Chemical constituents with anti-allergic activity from red peony root and a horticultural cultivar of *Paeonia lactiflora* and monoterpenoids profiles of peony related species

Yan-Hong SHI

Division of Pharmacognosy

Institute of Natural Medicine

University of Toyama

2016

Chemical constituents with anti-allergic activity from red peony root and a horticultural cultivar of *Paeonia lactiflora* and monoterpenoids profiles of peony related species

A THESIS

Submitted to University of Toyama

For the degree of

DOCTOR OF PHILOSCOPHY

In Pharmaceutical Science

Yan-Hong SHI

Division of Pharmacognosy

Institute of Natural Medicine

University of Toyama

Toyama, Japan

March, 2016

CONTENTS

General Introduction

Peony root (PR), called “Shakuyaku” in Japanese, is prescribed as the root of *Paeonia lactiflora* Pallas with no less than 2.0% of paeoniflorin in Japanese Pharmacopeia (JP) [The Ministry of Health, Labour and Welfare, 2011]. As an analgesic, antispasmodic, astringent etc., PR has been widely used in approximately one-third of generally-used Kampo formulas in Japan [Namba, 1993], such as Kakkonto (葛根湯), Tokishakuyakusan (当帰芍薬散), Keishibukuryogan (桂枝茯苓丸), etc. PR available in Japanese market is mainly imported from China and only a small part is produced domestically, for instance, in 2012, 1,407 tons of PR was imported and 81 tons was domestically-produced [http://www.nikkankyo.org]. In China, there are two kinds of PR, white peony root (WPR) and red peony root (RPR) which are used for different therapeutic purposes: WPR has been used for treatment of spasm, abdominal pain, blood deficiency, dizziness, etc.; whereas RPR has been used for treatment of blood stasis, gynecological and cardiovascular diseases, etc. [Jiangsu New Medical College, 1977; Li Shizhen, 1977]. In Chinese Pharmacopeia (CP), WPR is prescribed as the dried root of *P. lactiflora* which has been boiled and peeled before drying, while RPR is prescribed as the dried root of *P. lactiflora* or *P. veitchii* Lynch [The State Pharmacopeia Commission of China, 2010]. However, most of RPR are derived from *P. lactiflora*, the same botanical origin as WPR. We have previously demonstrated that *P. lactiflora* derived WPR and RPR are genetically and chemically different [Zhu et al., 2015]. WPR available in Chinese market and the imported PR in Japanese market are both derived from cultivated *P. lactiflora* produced in southern part of China such as Anhui, Zhejiang and Sichuan provinces, except for different processing methods. RPR derived from wild *P. lactiflora* is produced in northern part of China such as Inner Mongolia autonomous region, Liaoning and Heilongjiang

provinces, which has been occasionally used in Japan.

In the course of our studies to search for new resources of peony root and further to promote domestic production in Japan, more than 80 horticultural cultivars of *P. lactiflora*, harvested from Toyama Prefectural Medicinal Plants Center in Japan, have been genetically analyzed on the basis of nuclear DNA ITS sequence and chemically characterized on the basis of contents of 6 main components such as paeoniflorin, albiflorin, (+)-catechin, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG), gallic acid and paeonol [Yu et al., 2013; Zhu et al., 2013]. Several horticultural cultivars of RPR- and WPR-types of *P. lactiflora* were selected as promising candidates which have potential as medicinal resources of PR. The bioactive evaluation of such candidates is required.

According to bioactive screening data of crude drugs in the “Traditional Medicinal & Pharmaceutical Database” [http://dentomed.u-toyama.ac.jp/en/], RPR derived from *P. lactiflora* was found to have higher anti-allergic activity as inhibitory effect against immunoglobulin E (IgE)-mediated degranulation in rat basophil leukemia (RBL)-2H3 cells than WPR. However, to date the bioactive constituents responsible for the anti-allergic activity of RPR remain unclear. Recently, the anti-allergic activity of PR and the formulas containing PR has attracted much attention [Lee et al., 2008; Kageyama-Yahara et al., 2010]. Kampo formula of Kakkonto (葛根湯) and PR could prevent the development of allergic diarrhea in food-allergy mouse model, and one component of PGG had been revealed to suppress the surface expression of up-regulated IgE receptor (FcεRI) on mast cells derived from the food-allergy mouse colon [Kageyama-Yahara et al., 2010]. Lee et al. [2008] reported that 80% ethanol extract of the root of *P. lactiflora* and its constituents paeoniflorin and paeonol exhibited potent inhibitory effects against passive coetaneous anaphylaxis reaction induced by IgE-antigen complex and scratching

behaviors induced by compound 48/80. Murakami et al. [1996] reported that 1-*O*-(β-D-glucopyranosyl)-Paeonisuffrone isolated from *P. lactiflora* was found to inhibit histamine release from rat peritoneal exudates cell-induced antigen-antibody reaction.

Allergic reactions, mainly classified into four types, are the result of normally beneficial immune responses acting inappropriately, and sometimes cause inflammatory reactions and tissue damage [Rotti et al., 1988]. The prevalence and severity of allergic disease has increased dramatically during the past decades around the world, approximately 20% of the population have been affected in the developed countries [Li et al., 2008]. Type I allergic reactions are known to be triggered by binding of antigen (e.g. allergen) to specific IgE antibody which has been produced after initial stimulation with the antigen, and then the signal sensitizes mast cells and basophils via high-affinity FcεRI, which results in degranulation and the release of bioactive mediators such as histamine, β-hexosaminidase, arachidonic acid metabolites (Fig. 1) in the early-phase response [Turner et al., 1999; Siraganian et al., 2003; Galli et al., 2005; Ellmeier et al., 2011; Holowka et al., 2012].

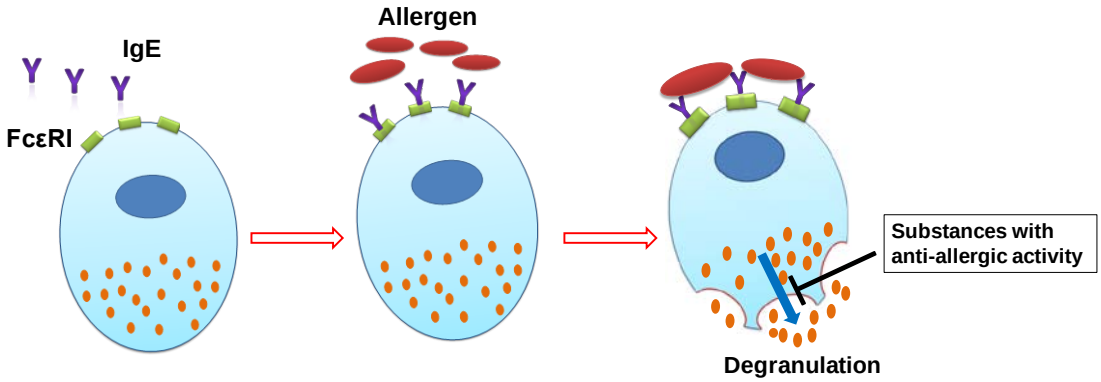


Fig. 1 Graphic scheme of IgE-mediated degranulation in mast cells or basophils

Nowadays, although several therapeutic approaches such as allergen-specific

immunotherapy, DNA vaccination, antihistamine drugs and steroid treatment have been used in allergic diseases, they produced undesirable side effects. Therefore, natural products as anti-allergic drug candidates with less cytotoxicity and adverse effects attracted much attention. β-hexosaminidase is considered as a degranulation marker, and inhibition of β-hexosaminidase release in RBL-2H3 cells that are physiologically very similar to mast cell but are a much more stable cell line in tissue culture, has been widely employed as an *in vitro* model of IgE-mediated degranulation for screening candidates with anti-allergic drugs from natural resources. Ginsenosides from *Panax ginseng* C.A. Meyer [Choo et al., 2003], xanthones from the pericarp of *Garcinia mangostana* L. [Itoh et al., 2008], flavonoids from *Fragaria ananassa* Duch and the peels of Japanese persimmon [Itoh et al., 2011], neoligans from the needles of *Pinus thunbergii* Parl. [Hong et al., 2014], sinomenine from *Sinomenium acutum* Rehder et Wilson [Huang et al., 2015], have been reported to have inhibitory effects against IgE-mediated degranulation in RBL-2H3 cells.

Previous phytochemical investigations on *Paeonia* species revealed a series of compounds including monoterpenoids, flavonoids, tannins, phenols and paeonols, etc. [Wu et al., 2010]. Monoterpenoids with “cage-like” pinane skeleton are considered as the representative and main bioactive constituents in peony root. Until now, more than 60 monoterpenoids have been isolated and identified from various *Paeonia* species [Shimizu et al., 1981; Yoshikawa et al., 1993; Liu et al., 2000; Wu et al., 2010; Shi et al., 2016]. Of the monoterpenoids obtained from *P. lactiflora*, diverse pharmacological effects have been reported, such as paeoniflorin with anti-allergic activity [Lee et al., 2008], paeoniflorin and 8-desbenzoylpaeoniflorin with anti-diabetic activity [Hsu et al. 1977], paeonidanin C and mudanpioside C with anti-inflammatory activity [Duan et al., 2009], paeonisuffrone and paeonisuffral with anti-oxidant activity [Yoshikawa et al., 2000], paeoniflorigenone, paeonilactone and

6-*O*-(β-D-glucopyranosyl)-lactinolide with anti-coagulative, sedative and analgesic activities [Shimazu et al., 1983; Hyashi et al., 1985; Murakami et al. 1996], etc. Several studies based on liquid chromatography coupled with time-of-flight mass spectrometry (LC-TOF-MS/MS) have been conducted to characterize the monoterpenoids composition in PR derived from *P. lactiflora*, however, all of them dealt with only one or a few predominant components such as paeoniflorin, albiflorin, oxypaeoniflorin, galloylpaeoniflorin and benzoylpaeoniflorin [Li et al., 2009; Liu et al., 2009; Wang et al., 2015]. Besides these main monoterpenoids, systematic study on distribution and contents variation of monoterpenoids in different types of PR has not been done.

The present study aims to elucidate bioactive constituents responsible for anti-allergic activity in RPR and in the roots of selected candidate cultivars of *P. lactiflora*, and further to elucidate the chemical composition and contents variation of monoterpenoids including those with anti-allergic activity in different type of PR and the related species. The following three parts of works are included.

The first, after investigating the anti-allergic activity of water extracts from crude drug samples of both WPR and RPR as inhibitory effects against 2, 4-dinitrophenylated bovine serum albumin (DNP-BSA) stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells, bioassay-guided fractionation on the RPR with the relatively strong activity was conducted and the isolated constituents were further evaluated for their anti-allergic activity (Chapter I).

The second, after comparing the anti-allergic activity of water extracts of the selected candidate cultivars, the promising candidate was subject to bioassay-guided fractionation for elucidating bioactive constituents (Chapter II).

The third, according to the above two parts, monoterpenoids, as the representative components in PR, were found to play important roles in the

anti-allergic activity of RPR. Therefore, monoterpenoids profiles of the different types of PR and the related species were investigated by using liquid chromatography coupled with ion trap and time-of-flight mass spectrometry (LC-IT-TOF-MS) (Chapter III).

Chapter Ⅰ

Chemical constituents with anti-allergic activity from red peony root, the root of *Paeonia lactiflora*

1.1 Introduction

In China, there are two kinds of peony root, white peony root (WPR) and red peony root (RPR). WPR and most of RPR are derived from the same botanical origin of *P. lactiflora,* but the two are quite different in their clinical application in the traditional Chinese medicine. WPR is often used for treatment of spasm, abdominal pain, blood deficiency, dizziness, etc.; whereas RPR is used for treatment of blood stasis, gynecological and cardiovascular diseases, etc. [Jiangsu New Medical College, 1977; Li Shizhen, 1977]. Although WPR and RPR have been used for different therapeutic purposes, modern pharmacological experiments suggested that both of them have the similar biological activities such as anti-inflammatory and anti-oxidant activities, relieving pain and convulsion, etc. [Wang et al., 2005; Wu et al., 2010]. To date, many papers have compared the chemical profiles of WPR and RPR derived from *P. lactiflora* [Wang et al., 2005; Luo et al., 2013; Wang et al., 2015]. In our previous studies, we demonstrated that *P. lactiflora* derived WPR and RPR are genetically and chemically different, RPR obviously contained a higher content of paeoniflorin and paeonol, but a lower content of albiflorin than the WPR samples [Zhu et al., 2015]. According to the data from the "Traditional Medicinal & Pharmaceutical Database" [http://dentomed.u-toyama.ac.jp/en/], RPR derived from *P. lactiflora* was found to possess higher anti-allergic activity as inhibitory effect against IgE-mediated degranulation in RBL-2H3 cells than WPR. However, the marker constituents responsible for the anti-allergic activity of RPR have not been studied.

In this Chapter, with the objectives to investigate the anti-allergic activity of RPR and to elucidate bioactive constituents responsible for this activity, bioassay-guided fractionation and isolation were conducted on the methanol extract of RPR and the bioassay was parallelly carried out by investigating inhibitory effects against

DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells.

1.2 Plant materials

Two crud drug samples of RPR (D12 and D27967) were purchased from Chifeng Rongxintang Pharmaceutical Co., Ltd., Inner Mongolia autonomous region of China in 2002 and 2013, respectively, which was clearly identified as RPR-type of *P. lactiflora* by genetic analysis of nrDNA ITS sequence. The crude drug sample of PR (D51) was purchased from Fukuda Co., Ltd., Nara prefecture of Japan in 2008, and it was also clearly identified as WPR-type of *P. lactiflora* [Zhu et al., 2015]. The vouchers (D12: TMPW No. 21565; D27967: TMPW No. 27967; D51: TMPW No. 25835) are deposited in the Museum of Materia Medica, Institute of Natural Medicine (TMPW), University of Toyama, Japan.

1.3 Results

1.3.1 Inhibitory effects of water extracts of PR against IgE-mediated degranulation in RBL-2H3 cells

According to the previous preliminary bioactivity screening experiment, three samples of PRR and PR (D27967, D12 and D51) were selected from 80 crude drugs registered in Japanese Pharmacopeia and further investigated their anti-allergic activity as inhibitory effect against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells.

As shown in Fig. 1.1, the water extract of D27967, RPR produced in Inner Mongolia autonomous region of China, showed potent anti-allergic activity. Therefore, D27967 was used as target to find out anti-allergic bioactive agents based on this pharmacological effect.

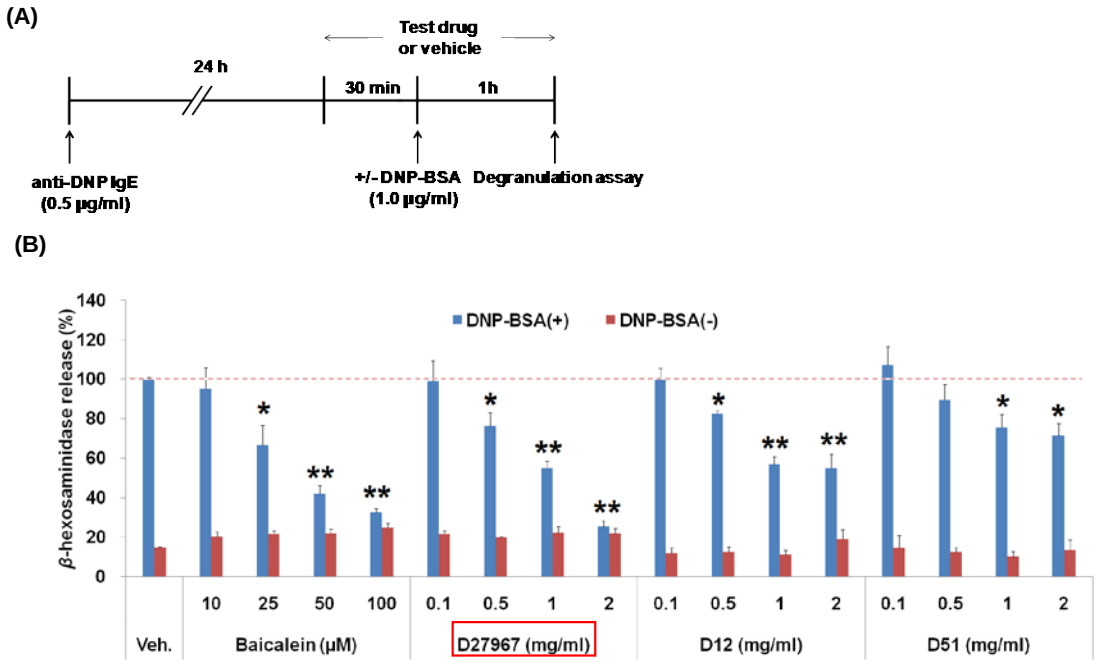


Fig. 1.1 Inhibitory effects of water extracts of RPR and PR on IgE-mediated degranulation by measuring β-hexosaminidase release in RBL-2H3 cells. (**A**) The time scheme for the degranulation assay. (**B**) RBL-2H3 cells were sensitized with 0.5 μg/ml anti-DNP IgE (24 h), and then were incubated with different water extracts of test samples for 30min. The cells were simulated with (Blue) or without (Red) 1.0 μg/ml DNP-BSA for 1 h, and β-hexosaminidase release was determined. The data are expressed as the mean ± S.D. (n=3). *$P< 0.05$; **$P< 0.01$ compared with the vehicle.

1.3.2 Inhibitory effects of methanol extract and related subfractions of D27967 against IgE-mediated degranulation in RBL-2H3 cells

Methanol extract of D27967 showed the relatively strong anti-allergic activity as inhibitory effects against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells (IC_{50}: 0.61 ± 0.02 mg/ml), compared to its water extract (IC_{50}: 0.90 ± 0.03 mg/ml) as shown in Fig. 1.2A. After fractionation with Daion HP-21 column chromatography (CC) eluted by a series of aqueous MeOH

solutions, 60% and 80% aqueous MeOH fractions were found to exhibit more promising inhibitory effects on β-hexosaminidase release (IC_{50}: 0.20 ± 0.01 and 0.12 ± 0.02 mg/ml, respectively, Fig. 1.2B). Therefore, bioassay-guided fractionation of the active 60% and 80% aqueous MeOH subfractions were further carried out to elucidate the active constituents.

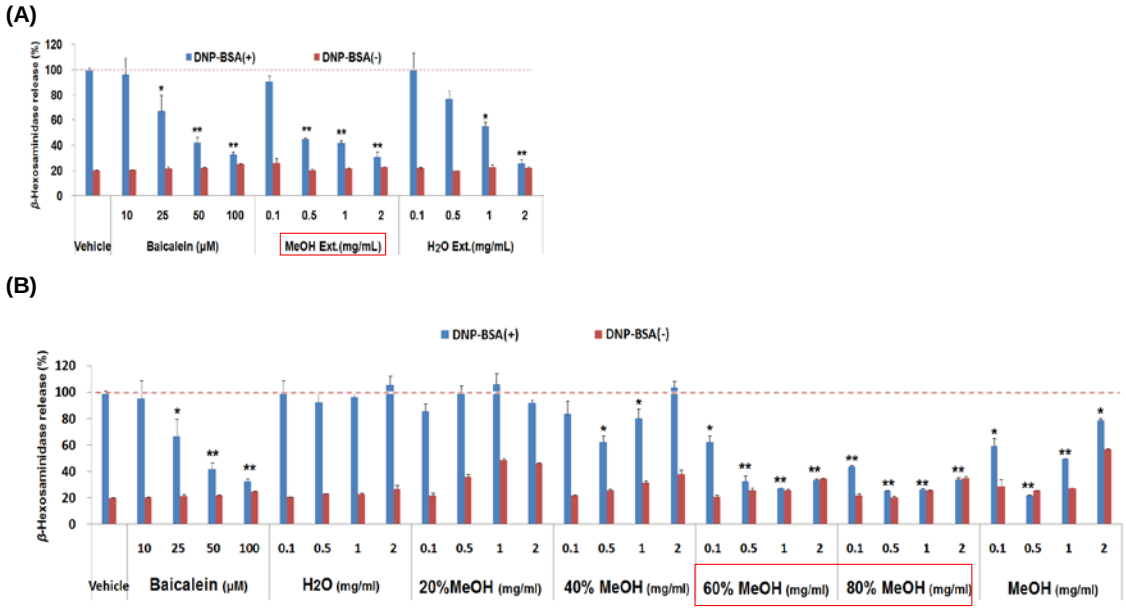


Fig. 1.2 Inhibitory effects of different extracts and related fractions of D27967 on IgE-mediated degranulation by measuring β-hexosaminidase release in RBL-2H3 cells. (**A**) Methanol and water extracts. (**B**) Related subfractions eluted from methanol extract. The data are expressed as the mean ± S.D. (n=3). **P< 0.05; **P<0.01* compared with the vehicle.

1.3.3 Isolation and structure determination

The above two active subfractions were subjected to a series of normal- and reverse-phase CC followed by the preparative HPLC to afford mainly monoterpenoids (**1**-**17**) including three new ones (**1**-**3**), five flavonoids (**19**-**23**), as well as six other types of compounds (**18**, **24**-**29**). The isolation procedure was illustrated in Chart 1.1 and the chemical structures of these isolated compounds were shown in Fig. 1.3.

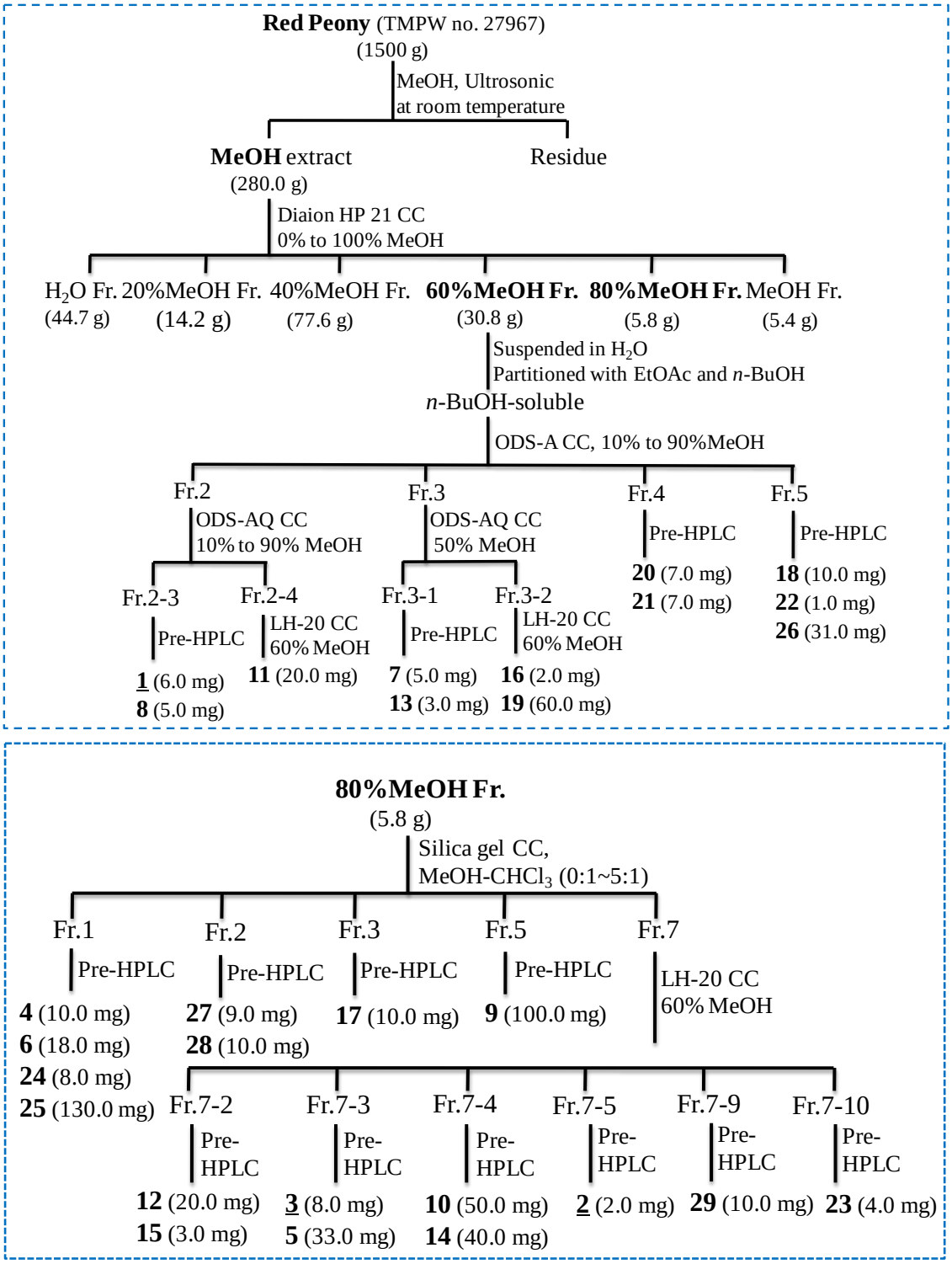
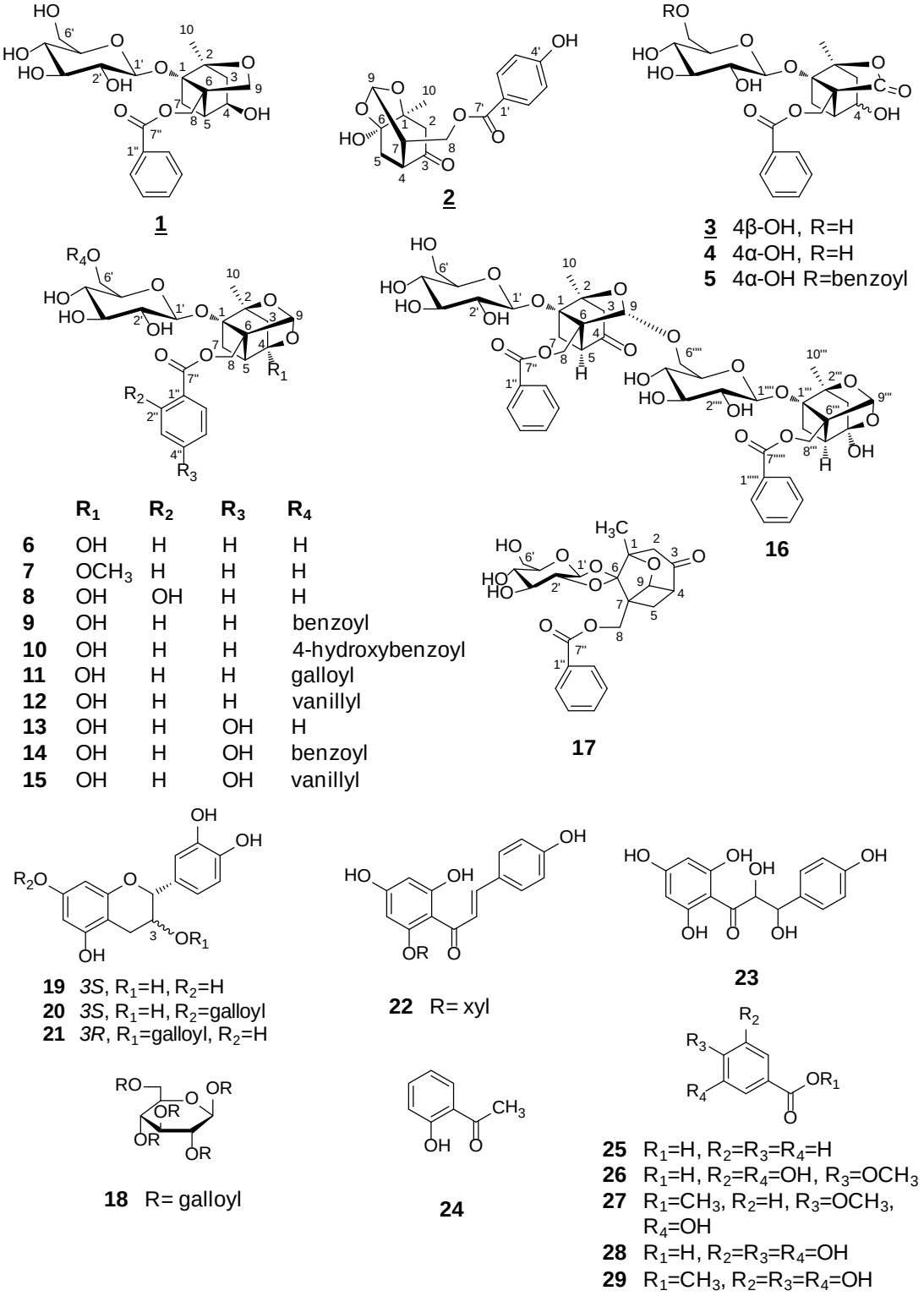


Chart 1.1 Isolation procedure of RPR (D27967) derived from *P. lactiflora*. New compounds are shown with underline.



	R_1	R_2	R_3	R_4
6	OH	H	H	H
7	OCH_3	H	H	H
8	OH	OH	H	H
9	OH	H	H	benzoyl
10	OH	H	H	4-hydroxybenzoyl
11	OH	H	H	galloyl
12	OH	H	H	vanillyl
13	OH	H	OH	H
14	OH	H	OH	benzoyl
15	OH	H	OH	vanillyl

Fig. 1.3 Structures of chemical constituents isolated from RPR (D27967) derived from *P. lactiflora*. New compounds are shown with underline.

1.3.4 Structure elucidation of the new compounds

Paeoniflorol (**1**)

White, amorphous powder; $[\alpha]_D^{20}$ -33.33^o (c = 0.13, MeOH). A quasimolecular ion at *m/z* 511.1812 $[M+HCOO]^-$ in the HR-ESI-MS spectrum suggested a molecular formula of $C_{23}H_{30}O_{10}$. The IR spectrum of **1** showed absorption bands at 3418 cm^{-1} (hydroxyl group), 1715 cm^{-1}(carbonyl group), and 1631 and 1456 cm^{-1} (aromatic ring). The ^{1}H-NMR spectrum of **1** (Table 1.1) showed signals of a tertiary methyl at δ_H 1.33 (3H, s, H-10), two methylenes at δ_H 1.84 (1H, d, J= 16.0 Hz, H-3β), 2.41 (1H, dd, J= 8.0, 16.0 Hz, H-3α), and at δ_H 1.73 (1H, d, J= 9.6 Hz, H-7α), 2.60 (1H, d, J= 1.2 Hz, H-7β), together with two oxymethylenes at δ_H 4.65, 4.73 (2H, J= 12.0 Hz, H-8) and δ_H 3.77, 4.78 (2H, J= 8.0 Hz, H-9). The ^{1}H-NMR of **1** also indicated a series of proton signals assignable to a glucopyranosyl moiety and a benzoyl moiety. In addition, the acid hydrolysis of **1** yielded β-D-glucopyranose. The ^{13}C-NMR spectrum of **1** showed signals at δ_C 21.7 (C-10), 45.5 (C-3), 28.2 (C-7) 67.3 (C-8) and 70.5 (C-9), which were in good accordance with those reported for 4α-hydroxyl derivative of paeonisuffrone (PSOH) [Murakami et al., 1996; Yoshikawa et al., 2000], except for the signals of C-1 and C-8. The deduced monoterpene skeleton was also supported by the cross peaks from the proton H-4 (δ_H 4.17) to H-3α (δ_H 2.41) and to H-5 (δ_H 2.59) in ^{1}H-^{1}H COSY, and the cross peak from H-4 (δ_H 4.17) to C-4 (δ_C 69.9) in HMQC spectrum. The downfield shift of C-1 (**1**: δ_C 87.9, PSOH: δ_C 82.6) and the long-range correlation between H-1' (δ_H 4.49) and C-1 in HMBC spectrum indicated that glucosylation at C-1 of the monoterpene skeleton. The downfield shift of C-8 (**1**: δ_C 67.3, PSOH: δ_C 64.9) and the long-range correlation between H-8 (δ_H 4.65 and δ_H 4.73) and C-7" in HMBC spectra confirmed that the benzoyl moiety was connected to C-8. The β-configuration of the hydrogen at C-4 of **1** was confirmed by the NOESY

cross signals between H-4 and H-3β, H-4 and H-7α, as well as between H-3β and H-7α (Fig. 1.4). Consequently, compound **1** was identified as 8-benzoyl-1-*O*-β-D-glucopyranosyl-4-hydroxyl derivative of paeonisuffrone, named as paeoniflorol.

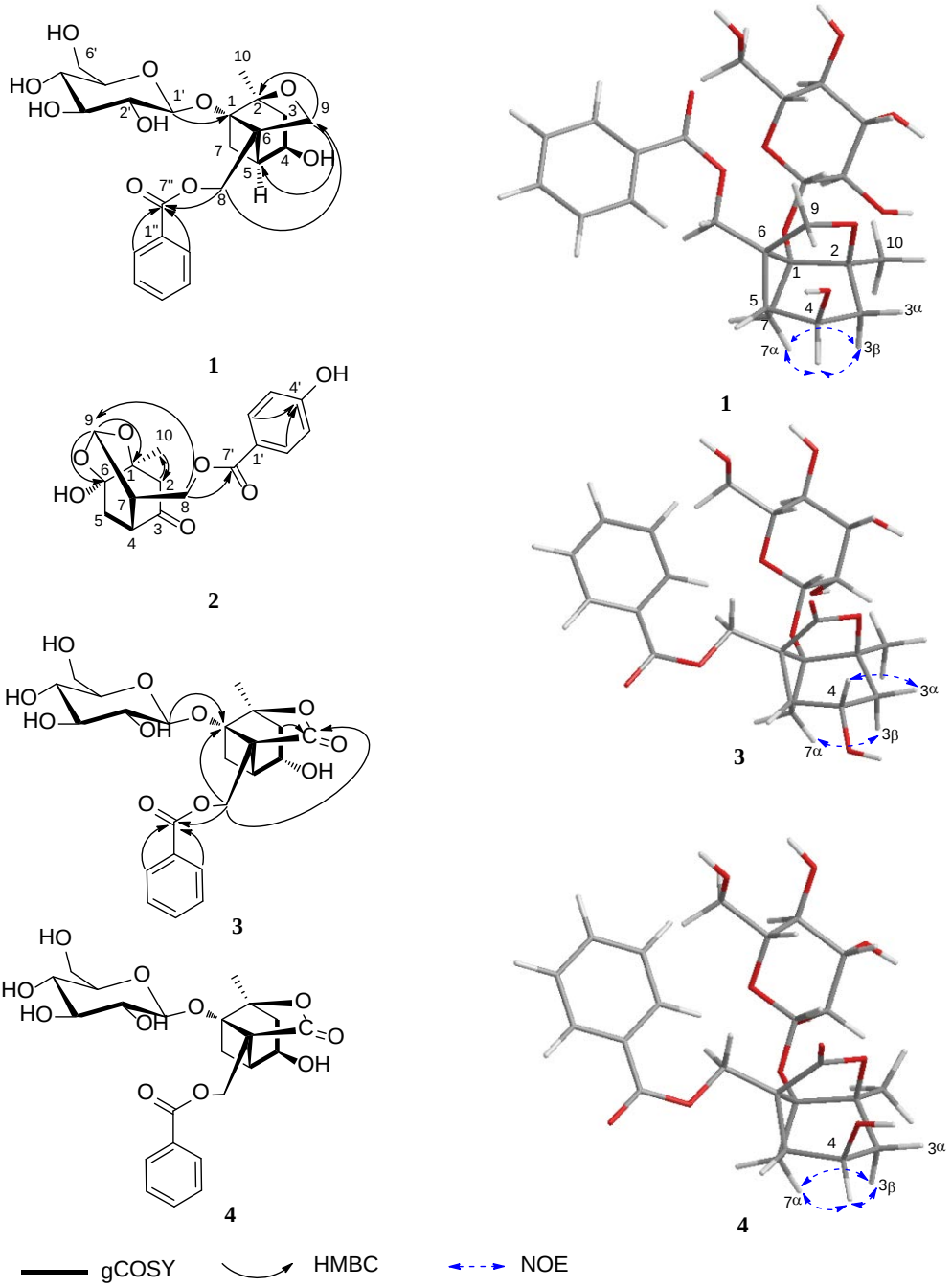


Fig. 1.4 Important 2D-NMR correlations of compounds **1**, **2**, **3** and **4**

Table 1.1 ^{13}C-NMR (100 MHz) and ^{1}H-NMR (400 MHz) spectroscopic data for **1-4** measured in CD_3OD.

	1		**2**		**3**		**4**	
Position	δ C	δ H (J in Hz)	δ C	δ H (J in Hz)	δ C	δ H (J in Hz)	δ C	δ H (J in Hz)
1	87.9		80.0		86.1		86.9	
2	91.3		48.1	2.53, d (16.0); 2.77, d (16.0)	92.4		93.5	
3	45.5	1.84, d (16.0); 2.41, dd (8.0, 16.0)	212.5		42.3	2.03, dd (8.0, 14.0); 2.39, d (14.0)	41.7	2.01, dd (1.6, 15.6); 2.40, dd (6.8, 15.6)
4	69.9	4.17, dd (4.0, 8.0)	47.9	2.88, d (7.6)	66.7	4.03, t (7.2)	68.4	4.26, dd (4.8, 6.4)
5	40.5	2.59, d (4.0)	35.6	2.28, dd (6.8, 13.2); 2.37, dd (5.2, 10.0)	40.3	2.77, d (7.2)	41.6	2.91, dd (5.2, 7.6)
6	59.0		102.8		58.7		56.9	
7	28.2	1.73, d (9.6); 2.60, d (1.2)	44.5	2.33, d (7.2)	25.8	2.35, dd (5.2, 12.0); 2.72, dd (7.2, 12.0)	28.5	2.04, d (11.2); 2.79, dd (8.0, 11.2)
8	67.3	4.65, d (12.0); 4.73, d (12.0)	63.7	3.99, dd (8.8, 11.6); 4.20, dd (6.0, 11.6)	61.3	4.69, d (12.0); 4.83, d (12.0)	62.0	4.67, d (12.4); 4.80, d (12.4)
9	70.5	3.77, d (8.0); 4.78, d (8.0)	101.0	5.39, s	176.5		178.0	
10	21.7	1.33, s	21.8	1.24, s	20.2	1.53, s	20.5	1.52, s
1-*O*-glucose								
1'	100.1	4.49, d (7.6)			100.1	4.54, d (8.0)	100.1	4.52, d (8.0)
2'	75.1	3.23, m			74.8	3.21, m	74.8	3.21, m
3'	77.9	3.37, m			78.0	3.23, m	78.0	3.24, m
4'	71.8	3.29, m			71.6	3.20, m	71.6	3.22, m
5'	78.1	3.31, m			78.1	3.32, m	78.2	3.30, m
6'	63.0	3.61, dd (4.8, 11.6); 3.84, dd (0.8, 11.6)			62.7	3.62, dd (4.8, 11.6); 3.85, d (11.6)	62.8	3.62, dd (4.4, 11.6); 3.84, d (11.6)
8-*O*-benzoyl								
***1''**	131.4		122.0		131.1		131.3	
***2''**	130.7	8.04, dd (1.6, 6.8)	132.8	7.86, dt (2.8, 8.4)	130.7	8.04, dd (1.2, 8.0)	130.8	8.07, dd (1.2, 8.0)
***3''**	129.7	7.48, dd (1.6, 7.6)	116.2	6.81, dt (2.8, 8.4)	129.7	7.49, t (8.0)	129.7	7.49, t (8.0)
***4''**	134.4	7.60, m	163.7		134.5	7.60, m	134.4	7.62, m
***5''**	129.7	7.48, dd (1.6, 7.6)	116.2	6.81, dt (2.8, 8.4)	129.7	7.49, t (8.0)	129.7	7.49, t (8.0)
***6''**	130.7	8.04, dd (1.6, 6.8)	132.8	7.86, dt (2.8, 8.4)	130.7	8.04, dd (1.2, 8.0)	130.8	8.07, dd (1.2, 8.0)
***7''**	168.2		167.7		167.8		167.9	

* Position No. is 1' to 7' in compound 2.

4'-hydroxypaeoniflorigenone (**2**)

White, amorphous powder; $[\alpha]_D^{20}$ +4.99^o (c = 0.10, MeOH). A quasimolecular ion at m/z 335.1124 $[M+H]^+$ in the HR-ESI-MS suggested a molecular formula of $C_{17}H_{18}O_7$. The IR spectrum of **2** indicated absorption bands at 3402 cm^{-1} (hydroxyl group), 1712 cm^{-1} (carbonyl group), and 1609 and 1514 cm^{-1} (aromatic ring). The ^{1}H-NMR spectrum of **2** (Table 1.1) showed signals of a tertiary methyl at δ_H 1.24 (3H, s, H-10), an oxymethylene at δ_H 3.99 (dd, J= 8.8, 11.6 Hz, H-8α) and δ_H 4.20 (J= 6.0, 11.6 Hz, H-8β), an acetal at δ_H 5.39 (1H, s, H-9), and as well as four aromatic protons at δ_H 6.81(2H, dt, J= 2.8, 8.4 Hz, H-3" and H-5") and δ_H 7.86 (2H, dt, J= 2.8, 8.4 Hz, H-2" and H-6"). The ^{13}C-NMR of **2** showed a set of diagnostic signals for a ketonic carbonyl (δ_C 212.5, C-3), an ester carbonyl (δ_C 167.7, C-7'), a methyl (δ_C 21.8, C-10), three methylenes (δ_C 35.6, C-5; δ_C 48.1, C-2; δ_C 63.7, C-8), three methines (δ_C 44.5, C-7; δ_C 47.9, C-4; δ_C 101.0, C-9), two quaternary carbons (δ_C 80.0, C-1; δ_C 102.8, C-6), and six aromatic carbons (δ_C 116.2~163.7), which closely resembled to those of paeoniflorigenone [Shimizu et al., 1983; Yoshikawa et al., 2000], except for the aromatic carbons. Both the ^{1}H and ^{13}C-NMR data indicated clearly the presence of a *p*-hydroxybenzoyl moiety. In addition, the correlation from H-8 to C-7' in HMBC spectrum confirmed that the *p*-hydroxylbenzoyl moiety was connected to C-8 (Fig. 1.4). Therefore, compound **2** was identified as 4'-hydroxypaeoniflorigenone.

4-*epi*-albiflorin (**3**)

White, amorphous powder; the HR-ESI-MS of **3** displayed a quasimolecular ion peak at m/z 525.1617 $[M+HCOO]^-$, which suggested a molecular formula of $C_{23}H_{28}O_{11}$. The IR spectrum of **3** indicated absorption bands at 3389 cm^{-1} (hydroxyl group), 1755 and 1715 cm^{-1} (carbonyl group), and 1631 and 1452 cm^{-1} (aromatic ring). The

^{1}H-NMR spectrum of **3** showed a methyl at δ_H 1.53 (3H, s, H-10), two methylenes at δ_H 2.03 (1H, dd, *J*= 8.0, 14.0 Hz, H-3β), 2.39 (1H, d, *J*= 14.0 Hz, H-3α) and at δ_H 2.35 (1H, dd, *J*= 5.2, 12.0 Hz, H-7α), 2.72 (1H, dd, *J*= 7.2, 12.0 Hz, H-7β), two methines at δ_H 4.03 (1H, t, *J*= 7.2 Hz, H-4) and at δ_H 2.77 (1H, d, *J*= 7.2 Hz, H-5), one oxymethylene at δ_H 4.69, 4.83 (2H, *J*= 12.0 Hz, H-8), five aromatic protons at δ_H 7.49 (2H, t, *J*= 8.0 Hz, H-3" and H-5"), δ_H 7.60 (1H, m, H-4") and δ_H 8.04 (2H, dd, *J*= 1.2, 8.0 Hz, H-2" and H-6"), together with a set of proton signals assignable to a glucopyranosyl moiety (Table 1.1). Correspondently, the ^{13}C-NMR spectrum displayed 23 carbon signals, which included characteristic signals of a methyl (δ_C 20.2, C-10), two methylenes (δ_C 42.3, C-3; δ_C 25.8, C-7), two methines (δ_C 66.7, C-4; δ_C 40.3, C-5), one oxymethylene (δ_C 61.3, C-8), two ester carbonyl (δ_C 176.5, C-9; δ_C 167.8, C-7"), six aromatic carbons (δ_C 131.1, C-1"; δ_C 130.7, C-2", 6"; δ_C 129.7, C-3", 5"; δ_C 134.5, C-4"), and six glucopyranosyl carbons (δ_C 100.1, 78.1, 78.0, 74.8, 71.6, 62.7) (Table 1). The 1D NMR (^{1}H and ^{13}C) and 2D NMR (HMQC, HMBC) data of **3** were closely similar to those of albiflorin (**4**), which suggested **3** had the same planar structure with **4** [Kaneda et al., 1972; Yen et al., 2007; Yean et al., 2008]. Carefully compared their NMR data, significant differences were found in the coupling constant of the methine proton H-4 (**3**: *J*= 7.2 Hz; **4**: *J*= 4.8, 6.4 Hz), as well as the chemical shifts of the methylene signals C-7 and H-7α (**3**: δ_C 25.8, δ_H 2.35; **4**: δ_C 28.5, δ_H 2.04), and the methine signals C-5 and H-5 (**3**: δ_C 40.3, δ_H 2.77; **4**: δ_C 41.6, δ_H 2.91), which suggested that **3** was an epimer of **4.** The stereochemistry of hydroxyl group at C-4 in **4** was confirmed to be α-configuration [Matsuda et al., 2002]. In the 1D different NOE spectrums of **4**, correspondently, the correlations from H-4 to H-3β and H-7α, as well as from H-3β to H-7α were observed (Fig. 1.4). In contrast, the NOE correlation between H-4 (δ_H 4.03) and H-3α was observed in **3**, suggesting the co-facial of these two protons with α-orientation. Moreover, the presence of NOE correlation between

H-3β and H-7α on the opposite side and the absence of correlation between H-4 and H-7α definitely indicated that the hydroxyl moiety at C-4 in **3** was β-configuration. Therefore, compound **3** was determined to be the epimer of albiflorin and named as 4-*epi*-albiflorin.

1.3.5 Identification of the known compounds

Albiflorin (**4**)

White, amorphous powder; $[\alpha]_D^{20}$ -19.54^o (*c* = 0.11, MeOH). The HR-ESI-MS displayed a quasimolecular ion peak at m/z 525.1652 $[M+HCOO]^-$, which suggested a molecular formula of $C_{23}H_{28}O_{11}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.07 (2H, dd, *J*= 1.2, 8.6 Hz, H-2" and H-6"), 7.62 (1H, m, H-4"), 7.49 (2H, t, *J*= 8.0 Hz, H-3" and H-5"), 4.80 (1H, d, *J*= 12.4 Hz, H-8α), 4.67 (1H, d, *J*= 12.4 Hz, H-8β), 4.52 (1H, d, *J*= 8.0 Hz, H-1'), 4.26 (1H, dd, *J*= 4.8, 6.4 Hz, H-4), 3.84 (1H, d, *J*= 11.6 Hz, H-6'α), 3.62 (1H, dd, *J*= 4.4, 11.6 Hz, H-6'β), 3.30 (1H, m, H-5'), 3.24 (1H, m, H-3'), 3.22 (1H, m, H-4'), 3.21 (1H, m, H-2'), 2.91 (1H, dd, *J*= 5.2, 7.6 Hz, H-5), 2.79 (1H, dd, *J*= 8.0, 11.2 Hz, H-7α), 2.04 (1H, d, *J*= 11.2 Hz, H-7β), 2.40 (1H, dd, *J*= 6.8, 15.6 Hz, H-3α), 2.01 (1H, dd, *J*= 1.6, 15.6 Hz, H-3β), 1.52 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 178.0 (C-9), 167.9 (C-7"), 134.4 (C-4"), 131.3 (C-1"), 130.8 (C-2", 6"), 129.7 (C-3", 5"), 100.1 (C-1'), 93.5 (C-2), 86.9 (C-1), 78.2 (C-5'), 78.0 (C-3'), 74.8 (C-2'), 71.6 (C-4'), 68.4 (C-4), 62.8 (C-6'), 62.0 (C-8), 56.9 (C-6), 41.7 (C-3), 41.6 (C-5), 28.5 (C-7), 20.5 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kaneda et al., 1972; Yen et al., 2007].

Paeonivayin (**5**)

White, amorphous powder; the HR-ESI-MS displayed a quasimolecular ion peak at

m/z 583.1815 $[M-H]^-$, which suggested a molecular formula of $C_{23}H_{28}O_{11}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.04 (4H, dd, *J*= 1.2, 8.4 Hz, H-2"/6" and H-2'"/6'"), 7.60 (1H, m, H-4'"), 7.58 (1H, m, H-4"), 7.49 (2H, t, *J*= 8.0 Hz, H-3'" and H-5'"), 7.45 (2H, t, *J*= 8.0 Hz, H-3" and H-5"), 4.74 (1H, d, *J*= 12.4 Hz, H-8α), 4.67 (1H, d, *J*= 12.4 Hz, H-8β), 4.54 (1H, d, *J*= 8.0 Hz, H-1'), 3.96 (1H, t, *J*= 4.8 Hz, H-4), 3.64 (1H, d, *J*= 11.6 Hz, H-6'α), 3.36 (1H, m, H-6'β), 3.31 (1H, m, H-5'), 3.30 (1H, m, H-3'), 3.28 (1H, m, H-4'), 3.26 (1H, m, H-2'), 2.80 (1H, d, *J*= 5.2 Hz, H-5), 2.73 (1H, dd, *J*= 8.0, 12.0 Hz, H-7α), 1.98 (1H, dd, *J*= 1.6, 15.6 Hz, H-3α), 1.87 (1H, dd, *J*= 15.6 Hz, H-3β), 1.72 (1H, d, *J*= 12.0 Hz, H-7β), 1.38 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 177.8 (C-9), 167.9 (C-7"), 167.5 (C-7'"), 134.5 (C-4"), 134.3 (C-4'"), 131.3 (C-1"), 131.2 (C-1'"), 130.7 (C-2", 6"), 130.6 (C-2'", 6'"), 129.7 (C-3", 5"), 129.6 (C-3'", 5'"), 99.9 (C-1'), 93.3 (C-2), 86.7 (C-1), 77.7 (C-5'), 75.2 (C-3'), 74.7 (C-2'), 71.9 (C-4'), 68.2 (C-4), 64.9 (C-6'), 61.8 (C-8), 56.4 (C-6), 41.4 (C-3), 41.4 (C-5), 28.0 (C-7), 20.5 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were closely similar with those of the reported values [Ma et al., 1999].

Paeoniflorin (**6**)

White, amorphous powder; a quasimolecular ion peak at m/z 525.1601 $[M+HCOO]^-$ in HR-ESI-MS suggested a molecular formula of $C_{23}H_{28}O_{11}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.05 (2H, dt, *J*= 1.6, 8.4 Hz, H-2" and H-6"), 7.60 (1H, dt, *J*= 7.6 Hz, H-4"), 7.47 (2H, dt, *J*= 7.6 Hz, H-3" and H-5"), 5.42 (1H, s, H-9), 4.74 (2H, dd, *J*= 2.8, 12.0 Hz, H-8), 4.52 (1H, d, *J*= 7.2 Hz, H-1'), 3.84 (1H, d, *J*= 12.0 Hz, H-6'α), 3.60 (1H, dd, *J*= 5.6, 12.0 Hz, H-6'β), 3.33 (1H, m, H-5'), 3.30 (1H, m, H-3'), 3.27 (1H, m, H-4'), 3.20 (1H, m, H-2'), 2.58 (1H, dd, *J*= 5.6 Hz, H-5), 2.49 (1H, dd, *J*= 6.8, 10.8 Hz, H-7α), 1.95 (1H, d, *J*= 10.8 Hz, H-7β), 2.18 (1H, d, *J*= 12.0 Hz, H-3α), 1.79 (1H, dd, *J*= 1.6, 12.0 Hz, H-3β), 1.36 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 168.0

(C-7"), 134.4 (C-4"), 131.2 (C-1"), 130.7 (C-2", 6"), 129.6 (C-3", 5"), 106.4 (C-4), 102.3 (C-9), 100.2 (C-1'), 89.3 (C-1), 87.2 (C-2), 78.0 (C-5'), 77.9 (C-3'), 75.0 (C-2'), 72.2 (C-6), 71.7 (C-4'), 62.8 (C-6'), 61.7 (C-8), 44.5 (C-3), 44.0 (C-5), 32.4 (C-7), 20.0 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were good accordance with those of the reported values [Kaneda et al., 1972].

4-*O*-methyl-paeoniflorin (**7**)

White, amorphous powder; a quasimolecular ion peak at m/z 493.1717 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{24}H_{30}O_{11}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.04 (2H, dd, *J*= 1.6, 7.2 Hz, H-2" and H-6"), 7.50 (1H, d, *J*= 7.2 Hz, H-4"), 7.48 (2H, dd, *J*= 1.6, 7.6 Hz, H-3" and H-5"), 5.45 (1H, s, H-9), 4.76 (2H, s, H-8), 4.52 (1H, d, *J*= 7.6 Hz, H-1'), 3.84 (1H, d, *J*= 12.0 Hz, H-6'α), 3.59 (1H, dd, *J*= 5.6, 12.0 Hz, H-6'β), 3.78 (3H, s, -OC<u>H</u>$_3$), 3.34 (1H, m, H-3'), 3.31 (1H, m, H-5'), 3.28 (1H, m, H-4'), 3.25 (1H, m, H-2'), 2.75 (1H, dd, *J*= 1.6, 5.6 Hz, H-5), 2.49 (1H, dd, *J*= 7.2, 10.8 Hz, H-7α), 1.90 (1H, d, *J*= 10.8 Hz, H-7β), 2.25 (1H, d, *J*= 12.4 Hz, H-3α), 1.94 (1H, dd, *J*= 1.2, 12.4 Hz, H-3β), 1.38 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 168.0 (C-7"), 134.5 (C-4"), 131.2 (C-1"), 130.6 (C-2", 6"), 129.7 (C-3", 5"), 109.5 (C-4), 102.5 (C-9), 100.2 (C-1'), 89.2 (C-1), 87.2 (C-2), 78.1 (C-5'), 78.0 (C-3'), 75.0 (C-2'), 71.8 (C-4'), 71.7 (C-6), 62.9 (C-6'), 61.7 (C-8), 42.3 (C-3), 41.0 (C-5), 23.4 (C-7), 19.6 (C-10), 51.4 (-O<u>C</u>H$_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Braca et al., 2008].

Salicylpaeoniflorin (**8**)

White, amorphous powder; a quasimolecular ion peak at m/z 495.1519 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{23}H_{28}O_{12}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.90 (1H, d, *J*= 7.6 Hz, H-6"), 7.49 (1H, dt, *J*= 6.8 Hz, H-4"), 6.93 (1H, d, *J*= 10.8

Hz, H-3"), 6.91 (1H, m, H-5"), 5.42 (1H, s, H-9), 4.80 (2H, d, *J*= 12.0 Hz, H-8), 4.52 (1H, d, *J*= 7.6 Hz, H-1'), 3.83 (1H, d, *J*= 12.0 Hz, H-6'α), 3.60 (1H, dd, *J*= 5.6, 12.0 Hz, H-6'β), 3.30 (1H, m, H-3'), 3.23 (1H, m, H-5'), 3.22 (1H, m, H-4'), 3.20 (1H, m, H-2'), 2.59 (1H, d, *J*= 5.6 Hz, H-5), 2.50 (1H, dd, *J*= 7.2, 10.8 Hz, H-7α), 1.95 (1H, d, *J*= 10.8 Hz, H-7β), 2.19 (1H, d, *J*= 12.8 Hz, H-3α), 1.78 (1H, dd, *J*= 1.2, 12.8 Hz, H-3β), 1.37 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 171.2 (C-7"), 162.8 (C-2"), 137.0 (C-4"), 131.2 (C-6"), 120.4 (C-5"), 118.4 (C-3"), 113.6 (C-1"), 106.4 (C-4), 102.2 (C-9), 100.2 (C-1'), 89.3 (C-1), 87.3 (C-2), 78.1 (C-5'), 78.0 (C-3'), 75.0 (C-2'), 71.8 (C-4'), 71.7 (C-6), 62.9 (C-6'), 62.1 (C-8), 44.5 (C-3), 44.0 (C-5), 23.4 (C-7), 19.6 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Okasaka et al., 2008].

Benzoylpaeoniflorin (**9**)

White, amorphous powder; a quasimolecular ion peak at m/z 583.1826 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{30}H_{32}O_{12}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.02 (2H, dd, *J*= 1.2, 8.0 Hz, H-2" and H-6"), 8.02 (2H, dd, *J*= 1.2, 8.0 Hz, H-2'" and H-6'"), 7.59 (1H, d, *J*= 7.2 Hz, H-4"), 7.56 (1H, d, *J*= 7.2 Hz, H-4'"), 7.45 (2H, d, *J*= 8.0 Hz, H-3" and H-5"), 7.42 (2H, d, *J*= 7.2 Hz, H-3'" and H-5'"), 5.46 (1H, s, H-9), 4.73 (2H, s, H-8), 4.61 (1H, d, *J*= 7.2 Hz, H-1'), 4.65 (1H, d, *J*= 11.2 Hz, H-6'α), 4.52 (1H, dd, *J*= 7.2, 11.2 Hz, H-6'β), 3.65 (1H, m, H-5'), 3.41 (1H, m, H-3'), 3.41 (1H, m, H-4'), 3.33 (1H, m, H-2'), 2.56 (1H, d, *J*= 6.4 Hz, H-5), 2.48 (1H, dd, *J*= 7.2, 10.8 Hz, H-7α), 1.70 (1H, d, *J*= 10.8 Hz, H-7β), 1.87 (1H, d, *J*= 12.8 Hz, H-3α), 1.72 (1H, d, *J*= 12.8 Hz, H-3β), 1.27 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 167.8 (C-7'"), 167.4 (C-7"), 134.4 (C-4"), 134.3 (C-4'"), 131.0 (C-1"), 130.8 (C-1'"), 130.5 (C-2", 6"), 130.5 (C-2'", 6'"), 129.5 (C-3", 5"), 129.4 (C-3'", 5'"), 106.0 (C-4), 102.0 (C-9), 99.8 (C-1'), 89.1 (C-1), 86.9 (C-2), 77.5 (C-5'), 74.9 (C-3'), 74.6 (C-2'), 71.8 (C-6), 71.6

(C-4'), 65.3 (C-6'), 61.5 (C-8), 44.2 (C-3), 43.6 (C-5), 22.8 (C-7), 19.5 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were good accordance with those of the reported values [Kaneda et al., 1972].

Mudanpioside C (**10**)

White, amorphous powder; a quasimolecular ion peak at m/z 599.1760 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{30}H_{32}O_{13}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.02 (2H, dd, *J*= 1.2, 7.6 Hz, H-2" and H-6"), 7.88 (2H, d, *J*= 6.8 Hz, H-2'" and H-6'"), 7.58 (1H, d, *J*= 7.6 Hz, H-4"), 7.45 (2H, dd, *J*= 1.6, 7.6 Hz, H-3" and H-5"), 6.82 (2H, d, *J*= 6.8 Hz, H-3'" and H-5'"), 5.40 (1H, s, H-9), 4.71 (2H, s, H-8), 4.56 (1H, d, *J*= 7.6 Hz, H-1'), 4.59 (1H, d, *J*= 11.2 Hz, H-6'α), 4.45 (1H, dd, *J*= 7.6, 11.2 Hz, H-6'β), 3.57 (1H, m, H-5'), 3.36 (1H, m, H-3'), 3.33 (1H, m, H-4'), 3.28 (1H, m, H-2'), 2.56 (1H, d, *J*= 5.2 Hz, H-5), 2.46 (1H, dd, *J*= 7.2, 10.8 Hz, H-7α), 1.72 (1H, d, *J*= 10.8 Hz, H-7β), 1.88 (1H, d, *J*= 12.4 Hz, H-3α), 1.71 (1H, d, *J*= 12.4 Hz, H-3β), 1.25 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 167.9 (C-7'"), 167.6 (C-7"), 163.3 (C-4'"), 134.4 (C-4"), 132.8 (C-2'", 6'"), 132.8 (C-1"), 130.6 (C-2", 6"), 129.6 (C-3", 5"), 122.0 (C-1'"), 116.2 (C-3'", 5'"), 106.2 (C-4), 102.1 (C-9), 99.9 (C-1'), 89.2 (C-1), 87.0 (C-2), 77.8 (C-3'), 75.2 (C-5'), 74.9 (C-2'), 72.0 (C-6), 71.9 (C-4'), 64.7 (C-6'), 61.5 (C-8), 44.4 (C-3), 43.8 (C-5), 22.7 (C-7), 19.5 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ding et al., 2012].

Galloylpaeoniflorin (**11**)

White, amorphous powder; a quasimolecular ion peak at m/z 631.1644 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{30}H_{32}O_{15}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.02 (2H, dt, *J*= 1.2, 7.2 Hz, H-2" and H-6"), 7.59 (1H, dt, *J*= 7.2 Hz, H-4"), 7.46

(2H, dt, *J*= 7.2 Hz, H-3'' and H-5''), 7.07 (2H, s, H-2''' and H-6'''), 5.39 (1H, s, H-9), 4.70 (2H, s, H-8), 4.53 (1H, d, *J*= 7.6 Hz, H-1'), 4.49 (1H, d, *J*= 12.0 Hz, H-6'α), 4.42 (1H, dd, *J*= 5.2, 12.0 Hz, H-6'β), 3.54 (1H, m, H-5'), 3.39 (1H, m, H-3'), 3.37 (1H, m, H-4'), 3.27 (1H, m, H-2'), 2.53 (1H, d, *J*= 6.4 Hz, H-5), 2.45 (1H, dd, *J*= 6.8, 10.8 Hz, H-7α), 1.73 (1H, d, *J*= 10.8 Hz, H-7β), 1.92 (1H, d, *J*= 12.4 Hz, H-3α), 1.69 (1H, d, *J*= 12.4 Hz, H-3β), 1.26 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 168.0 (C-7'''), 167.9 (C-7''), 146.5 (C-3''', 5'''), 139.9 (C-4'''), 134.4 (C-4''), 131.1 (C-1''), 130.6 (C-2'', 6''), 129.6 (C-3'', 5''), 121.4 (C-1'''), 110.1 (C-2''', 6'''), 106.3 (C-4), 102.1 (C-9), 100.0 (C-1'), 89.3 (C-1), 87.2 (C-2), 77.8 (C-3'), 75.2 (C-5'), 74.9 (C-2'), 72.0 (C-6), 71.9 (C-4'), 64.6 (C-6'), 61.6 (C-8), 44.4 (C-3), 43.8 (C-5), 22.9 (C-7), 19.5 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close similar with those of the reported values [Kang et al., 1991].

Mudanpioside J (**12**)

White, amorphous powder; a quasimolecular ion peak at m/z 629.1874 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{31}H_{34}O_{14}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.02 (2H, dt, *J*= 1.6, 7.2 Hz, H-2'' and H-6''), 7.59 (1H, m, H-4''), 7.58 (1H, m, H-2'''), 7.54 (1H, dd, *J*= 1.6, 8.4 Hz, H-6'''), 7.46 (2H, d, *J*= 7.2 Hz, H-3'' and H-5''), 6.84 (1H, d, *J*= 8.4 Hz, H-5'''), 5.39 (1H, s, H-9), 4.71 (2H, s, H-8), 4.57 (1H, d, *J*= 7.6 Hz, H-1'), 4.60 (1H, d, *J*= 12.0 Hz, H-6'α), 4.45 (1H, dd, *J*= 7.2, 12.0 Hz, H-6'β), 3.88 (3H, s, -OC$\underline{H}_3$), 3.58 (1H, m, H-5'), 3.33 (1H, m, H-3'), 3.32 (1H, m, H-4'), 3.28 (1H, m, H-2'), 2.51 (1H, d, *J*= 6.0 Hz, H-5), 2.45 (1H, d, *J*= 10.4 Hz, H-7α), 1.73 (1H, d, *J*= 10.4 Hz, H-7β), 1.88 (1H, d, *J*= 12.8 Hz, H-3α), 1.69 (1H, d, *J*= 12.8 Hz, H-3β), 1.26 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 167.9 (C-7'''), 167.6 (C-7''), 153.0 (C-4'''), 148.8 (C-3'''), 134.4 (C-4''), 131.1 (C-1''), 130.6 (C-2'', 6''), 129.6 (C-3'', 5''), 125.2 (C-6'''), 122.4 (C-1'''), 116.0 (C-5'''), 113.5 (C-2'''), 106.2 (C-4), 102.2 (C-9),

100.0 (C-1'), 89.2 (C-2), 87.1 (C-1), 77.8 (C-3'), 75.3 (C-5'), 74.9 (C-2'), 72.1 (C-6), 72.0 (C-4'), 65.0 (C-6'), 61.6 (C-8), 44.4 (C-3), 43.8 (C-5), 23.1 (C-7), 19.6 (C-10), 56.5 (-O<u>C</u>H_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ding et al., 2006].

Oxypaeoniflorin (**13**)

White, amorphous powder; a quasimolecular ion peak at m/z 495.1510 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{23}H_{28}O_{12}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.90 (2H, d, *J*= 8.8 Hz, H-2" and H-6"), 6.83 (1H, d, *J*= 8.8 Hz, H-3" and H-5"), 5.42 (1H, s, H-9), 4.69 (2H, s, H-8), 4.54 (1H, d, *J*= 7.6 Hz, H-1'), 3.85 (1H, d, *J*= 12.0 Hz, H-6'α), 3.62 (1H, dd, *J*= 5.6, 12.0 Hz, H-6'β), 3.35 (1H, m, H-5'), 3.31 (1H, m, H-3'), 3.27 (1H, m, H-4'), 3.23 (1H, m, H-2'), 2.57 (1H, d, *J*= 6.0 Hz, H-5), 2.50 (1H, dd, *J*= 5.6, 10.8 Hz, H-7α), 1.94 (1H, d, *J*= 10.8 Hz, H-7β), 2.18 (1H, d, *J*= 12.0 Hz, H-3α), 1.80 (1H, dd, *J*= 12.0 Hz, H-3β), 1.27 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 170.0 (C-7"), 163.5 (C-4"), 132.9 (C-2", 6"), 121.9 (C-1"), 116.2 (C-3", 5"), 106.3 (C-4), 102.2 (C-9), 100.1 (C-1'), 89.2 (C-1), 87.1 (C-2), 77.9 (C-5'), 77.8 (C-3'), 74.9 (C-2'), 71.8 (C-6), 71.6 (C-4'), 62.8 (C-6'), 61.2 (C-8), 44.5 (C-3), 43.8 (C-5), 23.4 (C-7), 19.6 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kaneda et al., 2008].

Benzoyloxypaeoniflorin (**14**)

White, amorphous powder; a quasimolecular ion peak at m/z 599.1760 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{30}H_{32}O_{13}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.03 (2H, dt, *J*= 7.2 Hz, H-2"' and H-6"'), 7.88 (2H, d, *J*= 8.8 Hz, H-2" and H-6"), 7.54 (1H, d, *J*= 7.6 Hz, H-4"'), 7.47 (2H, dt, *J*= 7.6 Hz, H-3"' and H-5"'), 6.82 (2H, d, *J*= 8.8 Hz, H-3" and H-5"), 5.37 (1H, s, H-9), 4.67 (2H, s, H-8), 4.64 (1H, d, *J*= 12.0

Hz, H-6'α), 4.56 (1H, d, *J*= 7.6 Hz, H-1'), 4.48 (1H, dd, *J*= 7.6, 12.0 Hz, H-6'β), 3.60 (1H, m, H-5'), 3.34 (1H, m, H-3'), 3.31 (1H, m, H-4'), 3.28 (1H, m, H-2'), 2.49 (1H, dd, *J*= 6.4 Hz, H-5), 2.45 (1H, dd, *J*= 10.2 Hz, H-7α), 1.70 (1H, m, H-7β), 1.83 (1H, d, *J*= 12.8 Hz, H-3α), 1.68 (1H, d, *J*= 12.8 Hz, H-3β), 1.24 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 167.9 (C-7'''), 167.6 (C-7''), 163.7 (C-4''), 134.5 (C-4'''), 134.5 (C-2'', 6''), 133.0 (C-1'''), 131.3 (C-2''', 6'''), 130.5 (C-3''', 5'''), 122.0 (C-1''), 116.2 (C-3'', 5''), 106.2 (C-4), 102.2 (C-9), 100.0 (C-1'), 89.2 (C-1), 87.0 (C-2), 77.8 (C-3'), 75.1 (C-5'), 74.9 (C-2'), 72.0 (C-6), 72.0 (C-4'), 65.1 (C-6'), 61.1 (C-8), 44.4 (C-3), 43.8 (C-5), 23.0 (C-7), 19.5 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ding et al., 2012].

6'-*O*-Vanillyloxypaeoniflorin (**15**)

White, amorphous powder; a quasimolecular ion peak at m/z 645.1551 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{31}H_{34}O_{15}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.03 (2H, d, *J*= 1.6, 7.2 Hz, H-2'' and H-6''), 7.59 (1H, m, H-2'''), 7.54 (1H, dd, *J*= 1.6, 8.4 Hz, H-6'''), 7.48 (2H, d, *J*= 7.2 Hz, H-3'' and H-5''), 6.88 (1H, d, *J*= 8.4 Hz, H-5'''), 5.40 (1H, s, H-9), 4.78 (2H, s, H-8), 4.64 (1H, d, *J*= 12.0 Hz, H-6'α), 4.58 (1H, d, *J*= 7.6 Hz, H-1'), 4.46 (1H, dd, *J*= 6.8, 12.0 Hz, H-6'β), 3.88 (3H, s, -OC$\underline{H}_3$), 3.50 (1H, m, H-5'), 3.33 (1H, m, H-3'), 3.32 (1H, m, H-4'), 3.28 (1H, m, H-2'), 2.53 (1H, d, *J*= 6.4 Hz, H-5), 2.50 (1H, d, *J*= 10.8 Hz, H-7α), 1.80 (1H, d, *J*= 10.8 Hz, H-7β), 1.93 (1H, d, *J*= 12.0 Hz, H-3α), 1.73 (1H, d, *J*= 12.0 Hz, H-3β), 1.24 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 167.9 (C-7'''), 167.0 (C-7''), 163.6 (C-4''), 153.3 (C-4'''), 148.4 (C-3'''), 132.2 (C-2'', 6''), 125.2 (C-6'''), 122.4 (C-1'''), 121.9 (C-1''), 116.2 (C-3'', 5''), 116.0 (C-5'''), 113.5 (C-2'''), 106.2 (C-4), 102.2 (C-9), 100.0 (C-1'), 89.2 (C-2), 87.1 (C-1), 77.8 (C-3'), 75.3 (C-5'), 74.9 (C-2'), 72.1 (C-6), 72.0 (C-4'), 65.0 (C-6'), 61.6 (C-8), 44.4 (C-3), 43.8 (C-5), 23.0 (C-7), 19.5 (C-10), 56.0 (-O$\underline{C}\underline{H}_3$). The above

^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [An et al., 2006].

Paeonidanin E (**16**)

White, amorphous powder; a quasimolecular ion peak at m/z 941.2952 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{46}H_{54}O_{21}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.06 (2H, m, H-2'' and H-6''), 8.06 (1H, m, H-2'''' and H-6''''), 7.60 (1H, m, H-4'' and H-4''''), 7.48 (2H, m, H-3'' and H-5''), 7.48 (1H, m, H-3'''' and H-5''''), 5.38 (1H, s, H-9'''), 5.32 (1H, s, H-9), 4.81 (2H, s, H-8), 4.60 (2H, m, H-8'''), 4.57 (1H, d, *J*= 7.2 Hz, H-1'), 4.19 (1H, d, *J*= 7.2 Hz, H-1''''), 3.88 (1H, dd, *J*= 2.0, 12.0 Hz, H-6'α), 3.65 (1H, m, H-6'β), 3.74 (1H, d, *J*= 12.0 Hz, H-6''''α), 3.57 (1H, m, H-6''''β), 3.35 (1H, m, H-5' and H-5''''), 3.33 (1H, m, H-3' and H-3''''), 3.32 (1H, m, H-4' and H-4''''), 3.21 (1H, m, H-2' and H-2''''), 2.94 (1H, m, H-5), 2.88 (1H, d, *J*= 18.4 Hz, H-3α), 2.54 (1H, d, *J*= 18.4 Hz, H-3β), 2.43 (1H, m, H-5'''), 2.45 (1H, d, *J*= 12.8 Hz, H-3'''α), 1.76 (1H, d, *J*= 12.8 Hz, H-3'''β), 2.21 (2H, m, H-7), 1.97 (2H, m, H-7'''), 1.42 (3H, s, H-10), 1.28 (3H, s, H-10'''); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 208.4 (C-4), 167.9 (C-7''''), 167.8 (C-7''), 134.7 (C-4''''), 134.4 (C-4''), 131.2 (C-1'', 1''''), 130.9 (C-2'''', 6''''), 130.7 (C-2'', 6''), 129.9 (C-3'''', 5''''), 129.7 (C-3'', 5''), 107.5 (C-9), 106.5 (C-4'''), 102.3 (C-9'''), 100.0 (C-1', 1''''), 89.1 (C-1), 88.8 (C-1'''), 87.6 (C-2), 87.3 (C-2'''), 78.1 (C-3', 3''''), 77.9 (C-5'), 76.7 (C-5''''), 75.0 (C-2'), 74.8 (C-2''''), 72.1 (C-6'''), 71.8 (C-4''''), 71.4 (C-4'), 69.5 (C-6''''), 65.2 (C-6), 63.8 (C-8), 62.9 (C-6'), 61.7 (C-8'''), 49.4 (C-3), 48.3 (C-5), 44.5 (C-3'''), 43.8 (C-5'''), 27.6 (C-7), 22.9 (C-7'''), 20.8 (C-10), 19.5 (C-10'''). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Duan et al., 2009].

Lactiflorin (**17**)

White, amorphous powder; a quasimolecular ion peak at m/z 461.1443 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{23}H_{26}O_{10}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.02 (2H, d, *J*= 7.6 Hz, H-2" and H-6"), 7.63 (1H, dt, *J*= 7.6 Hz, H-4"), 7.51 (1H, d, *J*= 7.6 Hz, H-3" and H-5"), 4.90 (1H, d, *J*= 4.4 Hz, H-9), 4.78 (2H, s, H-8), 4.62 (1H, d, *J*= 8.0 Hz, H-1'), 3.83 (1H, d, *J*= 11.2 Hz, H-6'α), 3.68 (1H, d, *J*= 11.2 Hz, H-6'β), 3.48 (1H, d, *J*= 9.6 Hz, H-3'), 3.45 (1H, m, H-4' and H-5'), 3.18 (1H, dd, *J*= 8.0, 9.6 Hz, H-2'), 2.77 (1H, m, H-4), 2.74 (1H, d, *J*= 18.0 Hz, H-2α), 2.53 (1H, d, *J*= 18.0 Hz, H-2β), 2.50 (1H, d, *J*= 13.2 Hz, H-5α), 2.17 (1H, d, *J*= 4.4, 13.2 Hz, H-5β), 1.50 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 218.6 (C-3), 167.6 (C-7"), 134.7 (C-4"), 130.8 (C-1"), 130.6 (C-2", 6"), 129.9 (C-3", 5"), 104.2 (C-6), 96.5 (C-1'), 86.7 (C-1), 81.7 (C-9), 81.7 (C-5'), 80.1 (C-2'), 76.2 (C-3'), 71.8 (C-4'), 63.8 (C-8), 62.3 (C-6'), 56.9 (C-7), 48.2 (C-2), 38.7 (C-4), 31.7 (C-5), 16.3 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Kaneda et al. 2008, Yean et al., 2008].

1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose (**18**)

White, amorphous solid; a quasimolecular ion peak at m/z 939.1114 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{41}H_{32}O_{26}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.12 (2H, s, H-2"" and H-6""), 7.06 (1H, s, H-2' and H-6'), 6.98 (2H, s, H-2"" and H-6""), 6.90 (2H, s, H-2" and H-6"), 6.75 (2H, s, H-2"' and H-6"'), 6.25 (1H, d, *J*= 8.4 Hz, H-1), 5.90 (1H, d, *J*= 9.6 Hz, H-3), 5.62 (1H, dd, *J*= 8.8, 9.6 Hz, H-2), 5.56 (1H, d, *J*= 9.6 Hz, H-4), 4.52 (1H, d, *J*= 10.8 Hz, H-6α), 4.39 (1H, dd, *J*= 8.8, 10.8 Hz, H-6β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 167.9 (C-7""), 167.2 (C-7"'), 167.0 (C-7"), 166.9 (C-7""), 166.2 (C-7'), 146.5 (C-2', 6'), 146.5 (C-2", 6"), 146.4 (C-2"", 6""), 146.3 (C-2"", 6""), 146.2 (C-2"', 6"'), 140.7 (C-4'), 140.3 (C-4""), 140.2 (C-4"), 140.1 (C-4"'), 140.0 (C-4""), 121.0 (C-1""), 120.3 (C-1"'), 120.2 (C-1"), 120.1 (C-1""),

119.7 (C-1'), 110.6 (C-3', 5'), 110.5 (C-3"", 5""), 110.4 (C-3""", 5"""), 110.4 (C-3", 5"), 110.3 (C-3"', 5"'), 110.3 (C-3""", 5"""), 89.1 (C-1), 74.4 (C-5), 74.1 (C-3), 72.2 (C-2), 69.8 (C-4), 63.1 (C-6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Nishizawa et al., 2009].

(+)-Catechin (**19**)

Yellowish, amorphous powder; a quasimolecular ion peak at m/z 289.0717 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{15}H_{14}O_6$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 6.85 (1H, d, *J*= 2.0 Hz, H-2'), 6.77 (1H, dt, *J*= 7.6 Hz, H-5'), 6.72 (1H, d, *J*= 7.6 Hz, H-6'), 5.94 (1H, d, *J*= 7.6 Hz, H-8), 5.88 (1H, d, *J*= 2.4 Hz, H-6), 4.59 (1H, d, *J*= 7.6 Hz, H-2), 3.99 (1H, ddd, *J*= 7.6, 7.6, 8.0 Hz, H-3), 2.86 (1H, dd, *J*= 5.6, 16.0 Hz, H-4α), 2.51 (1H, dd, *J*= 8.4, 16.0 Hz, H-4β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 157.7 (C-5), 157.5 (C-9), 156.8 (C-7), 146.1 (C-3', 4'), 132.1 (C-1'), 120.0 (C-6'), 116.1 (C-2'), 115.2 (C-5'), 100.7 (C-10), 96.3 (C-6), 95.5 (C-8), 82.7 (C-2), 68.7 (C-3), 28.4 (C-4). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Hosny et al., 2014].

(+)-Catechin-7-*O*-gallate (**20**)

Yellowish, amorphous powder; a quasimolecular ion peak at m/z 441.0813 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{22}H_{18}O_{10}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.18 (1H, d, *J*= 2.0 Hz, H-2" and H-6"), 6.83 (1H, d, *J*= 1.6 Hz, H-2'), 6.76 (1H, d, *J*= 8.0 Hz, H-5'), 6.71 (1H, dd, *J*= 1.6, 8.0 Hz, H-6'), 6.25 (1H, d, *J*= 2.4 Hz, H-8), 6.21 (1H, d, *J*= 2.0 Hz, H-6), 4.64 (1H, d, *J*= 7.6 Hz, H-2), 3.98 (1H, d, *J*= 7.2 Hz, H-3), 2.74 (1H, dd, *J*= 5.6, 16.0 Hz, H-4α), 2.47 (1H, dd, *J*= 8.4, 16.0 Hz, H-4β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 166.5 (C-7"), 158.1 (C-5), 151.7 (C-9), 157.0 (C-7), 146.7 (C-3", 5"), 146.4 (C-3'), 146.3 (C-4'), 140.7 (C-4"), 131.7 (C-1'), 120.3 (C-1"),

120.0 (C-6'), 116.1 (C-2'), 115.2 (C-5'), 110.5 (C-2", 6"), 106.5 (C-10), 103.5 (C-6), 101.6 (C-8), 83.1 (C-2), 68.2 (C-3), 28.7 (C-4). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Davis et al., 1996].

(-)-Epicatechin-7-*O*-gallate (**21**)

Yellowish, amorphous powder; a quasimolecular ion peak at m/z 441.0827 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{22}H_{18}O_{10}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 6.93 (1H, d, *J*= 2.0 Hz, H-2" and H-6"), 6.92 (1H, d, *J*= 1.6 Hz, H-2'), 6.80 (1H, d, *J*= 8.8 Hz, H-5'), 6.68 (1H, d, *J*= 8.8 Hz, H-6'), 5.95 (1H, d, *J*= 2.4 Hz, H-8), 5.94 (1H, d, *J*= 2.8 Hz, H-6), 5.52 (1H, d, *J*= 7.6 Hz, H-3), 5.02 (1H, s, H-2), 2.98 (1H, dd, *J*= 4.8, 15.6 Hz, H-4α), 2.83 (1H, dd, *J*= 2.4, 15.6 Hz, H-4β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 167.6 (C-7"), 157.9 (C-5), 157.9 (C-7), 157.3 (C-9), 146.4 (C-3", 5"), 146.0 (C-3'), 146.0 (C-4'), 139.8 (C-4"), 131.5 (C-1'), 121.5 (C-1"), 119.4 (C-6'), 116.0 (C-5'), 115.1 (C-2'), 110.2 (C-2", 6"), 99.4 (C-10), 96.6 (C-6), 95.9 (C-8), 78.7 (C-2), 70.0 (C-3), 26.9 (C-4). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Davis et al., 1996].

Narigenin chalcone 2'-*O*-xyloside (**22**)

Yellow, amorphous powder; a quasimolecular ion peak at m/z 403.0375 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{20}H_{20}O_9$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.02 (1H, d, *J*= 15.6 Hz, H-8), 7.64 (1H, d, *J*= 15.6 Hz, H-7), 7.60 (2H, *J*= 8.0 Hz, H-2 and H-6), 6.82 (2H, *J*= 8.0 Hz, H-3 and H-5), 6.20 (1H, d, *J*= 2.0 Hz, H-3'), 5.98 (1H, d, *J*= 2.4 Hz, H-5'), 5.13 (1H, d, *J*= 7.6 Hz, H-1"), 3.92 (1H, d, *J*= 15.6 Hz, H-5"α), 3.70 (1H, d, *J*= 6.4, 15.6 Hz, H-5"β), 3.50 (1H, m, 15.6 Hz, H-3"), 3.31 (1H, m, 15.6 Hz, H-4"), 3.29 (1H, m, 15.6 Hz, H-2"); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 194.5

(C-9), 167.9 (C-6'), 166.1 (C-2'), 161.9 (C-4'), 161.2 (C-4), 144.2 (C-7), 131.8 (C-2, 6), 128.6 (C-1), 126.0 (C-8), 116.9 (C-3, 5), 107.4 (C-1'), 101.9 (C-1''), 98.4 (C-5'), 95.7 (C-3'), 78.6 (C-3), 75.1 (C-2) 71.2 (C-4), 62.4 (C-5). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Yoshimura et al., 2009].

4,2',4',6',7,8-Hexahydroxy-7,(8)-dihydro-chalcone (**23**)

Yellow, amorphous powder; a quasimolecular ion peak at m/z 305.0735 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{15}H_{14}O_7$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.35 (2H, d, *J*= 8.4 Hz, H-2 and H-6), 6.83 (2H, d, *J*= 8.4 Hz, H-3 and H-5), 5.92 (1H, d, *J*= 1.6 Hz, H-3'), 5.88 (1H, d, *J*= 1.6 Hz, H-5'), 4.97 (1H, d, *J*= 12.0 Hz, H-8), 4.53 (1H, d, *J*= 12.0 Hz, H-7); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 198.5 (C-9), 168.7 (C-6'), 165.3 (C-2'), 164.5 (C-4'), 159.2 (C-4), 130.4 (C-2, 6), 129.2 (C-1), 116.1 (C-3, 5), 101.8 (C-1'), 97.3 (C-3'), 96.3 (C-5'), 84.9 (C-8), 73.6 (C-7). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Ahmed et al., 2007].

Paeonol (**24**)

White, amorphous powder; a quasimolecular ion peak at m/z 165.2045 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_9H_{10}O_3$. ^{1}H-NMR (CD_3OD, 500Hz) δ_H 7.78 (1H, dd, *J*= 4.0, 8.0 Hz, H-6), 6.50 (1H, dd, *J*= 5.0, 8.0 Hz, H-5), 6.41 (1H, d, *J*= 4.0 Hz, H-3), 3.83 (3H, s, -OC$\underline{H}_3$), 2.55 (3H, s, -COC$\underline{H}_3$); ^{13}C-NMR (CD_3OD, 125Hz) δ_c 200.9 (C-7), 167.7 (C-4), 166.2 (C-2), 134.0 (C-6), 115.0 (C-1), 108.4 (C-5), 101.7 (C-3), 56.1 (-O$\underline{C}H_3$), 26.4 (C-8). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Lee et al., 2005].

Benzoic acid (**25**)

Colorless, crystalline solid; ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.03 (2H, dd, *J*= 1.6, 7.6 Hz, H-2 and H-6), 7.57 (1H, m, H-4), 7.45 (2H, dd, *J*= 1.6, 7.6 Hz, H-3 and H-5); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 169.9 (C-7), 134.2 (C-4), 133.9 (C-1), 130.9 (C-2, 6), 129.6 (C-3, 5). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Youn et al., 2010].

3,5-Dihydroxy-4-methoxyl-benzoic acid (**26**)

Colorless, crystalline solid; a quasimolecular ion peak at m/z 183.1551 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_8H_8O_5$. ^{1}H-NMR (CD3OD, 400Hz) δ_H 7.05 (2H, s, H-2 and H-6), 3.81 (3H, s, -OC$\underline{H}_3$); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 169.0 (C-7), 146.5 (C-3, 5), 139.7 (C-4), 121.4 (C-1), 110.0 (C-2, 6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Youn et al., 2010].

Methyl 4-hydroxy-3-methoxybenzoate (**27**)

Colorless, crystalline solid; a quasimolecular ion peak at m/z 181.1730 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_9H_{10}O_4$. ^{1}H-NMR (CD3OD, 400Hz) δ_H 7.53 (1H, dd, *J*= 1.6, 7.6, H-6), 7.52 (1H, d, *J*= 1.6, H-2), 6.82 (1H, d, *J*= 7.6, H-5), 3.88 (3H, s, -COOC$\underline{H}_3$), 3.85 (3H, s, -OC$\underline{H}_3$); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 168.7 (C-7), 152.9 (C-4), 148.7 (C-3), 125.0 (C-1), 122.5 (C-6), 115.9 (C-5), 113.5 (C-2), 56.4 (-O$\underline{C}H_3$), 52.3 (-COO$\underline{C}H_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Hu et al., 2007].

Gallic acid (**28**)

White, amorphous powder; a quasimolecular ion peak at m/z 169.0165 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{15}H_{14}O_7$. ^{1}H-NMR (CD_3OD, 500Hz)

δ_H 7.05 (2H, s, H-2 and H-6); ^{13}C-NMR (CD_3OD, 125Hz) δ_c 170.4 (C-7), 146.4 (C-3, 5), 139.6 (C-4), 121.9 (C-1), 110.3 (C-2, 6). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Lee et al., 2005].

Methyl gallate (**29**)

White, amorphous powder; a quasimolecular ion peak at m/z 183.0327 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_8H_8O_5$. ^{1}H-NMR (CD_3OD, 500Hz) δ_H 7.04 (2H, s, H-2 and H-6), 3.81 (3H, s, -COOC<u>H</u>$_3$); ^{13}C-NMR (CD_3OD, 125Hz) δ_c 169.0 (C-7), 146.5 (C-3, 5), 139.7 (C-4), 121.4 (C-1), 100.0 (C-2, 6), (3H, s, -COO<u>C</u>H$_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Lee et al., 2005].

1.3.6 Inhibitory effects of isolated compounds against antigen-mediated degranulation in RBL-2H3 cells

The isolated 29 chemical constituents were further examined for their anti-allergic activity by testing inhibitory effects against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells, and their IC_{50} values are shown in Table 1.2. Cell viability under stimulation was evaluated by the Cell Counting kit-8 (CCK-8) assay, and none of the test compounds at a dose level up to 100 μM, nor the positive control Baicalein at a dose level up to 200 μM exhibited observable cytotoxcity. Four doses of all test compounds (10, 25, 50 and 100 μM) were taken for the assay inhibitory effect against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells. Among the 29 isolated constituents, sixteen compounds showed anti-allergic activities with IC_{50} ranging from 41.17 to 90.15 μM. Most of monoterpenoids especially with paeoniflorin-type

skeleton, PGG (**18**) and methyl gallate (**29**) showed moderate anti-allergic activity. Paeoniflorol (**1**) exhibited the most effectiveness. Compounds **8** and **11** showed relative strong activity among the paeoniflorin-type monoterpenoids (**6-15**), but albiflorin-type monoterpenoids (**3-5**) have no activity.

Table 1.2 Inhibitory effects of 29 compounds against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells

Compound No.	IC_{50} (μM)	Compound No.	IC_{50} (μM)
1	41.17 ± 3.2 [a]	**17**	59.88 ± 3.7
6	76.20 ± 5.5	**18**	62.00 ± 1.4
8	42.22 ± 1.8	**25**	88.92 ± 2.7
9	75.44 ± 2.5	**26**	90.15 ± 3.6
11	50.05 ± 3.9	**28**	78.11 ± 3.1
12	68.32 ± 2.7	**29**	50.12 ± 1.0
13	87.11 ± 1.1	**2-5**, **7**, **10**, **1**6,	NA [b]
14	77.51 ± 2.0	**19-24**, **27**	
15	76.68 ± 4.6	**Baicalein** [c]	37.78 ± 1.0

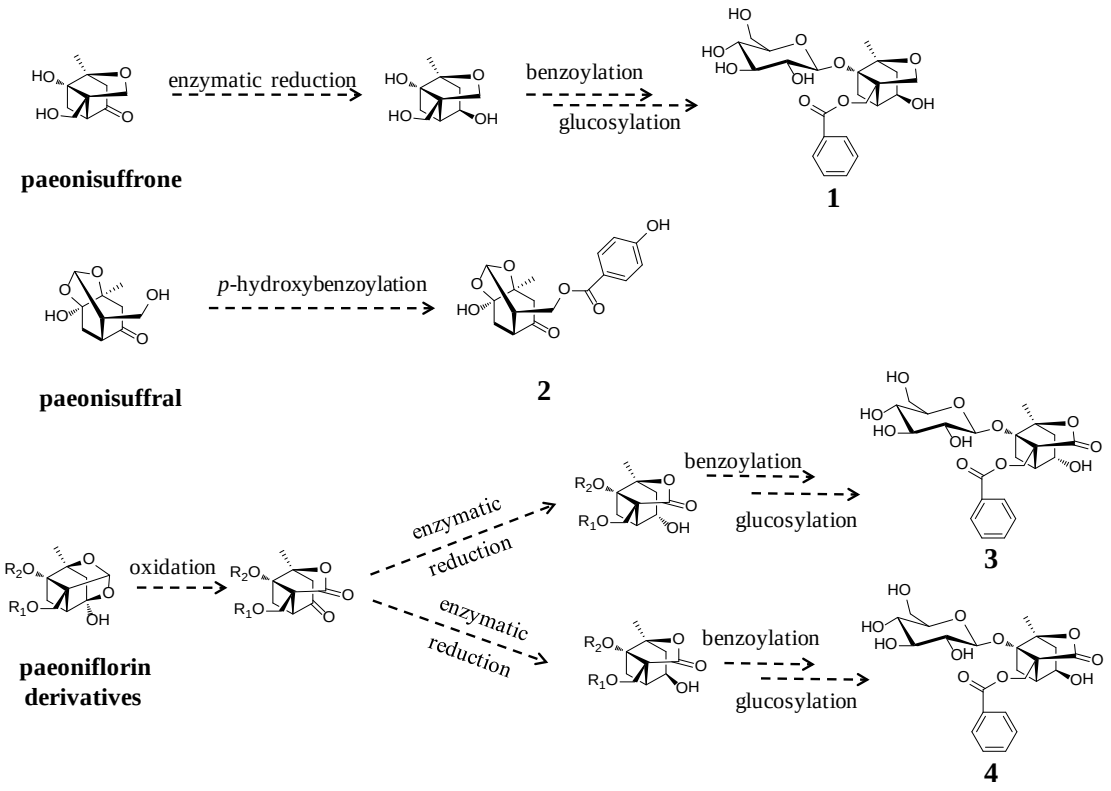
[a] The results are expressed as the mean ± S.D., IC_{50} values were calculated by Probit regression analysis of SPSS software (version 15.0);

[b] IC_{50} values are more than 100 μM; [c] Positive control [Matsuda et al., 2002].

1.4 Discussion

Phytochemical investigation on methanol extract of RPR derived from *P. lactiflora* led to isolation of 17 monoterpenoids. Monoterpenoids possessing “cage-like” pinane skeleton, such as paeoniflorin, albiflorin, paeonisuffrone, etc., have been only found in the genus *Paeonia*, the single genus in Paeoniaceae. Up to date, more than 60 monoterpenoids have been isolated and identified from *Paeonia* species. Although such unique monoterpenoids are presumed to be formed initially by the

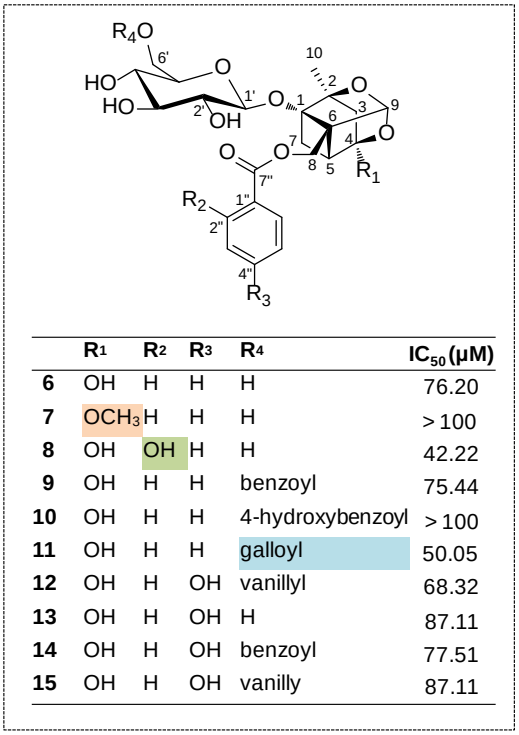
cyclization of geranyl pyrophosphate, followed by multi-steps of oxidation, cyclization, benzoylation, glycosylation, etc., experimental evidences of biosynthetic pathway in detail are scanty, and biogenetic relationship within various classics of monoterpenoids is still unclear. In the present study, an acetone soaking extract of *P. lactiflora* root was prepared and analyzed by LC-MS, and all of the three new compounds were detected, which suggested that these compounds are natural metabolites contained in this red peony root. From the viewpoint of biosynthesis, as shown in Scheme 1.1, paeoniflorol (**1**) is proposed to be derived from paeonisuffrone [Yoshikawa et al., 2000] by enzymatic reduction with alcohol dehydrogenase (ADH) which can catalyze the reduction of aldehydes and ketones to alcohols [Thmpson et al., 2008; Chung et al., 1999]. 4'-hydroxypaeoniflorigenone (**2**) can be derived from paeonisuffral [Yoshikawa et al., 2000] through *p*-hydroxybenzoylation of C8-OH. 4-*epi*-albiflorin (**3**) with β configuration of C4-OH is not isolated previously from *Paeonia* species, which is presumed to be formed via enzymatic reduction of keto-lactone derivatives of paeoniflorin [Keneda et al., 1972].



Scheme 1.1 Proposed biosynthesis pathways of compounds **1**, **2**, **3** and **4**

A primary structure-activity relationship of these isolated monoterpenoids was discussed. Compound **1** exhibited the most effectiveness with IC_{50} of 41.17 μM, followed by compounds **8** (IC_{50}: 42.22 μM) and **11** (IC_{50}: 50.05 μM), whereas compounds **2**-**5**, **7**, **10** and **16** showed no inhibitory activity. The results showed that the basic skeletons of compound **1** and the paeoniflorin-type (**6**-**15**) were important for the activity, compared to the albiflorin-type (**3**-**5**) with a lactone ring in the pinane aglycone. The similar tendency that the paeoniflorin-type skeleton is essential for the ameliorating effect on scopolamine-induced amnesia rather than the albiflorin-type has been observed previously [Abdel-Hefez et al., 1998]. Of the compounds with paeoniflorin-type skeleton (Fig. 1.5), hydroxyl group at C-4 of the aglycone was required for the activity, since methylation at this position resulted in complete losing of activity (**6** vs. **7**). Song et al. also reported that the methoxy group at C-4 was

deleterious for the anticomplement activity of the paeoniflorin type monoterpene glycosides [Song et al., 2014]. Moreover, compound **8** with *o*-hydroxybenzoyl moiety at C-8 was more effective than those with *p*-hydroxybenzoyl moiety (**6**, **9**-**15**). A galloyl group attaching to C-6' of glucose moiety seemed to enhance the activity (**11** vs. **9**-**10**, **12**).



	R1	R2	R3	R4	IC50 (μM)
6	OH	H	H	H	76.20
7	OCH_3	H	H	H	> 100
8	OH	OH	H	H	42.22
9	OH	H	H	benzoyl	75.44
10	OH	H	H	4-hydroxybenzoyl	> 100
11	OH	H	H	galloyl	50.05
12	OH	H	OH	vanillyl	68.32
13	OH	H	OH	H	87.11
14	OH	H	OH	benzoyl	77.51
15	OH	H	OH	vanilly	87.11

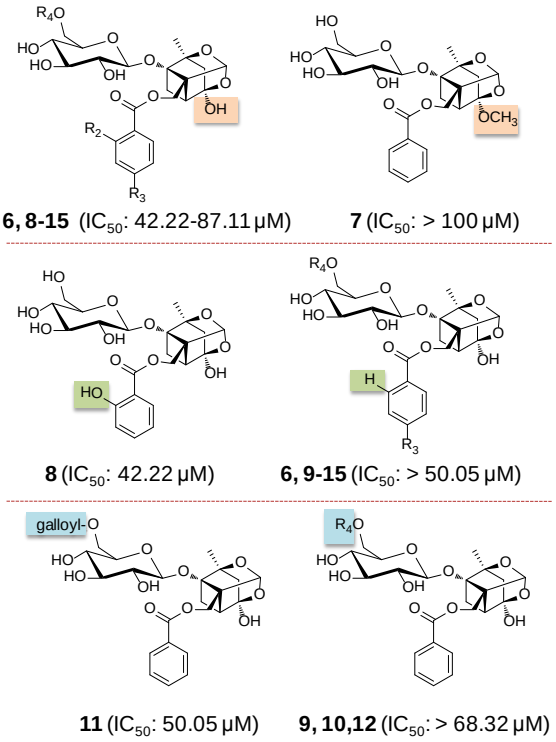


Fig. 1.5 Structure-activity relationship of paeoniflorin-type monoterpenoids

Hattori et al. [1985] and Shu et al. [1987] have reported that paeoniflorin (**6**), benzoylpaeoniflorin (**9**) and oxypaeoniflorin (**13**), the main monoterpenoids isolated from the root of *P. albiflora* Pall., were converted into paeonimetabolines I, II and III by human intestinal bacteria. 7*S*-paeonimetaboline I, as the major metabolic, had an appreciable suppressing effect on convulsions induced by pentylenetetrazole in rat [Namba et al., 1986; Shu et al., 1987]. In our studies, 4'-hydroxypaeoniflorigenone (**2**) which resembled to 7*S*-paeonimetaboline I, except for the additional

p-hydroxybenzoyl group, had no anti-allergic activity (IC_{50}: > 100μM) as inhibitory effect against IgE-mediated degranulation *in vitro*. Considering that PR decoction is generally used via oral intake, anti-allergic activity of the metabolites of monoterpenoids needs to be further studied.

Summary of Chapter Ⅰ

1) Methanol extract of the crud drug of RPR (D27967) produced in Inner Mongolia of China, was found to possess potent anti-allergic activity (IC_{50}: 0.61 ± 0.02 mg/ml) as inhibitory effects against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells. Subsequently, 60% and 80% aqueous MeOH sub-fractions eluted from methanolic extract were found to exhibit relatively strong inhibitory effects (IC_{50}: 0.20 ± 0.01 and 0.12 ± 0.02 mg/ml, respectively).

2) Bioassay-guided fractionation of the two active subfractions above led to isolation of seventeen monoterpenoids (**1**-**17**) including three new ones (**1**-**3**); as well as five flavonoids (**19**-**23**) and six other types of compounds (**18**, **24**-**29**).

3) Monoterpenoids, as the representative and main bioactive compounds in PR, are important for the anti-allergic activity. Paeoniflorol (**1**) and paeoniflorin-type monoterpenoids (**6**-**15**) showed moderate anti-allergic activity, but albiflorin-type monoterpenoids (**3**-**5**) had no activity.

4) Of the paeoniflorin-type monoterpenoids, hydroxyl group at C-4 was essential and *o*-hydroxybenzoyl moiety at C-8, a galloyl group attaching to C-6' of glucose were beneficial to the anti-allergic activity.

Chapter Ⅱ

Chemical constituents with anti-allergic activity from the root of Edulis Superba, a horticultural cultivar of *Paeonia lactiflora*

2.1 Introduction

In Japanese Pharmacopeia, PR is prescribed as the root of *P. lactiflora* with no less than 2.0% of paeoniflorin [The Ministry of Health, Labour and Welfare, 2011]. PR in Japanese market is mainly imported from China and only a small part is produced domestically; for instance, in 2012, 1,408 tons of peony roots was imported and 81 tons was domestically-produced [http://www.nikkankyo.org]. In the course of our study to search new resources of PR and further to promote domestic production, several horticultural cultivars of *P. lactiflora* were selected as promising candidates which have potential as medicinal resource on the basis of genetic and chemical analyses [Zhu et al., 2013; Yu et al., 2013]. However, the bioactive evaluation of these selected horticultural cultivars has not been studied.

In this Chapter, with the aim to elucidate the anti-allergic activity of the selected horticultural cultivars of *P. lactiflora* and to explore bioactive constituents of the active cultivar, chemical investigation as well as bioactivity evaluation on the active cultivar were conducted.

2.2 Plant materials

The roots of six cultivars of *P. lactiflora*, named Edulis Superba [エジュリスパーパー: S78 (2009) and S78N (2013)], Harunoyosooi (春の粧: S1, 2009), Rainbow (レインボー: S21, 2009), Kitasaisho (北宰相: S31, 2009), White-ivory (ホウイトアイボリー: S39, 2009), and Hanakago (花籠: S48, 2009) were harvested from Toyama Prefectural Medicinal Plants Center, Toyama, Japan. Their botanical sources were identified as *P. lactiflora* by genetic analysis of nrDNA-ITS sequence [Namba et al. 1993, Zhu el al., 2015]. Cultivars S1, S21, S39, S48, S78 and S78N were identified as RPR-type of *P. lactiflora*, while S31 was WPR-type of *P. lactiflora*. The vouchers (S1, S21, S31, S48, S78 and S78N) were deposited in the Museum of Materia Medica,

Institute of Natural Medicine, University of Toyama, Japan.

2.3 Results

2.3.1 Inhibitory effects of water extracts of six *P. lactiflora* cultivars against IgE-mediated degranulation in RBL-2H3 cells

Based on the previous genetic and chemical characteristics of PR as well as preliminary bioactivity screening experiment, six cultivars (S1, S21, S31, S39, S48, S78 and S78N) among 17 cultivars of *P. lactiflora* were screened and further investigated their anti-allergic activity as inhibitory effect against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells. As shown in Fig. 2.1, the root of “Edulis Superba” (S78N), a horticultural cultivar of RPR-type of *P. lactiflora*, showed relatively strong anti-allergic activity. Therefore, S78N was used as target to find out anti-allergic bioactive components in PR cultivar based on this pharmacological effect.

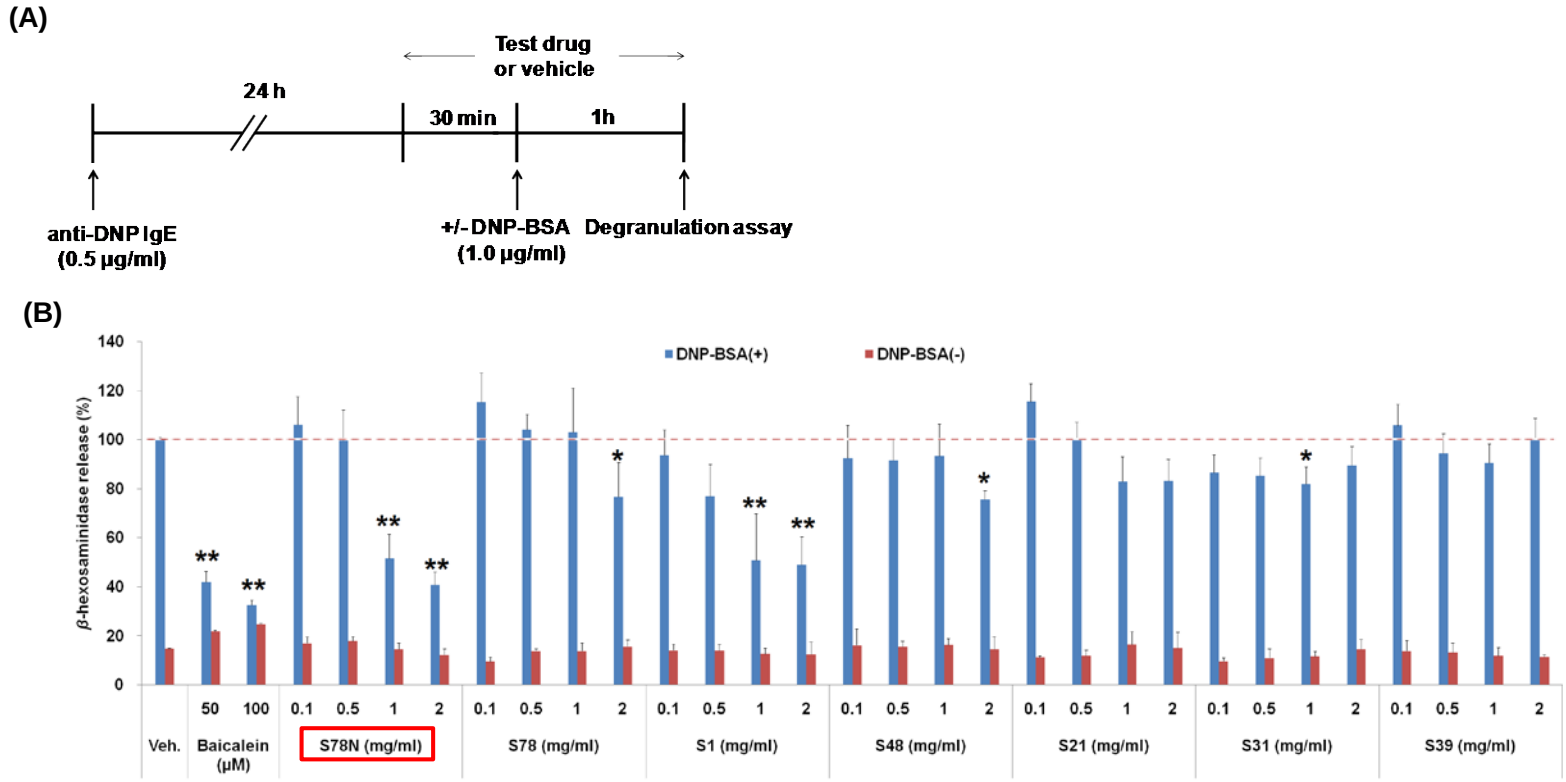


Fig. 2.1 Inhibitory effects of water extracts of the roots derived from *P. lactiflora* cultivars on IgE-mediated degranulation by measuring β-hexosaminidase release in RBL-2H3 cells. (**A**) The time scheme for the degranulation assay. (**B**) RBL-2H3 cells were sensitized with 0.5 μg/ml anti-DNP IgE (24 h), and then were incubated with different water extracts of test samples for 30min. The cells were simulated with (Blue) or without (Red) 1.0 μg/ml DNP-BSA for 1 h, and β-hexosaminidase release was determined. The data are expressed as the mean ± S.D. (n=3). *$P< 0.05$; **$P< 0.01$ compared with the vehicle.

2.3.2 Inhibitory effects of methanol extract and related subfractions of S78N against IgE-mediated degranulation in RBL-2H3 cells

Methanol extract from the root of Edulis Superba showed relatively strong anti-allergic activity as inhibitory effects against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells (IC_{50}: 0.72 ± 0.02 mg/ml), compared to water extract (IC_{50}: 0.97 ± 0.03 mg/ml). After partition with EtOAc, *n*-BuOH and H_2O from methanol extract, respectively, both EtOAc-soluble and *n*-BuOH-soluble subfractions exhibited potent inhibitory effects against β-hexosaminidase release (IC_{50}: 0.32 ± 0.01 mg/ml; 0.58 ± 0.01 mg/ml, respectively). Therefore, the two active subfractions were further investigated to elucidate the active constituents.

2.3.3 Isolation and structure determination

The above two active subfractions were subjected to a series of normal- and reverse-phase column chromatography, as well as the preparative HPLC to afford a new norneoligan glycoside (**30**) and 25 known compounds including five monoterpenoids (**4**, **6**, **8**, **13**, **31**), ten flavonoids (**19**-**21**, **32**-**38**), and ten other types of compounds (**18**, **24**-**25**, **28**-**29**, **39**-**43**). The isolation procedure was illustrated in Chart 2.1 and the structures of these isolated compounds were shown in Fig. 2.3.

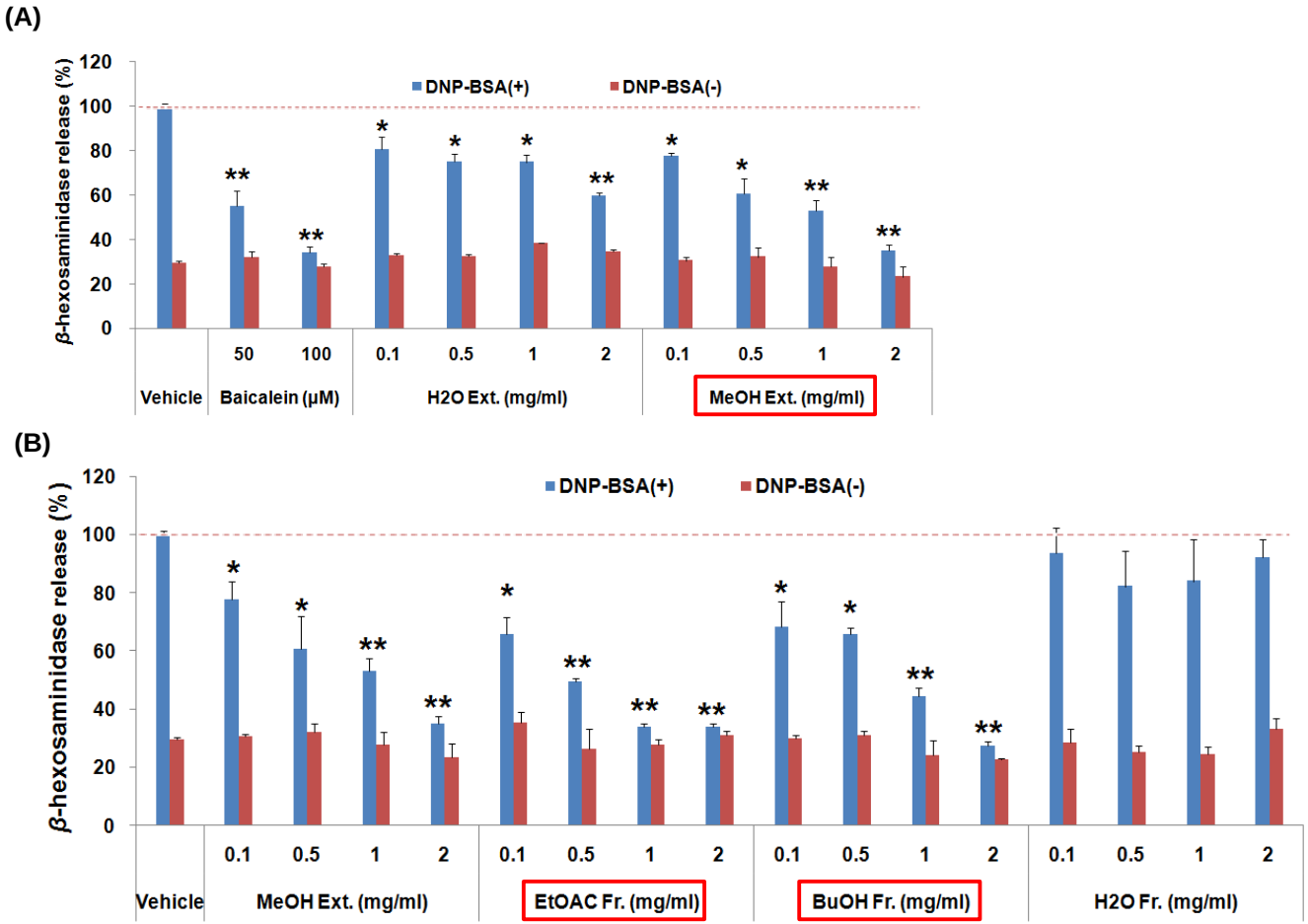


Fig. 2.2 Inhibitory effects of different extracts and related fractions of S78N on IgE-mediated degranulation by measuring β-hexosaminidase release in RBL-2H3 cells. (**A**) Methanol and water extracts. (**B**) Related subfractions partitioned from methanol extract. The data are expressed as the mean ± S.D. (n=3). **P< 0.05; **P<0.01* compared with the vehicle.

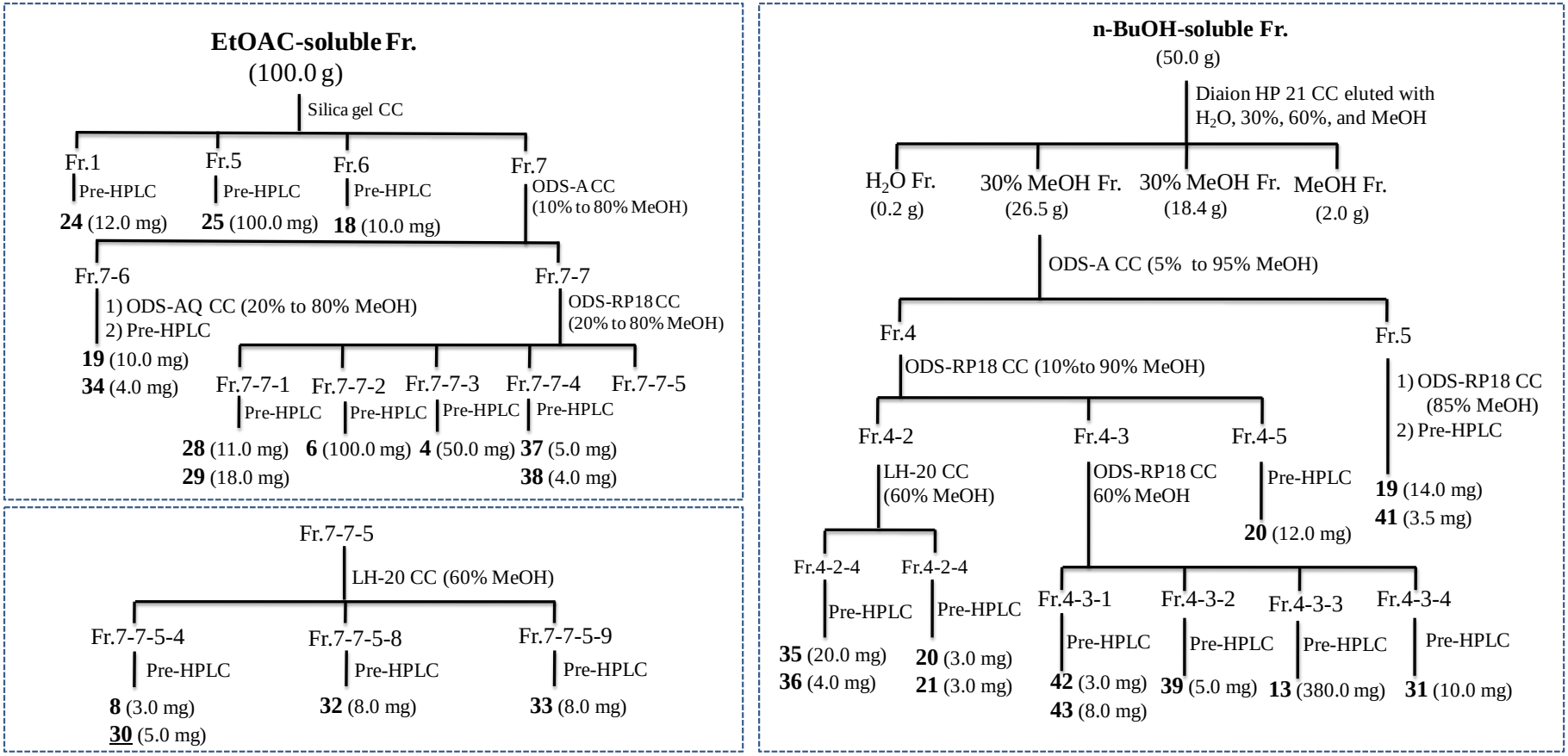


Chart 2.1 Isolation procedure of the root of Edulis Superba (S78N), a horticulture cultivar of *P. lactiflora*. New compound is shown with underline.

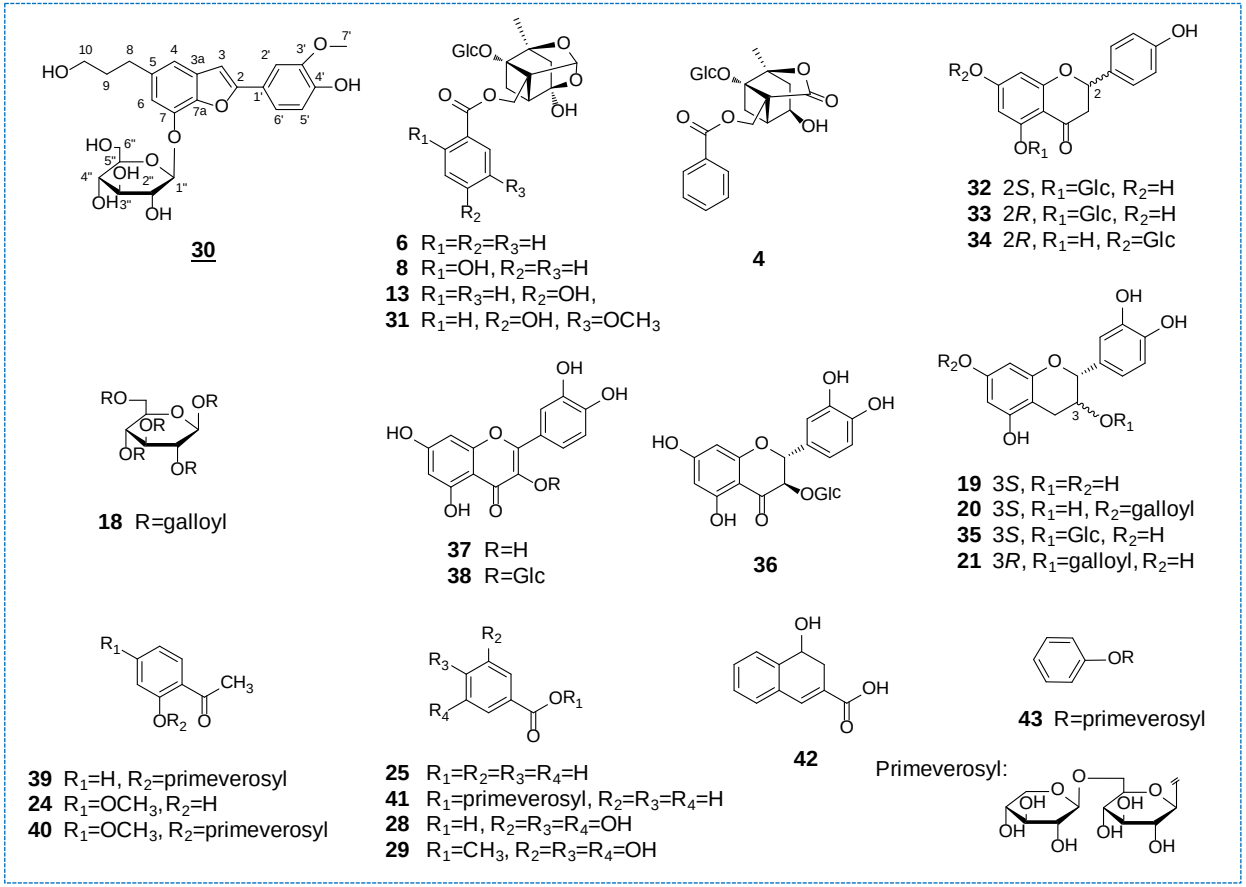


Fig. 2.3 Structures of isolated compounds from the root of Edulis Superba (S78N), a horticulture cultivar of *P. lactiflora*. New compound is shown with underline

2.3.4 Structure elucidation of the new compound

Paeonibenzofuran (**30**)

White, amorphous powder; $[\alpha]_D^{20}$ -44.77° (*c* = 0.11, MeOH). Quasimolecular ions at m/z 475.1632 $[M-H]^-$ and 521.1684 $[M+HCOO]^-$ in the HR-ESI-MS spectrum suggested a molecular formula of $C_{24}H_{28}O_{10}$. The IR spectrum showed absorption bands at 3397 cm^{-1} (hydroxyl group) and 1600, 1515 and 1485 cm^{-1} (aromatic ring). The ^{1}H-NMR spectrum of **30** (Table 2.1) presented signals attributable to a vinylic proton at δ_H 6.78 (1H, d, *J*= 1.2 Hz, H-3); a methoxyl signal at δ_H 3.86 (3H, s); a C3 fragment of $CH_2CH_2CH_2OH$ [δ_H 2.63 (2H, t, *J*= 8.0 Hz, H-8); δ_H 1.78 (2H, m, H-9); and δ_H 3.51 (2H, t, *J*= 6.0 Hz, H-10)]; and two sets of aromatic signals ascribable to a trisubstituted benzene ring [δ_H 6.77 (1H, d, *J*= 8.0 Hz, H-5'); δ_H 7.27 (1H, dd, *J*= 1.6, 8.0 Hz, H-6') and δ_H 7.34 (1H, d, *J*= 1.6 Hz, H-2')] and a tetra-substituted benzene ring [δ_H 6.83 (1H, br s, H-6) and δ_H 6.94 (1H, d, *J*= 1.6 Hz, H-4)]; together with a series of proton signals assignable to a glucopyranosyl moiety. In addition, the acid hydrolysis of **30** yielded β-D-glucopyranose. The ^{13}C-NMR spectrum displayed 24 carbon signals, which included characteristic signals of two vinylic carbons (δ_C 100.7, C-3; δ_C 158.1, C-2), a methoxyl signal (δ_C 56.6), a C3 fragment of $CH_2CH_2CH_2OH$ (δ_C 33.2, C-8; δ_C 35.8, C-9; δ_C 62.5, C-10), 12 aromatic carbons (δ_C 109.6~149.3), and a set of glucopyranosyl carbons (δ_C 62.2~102.6). The NMR data mentioned above closely resembled to those of 5-(3"-hydroxypropyl)-7-methyoxy-2-(3', 4'-dimethoxyphenyl) benzofuran [Arnone et al., 1988; Pauletti et al., 2000; Xiao et al., 2014; Cao et al., 2015], except for the absence of signals of two methoxyl protons, but a set of additional signals for a glucopyranosyl moiety. The long-range correlations between H-1" (δ_H 5.16) and C-7 (δ_C 143.3) in HMBC spectrum (Fig. 2.4) confirmed glucosylation at C-7 of the benzofuran skeleton. In addition, the correlation from

methoxyl protons (δ_H 3.86) to C-3' (δ_C 148.8) in the HMBC spectrum indicated that the methoxyl moiety was connected to C-3', which was also confirmed by the NOE enhancement signal of H-2' when irradiation at methoxyl protons. The correlations from H-9 to C-5 and from H-8 to C-4 and C-6 in the HMBC spectrum were obviously observed, which indicated that the C3 unit was connected to C-5. The correlations between H-8 and H-9, H-9 and H-10, as well as H-5' and H-6' were clearly observed in the ^{1}H-^{1}H COSY spectrum (Fig. 2.4). Consequently, compound **30** was identified as2-(4'-hydroxyl-3'-methxoyphenyl)-5-(3-hydroxypropyl)-benzofuran-7-*O*-β-D-gluco-pyranoside, and named paeonibenzofuran.

Table 2.1 ^{13}C-NMR (100 MHz) and ^{1}H-NMR (400 MHz) spectroscopic data of compound **30** measured in CD_3OD (δ in ppm).

	30 (Paeonibenzofuran)				
Position	δ C	δ H (*J* in Hz)	**Position**	δ C	δ H (*J* in Hz)
1			**1'**	123.8	
2	158.1		**2'**	109.6	7.34, d (1.6)
3	100.7	6.78, d (1.2)	**3'**	148.8	
3a	133.1		**4'**	149.3	
4	115.0	6.94, d (1.6)	**5'**	116.6	6.77, d (8.0)
5	139.1		**6'**	119.5	7.27, dd (1.6, 8.0)
6	113.0	6.83, br s	**1''**	102.6	5.16, d (6.8)
7	143.3		**2''**	75.0	3.50,dd (7.6,8.8)
7a	143.8		**3''**	78.1	3.59,dd (8.8,9.2)
8	33.2	2.63, t (8.0)	**4''**	71.4	3.35, m
9	35.8	1.78, m	**5''**	78.3	3.33, m
10	62.5	3.51, t (6.0)	**6''**	62.2	3.62,dd (5.2,12.0); 3.76,dd (1.2,12.0)
			OCH_3	56.6	3.86, s

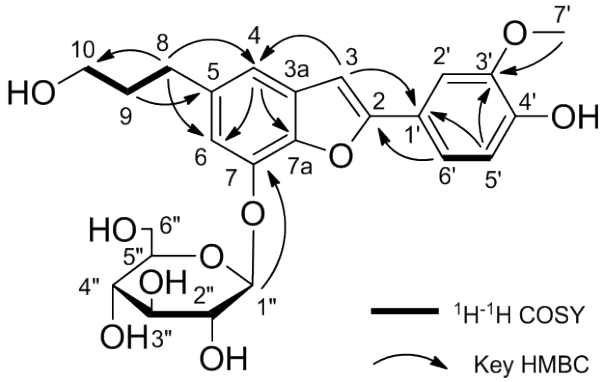


Fig. 2.4 Important 2D-NMR correlations of compound **30**

2.3.5 Identification of the known compounds

Mudanpioside E (**31**)

White, amorphous powder; a quasimolecular ion peak at m/z 525.1578 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{24}H_{30}O_{13}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.59 (2H, d, *J*= 1.6 Hz, H-2''), 7.56 (1H, m, H-6''), 6.84 (1H, m, H-5''), 5.41 (1H, s, H-9), 4.71 (2H, dd, *J*= 3.6, 12.0 Hz, H-8), 4.52 (1H, d, *J*= 7.6 Hz, H-1'), 3.90 (3H, s,-OC$\underline{H}_3$), 3.84 (1H, d, *J*= 12.0 Hz, H-6'α), 3.60 (1H, dd, *J*= 6.0, 12.0 Hz, H-6'β), 3.35 (1H, m, H-5'), 3.31 (1H, m, H-3'), 3.29 (1H, m, H-4'), 3.22 (1H, m, H-2'), 2.57 (1H, d, *J*= 5.6 Hz, H-5), 2.48 (1H, d, *J*= 10.8 Hz, H-7α), 1.94 (1H, d, *J*= 10.8 Hz, H-7β), 2.18 (1H, d, *J*= 12.4 Hz, H-3α), 1.79 (1H, dd, *J*= 12.4 Hz, H-3β), 1.36 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 168.0 (C-7''), 153.5 (C-4''), 148.8 (C-3''), 125.2 (C-1''), 122.2 (C-6''), 116.0 (C-2''), 113.7 (C-5''), 106.4 (C-4), 102.3 (C-9), 100.2 (C-1'), 89.3 (C-1), 87.3 (C-2), 78.1 (C-5'), 78.0 (C-3'), 75.0 (C-2'), 72.3 (C-6), 71.8 (C-4'), 62.9 (C-6'), 61.3 (C-8), 56.5 (-O$\underline{C}H_3$), 44.6 (C-3), 44.0 (C-5), 23.5 (C-7), 19.6 (C-10). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Tanaka et al., 2003].

(2*S*)-(-)-Narigenin-5-*O*-β-D-glucopyranoside (**32**)

Yellow, amorphous powder; a quasimolecular ion peak at m/z 433.1155 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{21}H_{22}O_{10}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.29 (2H, d, *J*= 8.8 Hz, H-2' and H-6'), 6.79 (2H, d, *J*= 8.8 Hz, H-3' and H-5'), 6.40 (1H, d, *J*= 2.4 Hz, H-8), 6.10 (1H, d, *J*= 2.4 Hz, H-6), 5.34 (1H, dd, *J*= 2.8, 12.8 Hz, H-2), 4.81 (1H, d, *J*= 7.2 Hz, H-1"), 3.91 (1H, d, *J*= 11.4 Hz, H-6"α), 3.71 (1H, dd, *J*= 7.2, 11.4 Hz, H-6"β), 3.57 (1H, m, H-5"), 3.52 (1H, m, H-3"), 3.48 (1H, m, H-4"), 3.42 (1H, m, H-2"), 3.07 (1H, d, *J*= 12.4, 16.0 Hz, H-3α), 2.65 (1H, dd, *J*= 2.8, 16.0 Hz, H-3β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 193.1 (C-4), 167.0 (C-5), 166.1 (C-7), 161.6 (C-4'), 159.0 (C-9), 131.1 (C-1'), 129.0 (C-2', 6'), 116.3 (C-3', 5'), 106.7 (C-10), 104.0 (C-8), 100.3 (C-1"), 99.5 (C-6), 80.3 (C-2), 78.5 (C-5"), 77.4 (C-3"), 74.6 (C-2"), 71.2 (C-4"), 62.5 (C-6"), 46.2 (C-3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Wang et al., 2009].

(2*R*)-(-)-Narigenin-5-*O*-β-D-glucopyranoside (**33**)

Yellow, amorphous powder; a quasimolecular ion peak at m/z 433.1165 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{21}H_{22}O_{10}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.28 (2H, d, *J*= 8.8 Hz, H-2' and H-6'), 6.80 (2H, d, *J*= 8.8 Hz, H-3' and H-5'), 6.45 (1H, d, *J*= 2.4 Hz, H-8), 6.11 (1H, d, *J*= 2.4 Hz, H-6), 5.29 (1H, dd, *J*= 2.1, 13.2 Hz, H-2), 4.75 (1H, d, *J*= 7.2 Hz, H-1"), 3.94 (1H, d, *J*= 12.0 Hz, H-6"α), 3.75 (1H, dd, *J*= 4.4, 12.0 Hz, H-6"β), 3.56 (1H, m, H-5"), 3.49 (1H, m, H-3"), 3.44 (1H, m, H-4"), 3.40 (1H, m, H-2"), 2.89 (1H, d, *J*= 13.2, 17.2 Hz, H-3α), 2.68 (1H, dd, *J*= 2.4, 17.2 Hz, H-3β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 193.1 (C-4), 167.0 (C-5), 166.1 (C-7), 161.6 (C-4'), 159.0 (C-9), 131.1 (C-1'), 129.0 (C-2', 6'), 116.3 (C-3', 5'), 107.1 (C-10), 104.0 (C-8), 100.3 (C-1"), 99.5 (C-6), 80.3 (C-2), 78.5 (C-5"), 77.4 (C-3"), 74.6 (C-2"), 71.2 (C-4"), 62.5 (C-6"), 46.3 (C-3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Wang et al., 2009].

(2*R*)-(-)-Narigenin-7-*O*-β-D-glucopyranoside (**34**)

Yellow, amorphous powder; a quasimolecular ion peak at m/z 433.1163 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{21}H_{22}O_{10}$. ^{1}H-NMR (DMSO, 400Hz) δ_H 10.66 (-CO<u>OH</u>), 9.56 (-C<u>OH</u>), 7.29 (2H, d, *J*= 8.4 Hz, H-2' and H-6'), 6.78 (2H, d, *J*= 8.4 Hz, H-3' and H-5'), 6.33 (1H, d, *J*= 1.2 Hz, H-8), 6.06 (1H, d, *J*= 1.2 Hz, H-6), 5.40 (1H, dd, *J*= 2.8, 12.4 Hz, H-2), 4.76 (1H, d, *J*= 6.8 Hz, H-1"), 4.59 (1H, dd, *J*= 6.0, 11.2 Hz, H-6"α), 3.70 (1H, dd, *J*= 4.8, 11.2 Hz, H-6"β), 3.70 (1H, m, H-5"), 3.54 (1H, m, H-3"), 3.48 (1H, m, H-4"), 3.34 (1H, m, H-2"), 3.10 (1H, d, *J*= 12.4, 16.8 Hz, H-3α), 2.58 (1H, dd, *J*= 2.8, 16.8 Hz, H-3β); ^{13}C-NMR (DMSO, 100Hz) δ_c 189.5 (C-4), 164.4 (C-7), 163.7 (C-9), 160.0 (C-5), 157.6 (C-4'), 129.1 (C-1'), 128.2 (C-2', 6'), 115.1 (C-3', 5'), 105.3 (C-10), 102.2 (C-1"), 98.6 (C-6), 97.6 (C-8), 78.2 (C-2), 77.4 (C-5"), 76.0 (C-3"), 73.4 (C-2"), 69.5 (C-4"), 60.6 (C-6"), 44.6 (C-3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Hosny et al., 2009].

(+)-Catechin-3-*O*-β-D-glucopyranoside (**35**)

Yellowish, amorphous powder; a quasimolecular ion peak at m/z 451.1248 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{21}H_{24}O_{11}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 6.81 (1H, d, *J*= 1.6 Hz, H-2'), 6.75 (1H, d, *J*= 8.0 Hz, H-5'), 6.70 (1H, dd, *J*= 2.0, 8.0 Hz, H-6'), 6.26 (1H, d, *J*= 2.0 Hz, H-8), 6.02 (1H, d, *J*= 2.0 Hz, H-6), 4.84 (1H, m, H-1"), 4.59 (1H, d, *J*= 7.6 Hz, H-2), 3.98 (1H, d, *J*= 7.2 Hz, H-3), 3.89 (1H, d, *J*= 12.4 Hz, H-6"α), 3.70 (1H, dd, *J*= 3.6, 12.4 Hz, H-6"β), 3.45 (1H, m, H-5"), 3.40 (1H, m, H-3"), 3.31 (1H, m, H-4"), 3.29 (1H, m, H-2"), 2.89 (1H, dd, *J*= 5.2, 16.0 Hz, H-4α), 2.57 (1H, dd, *J*= 8.4, 16.0 Hz, H-4β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 158.0 (C-5), 157.9 (C-7), 156.6 (C-9), 146.2 (C-3', 4'), 132.0 (C-1'), 119.9 (C-6'), 116.1 (C-2'), 115.2 (C-5'), 103.3 (C-10), 102.4 (C-1"), 98.1 (C-6), 96.8 (C-8), 83.1 (C-2), 78.2

(C-5''), 78.1 (C-3''), 74.8 (C-2''), 71.2 (C-4''), 68.5 (C-3), 60.6 (C-6''), 28.3 (C-4). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Hosny et al., 2009].

Taxifolin-3-*O*-β-D-glucopyranoside (**36**)

Yellowish, amorphous powder; a quasimolecular ion peak at m/z 465.1051 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{21}H_{22}O_{12}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 6.95 (1H, d, *J*= 1.6 Hz, H-2'), 6.84 (1H, dd, *J*= 1.6, 8.0 Hz, H-5'), 6.78 (1H, dd, *J*= 8.0 Hz, H-6'), 6.21 (1H, d, *J*= 2.4 Hz, H-8), 6.19 (1H, d, *J*= 2.4 Hz, H-6), 4.95 (1H, d, *J*= 6.0 Hz, H-3), 4.94 (1H, m, H-1''), 4.55 (1H, d, *J*= 2.0, 12.0 Hz, H-2), 3.86 (1H, dd, *J*= 6.4, 12.0 Hz, H-6''α), 3.66 (1H, dd, *J*= 9.2, 12.0 Hz, H-6''β), 3.44 (1H, m, H-5''), 3.39 (1H, m, H-3''), 3.34 (1H, m, H-4''), 3.29 (1H, m, H-2''); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 199.4 (C-4), 167.3 (C-5), 164.8 (C-7), 164.3 (C-9), 147.2 (C-3'), 146.4 (C-4'), 129.7 (C-1'), 121.0 (C-6'), 116.1 (C-2'), 116.0 (C-5'), 103.5 (C-10), 101.3 (C-1''), 98.3 (C-6), 97.0 (C-8), 83.4 (C-2), 78.3 (C-5''), 77.8 (C-3''), 74.8 (C-2''), 73.9 (C-3), 71.2 (C-4''), 62.4 (C-6''). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Choi et al., 2011].

Quercetin (**37**)

Yellow, amorphous needles; a quasimolecular ion peak at m/z 301.0418 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{15}H_{10}O_7$. ^{1}H-NMR (CD_3OD, 500Hz) δ_H 7.73 (1H, d, *J*= 2.0 Hz, H-2'), 7.62 (1H, dd, *J*= 2.0, 8.0 Hz, H-5'), 6.87 (1H, d, *J*= 8.0 Hz, H-6'), 6.38 (1H, d, *J*= 2.0 Hz, H-8), 6.18 (1H, d, *J*= 2.0 Hz, H-6); ^{13}C-NMR (CD_3OD, 125 Hz) δ_c 177.3 (C-4), 165.6 (C-7), 162.5 (C-5), 158.2 (C-9), 148.8 (C-2), 148.0 (C-4'), 146.2 (C-3'), 137.2 (C-3), 124.1 (C-1'), 121.7 (C-6'), 116.2 (C-2'), 116.0 (C-5'), 104.5 (C-10), 99.2 (C-8), 94.4 (C-6). The above ^{1}H- and ^{13}C-NMR

spectroscopic data were close accordance with those of the reported values [Lin et al., 1998].

Quercetin-3-*O*-β-D-glucopyranoside (**38**)

Yellow, amorphous needles; a quasimolecular ion peak at m/z 463.0901 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{21}H_{20}O_{12}$. ^{1}H-NMR (CD_3OD, 500Hz) δ_H 7.72 (1H, d, *J*= 2.0 Hz, H-2'), 7.59 (1H, dd, *J*= 2.0, 8.5 Hz, H-5'), 6.87 (1H, d, *J*= 8.5 Hz, H-6'), 6.38 (1H, d, *J*= 2.0 Hz, H-8), 6.18 (1H, d, *J*= 2.0 Hz, H-6), 5.25 (1H, d, *J*= 7.5 Hz, H-1''), 3.70 (1H, dd, *J*= 2.0, 12.0 Hz, H-6''α), 3.56 (1H, dd, *J*= 5.0, 12.0 Hz, H-6''β), 3.49 (1H, m, H-5''), 3.44 (1H, m, H-3''), 3.36 (1H, m, H-4''), 3.20 (1H, m, H-2''); ^{13}C-NMR (CD_3OD, 125 Hz) δ_c 179.6 (C-4), 166.1 (C-7), 163.1 (C-5), 159.1 (C-9), 158.5 (C-2), 149.9 (C-4'), 146.0 (C-3'), 135.7 (C-3), 132.2 (C-1'), 132.1 (C-6'), 117.6 (C-2'), 116.0 (C-5'), 105.8 (C-10), 104.3 (C-8), 99.9 (C-1''), 94.4 (C-6), 78.5 (C-3''), 78.2 (C-5''), 75.8 (C-2''), 71.3 (C-4''), 62.6 (C-6''). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Lin et al., 1998].

Neohancoside C (**39**)

White, amorphous powder; a quasimolecular ion peak at m/z 429.1398 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{19}H_{26}O_{11}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.65 (1H, dd, *J*= 2.0, 8.0 Hz, H-6), 7.56 (1H, m, H-4), 7.37 (1H, d, *J*= 8.0 Hz, H-3), 7.09 (1H, d, *J*= 8.0 Hz, H-5), 5.04 (1H, d, *J*= 7.6 Hz, H-1'), 4.28 (1H, d, *J*= 6.8 Hz, H-1''), 3.83 (1H, dd, *J*= 3.2, 12.0 Hz, H-5''α), 3.41 (1H, dd, *J*= 9.2, 12.0 Hz, H-5''β), 4.12 (1H, dd, *J*= 1.6, 11.2 Hz, H-6'α), 3.77 (1H, dd, *J*= 4.0, 11.2 Hz, H-6'β), 3.71 (1H, m, H-5'), 3.67 (1H, m, H-4''), 3.59 (1H, m, H-3''), 3.55 (1H, dd, *J*= 7.6, 8.8 Hz, H-2'), 3.46 (1H, dd, *J*= 4.0, 8.8 Hz, H-4'); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 202.5 (C-7), 158.0

(C-2), 135.4 (C-6), 130.7 (C-4), 130.3 (C-1), 123.3 (C-5), 117.7 (C-3), 105.0 (C-1"), 102.3 (C-1'), 78.1 (C-3'), 77.5 (C-3"), 74.9 (C-5'), 74.1 (C-2"), 72.6 (C-2'), 71.4 (C-4'), 69.5 (C-4", 6'), 66.7 (C-5"), 32.2 (C-8). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Konda et al., 1997].

Paeonolide (**40**)

White, amorphous powder; a quasimolecular ion peak at m/z 459.1531 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{20}H_{28}O_{12}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.74 (1H, d, *J*= 8.8 Hz, H-6), 6.84 (1H, d, *J*= 2.4 Hz, H-3), 6.65 (1H, dd, *J*= 2.4, 8.0 Hz, H-5), 5.05 (1H, d, *J*= 7.6 Hz, H-1'), 4.25 (1H, d, *J*= 7.6 Hz, H-1"), 4.12 (1H, dd, *J*= 1.6, 11.2 Hz, H-6'α), 3.77 (1H, dd, *J*= 4.0, 11.2 Hz, H-6'β), 3.85 (1H, d, *J*= 12.0 Hz, H-5"α), 3.43 (1H, d, *J*= 12.0 Hz, H-5"β), 3.78 (1H, m, H-5'), 3.68 (1H, m, H-4"), 3.57 (1H, m, H-3"), 3.51 (1H, m, H-2"), 3.49 (1H, dd, *J*= 7.6, 8.8 Hz, H-2'), 3.45 (1H, dd, *J*= 4.0, 8.8 Hz, H-4'), 3.87 (3H, s, -OC<u>H</u>$_3$); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 200.5 (C-7), 166.3 (C-4), 160.4 (C-2), 133.0 (C-6), 122.7 (C-1), 109.3 (C-5), 105.1 (C-1"), 102.9 (C-1'), 102.4 (C-3), 78.1 (C-3'), 77.4 (C-3"), 74.8 (C-5'), 74.2 (C-2"), 72.4 (C-2'), 71.3 (C-4'), 69.8 (C-4"), 69.5 (C-6'), 66.8 (C-5"), 32.2 (C-8), 56.5 (-O<u>C</u>H$_3$). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Konda et al., 1997].

Poacynose (**41**)

White, amorphous powder; a quasimolecular ion peak at m/z 459.1531 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{20}H_{28}O_{12}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 8.09 (2H, d, *J*= 8.0 Hz, H-2 and H-6), 7.63 (1H, d, *J*= 7.6 Hz, H-4), 7.49 (2H, dd, *J*= 2.0, 8.0 Hz, H-3 and H-5), 5.71 (1H, d, *J*= 7.6 Hz, H-1'), 4.32 (1H, d, *J*= 7.6 Hz, H-1"), 4.18 (1H, dd, *J*= 1.6, 12.0 Hz, H-6'α), 3.78 (1H, dd, *J*= 5.2, 11.2 Hz, H-6'β),

3.83 (1H, d, *J*= 12.0 Hz, H-5"α), 3.63 (1H, dd, *J*= 5.6, 12.0 Hz, H-5"β), 3.61 (1H, m, H-5'), 3.53 (1H, m, H-4"), 3.51 (1H, m, H-2"), 3.32 (1H, m, H-3"), 3.24 (1H, dd, *J*= 7.6, 8.8 Hz, H-2'), 3.19 (1H, dd, *J*= 4.0, 8.8 Hz, H-4'); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 166.7 (C-7), 134.7 (C-4), 131.0 (C-2, 6), 130.7 (C-1), 129.6 (C-3, 5), 104.5 (C-1"), 96.3 (C-1'), 77.9 (C-3'), 77.8 (C-3"), 75.0 (C-5'), 74.0 (C-2"), 71.4 (C-2'), 71.4 (C-4'), 70.8 (C-4"), 69.4 (C-6'), 62.6 (C-5"), 32.2 (C-8), 56.5 (-O<u>C</u>H_3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values (Morikawa et al., 2012).

3,4-Dihydro-4-hydoxynapthalene-2-carboxylic acid (**42**)

White, amorphous powder; ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.69 (1H, d, *J*= 8.0 Hz, H-6), 7.35 (1H, d, *J*= 8.0 Hz, H-8), 7.20 (1H, s, H-1), 7.10 (1H, dd, *J*= 1.2, 8.0 Hz, H-7), 7.02 (1H, dd, *J*= 1.2, 8.0 Hz, H-9), 3.84 (1H, dd, *J*= 4.0, 9.6 Hz, H-4), 3.51 (1H, dd, *J*= 3.6, 15.2 Hz, H-3α), 3.14 (1H, dd, *J*= 9.6, 15.2 Hz, H-3β); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 174.4 (C-11), 138.4 (C-10), 128.5 (C-9), 125.2 (C-1), 122.7 (C-6), 120.1 (C-7), 119.3 (C-8), 112.4 (C-5), 109.6 (C-2), 56.7 (C-4), 28.5 (C-3). The above ^{1}H- and ^{13}C-NMR spectroscopic data were close accordance with those of the reported values [Yang et al., 2011].

Phenyl-*O*-β-xylopyranosyl (1→6)-*O*-glucopyranoside (**43**)

White, amorphous powder; a quasimolecular ion peak at m/z 387.1537 $[M-H]^-$ in HR-ESI-MS suggested a molecular formula of $C_{17}H_{24}O_{10}$. ^{1}H-NMR (CD_3OD, 400Hz) δ_H 7.28 (2H, dd, *J*= 1.6, 7.6 Hz, H-2 and H-6), 7.09 (2H, dd, *J*= 1.6, 7.6 Hz, H-3 and H-5), 6.97 (1H, dd, *J*= 1.6, 7.6 Hz, H-4), 4.89 (1H, d, *J*= 7.6 Hz, H-1'), 4.30 (1H, d, *J*= 7.2 Hz, H-1"), 4.10 (1H, dd, *J*= 2.4, 12.0 Hz, H-6'α), 3.80 (1H, dd, *J*= 3.2, 12.0 Hz, H-6'β), 3.76 (1H, dd, *J*= 6.4, 8.8 Hz, H-5"α), 3.55 (1H, dd, *J*= 2.4, 8.8 Hz, H-5"β),

3.65 (1H, m, H-5'), 3.58 (1H, m, H-4"), 3.55 (1H, m, H-2"), 3.46 (1H, m, H-3"), 3.28 (1H, m, H-2'), 3.09 (1H, m, H-4'); ^{13}C-NMR (CD_3OD, 100Hz) δ_c 159.0 (C-1), 130.5 (C-2, 6), 133.3 (C-4), 117.8 (C-3, 5), 104.9 (C-1"), 102.0 (C-1'), 77.8 (C-3'), 77.3 (C-3"), 74.9 (C-5'), 74.1 (C-2"), 72.4 (C-2'), 71.5 (C-4'), 69.5 (C-4"), 69.3 (C-6'), 66.7 (C-5"). The above ^{1}H- and ^{13}C-NMR spectroscopic data were the same with those of the reported values [Chen et al., 2008].

2.3.6 Inhibitory effects of the isolated compounds against IgE-mediated degranulation in RBL-2H3 cells

The isolated 26 compounds were further examined for their anti-allergic activity as inhibitory effects against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells, and their IC_{50} values were shown in Table 2.2. Cell viability under stimulation was evaluated by the CCK-8 assay and all of the test compounds at several dose levels from 10.0 up to 100.0 μM showed no cytotoxicity. Among 26 isolated compounds, eighteen compounds exhibited anti-allergic activity with IC_{50} values ranging from 25.05 to 91.40 μM. Mudanpioside E (**31**) with paeoniflorin-type skeleton and quercetin (**37**) showed potent inhibitory activity against β-hexosaminidase release with IC_{50} values of 40.34 and 25.05 μM, respectively.

Table 2.2 Inhibitory effects of 26 isolated components against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells.

Compound	IC_{50} (μM)	Compound No.	IC_{50} (μM)
30	62.50 ± 1.8 [a]	**38**	42.55 ± 2.0
6	76.02 ± 1.4	**39**	65.44 ± 2.5
8	42.22 ± 2.9	**40**	48.84 ± 1.4
13	87.11 ± 1.7	**25**	88.92 ± 2.7
31	40.34 ± 3.2	**41**	68.71 ± 3.2
18	62.00 ± 1.4	**28**	78.11 ± 3.1
32	91.40 ± 1.5	**29**	47.72 ± 1.0
33	68.36 ± 1.3	**42**	78.51 ± 2.3
34	60.70 ± 1.1	**4**, **19-21**, **24**, **35-36**, **43**	NA [b]
37	25.05 ± 4.0	**Baicalein** [c]	37.78 ± 1.0

[a] The results are expressed as the mean ± S.D., IC_{50} values were calculated by Probit regression analysis of SPSS software (version 15.0);

[b] IC_{50} values are more than 100 μM; [c] Positive control [Matsuda et al., 2002; Murata et al., 2013].

2.4 Discussion

By comparing the IC_{50} value of these test compounds, as for the monoterpenoids which are considered as the main and characteristic constituents of PR, compounds **6**, **8**, **13** and **31** with paeoniflorin-type skeleton showed moderate activities, but albiflorin (**4**) with the lactone ring in the pinane aglycone exhibited no activity, which was consistent with our previous Chapter I that paeoniflorin-type skeleton is essential for the anti-allergic activity as inhibitory effect against IgE-mediated degranulation rather than albiflorin-type. Except for **31**, compounds **6**, **8** and **13** have also been isolated from RPR in the Chapter I. Of the four paeoniflorin derivatives, mudanpioside E (**31**) with 3-methoxy-4-hydroxybenzoyl moiety at C-8 showed the highest activity (IC_{50}: 40.34 μM), and salicylpaeoniflorin (**8**) with *o*-hydroxybenzoyl

moiety at C-8 exhibited higher activity than the two compounds with benzoyl (**6**) or *p*-hydroxybenzoyl moieties (**13**) at C-8. Quercetin (**37**) exhibited the most promising activity (IC_{50}: 25.05 μM), which was stronger than that of baicalein, the positive control (IC_{50}: 37.78 μM). The similar tendency has been previously reported that quercetin showed stronger activity against IgE-mediated allergic mediator release from human cultured mast cells than baicalein [Kimata et al., 2000]. In addition, the other flavonol of quercetin-3-*O*-β-D-glucopyranoside (**38**) also exhibited highly inhibitory activity, followed by three flavanones (**32-34**), whereas four favan-3-ols (**19-21**, **35**) and a flavanonol (**36**) showed no activity. These results suggested that the vinylic structure at C2-C3 of flavonols was important for the activity (**37**, **38** vs. **36**), and glycosylation of hydroxyl group at C-3 in flavonols reduced the inhibitory activity (**37** vs. **38**). Flavanones having C-2 (*R*) configuration was more active than that of C-2 (*S)* configuration (**33**, **34** vs. **32**). Among the three paeonol derivatives (**24**, **39-40**), compounds (**39** and **40**) with primeverosyl moiety at C-4 of aglycone exhibited inhibitory activity, but paeonol (**24**) had no activity.

Up to date, several studies have reported that paeoniflorin (**6**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**18**), paeonol (**24**) showed potential anti-allergic activity both *in vitro* and *in vivo* [Lee et al., 2008; Kageyama-Yahara et al., 2010]. In the present study, several other compounds belonging to monoterpenoids and flavonol, such as mudanpioside E (**31**), salicylpaeoniflorin (**8**) and quercetin (**37**), etc., are also documented to make contribution to the anti-allergic activity of PR as inhibitory effect against IgE-mediated degranulation in RBL-2H3 cells.

Summary of Chapter Ⅱ

1) Methanol extract of the root of Edulis Superba (S78N), a horticultural cultivar among 17 cultivars of *P. lactiflora*, was found to possess relatively strong anti-allergic activity (IC_{50}: 0.72 ± 0.02 mg/ml) as inhibitory effects against IgE-mediated degranulation. Subsequently, the EtOAc and *n*-BuOH soluble subfractions partitioned from methanol extract showed the most promising anti-allergic activity (IC_{50}: 0.32 mg/ml; 0.58 mg/ml, respectively).

2) Bioassay-guided fractionation on the two active subfractions led to the isolation of a new norneoligan, named paeonibenzofuran (**30**), and 25 known compounds including five monoterpenoids (**4**, **6**, **8**, **13**, **31**), ten flavonoids (**19**-**21**, **32**-**38**), ten of other types of compounds (**18**, **24**-**25**, **28**-**29**, **39**-**43**).

3) Mudanpioside E (**31**) with paeoniflorin-type skeleton and quercetin (**37**) showed stronger inhibitory activity against β-hexosaminidase release with IC_{50} values of 40.34 and 25.05 μM, respectively.

Chapter Ⅲ

Characterization and quantification of monoterpenoids in different types of peony root and the related species in sect. *Paeonia* by LC-ESI-IT-TOF-MS

3.1 Introduction

According to the results of the phytochemical and biological investigations in Chapter I and II, monoterpenoids are not only the main and characteristic components in PR, but also the important components responsible for the anti-allergic activity as inhibitory effects against IgE-mediated degranulation, especially paeoniflorin-type monoterpenoids such as salicylpaeoniflorin (**8**), galloylpaeoniflorin (**11**), mudanpioside E (**31**), etc. Until now, more than 60 monoterpenoids have been isolated and identified from various *Paeonia* species [He et al., 2010; Wu et al., 2010]. Several studies based on liquid chromatography coupled with time-of-flight mass spectrometry (LC-TOF-MS) dealt with only one or a few of predominate components such as paeoniflorin, albiflorin, oxypaeoniflorin, galloylpaeoniflorin and benzoylpaeoniflorin [Lin et al., 2009; Liu et al., 2009; Wang et al., 2015]. Detailed investigations on chemical distribution and characterization of monoterpenoids in PR and the related species such as *P. lactiflora*, *P. veitchii*, *P. anomala* Linn. and *P. japonica* Miyabe et Takeda belonging to sect. *Paeonia* remains unclear.

In the Chapter III, a high performance liquid chromatography electrospray ionization coupled with ion trap and time-of-flight mass spectrometry (HPLC-IT-TOF-MS) method was developed and validated for characterization and quantification of monoterpenoids in different types of PR and the related species in sect. *Paeonia*. MS/MS fragmentation pathways of monoterpenoids with paeoniflorin-, albiflorin- and sulfonated paeoniflorin-types of skeletons were reconstructed by using the authenticated compounds to provide basic clues for subsequent elucidation of monoterpenoids profiles. Subsequently, monoterpenoids profiling was investigated to clarify the chemical characteristics of the four species, and to found the possible marker constituents for their discrimination. Furthermore, quantification analysis of

15 monoterpenoids including those with anti-allergic activity was investigated to evaluate their contents variation in different types of PR and the related species.

3.2 Results and discussion

3.2.1 Fragmentation pathways of monoterpenoids

The MS/MS fragmentation patterns of paeoniflorin, albiflorin and galloylpaoniflorin have been reported, which provided useful information for further assignment of monoterpenoids by using mass techniques [Lin et al., 2009; Liu et al., 2009; Wang et al., 2015]. In order to reveal fragmentation patterns of monoterpenoids with different types of skeletons, eleven reference compounds, including eight with paeoniflorin-type skeleton and three with albiflorin-type skeleton were preferentially analyzed by ESI-IT-TOF-MS/MS.

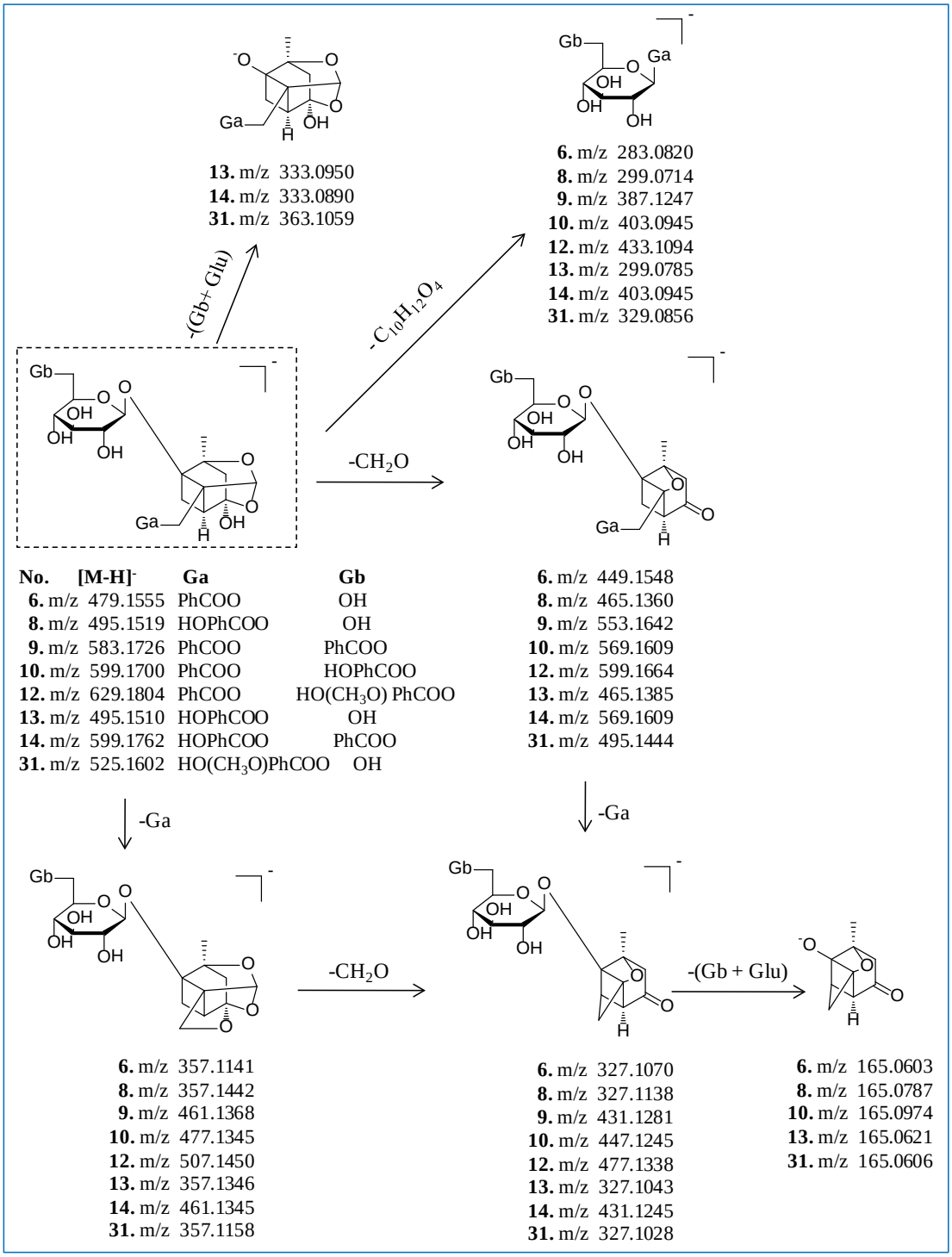
3.2.1.1 Monoterpenoids with paeoniflorin-type skeleton (PFs)

Of the eight PFs (**6**, **8**-**10, 12**-**14**, **31**), the predominant fragments from successive or simultaneous losses of the moiety at the C-8 position of pinane skeleton (Ga) and a CH_2O unit, $[M–H–Ga]^-$ and $[M–H–30]^-$ ions were clearly observed. Most previous studies reported that lost of the 30 dalton (Da) was due to dissociation of a terminal CH_2O (formaldehyde) unit from glucose moiety [Xu et al., 2006; Dong et al., 2007; Li et al., 2007; Lin et al., 2009; Liu et al., 2009]. However, this characteristic ion was also clearly detected in the MS/MS spectra of the four compounds (**9**, **10**, **12** and **14**) without free CH_2O unit in the glucose moiety. Upon the structural features of the eight PFs, it is more reasonable that the loss of 30 mass units was due to degradation of acetal group from the paeoniflorin-type aglycone [Ye et al., 2007; Wu et al., 2012], but not from the glucose moiety. In addition, the characteristic product ions resulted

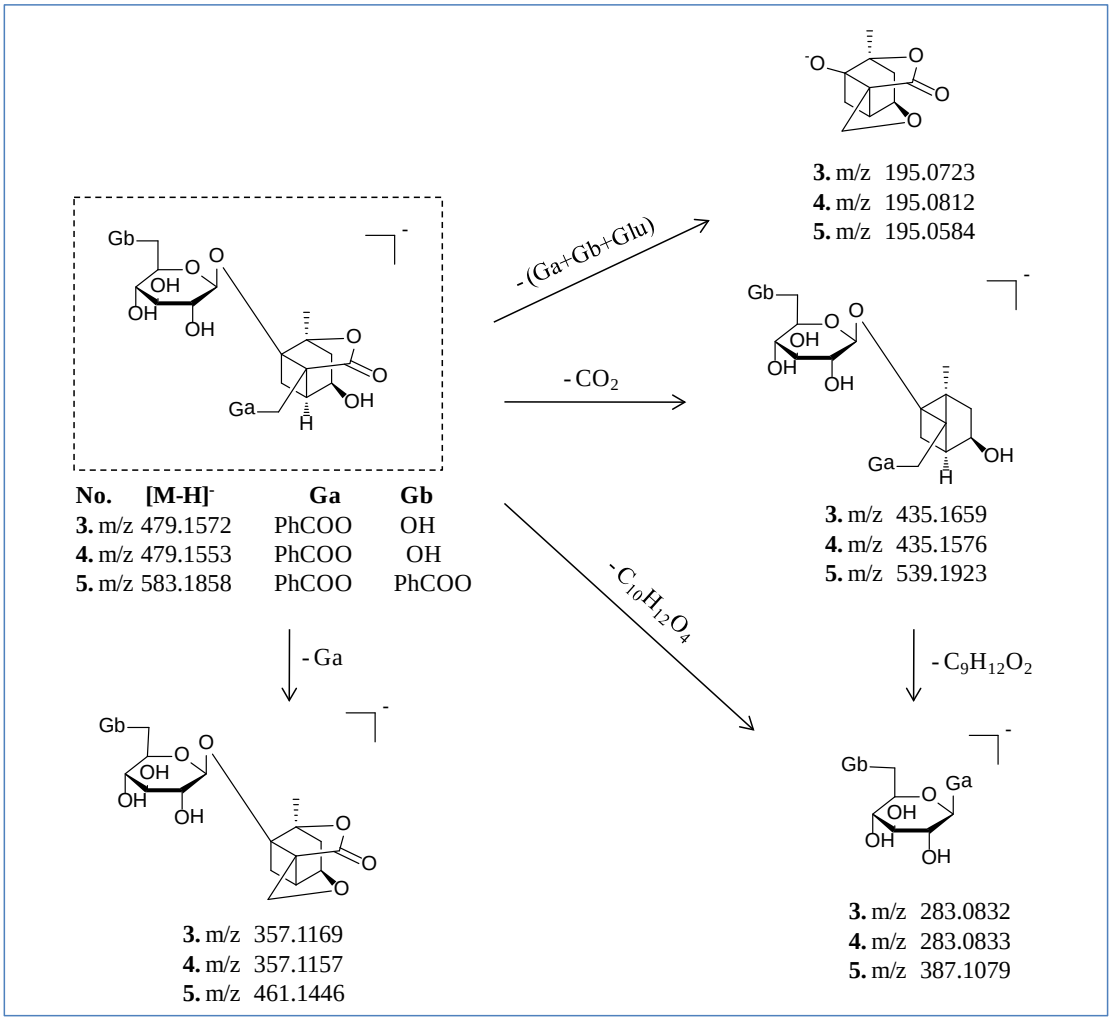
from the pinane skeleton at *m/z* 165 that was assignable to fragment of the core skeleton, and the product ion of $[M–H–C_{10}H_{12}O_4]^-$ were observed in most of PFs. Based on the above evidences achieved from the MS/MS spectra of eight PFs, the proposed fragmentation pathways for precursor ions $[M–H]^-$ of PFs were summarized in Scheme 3.1.

3.2.1.2 Monoterpenoids with albiflorin-type skeleton (AFs)

Of the three AFs (**3**-**5**), the MS/MS spectra showed a predominant product ion $[M–H–44]^-$ at *m/z* 435 in **3** and **4**, as well as at *m/z* 539 in **5**, which was attributed to the loss of CO_2 (44 Da) from the lactone ring of pinane skeleton [Lin et al., 2009; Liu et al., 2009; Wang et al., 2015]. The characteristic product ion arised from albiflorin-type aglycone at *m/z* 195 were clearly detected in all of AFs, which was able to use for identification AFs. Additionally, $[M–H–Ga]^-$ (*m/z* 357 in **3** and **4**; *m/z* 461 in **5**) and $[M–H–C_{10}H_{12}O_4]^-$ (*m/z* 283 in **3** and **4**; *m/z* 387 in **5**) were obviously observed in all the three AFs (Scheme 3.2).



Scheme 3.1 Proposed fragmentation pathway of paeoniflorin-type monoterpenoids (PFs)



Scheme 3.2 Proposed fragmentation pathway of albiflorin-type monoterpenoids (AFs)

3.2.2 Monoterpenoids profiles of different types of PR and the related species

Monoterpenoids profiles of the different types of PR and the roots of the related four *Paeonia* species, including *P. lactiflora*, *P. veitchii*, *P. anomala* and *P. japonica* were analyzed and compared by using LC-ESI-IT-TOF-MS. Negative ion mode was preferred to use for analysis owing to its high sensitivity of signals from most compounds, as well as low background noise.

In the typical total ion chromatograms of *Paeonia* samples (Fig. 3.1), fifteen monoterpenoids (**1**, **3-5**, **6-14**, **17**, **31**) were unambiguously assigned by comparing with the respective reference standards that isolated in Chapter I and II, and nine

peaks were tentatively identified according to the clarified MS/MS fragmentation patterns as well as the information in reported literatures [Lin et al., 2009; Liu et al., 2009; Xu et al., 2006; Yan et al., 2012]. The detail information of the nine identified monoterpenoids (**PF1**-**PF5**, **AF1**-**AF2**, **M1**-**M2**) were summarized in Table 3.1.

It is noteworthy that more than half of commercial WPR samples collected from Chinese market showed significant difference in the total ion chromatograms (Fig. 3.1c), and some observable specific peaks were detected. We previously reported that a peak of paeoniflorin sulfonate (**PS4**) was detected in sulfur-fumigated WPR [Zhu et al., 2015]. Yan Z. et al. have reported that the sulfuric aglycone ion at *m/z* 259 is a characteristic signal for identification of sulfonated monoterpenoids in peony root [Yan et al., 2012]. In the present study, beside the peak of **PS4**, five other conspicuous peaks were tentatively identified as oxypaeoniflorin sulfonate (**PS1**), mudanpioside E sulfonate (**PS2**), isomaltopaeoniflorin sulfonate (**PS3**), galloylpaeoniflorin sulfonate (**PS5**) and benzoyloxypaeoniflorin sulfonate (**PS6**) according to their characteristic MS/MS profiles and UV spectra [Lin et al., 2009; Yan et al., 2012]. The MS/MS spectra of the six main sulfonated monoterpenoids were carefully analyzed and their fragmentation patterns were summarized in the Scheme 3. The characteristic losses of the 46 Da unit that was supposed to be generated via degradation of acetal group (CH_2O_2) from the sulfonated PF-type skeleton were observed. Besides the ion of sulfonated pinane skeleton at *m/z* 259, the product ion of $[259–CH_2O_2]^-$ at *m/z* 213 detected in all PSs was also a characteristic ion that can be used to identify sulfonated paeoniflorin-type monoterpenoids (PSs).

Finally, a total of thirty peaks assigned as monoterpenoids, which included 15 PFs, 5 AFs, and 6 PSs as well as 4 other types of monoterpenoids. The details of these identified monoterpenoids were summarized in Table 3.1. Five monoterpenoids were found to be predominant compounds in all the samples, which were paeoniflorin (**6**),

albiflorin (**4**), benzoylpaeoniflorin (**9**), galloylpaeoniflorin (**11**) and oxypaeoniflorin (**13**), but their relative contents varied considerably among different types of PR as well as the different species. In addition, some specific and distinctive marker constituents for discrimination in different species were also observed. Mudanpioside C (**10**) was the characteristic constituent of *P. lactiflora*, whereas 4-*O*-methyl-paeoniflorin (**7**) was only detected in *P. veitchii* and *P. anomala*.

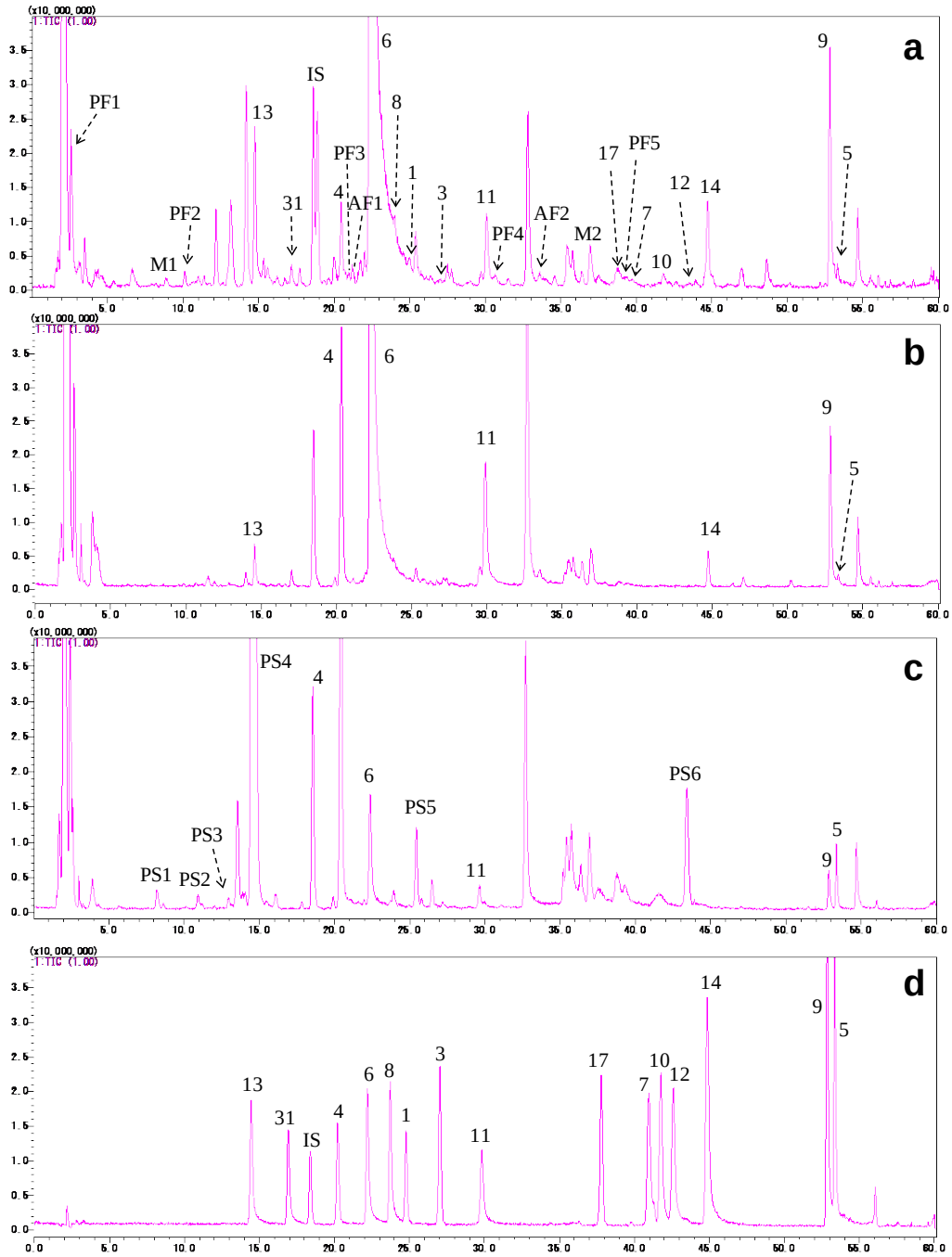
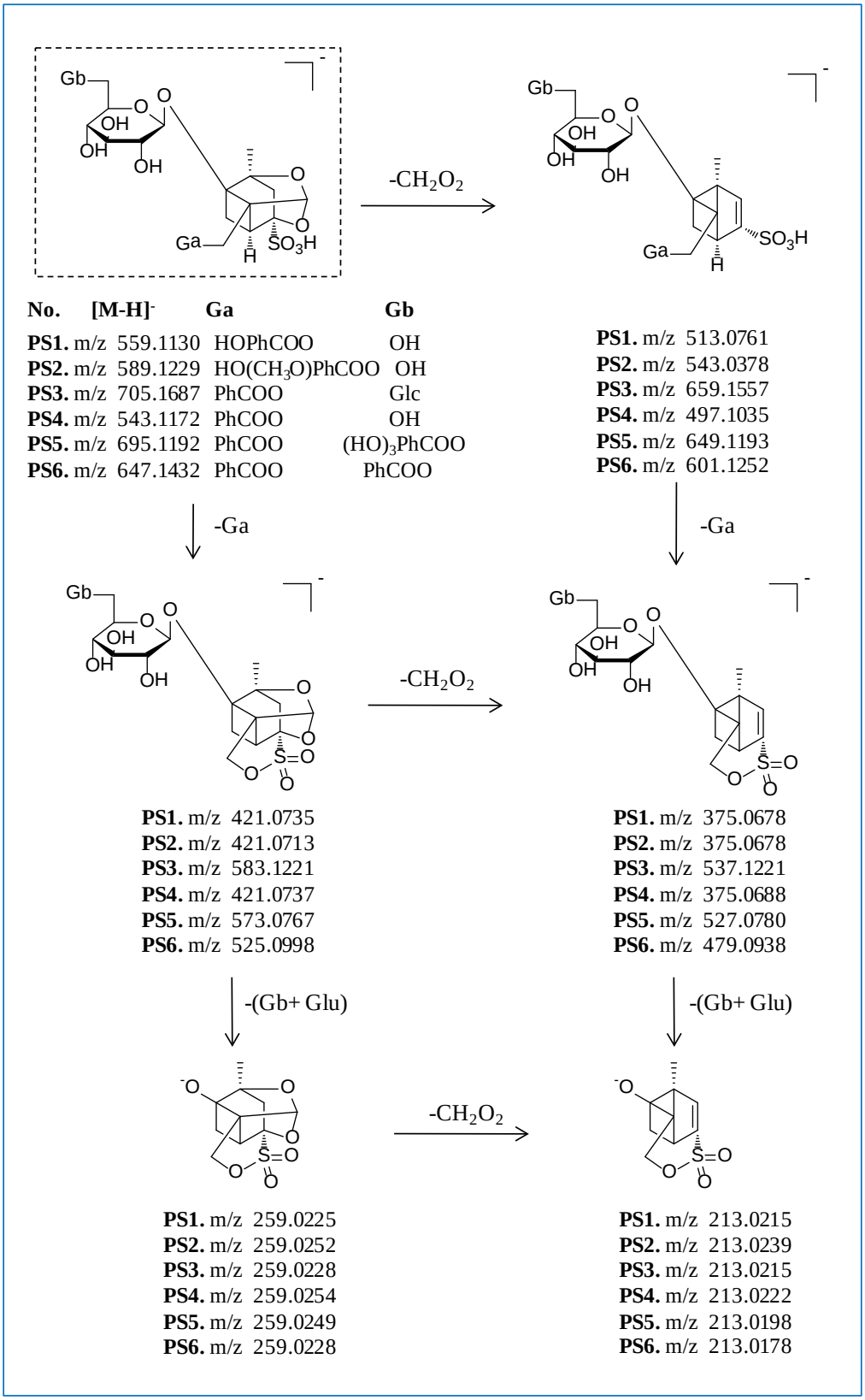


Fig. 3.1 Representative total ion chromatograms of peony root in negative ion mode by LC-ESI-IT-TOF-MS. **a**: a plant specimen of RPR-type of *P. lactiflora* (P1); **b**: a commercial sample of Japanese PR derived from WPR-type of *P. lactiflora* (D52); **c**: a commercial sample of Chinese WPR processed by sulfur-fumigation (D24); **d**: a mixed standard of 15 monoterpenoids. IS (internal standard), IS: geniposide. 1-31: unambiguously identified compounds listed in Chapter I and II. PF: paeoniflorin-type monoterpenoids; AF: albiflorin-type monoterpenoids; PS: sulfonated paeoniflorin-type monoterpenoids. The details of these identified compounds were summarized in Table 3.1



No.	$[M-H]^-$	Ga	Gb
PS1.	m/z 559.1130	HOPhCOO	OH
PS2.	m/z 589.1229	$HO(CH_3O)PhCOO$	OH
PS3.	m/z 705.1687	PhCOO	Glc
PS4.	m/z 543.1172	PhCOO	OH
PS5.	m/z 695.1192	PhCOO	$(HO)_3PhCOO$
PS6.	m/z 647.1432	PhCOO	PhCOO

PS1. m/z 513.0761
PS2. m/z 543.0378
PS3. m/z 659.1557
PS4. m/z 497.1035
PS5. m/z 649.1193
PS6. m/z 601.1252

PS1. m/z 421.0735
PS2. m/z 421.0713
PS3. m/z 583.1221
PS4. m/z 421.0737
PS5. m/z 573.0767
PS6. m/z 525.0998

PS1. m/z 375.0678
PS2. m/z 375.0678
PS3. m/z 537.1221
PS4. m/z 375.0688
PS5. m/z 527.0780
PS6. m/z 479.0938

PS1. m/z 259.0225
PS2. m/z 259.0252
PS3. m/z 259.0228
PS4. m/z 259.0254
PS5. m/z 259.0249
PS6. m/z 259.0228

PS1. m/z 213.0215
PS2. m/z 213.0239
PS3. m/z 213.0215
PS4. m/z 213.0222
PS5. m/z 213.0198
PS6. m/z 213.0178

Scheme 3.3 Proposed fragmentation pathway of sulfonated paeoniflorin-type monoterpenoids (PSs)

Table 3.1 Characterization of unambiguously and tentatively identified thirty monoterpenoids from the root of *P. lactiflora* using LC-ESI-IT-TOF-MS.

	Monoterpenoids	t_R (min)	Assiged identity	Molecular formular	UV λmax (nm)	[M-H]$^-$ *m/z*			[M+HCOO]$^-$ *m/z*	[2M-H]$^-$ *m/z*	MS/MS data (measured from [M-H]$^-$)
						Mean mesured mass (Da)	Theoretical extract	Mass accuracy			
PFs	**6***	22.432	Paeoniflorin	$C_{23}H_{28}O_{11}$	232	479.1555	479.1553	0.4	525.1601	959.3080	449.1548, 357.1141, 327.1070, 283.0820, 165.0603
	7*	41.778	4-*O* -Methyl-paeoniflorin	$C_{24}H_{30}O_{11}$	232	493.1717	493.1710	1.4	539.1733		493.1647, 431.1156, 371.1332, 283.0746
	8*	23.905	Salicylpaeoniflorin	$C_{23}H_{28}O_{12}$	232	495.1519	495.1503	3.2			465.1360, 357.1442, 327.1138, 299.0714, 165.0787
	9*	53.257	Benzoylpaeoniflorin	$C_{30}H_{32}O_{12}$	232	583.1826	583.1816	1.7	629.1811	1168.3596	553.1642, 461.1368, 431.1281, 387.1247, 265.0687
	10*	42.602	Mudanpioside C	$C_{30}H_{32}O_{13}$	232, 258	599.1760	599.1765	-0.8	645.1745	1199.3489	569.1609, 477.1348, 447.1245, 403.0945, 281.0624. 165.0974
	11*	30.527	Galloylpaeoniflorin	$C_{30}H_{32}O_{15}$	217, 275	631.1644	631.1663	-3.0			613.1531, 509.1229, 491.1189, 479.1132, 271.0460, 211.0264. 124.0196
	12*	43.420	Mudanpioside J	$C_{31}H_{34}O_{14}$	220, 258	629.1874	629.1870	0.6	675.1884	1259.1552	599.1664, 507.1450, 477.1338, 433.1094
	13*	14.687	Oxypaeoniflorin	$C_{23}H_{28}O_{12}$	258	495.1510	495.1503	1.4	531.1424	991.3068	465.11385, 357.1346, 333.0950, 327.1093, 165.0621
	14*	45.710	Benzoyloxypaeoniflorin	$C_{30}H_{32}O_{13}$	232, 258	599.1762	599.1765	-0.5	645.1725	1199.3681	569.1609, 461.1345, 431.1245, 403.0945, 333.0903. 281.0638
	31*	17.213	Mudanpioside E	$C_{24}H_{30}O_{13}$	215, 270	525.1602	525.1608	-1.1	571.1590		495.1444, 363.1059, 357.1158,329.0856, 327.1028, 167.0366. 165.0606
	PF1	2.458	Desbenzoylpaeoniflorin	$C_{16}H_{24}O_{10}$	210	375.1293	375.1291	0.5	421.1291		345.1141, 165.0725
	PF2	10.968	6'-*O* -Galloyl-desbenzoylpaeoniflorin	$C_{23}H_{28}O_{14}$	211, 276	527.1363	527.1401	-7.2			497.1008, 302.0501, 221.0216, 191.6058
	PF3	20.815	Isomaltopaeoniflorin	$C_{29}H_{38}O_{16}$	210, 275	641.2061	641.2082	-3.3	687.2037		611.1385, 593.1754, 519.3011, 489.1551, 283.4978
	PF4	31.257	Galloylpaeoniflorin isomer	$C_{30}H_{32}O_{15}$	217, 275	631.1652	631.1663	-1.7			613.1497, 491.1073, 271.0465
	PF5	39.628	3', 6'-Di-*O* -galloylpaeoniflorin	$C_{37}H_{36}O_{19}$	215, 268	783.1805	783.1773	4.1			631.0524, 509.0459, 465.1473, 313.0448, 169.0149
AFs	**3***	27.135	4-*epi* -Albiflorin	$C_{23}H_{28}O_{11}$	232, 275	479.1572	479.1553	4.0	525.1638		435.1659, 357.1169, 283.0832, 195.0812
	4*	20.515	Albiflorin	$C_{23}H_{28}O_{11}$	232, 275	479.1553	479.1553	0.0	525.1652		435.1576, 357.1157, 283.0833, 195.0812
	5*	53.672	Paeonivayin	$C_{30}H_{32}O_{12}$	232, 275	583.1815	583.1816	-0.2	629.1930		539.1923, 461.1446, 387.1079, 195.0813
	AF1	20.815	6'-*O* -glucopyranosylalbiflorin	$C_{29}H_{38}O_{16}$	210, 275	641.2081	641.2082	-0.5	687.1994		597.1874, 515.2982, 471.1428, 252.5066, 195.0438
	AF2	35.563	Galloylalbiflorin	$C_{30}H_{32}O_{15}$	217, 275	631.1652	631.1663	-1.7			587.1694, 525.1554, 479.1495, 357.1163, 327.0740
PSs	**PS1**	8.373	Oxypaeoniflorin sulfonate	$C_{23}H_{28}O_{14}S$	257	559.1130	559.1122	1.4			513.0761, 421.0735, 375.0678, 259.0225, 213.0215
	PS2	11.287	Mudanpioside E sulfonate	$C_{24}H_{30}O_{15}S$	257	589.1229	589.1227	0.3			543.0378, 421.0713, 375.0678, 259.0252, 213.0239
	PS3	13.820	Isomaltopaeoniflorin sulfonate	$C_{29}H_{38}O_{18}S$	258	705.1687	705.1701	-2.0			659.1557, 583.1221, 537.1221, 421.0774, 375.0686, 259.0228. 213.0215
	PS4	14.863	Paeoniflorin sulfonate	$C_{23}H_{28}O_{13}S$	232	543.1174	543.1172	0.4			497.1035, 421.0737, 375.0688, 259.0254, 213.0222
	PS5	25.803	Galloylapeoniflorin sulfonate	$C_{30}H_{32}O_{17}S$	275	695.1296	695.1282	2.0			649.1193, 573.0767, 527.0780, 259.0249, 213.0198
	PS6	44.582	Benzoylpaeoniflorin sulfonate	$C_{30}H_{32}O_{14}S$	275	647.1432	647.1435	-0.5			601.1252, 525.0998, 479.0938, 437.8443, 381.0590, 259.0228, 213.0178
Others	**1***	25.063	Paeoniflorol	$C_{23}H_{28}O_{10}$	230	465.1749	465.1761	-2.6	511.1861		411.7129, 343.1349, 283.0840, 119.4497
	17*	38.3	Lactiflorin	$C_{23}H_{26}O_{10}$	231	461.1443	461.1448	-1.1	507.1483		339.1044, 229.3689, 177.0539
	M1	9.958	Mudanpioside F	$C_{16}H_{24}O_8$	210, 260	343.1386	343.1393	-2.0	389.1374		343.1361, 205.5758
	M2	36.733	Albiflorin R1	$C_{23}H_{28}O_{11}$	220, 270	479.1541	479.1553	-2.5			357.1158, 327.1022, 121.1340

* Compared with reference standards.

3.2.3 Quantitative analysis of 15 monoterpenoids

3.2.3.1 Method validation

The contents of the fifteen monoterpenoids, including ten PFs (**6-14**, **31)**, three AFs (**3-5**) and two others (**1**, **17**), were simultaneously determined by LC-ESI-IT-TOF-MS. Geniposide (**IS**) was used as the internal standard to improve precision and robustness of the developed method. As shown in Table 3.2, all calibration curves of the 15 monoterpenoids showed good linearity (R^2 > 0.9985) within relatively wide range of concentrations (from 0.052 to 58.5 μg/ml). The LODs and LOQs were lower than 0.072 and 0.239 ng/ml, respectively. Repeatability, stability, inter- and intra-day precision of the developed method was also validated for each analyte, and the RSD values were less than 3.0%. The recoveries of 15 monoterpenoids were measured from 98.5 to 103.6%, and the RSD values were less than 3.0%.

3.2.3.2 Chemical comparison on the basis of contents of 15 monoterpenoids

The contents of 15 monoterpenoids were simultaneously quantified on 56 *Paeonia* samples including plant specimens from four different species and crude drugs of RPR, WPR and PR available in Chinese and Japanese markets. The results (Table 3.3 and Fig. 3.2A) showed that total content of the 15 monoterpenoids, mainly composed of PFs, was obviously higher in *P. lactiflora* (26.34-65.03 mg/g, except for sulfur-fumigated WPR samples) and *P. veitchii* (33.77-74.49 mg/g), which are the two officially recorded botanical sources of PR, than in *P. japonica* (30.36 mg/g) and *P. anomala* (11.20-17.71 mg/g). Paeoniflorin (**6**), as the predominant component in PR, also showed an comparatively higher content in *P. lactiflora* and *P. veitchii* than other two species (Fig. 3.2B). With the commercial samples derived from *P. lactiflora*, we

have previously demonstrated that RPR produced in northern parts of China had an comparatively higher content of **6**, but a lower content of albiflorin (**4**) than WPR/PR produced in southern parts of China and most of PR produced in Japan [Zhu et al., 2015]. Meanwhile, other paeoniflorin-type monoterpenoids such as salicylpaeoniflorin (**8**), mudanpioside C (**10**), mudanpioside J (**12**), oxypaeoniflorin (**13**) and benzoyloxypaeoniflorin (**14**) also with comparatively higher contents in RPR, but the contents of 4-*epi*-albiflorin (**3**) and paeonivayin (**5**) with albiflorin-type skeleton were comparatively lower in RPR than in WPR/PR. As for the two other types of monoterpenoids, paeoniflorol (**1**) was found to be with higher content in RPR and the cultivars of *P. lactiflora*, whereas lactiflorin (**17**) was with obviously high content in WPR/PR. Among the 11 WPR samples collected from Chinese market, eight samples contained less than 20.0 mg/g of paeoniflorin; especially, three WPR samples (D18, D22 and D24) were with extremely low content (< 1.28 mg/g). In the eight samples, paeoniflorin sulfonate as well as several other sulfonated paeoniflorin-type monoterpenoids were clearly detected, indicating such samples were processed by sulfur-fumigation which resulted in low contents of paeoniflorin and other PFs.

In China, sulfur-fumigation is a conventional method used in post-harvest processing of the crude drugs for the purpose of keeping white looks, inhibiting bacterial growth and preventing insects [Wang et al., 2009; Kan et al., 2011]. However, such processing method may result in changes of chemical profiles of the processed crude drugs, and the influence of sulfur-fumigation on their efficacy and safety remains unclear. Therefore, comprehensive investigations on chemical profiles, toxicities and adverse effects, pharmacokinetics and pharmacological activities of sulfur-fumigated crude drugs are necessary.

The samples derived from *P. veitchii* had the highest contents of **8** and **11**,

clearly differed from those derived from other three species. In particular, the content of **11** was more than twice as that in other species. In addition, the minor monoterpenoids of **13** and **14** had very low contents, and **10** could not be detected in *P. veitchii*, whereas *P. lactiflora* showed relatively high contents of these three compounds, which were found to be characteristic feature of *P. lactiflora*. Of the roots of *P. anomala* (P4-P15), most of detected monoterpenoids showed very low contents. 4-*O*-methyl-paeoniflorin (**P2**) was only detected in *P. veitchii* and *P. anomala*. In addition, monoterpenoids composition of the respective *P. lactiflora* cultivar showed high similarity despite RPR-type or WPR-type, which differed from situation between the commercial WPR and RPR.

3.2.4 Comparison of *Paeonia* samples by principle component analysis (PCA)

PCA, as an unsupervised pattern recognition method, is one of the multivariate statistical procedures that were used for reducing the dimensionality of numerical datasets as well as for identifying the most significant traits in the datasets [He et al., 2014; Guo et al., 2016]. PCA was employed based on the normalized peak areas of 21 monoterpenoids including the fifteen quantified monoterpenoids and the six sulfonated paeoniflorin-type monoterpenoids.

The scores plot of PCA (Fig. 3.3A), where a two-component PCA model cumulatively accounted for 66.40% (PC1, 39.15%; PC2, 27.25%) of the total variables, showed that the samples were successfully classified into five separated groups. Besides the respective groups of *P. veitchii* and *P. anomala*, samples derived *P. lactiflora* were clearly classified into three groups that were RPR group, WPR/PR group and sulfur-fumigated WPR group. The characteristic monoterpenoids composition of the respective group could be easily observed by the integrated

information from both the scores plot and the loading plot (Fig. 3.3B). *P. veitchii* group was characterized by the relatively high contents of **7**, **8**, **11** and **17**. Paeoniflorin-type monoterpenoids **10** and **14** showed obviously high contents in RPR group, which could be served as maker constituents to discriminate RPR from WPR/PR. Sulfonated Paeoniflorin-type monoterpenoids (**PS1**-**PS6**) were the indicative constituents of the sulfur-fumigated WPR samples.

According to the results of the bioactive investigation in Chapter I and II, paeoniflorin-type monoterpenoids such as salicylpaeoniflorin (**8**) and galloylpaeoniflorin (**11**) from RPR, mudanpioside E (**31**) from Edulis Superba, and other type monoterpenoids of paeoniflorol (**1**) from RPR are the important components responsible for the anti-allergic activity. The quantitative analysis of 15 monoterpenoids showed that RPR had the obviously higher contents of those bioactive monoterpenoids than WPR, which partially supported that RPR had stronger anti-allergic activity than WPR as inhibitory effect against IgE-mediated degranulation. Edulis Spuperba (S78N), a horticultural cultivar of RPR-type of *P. lactiflora*, has been selected as candidate for the development of new resource of RPR and also had the relatively high contents of those active monoterpenoids.

In addition, flavonoids components such as quercetin (**37**) and quercetin-3-*O*-β-D-glucopyranoside (**38**) had relatively strong anti-allergic activity; however, the contents of these components were relatively low. Therefore, the contribution of these components to the anti-allergic effect of RPR is thought to be less than that of above monoterpenoids.

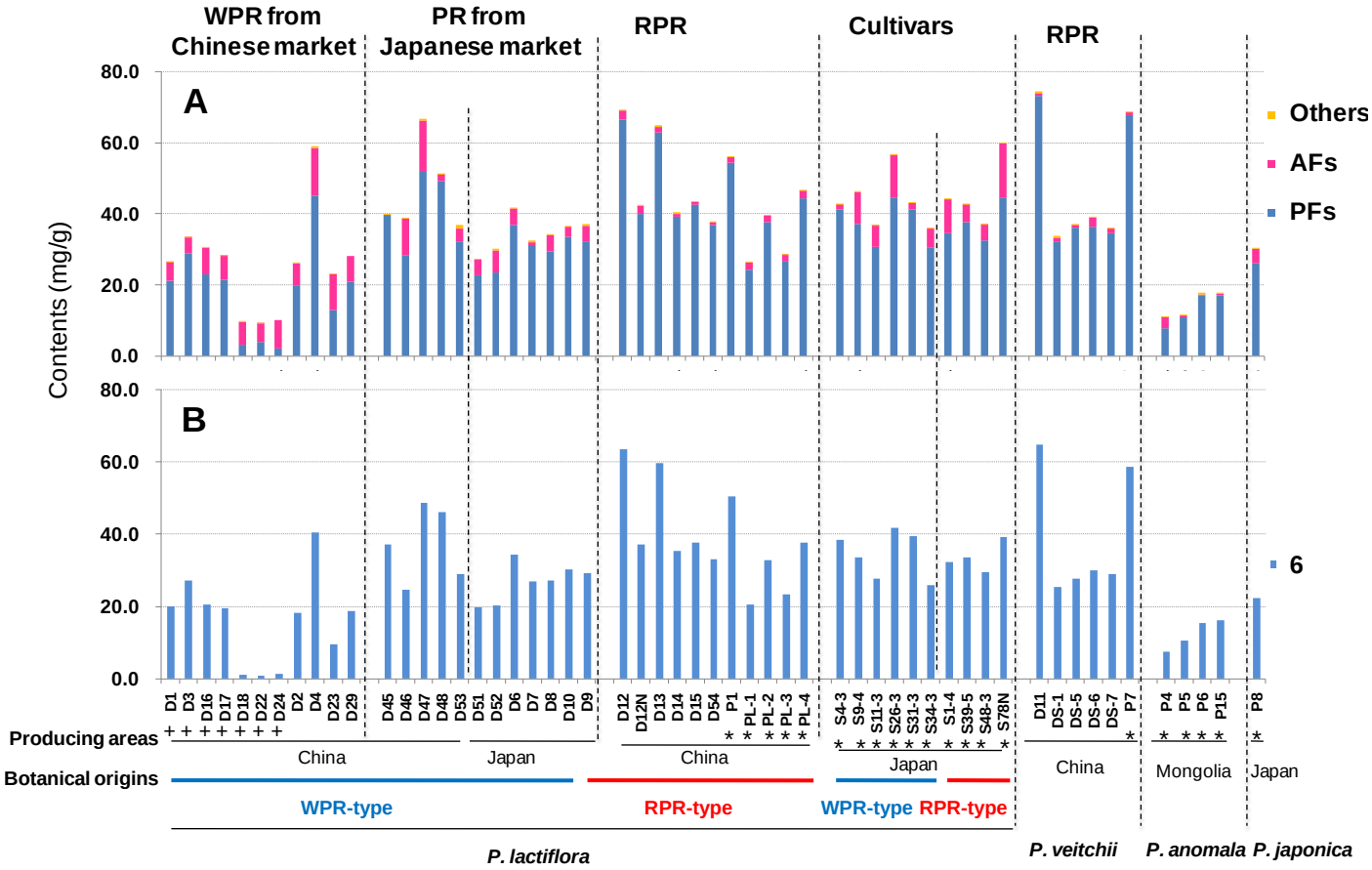
Table 3.2 Regression equations, correlation coefficients, linearity ranges, limit of detection (LOD), limit of quantitative (LOQ), intra- and inter-day precisions, repeatability, stability and recovery for 15 monoterpenoids.

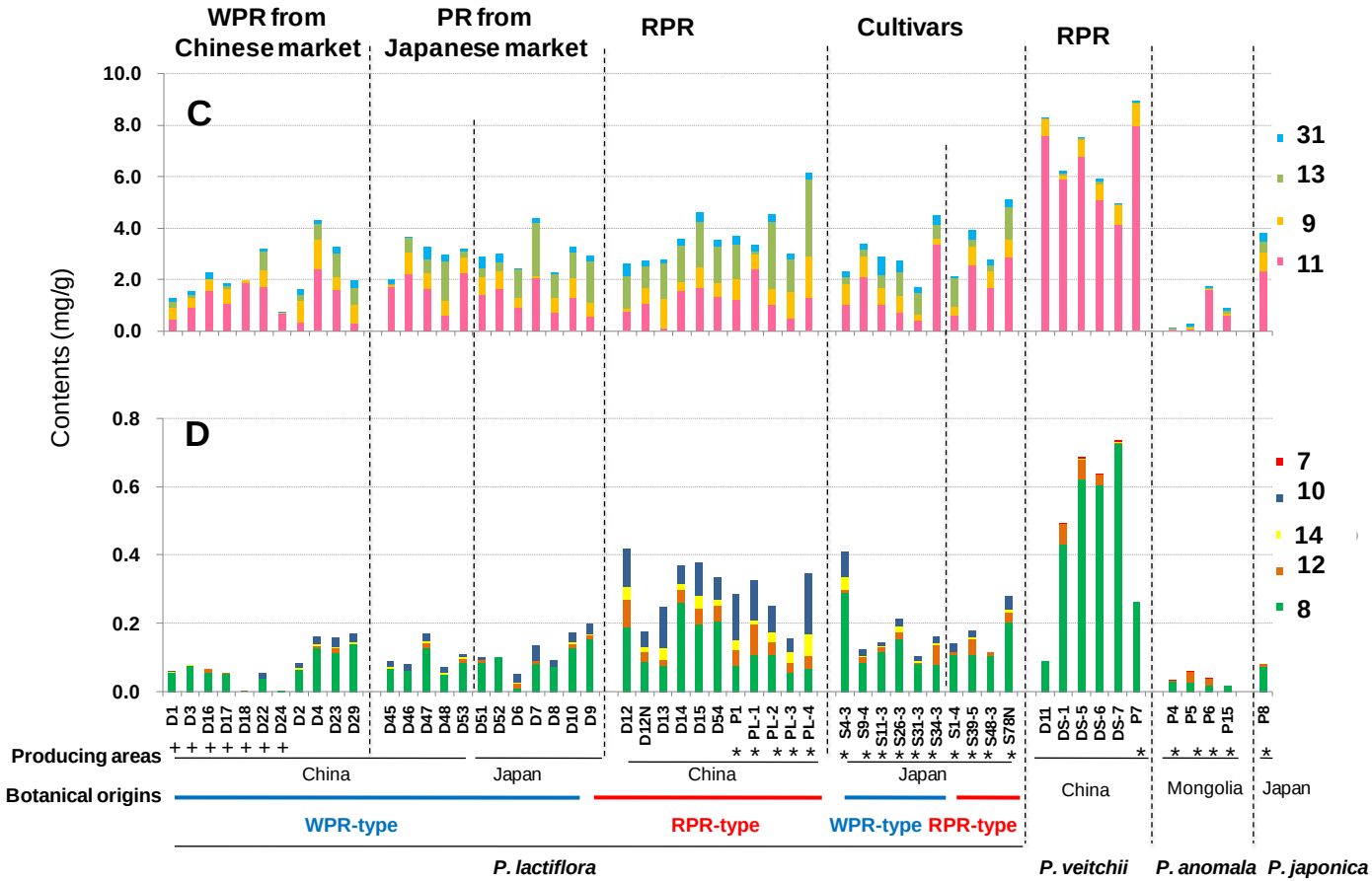
Monoterpenoids	Rentention time (min)	Measured ions (m/z)	Calibration curves equation [a]	R^2	Linear range (μg/mL)	LOD [b] (ng/mL)	LOQ [c] (ng/mL)	Precision (RSD%)		Repeatability (RSD%) n=9	Stability (RSD%) n=6	Recovery (%) n= 3	RSD %
								Intra-day n=3	Inter-day n=3				
Paeoniflorin **(6)**	22.465	525.1538	y = 0.0363 x + 0.0091	0.9995	0.056- 56.000	0.004	0.014	2.36	0.49	1.62	0.47	98.5	2.87
4-Methyl-paeoniflorin (**7**)	41.784	539.1699	y = 0.0581 x + 0.0137	0.9995	0.056- 55.500	0.058	0.193	1.80	1.56	1.73	2.26	101.3	2.40
Salicylpaeoniflorin (**8**)	24.015	495.1428	y = 0.0519 x + 0.0143	0.9985	0.055- 55.000	0.011	0.037	2.27	1.96	1.63	1.08	99.6	1.10
Benzoylpaeoniflorin (**9**)	53.197	629.1790	y = 0.0626 x + 0.0182	0.9994	0.056- 56.000	0.005	0.015	2.03	1.31	1.84	1.40	100.5	1.14
Mudanpioside C **(10)**	42.660	599.1677	y = 0.0520 x + 0.0021	0.9997	0.055- 54.500	0.047	0.016	1.39	0.51	2.02	1.98	102.2	1.28
Galloylpaeoniflorin (**11**)	30.474	631.1575	y = 0.0384 x + 0.0034	0.9998	0.059- 58.500	0.005	0.016	0.85	1.30	0.85	0.95	102.3	2.83
Mudanpioside J (**12**)	43.474	629.1779	y = 0.0499 x + 0.0086	0.9997	0.057- 56.500	0.072	0.239	0.72	0.73	2.17	2.73	100.7	1.59
Oxypaeoniflorin (**13**)	14.681	495.1427	y = 0.0322 x + 0.0062	0.9998	0.054- 54.000	0.015	0.049	0.68	0.67	0.65	0.72	99.0	1.18
Benzoyloxypaeoniflorin (**14**)	45.744	599.1685	y = 0.0675 x + 0.0159	0.9996	0.052- 52.000	0.031	0.102	0.31	0.77	2.63	2.96	101.7	2.44
Mudanpioside E (**31**)	17.138	525.1534	y = 0.0293 x + 0.0079	0.9993	0.058- 57.500	0.014	0.046	1.99	2.20	2.16	2.17	101.6	1.42
4-*epi* -Albiflorin (**3**)	27.420	525.1539	y = 0.0483 x + 0.0004	1.0000	0.057- 56.500	0.027	0.090	0.48	1.80	1.83	2.04	98.9	1.74
Albiflorin **(4)**	20.454	525.1530	y = 0.0302 x + 0.0084	0.9992	0.053- 53.000	0.005	0.018	0.46	0.98	1.56	1.86	99.4	1.70
Paeonivayin (**5**)	53.658	629.1791	y = 0.0482 x + 0.0004	0.9992	0.057- 57.000	0.058	0.019	0.36	0.91	1.32	1.38	103.6	0.96
Paeoniflorol **(1)**	25.063	511.1746	y = 0.0504 x + 0.0143	0.9992	0.057- 57.500	0.009	0.030	1.98	2.45	2.33	2.74	100.4	1.45
Lactiflorin (**17**)	38.340	507.1435	y = 0.0246 x + 0.0082	0.9990	0.054- 54.000	0.007	0.024	1.80	2.28	1.65	1.67	101.5	1.60

[a] y: ratio of the peak areas of analytes to the peak area of IS; x: concentration of standard (μg/ml)

[b] Limit of detection (S/N = 3)

[c] Limit of quantification (S/N = 10)


WPR from Chinese market
PR from Japanese market
RPR
Cultivars
RPR
A
B
Contents (mg/g)
80.0
60.0
40.0
20.0
0.0
Others
AFs
PFs
6
D1 D3 D16 D17 D18 D22 D24 D2 D4 D23 D29
D45 D46 D47 D48 D53 D51 D52 D6 D7 D8 D10 D9
D12 D12N D13 D14 D15 D54 P1 PL-1 PL-2 PL-3 PL-4
S4-3 S9-4 S11-3 S26-3 S31-3 S34-3 S1-4 S39-5 S48-3 S78N
D11 DS-1 DS-5 DS-6 DS-7 P7
P4 P5 P6 P15
P8
Producing areas
China
Japan
China
Japan
China
Mongolia
Japan
Botanical origins
WPR-type
RPR-type
WPR-type
RPR-type
P. lactiflora
P. veitchii
P. anomala
P. japonica


WPR from Chinese market
PR from Japanese market
RPR
Cultivars
RPR
C
D
Contents (mg/g)
10.0
8.0
6.0
4.0
2.0
0.0
0.8
0.6
0.4
0.2
0.0
31
13
9
11
7
10
14
12
8
D1 D3 D16 D17 D18 D22 D24 D2 D4 D23 D29
D45 D46 D47 D48 D53 D51 D52 D6 D7 D8 D10 D9
D12 D12N D13 D14 D15 D54 P1 PL-1 PL-2 PL-3 PL-4
S4-3 S9-4 S11-3 S26-3 S31-3 S34-3 S1-4 S39-5 S48-3 S78N
D11 DS-1 DS-5 DS-6 DS-7 P7
P4 P5 P6 P15
P8
Producing areas
China
Japan
China
Japan
China
Mongolia
Japan
Botanical origins
WPR-type
RPR-type
WPR-type
RPR-type
P. lactiflora
P. veitchii
P. anomala
P. japonica

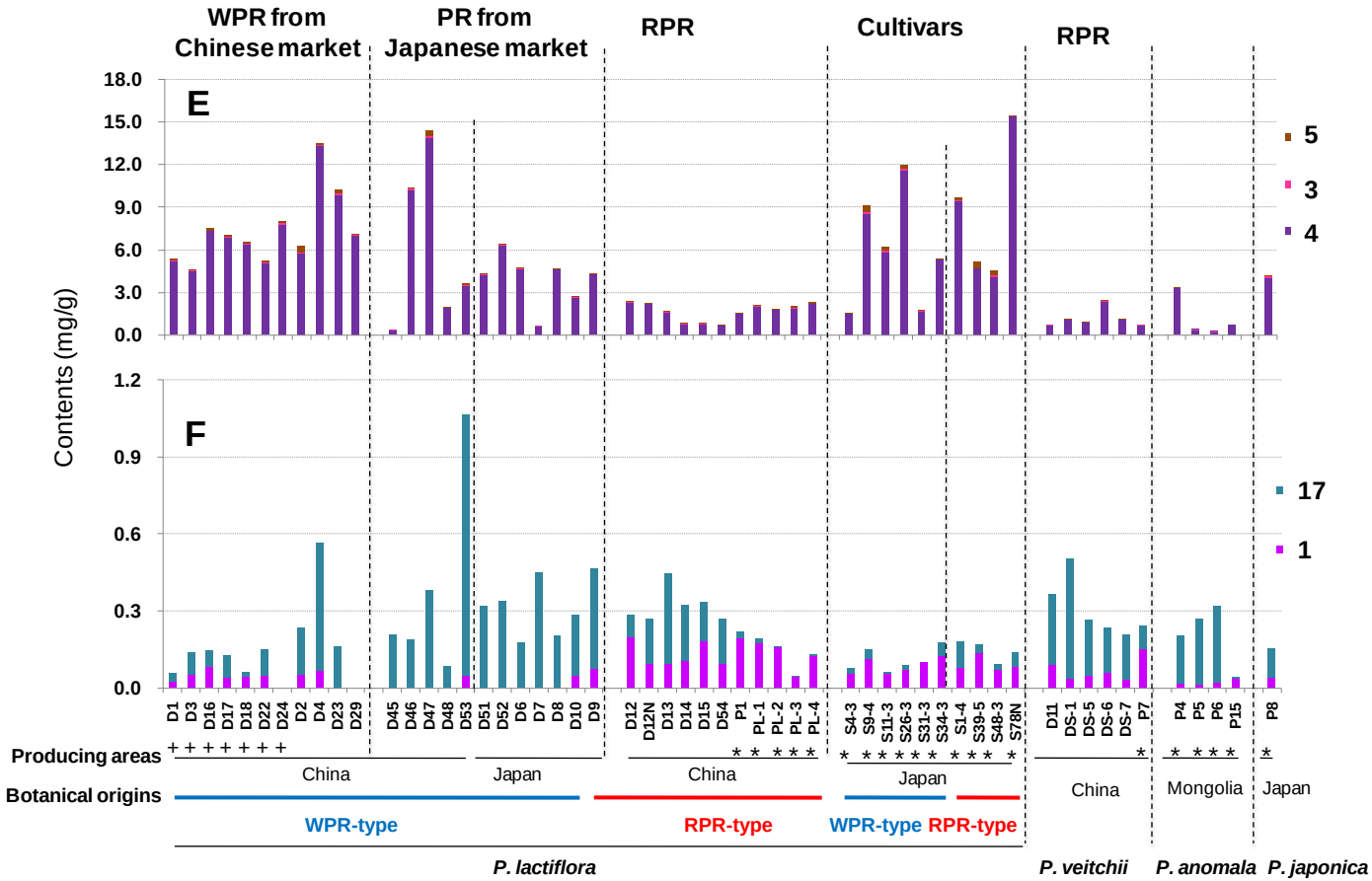


Fig. 3.2 Contents of 15 monoterpenoids in the different types of peony root and the roots of four related species including *P. lactiflora*, *P. veitchii*, *P. anomala* and *P. japonica*. **A**: total contents of 15 monoterpenoids; **B**: content of paeoniflorin (**6**); **C** and **D**: contents of other PFs (**7**-**14**, **31**); **E**: contents of AFs (**3**-**5**); **F**: contents of other monoterpenoids (**1**, **17**). *: plant specimens; +: samples processed by sulfur-fumigation.

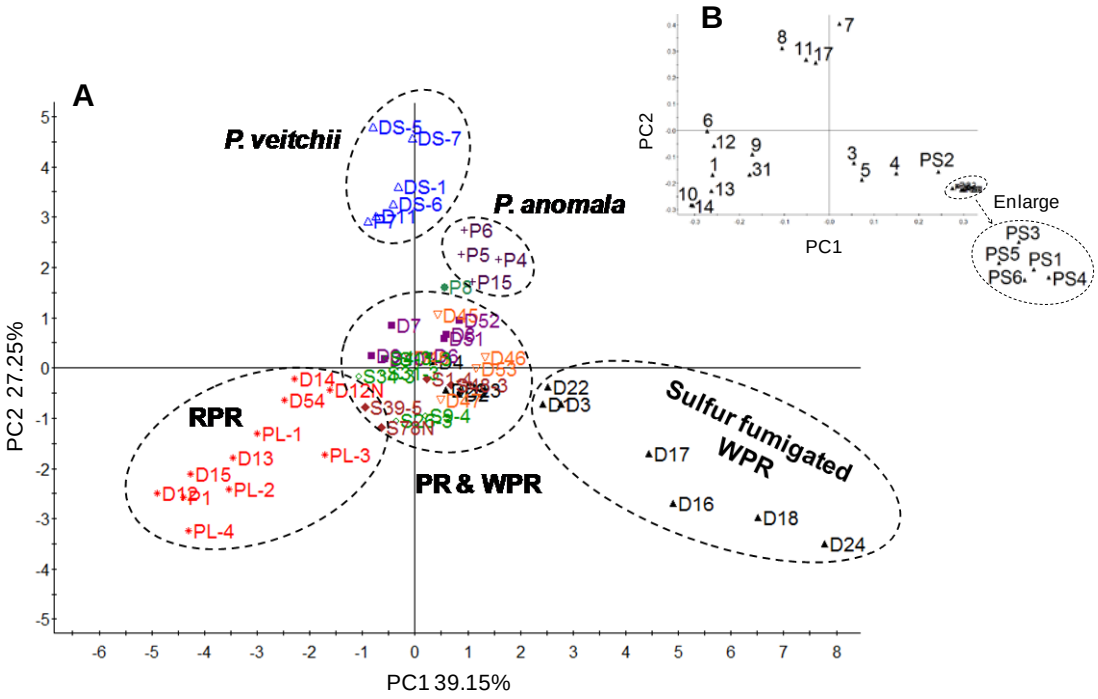


Fig. 3.3 Results of Principal component analysis (PCA) using the normalized peak areas of 15 monoterpenoids and 6 sulfonated paeoniflorin-type monoterpenoids in 56 *Paeonia* samples. (**A**) Scores plot, *open inverted triangle*: PR derived from *P. lactiflora* produced in China; *closed triangle*: sulfonated WPR in Chinese market; *closed box*: PR derived from *P. lactiflora* produced in Japan; *star*: RPR-type of *P. lactiflora* and its derivative RPR produced in Inner Mongolia, China; *open circle*: WPR-type of *P. lactiflora* cultivar cultivated in Japan; *closed diamond*: RPR-type of *P. lactiflora* cultivar cultivated in Japan; *open triangle*: RPR derived from *P. veitchii*; *cross*: *P. anomala*; *closed circle*: *P. japonica*. (**B**) Loading plot, the markers represent compounds listed in Table 3.1.

Table **3.3** Contents (mg/g) of 15 monoterpenoids in different types of peony root and the roots of four related species including *P. lactiflora*, *P. veitchii*, *P. anomala* and *P. japonica*.

			Contents (mg/g)																
			PFs									AFs				Others			
	Code No.	Botanical origions [d]	6	7	8	9	10	11	12	13	14	31	3	4	5	1	17	Total
WPR producted in China	D1 [a]	WPR-PL	19.9522	ND [b]	0.0551	0.4783	0.0032	0.4214	0.0024	0.2368	0.0008	0.1290	0.0611	5.1650	0.1205	0.0262	0.0303	26.68
	D3 [a]	WPR-PL	27.2188	ND	0.0765	0.3966	Trace [c]	0.8936	0.0007	0.1302	0.0002	0.1303	0.0735	4.4997	0.0768	0.0502	0.0850	33.63
	D16 [a]	WPR-PL	20.6398	ND	0.0558	0.4568	Trace	1.5709	0.0111	Trace	Trace	0.2362	0.0056	7.3746	0.1893	0.0841	0.0651	30.69
	D17 [a]	WPR-PL	19.4106	ND	0.0558	0.5113	Trace	1.0986	0.0004	0.1272	Trace	0.0912	0.0730	6.8556	0.1217	0.0415	0.0845	28.47
	D18 [a]	WPR-PL	1.0795	ND	Trace	0.1290	0.0034	1.8282	Trace	Trace	0.0002	Trace	0.0667	6.3677	0.1722	0.0442	0.0182	9.71
	D22 [a]	WPR-PL	0.7161	ND	0.0376	0.6517	0.0158	1.6976	0.0027	0.7351	0.0001	0.1336	0.0066	5.0689	0.1235	0.0517	0.0990	9.34
	D24 [a]	WPR-PL	1.2817	ND	0.0034	0.0794	Trace	0.6763	Trace	Trace	Trace	0.0049	0.0985	7.7771	0.1326	Trace	Trace	10.05
	D2	WPR-PL	18.1872	ND	0.0625	0.8492	0.0114	0.3179	Trace	0.2560	0.0076	0.1895	0.0518	5.7261	0.4461	0.0499	0.1838	26.34
	D4	WPR-PL	40.6239	ND	0.1294	1.1244	0.0228	2.4169	0.0052	0.5878	0.0045	0.1697	0.1626	13.2367	0.1527	0.0635	0.4997	59.20
	D23	WPR-PL	9.4589	ND	0.1125	0.5205	0.0247	1.5801	0.0146	0.9251	0.0058	0.2441	0.0795	9.8495	0.2544	Trace	0.1632	23.23
	D29	WPR-PL	18.8127	ND	0.1406	0.7145	0.0261	0.2968	Trace	0.6460	0.0042	0.2788	0.1055	6.9372	0.0518	Trace	0.0000	28.01
PR produced in China	D45	WPR-PL	37.2556	ND	0.0653	0.0865	0.0161	1.6925	Trace	0.0680	0.0064	0.1685	0.0642	0.3652	Trace	ND	0.2100	40.00
	D46	WPR-PL	24.5805	ND	0.0611	0.8799	0.0186	2.1853	Trace	0.5707	Trace	0.0072	0.1841	10.1361	0.0659	ND	0.1854	38.87
	D47	WPR-PL	48.5917	ND	0.1285	0.6655	0.0218	1.6156	0.0133	0.5054	0.0069	0.4564	0.1272	13.8726	0.3976	ND	0.3820	66.78
	D48	WPR-PL	46.2621	ND	0.0501	0.5668	0.0166	0.6177	Trace	1.5188	0.0061	0.2524	0.0494	1.9043	0.0117	ND	0.0834	51.34
	D53	WPR-PL	28.9685	ND	0.0862	0.5731	0.0105	2.2676	0.0115	0.2500	0.0030	0.1126	0.0667	3.4930	0.1178	0.0473	1.0167	37.02
PR produced in Japan	D51	WPR-PL	19.7788	ND	0.0852	0.7081	0.0069	1.3932	0.0092	0.3674	Trace	0.4041	0.0604	4.1958	0.0542	ND	0.3202	27.38
	D52	WPR-PL	20.2530	ND	0.1022	0.6908	Trace	1.6124	Trace	0.3722	Trace	0.3274	0.0092	6.3754	0.0432	ND	0.3362	30.12
	D6	WPR-PL	34.3078	ND	0.0104	0.3813	0.0246	0.9193	0.0120	1.0957	0.0038	0.0204	0.0564	4.6325	0.0455	ND	0.1763	41.69
	D7	WPR-PL	26.7963	ND	0.0818	0.0711	0.0428	2.0568	0.0092	2.0965	Trace	0.1687	0.0288	0.6136	Trace	ND	0.4490	32.41
	D8	WPR-PL	27.0581	ND	0.0724	0.6070	0.0188	0.7086	Trace	0.8929	Trace	0.0487	Trace	4.6306	0.0703	ND	0.2057	34.31
	D10	WPR-PL	30.2003	ND	0.1303	0.7488	0.0282	1.3161	0.0090	1.0045	0.0061	0.2286	0.0552	2.6546	0.0234	0.0485	0.2367	36.69
	D9	RPR-PL	29.1662	ND	0.1539	0.5095	0.0283	0.5952	0.0091	1.6091	0.0070	0.2203	Trace	4.2818	0.0339	0.0766	0.3893	37.08
RPR produced in China	D12	RPR-PL	63.6020	ND	0.1889	0.1292	0.1109	0.7580	0.0820	1.2546	0.0369	0.4637	0.0877	2.3271	0.0131	0.1978	0.0854	69.34
	D12N	RPR-PL	37.0957	ND	0.0882	0.6107	0.0425	1.0671	0.0280	0.8550	0.0166	0.2167	0.0260	2.1821	0.0183	0.0948	0.1736	42.52
	D13	RPR-PL	59.8307	ND	0.0788	1.1427	0.1206	0.1057	0.0141	1.3742	0.0353	0.1397	0.0748	1.6118	0.0499	0.0939	0.3530	65.03
	D14	RPR-PL	35.2919	ND	0.2611	0.3670	0.0542	1.5621	0.0383	1.4053	0.0177	0.2229	0.0355	0.7986	0.0220	0.1096	0.2149	40.40
	D15	RPR-PL	37.5644	ND	0.1976	0.7731	0.0975	1.6655	0.0486	1.8082	0.0340	0.3627	0.0709	0.7596	0.0178	0.1843	0.1491	43.73
	D54	RPR-PL	32.9922	ND	0.2080	0.5507	0.0663	1.3204	0.0418	1.4175	0.0190	0.2357	0.0320	0.7114	0.0206	0.0933	0.1758	37.88
	D11	PV	64.9791	Trace	0.0917	0.6543	ND	7.5476	Trace	0.0647	Trace	0.0309	0.0785	0.6765	Trace	0.0887	0.2771	74.49
	DS-1	PV	25.4056	0.0018	0.4334	0.1341	ND	5.8718	0.0600	0.1100	Trace	0.1238	0.0435	1.0806	0.0072	0.0359	0.4659	33.77
	DS-5	PV	27.7876	0.0050	0.6218	0.6316	ND	6.7813	0.0607	0.0832	0.0008	0.0379	0.0146	0.8901	0.0339	0.0513	0.2147	37.21
	DS-6	PV	29.9585	0.0018	0.6073	0.6102	ND	5.0770	0.0296	0.1118	Trace	0.1108	0.0232	2.4180	0.0243	0.0587	0.1753	39.21
	DS-7	PV	28.9500	0.0044	0.7249	0.7984	ND	4.1050	0.0062	0.0488	0.0004	0.0332	Trace	1.0985	0.0333	0.0343	0.1746	36.01
Plant specimens (wild)	P7	PV	58.7083	Trace	0.2614	0.8624	ND	7.9484	Trace	0.0653	Trace	0.0627	0.0707	0.6688	Trace	0.1527	0.0888	68.89
	P1	RPR-PL	50.4379	ND	0.0739	0.8011	0.1349	1.2358	0.0490	1.3255	0.0284	0.3237	0.0439	1.5294	0.0288	0.1929	0.0259	56.23
	PL-1	RPR-PL	20.5050	ND	0.1076	0.5313	0.1175	2.4207	0.0895	0.1480	0.0130	0.2590	0.0210	2.0432	0.0640	0.1770	0.0170	26.51
	PL-2	RPR-PL	32.8699	ND	0.1081	0.5510	0.0778	1.0631	0.0373	2.6290	0.0285	0.3069	0.0604	1.7502	0.0397	0.1574	0.0057	39.69
	PL-3	RPR-PL	23.3783	ND	0.0553	1.0077	0.0382	0.5058	0.0314	1.2607	0.0304	0.2107	0.1003	1.8112	0.0984	0.0440	0.0023	28.57
	PL-4	RPR-PL	37.8089	ND	0.0687	1.6343	0.1784	1.2856	0.0357	2.9402	0.0630	0.2581	0.0315	2.1861	0.1758	0.1263	0.0074	46.80
	P4	PA	7.4225	0.0017	0.0278	0.0166	ND	0.0464	0.0038	0.0392	ND	0.0581	Trace	3.3652	0.0114	0.0182	0.1860	11.20
	P5	PA	10.4752	0.0016	0.0277	0.1109	ND	0.0523	0.0313	0.0224	ND	0.1223	0.0216	0.3852	Trace	0.0131	0.2551	11.52
	P6	PA	15.3336	0.0018	0.0179	0.0150	ND	1.6054	0.0215	0.0290	ND	0.0814	0.0194	0.2616	Trace	0.0222	0.2961	17.71
	P15	PA	16.0300	Trace	0.0180	0.1086	ND	0.5986	Trace	0.0944	ND	0.0921	Trace	0.7252	Trace	0.0371	0.0082	17.71
	P8	PJ	22.1916	ND	0.0727	0.7345	ND	2.3243	0.0080	0.4049	ND	0.3114	0.1173	4.0080	0.0305	0.0392	0.1168	30.36
Plant specimens (cultivars)																		
WPR-PL	S4-3	Harunoniji	38.4613	ND	0.2890	0.8533	0.0735	1.0025	0.0119	0.2556	0.0357	0.1967	0.0445	1.5100	0.0107	0.0548	0.0221	42.82
	S9-4	Bridal icing	33.5716	ND	0.0859	0.7935	0.0176	2.1070	0.0153	0.2615	0.0046	0.2450	0.1044	8.5341	0.4413	0.1167	0.0362	46.33
	S11-3	Meigetsu	27.6220	ND	0.1165	0.6706	0.0107	1.0279	0.0140	0.4528	0.0040	0.7207	0.1121	5.8250	0.2546	0.0552	0.0056	36.89
	S26-3	Shirayuki	41.7715	ND	0.1543	0.6612	0.0230	0.7254	0.0199	0.9049	0.0159	0.4506	0.1457	11.5458	0.2986	0.0735	0.0151	56.81
	S31-3	Kitasaishou	39.5789	ND	0.0788	0.2840	0.0152	0.3848	0.0064	0.8260	0.0049	0.2159	0.0026	1.7274	0.0508	0.1024	Tra ce	43.28
	S34-3	Bonten	25.7849	ND	0.0813	0.2658	0.0212	3.3417	0.0554	0.5526	0.0049	0.3413	0.0447	5.3552	0.0177	0.1251	0.0515	36.04
RPR-PL	S1-4	Harunoyosooi	32.2420	ND	0.1064	0.3026	0.0247	0.6328	0.0098	1.1045	0.0022	0.0803	0.0350	9.4891	0.1436	0.0793	0.0993	44.35
	S39-5	White-Ivory	33.5077	ND	0.1061	0.7247	0.0196	2.5474	0.0461	0.3013	0.0054	0.3466	Trace	4.6708	0.4709	0.1374	0.0310	42.91
	S48-3	Hanakago	29.6023	ND	0.1049	0.6574	Trace	1.6574	0.0118	0.2445	Trace	0.2267	0.1727	4.0513	0.3315	0.0714	0.0207	37.15
	S78N	Edulis Superba	39.1449	ND	0.2013	0.6880	0.0371	2.8490	0.0300	1.2879	0.0092	0.3041	0.0631	15.3102	0.0300	0.0827	0.0585	60.10

[a] Samples were processed by sulfur-fumigation

[b] ND: below the LOD

[c] Trace: bleow the LOQ

[d] WPR-PL: WPR-type of *P. lactiflora* ; RPR-PL: RPR-type of *P. lactiflora* ; PV: *P. veitchii* ; PA: *P. anomala* ; PJ: *P. Japonica*

Summary of Chapter Ⅲ

1) MS/MS fragmentation pathways of PF-, AF- and PS-types of monoterpenoids showed the different specific neutral losses patterns and characteristic product ions which were used to identify different types of monoterpenoids and also provided basic clues for monoterpenoids profiles. 30 monoterpenoids were unambiguously and tentatively identified.

2) Monoterpenoids composition of *P. lactiflora* and *P. veitchii* obviously differed from *P. anomala* and *P. japonica*. Besides the predominant common monoterpenoids (**4**, **6**, **9**, **11**, **13**, **17**) showed in all the four species, 4-*O*-methyl-paeoniflorin (**7**) was only detected in *P. veitchii* and *P. anomala*, whereas mudanpioside C (**10**) only was observed in *P. lactiflora*. These specific and distinctive constituents could be used as potential markers for identification and discrimination of different types of PR and the related species.

3) Total contents of fifteen monoterpenoids were obviously higher in *P. lactiflora* and *P. veitchii* than in *P. japonica* and *P. anomala*.

4) Among the commercial samples derived from *P. lactiflora*, RPR showed obviously high contents of paeoniflorin-type monoterpenoids such as compounds **8**, **10**, **12**, **13** and **14**, but low contents of albiflorin-type monoterpenoids (**3**-**5**). *P. veitchii* had the highest content of salicylpaeoniflorin (**8**) and galloylpaeoniflorin (**11**), clearly differed from the samples derived from other three species.

5) Monoterpenoids composition between RPR-type and WPR-type of *P. lactiflora* cultivars showed high similarities, which differed from the situation between the commercial WPR and RPR.

Conclusion

1) In the present study, 43 compounds, including three new monoterpenoids (**1**-**3**) from the crude drug sample of RPR derived from *P. lactiflora* and one new norneolignan (**30**) from the root of RPR-type of *P. lactiflora* cultivar were afforded. They are mainly monoterpenoids, as well as flavonoids, lignan and other types of compounds.
2) Monoterpenoids, as the representative components in PR, especially with paeoniflorin-type skeleton exhibited moderate inhibitory effect against IgE-mediated degranulation. Among them, paeoniflorol (**1**), salicylpaeoniflorin (**8**) and galloylpaeoniflorin (**11**) from RPR, and mudanpioside E (**31**) from Edulis Superba showed stronger activity than others.
3) Monoterpenoids profiling clarified the characteristics of the four species in sect. *Paeonia*, and the marker constituents for their discrimination. Quantification of fifteen monoterpenoids revealed considerable contents variation in the representative monoterpenoids, and the total contents in *P. lactiflora* and *P. veitchii* were obviously higher than in *P. anomala* and *P. japonica*. The four species as well as the different types of PR showed their feature composition of the paeoniflorin-type monoterpenoids.
4) RPR showed higher anti-allergic activity than WPR. The active constituents in RPR responsible for the anti-allergic effect as well as its monoterpenoids profiles were elucidated.
5) Edulis Superba, the horticultural cultivar of *P. lactiflora*, was selected as a candidate of new resource of RPR with anti-allergic activity, and its active constituents and monoterpenoids profiling have been evaluated.

General Experimental

General Experimental

4.1 Experiment on Chapter I and II

4.1.1 Experimental instrumentation and chemical reagents

NMR spectra were recorded on a JEOL ECX-400 spectrometer (^{1}H, 400MH_Z; ^{13}C, 100M H_Z) with TMS as an internal standard. ESI-MS spectra were measured by Agilent HPLC system with ion-trap mass spectrometer fitted with an electrospray ionization source (Bruker Daltonics Inc., Germany). HR-ESI-MS spectra were employed by Accella HPLC system equipped with an Orbitrap-EX mass spectrometer (Thermo-Fisher Scientific Inc., USA). IR spectra were measured by using a JASCO FT/IR-460 Plus spectrophotometer (JASCO Internal Co., Ltd., Japan). Optical rotations were detected by using a JASCO P2100 digital polarimeter (JASCO Internal Co., Ltd., Japan). Column chromatography (CC) was performed with normal-phase (Wakogel® C-200 and 300HG, Wako Pure Chemical Industries, Ltd., Japan), reverse-phase [ODS-A (12 nm, s-150 μm) and ODS-AQ-HG (12nm, s-50 μm), YMC Co., Ltd., Japan], and Sephadex LH-20 (GE Healthcare Life Sciences, Sweden). Preparative HPLC was performed on a Waters HPLC system (Waters Co., Ltd., USA) equipped with a Delta 600 pump and 2489 UV detector by using YMC-Pack ODS-A column (5μm, 250 × 20 mm i.d.). TLC was carried out to monitor fractions on precoated silica gel (60F_{254}) or RP-18 (F_{254}) plates (0.50 mm thickness, Merck, Germany). Spots were visualized under UV (254 and 360 nm) and/or monitored by spraying with the solution of 10% sulfuric acid in ethanol (v/v). A HPLC system (Shimadzu, Kyoto, Japan) consisting of an LC-20AD binary pump, DGU-14A degasser, SIL-20AC autosampler, and a CTO-20AC column oven were employed for chemical purity determination and sugar analysis. HPLC grade of formic acid, acetonitrile and ultrapure water; and all solvents with analytical grade used for

extraction and isolation; as well as several chemical reagents such as L-cysteine methyl ester hydrochloride, phenyl isothiocyanate, L-(-)-glucose (purity: ≥ 98%), D-(+)-glucose (purity: ≥ 98%), D-(-)-xylose (purity: ≥ 98%), L-(-)-xylose (purity: ≥ 97%) and Pyridine were purchased from Wako Pure Chemical Industries, Ltd.

4.1.2 Extraction and isolation of compound from D27967 (Chapter I)

The dried roots (1.5 kg) were cut into small pieces and then extracted repeatly with methanol (3 × 2.5 L) by ultrasonic for 2 h at room temperature. The MeOH-extract (280.0 g) was obtained after removing the solvent under vacuum, and then was subjected to Daion HP 21 CC eluted with a gradient of H_2O and MeOH as 0, 20%, 40%, 60%, 80% and 100% MeOH (v/v), respectively. The anti-allergic activity assay showed that the 60% and 80% aqueous MeOH-elutions had significant inhibitory activities against β-hexosaminidase release in IgE-sensitized RBL-2H3 cells, so the two elutions were further isolated for tracking the active constituents. The 60% MeOH-elution (30.8 g) was suspended in water and partitioned with EtOAc and *n*-BuOH, respectively. The *n*-BuOH-soluble fraction was then subjected to YMC-ODS-A CC eluted with MeOH-H_2O (1:9 to 1:0, v/v) to obtain six fractions (60MBF-1 to 6). The 60MBF-2 was further subjected to YMC-ODS AQ CC eluted with MeOH-H_2O (1:9 to 1:0, v/v) to give seven fractions (60MBF-2-1 to 7). 60MBF-2-3 was separated by using preparative HPLC eluted with isocratic acetonitrile (CH_3CN)-H_2O (15:85, v/v) to give **1** (6.0 mg) and **8** (5.0 mg). Compound **11** (20.0 mg) was obtained from 60MBF-2-4 by using Sephadex LH-20 CC with MeOH-H_2O (6:4, v/v). The 60MBF-3 was separated by YMC-ODS-A CC eluted with MeOH-H_2O (1:1, v/v) to give four fractions (60MBF-3-1 to 4). Compounds **7** (5.0 mg) and **13** (3.0 mg) were purified from 60MBF-3-1 by using preparative HPLC with

isocratic elution of CH_3CN-H_2O (15:85, v/v). The 60MBF-3-2 was further separated by Sephadex LH-20 CC eluted with MeOH-H_2O (6:4, v/v) to give **16** (2.0 mg) and **19** (60.0 mg). Compounds **20** (7.0 mg) and **21** (7.0mg) were purified from 60MBF-4 by using preparative HPLC eluted with isocratic CH_3CN-H_2O (17:83, v/v). The 60MBF-5 was separated by using preparative HPLC eluted with CH_3CN-H_2O (12:88, v/v) to give **18** (10.0 mg), **22** (1.0 mg) and **26** (31.0 mg).

In addition, 80% MeOH-elution (5.9 g) was subjected to a normal phase silica gel CC eluted by CH_3Cl-MeOH with gradient condition from 1:0 to 1:5 (v/v) to give seven fractions (80MF-1 to 7). The 80MF-1 was purified by using the preparative HPLC with CH_3CN-H_2O (30:70, v/v) to give **4** (10.0 mg), **6** (18.0 mg), **24** (8.0 mg) and **25** (130.0 mg). The 80MF-2 was separated by using preparative HPLC eluted with CH_3CN-H_2O (30:70, v/v) to obtain **27** (9.0 mg), and **28** (10.0 mg). Compounds **17** from 80MF-3 (10.0 mg) and **9** (100.0 mg) from 80MF-5 were purified under the same pre-HPLC condition. The 80MF-7 was further separated by Sephadex LH-20 CC with MeOH-H_2O (6:4, v/v) to give ten subfractions (80MF-7-1 to 10). The isolation of **12** (20.0 mg) and **15** (5.0 mg) from 80MF-7-2, **10** (50.0 mg) and **14** (40.0 mg) from 80MF-7-4 were achieved by using preparative HPLC with CH_3CN-H_2O (30:70, v/v). The isolation of **3** (8.0 mg) and **5** (33.0 mg) from 80MF-7-3, and **2** (2.0 mg) from 80MF-7-5, **29** (10.0 mg) from 80MF-7-9 and **23** (4.0 mg) from 80MF-7-10 were also achieved on the preparative HPLC eluted with CH_3CN-H_2O (20:80, v/v).

4.1.3 Extraction and isolation of compound from S78N (Chapter II)

The dried root (1.2 kg) was cut into small pieces and then extracted repeatedly with methanol (3 × 2.0 L) by ultrasonic for 2 h at room temperature. The MeOH extract (330.0 g) was obtained after removing the solvent under vacuum, and then was

suspended in water and partitioned with EtOAc and *n*-BuOH, respectively. The anti-allergic activity assay showed that the EtOAc and *n*-BuOH-soluble fractions had potent activities against β-hexosaminidase release in IgE-sensitized RBL-2H3 cells, so the two elutions were further isolated for tracking the active constituents. EtOAc-soluble fraction (100.0 g) was subjected to normal phase silica gel CC using $CHCl_3$-MeOH gradient elution from 100:1 to 2:1 (v/v) to obtain eight fractions (EF-1~8). EF-1 was separated by preparative HPLC eluted with isocratic acetonitrile (CH_3CN)-H_2O (30:70, v/v) to give **24** (12.0 mg). Compounds **25** (100.0 mg) from EF-5 and **7** (10.0 mg) from EF-6 were obtained by using preparative HPLC eluted with isocratic CH_3CN-H_2O (30:70, v/v). EF-7 was subjected to YMC-ODS-A CC and eluted with MeOH-H_2O (1:9 to 1:0, v/v) to give eight fractions (EF-7-1~8). EF-7-6 was further separated by YMC-ODS-AQ CC eluted with MeOH-H_2O (2:8 to 1:0, v/v) to get five fractions (EF-7-6-1~5). Compounds **34** (4.0 mg) from EF-7-6-4, and **19** (10.0 mg) from EF-7-6-2 were obtained by using preparative HPLC eluted with CH_3CN-H_2O (15:85, v/v). EF-7-7 was subjected to YMC-ODS-Rp18 CC eluted with MeOH-H_2O (2:8 to 1:1, v/v) to obtain seven fractions (EF-7-7-1~7). The EF-7-7-1 was purified by using preparative HPLC eluted with CH_3CN-H_2O (10:90, v/v) to give **28** (11.0 mg) and **29** (8.0 mg). Compounds **6** (100.0 mg) and **4** (50.0 mg) were purified from EF-7-7-2 and EF-7-7-3 by using preparative HPLC eluted with CH_3CN-H_2O (15:85, v/v). Compounds **37** (5.0 mg) and **38** (4.0 mg) were purified from EF-7-7-4 by using the preparative HPLC eluted with CH_3CN-H_2O (12:88, v/v). EF-7-7-5 was subjected to Sephadex LH-20 CC eluted with MeOH-H_2O (6:4, v/v) to give thirteen fractions (EF-7-7-5-1~13), compounds **30** (5.0 mg) and **8** (3.0 mg) were obtained from EF-7-7-5-4 by using preparative HPLC eluted with CH_3CN-H_2O (25:75, v/v). Compounds **32** (8.0 mg) and **33** (8.0 mg) were obtained from EF-7-7-5-8 and EF-7-7-5-9 by using the preparative HPLC eluted with CH_3CN-H_2O (20:80, v/v),

respectively. In addition, the *n*-BuOH-soluble fractions (50.0 g) was subjected to Daion HP-21 CC eluted with a gradient of H_2O and MeOH as 0%, 30%, 60% and 100% MeOH (v/v), respectively. 30%MeOH-soluble layer was subjected to YMC-ODS-A CC eluted with MeOH-H_2O (5:95 to 90:10, v/v) to obtain eight fractions (30MF-1~8). 30MF-4 was further subjected to YMC-ODS-RP18 CC eluted with MeOH-H_2O (1:9 to 6:4, v/v) to give six fractions (30MF-4-1~6). 30MF-4-2 was separated by using Sephadex LH-20 CC eluted with MeOH-H_2O (6:4, v/v) to give seven fractions (30MF-4-2-1~7). Compounds **20** (3.0 mg) and **21** (3.0 mg) were purified from 30MF-4-2-5, **35** (20.0 mg) and **36** (4.0 mg) from 30MF-4-2-4 by using preparative HPLC eluted with CH_3CN-H_2O (12:88, v/v). 30MF-4-3 was further eluted with MeOH-H_2O (1:9 to 6:4, v/v) by using YMC-ODS-AQ CC to give four fractions (30MF-4-3-1~4). Compounds **42** (3.0 mg) and **43** (8.0 mg) were purified from 30MF-4-3-1 by using preparative HPLC eluted with CH_3CN-H_2O (12:88, v/v). Compounds **39** (5.0 mg) and **13** (380.0 mg) were obtained from 30MF-4-3-2 and 30MF-4-3-3 by using preparative HPLC eluted with CH_3CN-H_2O (13:87, v/v), respectively. 30MF-4-3-4 was subjected to the preparative HPLC eluted with CH_3CN-H_2O (13:87, v/v) to achieve **31** (10.0 mg). Compound **40** (17.0 mg) were purified from 30MF-4-4 and 30MF-4-5 by using preparative HPLC eluted with CH_3CN-H_2O (13:87, v/v). Furthermore, 30MF-5 was further subjected to YMC-ODS-AQ CC eluted with MeOH-H_2O (1:9 to 1:0, v/v) to give nine fractions (30MF-5-1~9). 30MF-5-8 was further separated by using YMC-ODS-AQ CC eluted with MeOH-H_2O (2:8 to 1:1, v/v) to give 6 fractions (30MF-5-8-1~6). 30MF-5-8-3 was purified by using the preparative HPLC eluted with CH_3CN-H_2O (13:87, v/v) to afford **40** (35.0 mg). 30MF-5-8-6 was subjected to YMC-ODS-RP18 CC eluted with MeOH-H_2O (15:85, v/v) to obtain six fractions (30MF-5-8-6-1~6). Compounds **41** (3.5 mg) and **19** (14.0 mg) were obtained from 30MF-5-8-6-3 and 30MF-5-8-6-6 by

using preparative HPLC eluted with CH_3CN-0.1% acetic acid in H_2O (12:88, v/v), respectively.

4.1.4 Acidic hydrolysis and sugar analysis of new compounds

Acid hydrolysis of new compounds **1**-**3** and **30** were carried out using a procedure similar to previous report [Tanaka et al. 2007]. Briefly, each new compound (1.0 mg) was hydrolyzed by heating in 1.0 M HCl (0.2 ml) at 90°C in a stoppered vial for 2 h and neutralized with Amberlite IRA-400. After drying in vacuum, the residue was dissolved in pyridine (0.1 ml) containing L-cysteine methyl ester hydrochloride (0.5 mg) and heated at 60 °C for 1 h, and then 0.1 ml solution of phenyl isothiocyanate (0.5 mg) was added to the mixture and heated at 60 °C for 1 h. The reaction solution was directly analyzed by HPLC-DAD system to determine the D/L configuration of sugar moieties by comparing their retention time (t_R) with these authentic D- and L-glucose, D- and L-xylose derivatives (t_R: D-glucose, 21.05 min; L-glucose, 22.02 min; D-xylose, 25.10 min; and L-xylose, 27.05 min).

4.1.5 Cell culture and treatment

4.1.5.1 Measurement of β-hexosaminidase release in IgE-sensitized RBL-2H3 cells

The inhibitory effects of the test samples against DNP-BSA stimulated β-hexosaminidase release in IgE-sensitized RBL-2H3 cells (Cell No. RCB2782, RIKEN Cell Bank, Tsukuba, Japan) were evaluated by a previously reported method [Matsuda et al. 2002]. Briefly, RBL-2H3 cells were dispended into 96-well culture plates at a concentration of 4×10^5 cells/well using Eagle's Minimum Essential Medium (MEM, Gibco, Thermo Fisher Scientific Inc., USA) supplementing with 10%

Fetal Bovine Serum (FBS, 12003C, Sigma-Aldrich Co., LLC., USA), penicillin (100U/ml, Sigma-Aldrich) and streptomycin (100 μg/ml, Sigma-Aldrich) and cultured overnight at 37°C in 5% CO_2. After washing with phosphate-buffered saline (PBS, Gibco), the cells were sensitized with 0.5 μg/ml of anti-DNP IgE (D8406, Sigma-Aldrich) for 24 h, washed twice with 200 μl Siraganian buffer [119 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 25 mM piperazine-*N,N'-bis* (2-ethanesulfonic acid) (PIPES), 40 mM NaOH; pH 7.2] and incubated in 160 μl Siraganian (+) buffer [Added 5.6 mM glucose, 1 mM $CaCl_2$ and 0.1 % bovine serum albumin (BSA, A7030, Sigma-Aldrich) in Siraganian buffer; pH 7.2] for 10 min at 37 °C. Subsequently, 50 μl of test sample solution was added into each well and incubated for 30 min, followed by 50 μl DNP-BSA (1.0 μg/ml, A23018, Thermo Fisher Scientific Inc.) as an antigen for 1 h to activate cells and to evoke allergic reactions (degranulation). The reaction was stopped by cooling in an ice-bath for 10 min, and 100 μl of the supernatant were transferred to 96-well plate and incubated with 50 μl of substrate (3.3 mM *p*-nitrophenyl-N-acetyl-β-D-glucosaminide) for 1 h at 37 °C.The reaction was stopped by adding 100 μl of alkaline buffer (0.1 M $NaHCO_3$ and 0.1 M Na_2CO_3; pH 10) and cooled in an ice-bath for 10 min. The absorbance at 405 nm was measured using microplate reader (Infinite® F200, Swizerland). The test samples were dissolved in dimethylsulfoxide (DMSO) and Siraganian (+) buffer (final DMSO concentration was less than 0.1%).

The inhibitory activity against β-hexosaminidase release of the test samples was calculated using the following equation:

$$\text{Inhibition (\%)} = [1 - (A_t - A_b - A_n) / (A_c - A_n)] \times 100$$

A_t: test, DNP-BSA (+), sample (+) A_b: blank, DNP-BSA (-), sample (+)

A_n: normal, DNP-BSA (-), sample (-) A_c: control, DNP-BSA (+), sample (-)

The results were expressed as mean ± S.D. for three independent experiments.

One-way ANOVA followed by post-hoc Dunnett's test or Chi-square test were used for statistical analysis, and IC_{50} values were calculated by using SPSS software (version 15.0, IBM, USA).

4.1.5.2 Measurement of cell viability

The cytotoxicity of extract, fractions and isolated compounds on RBL-2H3 cells was evaluated with CCK-8 (Dojindo laboratories Co. Ltd., Japan) according to manufacturer's instructions. Briefly, anti-DNP IgE-sensitized cells were treated with various sample solutions and stimulated by DNP-BSA as described above. After wash with PBS, 100 μl 10% FBS-MEM containing 10 μl WST-8 was added to each well and incubated for 3 h. Then, the absorbance at 450 nm was measured by using a microplate reader. The percentage of cell viability was calculated according to the following formula: Cell viability (%) = (Mean absorbance of test samples / Mean absorbance of vehicle control) × 100.

4.2 Experiment on Chapter III

4.2.1 Plant and crude drug materials

Eleven specimens of four *Paeonia* species including *P. lactiflora, P. veitchii, P. anomala* and *P. japonica* were collected from China, Japan and Mongolia. Eleven horticultural cultivars of *P. lactiflora* were harvested from four to eight years in Toyama Prefectural Medicinal Plants Center, Japan (Table S1). Twenty-nine commercial samples of PR were derived from *P. lactiflora* including WPR and RPR produced in China and PR produced in Japan. Five samples of *P. veitchii* were collected or purchased from Chinese and Japanese markets (Table S2). Their botanical origins were identified by genetic analysis of nrDNA ITS sequence [Zhu et al., 2015]. The vouchers are deposited in the Museum of Materia Medica, Institute of Natural Medicine (TMPW), University of Toyama, Japan.

4.2.2 Chemicals and reagents

The following 15 monoterpenoids were isolated and purified from the root of *P. lactiflora* in our previous studies [Shi et al., 2016a; Shi et al., 2016b]: paeoniflorin (**6**), 4-*O*-methyl-paeoniflorin (**7**), salicylpaeoniflorin (**8**), benzoylpaeoniflorin (**9**), mudanpioside C (**10**), galloylpaeoniflorin (**11**), mudanpioside J (**12**), oxypaeoniflorin (**13**), benzoyloxypaeoniflorin (**14**) and mudanpioside E (**31**) with paeoniflorin-type skeleton; 4-*epi*-albiflorin (**3**), albiflorin (**4**) and paeonivayin (**5**) with albiflorin-type skeleton; paeoniflorol (**1**), lactiflorin (**17**) with other type skeletons. The purities of them were determined to be higher than 98.0% through HPLC-DAD analysis. Geniposide (**IS**) used as internal standard was purchased from Wako Pure Chemical Industries, Ltd., Japan. HPLC-grade acetonitrile, methanol and ultrapure water, and analytical-grade ethanol for extraction were purchased from Wako Pure Chemical

Industries, Ltd.

4.2.3 Preparation of standard solutions

Each of 15 reference compounds was accurately weighted and then dissolved in ethanol-water (75:25, v/v) to prepare the standard stock solutions of 1.0 mg/ml. A series of standard solutions (0.01, 0.1, 1.0 and 10.0 μg/ml) were prepared by appropriate dilution of the stock solutions to make calibration curves. The working solutions were stored at 4 ºC for further analysis.

4.2.4 Sample preparation

The sample solution was prepared according to the previously reported method [Zhu et al., 2015]. The respective samples were pulverized and then screened through a 300 μm sieve to obtain homogeneous fine powder. 0.3 g of the fine powder was accurately weighted and extracted with 75% ethanol (9 ml, 8 ml × 2) by ultrasonication at room temperature for 30 min, mixed periodically by vortex to obtain full extraction. The supernatant was then obtained by centrifugation at 2,500 rpm (Kubota 3740, Japan) for 15 min. Supernatants were combined into a 25.0 ml volumetric flask and finally filled with 75% ethanol to the volume. After filtration through a 0.22 μm Millipore filter unit (Advantec, Japan), 1.0 μl of this solution was injected into the LC-MS-IT-TOF system for analysis.

4.2.5 HPLC conditions

HPLC system (Shimadzu, Kyoto, Japan) consisted of an LC-20AD binary pump, a DGU-14A degasser, a SIL-20AC autosampler, a CTO-20AC column oven and a SPD-M20A DAD. The mobile phase consisted of acetonitrile containing 0.1% formic

acid (v/v) (A) and water containing 0.1% formic acid (v/v) (B). The sample solutions were analyzed on a YMC Pack ODS-AQ column (3 μm, 2.0 mm × 150 mm) with a gradient elution system as followns: 0-10min, 5%-10% A; 10-20min, 10%-15% A; 20-25min, 15% A; 25-30min, 15%-20% A; 30-35min, 20% A; 35-45min, 20%-25% A, 45-50min, 25%-40% A, 50-60min, 40%-90% A. The flow rate was 0.2 ml/min, and the column temperature was set at 40 °C. The DAD detector was monitored at 254 nm, and the on-line UV spectra were recorded in the range of 190-600 nm.

4.2.6 ESI-IT-TOF-MS analysis

A hybrid ion trap time-of-flight (IT-TOF) mass spectrometer (Shimadzu, Tokyo, Japan) equipped with electrospray ionization (ESI) source interface was connected to the above Shimadzu HPLC system. TOF mass spectrometer was calibrated using a lock-mass trifluoroacetic acid sodium solution to increase mass accuracy. The optimized mass parameters in the negative mode were set as follows: Detector voltage, 1.75 kV; spray voltage, (-)-3.5 kV; nebulizing gas (N_2) flow, 1.5 l/min; dry gas (N_2) pressure, 100 kPa; curved desolvation line (CDL) temperature, 200°C; heat block temperature, 200°C. Molecular weight acquisition was performed from *m/z* 100 to 2000 for MS and *m/z* 100 to 1000 for MS/MS. The ion accumulation time was set at 30 ms, and the collision energy of collision-induced dissociation (CID) was set as 50%. Data acquisition and processing were performed with the LCMS solution version 3.41 software package (Shimadzu, Tokyo, Japan).

4.2.7 Statistical and multivariate analysis

The data matrix (21 markers × 56 samples) was employed using unsupervised

principal component analysis (PCA). PCA was performed using SIMCA-P (version 11.5, Umetrics, Sweden) to generate an overview for group clustering.

Table **S1** Plant specimens used in the Chapter III

	Plant	Location for collecting plant	W/C [a]	Voucher No.	Code No.	Collection Date
Speciemens	*Paeonia lactiflora* Pallas	Duolun, Inner Mongolia, China	W	Wei S.L., 2007-1	P1	2007.01.28
	P. lactiflora	Arong, Inner Mongolia, China	W	Komatsu K. et al. HJN67	PL-1	2012.07.20
	P. lactiflora	Arong, Inner Mongolia, China	W	Komatsu K. et al. HJN70	PL-2	2012.07.20
	P. lactiflora	Chifeng, Inner Mongolia, China	W	Komatsu K. et al. HJN252	PL-3	2012.08.04
	P. lactiflora	Duolun, Inner Mongolia, China	W	Komatsu K. et al. HJN295	PL-4	2012.08.05
	P. anomala Linn.	Tuluugiyn davas-Hutag-Ondor, Bulgan, Mongolia	W	Komatsu K. et al., M505	P4	2002.07.20
	P. anomala	Jingiin halzan-Bayannuul, Uvs, Mongolia	W	Komatsu K. et al., M755-1	P5	2002.07.29
	P. anomala	Jingiin halzan-Bayannuul, Uvs, Mongolia	W	Komatsu K. et al., M755-2	P6	2002.07.29
	P. anomala	Bayanchandmani, Tov, Mongolia	W	Komatsu K. et al., MII 127	P15	2015.08.01
	P. veitchii Lynch	Ganzi, Sichuan, China	W	Komatsu K. et al., S-1	P7	1996.07.10
	P. japonica Miyabe et Takeda	Toyama, Japan	C	Murakami M., MM-4	P8	2008.12.04
Cultivars of *P. lactiflora*	Harunoniji	TPMPC [b], Toyama, Japan	C		S4-3	2009
	Bridalicing	TPMPC, Toyama, Japan	C		S9-3	2009
	Meigetsu	TPMPC, Toyama, Japan	C		S11-3	2009
	Shirayuki	TPMPC, Toyama, Japan	C		S26-3	2009
	Kitasaishou	TPMPC, Toyama, Japan	C		S31-4	2010
	Bonten	TPMPC, Toyama, Japan	C		S34-3	2009
	Harunoyosooi	TPMPC, Toyama, Japan	C		S1-3	2009
	White-Ivory	TPMPC, Toyama, Japan	C		S39-4	2010
	Hanakago	TPMPC, Toyama, Japan	C		S48-5	2011
	Edulis Superba	TPMPC, Toyama, Japan	C		S78N	2013

[a] W: Wild; C: Cultivated

[b] TPMPC: Toyama Prefectural Medicinal Plants Center

Table **S2** Crude drug samples analyzed in Chapter III

Drug Name	Producing area	Obtained from	Code No.	Identity [a]	Collection Date	TMPW No.
WPR	Bozhou, Anhui, China	Bozhou, Anhui, China	D1	WPR-PL	2006.05.02	25071
WPR	Bozhou, Anhui, China	Bozhou, Anhui, China	D2	WPR-PL	2006.05.02	25072
WPR	Bozhou, Anhui, China	Bozhou, Anhui, China	D3	WPR-PL	2006.10.17	25244
WPR	Bozhou, Anhui, China	Bozhou, Anhui, China	D17	WPR-PL	2006.05.02	25073
WPR	Anhui, China	Hohhot, Inner Mongolia, China	D23	WPR-PL	2010.10.14	26975
WPR	Zhongjiang, Sichuan, China	Jihuang, Zhongjiang, Sichuan, China	D4	WPR-PL	2002.09.23	25820
WPR	Zhejiang, China	Cenxi, Guangxi, China	D18	WPR-PL	2008.09.06	25973
WPR	Hangzhou, Zhejiang, China	Hohhot, Inner Mongolia, China	D22	WPR-PL	2010.10.13	26974
WPR	Panan, Zhejiang, China	Panan, Zhejiang, China	D29	WPR-PL	2009.08.05	26620
WPR	Jiangsu, China	Hohhot, Inner Mongolia, China	D24	WPR-PL	2010.10.16	26976
RPR	Sichuan, China	Cenxi, Guangxi, China	D16	RPR-PL	2008.09.06	25974
RPR	Inner Mongolia, China	Chifeng, Inner Mongolia, China	D12	RPR-PL	2002.09.14	21565
RPR	Inner Mongolia, China	Chifeng, Inner Mongolia, China	D12N	RPR-PL	2013.10.30	27967
RPR	Inner Mongolia, China	Bozhou, Anhui, China	D13	RPR-PL	2006.05.01	25047
RPR	China	Uchida Wakanyaku Co., Ltd., Tokyo, Japan	D14	RPR-PL	2008.10.07	26401
RPR	Inner Mongolia, China	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D15	RPR-PL	2008.10.16	26406
RPR	China	Matsuura Co., Ltd., Nagoya, Japan	D54	RPR-PL	2012.10.04	27893
RPR	Ganzi, Sichuan, China	Ganzi, Sichuan, China	D11	PV	1996.07.11	17304
RPR	Sichuan, China	Hehuachi, Sichuan, China	DS-1	PV	1995.09.17	16306
RPR	Aba, Sichuan, China	Chengdu, Sichuan, China	DS-5	PV	2014.09.22	28405
RPR	Lixian, Sichuan, China	Lixian, Sichuan, China	DS-6	PV	2014.09.22	28396
RPR	Maerkang, Sichuan, China	Lixian, Sichuan, China	DS-7	PV	2014.09.22	28397
PR	Zhejiang, China	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D45	WPR-PL	2012.09.04	27887
PR	Sichuan, China	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D46	WPR-PL	2012.09.04	27888
PR	Anhui, China	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D47	WPR-PL	2012.09.04	27889
PR	China	Uchida Wakanyaku Co., Ltd., Tokyo, Japan	D48	WPR-PL	2012.09.04	27890
PR	China	Matsuura Co., Ltd., Nagoya, Japan	D53	WPR-PL	2012.10.04	27892
PR (Bonten)	Toyama, Japan	Nara, Japan	D51	WPR-PL	2008.02.15	25835
PR (Bonten)	Toyama, Japan	Nara, Japan	D52	WPR-PL	2008.02.15	25836
PR	Niigata, Japan	Uchida Wakanyaku Co., Ltd., Tokyo, Japan	D6	WPR-PL	2008.10.07	26400
PR (Yamato shakuyaku)	Nara, Japan	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D7	WPR-PL	2008.02.14	25818
PR (Yamato shakuyaku)	Hokkaido and Nagano, Japan	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D8	WPR-PL	2008.02.14	25819
PR (Yamato shakuyaku)	Nara, Japan	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D10	WPR-PL	2008.10.31	26398
PR (Yamato shakuyaku)	Nara, Japan	Tochimoto Tenkaido Co., Ltd., Osaka, Japan	D9	RPR-PL	2008.10.31	26107

[a] The botanical origion of each sample was identified by ITS sequence. WPR idicates WPR-type of *P. lactiflora* , RPR indicates RPR-type of *P. lactiflora* . PV represents *P. veitchii*.

References

Abdel-Hefez AA, Meselhy MR, Nakamura N, Hattori M, Watanabe H, Murakami Y, El-Gendy MA, Mahfouz NM, Mohamed TA. (1998) Effects of paeoniflorin derivatives on scopolamine-induced amnesia using a passive avoidance task in mice: structure-activity relationship. Biol Pharm Bull 21: 1174-1179.

Ahmed M, Al-howiriny TA. (2007) Two new hydroxyl chalcone derivatives from *Thymus cilicicus*. Zeitschrift für Naturforschung B 62: 121-124.

An RB, Kim HC, Lee SH, Jeong GS, Sohn DH, Park H, Kwon DY, Lee JH, Kim YC. (2006) A new monoterpene glycoside and antibacterial monoterpene glycosides from *Paeonia suffruticosa*. Arch Pharm Res 29: 815-820.

Arnone A, Di Modugno V, Nasini G, Venturini I. (1988) Isolation and structure determination of new active neolignans and norneoligans from *Ratanhia*. Gazz Chim Ital 118: 675-682

Braca A, Kiem PV, Yen PH, Nhiem NX, Quang TH, Cuong NX, Minh CV. (2008) New monoterpene glycosides from *Paeonia lactiflora*. Fitoterapia 79: 117-120.

Cao TQ, Tran MH, Kim JA, Tran PT, Lee JH, Woo MH, Lee HK, Min BS. (2015) Inhibitory effects of compounds from *Styrax obassia* on NO production. Bioorg Med Chem Lett 25: 5087-5091.

Chen B, Liu Y, Liu HW, Wang LN, Yang BF, Yao XS. (2008) Iridoid and aromatic glycosides from *Scrophularia ningpoesis* Hemsl. and their inhibition of $[Ca^{2+}]_i$ increase induced by KCL. Chem Biodivers 5: 1723-1735.

Choi SE, Park KH, Han BH, Jeong MS, Seo SJ, Lee DI, Joo SS, Lee MW. (2011) Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by phenolic compounds from roots of *Rhododendron mucronulatum*. Phytothe Res 25: 1301-1305.

Choo MK, Park EK, Han MJ, Kim DH. (2003) Antiallergic activity of Genseng and its ginsenosides. Planta Med 69: 518-522.

Chuang MJ, Sohng JK, Choi DJ, Park YI. (2013) Inhibitory effect of phloretin and biochanin A on IgE-mediated allergic responses in rat basophilic leukemia RBL-2H3 cells. Life Sci 93:

401-408.

Chung HJ, Ferl RJ. (1999) Arabidopsis alcohol dehydrogenase expression in both shoots and roots is conditioned by root growth environment. Plant Physiol 121: 429-436.

Davis AL, Cai Y, Davis AP, Lewis JR. (1996) ^{1}H and ^{13}C NMR assignments of some green tea polyphenols. Magn Reson Chem 34: 887-890.

Ding LQ, Jian ZH, Liu Y, Chen LX, Zhao Q, Yao XS, Zhao F, Qiu F. (2012) Monoterpene inhibitors of NO production from *Paeonia suffruticosa*. Fitoterapia 83: 1598-1603.

Dong H, Liu Z, Song F, Yu Z, Li H, Liu S. (2007) Structural analysis of monoterpene glycosides extracted from *Paeonia Lactiflorin* Pall. using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry and high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 21: 3193-3199.

Duan JW, Yang JY, Chen LX, Zhang LJ, Jiang ZH, Cai XD, Zhang X, Qiu F. (2009) Monoterpenes from *Paeonia albiflora* and their inhibitory activity on nitric oxide production by lipopolysaccharide-actived microglia. J Nat Prod 72: 1579-1584.

Ellmeier W, Abramova A, Schebesta A. (2011) Tec family kinases: regulation of FcεRI-mediated mast cell activation. FEBS J 278: 1990-2000.

Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CMM, Tsai M. (2005) Mast cells “tunable” effector and immunoregulatory cells: recent advances. Annu Rev Immunol 23: 749-786.

Guo L, Zeng SL, Zhang Y, Li P, Liu EH (2016) Comparative analysis of steroidal saponins in four Discoreae herbs by high performance liquid chromatography coupled with mass spectrometry. J Pharm Biomed Anal 117: 91-98.

Hattori M, Shu YZ, Shimizu M, Hayashi T, Morita N, Kobashi K, Xu GJ, Namba T. (1985) Metabolism of paeoniflorin and related compounds by human intestinal bacteria. Chem Pharm Bull 33: 3838-3846.

Hayashi T, Shinbo T, Shimizu M, Arisawa M, Morita N, Kimura M, Matsuda S, Kikuchi T. (1985) Paeonilactone-A, -B and -C, new monoterpenoids from paeony root. Tetrahedron Lett 26: 3699-3702.

He C, Peng B, Dan Y, Peng Y, Xiao PG. (2014) Chemical taxonomy of tree peony species from China based on root cortex metabolic fingerprint. Phytochemistry 107: 69-79.

He CN, Peng Y, Zhang YC, Xu LJ, Gu J, Xiao PG. (2010) Phytochemical and biological studies of Paeoniaceae. Chem Biodivers 7: 805-838.

Holowka D, Calloway N, Cohen R, Gadi D, Lee J, Smith NL, Barid B. (2012) Roles for Ga^{2+} , mobilization and regulation in mast cell finctions. Front Immunol 3: 1-10.

Hong SS, Jeong W, Kim JK, Kwon JG, Lee JY, Ahn EK, Oh J, Seo DW, Oh JS. (2014) Neolignan inhibitors of antigen-induced degranulation in RBL-2H3 cells from the needles of *Pinus thunbergii*. Fitoterapia 99: 347-351.

Hosny M, Zheng MS, Zhang H, Chang HW, Woo MH, Son JK, Lee SKS. (2014) (-)-Catechin glycosides from *Ulmus davidiana*. Arch Pharm Res 37: 698-705.

Hu HB, Jian YF, Cao H, Zheng XD. (2007) Phenolic compounds from *Elsholtzia bodinieri* Van't. J Chin Chem Soc 54: 1189-1194.

Huang L, Li T, Zhou H, Qiu P, Wu J, Liu L. (2015) Sinomenine potentials degranulation of RBL-2H3 basophils via up-regulation of phospholipase A_2 phosphorylation by Annexin A1 cleavage and ERK phosphorylation without influencing on calcium mobilization. Int Immunopharmacol 28: 945-951.

Itoh T, Ohguchi K, Linuma M, Nozawa Y, Akao Y. (2008) Inhibitory effect of xanthones isolated from the pericarp of *Garcinia mangostana* L. on rat basophilic leukemia RBL-2H3 cell degranulation. Bioorgan Med Chem 16: 4500-4508.

Itoh T, Ohguchi K, Nakajima C, Oyama M, Linuma M, Nozawa Y, Akao Y, Ito M. (2011) Inhibitory effects of flavonoid glycosides isolated from the peel of Japanese persimmon (*Diospyros kaki Fuyu*) on antigen-stimulated degranulation in rat basophil leukaemia RBL-2H3 cells. Food Chem 126: 289-294.

Japan Kampo Medicinal Manufacturers Association. (2015) Survey reports on annual consumption of herbal drugs (3). (http://www.nikkankyo.org)

Jiangsu New Medica College. (1977) Dictionary of Chinese Materia Medica, Shanghai Science and Technology Press, Shanghai, p.1127.

Jiangsu New Medical College. (1999) Glossary of Chinese Herbal Medicines. Shanghai,

Shanghai Science and Technology Press, 1093-1095.

Juan YC, Chang CC, Tsai WJ, Lin YL, Hsu YS, Liu HK. (2011) Pharmacological evaluation of insulin mimetic novel suppressors of PEPCK gene transcription from Paeoniae Rubra Radix. J Ethnopharmacol 137: 592-600.

Kageyama-Yahara N, Suehiro Y, Maeda F, Kageyama S, Fukuoka J, Katagiri T, Yamamoto T, Kadowaki M. (2010) Pentagalloylglucose down-regulates mast cell surface FcεRI expression *in vitro* and *in vivo*. FEBS Lett 584: 111-118.

Kan WLT, Ma B, Lin G. (2011) Sulfur fumigation processing of traditional Chinese medicinal herbs: beneficial or detrimental ? Front Pharmacol 2: 84.

Kaneda M, Iitaka Y, Shibata S. (1972) Chemical studies on the oriental plant drugs-XXXIII: The absolute structures of paeoniflorin, albiflorin, oxypaeoniflorin and benzoylpaeoniflorin isolated from Chinese Paeony Root. Tetrahedron 28: 4309-4317.

Kang SS, Shin KH, Chi HJ. (1991) Galloylpaeoniflorin, A new acylated monoterpene glucoside from Paeony Root. Arch Pharm Res 14: 52-54.

Kawakami T, Galli SJ. (2002) Regulation of mast-cell and basophil function and survival by IgE. Nat Rev Imunol 2: 773-786.

Kee JY, Inujima A, Andoh T, Tanaka T, Li F, Kuraishi Y, Sakurai H, Shibahara N, Saiki I, Koizumi K. (2015) Inhibitory effect of *Moutan Cortex* aqueous fraction on mast cell-mediated allergic inflammation. J Nat Med 69: 209-217.

Kim K, Kim Y, Kim HY, Jeoung D. (2008) Inhibitory mechanism of anti-allergic peptides in RBL-2H3 cells. Eur J Pharmacol 581: 191-203.

Kim SH, Jun CD, Suk K, Choi BJ, Park S, Lee SH, Shin HY, Kim DK, Shin TY. (2006) Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cell. Toxicol Sci 91: 123-131.

Kimata M, Shichijo M, Miura T, Serizawa I, Inagaki N, Nagai H. (2000) Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. Clin Exp Allergy 30: 501-508

Konda Y, Iwasaki Y, Takahata S, Arima S, Toida T, Kaji E, Takeda K, Harigaya Y (1997) Synthesis of a new phenol glycoside, neohancoside C from *Cynanchum hancockianum*. Chem

Pharm Bull 45: 626-630.

Lee B, Shin YW, Bae EA, Han SJ, Kim JS, Kang SS, Kim DH. (2008) Antiallergic effects of the root of *Paeoniae lactiflora* and its constituents paeoniflorin and paeonol. Arch Pharm Res 31: 445-450.

Lee SC, Kwon YS, Son KH, Kim SH, Heo MY. (2005) Antioxidative constituents from *Paeonia lactiflora*. Arch Pharm Res 28: 775-783.

Li R, Wang X, Zhou Y, Cai M, Ding L. (2007) Analysis of sodium adduct paeoniflorin, albiflorin and their derivatives by (+) ESI-MS^n, DFT calculations and computer-aided mass spectrometry analysis program. J Mass Spectrom 42: 335-345.

Li Shizhen. (1977) *Bencao Gangmu*: Compendium of Materia Medica, vol 2, People's Medical Publishing House, Beijing, p.96-148.

Lin HC. (1998) Two novel compounds from *Paeonia suffruticosa*. J Nat Prod 61: 343-346.

Lin SL, Song JZ, Choi FFK., Liu H, Qiao CF, Zhou Y, Han QB, Xu HX. (2009) Chemical profiling of Radix Paeoniae evaluated by ultra-performance liquid chromatography/ photo-diode-array/ quadruple time-of-flight mass spectrometry. J Pharm Biomed Anal 49: 253-266.

Liu EH, Qi LW, Li B, Peng YB, Li P, Li CY, Cao J. (2009) High-speed separation and characterization of major constituents in Radix Paeoniae Rubra by fast high-performance liquid chromatography coupled with diode-array detection and time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 23: 119-130.

Luo NC, Ding W, Wu J, Qian DW, Li ZH, Qian YF, Guo JM, Duan JA. (2013) UPLC-Q-TOF/MS coupled with multivariate statistical analysis as a powerful technique for rapidly exploring potential chemical marers to differentiate between Radix Paeoniae Alba and Radix Paeoniae Rubra. Nat Prod Commun 8: 487-491.

Ma YB, Wu DG, Liu JK. (1999) Paeonivayin, A new monoterpene glycoside from *Paeonia delavayi*. Chin Chem Lett 10: 771-774.

Matsuda H, Morikawa T, Ueda K, Managi H, Yoshikawa M. (2002) Structural requirements of flavonoids for inhibition of antigen-induced degranulation, TNF-α and IL-4 production from RBL-2H3 Cells. Bioorg Med Chem 10: 3123-3128.

Matsumoto T, Nakamura S, Fujimoto K, Ohta T, Ogawa K, Yoshikawa M, Onishi E, Fukaya M, Matsuda H. (2015) Structure of diarylheptanoids with antiallergic activity from the rhizomes of *Curcuma comosa*. J Nat Med 69: 142-147.

Morikawa T, Imura K, Miyake S, Ninomiya K, Matsuda H, Yamashita C, Muraoka O, Hayakawa T, Yoshikawa M. (2012) Promoting the effect of chemical constituents from the flower of *Poaynum henderonii* on adipogenesis in 3T3-L1 cells. J Nat Med 66: 39-48.

Murakami N, Saka M, Shimada H, Matsuda H, Yamahara J, Yoshikawa M. (1996) New bioactive monoterpene glycosides from Paeoniae Radix. Chem Pharm Bull 44: 1279-1281.

Murata K, Takano S, Masuda M, Iinuma M, Matsuda H. (2013) Anti-degranulating activity in rat basophil leukemia RBL-2H3 cells of flavanone glycosides and their aglycones in citrus fruits. J Nat Med 67: 643-646.

Namba T, Hattori M, Shu YZ, Ishige A, Sugimoto A, Sekiguchi K. (1986) Abstracts of Papers, 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, p. 214.

Namba T. (1993) The Encyclopedia of Wakan-Yaku (Traditional Sino-Japnese Medicines) with color Pictures, 2nd ed. Osaka Vol. I, Hokusha Publishing Co., Ltd., 102-104.

Nishizawa M, Yamagishi T, Nonaka GI, Nishioka I, Nagasawa T, Oura H. (1983) Tannins and related compounds.XII: Isolation and characterization of galloylglucose from Paeoniae Radix and their effect on urea-nitrogen concentration in rat serum. Chem Pharm Bull 31: 2593-2600.

Okasaka M, Kashiwada Y, Kodzhimatov O, Ashrmetov O, Takaishi Y. (2008) Monoterpene glycosides from *Paeonia hybrida*. Phytochemistry 69: 1767-1772.

Pauletti PM, Araújo AR, Young MCM, Giesbrecht AM, Bolzani VS. (2000) *nor*-Lignans from the leaves of *Styrax ferrugineus* (Styracaceae) with antibacterial and antifungal activity. Phytochemistry 55: 597-601.

Roitt IM, Brosttoff J, Male DK. (1998) Immunology, Fifth edition. Morsby, ISBN: 0723421781.

Sato A, Tamura H. (2015) High antiallergic activity of 5, 6, 4′-trihydroxy-7, 8, 3′-trimethoxyflavone and 5, 6-dihydroxy-7, 8, 3′, 4′-tetramethoxyflavone from eau de cologne mint (*Mentha* × *pipertia citrata*). Fitoterapia 102: 74-83.

Shi YH, Zhu S, Tamura T, Kadowaki M, Wang ZT, Yoshimatsu K, Komatsu K. (2016a)

Chemical constituents with anti-allergic activity from the root of Edulis Superba, a horticultural cultivar of *Paeonia lactiflora*. J Nat Med 70: 234-240.

Shi YH, Zhu S, Ge YW, He YM, Kazuma K, Wang ZT, Yoshimatsu K, Komatsu K. (2016b) Monoterpene derivatives with anti-allergic activity from red peony root, the root of *Paeonia lactiflora*. Fitoteriapia 108: 55-61.

Shimizu M, Hayashi T, Morita N, Kiuchi F, Noguchi H, Iitaa Y, Sankawa U. (1983) The structure of paeoniflorigenone, a new monoterpene isolated from Paeoniae Radix. Chem Pharm Bull 31: 577-583.

Shu YZ, Hattori M, Akao T, Kobashi K, Kagei K, Fukuyama K, Tsukihaya T, Namba T. (1987) Metabolism of paeoniflorin and related compounds by human intestinal bacteria II: structures of 7*S* and 7*R*-paeonimetabolines I and II formed by *Bacteriodes fragilis* and *Lactobacills brevis*. Chem Pharm Bull 35: 3726-3733.

Song WH, Cheng ZH, Chen DF. (2014) Anticomplement monoterpenoid glucosides from the root bark of *Paeonia suffruticosa*. J Nat Prod 77: 42-48.

Tanaka T, Fukumori M, Ochi T, Kouno I. (2003) Paeonians A-E, new dimeric and monomeric ellagitannons from the fruits of *Paeonia lactiflora*. J Nat Prod 66: 759-763.

Tanaka T, Nakashima T, Ueda T, Tomii K, Kouno I. (2007) Facile Discrimination of aldose enantiomers by reversed-phase HPLC. Chem Pharm Bull 55: 899-901.

The Ministry of Health, Labour and Welfare. (2011) The Japanese Pharmacopoeia (English version), 16th ed. p. 1704.

The State Pharmacopeia Commission of China. (2010) Pharmacopeia of the People's Republic of China, vol 1 (English version). Beijing, China Medical Science Press, p. 306-308.

Thompson CE, Fernandes CL, de Souza ON, de Freitas LB, Salzano FM. (2010) Evaluation of the impact of functional diversification on Poaceae, Brassicaceae, Fabaceae, and Pinaceae alcohol dehydrogenase enzymes. J Mol Model 16: 919-928.

Traditional Medical & Pharmaceutical Database: http://dentomed.u-toyama.ac.jp/en/

Turner H, Kinet JP. (1999) Signaling through the high-affinity IgE receptor FcεRI. Nature 402: 24-30.

Wang LB, Gao HY, Morikawa T, Sun BH, Huang J, Yoshikawa M, Wu LJ. (2008) Flavonones from *Helichrysi flos* syn. Chin J Nat Med 5: 357-360.

Wang Q, Liang Z, Peng Y, Hou JL, Wei SL, Zhao ZZ, Wang WQ. (2015) Whole transverse section and specific-tissue analysis of secondary metabolites in seven different grades of root of *Paeonia lactiflora* using laser microdissection and liquid chromatography-quadruple time of flight-mass spectrometry. J Pharm Biomed Anal 103: 7-16.

Wang Q, Liu RX, Guo HZ, Ye M, Huo CY, Bi KS, Guo DA. (2005) Simultaneous LC determination of major constituents in red and white peony root. Chromatographia 62: 581-588.

Wang XH, Xie PS, Lam CW, Yan YZ, Yu QX. (2009) Study of the destructive effect to inherent quality of *Agenlicae dahuricae* radix (Baizhi) by sulfur-fumigated process using chromatographic fingerprinting analysis. J Pharm Biomed Anal 49: 1221-1225.

Wang Y, Wang P, Xu CH, Sun SQ, Zhou Q, Shi Z, Li J, Chen T, Li Z, Cui WL. (2015) Discrimination and chemical characterization and different *Paeonia lactiflora* (Radix Paeoniae Alba and Radix Paeoniae Rubra) by infrared macro-fingerprint analysis-through-separation. J Mol Struct 1099: 68-76.

Wu J, Shen H, Xu J, Zhu LY, Jia XB, Li SL. (2012) Detection of sulfur-fumigation Paeoniae Alba Radix in complex preparations by high performance liquid chromatography tandem mass spectrometry. Molecules 17: 8938-8954.

Xiao HH, Dai Y, Wong MS, Yao XS. (2014) New lignans from the bioactive fraction of *Sambucus williamsii* Hance and profieration activities on osteoblastic-like UMR106 cells. Fitoterapia 94: 29-35.

Xu SJ, Yang L, Zeng X, Zhang M, Wang ZT. (2006) Characterization of compounds in Chinese herbal drug Mu-Dan-Pi by liquid chromatography coupled to electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 20: 3275-3288.

Yan Z, Chen C, Xie X, Fu B, Yang X. (2012) Rapid screening and quantification of sulfonate derivatives in white peony root by UHPLC-MS-MS. Anal Bioanal Chem 402: 2173-2182.

Yang F, Chen R, Feng L, Li HD, Zhang H. (2011) Two new alkaloids from the aerial part of *Peganum nigellastrum*. Helvetica Chimica Acta 94: 514-518.

Ye M, Liu SH, Jiang Z, Lee Y, Tilton R, Cheng YC. (2007) Liquid chromatography/ mass spectrometry analysis of PHY906, a Chinese medicine formulation for cancer therapy. Rapid Commun Mass Spectrom 21: 3593-3607.

Yean MH, Lee JY, Kim JS, Kang SS. (2008) Phytochemical studies on Paeoniae Radix (1)-monoterpene glucosides. Kor J Pharmacogn 39: 19-27.

Yen PH, Kiem PV, Nhiem NX, Tung NH, Quang TH, Minh CV, Kim JW, Choi EM, Kim YH. (2007) A new monoterpene glycoside from the roots of *Paeonia lactiflora* increase the differentiation of osteoblastic MC3T3-E1. Arch Pharm Res 30: 1179-1185.

Yoshikawa M, Ohta T, Kawaguchi A, Matsuda H. (2000) Bioactive constituents of chinese natural medicines. V: Radical scavenging effect of Moutan Cortex (1): absolute stereostructures of two monoterpenes, Paeonisuffrone and Paeonisuffral. Chem Pharm Bull 48: 1327-1331.

Yoshimura M, Sano A, Kamei JI, Obata A. (2009) Identification and quantitative of metabolites of orally administered naringenin chalcone in rats. J Agric Food Chem 57: 6432-6437.

Youn UJ, Lee YJ, Jeon HR, Shin HJ, Son YM, Nam JW, Han AR, Seo EK. (2010) A pyridyl alkaloid and benzoic acid derivatives from the rhizomes of *Anemarrhea asphodeloids*. Nat Prod Sci 16: 203-206.

Yu XL, Zhu S, Wu YQ, Komatsu K, Murakami M, Tamura T. (2013) Chemical diversity of Peony Root (2)-Chemical difference between White and Red Peony roots and the potential of horticultural cultivars of *Paeonia lactiflora* as medicinal resources. The 133rd Annual Meeting of the Pharmaceutical Society of Japan, Proceedings: 2: 219 (Yokohama)

Zhu S, Yu XL, Shiraishi F, Komatsu K, Murakami M, Tamura T. (2013) Genetic characterization of White/Red Peony roots and the horticultural cultivars of *Paeonia lactiflora* by nuclear rDNA ITS sequences. The 133rd Annual Meeting of the Pharmaceutical Society of Japan, Proceedings: 2: 219 (Yokohama)

Zhu S, Yu XL, Wu YQ, Shiraishi F, Kawahara N, Komatsu K. (2015) Genetic and chemical characterization of white and red peony root derived from *Paeonia lactiflora*. J Nat Med 69: 35-45.

List of Publications

[1] Shi YH, Zhu S, Ge YW, He YM, Kazuma K, Wang ZT, Yoshimatsu K, Komatsu K. Monoterpene derivatives with anti-allergic activity from red peony root, the root of *Paeonia lactiflora*. Fitoterapia 108 (2016) 55-61.

[2] Shi YH, Zhu S, Tamura T, Kadowaki M, Wang ZT, Yoshimatsu K, Komatsu K. Chemical constituents with anti-allergic activity from the root of Edulis Superba, a horticultural cultivar of *Paeonia lactiflora*. J Nat Med 70 (2016) 234-240.

[3] Shi YH, Zhu S, Toume K, Wang ZT, Javzan B, Komatsu K. Characterization and quantification of monoterpenoids in different types of peony root and the related species in sect. *Paeonia* by LC-ESI-IT-TOF-MS. (Submitted).

Acknowledgements

First of all, I would like to express my sincere thanks and gratitude to my supervisor Prof. Katsuko KOMATSU, Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama for her excellent supervision, suggestions and encouragements throughout my research works.

I am especially grateful to Assistant Prof. Shu ZHU, Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama for her continuous guidance, strong and generous support, excellent suggestions and comments throughout the period of my study in Toyama.

I am sincerely thankful to Associate Prof. Kazufumi TOUME from Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama for providing help of LC-MS analysis and chemical structure elucidation.

I am highly grateful to Prof. Zhengtao WANG, Institute of Chinese Materia, Shanghai University of Traditional Chinese Medicine, who recommended me to study in Japan, and provided valuable suggestions and encouragements throughout the period of studies in Toyama. I am also very grateful to Prof. Li YANG and Prof. Rui WANG for their support and kind help.

My thanks are extended to Prof. Hiroyuki Morita, Division of Natural Products Chemistry, Institute of Natural Medicine, University of Toyama and Prof. Fumiya Kurosaki, Lab of Pharmacognosy, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama for their comments and suggestions for reviewing my

thesis.

I am greatly indebted to all the members in Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama, especially Mrs. Masako YUKI, Dr. Yue-Wei GE, Dr. Yu-Min HE, Ms. Xiao-Ting WU, Mr. Kenichiro HOTTA, Ms. Mai ASANUMA, Ms. Aimi SHIRAKAWA, Ms. Yuki TOMITA, Ms. Zhi-Yan HOU for their irreplaceable friendship and kind help in not only laboratory items but also all the aspects of daily life.

I would like to express my special thanks to the Campus Asian Program of University of Toyama for affording the scholarship on the first two years of my study and the Ministry of Education, Culture, Sports, Science and Technology of Japan for affording the scholarship on the last year of my Ph.D. study in Japan.

Finally, I would like to express my heartfelt thanks and gratitude to my parents, my wife, my brother, sisters and relatives for their wholehearted love, enthusiasm support and encouragement in every step of my life.

Yan-Hong SHI

Feb., 2016