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A plasma metabonomic investigation into the intervention of volatile oil of *Magnolia biondii* Pamp on rat model of acute inflammation

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ABSTRACT

Ethnopharmacological relevance: The dried flower buds of *Magnolia biondii* Pamp (Magnoliaceae) possesses significant anti-inflammatory activities.
Aim of the study: Volatile oil in *Magnolia biondii* Pamp (VOMbP) is considered to be important pharmacologically active individuals against acute inflammation, but its exact anti-inflammatory mechanism remains elusive. In this study, we aimed to investigate the intervention of VOMbP on rats with acute inflammation and explore the possible anti-inflammatory mechanisms of VOMbP with metabonomic strategy.
Materials and methods: Acute inflammation was induced by subcutaneously injection of carrageenan in the rats. Plasma was analyzed using gas chromatography–mass spectrometry (GC–MS), based on which the principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA) models were established for metabonomic analysis.
Results: It was revealed that the pretreatment of VOMbP in acute inflammatory rats induces a substantial and characteristic change in their metabolic profiles. Some significantly changed metabolites, including hexadecanoic acid, linoleic acid, oleic acid, stearic acid, and cholesterol, were found to be reasonable in explaining the anti-inflammatory mechanism of VOMbP.
Conclusions: In all, it is likely that VOMbP intervenes the metabolic process of inflammatory rats by affecting the fatty acid and cholesterol metabolism. Our work also indicated that the metabonomics method is a promising tool for performing intervention and mechanism research of traditional Chinese medicines.

1. Introduction

The dried flower buds of *Magnolia biondii* Pamp (Magnoliaceae), commonly known as Xin-Yi in China, exists in many Chinese medicine prescriptions (The State Commission of Chinese Pharmacopoeia, 2010). Pharmacological tests have revealed that *Magnolia biondii* Pamp possesses significant anti-inflammatory activities (Masayasu et al., 1985; Shen et al., 2008). Volatile oil in *Magnolia biondii* Pamp (VOMbP), is considered to be one important group of the pharmacologically active individuals against acute inflammation. It can reduce local vascular permeability of the inflammation, inhibit inflammatory exudation and leukocyte swim, and inhibit the production and release of some inflammatory mediators including prostaglandins E_2 (PGE_2), interleukin-1 (IL-1) and NO (Lim et al., 2002; Wang et al., 2000, 2005; Xiong et al., 2006).

VOMbP contains tens of volatile components including monoterpene, sesquiterpene and iridoid, *etc.*, some of which have been qualitatively and quantitatively analyzed by gas chromatography-mass spectrometry (GC–MS) (Zhao and Liang, 2007). However, complete separation of these chemical components is difficult to achieve due to the existence of overlapped or embedded peaks, even if rigorous conditions are imposed on the chromatographic separation process. In our previous work, GC–MS combined with chemometric methods was applied to identify and determine the components in VOMbP and a total of 80 components were finally identified after the overlapping peak clusters were resolved by chemometric methods (Qu et al., 2009).

However, the exact anti-inflammatory mechanism of VOMbP still remains elusive. In 2000, VOMbP was reported to inhibit phospholipases A2 (PLA_2) *in vivo* (Wang et al., 2000), which is an important enzyme in inflammatory responses. To the best of our knowledge, no further literatures that focus on anti-inflammatory

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mechanism of VOMbP appear after that. As was shown in the development of anti-inflammatory drugs, specific single-target inhibitors cannot sufficiently control the balance of metabolic network and may cause side effects (Khanna et al., 2005), hence VOMbP may find its role in intervening multi-targets and keeping the balance in inflammation treatment, since it possesses many bioactive components which may affect various pathways. Nevertheless, due to its complex constituents, it is not easy to explore the anti-inflammatory mechanism of VOMbP using traditional ways.

Metabonomics, a new member of systems biology, has nowadays attracted more and more attentions in the fields of disease diagnosis, biomarker screening, pharmaceutical discovery and toxicity evaluation (Nicholson et al., 2002; Claudino et al., 2007). It can reveal various metabolic characteristics of normal, pathological or drug-administrated subjects and be used to study the mechanism of drugs action (Zhao et al., 2008; Sun et al., 2010). Since traditional Chinese medicine (TCM) is based on "holism" philosophy instead of "reductionism", it is philosophically conceivable that metabonomic techniques may play some roles in providing important information to TCM (Qiu, 2007).

As we all know, NMR spectroscopy (Liu et al., 2010), LC–MS (Sangster et al., 2007), CE–MS (Ullsten et al., 2006) and GC–MS (Yu et al., 2007) have been widely used in metabonomics studies. Among them, GC–MS has been proved of high selectivity and reproducibility. Compared with LC–MS and NMR-based metabonomic approaches, the availability of many structure databases can be the advantage for GC–MS-based metabonomic study (A et al., 2005; Koek et al., 2006). In the present study, GC–MS-based metabonomics was applied to investigate the intervention of VOMbP on metabolic profiles of rats with carrageenan (a pro-inflammatory agent)-induced acute inflammation, so as to explore the possible anti-inflammatory mechanisms of VOMbP on acute inflammation, based on the significantly altered metabolites revealed in this study.

2. Experiment

2.1. Materials and preparation of VOMbP

The dried flower buds of *Magnolia biondii* Pamp were purchased from Lei-Yun-Shang pharmacy (Shanghai, China). Carrageenan, N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), trimethylchlorosilane (TMCS), methoxyamine and ribitol (used as internal standard) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Prednisone was purchased from Yi-Feng super drugstore (Shanghai, China). Anhydrous sodium sulphate, methanol and pyridine were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Tween 80 was purchased from Shanghai Genebase Gene-Tech Co. (Shanghai, China).

The VOMbP used in this study was prepared according to the Chinese pharmacopoeia (The State Commission of Chinese Pharmacopoeia, 2010). 150 g *Magnolia biondii* Pamp was grounded into powder, and then put into extracting apparatus and subjected to hydro-distillation for 5 h. After that, about 3.6 mL canary clear oil-like volatile oil was obtained. Next, the volatile oil was dried over anhydrous sodium sulphate until the last traces of water were removed to give VOMbP and then stored in a dark glass bottle at 4 °C for analysis. VOMbP was dissolved in Tween 80 (2%, v/v). Prednisone and carrageenan were dissolved in normal saline (0.9% NaCl, w/v).

2.2. In vivo experiments protocol

Sprague–Dawley rats (180–210 g, male) were purchased from the Sippr-Bk Lab. Animal Ltd. Co. (Shanghai, China) and fed with certified standard diet and tap water *ad libitum*. Temperature and humidity were regulated at 21–23 °C and 40–60%, respectively. A 12 h light/dark cycle was established. The study protocol was in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the United States National Institute of Health and was approved by the Animal Ethic Review Committees of Second Military Medical University. After one week of acclimatization, the rats were randomly divided into five groups (each consists of 5 rats): (A) control group; (B) acute inflammation group; (C) low dose group of VOMbP; (D) high dose group of VOMbP; (E) prednisone group. Group A and B received vehicle (Tween 80), Groups C and D received VOMbP (0.07 mL/kg/day and 0.14 mL/kg/day, respectively) and Group E received prednisone (5 mg/kg/day). Drugs and vehicle were orally administrated one time for 3 successive days. Acute inflammation was induced 0.5 h after gavage at day three. Rats in group B, C, D and E were injected subcutaneously with 0.1 mL 1.0% carrageenan in the sub-plantar region of right hind paw. Oedema of the paw was calculated by the difference of its volume before and 3 h after carrageenan injection (Arumugam et al., 2008). After that, 1.0 mL blood samples of group A to D were collected into heparinized tubes, and then centrifugated at 3000 × *g* for 10 min. Then the plasma was separated and stored at −80 °C until use.

2.3. Sample preparation

Prior to analysis, the plasma samples were thawed at room temperature. A 900 μL methanol was added into 100 μL of plasma to precipitate the protein, and the mixture was shaken vigorously for 60 s. A 20 μL ribitol solution (0.2 mg/mL) as the internal standard was added into the mixed sample. The mixture was then ultrasonically extracted for 10 min, followed by centrifugation (3000 × *g*) for another 10 min. Then, 400 μL supernatant was transferred to the GC vial and evaporated to dryness under a stream of nitrogen gas. The chemical derivatization of the plasma metabolites was carried out using the combination of methoxymation and silylation. A 100 μL aliquot of methoxyamine pyridine solution (15 μg/μL) was added to the vial. The methoxymation was performed at 70 °C for 1 h. Then, 180 μL of MSTFA with 1% TMCS as catalyst was added to the vial. After the silylation at 70 °C for 1 h, the sample solution was transferred to the GC microvial for GC–MS analysis after filtration. The derivatization procedure, according to the reference (Yu et al., 2007) and with minor revision, had been validated on other known chemicals (results not shown).

2.4. GC–MS analysis

The GC–MS analysis was carried out on a Varian 450 GC connected to a 240 MSD iron trap mass spectrometer (Varian Corporation, Palo Alto, USA) equipped with a VF-5 MS capillary column (30 m × 0.15 mm I.D.; 0.15 μm). The injector temperature was set at 270 °C. Helium was used as carrier gas at a constant flow rate of 1 mL/min through the column. The column temperature was initially kept at 70 °C for 2 min, and then increased from 70 to 300 °C at 10 °C/min, where it was held for 10 min. The MS trap temperature was set at 200 °C and the ion source temperature was 220 °C. Full-scan mass range of 50–600 *m*/*z* was acquired. The mass accuracy of the instrument was 0.1 atomic mass unit (amu). GC-MSD workstation software was used for auto-acquisition of GC total ion current (TIC) chromatograms and mass fragmentation patterns composed of split molecular ions. Identification of the metabolites in the GC–MS spectra was based on the comparison of the experimental mass spectrum with that in the NIST (National Institute of Standards and Technology) mass spectra library.

2.5. Data processing and pattern recognition

The acquired GC–MS data was first converted into NetCDF format and then subjected to noise reduction and baseline correction

using our custom scripts in MATLAB (version 6.5, MathWorks, Inc., USA). The peaks with intensities higher than threefold of the signal-to-noise (S/N) ratio and consistently present in each sample were recorded. All known artifact peaks, such as peaks due to column bleed and MSTFA artifact peaks, were excluded and not into the following data analysis.

Then, for every sample, the relative intensity of each selected peak was normalized against that of the internal standard so that each sample was represented by a collection of the variables to characterize its biochemical pattern. Next, a data matrix of relative intensities of the commensal peaks from all samples was generated and then mean-centered and pareto-scaled prior to multivariate statistical analysis. The mean-centering procedure subtracts the column mean from each value in the column so that each column of the matrix can be given a mean of zero; the pareto-scaling procedure divides the values in each column by the square root of its standard deviation, which amplifies the contribution of lower concentration metabolites. The obtained matrix was then employed for pattern recognition. The unsupervised (principal component analysis, PCA) and supervised (partial least squares-discriminate analysis, PLS-DA) statistical methods were applied to investigate the metabolic profiles of the rats. *T*-test was employed to analyze the metabolite levels among different groups, and a *p*-value of <0.05 was taken to indicate statistical significance. All the above were performed with in-house algorithms running on Matlab and PLS-Toolbox 4.2 (Eigenvector Research, Inc., Copyright (C) 2008).

3. Results and discussion

3.1. Evaluation of the anti-inflammatory activities of VOMbP

Fig. 1 shows the anti-inflammatory effects of VOMbP on carrageenan-induced hind paw oedema in rats. It can be seen that though the strongest inhibition was found in prednisone group, VOMbP administration of 0.07 and 0.14 mL/kg inhibit the paw oedema positively as they caused significant reductions in paw oedema ($p < 0.05$ and $p < 0.01$, respectively).

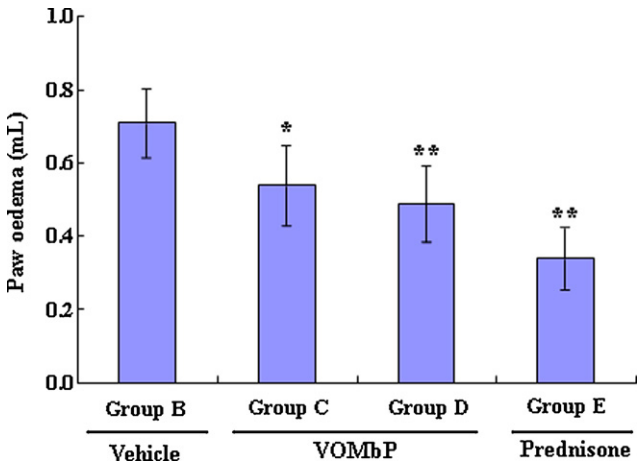

Fig. 1. Effect of oral treatment with VOMbP and prednisone on carrageenan-induced hind paw oedema in rats (mL ± SD). Oedema of the paw was calculated by the difference of its volume before and 3 h after carrageenan injection. Group B (acute inflammatory group) received vehicle, Groups C and D received VOMbP (0.07 mL/kg/day and 0.14 mL/kg/day, respectively) and Group E received prednisone (5 mg/kg/day). Significant differences were based on two-detailed *T*-test $^{*}p < 0.05$ and $^{**}p < 0.01$ with respect to Group B.

3.2. GC–MS analysis

Typical GC–MS TIC chromatograms of the plasma samples from control, acute inflammation and VOMbP groups were shown in Fig. 2. After peak picking and alignment, 49 endogenous metabolites in the plasma samples were identified by NIST library. These metabolites, including fatty acids, organic acids, carbohydrates and amino acids, etc., are known to be involved in multiple biochemical processes (Postic et al., 2004), as will be discussed later.

Precision and repeatability of the applied GC–MS method was evaluated prior to the analysis of the experimental samples. Precision of this method was evaluated by analyzing six independently processed replicates and then calculating the relative standard deviations (R.S.D) of retention times and peak areas for each peak

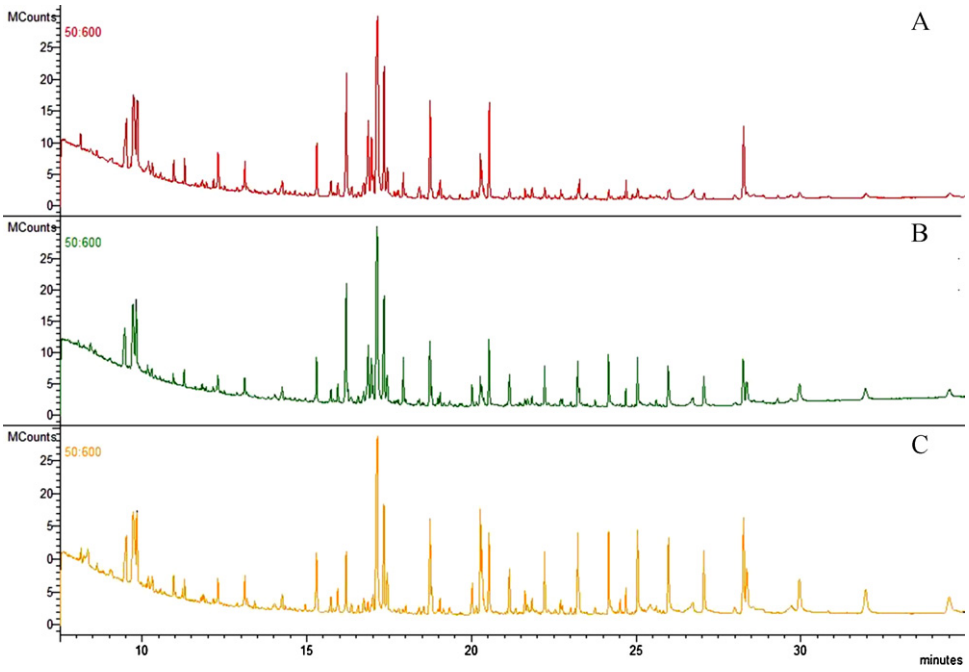

Fig. 2. Representative GC–MS TIC chromatograms of the plasma samples from the three groups. (A) Control group, (B) acute inflammation group, (C) VOMbP group (0.14 mL/kg/day).

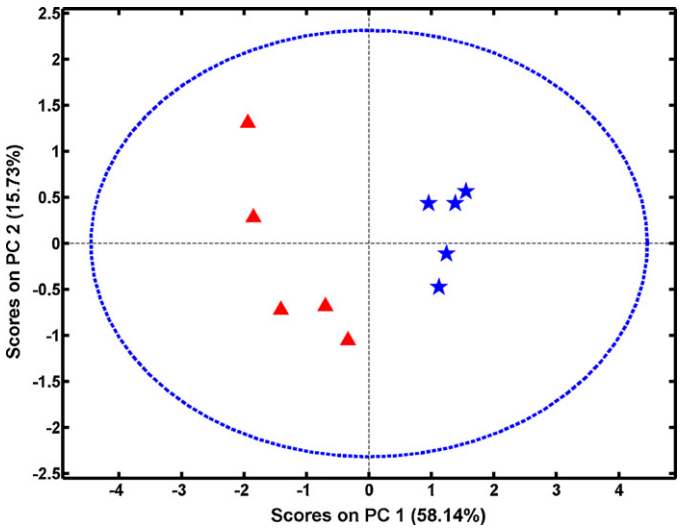

Fig. 3. Score plot from PCA model classifying control group (▲) and acute inflammation group (★).

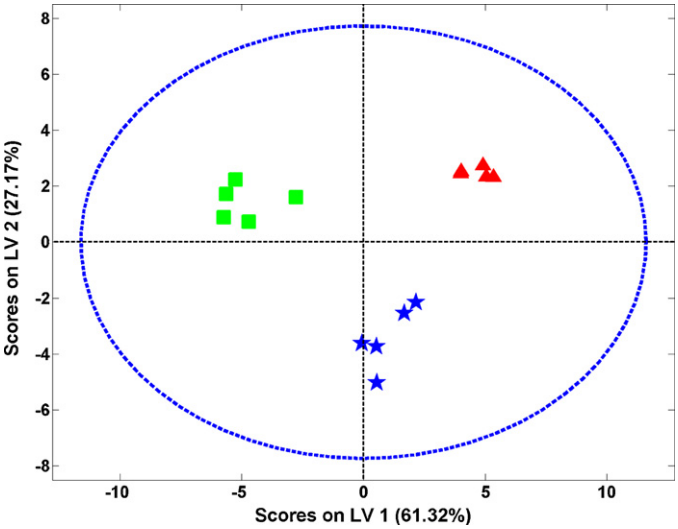

Fig. 4. Score plot from PLS-DA model classifying control (▲), acute inflammation (★) and VOMbP group (high dose) (■).

in these injections. The R.S.D ranged from 0.17% to 0.86% for the retention times and ranged from 1.43% to 4.95% for the peak areas. Repeatability of this method was investigated by preparing six parallel samples using the same preparation protocol, and then the R.S.D of the retention times and peak areas were calculated. The R.S.D. of metabolites' retention times and peak areas ranged from 0.36% to 1.24% and from 3.22% to 7.04%, respectively.

3.3. Intervention of VOMbP on metabolic pattern of acute inflammatory rats

At first, we carried out a PCA analysis on the data of control and acute inflammation groups, to visualize general clustering, trends, or outliers among the observations, and also to know whether these two groups could be distinguished based on their metabolic profiles provided by GC–MS. As shown in the PCA score plot (Fig. 3), even from this unsupervised analysis, a separation between the control and acute inflammation group was clearly seen, indicating that they had completely different metabolic profiles.

Next, in order to examine whether VOMbP was possible to influence the metabolic pattern of acute inflammation model, and to further refine the metabolites differentially produced by carrageenan or VOMbP, PLS-DA was conducted. PLS-DA is a supervised method based on PLS in which a "dummy" Y-variable containing class membership information is correlated to the GC–MS data matrix (X-variable), and the resulting projection model gives latent variables (LVs) that focus on maximum separation (discrimination) (Eriksson et al., 2004). In order to establish a reliable PLS-DA model, we used leave-one-out cross validation procedure to select the number of LVs. According to the root-mean-square error of cross validation (RMSECV) curve, it was revealed that three LVs were sufficient to understand 96.04% of the variation in Y using 92.06% of the GC–MS data (Table 1), hence three LVs were selected in our model. In order to validate the prediction accuracy of the model, we performed a 4-fold cross-validation where in each trial 75% of the samples were used as calibration data set to construct a model, and the remaining 25% were applied as the independent test data set. This process was repeated until every sample served as an independent test sample once and only once. No misclassified samples were returned from the predicted Y values, which further guaranteed the models.

Table 1
Percent variance captured by the PLS-DA model.

LVs	V_x (%)	V_x (cum, %)	V_Y (%)	V_Y (cum, %)
1	61.32	61.32	46.92	46.92
2	27.17	88.49	47.52	94.44
3	3.57	92.06	1.60	96.04

V_x, V_Y: variation explained by each LV; V_x (cum), V_Y (cum): cumulative variation explained.

From the score plot (Fig. 4) of the PLS-DA model, a clear separation among the control, acute inflammation and VOMbP group was easily seen, where LV_1 and LV_2 explains 61.32% and 27.17% of the variance in the data, respectively. All the samples fell inside the 95% confidence interval, which was represented by an ellipse in Fig. 4. VOMbP group was located at the opposite direction along LV_1 comparing to the other two groups (without VOMbP pretreatment); moreover, this group was even showing a trend to return to the control group, as both the VOMbP group and the control group were at the same side on LV_2, opposite to the acute inflammation group. The above results, which were also in accordance with the results in Fig. 1, indicated that there is really no doubt that the pretreatment of VOMbP in acute inflammatory rats induces substantial and characteristic changes in their metabolic profiles.

3.4. Alteration of the marker metabolites in acute inflammation group

In PCA loading plot (Fig. 5), the individual metabolites were prioritized according to their respective contributions to the discrimination between control and acute inflammation group. A number of metabolites, which were the furthest from the origin by visual inspection, were thus found to possibly contribute to the separation. Importantly, directions in the score plot correspond to directions in the loading plot (Trygg et al., 2007). Therefore, it can be seen that in acute inflammation group, levels of urea (9.508 min), cadaverine (9.742 min), lactic acid (10.559 min), furanose (16.875 min and 16.969 min) and arachidonic acid (AA, 21.621 min) increased significantly since they were positively covariant to the acute inflammation group as shown in Fig. 3. Conversely, metabolites such as hexadecanoic acid (18.759 min), cholesterol (28.273 min), L-altrose (15.308 min), linoleic acid (20.276 min) and stearic acid (20.543 min) had remarkably reduced levels comparing to the control group. The alteration in levels of these metabolites in acute inflammation group man-

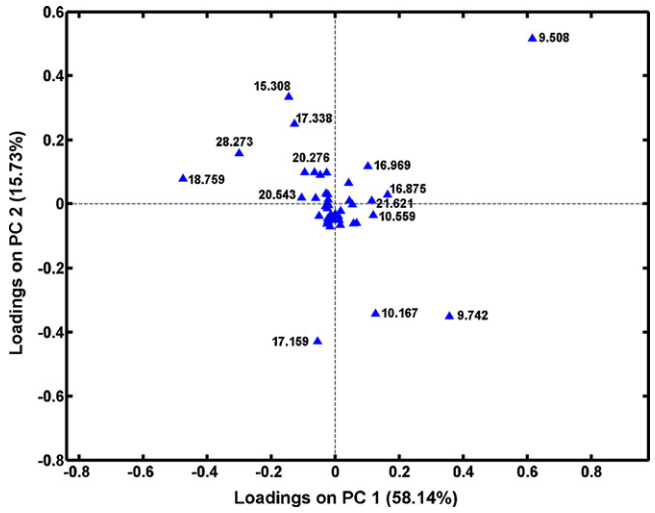

Fig. 5. Loading plot from PCA model classifying control and acute inflammation group.

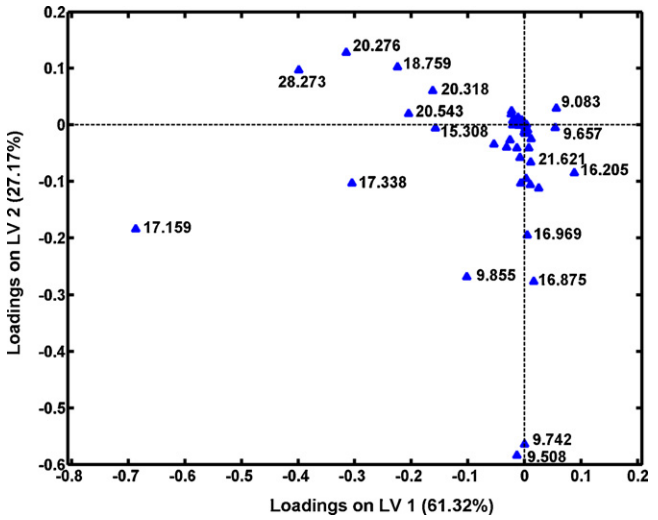

Fig. 6. Loading plot from PLS-DA model classifying control, acute inflammation and VOMbP group.

ifested the characteristics of metabolic profile in inflammatory responses.

Cadaverine, a foul-smelling biogenic diamine, is synthesized by bacterial decarboxylation of lysine during protein hydrolysis, *e.g.* during putrefaction of animal tissue (Osborne and Seidel, 1990). In a recent report, 5-aminovaleric acid, a production from the metabolism of cadaverine, was found to have elevated level in the urine of interleukin-10-deficient ($IL10^{-/-}$) mice that were with intestinal inflammation. The authors suspected that this may be caused by inflammation-induced tissue damage that increases the levels of lysine, resulting in enhanced synthesis of cadaverine (Lin et al., 2010).

Urea is the principal end product of protein catabolism. The increased urea level in acute inflammation group may be related to the protein accumulation and the interference of amino acid metabolism. Ning et al. (2007) also reported an increased urea level in plasma of rabbits during the stage of early inflammatory reaction after traumatic hemorrhagic shock.

AA, a polyunsaturated essential fatty acid, is well known for mediating inflammation. Dietary linoleic acid (another unsaturated essential fatty acid) is lengthened and desaturated to form AA, and then the latter is present in the phospholipid fats in the cell membrane. In response to many (*e.g.* carrageenan-induced) inflammatory stimuli, particular phospholipase (PLA_2) specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing AA. AA can then be oxygenated and further modified to form eicosanoids including prostaglandins, thromboxanes and leukotrienes which act as regulators of inflammation process (Dennis, 1994; Baynes and Dominiczak, 2005). In our study, an increased level of AA and decreased level of linoleic acid were observed in acute inflammatory group, this may be attributed to a large amount of linoleic acid consumption in forming AA. Pacheco et al. (1987) also reported higher level of AA and lower level of oleic acid (another mono-unsaturated fatty acid) in the colon mucosa of inflammatory bowel disease (IBD) patients compared with that of healthy subjects, which was quite consistent with our findings.

In the present study, cholesterol was found to have notably reduced level in acute inflammatory group. Cholesterol accounts for 99% of all sterols in mammals and plays multiple important biological roles. It is a major constituent of cell membranes where it is required to establish proper membrane permeability and fluidity. In healthy individuals, about thirty percent of blood cholesterol is carried by high-density lipoprotein (HDL) (Berrougui et al., 2007). Recently, it was reported that inflammation impairs reverse cholesterol transport (RCT), a major atheroprotective function of HDL, where cholesterol movement from macrophage to plasma was reduced, leading to decreased cholesterol level in the plasma of inflammation model (McGillicuddy et al., 2009). According to the authors, this may reflect reduced capacity of inflammatory HDL particles to efficiently accept cholesterol from macrophages.

As to other metabolites marked in Fig. 5, the decline in level of L-altrose in acute inflammation group may be related to increased energy requirement and temporary imbalance between catabolism and anabolism in acute inflammation state. α-(β-) and β-(α-)Furanose, however, simultaneously had elevated levels in acute inflammation group, though the configurations of these two carbohydrates could not be determined using the current MS as they have identical mass spectra. Lactic acid, an important organic acid, also had increased level in acute inflammation group. All the above still need further investigations. The two metabolites in 17.159 min and 17.338 min failed to be identified after searching the NIST library, whose poor matching results may be caused by some coeluting peaks or impurities within them. However, it seems that they have quite similar mass spectra with those of glucose and galactose, respectively, and further studies are still awaited on their identification and explanation.

3.5. Possible anti-inflammatory mechanisms of VOMbP

The loading plot from PLS-DA model was shown in Fig. 6. Similarly, the metabolites which are far from the origin are regarded as important variables. Altogether, the marker metabolites revealed in this study were listed in Table 2, where relative intensities of these metabolites were subjected to pair-wise comparison by calculating the *p*-values between control and acute inflammation group, and between VOMbP and acute inflammation group, respectively. In VOMbP group, as expected, all the metabolites listed in Table 2 had reversed levels comparing to those in the acute inflammation group. The recovery of these metabolites after VOMbP pretreatment was possibly involved in the anti-inflammatory activation of VOMbP.

Among these marker metabolites, five metabolites, namely hexadecanoic acid, linoleic acid, oleic acid, stearic acid, and cholesterol, may be mostly related to the anti-inflammatory activity of VOMbP, as in the PLS-DA loading plot (Fig. 6), they positively covariant to the VOMbP pretreatment group as shown in the score plot (Fig. 4).

Table 2
Summary of the marker metabolites revealed in this study.*

Metabolites	R.T. (min)	Match percent (%)	Chemical class	Acute Inflammation group	VOMbP group	Pathways
Urea	9.508	90	Others	$\uparrow^{\#\#}$	$\downarrow^{**}$	Purine metabolism
Cadaverine	9.742	86	Polyamines	$\uparrow^{\#}$	$\downarrow^{*}$	Lysine degradation
Lactic acid	10.559	83	Organic acids	$\uparrow^{\#\#}$	$\downarrow^{**}$	Pyruvate metabolism
L-Altrose	15.308	92	Carbohydrates	$\downarrow^{\#}$	$\uparrow^{**}$	Unknown
α-(β-)Furanose	16.875	88	Carbohydrates	$\uparrow^{\#\#}$	$\downarrow^{**}$	Unknown
β-(α-)Furanose	16.969	86	Carbohydrates	$\uparrow^{\#}$	$\downarrow^{**}$	Unknown
Hexadecanoic acid	18.759	96	Saturated fatty acids	$\downarrow^{\#\#}$	$\uparrow^{**}$	Fatty acid biosynthesis
Linoleic acid	20.276	91	Unsaturated fatty acids	$\downarrow^{\#\#}$	$\uparrow^{**}$	Linoleic acid metabolism
Oleic acid	20.318	86	Unsaturated fatty acids	$\downarrow^{\#}$	$\uparrow^{**}$	Fatty acid biosynthesis
Stearic acid	20.543	92	Saturated fatty acids	$\downarrow^{\#\#}$	$\uparrow^{**}$	Fatty acid biosynthesis
Arachidonic acid	21.621	85	Unsaturated fatty acids	$\uparrow^{\#\#}$	$\downarrow^{**}$	Arachidonic acid metabolism
Cholesterol	28.273	95	Lipids	$\downarrow^{\#\#}$	$\uparrow^{**}$	Steroid biosynthesis

* The up-(or down-) regulation of the arrows represent the relatively increased (or decreased) levels of the metabolites in acute inflammation group or VOMbP group. Significant differences compared with control group ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$); significant differences compared with acute inflammation group ($^{*}p < 0.05$, $^{**}p < 0.01$).

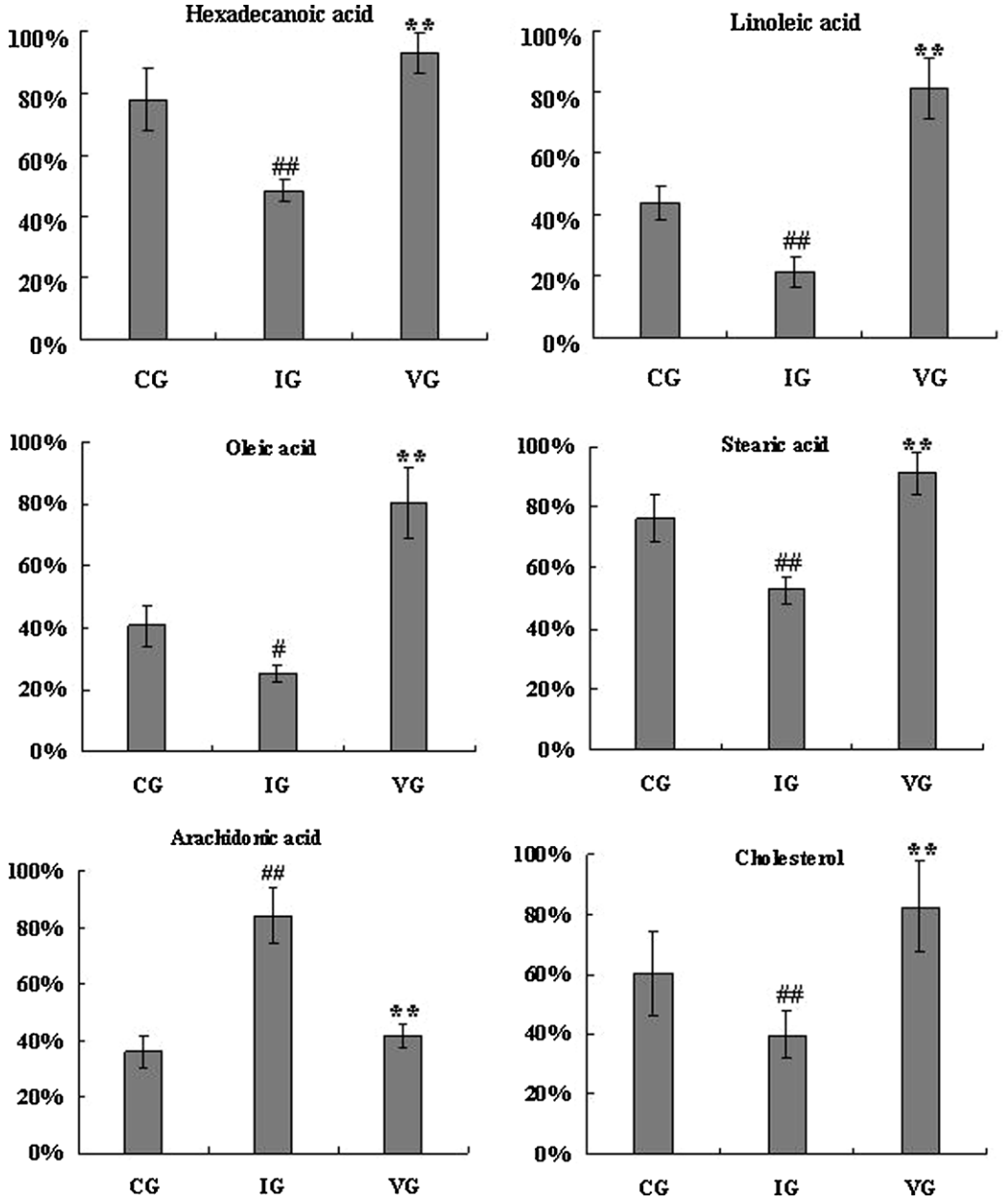

Fig. 7. Relative contents of the marker metabolites possibly related with anti-inflammatory activity of VOMbP. CG: control group, IG: acute inflammation group, VG: VOMbP group. The data were expressed as mean ± SD. Significant differences compared with CG ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$), significant differences compared with IG ($^{*}p < 0.05$, $^{**}p < 0.01$).

Based on these findings, it is likely that the anti-inflammatory activity of VOMbP is related to the fatty acid metabolism and cholesterol biosynthesis and transport. As described above, AA plays a central role in inflammation related to injury and many diseased states; linoleic acid is a polyunsaturated fatty acid used in the biosynthesis of AA. As to the other three fatty acids, stearic acid is the saturated derivative from hydrogenation of double bond of oleic acid (Dangi et al., 2010), and hexadecanoic acid is one of the most common saturated fatty acids found in animals and plants (Beare-Rogers et al., 2001). These are not only the main energy source as nutrients, but also signaling molecules in various cellular processes. In all, the alteration in the fatty acid profile in acute inflammation group may partly explain the increased synthesis of eicosanoids in acute inflammatory group; and after administration of VOMbP, however, the AA level was significantly decreased, so it could be expected that the pro-inflammatory eicosanoids levels were reduced which results in a reduction in inflammation. At the same time, the administration of VOMbP leads to an increase in cholesterol level, indicating recovered synthesis and transport of cholesterol that may be related to recovered RCT as described above. Fig. 7 displayed the relative intensities of the above five metabolites together with AA in all the three groups, which shows that after the administration of VOMbP, the levels of these metabolites had tendency to come back from the model group to the control group.

As mentioned above, the exact mechanisms by which VOMbP inhibits inflammation remain incompletely characterized up to now. Fortunately, our findings from the view of metabonomics may provide some clues for future research. Firstly, it is likely that VOMbP leads to a restore of the fatty acids metabolism network to the normal state, by regulating some key enzymes including PLA_2 (Wang's report of VOMbP inhibiting PLA_2 activitiy (Wang et al., 2000) is quite supportive to our results on AA metabolism). Also, myeloperoxidase (MPO), the abundant phagocyte-derived hemoprotein released during phagocyte activation, was recently found to play important role in modulating biologically active AA and linoleic acid metabolites during acute inflammation (Kubala et al., 2010). In an earlier study, a marked increase of MPO was induced by carrageenan injection to the footpad of the mice; and the administration of geranium oils, another essential oil which is also known for treating inflammatory diseases, suppressed the increase of MPO value in the inflammation group significantly (Maruyama et al., 2006). Therefore, our findings to some extent indicate the importance of fatty acid metabolism in examining the anti-inflammatory mechanism of VOMbP.

In addition, liver X receptors (LXRs), a super-family of ligand-activated transcription factors, were found to play an important role in the regulation of lipid metabolism and inflammatory response (Torocsik et al., 2009). It has been revealed that activation of LXRs reduces inflammation (Fowler et al., 2003), inhibits inflammatory gene expression (Joseph et al., 2002), and affects the production and secretion of inflammatory mediators (Ogawa et al., 2005). In our study, the levels of some fatty acids including hexadecanoic acid, linoleic acid, oleic acid and stearic acid increased in the VOMbP pretreated group; we thus assume that these metabolites may be regarded to act as LXRs activators, since endogenous activators derived from the metabolism of unsaturated fatty acids can activate LXRs (Torocsik et al., 2009). Remarkably, the activation of LXRs results in cholesterol efflux from macrophages, and the LXRs target genes are known to transport lipid molecules across the cell membrane and are involved in RCT (Torocsik et al., 2009); hence the elevation of cholesterol level in VOMbP pretreated group may be related to the activation of LXRs as well. Therefore, according to our results, the LXRs signaling pathway should also be paid enough attention in carrying out investigations on the anti-inflammatory mechanism of VOMbP.

4. Conclusion

In this study, a GC–MS based metabonomics method was employed to evaluate the intervention of VOMbP on rat model of acute inflammation. VOMbP was confirmed to influence the metabolic pattern of acute inflammation model, and the VOMbP pretreated group even exhibited a tendency of recovering to healthy state. A couple of fatty acids together with cholesterol were considered to be mostly involved in the anti-inflammatory mechanism of VOMbP. Fatty acid metabolism as well as cholesterol synthesis and transport pathway, seem to be important in anti-inflammatory mechanism of VOMbP. This work also indicated that the metabonomics method is a promising tool for performing intervention and mechanism research of traditional Chinese medicines.

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References

A, J.Y., Trygg, J., Gullberg, J., Johansson, A.I., Jonsson, P., Antti, H., Marklund, S.L., Moritz, T., 2005. Extraction and GC/MS analysis of the human blood plasma metabolome. Analytical Chemistry 77, 8086–8094.

Arumugam, P., Priya, N.G., Subathra, M., Ramesh, A., 2008. Anti-inflammatory activity of four solvent fractions of ethanol extract of Mentha spicata L. investigated on acute and chronic inflammation induced rats. Environmental Toxicology and Pharmacology 26, 92–95.

Baynes, J.W., Dominiczak, M.H., 2005. Medical Biochemistry, 2nd ed. Elsevier, Mosby, p. 555.

Beare-Rogers, J., Dieffenbacher, A., Holm, J.V., 2001. Lexicon of lipid nutrition (IUPAC Technical Report). Pure and Applied Chemistry 73, 685–744.

Berrougui, H., Isabelle, M., Cloutier, M., Grenier, G., Khalil, A., 2007. Age-related impairment of HDL-mediated cholesterol efflux. The Journal of Lipid Research 48, 328–336.

Claudino, W.M., Quattrone, A., Biganzoli, L., Pestrin, M., Bertini, I., Di, L.A., 2007. Metabolomics: available results, current research projects in breast cancer, and future applications. Journal of Clinical Oncology 25, 2840–2846.

Dangi, B., Obeng, M., Nauroth, J.M., Chung, G., Bailey-Hall, E., Hallenbeck, T., Arterburn, L.M., 2010. Metabolism and biological production of resolvins derived from docosapentaenoic acid (DPAn-6). Biochemical Pharmacology 79, 251–260.

Dennis, E.A., 1994. Diversity of group types, regulation, and function of phospholipase A2. The Journal of Biological Chemistry 269, 13057–13060.

Eriksson, L., Antti, H., Gottfries, J., Holmes, E., Johansson, E., Lindgren, F., Long, I., Lundstedt, T., Trygg, J., Wold, S., 2004. Using chemometrics for navigating in the large data sets of genomics, proteomics, and metabonomics (gpm). Analytical and Bioanalytical Chemistry 380, 419–429.

Fowler, A.J, Sheu, M.Y., Schmuth, M., Kao, J., Fluhr, J.W., Rhein, L., Collins, J.L., Willson, T.M., Mangelsdorf, D.J., Elias, P.M., Feingold, K.R., 2003. Liver X receptor activators display anti-inflammatory activity in irritant and allergic contact dermatitis models: liver-X-receptor-specific inhibition of inflammation and primary cytokine production. Journal of Investigative Dermatology 120, 246–255.

Joseph, S.B., McKilligin, E., Pei, L., Watson, M.A., Collins, A.R., Laffitte, B.A., Chen, M., Noh, G., Goodman, J., Hagger, G.N., Tran, J., Tippin, T.K., Wang, X., Lusis, A.J., Hsueh, W.A., Law, R.E., Collins, J.L., Willso, T.M., Tontonoz, P., 2002. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. In: Proceedings of the National Academy of Sciences of United States of America , 99, pp. 7604–7609.

Khanna, D., Khanna, P.P., Furst, D.E., 2005. COX-2 controversy: where are we and where do we go from here? Inflammopharmacology 13, 395–402.

Koek, M.M., Muilwijk, B., Van der Werf, M.J., Hankemeier, T., 2006. Microbial metabolomics with gas chromatography/mass spectrometry. Analytical Chemistry 78, 1272–1281.

Kubala, L., Schmelzer, K.R., Klinke, A., Kolarova, H., Baldus, S., Hammock, B.D., Eiserich, J.P., 2010. Modulation of arachidonic and linoleic acid metabolites in myeloperoxidase-deficient mice during acute inflammation. Free Radical Biology Medicine 48, 1311–1320.

Lim, S.S., Shin, K.H., Ban, H.S., Kim, Y.P., Jung, S.H., Kim, Y.J., Ohuchi, K., 2002. Effect of the essential oil from the flowers of Magnolia sieboldii on the lipopolysaccharide-induced production of nitric oxide and prostaglandin E2 by rat peritoneal macrophages. Planta Medica 68, 459–462.

Lin, H.M., Barnett, M.P., Roy, N.C., Joyce, N.I., Zhu, S., Armstrong, K., Helsby, N.A., Ferguson, L.R., Rowan, D.D., 2010. Metabolomic analysis identifies inflamma-

tory and noninflammatory metabolic effects of genetic modification in a mouse model of Crohn's disease. Journal of Proteome Research 9, 1965–1975.
Liu, Y.R., Huang, R.Q., Liu, L.J., Peng, J.N., Xiao, B.K., Yang, J.Y., Miao, Z.C., Huang, H.L., 2010. Metabonomics study of urine from Sprague–Dawley rats exposed to Huang-yao-zi using ^{1}H NMR spectroscopy. Journal of Pharmaceutical and Biomedical Analysis 52, 136–141.
Maruyama, N., Ishibashi, H., Hu, W.M., Morofuji, S., Inouye, S., Yamaguchi, H., Abe, S., 2006. Suppression of carrageenan- and collagen II-induced inflammation in mice by geranium oil. Mediators of Inflammation 2006, 1–7.
Masayasu, K., Jun, S., Tadashi, Y., Masao, Y., Tohru, K., Shigetoshi, K., Satako, M., 1985. Anti-inflammatory effect of neolignans newly isolated from the crude drug "Shin-i" (Flos Magnoliae). Planta Medica 51, 291–293.
McGillicuddy, F.C., Moya, M.L., Hinkle, C.C., Joshi, M.R., Chiquoine, E.H., Billheimer, J.T., Rothblat, G.H., Reilly, M.P., 2009. Inflammation impairs reverse cholesterol transport *in vivo*. Circulation 119, 1135–1145.
Nicholson, J.K., Connelly, J., Lindon, J.C., Holmes, E., 2002. Metabonomics: a platform for studying drug toxicity and gene function. Nature Reviews Drug Discovery 1, 153–161.
Ning, J.L., Lai, X.N., Ge, H.J., Wang, S.L., Mo, L.W., Wang, L.L., Zhu, J.W., 2007. Effects of controlled hypothermia on inflammatory reaction and organ function after traumatic hemorrhagic shock in rabbits. Journal of Traumatic Surgery 9, 20–23.
Ogawa, S., Lozach, J., Benner, C., Pascual, G., Tangirala, R.K., Westin, S., Hoffmann, A., Subramaniam, S., David, M., Rosenfeld, M.G., Glass, C.K., 2005. Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. Cell 122, 707–721.
Osborne, D.L., Seidel, E.R., 1990. Gastrointestinal luminal polyamines: cellular accumulation and enterohepatic circulation. American Journal of Physiology 258, G576–G584.
Pacheco, S., Hillier, K., Smith, C., 1987. Increased arachidonic acid levels in phospholipids of human colonic mucosa in inflammatory bowel disease. Clinical Science (London) 73, 361–364.
Postic, C., Dentin, R., Girard, J., 2004. Role of the liver in the control of carbohydrate and lipid homeostasis. Diabetes & Metabolism 30, 398–408.
Qiu, J., 2007. Traditional medicine: a culture in the balance. Nature 448, 126–128.
Qu, L.P., Qi, Y.P., Fan, G.R., Wu, Y.T., 2009. Determination of the volatile oil of *Magnolia biondii* Pamp by gas chromatography–mass spectrometry combined with chemometric techniques. Chromatographia 70, 905–914.
Sangster, T.P., Wingate, J.E., Burton, L., Teichert, F., Wilson, I.D., 2007. Investigation of analytical variation in metabonomic analysis using liquid chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry 21, 2965–2970.
Shen, Y., Li, C.G., Zhou, S.F., Pang, E., Story, D.F., Xue, C.C., 2008. Chemistry and bioactivity of Flos Magnoliae, a Chinese herb for rhinitis and sinusitis. Current Medicinal Chemistry 15, 1616–1627.
Sun, C.H., Teng, Y., Li, G.Z., Yoshioka, S., Yokota, J., Miyamura, M., Fang, H.Z., Zhang, Y., 2010. Metabonomics study of the protective effects of Lonicera japonica extract on acute liver injury in dimethylnitrosamine treated rats. Journal of Pharmaceutical and Biomedical Analysis 53, 98–102.
The State Commission of Chinese Pharmacopoeia, 2010. Pharmacopoeia of People's Republic of China, Part I. China Medical Science Press, Beijing.
Torocsik, D., Szanto, A., Nagy, L., 2009. Oxysterol signaling links cholesterol metabolism and inflammation via the liver X receptor in macrophages. Molecular Aspects of Medicine 30, 134–152.
Trygg, J., Holmes, E., Lundstedt, T., 2007. Chemometrics in metabonomics. Journal of Proteome Research 6, 469–479.
Ullsten, S., Danielsson, R., Backstrom, D., Sjoberg, P., Bergquist, J., 2006. Urine profiling using capillary electrophoresis-mass spectrometry and multivariate data analysis. Journal of Chromatography A 1117, 87–93.
Wang, W.K., Wang, Y.M., Zhang, Y., Shen, Y.J., Qi, Y., 2000. Anti-inflammatory mechanism of the volatile oil of Flos Magnoliae. Journal of Shanxi Agricultural University 20, 324–326.
Wang, W.K., Shen, Y.J., Qi, Y., Song, J.X., 2005. Volatile oil of flos magnoliae biondii pamp for the foot tumefaction and the expression of prostaglandin E2 in joint tissue in rats with adjuvant arthritis. Chinese Journal of Clinical Rehabilitation 9, 210–211.
Xiong, T.Q., Qin, X.H., Pang, L., Shen, Y.J., 2006. Experimental study of VOMbP on allergic rhinitis. Study Journal of Traditional Chinese Medicine 24, 1031–1032.
Yu, K., Sheng, G.P., Sheng, J.F., Chen, Y.M., Xu, W., Liu, X.L., Cao, H.C., Qu, H.B., Cheng, Y.Y., Li, L.J., 2007. A metabonomic investigation on the biochemical perturbation in liver failure patients caused by hepatitis B virus. Journal of Proteome Research 6, 2413–2419.
Zhao, O., Liang, Y.Z., 2007. Volatile oil obtained from Yulan Magnolia flower bud with different methods. Journal of Chinese Mass Spectrometry Society 28, 106–113.
Zhao, X.J., Zhang, Y., Meng, X.L., Yin, P.Y., Deng, C., Chen, J., Wang, Z., Xu, G.W., 2008. Effect of a traditional Chinese medicine preparation Xindi soft capsule on rat model of acute blood stasis: a urinary metabonomics study based on liquid chromatography–mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 873, 151–158.