5.8
K. henryi Dummer	Sapindaceae	M-40	17.2
M. pulchra Kurz var. *microphylla* Dunn	Fabaceae	M-53	7.6
N. sericea (Blume) Koidz. var. *aurata* (Hayata) Hatusima	Magnoliaceae	M-46	13.2
P. moluccanum Miq	Pittosporaceae	M-45	9.8
S. warburgii O. Seem	Salicaceae	M-48	8.3
S. formosanum (Hayata) Moti	Myrtaceae	M-39	14.7