COMPARISON ON ANTIOXIDANT ACTIVITY OF KOREAN TEAS

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Summary

Scavenging or trapping agents are compounds that physically react with the activated (electrophilic) forms of carcinogens and oxygen free radicals. In the current investigation, green tea, oolong tea and black tea and four kinds of flower teas, which have been processed at Korea in 2004 were selected for study, since 80% ethyl alcohol and ethyl acetate-soluble extracts of the teas were found to exhibit antioxidant activity based on scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. Flavonoids with strong antioxidant activity, were isolated from flowers of *Prunus mume* and *Chrysanthemum zawadshii.*

Key Words: Antioxidant, *Camellia sinensis*, *Prunus mume*, *Chrysanthemum zawadshii*, Flavonoids, DPPH

Introduction

Antioxidants have the potential to inhibit mutations in genes currently known to be associated with tumorigenes. Currently, naturally occurring polyphenols have received considerable attention as potential chemopreventive agents. Their principal action as antioxidants is to protect against oxidative damage, including lipid peroxidation and DNA strand breaks[1)]. Also many flavonoids exhibit antioxidant activities through their ability to scavenge oxygen radicals, inclusive of singlet oxygen, peroxy radicals, superoxide anion, and hydroxyl radicals[2-5)].

As a part of our study on the biological activity of Korean teas, ethyl acetate-soluble extract of green tea exhibited the most potent anti-oxidant activity than oolong tea and black tea. Two strong antioxidant flavonoids **1** and **2** from the flowers of *Prunus mume* and *Chrysanthemum zawadshi* were isolated using activity monitoring assay.

Material and Methods

Chemicals: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from sigma Chemicals Co. All other chemicals were purchased from commercial sources and were of the highest purity available.

Tea samples: Tea leaves were harvested and prepared at Bosung, Chunnam, and flower teas of *Prunus mume*, *Chrysanthemum morifolium*, *Chrysanthemum zawadshii*, *Rosa multiflora* were collected at Kyungki province at Korea in 2004 and dried. Ground teas (each 50g) were macerated with 200 ml of 80% ethanol overnight. Each ethanol-soluble extract was filtered and evaporated, after then ethyl acetate

and water (1:1 volume) were added to separate two layers, and ethyl acetate-soluble fraction, upper layer was evaporated to dryness and used for biological tests and isolation of bioactive compounds.

Assay for DPPH Free Radical Scavenging Activity: Reaction mixture containing test samples (5 μl, dissolved in DMSO) and 316 μM DPPH ethanol solution (95 μl, final DPPH concentration is 300 μM) in 48-well micro-filter plates were incubated at 37°C for 30 min and absorbance was measured at 515 nm. Percent inhibition by sample treatment was determined by comparison with a DMSO-treated control group. IC_{50} values denote the concentration of sample is required to scavenge 50% DPPH free radicals[6].

Results

Alcoholic extracts of teas were evaluated initially for antioxidant activity at a final concentration of 100 μg/ml using DPPH free radical scavenging test system. The preliminary distribution pattern of antioxidant activity in tea extracts was categorized as active (>80% inhibition), moderately active (50-80% inhibition), inactive (>50% inhibition). For the active flower tea extracts showing over 80% inhibition in this primary screen, isolation and structure identification of bioactive compounds were determined. As a result, 80% ethanol-soluble extract from teas showed antioxidant activity (Table 1).

Table 1. Evaluation of Antioxidant Activity of 80% Alcoholic Extracts of Teas

Teas	Family	Part used	DPPH (% Inhibition)
Green tea (GT)	Theaceae	Leaves	88.9
Oolong tea (OT)	Theaceae	Leaves	83.1
Black tea (BT)	Theaceae	Leaves	78.2
Prunus mume (PM)	Rosaceae	Flower	80.6
Chrysanthemum morifolium (CM)	Compositae	Flower	75.6
Chrysanthemum zawadshii (CZ)	Compositae	Flower	81.3
Rosa multiflora (RM)	Rosaceae	Flower	59.3

The extracts were re-extracted with ethyl acetate and water. The ethyl acetate-soluble extracts of green tea and oolong tea, and flower extracts of *Prunus mume* and *Chrysanthemum zawadshii* showed strong antioxidant activities (Figure 1). The ethyl acetate-soluble extracts of *Prunus mume* and *Chrysanthemum zawadshii* were subjected individually to a series of activity-guided chromatographic separation steps to afford compounds **1** and **2** (Figure 2). Two pure isolates gave characteristic flavonoid color reactions, and UV, ^{1}H NMR and ^{13}C NMR spectral data of compounds were characterized as quercetin (**1**) and luteolin (**2**). These compounds showed significant antioxidant activity in a concentration-dependent manner, and the IC_{50} of compounds **1** and **2** were 17.4 and 11.9 μg/ml, respectively (Table 2). It is known the radical scavenging activity is potential with 3,4-disubstitution or 2,3,4-trisubstitution in the B ring.

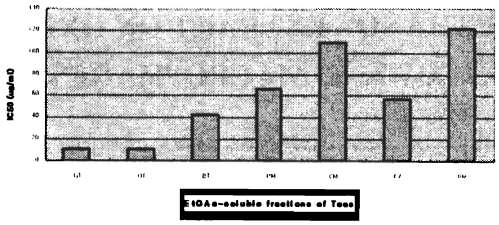
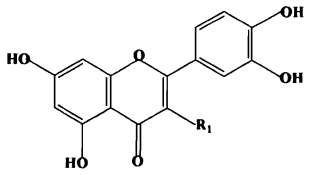


Figure 1. Antioxidant activity of ethylacetate-soluble fractions of teas: Activity criteria: IC_{50} (μg/ml): strong active; <20, moderately active; 20-100, weakly active; 100-200



Compound 1, R_1=OH; Compound 2, R_1=H

Figure 2. Structure of Compounds **1**and **2**

Table 2. Antioxidant Activity of Compounds **1** and **2**

Compounds	Source of Teas	DPPH (IC_{50}: μg/ml)
1	*Prunus mume*	17.4
2	*Chrysanthemum zawadshii*	11.9
Ascorbic acid		22.4
BHA		20.6

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