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Biological activities of ethanolic extract from *Robinia pseudoacacia* L. flower

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Abstract Biological activities such as antioxidant, anticoagulant, and α-glucosidase inhibitory effects of 40% (v/v) ethanolic extract from black locust (*Robinia pseudoacacia* L.) flower were investigated. The polyphenol content of the black locust flower extract was 39.8±0.5 mg gallic acid equivalents/g. The flower extract represented antioxidant effects such as free radical, cationic radical, and nitrite scavenging abilities as well as reducing power. Also the flower extract inhibited α-glucosidase activity and common pathway in plasma coagulation system.

Keywords Anticoagulant · Antioxidant · Black locust (*Robinia pseudoacacia* L.) flower · α-Glucosidase inhibitory · Polyphenol

Introduction

Black locust (*Robinia pseudoacacia* L.) belonging to Fabaceae family is originally native to North America and widely distributed throughout Korea [1]. The black locust flower (BLF), which has been used as a food additive and traditional medicine, is considered very important as the main raw material for honey harvesting [1,2]. The BLF has been known to have diuretic, sedative and anti-inflammatory effects [3,4]. The BLF contains a lot of ascorbic acid and phenolics, which have excellent antioxidant effects, and also has a relatively high content of free sugars and minerals [5]. Flavone glycosides such as acacetin contained in BLF are reported to have antimicrobial and antioxidant activities [6]. Accordingly, the black locust flower extract (BLFE) showed antioxidant, antimicrobial, and DNA damage protective effects [1,7].

In this study, an antioxidant effect of 40% (v/v) ethanolic extract from BLF was comprehensively evaluated by measuring its free radical, cationic radical, and nitrite scavenging abilities as well as reducing power. In addition, by elucidating the α-glucosidase inhibitory and anticoagulant effects of BLFE, we intend to provide basic data for using the extract as a bioactive food material.

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Materials and Methods

Polyphenol content of black locust flower extract

Black locust (*Robinia pseudoacacia* L.) flowers, collected from Ganghwa island (Incheon, Republic of Korea), were dried, finely powdered, and filtered through a 200 mesh sieve. The flower powder was extracted at 45 °C for 2 h by using 40% (v/v) ethanol solution, and centrifuged (3,000×g, 10 min), thus obtaining the BLFE. The polyphenol content of BLFE was determined as previously described [8]. The BLFE was mixed with Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), and reacted at room temperature for 5 min. After 10% Na_2CO_3 solution was added and incubated for 60 min, absorbance was measured at a wavelength of 725 nm. The polyphenol content of BLFE was represented as gallic acid equivalents.

Antioxidant effects of black locust flower extract

The free radical scavenging activity of BLFE was determined as previously described by Blois [9]. The BLFE was incubated with 0.2 mM 2,2-diphenyl-picryl-hydrazyl (DPPH, Sigma-Aldrich) solution at room temperature for 30 min, and absorbance was then measured at a wavelength of 517 nm.

The cation radical scavenging activity of BLFE was determined as previously described by Re et al. [10]. A mixture of 7.5 mM

2,2'-azinobis-(3-ethyl-benzothiazoline)-sulfonic acid (ABTS, Sigma-Aldrich) and 2.5 mM potassium persulfate was incubated in the dark for 15 h, and then diluted so that absorbance at a wavelength of 414 nm was 1.500±0.100. The BLFE was incubated with the diluted ABTS solution at room temperature for 90 min, and absorbance was then measured at a wavelength of 414 nm.

The nitrite scavenging activity of BLFE was determined as previously described by Gray and Dugan [11]. The BLFE was mixed with 1 mM $NaNO_2$ and 0.2 M citrate buffer (pH 1.2), and then incubated at 37 °C for 60 min. After Griess reagent was added and incubated at room temperature for 20 min, absorbance was measured at a wavelength of 520 nm.

The reducing power of BLFE was determined as previously described by Oyaizu [12]. The BLFE was mixed with 1% $K_3Fe(CN)_6$ and 0.2 M phosphate buffer (pH 6.8), and incubated at 50 °C for 20 min. After 10% trichloroacetic acid was added and centrifuged (3,000×g, 10 min), the supernatant was mixed with 0.1% $FeCl_3$ and absorbance was measured at a wavelength of 700 nm.

Anticoagulant and α-glucosidase inhibitory effects of black locust flower extract

Human normal plasma, thrombin, aPTT-XL and thromboplastin reagents were purchased from Thermo Fisher Scientific (Middletown, VA, USA). Anticoagulant effect of BLFE was measured using a blood coagulation analyzer (CM2, Behnk Elektronik, Norderstedt, Germany) as previously described by Fox et al. [13]. All experiments for anticoagulant activity assay were performed at 37 °C. A mixture of BLFE, human plasma and aPTT-XL reagent was preincubated for 3 min, and 20 mM $CaCl_2$ was then added to measure activated partial thromboplastin time (aPTT). A mixture of BLFE and human plasma was preincubated for 3 min, and thromboplastin reagent was then added to measure prothrombin time (PT). A mixture of BLFE, human plasma and 20 mM $CaCl_2$ was preincubated for 3 min, and then 0.5 U thrombin was added to measure thrombin time (TT).

Alpha-glucosidase inhibitory effect of BLFE was determined as previously described by Kim [14]. The BLFE was mixed with 0.4 U α-glucosidase (Sigma-Aldrich) and 0.2 M phosphate buffer (pH 6.8), and then preincubated at 37 °C for 10 min. After 3 mM *p*-nitrophenol-α-D-glucose (PNPG, Sigma-Aldrich) was added and incubated at room temperature for 10 min, 0.1 M Na_2CO_3 was added and absorbance was measured at a wavelength of 405 nm.

Results and Discussion

The polyphenol content of 40% (v/v) ethanolic extract from BLF was 39.8±0.5 mg gallic acid equivalent (GAE)/g-extract (Table 1). When the polyphenol content of BLFE was compared with that of extracts from other medicinal plants, it was lower than 186.2 mg/g [15] of lotus (*Nelumbo nucifera*) leaf, 106.9 mg/g of mugwort (*Artemisia princeps* Pampanini) [16] and 64.1 mg/g of Du-zhong (*Eucommia ulmoides* Oliver) leaf [17], but it was higher than 10.3 mg/g of ginseng (*Panax ginseng*) root [18] and 10.4 mg/g of notoginseng (*Panax notoginseng*) root [18]. The polyphenol content varies greatly from plant type as well as from parts and organs within the same plant [19,20]. In the previous study, the polyphenol contents of BLFE were reported to be 9.03 mg/g in the hot water extraction, and 8.78 mg/g in the ethanol extraction [1]. The polyphenol content of the 40% ethanolic extract in this study was found to be higher, which is because extraction with an aqueous ethanol solution facilitates the penetration of the solvent into cell membrane of plants rather than using water or ethanol alone [21]. Also, when the polyphenol content of BLFE was compared with that of extracts from other flowers, it was lower than 186.14 mg/g of magnolia (*Magnolia denudata*) [22] and 63.96 mg/g of cutleaf evening-primrose (*Oenothera laciniata*) [23], but it was similar to 32.3 mg/g of Chinese fringetree (*Chionanthus retusa*) [24] and 30.6 mg/g of Korean rhododendron (*Rhododendron mucronulatum*) [25]. Polyphenols, which are representative biologically active substances of plants, are known to have antimicrobial, anticoagulant and α-glucosidase inhibitory effects as well as antioxidant activity [26-28].

Table 1 Yield and polyphenol content of 40% ethanolic extract from *Robinia pseudoacacia* L. flower

Yield (%)	polyphenol content[2] (mg GAE/g-extract)
50.8±0.8[1]	39.8±0.5[1]

[1]Data represented means and SD of triplicate measurements
[2]Polyphenol content was expressed as gallic acid equivalents (GAE)

The BLFE scavenged free and cation radicals in a concentration-dependent manner (Fig. 1A). The IC_{50} values of BLFE, which is the concentration at which 50% of DPPH or ABTS radicals were scavenged, were 2,920.2 and 961.4 μg/mL, respectively. The IC_{50} value for DPPH radical scavenging of BLFE was higher than 205.1 μg/mL of lotus leaf extract [15], 297.1 μg/mL of mugwort extract [16] and 574.2 μg/mL of Du-zhong leaf extract [17], and was similar to 2,103.1 μg/mL of Du-zhong bark extract [17]. DPPH free radical scavenging ability of each plant extract was proportional to the polyphenol contents. The IC_{50} value for ABTS radical scavenging of BLFE was higher than 274.1 μg/mL of lotus leaf extract [15], 226.1 μg/mL of mugwort extract [16] and 560.6 μg/mL of Du-zhong leaf extract [17], and was lower than 1,357.4 μg/mL of Du-zhong bark extract [17] and 4,762.8 μg/mL of ginseng root extract [18]. The BLFE had better scavenging ability against ABTS cation radicals than that against DPPH free radicals. In addition, the BLFE effectively scavenged nitrite which is the source of carcinogenic nitrosamine (Fig. 1A). The IC_{50} value of BLFE, which is the concentration at which 50% of nitrite were scavenged, was 816.1 μg/mL. The value was higher than 580.3 μg/mL of lotus leaf extract [15], but was lower than 976.1 μg/mL of mugwort extract [16], 2,329.2 μg/mL of Du-zhong leaf extract

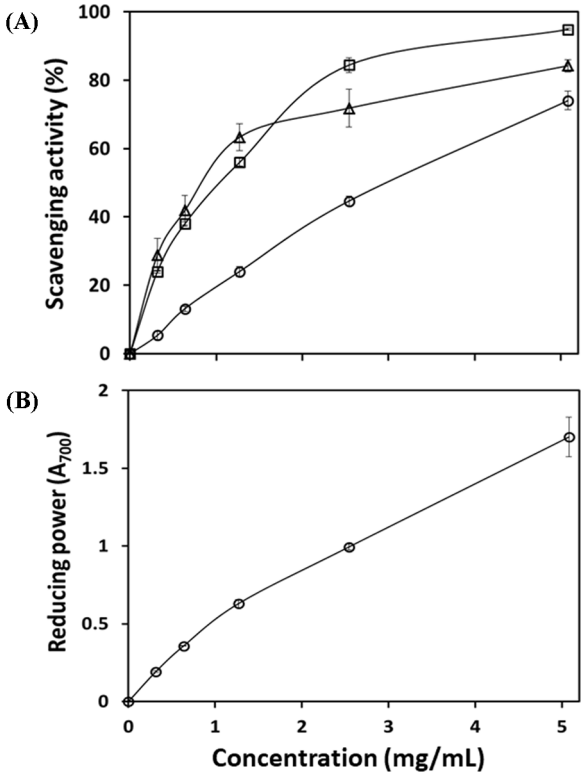

Fig. 1 (A) DPPH free radical (-○-), ABTS cation radical (-□-) and nitrite (-△-) scavenging activities, and (B) Reducing power of 40% ethanolic extract from *Robinia pseudoacacia* L. flower. Data were means and SD of triplicate measurements

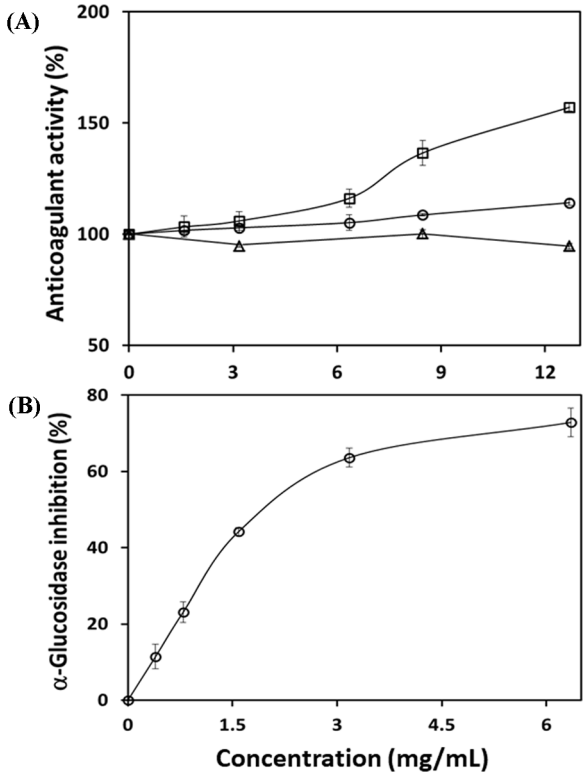

Fig. 2 (A) Effect of 40% ethanolic extract from *Robinia pseudoacacia* L. flower on TT (-□-), PT (-○-) and aPTT (-△-) in plasma coagulation system, and (B) α-glucosidase inhibitory activity of 40% ethanolic extract from *Robinia pseudoacacia* L. flower. Data were means and SD of triplicate measurements

[17] and 5,467.6 μg/mL of Du-zhong bark extract [17]. The nitrite scavenging ability of BLFE is considered to be quite good considering its relatively low polyphenol content.

The reducing power increased in proportion to the concentration of BLFE (Fig. 1B). The IC_{50} value for reducing power, which is the concentration at which the absorbance reaches 0.500, of BLFE was 970.3 μg/mL. The value is higher than 238.1 μg/mL of lotus leaf extract [15], 178.6 μg/mL of mugwort extract [16] and 319.9 μg/mL of Du-zhong leaf extract [17]. The reducing power of each plant extract showed a proportional relationship with the polyphenol content.

The IC_{50} values for DPPH radical scavenging, ABTS radical scavenging, nitrite scavenging, and reducing power of L-ascorbic acid as a positive control were 39.7, 39.9, 259.8, and 61.0 μg/mL, respectively. The antioxidant activities of BLFE were much lower than those of L-ascorbic acid, but considering that L-ascorbic acid is a single compound and the BLFE is a mixture containing a large amount of various compounds, the BLFE shows potential as an antioxidant.

The BLFE inhibited the common pathway in blood coagulation system in proportion to its concentration (Fig. 2A). The BLFE at a concentration of 12.7 mg/mL delayed TT, which means a coagulation time in the common pathway, by 1.57-fold. It is judged that the BLFE suppresses the plasma coagulation, primarily by inhibiting the common pathway. The anticoagulant effect of BLFE is considered to be rather low compared with the reports that lotus leaf extract delayed TT by 2.2-fold at a concentration of 3.40 mg/mL [15], mugwort extract did TT by 1.8-fold at a concentration of 3.22 mg/mL [16], and mistletoe (*Viscum album* var. coloratum) extract did TT by 1.7-fold at a concentration of 7.73 mg/mL [29]. It is reported that polyphenols such as cyanidin, quercetin, silybin, catechin and epicatechin inhibited thrombin activity, and polyphenol aglycones act as a competitive inhibitor against thrombin [30].

The BLFE inhibited α-glucosidase enzymatic activity in proportion to its concentration (Fig. 2B). The IC_{50} value of BLFE, which is the concentration at which 50% of α-glucosidase activity were inhibited, was 2.39 mg/mL. Natural products that inhibit the activity of α-glucosidase are known to contribute to diabetes management by suppressing blood sugar rise without any toxicity [31]. The IC_{50} values for α-glucosidase inhibition of extracts from *Musa* spp. (Baxijiao) and *Alnus firma* flowers were reported to be 343.1 μg/mL [32] and 137.4 μg/mL [33], respectively. In addition, mistletoe extract inhibited α-glucosidase activity by 46.8% at a concentration of 480.0 μg/mL [29], lotus leaf extract at 68.7 μg/

mL did 63.1% [15], and fingerroot (*Boesenbergia rotunda*) root extract at 250 μg/mL did 60.7% [34]. Polyphenols from many plant extracts have α-glucosidase inhibitory activity [28], and in particular, polyphenols with galloyl moieties have been found to have very superior inhibitory activity compared to polyphenols without them [35].

In conclusion, 40% ethanolic extract from BLF had antioxidant, anticoagulant and α-glucosidase inhibitory activities.

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