Effective screening for the anti-hypertensive of selected herbs used in the traditional Korean medicines

Ji Yeon Kim¹ · Doram Kim² · Oran Kwon²

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Abstract In this study, Korean medicinal herb extracts were investigated for their in vitro effect on anti-hypertensive activities, including nitric oxide (NO) production in the human umbilical vein endothelial cell line EA.hy529 and angiotensin-converting enzyme (ACE) activity. Traditional remedies related to the treatment of hypertension were identified using DongUiBoGam, the most comprehensive source for Korean traditional pharmacopoeia. Herbal remedies were selected by translating the symptom terminology into westernized medicinal terms using the Korean classification of disease in Oriental medicine and Korea Traditional Knowledge Portal. Seven types of herb extracts were selected and screened for in vitro anti-hypertensive activities. Among the extracts, those of Evodiae fructus, Bambusae caulis in Taeniam, Sophorae fructus, Melonis calyx, and Schizonepeta rhizome produced significant increases in NO production, although their ability to inhibit ACE were weaker. Using Fourier transform ion cyclotron resonance mass spectrometry, the major flavonoids exhibiting anti-hypertensive activities were identified: quercetin, apigenin, and luteolin. Our results indicate that these medicinal herbs are potent natural anti-hypertensive agents that can be developed for clinical therapies.

In addition, our systematic approach to identifying candidate herbal remedies in the traditional Korean pharmacopoeia can be used to search for additional traditional remedies, such as for hypoglycemia and obesity.

Keywords Angiotensin-converting enzyme inhibition · Anti-hypertensive activity · DongUiBoGam · Medicinal herb · Nitric oxide · Traditional Korean pharmacopoeia

Introduction

Hypertension is a persistent elevation of blood pressure over 140/90 mm Hg, and it is considered as one of the key risk factors for the development of cardiovascular disease (WHO 2013). Blood pressure is regulated through the integration of cardiac, neuronal, humoral, and vascular mechanisms. Among the interacting homeostatic regulators identified in the pathogenesis of hypertension, those of the renin–angiotensin system and autonomic nervous system primarily protect the body from hypotension. Several angiotensin-converting enzyme (ACE) inhibitors are used as synthetic drugs; however, such agents usually produce adverse side effects (Puchalska et al. 2015). Endothelium-derived relaxing factor (EDRF) is an unstable humoral mediator released from arteries and veins, and it plays a pivotal role in establishing the baroreceptor set point (Matsuda et al. 1995; Liu and Huang 2008). Certain investigations have identified EDRF as nitric oxide (NO) after comparing their biological and chemical properties (Ignarro et al. 1987; Furchgott and Vanhoutte 1989). The involvement of NO in blood pressure regulation was clearly indicated by the reduced vascular responsiveness to normal vasodilatory stimuli with decreased NO bioavailability by treatment with pharmacologic inhibitors of...
endothelial nitric oxide synthase (eNOS), such as L-nitro-arginine and L-N-arginine-methyl-ester (Sakuma et al. 1992; Liu and Huang 2008).

Dietary interventions with food supplements may improve eNOS function; thus, they might be a promising strategy to inhibiting ACE activity or restoring NO bioavailability (Mizuno et al. 2010). As food supplements, medicinal plants may be appropriate candidates. Most of the medicinal dietary plants have been in use for a long time and are known to be safer than isolated active compounds (Fabricant and Farnsworth 2001). DongUiBoGam is an encyclopedia of medical knowledge and treatment techniques compiled in Korea in 1613, and it covers the majority of oriental pharmacopoeia and includes all of the medicinal information of that time period. In addition, DongUiBoGam has been widely used in Oriental medicine up to the present day. Thus, a number of studies investigating the standardization and objectification of symptoms of Oriental medicine have focused on DongUiBoGam (Cha et al. 2007; Lee et al. 2014). However, traditional herbal remedies represent knowledge based on experience; thus, their efficacy should be established through modern scientific techniques. Nonetheless, DongUiBoGam may be an appropriate oriental pharmaceutical literature source for screening medicinal herbs, and we envision that there is great potential for DongUiBoGam herbs that may contain eNOS activators or ACE inhibitors.

In this study, we searched candidate anti-hypertensive herbs by translating the efficacy terminology of DongUiBoGam remedies and have performed in vitro assays quantify eNOS activation or ACE inhibition. In addition, we characterized anti-hypertensive chemical profiles using 15Tesla Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for each extract.

Materials and methods

Herbal material

Using the Korean Classification of Disease in Oriental Medicine (KCDOM, National Statistic Office, Korea) and Korea Traditional Knowledge Portal (KTKP, Korean Intellectual Property Office and Korea Institute of Oriental Medicine, Korea), we identified terminology related to hypertension used in the traditional Korean pharmacopoeia DongUiBoGam (Table 1) and identified traditional Korean herbal remedies used to treat hypertensive symptoms (Table 2). Among the herbs used in the anti-hypertensive herbal remedies, we selected seven types of plants based on their obtainability, potential application as food or food supplements, and preferences of several doctors of Oriental medicine (Table 3). The selected herbs were obtained from the Plant Extract Bank (Daejeon, Korea), which includes plants collected throughout the empire of Korea, and the samples were authenticated. The plant extracts were prepared using plants that were dried according to the institute’s standard protocol. Briefly, plant materials were divided into parts and dried at room temperature in the shade for 5 days. Hot water or ethanol extracts were prepared in a ratio of 1:10 using a traditional herb extractor for 3 h. Subsequently, the solution was filtered and freeze dried, and the extracts were concentrated under decompression at 45 °C and maintained at −4 °C.

Measurement of nitric oxide production using the EA.hy529 cell line

The immortalized human umbilical vein endothelial cell line EA.hy529 was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were grown in Dulbecco’s modified Eagle medium supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin and incubated in a humidified atmosphere with 5 % CO2 at 37 °C. Cell viability was confirmed using a tetrazolium colorimetric (MTT) assay. For the NO production assay, confluent cells in 12-well plates were serum-starved overnight and treated with the respective samples in Ca2+-containing phosphate-buffered saline for 10 min at 37 °C. Confluent cells were pre-incubated with their respective samples for 10 min prior to treatment with 1 μM DAF-2 (Cayman Chemical, Ann Arbor, MI, USA) for 5 min at 37 °C in the dark. Aliquots of the solutions were sampled, and fluorescence was measured using a Thermo Scientific fluorometer (Barrington, IL, USA) at 495 nm excitation and 515 nm emission wavelengths. NO production was normalized with respect to the protein content in each well, and the samples were assayed in triplicate.

Angiotensin-converting enzyme inhibitory activity

The ACE inhibitory activities of the herb extracts were assayed using published methods with slight modifications (Cushman and Cheung 1971). Briefly, 50 μL of each test sample was added to 500 μL 100 mM sodium borate buffer (pH 8.3) containing the substrate 4 mM hippuryl-L-histidyl-L-leucine. The solutions were incubated at 37 °C for 10 min before the addition of ACE, and the enzyme reactions were initiated by adding 20 μL ACE (0.1 U/mL) to the pre-incubated solution mixture. The samples were then incubated at 37 °C for 30 min, and the enzyme reaction was stopped by adding 1 M HCl and 1.5 mL ethyl acetate. After centrifugation at 1000×g for 5 min, 1 mL supernatant was collected, and ethyl acetate was evaporated at 120 °C for 30 min using a heating block (DRI-
The residue was redissolved in distilled water, and absorbance was measured at 228 nm. The ACE inhibitory activities were calculated as the percentage of inhibition.

### Identification of anti-hypertensive compounds using \(^{15}\text{T}\) FT-ICR-MS

Mass spectra were obtained with \(^{15}\text{T}\) FT-ICR mass spectrometers (built at the Korea Basic Science Institute, Daejeon, Korea) for the analysis of phytochemicals in the respective herbal extracts. The baseline noise from the spectrum was calculated using the program DataAnalysis (Bruker Daltonics, Billerica, MA, USA), and the crude samples were diluted to 1 mg/mL in a 100 % methanol solution before analysis. The analyses were performed with

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#### Table 1  Traditional Korean herbal remedies used for hypertension symptoms in *DongUiBoGam*

| Oriental symptom | Traditional Korean herbal remedies |
|------------------|-----------------------------------|
| Ganyanghyeonhun  | Dizziness caused by liver-yang: dizziness caused by hyperactivity of the liver | Corni fructus |
| Ganhwasangyeom   | Flaming-up of fire of the liver: liver inflammation | ND |
| Ganheonohwa      | Liver-yang turning into wind: liver hyperactivity transformed into stroke | ND |
| Gansinhyusun     | Eum deficiency of the liver and kidney: Vital essence deficiency in the liver and kidney | Rehmanniae radix, Corni fructus |
| Gansineumheo     | Edema, hypertensive heart disease, chronic ischemic heart disease, heart failure | Melonis calyx, Astragal radix, Oenanthis herba, Convallariae herba |
| Bujong           | Fire and phlegm (syndrome) | Trichosanthis radix |
| Yeoldam          | Dizziness; vertigo | Sesami semen, Cassiae semen, Cinnamomi cortex, Rauwolfia radix, Farfarae flos, Sophorae fructus, Rehmanniae radix, Angelicae gigantis radix, Pinelliae rhizoma* |
| Hyeonhun         | | |
| Yukhyeo          | Epistaxis | Puerariae radix, Bambusae caulis in Taeniam, Pruni radix, Allii tuberosi, Polygoni folium, Agrimoniae herba, Phragmites flos, Stachys folium, Iridis flos/semen, Imperatae flos, Auriculariae polypor, Granati flos, Oenanthis herba, Myricae cortex, Phragmites folium, Salviae radix, Cirsii herba, Rosa multiflora, Broussonetiae folium, Cuscutae herba, Capsellae herba, Plantaginis folium, Allii fistulosi, Teucrii herba, Trachycarpi petioli, Abelmoschi flos, Dioscoreae bulbiferae tuber, Crassirhizome rhizoma |
| Dutong           | Headache | Puerariae radix, Rauwolfia radix, Chrysanthemi flos, Ligustici rhizoma, Isatidis radix/folium, Centipediae herba, Blumeae cum pini ramulu, Osterici radix, Sedi herba, Sophorae fructus, Raphani radix, Saposhnikoviae radix Angelicae gigantis radix, Pruni radix/folium, Menthae herba, Asari radix, Codonopsis radix, Evodiae fructus, Coriandri fructus, Pterocarpus lignum, Usnea, Allii fistulosi, Piperis longi fructus, Schizonepeta rhizome |
| Hyeolheodutong   | | Chebulae fructus |
| Hyeolheodutong   | | Puerariae radix, Saposhnikoviae radix |
| Pungdam          | Hepatic phlegm | |
| Hanggang         | Neck stiffness; neck rigidity; rigidity of the neck; rigid neck | Puerariae radix, Rauwolfia radix |
| Simheojeung      | Symptoms caused by the lack of heart-energy and heart blood | ND |
| Jungpungneojjeung| Signs of stroke | ND |

*ND* not defined

#### Table 2  Herbal material used for in vitro screening

| Name of medicinal herbs | Part used | Abbreviation |
|-------------------------|-----------|--------------|
| Chrysanthemi flos       | Flower    | ChF          |
| Corni fructus           | Fruit     | CF           |
| Evodiae fructus         | Fruit     | EF           |
| Bambusae caulis in Taeniam | Endodermis | BCT         |
| Sophorae fructus        | Fruit     | SF           |
| Melonis calyx           | Fruit     | MC           |
| Schizonepeta rhizome    | Root      | SR           |

BATHSTM 17610-26, Barnstead Thermolyne, Dubuque, IA, USA). The residue was redissolved in distilled water, and absorbance was measured at 228 nm. The ACE inhibitory activities were calculated as the percentage of inhibition.
electrospray ionization (ESI), and the samples were directly injected using a syringe pump at a flow rate of 40–70 µL/h. ESI needle voltage was set to −3.8 kV. All samples were analyzed in positive ionization mode with broadband detection. Ions were accumulated in a hexapole ion trap for 2.0 s before being introduced into the ICR cell. At least 200 scans were accumulated and averaged to improve the signal-to-noise ratio of the resulting spectra. 4 M words of data were recorded per broadband mass scan. The lower mass limit was set to \( m/z = 60 \) Da, the upper mass limit was set to \( m/z = 1500 \) Da. The molecular formula was calculated using the peak intensity and compared with the generated molecular formula and predicted isotope peak. Among all of the identified phytochemicals, the molecular formula of the compound exhibiting anti-hypertensive activity was identified by searching for the structure using the in-house integrated database of the Bioinformatics and Molecular Design Research Center in which chemical structures were combined with their pharmacological information (Seoul, Korea).

### Statistical analysis

All of the results are expressed as the mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) and post hoc Dunnett’s multiple range tests were applied. The significance was set at \( P < 0.05 \) for all of the statistical analyses and post hoc comparisons, and the statistical analyses were performed using the Statistical Analysis Systems package version 9.2 (SAS Institute, Cary, NC, USA).

### Results

#### Identification of symptoms and selection of plants for in vitro investigation

As a first step, we identified symptoms that appeared in traditional Korean herbal remedies. Compared with modern scientific techniques that utilize the concept of biomarkers, traditional remedies rely on disease symptoms to predict risk signs, disease progression, and disease susceptibility to a specific treatment. Therefore, we focused on identifying symptoms related to hypertension to identify traditional Korean herbal remedies in DongUiBoGam and relied on two data sources for this work, KCDOM and KTKP, because of their reliability, suitability, and accessibility. The KCDOM and KTKP are reliable data sources that have been established and managed by governmental offices. The KCDOM was established by the National Statistic Office to provide statistical data on disease, death, and health among Koreans. The KTKP is a web-based database established by the Korean Intellectual Property Office and Korea Institute of Oriental Medicine to protect Korean traditional knowledge. Both databases provide information on diseases recorded in historical documents of traditional Korean medicine and detail the relationship of such treatments to the corresponding Western medicine treatment, with free online content. Therefore, we searched the terminology for symptoms corresponding to hypertension using these two data sources. We identified seventeen symptoms relevant to hypertension using the KCDOM and KTKP (Table 1), and these symptoms were mostly related to liver function or stroke symptoms, such as dizziness, which may be caused by liver hyperactivity, ischemic heart disease or headache, or stroke symptoms. Using these symptoms, we searched DongUiBoGam for plant materials used as herbal medicine for hypertension. This search was performed with the “Treatise on Asian Herbal Medicine,” which is published by the Natural Products Research

| Herbs | Anti-hypertensive compounds |
|-------|-----------------------------|
| Evodiae fructus | 3,3',4',5,7-Pentahydroxyflavone, Quercetin, Rutacarpine, 4',5,7-Trihydroxyflavone, Apigenin, 3',4',5,7-Tetrahydroxyflavone, Luteolin, 3',4',5,7-Tetrahydroxy-3',8-dimethoxyflavone, Oleuropein, 5,11(13)-Eudesmadien-12,8-olide, 3-(3,4-Dihydroxyphenyl)-2-propen-1-ol, 3',4',5,6,7-Pentahydroxyisoflavone, 2,6,10,14-Phytatetraene-1,13-diol |
| Bambusae caulis in Taeniam | 4',5,7-Trihydroxyflavone, Apigenin, 3',4',5,6,7-Pentahydroxyisoflavone, Oleuropein, Osoic acid, 3',4',6,7,8-Pentahydroxyisoflavone, Debenzoylpaesonilin |
| Sophorae fructus | 4',5,7-Trihydroxyflavone, Apigenin, 3',4',5,7-Tetrahydroxyflavone, Luteolin, Cochimnicin I, 3-(3,4-Dihydroxyphenyl)-2-propen-1-ol |
| Melonis calyx | Fenfangjine H, 3',4',5,7-Tetrahydroxyflavone, Luteolin, 2,6,10,14-Phytatetraene-1,13-diol, 3',4',5,6,7-Pentahydroxyisoflavone |
| Schizonepeta rhizome | Osoic acid, 3',4',5,7-Tetrahydroxyflavone, Luteolin, 5,11(13)-Eudesmadien-12,8-olide |

Table 3: Anti-hypertensive compounds identified in the Korean traditional pharmacopoeia
Institute of Seoul National University and KTKP, in the Chapter "DongUiBoGam." We identified 13 types of herbal remedies as well as the various herbs used in those remedies (Table 1). Of these, we selected plant extracts that could be obtained through the Plant Extract Bank (Table 2). The selected plants were as follows: Chrysanthemi flos, Corni fructus, Evodiae fructus, Bambusae caulis in Taeniam, Sophorae fructus, Melonis calyx, and Schizonepeta rhizome. These plants are still in use in the modern era as herbal medicines in Korea.

In vitro assessment of the effect of selected medicinal herb extracts on NO production or ACE inhibition

To compare the efficacy of herbs commonly used as anti-hypertensive remedies, we first examined NO production using the EA.hy529 cell line, an immortalized human umbilical vein endothelial cell line. EA.hy529 cells were treated with 500 μg/mL aqueous extracts of Chrysanthemi Flos (ChF), Corni Fructus, Evodiae Fructus (EF), Bambusae Caulis in Taeniam (BCT), Sophorae fructus (SF), Melonis calyx (MC), and Schizonepeta rhizome (SR), and cell viability was measured with an MTT assay (data not shown). The NO production in EA.hy529 was quantified with DAF-2 fluorescence. As shown in Fig. 1, the NO production of five extracts (EF, BCT, SF, MC, and SR) in EA.hy529 cells was significantly increased relative to the PBS, which was used as the control (P < 0.001). In particular, EF produced a 15-fold increase in NO production relative to the vehicle control, and the other extracts also increased NO production by approximately 7–15 fold, and the results were significant (P < 0.001).

An ACE inhibition assay was performed to measure the ability of the 7 extracts to inhibit ACE activity (Fig. 2). ChF and BCT slightly inhibited ACE activities (approximately 22%) relative to the positive control; however, none of the other extracts exhibited inhibitory activities.

Anti-hypertensive components identified in herb extracts

To investigate the anti-hypertensive compounds in the herbs that exhibited high NO production activity (EF, BCT, SF, MC, and SR), we analyzed their phytochemical profiles using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Each plant extract was dissolved in methanol and was investigated by direct-infusion ESI-FT-ICR-MS. Using the detected MS chromatogram, each compound was matched to our internal database, and the compounds exhibiting anti-hypertensive activities were identified. Flavonoids such as quercetin, luteolin, and apigenin were common among the compounds exhibiting strong NO production (Table 3). For EF, the anti-hypertensive compounds included quercetin, rutacearpine, apigenin, luteolin, tetrahydroxydimethoxyflavone, and oleuropein. For BCT, the anti-hypertensive compounds included osoic acid, pentahydroxyisoflavone, debenzoyl-paoniflorin, apigenin, and oleuropein. For SF, the anti-
Western disease (Kim 2003). In this study, this problem might not be entirely consistent with a single and Western medicine place different values on disease and issues involves interpretative difficulties. Oriental medicine herbal remedies in historical literature, one of the primary remedies. However, when searching traditional Korean demonstrate the efficacy of traditional Korean herbal remedies for treatments to relieve such symptoms. We primarily relied on two data sources for this work: the KCDOM and KTKP. The KCDOM was established by the National Statistical Office to provide a statistical data on disease, death, and health of Koreans. In the KCDOM, corresponding symptoms in Oriental medicine are classified according to the names of diseases in Western medicine. The KTKP is a website established by the Korean Intellectual Property Office and Korea Institute of Oriental Medicine to protect Korean traditional knowledge. The KTKP provides information on diseases recorded in historical documents of traditional Korean medicine and describes the relationship of such treatments to the corresponding treatment in Western medicine. Thus, we examined a wide range of possible oriental symptoms relevant to hypertension. Subsequently, we identified herbal remedies for treating symptoms related to hypertension using DongUiBoGam, the most prominent Korean traditional pharmacopoeia. According to DongUiBoGam, thirteen types of herbal remedies were relevant, and the remedies consisted of various medicinal plants used either singly or as mixtures of up to 23 plants.

We selected 7 types of medicinal herbs and screened their anti-hypertensive activities using the NO production capacity in the human umbilical vein endothelial cell line EA.hy529 and ACE inhibitory activity. As shown in Fig. 1, of the seven medicinal plant extracts, EF, BCT, SF, MC, and SR significantly increased NO production. These results suggest that the extracts could exert endothelium-dependent vascular relaxation through the NO signaling pathway in humans. EF exhibited the highest NO production capacity in the EA.hy529 cell line among the seven selected extracts. EF is the dried unripened fruit of Evodia officinalis Dode, and it has been used for the treatment of gastrointestinal disorders, headache, and amenorrhea and prescribed as a cardiotonic, or central stimulant with transient hypertensive effect (Rebhun et al. 2015). This herb is reported to exhibit anti-inflammatory (Ko et al. 2007), anti-nociceptive (Kobayashi 2003), and angiotensin II receptor binding inhibitory activities (Lee et al. 1998), although the active compound remains uncertain. To our knowledge, this is the first study to report EF as a potent stimulator of NO production in human umbilical vein endothelial cells.

BCT is another herb identified as a potent activator of eNOS. BCT is the green middle layer of the stems of Phyllostachys nigra var. henonis or Phyllostachys bambusoides, and it can be obtained by scraping off the bark from bamboo stems, cutting the stems into slices, binding the slices together, and drying them in shade. BCT has been used as a treatment for cardiovascular disease and hypertension and has also been recorded to relieve fever, vomiting, stomachache, and diarrhea (Jiao et al. 2007). Recent studies have focused on the anti-inflammatory and antioxidant activities of BCT extract, which exhibits anti-inflammatory effects through the suppression of NF-κB activation or heme oxygenase-1 expression via Nrf-2 and p-38 MAPK signaling in macrophages (Kim et al. 2013).

SF has been used as a traditional herbal remedy for the treatment of hemorrhoids, hypertension, and odontalgia and contains high levels of flavonoids such as sophoricoside, genistein, rutin, quercetin, and kaempferol (Zhi et al. 2015). Recently, the isoflavone extract obtained from SF was investigated in the treatment of menopause syndrome (Joo et al. 2005). Although SF has been characterized to contain high levels of flavonoids, there have been no studies to date on their NO production capacities.

MC and SZ have are both indicated in DongUiBoGam for the treatment of symptoms related to hypertension; however, few studies have reported the beneficial effects of these herbs with respect to hypertension therapy.

Although NO plays an important role in relaxing vascular smooth muscles, vascular relaxation by NO production is only one local effect among diverse mechanisms of vascular relaxation (Qian and Fulton 2013); therefore, using NO production alone is insufficient to screen these materials for vasorelaxant effects. To clarify the vasorelaxant effect of these plant materials, further study is required to identify additional pharmacological actions of the plant materials. We investigated ACE inhibition of the aqueous extracts of seven materials using an in vitro cell-free system, and only ChF and BCT exhibited an inhibitory effect (22%).

To identify anti-hypertensive components in these plants, the chemical profiles of the extracts were analyzed using FT-ICR-MS, which exhibits excellent resolving
power, mass accuracy, and sensitivity (Hur et al. 2009). Using our internal database, well-known flavonoids, such as quercetin, luteolin, and apigenin, were identified in the five anti-hypertensive plant extracts. Epidemiologic and intervention studies suggest that the increased dietary intake of flavonoids decreases the risk of cardiovascular disease (Hertog et al. 1993). The protective effects of flavonoids in relation to cardiovascular disease can be partially attributed to the beneficial effects of flavonoids on endothelial function (Vita 2005). In the present study, we revealed that these anti-hypertensive medicinal herbs contained flavonoids such as quercetin, luteolin, and apigenin. According to a recent study of the structure-expression relationships of flavonoids (at physiological concentrations), glycosylation and double bonds were highly affected by eNOS activation. Among the various flavonoids, luteolin and apigenin exhibited the strongest effect on the up-regulation of eNOS mRNA expression (Martinez-Fernandez et al. 2015). Besides up-regulation of eNOS mRNA, luteolin induced vasorelaxation in aortic rings dose-dependently (Si et al. 2014). Quercetin has been reported to induce a rapid, concentration-dependent phosphorylation of eNOS at serine 1179 via Akt-dependent, cyclic AMP protein kinase A-mediated pathway to enhance the production of NO and to promote vasodilation (Li et al. 2012). Although fewer reports were published for apigenin and eNOS activation compared to luteolin and quercetin, apigenin also activated eNOS calcium-dependent manner mediated by phosphatidylinositol 3-kinase and Akt pathways (Chen et al. 2010). Because of the role of NO in preventing hypertension, promoting endogenous NO bioavailability has been regarded as an important strategy to maintain healthy blood pressure status. In this regard, five medicinal plants retrieved from DongUiBoGam are particularly attractive, although the concentrations of major flavonoids including luteolin, apigenin, and quercetin identified using FT-ICR-MS were not quantified.

In summary, we conducted in vitro tests on the aqueous extract of seven out of 17 herbs identified as possessing anti-hypertensive effects. Among the 7 medicinal herb extracts, EF exhibited the highest NO production capacity in the EA.hy529 cell line, and an additional four herb extracts, BCT, SF, MC, and SR, exhibited NO production capacities and weak ACE inhibitory activities. Using FT-ICR-MS, the major flavonoids with anti-hypertensive activities were identified, and they included quercetin, apigenin, and luteolin. Further investigations are recommended to elucidate the mechanism by which these compounds induce eNOS activation, and animal or human clinical trials should be conducted to further develop these compounds as functional ingredients in food supplements or as herbal drugs. Moreover, the present work, which describes a systematic approach for the translation of DongUiBoGam terminology, can be used in the search of traditional Korean herbal remedies for other diseases, including obesity, diabetes, and inflammation. Additionally, this approach may be useful in the development of functional foods because it can rapidly and systematically screen the numerous traditional Korean herbal remedies for compounds that have a high potential for use as functional foods.

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