Monoamine Oxidase and Dopamine β-Hydroxylase Inhibitors from the Fruits of *Gardenia jasminoides*

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Abstract

This research was designed to determine what components of *Gardenia jasminoides* play a major role in inhibiting the enzymes related antidepressant activity of this plant. In our previous research, the ethyl acetate fraction of *G. jasminoides* fruits inhibited the activities of both monoamine oxidase-A (MAO-A) and monoamine oxidase-B (MAO-B), and oral administration of the ethanolic extract slightly increased serotonin concentrations in the brain tissues of rats and decreased MAO-B activity. In addition, we found through *in vitro* screening test that the ethyl acetate fraction showed modest inhibitory activity on dopamine-β hydroxylase (DBH). The bioassay-guided fractionation led to the isolation of two iridoid glycosides, geniposide (223 μmol/L) and 6′-O-trans-p-coumaroylgeniposide (127 μmol/L), were selective MAO-B inhibitor. Especially, 6′-O-trans-p-coumaroylgeniposide exhibited more selective MAO-B inhibition than deprenyl, well-known MAO-B inhibitor for the treatment of early-stage Parkinson’s disease. The inhibitory activity of 3,5-dihydroxy-1,7-bis(4-hydroxyphenyl) heptanes (4), and ursolic acid (5), from the ethyl acetate fraction of *G. jasminoides* fruits. The isolated compounds showed different inhibitory potentials against MAO-A, -B, and DBH. Protocatechuic acid showed potent inhibition against MAO-B (IC50 300 μmol/L) and DBH (334 μmol/L), exhibiting weak MAO-A inhibition (2.41 mmol/L). Two irridoid glycosides, geniposide (223 μmol/L) and 6′-O-trans-p-coumaroylgeniposide (127 μmol/L), were selective MAO-B inhibitor. Especially, 6′-O-trans-p-coumaroylgeniposide exhibited more selective MAO-B inhibition than deprenyl, well-known MAO-B inhibitor for the treatment of early-stage Parkinson’s disease. The inhibitory activity of 3,5-dihydroxy-1,7-bis(4-hydroxyphenyl) heptane was strong for MAO-B (196 μmol/L), modest for MAO-A (400 μmol/L), and weak for DBH (941 μmol/L). Ursolic acid exhibited significant inhibition of DBH (214 μmol/L), weak inhibition of MAO-B (780 μmol/L), and no inhibition against MAO-A. Consequently, *G. jasminoides* fruits are considerable for development of biofunctional food materials for the combination treatment of depression and neurodegenerative disorders.

Key Words: *Gardenia jasminoides*, Rubiaceae, Monoamine oxidase inhibitor, Dopamine β-hydroxylase inhibitor

INTRODUCTION

*Gardenia* is a popular ornamental shrub found worldwide. The fruits of *Gardenia jasminoides* (Rubiaceae) (Korean herbal name is Chi Za) have been used as a natural yellow colorant in foods and also in traditional medicine for the treatment of liver and bladder disorders and inflammatory disease. The major effective constituents of Gardenia fruits, iridoid glycosides, flavonoids, and carotenoids, are responsible for the biological activities such as hypoglycemic activity, anti-tumor effect, anti-angiogenic activity, anti-thrombotic effect, and antioxidant activity (Miura et al., 1996; Pharm et al., 2000; Suzuki et al., 2001; Koo et al., 2004; Peng et al., 2005). In our previous research, cold drugs inhibited the activity of MAO (Hwang et al., 1999) and especially, the total methanolic extract of the fruit of *G. jasminoides* exhibited a significant inhibition on MAO activity (IC50 value of MAO-A is 1.23 mg/ml; MAO-B is 1.34 mg/ml) (Hwang and Lim, 2003). The ethyl acetate fraction of *G. jasminoides* fruits showed significant activities in *in vitro* assays on both MAO-A and MAO-B (IC50 value of MAO-A is 0.72 mg/ml; MAO-B is 0.77 mg/ml), and oral administration of the ethanolic extract slightly increased serotonin concentrations in the brain tissues of rats and decreased MAO-B activity (Hwang and Park, 2007). This tendency is similar to the activity of deprenyl which is a well-known MAO inhibitor having antidepressant effects. In addition, we found through *in vitro* screening test that the ethyl acetate fraction showed modest inhibitory activity on DBH. It is well known that major depression is related to the deficit of monoamine at critical synapses in the central nervous system whereas Parkinson’s disease (PD) is mainly due to a deficit of dopamine.

This research was designed to determine what components of *G. jasminoides* play a major role in inhibiting those enzymes.
MATERIALS AND METHODS

General experimental procedures

NMR experiments were performed on a Bruker/Advance-500 (500 MHz), a Bruker/Advance-400 (400 MHz) or a Varian-Gemini-2000 (300 MHz) spectrometer. The chemical shifts are reported in ppm and the coupling constants (J values) are reported in hertz. Exact masses were measured using a Hewlett Packard 5890 Series II mass spectrometer. Column chromatography was carried out on silica gel 60 (0.063-0.200 mm; Merck 7734) and ODS gel (12 nm, S-150 μm; YMC* GEL ODS-A AA12SA5). TLC analyses were carried out on silica gel 60 F254 (Merck 7734) and RP-18 F254s (Merck 15685) plates. Compounds on the TLC plates were detected using UV light and a 10% H₂SO₄/water spraying reagent. After spraying, the TLC plate was heated at 110°C for 1-2 minutes. In the bioassay experiments, the UV absorbance was measured by a UVIKON XS UV/Vis spectrometer.

Plant material

The fruits of Gardenia jasminoides that were collected in Muju, Jeollabukdo Province, Korea were purchased from a store at Kyungdong Market in Seoul and authenticated by Dr. Hyung Jun Ji, an emeritus professor of Seoul National University. A voucher specimen (NP20-017) has been deposited in the specimen room of Duksung Women’s University, Seoul, Korea.

Animals

Male Sprague-Dawley rats weighing 180-200 g were obtained from the Orient Animal Laboratory (Seoul, Korea) and were maintained on a 12 hour light-dark cycle (light phase: 06:30-18:30) in a temperature-controlled environment (22 ± 1°C) with free access to food and water. Experiment began after 10 day period of acclimatization. All procedures were approved by the KonKuk University Animal Care and Use Committee. They complied with the Guide for the Care and Use of Laboratory Animals, Bio - Food and Drug Research Center KunKuk University.

Extraction and bioassay-guided fractionation

The powdered sample of the fruits (10 kg) was extracted 3 times with 30 L of an 80% MeOH solution over one month at room temperature. The 80% MeOH extract (3.31 kg) was suspended in water and extracted with n-hexane and ETOAc, sequentially. The ETOAc layer was evaporated to yield the ETOAc residue (140.54 g).

The ETOAc residue (32.54 g) was submitted to a silica gel column (60-200 μm, 5×35 cm) using a step gradient of CHCl₃-MeOH 10:1 (2.5 L) and 3:1 (2 L) to yield fractions (1 and 2). 

Kim et al.  MAO Inhibitors from Gardenia jasminoides