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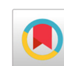

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Phytochemical analysis and antioxidant properties of mixtures containing Platycodonis Radix, Codonopsidis Radix, Paeoniae Radix, and Lonicerae Flos

Hak-Dong Lee[1,2] · Neil Patrick Uy[1] · Wang Kwon Kim[3] · Sanghyun Lee[1,2]

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Abstract Medicinal plants, rich sources of bioactive compounds such as *Platycodon grandiflorus, Codonopsis lanceolata*, *Paeonia japonica*, and *Lonicera japonica*, have been an integral part of traditional medicine for centuries. Therefore, this study aims to explore the botanical and chemical profiles of key Korean medicinal plants: steamed Platycodonis Radix, steamed Codonopsidis Radix, non-steamed Paeoniae Radix (NPR), and non-steamed Lonicerae Flos (NLF), investigating their constituents and antioxidant potential. Assessments of total polyphenol content (TPC) and total flavonoid content (TFC) revealed considerable variation among the samples, with higher TPC generally correlating with increased TFC. Among the samples, NLF exhibited the highest TPC and TFC, indicative of its rich polyphenol and flavonoid content. Antioxidant assays using 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) methods identified NPR and NLF as promising natural antioxidants, though less potent than ascorbic acid. High-performance liquid chromatography analysis revealed key compounds in each sample, with paeoniflorin particularly abundant in NLF, probably contributing to its antioxidant activity. These findings offer fundamental insights into developing functional foods and Chinese medicinal herb mixtures and serve as a valuable reference for future research on traditional herbal medicine analysis and product formulation.

Keywords Antioxidant activity · Codonopsidis Radix · Lonicerae Flos · Paeoniae Radix · Phytochemical · Platycodonis Radix

Hak-Dong Lee and Neil Patrick Uy equally contributed to this article.

Sanghyun Lee (✉)
E-mail: slee@cau.ac.kr

[1]Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea

[2]Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

[3]Korea Best One Corp., Chuncheon 24341, Republic of Korea

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Introduction

Medicinal plants, rich in bioactive compounds, have been used for centuries to treat and prevent diseases [1,2]. The term 'medicinal herb' pertains specifically to herbaceous plants with medicinal properties. Herbal medicines, derived from whole plants or specific plant parts, are typically consumed raw, dried, or decocted with minimal processing. Over 400 plant species serve as the raw materials for several herbal medicines. Medicinal plants can be categorized into two main growth forms: woody and herbaceous. Woody plants, such as trees and shrubs, develop permanent, lignified stem tissue (wood), enabling them to persist for many years. Conversely, herbaceous plants have nonwoody stems that die back at the end of the growing season or after a few years. Additionally, they can be further categorized as annuals, biennials, or perennials based on their lifespan [3].

According to myth, the medicinal botanical technology of Korea originated with the story of King Hwan Woong, who used wormwood and garlic during the Three Kingdoms period [4]. Subsequently, many medical books emerged, primarily influenced by Chinese medicine, alongside many independent writings. Among these, Heo Jun's Donguibogam (Eastern Medical Encyclopedia) stands out as an excellent Korean medical encyclopedia and is acknowledged in China and Japan [5].

Among the widely used medicinal plants in Korea are *Platycodon*

grandiflorus, *Codonopsis lanceolata*, *Paeonia japonica*, and *Lonicera japonica*. *P. grandiflorus* (Jacq.) A. DC.—a perennial plant belonging to the family Campanulaceae—is commonly found in the wild. It grows from 40-100 cm high, characterized by a thick root system and one or several stems. During July and August, it produces purple or white flowers arranged in single or multiple bell-shaped clusters at the end of the primary stem, each with a five-pronged tip. Platycodonis Radix—the root of *P. grandiflorus*—contains essential constituents such as triterpenoid types of platycodin E, platycodin D_3, and platycodin D [6,7]. *C. lanceolata* (Siebold & Zucc.) Benth. & Hook.f. ex Trautv., identified as a perennial vine, grows 2-5 m long, and it is characterized by four leaves that appear to be clustered at the end of a short branch. When damaged, it releases a white exudate with a distinct aroma. The leaves are hairless, and the bell-shaped purple flowers bloom in August and September, with the fruits maturing between October and November. Codonopsidis Radix refers to the root of *C. lanceolata*, containing important constituents such as tangshenoside I, lobetyolin, and lancemaside A [8]. *P. japonica* (Makino) Miyabe & Takeda, identified as a perennial herb, features leaf blades composed of several 1-lobed to 3-lobed leaf blades, which are long elliptic or ovate in shape. The base is distinct from the petiole, with burnt edges, and the back is whitish. The flowers are white, blooming in April and May, lacking bracts at the base, with stalks shorter than the leaves. Paeoniae Radix—the root of *P. japonica*—contains important constituents such as albiflorin, paeoniflorin, and paeonol [9-11]. *L. japonica* Thunb., a perennial vine in the Caprifoliaceae family, is semi-evergreen; hence, it is called 'honeysuckle' due to its blue leaves even in winter. Its fragrant white flowers bloom in May and June, initially with a light reddish tinge that later turns yellow. The fruit is a round berry that ripens in October or November. Finally, Lonicerae Flos refers to the bud or flower of *L. japonica* that has just begun flowering. The essential constituents of Lonicerae Flos include secologanin, 3,4-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid [12,13]. Although used for centuries already, there is still scant data about these plants as well as the effects of combining them.

Therefore, this study aims to conduct phytochemical analysis and evaluate antioxidant activity to provide foundational data for developing healthy functional foods through the mixture of various medicinal plants.

Materials and Methods

Plant material

Four types of medicinal plants namely Platycodonis Radix, Codonopsidis Radix, Paeoniae Radix, and Lonicerae Flos were supplied by Korea Best Co., Chuncheon, Republic of Korea (Fig. 1). All samples were deposited at Korea Best One Corp., Chuncheon, Republic of Korea.

Fig. 1 The samples of SPR (A), SCR (B), NPR (C), and NLF (D). SPR, steamed Platycodonis Radix; SCR, steamed Codonopsidis Radix; NPR, non-steamed Paeoniae Radix; and NLF, non-steamed Lonicerae Flos

Instruments and reagents

High-performance liquid chromatography (HPLC) was conducted using the Waters Alliance e2695 separation module (Milford, MA, USA) and Waters 2998 photodiode array detector. HPLC-grade solvents, including water, phosphoric acid, and acetonitrile (ACN), were procured from Scharlau (Barcelona, Spain) and Honeywell (Burdick and Jackson, Muskegon, MI, USA). Platycodin E (**1**), platycodin D_3 (**2**), platycodin D (**3**), tangshenoside I (**4**), lobetyolin (**5**), lancemaside A (**6**), albiflorin (**7**), paeoniflorin (**8**), paeonol (**9**), secologanin (**10**), 3,4-dicaffeoylquinic acid (**11**), and 3,5-dicaffeoylquinic acid (**12**) were sourced from the Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Republic of Korea (Fig. 2). Steam treatment was performed using an Electric Steam Generator (DSE-3) with Automatic Controller (Deoksan Boiler Co., Siheung, Republic of Korea).

Sample preparation and steam treatment

Two sample types, one subjected to steam treatment (Platycodonis Radix and Codonopsidis Radix) and the other without steam treatment (Paeoniae Radix and Lonicerae Flos), were prepared by Korea Best One Co., Republic of Korea (Fig. 1). The steamed Platycodonis Radix (SPR), steamed Codonopsidis Radix (SCR), non-steamed Paeoniae Radix (NPR), and non-steamed Lonicerae Flos (NLF), were extracted with water using a reflux system (10 g/200 mL, 5 h). Mixtures of SPR, SCR, and NPR (at ratios of 1:1:1 and 2:2:1) and mixtures of SPR, SCR, and NLF (at ratios of 1:1:1 and 2:2:1) were provided by Korea Best One Co., Republic of Korea. The mixtures were dissolved in 80% MeOH (50 mg/

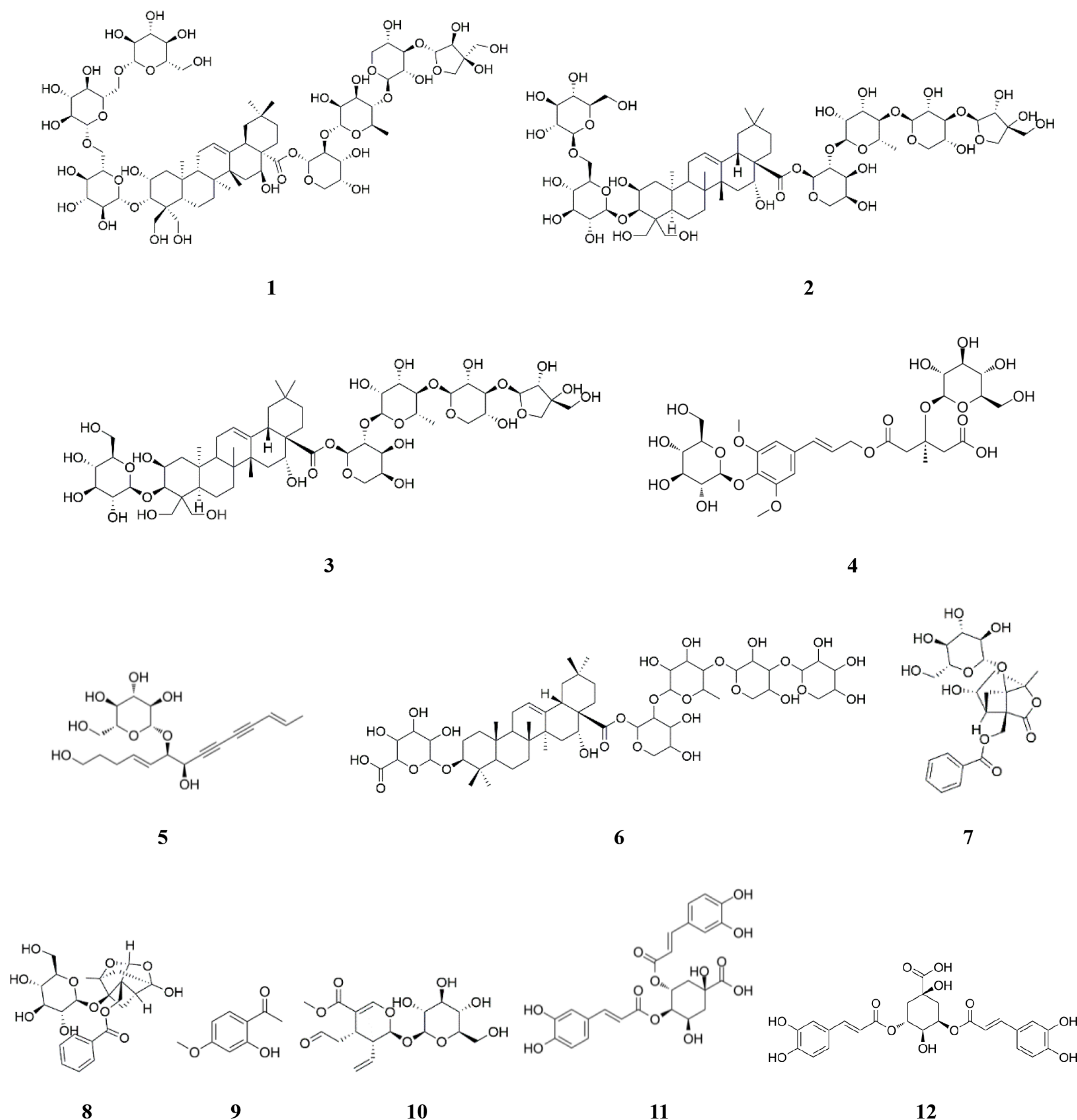

Fig. 2 Chemical structures of platycodin E (**1**), platycodin D_3 (**2**), platycodin D (**3**), tangshenoside I (**4**), lobetyolin (**5**), lancemaside A (**6**), albiflorin (**7**), paeoniflorin (**8**), paeonol (**9**), secologanin (**10**), 3,4-dicaffeoylquinic acid (**11**), and 3,5-dicaffeoylquinic acid (**12**)

mL) and filtered through a syringe filter (PVDF, 0.22 μm). Stock solutions of the standards were prepared by dissolution in 80% MeOH. Working solutions were prepared by diluting the stock solution to the desired concentrations for the standard calibration curves. The condition of steam treatment of SPR and SCR is shown at Table 1.

Analysis of the total polyphenol content (TPC) and total flavonoid content (TFC)

The TPC and TFC in the extract of the four raw materials and mixtures were determined following established procedures [14]. To perform a total polyphenol analysis, 60 μL of the extract was combined with 60 μL of 15% Na_2CO_3 (Daejung Chemicals, Siheung, Korea) and allowed to react for 30 min. The phenol reagent used was 2N Folin-Ciocalteu (Sigma-Aldrich,St. Louis, USA). To measure the total flavonoids, 100 μL of 2% $AlCl_3$ was added to 100 μL of the extract, and the mixture was incubated for 10 min. Absorbance measurements for total polyphenols and flavonoids were conducted at 760 and 430 nm, respectively, using microplate readers. The corresponding calibration curves were

Table 1 Condition of steam treatment of SPR and SCR

Process	Temperature (°C)	Time	Condition
Preheating	100	30-40 min	Wet
1st steaming	100-105	1 h	Wet
Dryness	55-60	3 days	Dehumidified and dry
2nd steaming	100-105	3 h	Wet
Dryness	55-60	3 days	Dehumidified and dry
3rd steaming	100-105	3 h	Wet
Dryness	55-60	3 days	Dehumidified and dry

established using gallic acid and quercetin as standards. Each experimental procedure was conducted at room temperature.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Plant extracts are often assessed for antioxidant activity using the DPPH technique [15]. Using the methodology outlined in a prior study [16], the antioxidant activity of the extracts from the four raw materials and mixtures was evaluated in this experiment. Initially, 10 μL from each experimental group was mixed with 200 μL of 0.2 mM DPPH dissolved in 95% ethanol in an E.P. tube, vortexed, and then allowed to incubate for 30 min in the dark. The concentration of residual radicals was measured using a microplate reader set at 514 nm. A positive control of ascorbic acid (Sigma-Aldrich) was used. The concentration needed to reduce the absorbance of the control group by 50% using only the solvent was used to express the scavenging capacity (IC_{50}) of the extract against DPPH.

DPPH radical-scavenging activity (%)
= (Blank O.D – Sample O.D) / Blank O.D ×100

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

A previously published method was modified to assess the ABTS radical scavenging activity of extract from four raw materials and their mixtures [17]. In the experimental setup, distilled water (pH 7.4) was diluted to achieve an absorbance value of 1.00±0.04 by mixing 7.4 mM ABTS and 2.6 mM potassium persulfate that had been dissolved in it in a 1:1 ratio. Following a 24-h dark incubation period, 10 μL of each concentration-prepared sample was added to 200 μL of the radical stock solution. After allowing the mixture to stand for 30 minutes, the concentrations of residual radicals were determined using a microplate reader set to 734 nm. Ascorbic acid served as a positive control. The scavenging capacity (IC_{50}) of the extract against ABTS was calculated as the concentration necessary to reduce the absorbance of the control group by 50% using only the solvent.

ABTS radical-scavenging activity (%)
= (Blank O.D – Sample O.D) / Blank O.D ×100

HPLC analysis and conditions

A reversed-phase HPLC system with a YMC J'sphere ODS-H80 column (250 mm × 4.6 mm, 4 μm) at 30 °C was employed for the comparative analysis. An injection volume of 10 μL was monitored at 205 and 230 nm. The flow rate was set to 0.95 mL/min. Gradient elution was conducted using a mobile phase consisting of 0.1% phosphoric acid in water (A) and ACN (B). 90% A was used from 0 to 5 min, 78% A from 5 to 10 min, with A maintained from 10 to 15 min. From 15 to 33 min, 70% A was used, and from 33 to 37 min, 0% A was used and maintained that way until 43 min. From 43 to 45 min, 90% A was used until the end of the run at 60 min. The calibration curve of the standard compound was fitted, and the standard deviation values of the intercept (σ) and the slope (S) were used to calculate the limit of detection (LOD) and limit of quantification (LOQ). The LOD represents the lowest amount or concentration of the analyte that can be reliably distinguished from the baseline, whereas the LOQ represents the minimum amount of the analyte that can be quantified with acceptable repeatability and accuracy. The LOD and LOQ values were derived using the formulas: $LOD = 3.3 (\sigma/S)$ and $LOQ = 10 (\sigma/S)$.

Calibration curves

A calibration curve for each standard compound was generated by plotting the concentrations of the standard solutions against the corresponding peak areas (Table 2). The correlation coefficient (r^2) was employed to evaluate the linearity of the calibration curve, and the calibration curve was subsequently used to determine the standard concentrations in the samples. Calibration functions were calculated from the peak area (Y) and concentration (X, mg/mL), with mean ± standard deviation (n =3).

Results and Discussion

TPC and TFC

The TPC and TFC of the raw materials and mixtures were assessed using a colorimetric method. Table 3 shows the overall results of the analysis. As expected, TPC values generally exceeded TFC values across all samples. This is because TPC

Table 2 Calibration curves for compounds **1**–**12**

Compound	Concentrations (μg/mL)	Calibration equation	Correlation factor, r^2
Platycodin E (**1**)	5–500	Y = 1966.4X + 542.15	1.0000
Platycodin D_3 (**2**)	5–500	Y = 2028.5X + 601.53	1.0000
Platycodin D (**3**)	5–500	Y = 1383.4X + 410.35	1.0000
Tangshenoside I (**4**)	-	-	-
Lobetyolin (**5**)	8–500	Y = 6654.9X + 14175	0.9997
Lancemaside A (**6**)	8–500	Y = 1711.5X + 789.27	1.0000
Albiflorin (**7**)	2–166	Y = 9161.8X + 3912.1	0.9999
Paeoniflorin (**8**)	20–500	Y = 14110X - 39486	0.9996
Paeonol (**9**)	2–166	Y = 28440X + 7334.5	1.0000
Secologanin (**10**)	20–500	Y = 7214.5X + 8069.1	1.0000
3,4-Dicaffeoylquinic acid (**11**)	20–500	Y =10702X + 5738	1.0000
3,5-Dicaffeoylquinic acid (**12**)	20–500	Y = 17572X - 20194	1.0000

measures the total polyphenol content, including different classes of polyphenols, while TFC specifically quantifies the flavonoid content, which is a subclass of polyphenols [18,19]. Among the raw materials, NLF exhibited the highest TPC (16.17 mg TAE/mL), followed by NPR (10.83 mg TAE/mL), SCR (6.54 mg TAE/mL), and SPR (4.87 mg TAE/mL). A similar trend was observed for TFC values, with NLF showing the highest TFC value (3.50 mg QAE/mL) and SPR the lowest (0.08 mg QAE/mL). A positive correlation between TPC and TFC is evident, suggesting that samples with higher polyphenol content tend to exhibit higher flavonoid content [20]. This correlation is evident in the data, where the NLF sample, having the highest TPC value, also displays the highest TFC value.

When comparing samples with different compositions, such as SPR, SCR, and NPR vs. SPR, SCR, and NLF, variations were observed in TPC and TFC values. This suggests that the sample composition, including the types and proportions of plant materials used, can influence total polyphenol and flavonoid contents [21]. These findings suggest that the NLF sample may be particularly rich in total polyphenols and flavonoid contents compared to other samples. Consequently, understanding the polyphenolic composition of each sample could provide insight into their potential health benefits and applications.

ABTS and DPPH assays

The antioxidant potential of each sample was assessed using the DPPH and ABTS assays (Table 4). The samples were tested by comparing them to ascorbic acid (AA), a commonly used positive standard for antioxidant activity. The results showed clear trends in the antioxidant properties of the samples, particularly samples NPR and NLF, which showed good promising efficacy as natural antioxidants compared to the other samples, as evidenced by their IC_{50} values in DPPH and ABTS assays. However, despite their potential efficacy, NPR and NLF demonstrated lower activity compared to AA, consistent with the anticipated superior antioxidant activity of AA as a positive control. Conversely, samples SPR and SCR showed negligible antioxidant activity in both assays.

Mixtures of NPR and NLF with other samples also exhibited different levels of antioxidant activity, suggesting potential synergistic or antagonistic effects. These mixtures consist of raw materials combined in different ratios. As observed, mixtures with lower ratios of SCR and SPR displayed enhanced antioxidant potential compared to their counterparts with higher ratios. These trends aligned with the observed polyphenol content in the TPC

Table 3 Total polyphenol and flavonoid contents of SPR, SCR, NPR, NLF, and their mixtures

Sample	TPC (mg TAE/mL)	TFC (mg QAE/mL)
SPR	4.87±0.55	0.08±0.08
SCR	6.54±0.15	0.19±0.18
NPR	10.83±2.14	0.83±0.82
NLF	16.17±1.75	3.50±0.21
SPR, SCR, and NPR (1:1:1)	4.61±0.36	0.05±0.26
SPR, SCR, and NPR (2:2:1)	4.72 ±0.34	0.12±0.28
SPR, SCR, and NLF (1:1:1)	6.94±0.23	0.36±0.40
SPR, SCR, and NLF (2:2:1)	6.58±0.50	0.40±0.34

SPR, steamed Platycodonis Radix; SCR, steamed Codonopsidis Radix; NPR, non-steamed Paeoniae Radix; and NLF, non-steamed Lonicerae Flos

Table 4 DPPH and ABTS radical scavenging activities in SPR, SCR, NPR, NLF, and their mixtures

Sample	IC_{50} (mg/mL)	
	DPPH	ABTS
SPR	ND	ND
SCR	ND	ND
NPR	1.51±0.02	1.69±0.20
NLF	2.10±0.63	5.69±2.89
SPR, SCR, and NPR (1:1:1)	6.41±0.01	7.71±0.22
SPR, SCR, and NPR (2:2:1)	7.49±0.07	ND
SPR, SCR, and NLF (1:1:1)	7.35±0.21	ND
SPR, SCR, and NLF (2:2:1)	7.01±0.42	ND
AA	0.12±0.00	0.12±0.00

ND, not determined; AA, ascorbic acid; SPR, steamed Platycodonis Radix; SCR, steamed Codonopsidis Radix; NPR, non-steamed Paeoniae Radix; and NLF, non-steamed Lonicerae Flos

assay, suggesting a correlation between polyphenol content and antioxidant activity. While NPR and NLF exhibit potential as natural antioxidants, further research is necessary to elucidate their specific mechanisms of action and potential health benefits. These findings highlight the importance of employing appropriate positive controls, such as AA, in antioxidant assays for comparative analysis and interpretation of results.

HPLC analysis

In order to elucidate the compounds responsible for the antioxidant potential exhibited by the samples, HPLC analysis was carried out (Tables 5, 6). Each raw material was quantified based on three commonly found compounds in the sample (SPR, SCR, NPR, and NLF). In varying concentrations, all three chemicals were detected in each raw material. In SPR, the most abundant component was platycodin D (**3**), followed by platycodin E (**1**) and platycodin D_3 (**2**). In SCR, tangshenoside (**4**) was found only at negligible levels, whereas lobetyolin (**5**) was the most abundant, followed by lancemaside A (**6**). In NLF, the most abundant compound was 3,4-dicaffeoylquinic acid (**11**), with secologanin (**10**) and 3,5-dicaffeoylquinic acid (**12**) following. Finally, for NPR, paeoniflorin (**8**) was found in the sample exceptionally abundant, whereas paeonol (**9**) and albiflorin (**7**) were present in smaller amounts (Figs. 3, 4). Previous assays demonstrated that NLF and NPR, particularly in mixtures with lower SPR and SCR ratios (higher NLF and NPR ratios), outperformed their counterparts. From the HPLC analysis results, we can infer that paeoniflorin (**8**) contributes to the antioxidant activity observed in NLF and NLF mixtures with low SPR and SCR ratios. Paeoniflorin (**8**) is a phytochemical present in Paeonia sp. and is recognized for its antioxidant properties [22]. However, paeoniflorin (**8**) is not classified as a polyphenol, indicating the possibility of other compounds present in NLF and NPR that were detected in the TPC and TFC assays. The LOD and LOQ values

Table 5 Phytochemical constituent content in SPR, SCR, NPR, and NLF

Compound	Contents (mg/g DW)			
	SPR	SCR	NPR	NLF
Platycodin E (**1**)	1.02±0.01			
Platycodin D_3 (**2**)	0.45±0.02			
Platycodin D (**3**)	1.31±0.01			
Tangshenoside I (**4**)		tr		
Lobetyolin (**5**)		1.62±0.01		
Lancemaside A (**6**)		1.22±0.01		
Albiflorin (**7**)			0.48±0.01	
Paeoniflorin (**8**)			35.00±0.05	
Paeonol (**9**)			0.12±0.00	
Secologanin (**10**)				2.47±0.01
3,4-Dicaffeoylquinic acid (**11**)				4.25±0.00
3,5-Dicaffeoylquinic acid (**12**)				1.91±0.00

tr, trace; SPR, steamed Platycodonis Radix; SCR, steamed Codonopsidis Radix; NPR, non-steamed Paeoniae Radix; and NLF, non-steamed Lonicerae Flos

Table 6 Marker compound content in mixtures of SPR, SCR, NPR, and NLF

Compound	Contents (mg/g)			
	SPR, SCR, and NPR		SPR, SCR, and NLF	
	1:1:1	2:2:1	1:1:1	2:2:1
Platycodin D_3 (**2**)	0.049±0.002	0.052±0.002	0.156±0.003	0.166±0.001
Lobetyolin (**5**)	0.538±0.006	0.704±0.007	0.585±0.002	0.614±0.001
Paeoniflorin (**8**)	2.363±0.005	1.051±0.005	-	-
3,4-Dicaffeoylquinic acid (**11**)	-	-	1.342±0.002	0.362±0.001

-: Not applicable
SPR, steamed Platycodonis Radix; SCR, steamed Codonopsidis Radix; NPR, non-steamed Paeoniae Radix; and NLF, non-steamed Lonicerae Flos

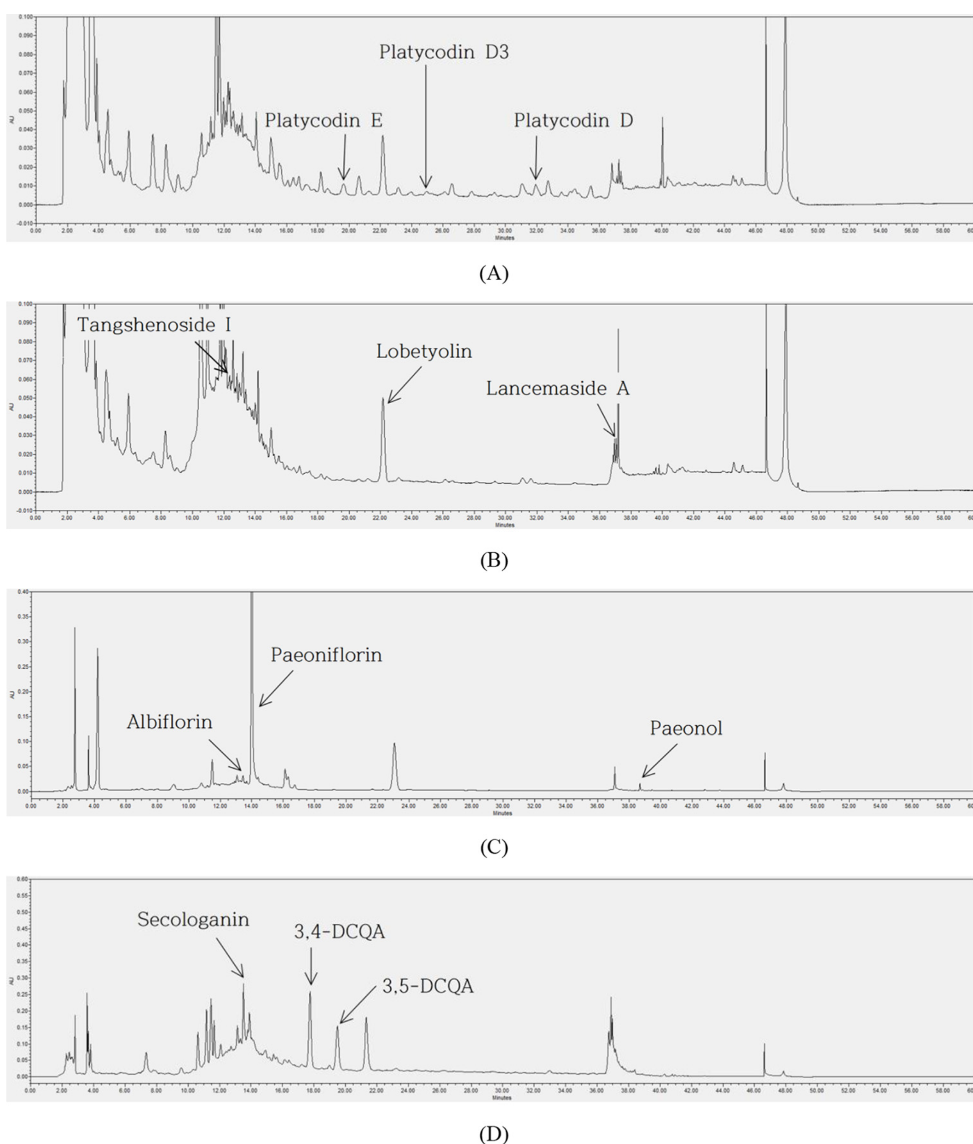

Fig. 3 HPLC chromatograms of SPR (A) and SCR (B) at 205 nm; NPR (C) and NLF (D) at 230 nm. SPR, steamed Platycodonis Radix; SCR, steamed Codonopsidis Radix; NPR, non-steamed Paeoniae Radix; and NLF, non-steamed Lonicerae Flos

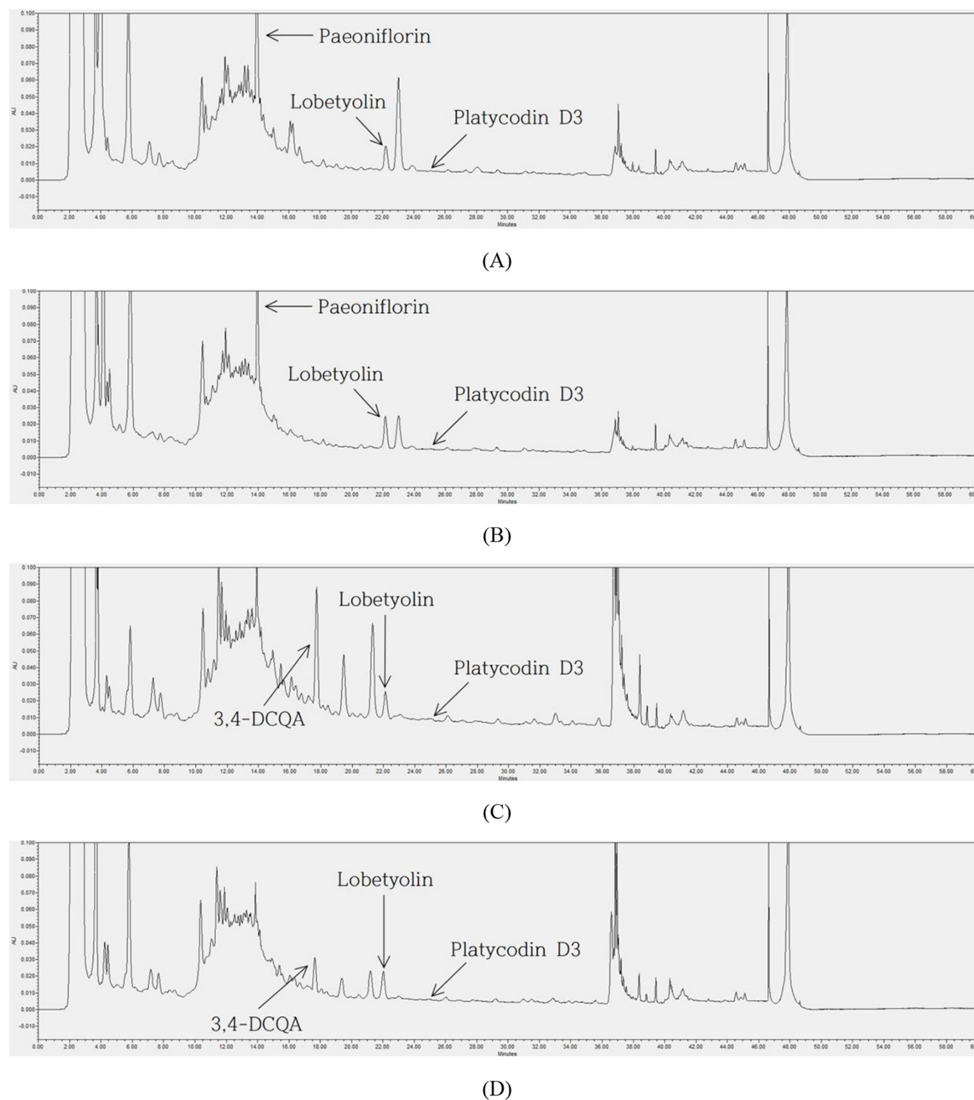

Fig. 4 HPLC chromatograms of the mixture of SPR, SCR, and NPR [1:1:1 (A) and 2:2:1 (B)] and the mixture of SPR, SCR, and NLF [1:1:1 (C) and 2:2:1 (D)] at 205 nm. SPR, steamed Platycodonis Radix; SCR, steamed Codonopsidis Radix; NPR, non-steamed Paeoniae Radix; and NLF, non-steamed Lonicerae Flos

for platycodin D_3 are 1.63 and 4.90 μg/mL, respectively, while for lancemaside A, the LOD and LOQ values are 2.61 and 7.82 μg/mL, respectively. Although the peaks for these substances appear smaller, they have areas above the LOQ values, ensuring that they can be accurately quantified.

In a previous study by Yuk et al. [23], the effects of various cooking methods on the phytochemicals and antioxidant activity of Platycodon Radix were investigated. Their findings indicated that steaming can enhance the polyphenolic and flavonoid content of Platyocdon Radix. Similarly, a study by Kim et al. [24] examined the effects of a complex steaming process on the phytochemical profile and antioxidant activity of Codonopsis Radix. Their findings revealed that the complex steaming process increased the polyphenol and flavonoid contents of Codonopsis Radix, thereby increasing its antioxidant activity.

Although these studies compared steamed and non-steamed samples of the same plant species, the present study excluded this part. Based on a review of existing literature, it was determined that steaming Platycodon Radix and Codonopsis Radix should theoretically enhance their phytochemical content and antioxidant activities of the plant sample. Conversely, Paeoniae Radix and Lonicerae Flos, were not steamed, as there is no rationale for doing so. The findings of this study demonstrate that steaming is unnecessary to increase their antioxidant activities.

The findings of this study provide a foundation for developing Korean herbal mixtures for product formulation. Additionally, this study serves as a reference for future researchers who wish to analyze Korean medicinal herbs with the incorporation of more

sophisticated characterization techniques and adding other in vitro and in vivo experiments.

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