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In-vitro antioxidant activity of flavonoids from *Acer okamotoanum*

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Abstract

Degenerative diseases are commonly associated with excess free radicals. *Acer okamotoanum*, a plant endemic to Korea, is reported to have anti-oxidant, anti-cancer, and anti-viral activities. We previously isolated flavonoids from the ethyl acetate fraction of *A. okamotoanum* such as quercitrin (QU), isoquercitrin (IQ), and afzelin (AF). In the present study, the *in vitro* antioxidant activity of flavonoids such as QU, IQ, and AF isolated from the ethyl acetate fraction of *A. okamotoanum* were investigated by measuring the free radical scavenging activity including 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl radical ($^{\cdot}OH$), and superoxide anion (O_2^{-}). The flavonoids (QU, IQ, and AF) concentration-dependently showed a DPPH radical scavenging activity. In particular, QU and IQ showed a higher DPPH radical scavenging activity than that of AF. In addition, the flavonoids (QU, IQ, and AF) at 10 μg/mL showed over an 80% scavenging effect against $^{\cdot}OH$ radical production. Furthermore, the O_2^{-} radical scavenging activity of the flavonoids, QU, IQ, and AF increased in a dose-dependent manner. Particularly, IQ exerted the strongest scavenging activities against $^{\cdot}OH$ and O_2^{-} radicals among the other flavonoids. These results indicate that the flavonoids from *A. okamotoanum*, in particular IQ, would have a protective activity against oxidative stress induced by free radicals, and potentially be considered as a natural antioxidant agent.

Keywords: afzelin, anti-oxidant, free radical, isoquercitrin, quercitrin

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Introduction

Degenerative diseases such as cancer, diabetes, obesity, and Alzheimer's disease are associated with the over-production of free radicals in the body (Di Domenico et al., 2015). The accumulation of free radical in the cells damages biological molecules such as proteins, lipids, and deoxyribonucleic acid (Stanner et al., 2004; Halliwell, 2012). Hence, to remove free radicals, antioxidant enzymes in the body are activated including superoxide dismutase, catalase, and reduced glutathione. In addition, the consumption of dietary antioxidants can also provide added protection from free radicals (Halliwell,

2012). Dietary antioxidants are known to reduce the risk of several diseases caused by free radicals by supplying electrons to damaged cells (Halliwell, 2012). Secondary metabolites obtained from plants such as polyphenols, flavonoids, and vitamin C have been shown to confer antioxidant effects, and are regarded to confer lower side effects and toxicity, compared with other synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and tert-butylhydroquinone (Liao and Yin, 2000; Stanner et al., 2004). Therefore, natural antioxidants from plants have been consistently studied as preventive therapy for degenerative diseases.

Acer okamotoanum is a plant endemic in Korea reported to have various biological activities including anti-cancer, anti-oxidant, and cognitive improvement effects (Jin et al., 2008; Takayama et al., 2013; Choi et al., 2017). Previous studies show that *A. okamotoanum* contains several active compounds such as flavonol glycoside gallate ester, cleomiscosins A and C (Kim et al., 1998; Jin et al., 2007). In addition, we have previously isolated flavonoids (Fig. 1) from the ethyl acetate fraction of *A. okamotoanum* such as quercitrin (QU), isoquercitrin (IQ), and afzelin (AF) (Lee et al., 2018), however, their free radical scavenging activities are yet to be determined. Therefore, in this study, we investigated the *in vitro* anti-oxidant activities of the flavonoids isolated from *A. okamotoanum*, namely, QU, IQ, and AF, by measuring their scavenging activities against the free radicals 1,1-dephenyl-2-picrylhydrazyl (DPPH), hydroxyl radical ($^{\cdot}OH$), and superoxide anion (O_2^-).

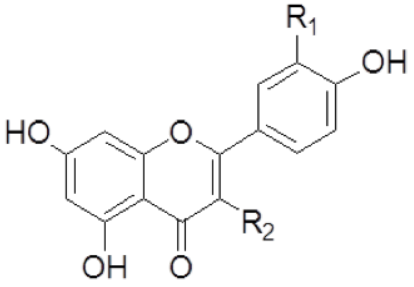
Materials and Methods

Preparation of flavonoids

QU, IQ, and AF were isolated from the ethyl acetate fraction of the aerial parts of *A. okamotoanum* by open column chromatography and were identified by spectroscopic analysis (Lee et al., 2018).

Reagents

DPPH and 2-deoxy-ribose were purchased from Sigma (St. Louis, MO, USA) and H_2O_2 was purchased from Junsei (Tokyo, Japan). $FeSO_4 \cdot 7H_2O$ was purchased from Daejung Chemicals & Metals Co. Ltd (Siheung, Korea), EDTA disodium salt dehydrate and phosphoric acid were obtained from Samchun Pure Chemical Co. Ltd (Pyeongtaek, Korea). The



Compound	R_1	R_2
QU	OH	*O*-Rham
IQ	OH	*O*-Glc
AF	H	*O*-Rham

Fig. 1. The structures of flavonoids from *Acer okamotoanum*.
QU, quercitrin; IQ, isoquercitrin; AF, afzelin; *O*-Rham, *O*-Rhamnoside; *O*-Glc, *O*-glucoside.

thiobarbituric acid (TBA) was from Acros Organics (New Jersey, USA), trichloroacetic acid (TCA) was from purchased Kanto Chemical Co. Inc (Tokyo, Japan). Phenezine methosulfate (PMS), NADH disodium salt, and nitrotetrazolium blue chloride (NBT) were from purchased Bio Basic Co. (Toronto, Canada).

DPPH radical scavenging activity

The DPPH radical scavenging activity were determined according to the method described by Hatano et al. (1989). Each sample was added to DPPH solution in the 96 well plate, and then incubated for 30 min at room temperature in the absence of light. The absorbance was measured at 540 nm using a microplate reader (Thermo Fisher Scientific, Vantaa, Finland). The DPPH radical scavenging activity was expressed as IC_{50} and a percentage (%) compared to the control as follow.

DPPH scavenging activity (%) = $(Abs_c - Abs_s)/Abs_c \times 100$

Abs_c: Absorbance of control, Abs_s: Absorbance of sample

Hydroxyl radical ($^{\cdot}OH$) scavenging activity

Hydroxyl radical ($^{\cdot}OH$) scavenging activity was determined according to the method described by Gutteridge (1987). Each sample was added to the reaction mixture containing 10 mM $FeSO_4 \cdot 7H_2O$-EDTA, 10 mM 2-deoxyribose, and 10 mM H_2O_2, and then incubated for 4 h at 37ºC without light. After, 1% TBA solution and 2.8% TCA solution were added to the mixture and heated for 20 min at 100ºC. The absorbance was measured at 490 nm using a microplate reader (Thermo Fisher Scientific, Vantaa, Finland). The $^{\cdot}OH$ radical scavenging activity was recorded as a percentage (%) compared to the control.

$^{\cdot}OH$ scavenging activity (%) = $(Abs_c - Abs_s)/Abs_c \times 100$

Abs_c: Absorbance of control, Abs_s: Absorbance of sample

Superoxide anion (O_2^-) scavenging activity

The O_2^- radical scavenging activities were measured according to the method described by Ewing and Janero (1995). Each sample was added to 0.1 M Tris-Hcl (pH 7.4), 100 μM PMS, 500 μM NBT, and 500 μM NADH, and then incubated for 10 min at room temperature without light. The absorbance was measured at 560 nm using a microplate reader (Thermo Fisher Scientific, Vantaa, Finland). The O_2^- radical scavenging activity was recorded as a percentage (%) compared to the control.

O_2^- scavenging activity (%) = $(Abs_c - Abs_s)/Abs_c \times 100$

Abs_c: Absorbance of control, Abs_s: Absorbance of sample

Statistical analysis

Data were presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) followed with Duncan's multiple test was used for statistical analysis. $p < 0.05$ was considered statistically significant.

Results and Discussion

Scavenging of free radicals is vital in the prevention of the deleterious effects caused by the accumulation of free radicals that often leads to various degenerative diseases such as diabetes, cardiovascular disease and Alzheimer's disease (Di Domenico et al., 2015; Singh et al., 2015). Numerous studies have reported the antioxidant activities of different extracts and