

Tropical Journal of Pharmaceutical Research June 2016; 15 (6): 1235-1241

ISSN: 1596-5996 (print); 1596-9827 (electronic)

© Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

All rights reserved.

Available online at <http://www.tjpr.org><http://dx.doi.org/10.4314/tjpr.v15i6.16>

Original Research Article

Isolation and identification of two galangin metabolites from rat urine and determination of their *in vitro* hypolipidemic activity

Xuguang Zhang^{1,2}, Shouqian Cheng^{1,2}, Hailong Li^{1,2}, Xiaopo Zhang², Feng Chen^{1,2}, Youbin Li^{1,2}, Junqing Zhang^{1,2*} and Yinfeng Tan^{1,2}

¹Hainan Provincial Key Laboratory of R&D of Tropical Herbs, ²School of Pharmaceutical Science, Hainan Medical University, Haikou, 571101, PR China

*For correspondence: **Email:** jqzhang2011@163.com, secondmessenger@163.com; **Tel:** +86 898 66895337; **Fax:** +86 898 66895337

Received: 24 November 2015

Revised accepted: 14 May 2016

Abstract

Purpose: To investigate the lipid-lowering activity of two metabolites of galangin, namely, galangin-3-O- β -D-glucuronic acid (GG-1) and galangin-7-O- β -D-glucuronic acid (GG-2).

Methods: Female Sprague-Dawley rats were orally administered with galangin. The two metabolites of galangin were isolated from urine sample and purified using Sephadex LH-20 and semi-preparative high performance liquid chromatography (HPLC). The structures of the metabolites were identified by analyzing spectroscopic data. Hypolipidemic activity was evaluated in HepG2 cells. The down- or up-regulation of lipogenic genes was detected using real-time quantitative polymerase chain reaction (qPCR).

Results: Both metabolites of galangin showed hypolipidemic activity. These activities are closely associated with the down-regulation of lipogenic genes such as SREBP-1a, SREBP-1c, and SREBP-2 transcription factors, and the downstream genes such as FAS, ACC, and HMGR were revealed by real-time qPCR data.

Conclusion: The results show that both metabolites possess better lipid-lowering activities than galangin. These hypolipidemic activities are closely associated with inhibiting key genes or proteins that regulated the biosynthesis of both cholesterol and triglycerides.

Keywords: Galangin, Galangin-3-O- β -D-glucuronic acid, Galangin-7-O- β -D-glucuronic acid, Hypolipidemic, Lipogenic genes, Metabolites

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Obesity is becoming a serious threat to human beings [1]. More accumulation of lipids in liver is a key cause for insulin resistance [2]. Therefore, decreasing lipid deposition in liver through inhibition of lipogenesis or stimulation of lipolysis is an effective way for the prevention and treatment of obesity and diabetes [3-4]. Natural products such as cordycepin [5], resveratrol [6],

and berberine [7] have showed ability in suppressing lipogenesis and accumulation of fat, and are considered to be the potential candidates in developing new therapeutic agents.

Galangin, a natural flavonoid obtained from *Alpinia officinarum*, has been found to possess various biological activities [8,9]. Specifically, this compound showed a significant decrease in

serum lipids, liver weight, peroxidation of lipids, and accumulation of hepatic triglycerides, which suggested that galangin has the potential for controlling obesity [10]. Interestingly, previous pharmacokinetic studies have found two metabolites of galangin, and are considered as the effective *in vivo* components [11-12]. However, the limitations in developing new therapeutic agent from galangin are mainly due to its low bioavailability. Therefore, the metabolites obtained from urine samples may be considered as the better candidates. From this approach, two metabolites namely galangin-3-O- β -D-glucuronic acid (GG-1) and galangin-7-O- β -D-glucuronic acid (GG-2) were obtained from the rat urine. In addition, the effects of these two metabolites on lipid accumulation in HepG2 cells and its hypolipidemic mechanisms were investigated to find potential utility in the prevention and treatment of obesity.

EXPERIMENTAL

General experimental procedures

HepG2 cells were obtained from the American Type Culture Collection (Manassas, Virginia, USA) and China Union Medical University. ^1H , ^{13}C -nuclear magnetic resonance (NMR) experiments were performed on Bruker spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C (tetramethylsilane was used as an internal standard). Chemical shifts were expressed in δ ppm referenced to solvent peaks at δH 2.50 and δC 39.6 in dimethyl sulfoxide (DMSO)- d_6 , and coupling constants were in Hz. Electrospray ionization-mass spectrometry (ESI-MS) was obtained from a Thermo Scientific LTQ-Orbitrap XL instrument (Bremen, Germany). Sephadex LH-20 (GE Health care) was used for column chromatography. High performance liquid chromatography (HPLC) was performed on LC-6AD equipped with an ultra violet (UV) detector of SPD-10A (Shimadzu), and a column of Zorbax SB-phenyl (250 \times 9.4mm, 5 μm , Agilent Technologies Co., Ltd). Mixtures of methanol (CH_3OH)/water (H_2O) were used as an eluent. All solvents used were of analytical grade.

Reagents

Galangin was extracted from *Alpinia officinarum* in laboratory previously. The chemical structure of galangin was established by analyzing NMR data and compared with literature. The purity of galangin (> 95 %) was determined using HPLC equipped with a UV detector with an Agilent eclipse XDB-C18 column (5 μm , 4.6 \times 250 mm). Then, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-

diphenyltetrazolium bromide (MTT) was obtained from Sigma-Aldrich (Saint Louis, Missouri, USA).

Animals

All animal experiments were performed per International Guidelines for Care and Use of Laboratory Animals [20] and approved by the animal ethics committee of Hainan Medical University (reg. no. 201506017/HMU). Female Sprague-Dawley rats (240 to 300 g) were purchased from DongChuang Laboratory Animal Service Department (Changsha, China). Commercial rat chow was available *ad libitum* except for an overnight fasting period before dosing. All rats were provided with free access to H_2O .

Urine collection

Rats were housed separately in rat metabolic cages. Urine samples were collected before and after 0-24 h oral dose of galangin (90 mg/kg). For the oral administration, 30 mg/mL of galangin solution was dissolved in distilled H_2O containing 2 % (w/v) tween-80.

Extraction and isolation of two galangin metabolites

The collected urine samples were pooled. Then, 300 mL of pooled urine samples was diluted with 700 mL of distilled H_2O to give 1000 mL of solvent. Then, the solvent was partitioned twice with 300 mL of ethyl acetate and n-butanol to give two extracts, respectively. These extracts were concentrated successively under reduced pressure to obtain the ethyl acetate (2.0 g) and n-butanol (5.0 g). The n-butanol extract was separated with Sephadex LH-20 using CH_3OH as an eluent to yield six fractions (Fr.1 to Fr.6). Fr.5 (800 mg) was further purified by semi-preparative HPLC with $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (40:60) as an eluent to give compounds 1 (6.0 mg), and 2 (8.0 mg).

Cell-based lipid accumulation assay

HepG2 cells were maintained in a Dulbecco's modified eagles medium (DMEM, Gibco, Grand Island, New York, USA) supplemented with 10 % fetal bovine serum (Gibco) and 100 $\mu\text{g}/\text{mL}$ of penicillin/streptomycin (Gibco). After reaching 70-80 % confluence, the cells were incubated in DMEM and 100 μM of oleic acid (OA, Sigma-Aldrich, Shanghai, China) for 12 h, then the cells were treated with 10 μM of indicated compounds or marketed antihyperlipidemic drug simvastatin (Sigma-Aldrich) in DMEM and 100 μM of OA or with DMEM and 100 μM of OA alone for another

6 h. Subsequently, the cells were subjected to oil-red O staining, total cholesterol and triglycerides were determined as described previously [19]. Each experiment was repeated for three times.

MTT assay

HepG2 cells were cultured in a 24-well plate. After reaching confluence, the cells were incubated for 48 h in presence of GG-1, GG-2, and galangin. Subsequently, the culture medium was removed and replaced with 500 μ L of fresh culture medium containing 10 % sterile filtered MTT. After 3 h, the formed insoluble formazan crystals were dissolved in 500 μ L of isopropanol per well and absorbance was measured at 570 nm using the 630 nm reading as a reference. The inhibition of growth due to tested compounds was expressed as a percentage of viable cells in experimental wells than in control wells.

Real-time quantitative polymerase chain reaction (qPCR)

The mRNA levels of lipid metabolism-related genes were determined using real-time qPCR. Total RNA extraction, cDNA synthesis, and quantitative PCR assays were all performed as described previously [20]. Samples were cycled 40 times using a fast applied biosystems (ABI)-7500 sequence detector (Foster City, USA). ABI-7500 cycle conditions were as follows: denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C for 15 s, annealing at 60 °C for 30 s, and holding at 72 °C for 30 s. Cycle threshold was calculated under default settings of real-time sequence detection software (Applied Biosystems). Three independent biological replicates were analyzed to check the reproducibility of the data.

Statistical analysis

Data are presented as mean \pm standard deviation. One-way analysis of variance was used to determine the significant difference between both groups. Modified Student's t-test with the Bonferroni correction was used to compare the difference between individual groups. $P < 0.05$ was considered as statistically significant.

RESULTS

Spectral data for GG-1, GG-2, and galangin

GG-1: ESI-MS m/z 444.9 [M-H]⁻; ¹H-NMR (400 MHz, DMSO-*d*₆) δ : 12.38 (1H, br.s, 5-OH), 8.13 (2H, d, $J=7.5$ Hz, H-2', 6'), 7.52 (3H, m, H-3', 4', 5'), 6.26 (1H, br.s, H-8), and 6.08 (1H, br.s, H-6), and 5.54 (1H, d, $J=7.2$ Hz, H-1''); and ¹³C-NMR (100 MHz, DMSO-*d*₆) δ : 178.1 (C-4), 172.2 (C-6''), 163.0 (C-7), 162.6 (C-5), 157.0 (C-9), 156.3 (C-2), 134.5 (C-3), 131.1 (C-1'), 130.9 (C-4'), 129.6 (C-2', 6'), 128.6 (C-3', 5'), 104.3 (C-10), 99.2 (C-6), 94.3 (C-8), 76.7 (C-3''), 74.8 (C-5''), 74.3 (C-2''), and 72.5 (C-4'').

GG-2: ESI-MS m/z 444.9 [M-H]⁻; ¹H-NMR (400 MHz, DMSO-*d*₆) δ : 12.38 (1H, br.s, 5-OH), 8.10 (2H, d, $J=7.5$ Hz, H-2', 6'), 7.52 (3H, m, H-3', 4', 5'), 6.85 (1H, br.s, H-8'), 6.44 (1H, br.s, H-6''), and 5.13 (1H, d, $J=7.2$ Hz, H-1''); and ¹³C-NMR (100 MHz, DMSO-*d*₆) δ : 178.1 (C-4), 172.1 (C-6''), 162.6 (C-5), 160.8 (C-7), 156.5 (C-9), 146.8 (C-2), 132.1 (C-3), 131.4 (C-1'), 130.6 (C-4'), 129.0 (C-2', 6'), 128.1 (C-3', 5'), 105.3 (C-10), 99.4 (C-6), 94.8 (C-8), 76.7 (C-3''), 74.7 (C-5''), 73.3 (C-2''), and 72.3 (C-4'').

Galangin: ESI-MS m/z 268.9 [M-H]⁻; ¹H-NMR (400 MHz, DMSO-*d*₆) δ : 12.36 (1H, br.s, 5-OH), 8.14 (2H, m, $J=7.5$ Hz, H-4'), 7.50-7.55 (3H, m, H-3', 4', 5'), 6.45 (1H, br.s, H-8'), and 6.20 (1H, br.s, H-6'); and ¹³C-NMR (100 MHz, DMSO-*d*₆) δ : 176.3 (C-4), 164.3 (C-7), 160.8 (C-5), 156.5 (C-9), 145.8 (C-2), 137.1 (C-3), 131.4 (C-1'), 130.0 (C-4'), 128.6 (C-2', 6'), 127.6 (C-3', 5'), 103.3 (C-10), 98.4 (C-6), and 93.6 (C-8).

Characteristic features of the two galangin metabolites

Compound 1

Compound **1** was obtained as a brown powder. The spectral data of ¹H-NMR were resonated at δ : 12.38 (1H, br.s, 5-OH), 8.13 (2H, d, $J=7.5$ Hz, H-2', 6'), 7.52 (3H, m, H-3', 4', 5'), 6.26 (1H, br.s, H-8), and 6.08 (1H, br.s, H-6) indicated compound **1** as a flavonoid. The NMR spectral data of compound **1** were similar to that of galangin. Further analysis of NMR data showed that a glucuronic acid group existed in compound **1**. Comparing the ¹³C-NMR data of compound **1** with galangin revealed the downfield shifts of C-2 and C-4 were +10.6 and 1.8, respectively, whereas the upfield shift of C-3 (-1.6 ppm) indicating the glucuronic acid group was located at C-3. Therefore, compound **1** was identified as GG-1.

Compound **2** was also obtained as a brown powder. The spectral data of NMR and MS were identical to compound **1**. The connection of glucuronic acid group to the aglycone was also determined in a same manner. Comparing the ¹³C-NMR data of compound **2** with galangin revealed the downfield shifts of C-6 and C-8 were +1.2 and 1.1, respectively, whereas the upfield shift of C-7 (-3.4 ppm) indicating the

glucuronic acid group was located at C-7. Therefore, compound **2** was identified as GG-2.

GG-1 and GG-2 inhibit lipid accumulation in HepG2 cells

To evaluate the effect of GG-1 and GG-2 on lipid metabolism, OA-elicited neutral lipid accumulation in HepG2 cells was used. The intracellular lipid content was determined by oil-red O staining and specific kits for accumulation of lipid, total cholesterol, and triglycerides.

Supplementation with OA significantly increased accumulation of lipid in HepG2 cells (Figure 2). Treatment with GG-1 and GG-2 decreased OA-elicited neutral lipid accumulation (Figure 2A) as well as intracellular contents of triglyceride (Figure 2B) and total cholesterol (Figure 2C) in a dose-dependent manner. The inhibitory efficiency of both compounds was higher than that of galangin. The MTT assay showed that the inhibitory effect of GG-1 and GG-2 on lipid metabolism was independent of its cytotoxic effects on HepG2 cells (Figure 3).

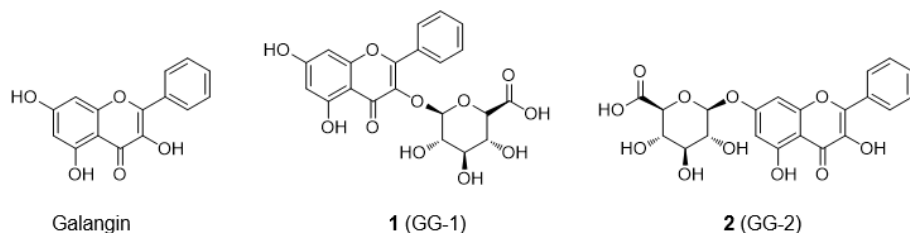


Figure 1: Chemical structures of galangin, **1** (GG-1), and **2** (GG-2)

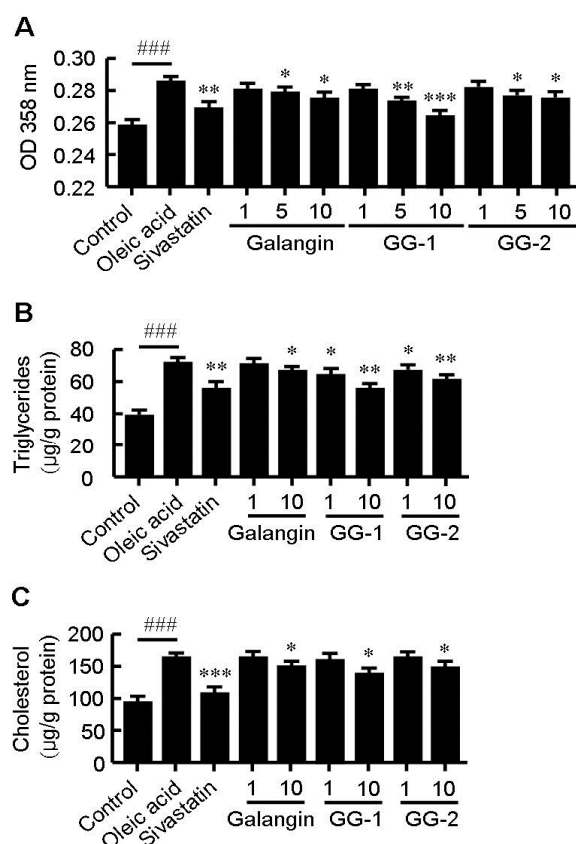


Figure 2: Effects of GG-1 and GG-2 on lipid accumulation. HepG2 cells were treated with galangin, GG-1, and GG-2 (μM as indicated) or simvastatin ($10 \mu\text{M}$) in DMEM containing $100 \mu\text{M}$ of oleic acid or with serum-free DMEM alone (blank) for 24 h. The optical density of 358 nm was obtained after oil-red O staining (**A**), intracellular levels of triglyceride (**B**), and total cholesterol (**C**) were determined. Values are represented as mean \pm SD. Results are represented in three independent experiments. ^{###} $P < 0.001$ vs blank group; ^{*} $P < 0.05$, ^{**} $P < 0.01$, and ^{***} $P < 0.001$ vs oleic acid group

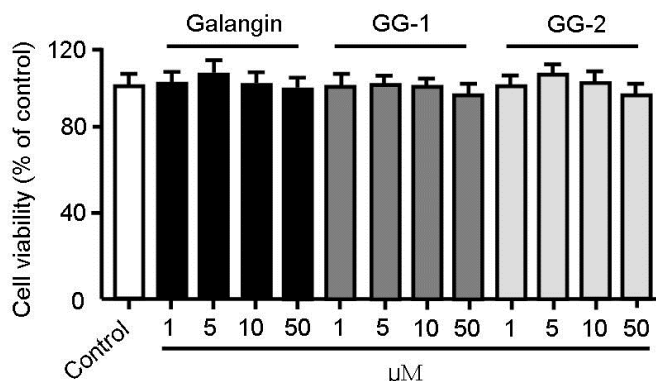


Figure 3. Effect of GG-1 and GG-2 on cell viability was determined by MTT assay. The inhibition of cell viability was expressed as a percentage of viable cells in experimental wells than in control wells. Values are represented as mean \pm SD. Results are represented in three independent experiments

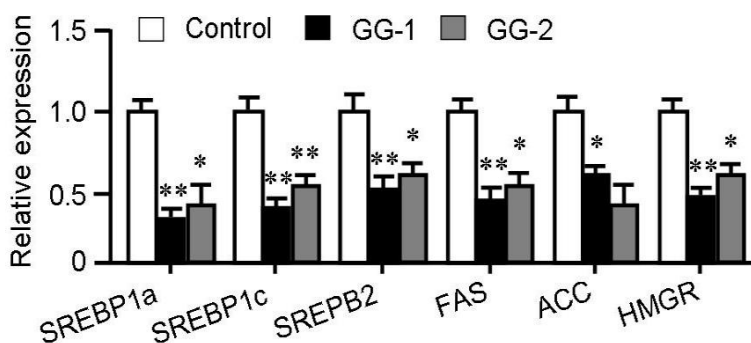


Figure 4: Effects of GG-1 and GG-2 on expression of SREBP-1a, SERBP-1c, and SREBP-2, and mRNA levels of FAS, ACC, and HMGR were analyzed using real-time qPCR. The expression level of genes was normalized to β -actin mRNA levels. Values are presented as mean \pm SD. Results are represented in three independent experiments; * $p < 0.05$, ** $p < 0.01$ vs control group

GG-1 and GG-2 decreased transcription of lipogenesis-related transcription factors and its targeted genes

Real-time qPCR showed that treatment with 10 μ M of GG-1 and GG-2 significantly decreased the expression of lipogenic genes such as SREBP-1a, SREBP-1c, and SREBP-2 transcription factors and its downstream genes such as FAS, ACC, and HMGR. GG-1 is more potent in regulation of these lipogenic genes than GG-2 (Figure 4).

DISCUSSION

Herbal medicines have been used in the treatment of obesity for past 100 decades [21,22]. Natural compounds with interesting structures and lipid regulating activities have attracted numerous attentions from chemists and pharmacologists [23]. Statins are the

representative compound originated from natural sources with antihyperlipidemic activity [24]. Other compounds such as cordycepin, resveratrol, and berberine are deemed as the effective regulators of lipid metabolism. Galangin has also been proved to have the ability in lowering the lipid levels in liver and blood [10].

However, most natural products are not used as a drug when isolated from natural sources [25]. There are many factors influencing the drug ability. Among all, low biological availability is considered to be the most important factor [26]. Many methods are used to resolve this problem. Finding metabolites from natural products of biological samples have proved to be an effective process in drug discovery [27,28].

Previous studies have found that GG-1 and GG-2 are the main metabolites of blood and urine samples when rats were orally administered with

galangin. Therefore, GG-1 and GG-2 are considered to be the active *in vivo* constituents. To verify this hypothesis, the lipid lowering activity of these metabolites has been investigated. The results have demonstrated that GG-1 and GG-2 possess lipid lowering activity. Significantly, these two metabolites showed better hypolipidemic activities than galangin. This may be attributed to the presence of glucuronic acid group in the carbon skeleton. The potential mechanisms have also been investigated and are found to be closely associated with inhibiting key genes or proteins that regulated the biosynthesis of both cholesterol and triglycerides.

CONCLUSION

The findings of this study indicate that both metabolites possess better lipid-lowering activities than galangin. These hypolipidemic activities are closely associated with inhibiting key genes or proteins that regulate the biosynthesis of both cholesterol and triglycerides.

ACKNOWLEDGEMENT

The authors appreciated the financial support provided by the National Natural Science Fund of China (no. 81560721), Hainan Special Plan for the Modernization of Chinese Medicines (no. 2015ZY06), and Hainan Science and Technology Major Project (nos. ZDZX2013008-3 and ZDXM 2015078).

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. Medina-Escobedo M, Alcocer-Dzul R, López-López J, Salha-Villanueva J. Obesity as a risk factor for metabolic disorders in adults with urolithiasis. *Rev Med Inst Mex Seguro Soc* 2015; 53: 692-697.
2. Pin M, Štimac D. Correlation between obesity and pancreatic cancer. *Lijec Vjesn* 2015; 137: 236-240.
3. Nuffer WA, Trujillo JM. Liraglutide: A new option for the treatment of obesity. *Pharmacotherapy* 2015; 35: 926-934.
4. Laragione T, Gulko PS. Liver X receptor regulates rheumatoid arthritis fibroblast-like synoviocyte invasiveness, matrix metalloproteinase 2 activation, interleukin-6 and CXCL10. *Mol Med* 2012; 18: 1009-1017.
5. Ma L, Zhang S, Du M. Cordycepin from *Cordyceps militaris* prevents hyperglycemia in alloxan-induced diabetic mice. *Nutr Res* 2015; 35: 431-439.
6. Aguirre L, Fernández-Quintela A, Arias N, Portillo M. Resveratrol: anti-obesity mechanisms of action. *Molecules* 2014; 19: 18632-18655.
7. Jung YC, Kim ME, Yoon JH, Park PR, Youn HY, Lee HW, Lee JS. Anti-inflammatory effects of galangin on lipopolysaccharide-activated macrophages via ERK and NF- κ B pathway regulation. *Immunopharmacol Immunotoxicol* 2014; 36: 426-432.
8. Huo SX, Liu XM, Ge CH, Gao L, Peng XM, Zhao PP, Yan M. The effects of galangin on a mouse model of vitiligo induced by hydroquinone. *Phytother Res* 2014; 28: 1533-1538.
9. Zhang Z, Zhang H, Li B, Meng X, Wang J, Zhang Y, Yao S, Ma Q, Jin L, Yang J, Wang W, Ning G. Berberine activates thermogenesis in white and brown adipose tissue. *Nat Commun* 2014; 5: 5493.
10. Kumar S, Alagawadi KR. Anti-obesity effects of galangin, a pancreatic lipase inhibitor in cafeteria diet fed female rats. *Pharm Biol* 2013; 51: 607-613.
11. Chen F, Tan YF, Li HL, Qin ZM, Cai HD, Lai WY, Zhang XP, Li YH, Guan WW, Li YB, Zhang JQ. Differential systemic exposure to galangin after oral and intravenous administration to rats. *Chem Cent J* 2015; 9: 14-24.
12. Zhang JQ, Wang Y, Li HL, Wen Q, Hang Y, Zeng NK, Lai WY, Wei N, Cheng SQ, Kang SL, Chen F, Li YB. Simultaneous quantification of seventeen bioactive components in rhizome and aerial parts of *Alpinia officinarum* Hance using LC-MS/MS. *Anal. Methods* 2015; 7: 4919-4926.
13. Kim A, Choi J, Htwe KM, Chin YW, Kim J, Yoon KD. Flavonoid glycosides from the aerial parts of *Acacia pennata* in Myanmar. *Phytochemistry* 2015; 118: 17-22.
14. Gao J, Yang H, Chen J, Fang J, Chen C, Liang R, Yang G, Wu H, Wu C, Li S. Analysis of serum metabolites for the discovery of amino acid biomarkers and the effect of galangin on cerebral ischemia. *Mol Biosyst* 2013; 9: 2311-2321.
15. Guerrero L, Margalef M, Pons Z, Quiñones M, Arola L, Arola-Arnal A, Muguerza B. Serum metabolites of proanthocyanidin-administered rats decrease lipid synthesis in HepG2 cells. *J Nutr Biochem* 2013; 24: 2092-2099.
16. Zhang T, Yamamoto N, Ashida H. Chalcones suppress fatty acid-induced lipid accumulation through a LKB1/AMPK signaling pathway in HepG2 cells. *Food Funct* 2014; 5: 1134-1141.
17. Lee J, Hong SW, Park SE, Rhee EJ, Park CY, Oh KW, Park SW, Lee WY. AMP-activated protein kinase suppresses the expression of LXR/SREBP-1 signaling-

- induced ANGPTL8 in HepG2 cells. *Mol Cell Endocrinol* 2015; 414: 148-155.
18. Wong TY, Lin SM, Leung LK. The Flavone Luteolin Suppresses SREBP-2 Expression and Post-Translational Activation in Hepatic Cells. *PLoS One* 2015; 10: e0135637.
 19. Hao S, Xiao Y, Lin Y, Mo Z, Chen Y, Peng X, Xiang C, Li Y, Li W. Chlorogenic acid-enriched extract from *Eucommia ulmoides* leaves inhibits hepatic lipid accumulation through regulation of cholesterol metabolism in HepG2 cells. *Pharm Biol* 2015; 7: 1-9.
 20. Hong Q, Xia C, Xiangying H, Quan Y. Capsinoids suppress fat accumulation via lipid metabolism. *Mol Med Rep* 2015; 11: 1669-1674.
 21. Neves BJ, Andrade CH, Cravo PV. Natural products as leads in schistosome drug discovery. *Molecules* 2015; 20: 1872-1903.
 22. Gerwick WH, Moore BS. Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. *Chem Biol* 2012; 19: 85-98.
 23. Jia Y, Shen J, Li X, Xie H, Wang J, Luo J, Wang KD, Liu Q, Kong L. Identification and analysis of gastrodin and its five metabolites using ultra-fast liquid chromatography electrospray ionization tandem mass spectrometry to investigate influence of multiple-dose and food. *J Chromatogr A* 2014; 1358: 110-116.
 24. Endo-Umeda K, Yasuda K, Sugita K, Honda A, Ohta M, Ishikawa M, Hashimoto Y, Sakaki T, Makishima M. 7-Dehydrocholesterol metabolites produced by sterol 27-hydroxylase (CYP27A1) modulate liver X receptor activity. *J Steroid Biochem Mol Biol* 2014; 140: 7-16.