JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Influence of Gastrointestinal Digestion and Edible Plant Combination on Oral Bioavailability of Triterpene Saponins, Using a Biomimetic Digestion and Absorption System and Determination by HPLC

Shun-Xing Li,*[,†,‡] Yang Mu,[†] and Feng-Ying Zheng[†,‡]

[†]Department of Chemistry and Environmental Science and [‡]Fujian Province University Key Laboratory of Modern Analytical Science and Separation Technology, Minnan Normal University, Zhangzhou, China 363000

ABSTRACT: Saponins have many biological activities, but their overload could cause toxicity to the human body. Bionic gastrointestinal digestion and monolayer liposome extraction were used for oral bioavailability assessment of triterpene saponins (notoginsenoside R1, ginsenosides Rb1 and Rd1) in an edible herb (San-Chi) and its compound herbal medicine (Pien Tze Huang, PZH). The concentrations of affinity-monolayer liposome saponins in the chyme were determined by HPLC and used for oral bioavailability assessment. With the digestion of San-Chi and PZH from the stomach to the intestine, the release of saponins in their chyme was increased. The intestinal absorption ratios of N-R1, G-Rb1, G-Rd1, and total saponins from San-Chi were 86.57, 18.56, 73.30, and 40.20%, respectively, which were more than those from PZH (i.e., 19.56, 10.11, 30.11, and 16.08%). The oral bioavailability of saponins was controlled by saponin species, gastrointestinal digestion, and edible plants combination.

KEYWORDS: *saponins, oral bioavailability, gastrointestinal digestion, edible plants combination*

INTRODUCTION

Saponins are a heterogeneous group of triterpene or steroidal glycosides that are widely distributed among food plants.[1] San-Chi is a kind of edible herb, known as *Acanthopanax Gracilistylus*, *stephania sinica*, and *pseudoginsen radix*. As a famous compound medicine in China, even Southeast Asia, Pien Tze Huang (PZH) was the combination of San-Chi, cow bezoar (dried cattle gall bladder stones), snake's gall bladder, and musk.[2] PZH was used for the investigation of the influence of edible plant combination. Three triterpene saponin species, including notoginsenoside R1 (N-R1), ginsenoside Rb1 (G-Rb1), and ginsenoside Rd1 (G-Rd1), are the principal active constituents of San-Chi and PZH.[3,4] These three species of saponins could be used as an anti-inflammatory,[5] for neuro-protection,[6] to attenuate fibrosis of myocardial and renal tubulointerstitial,[7] for cardiovascular and liver protection,[8−10] and for immune stimulation.[11] However, saponin overload could cause gastrointestinal tract irritation, intestinal disorders, violent convulsions, and paralysis.[12] Consequently, the oral bioavailability of saponins is important for the consumer, and the safe dose of saponins should be assessed.

Most of the research on saponins in edible plants is focused on determination, isolation, characterization, and pharmacology.[13−15] Oral administration is the most convenient route for medical delivery. The published papers do not give information on the amount of oral bioavailability for reported saponins. With respect to the potential toxicity of saponins, it is necessary to plan the dose of edible plants. All of these problems should be given appropriate attention to the bioavailability assessment of saponins in edible plants. The design of a valid method for oral bioavailability assessment of saponins in edible plants is important.

Currently, saponin bioavailability has been assessed by laboratory animal in vivo studies.[16−19] However, animal experimentation is limited by the uncertainties with regard to the differences in the metabolism between animals and man. Both Caco-2 cell monolayer and everted gut sac have been used for in vitro studies,[20,21] but such methods are costly, time-consuming, and complicated. The major obstacle to rapidly assessing the oral bioavailability of bioactive compositions is lack of experimental model systems for replacing in vivo and in vitro studies.[19,22−24] Because edible plants, including San-Chi and PZH, have been have been used for a long time, their absorption examinations of saponins could provide useful information.[25,26] Herein, a biomimetic digestion system of gastrointestinal tracts has been proposed by us.[27] Similar to the biomembrane between the gastrointestinal tract and blood vessels, monolayer liposome is used as a biomembrane model. The content of affinity-monolayer liposome saponins (AMLS) could be the criterion for oral bioavailability assessment of saponins in the chyme.

The oral bioavailability of saponins is very low, but the underlying mechanisms remain unknown.[28] It might be due to low intestinal absorption or high gastrointestinal metabolism. Furthermore, edible plants are usually used together, that is, edible plant combinations. Therefore, the present study aims to investigate the effect of gastrointestinal digestion and edible plant combination on saponins' bioavailability. High-performance liquid chromatography (HPLC) was used for the detection and quantification of N-R1, G-Rb1, and G-Rd1 in San-Chi, PZH, and their chymes.

MATERIALS AND METHODS

Chemicals. HPLC grade acetonitrile (purity ≥ 99.9%) was purchased from Merck KGaA (Darmstadt, Germany). PTZ and San-

Received: July 7, 2013
Revised: October 3, 2013
Accepted: October 7, 2013
Published: October 7, 2013

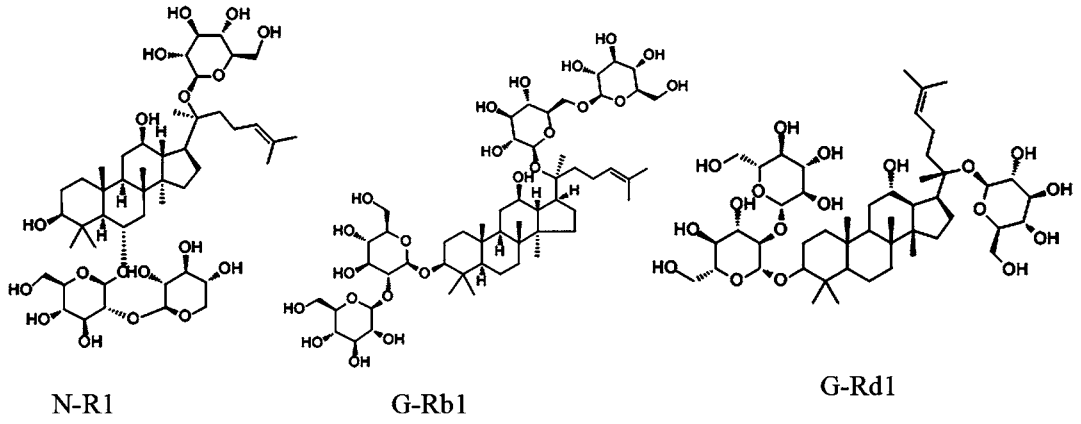

Figure 1. Structures of N-R1, G-Rb1, and G-Rd1.

Chi were bought from Pien Tze Huang Pharmaceutical Co., Ltd. (Zhang Zhou, China); their origins were authenticated by Professor Hong Fei. Certified materials, N-R1, G-Rb1, and G-Rd1 were supported by the National Standard Substance Center in China, and their structures are shown in Figure 1, and 20 mg of them was dissolved by MeOH for the preparation of standard solutions, respectively. Ethanol, uric acid, mucin, bovine albumin, pepsin, pancreatin, lipase, bile, and lecithin were all of analytical reagent grade and purchased from Sigma (St. Louis, MO, USA). All sample preparations were prepared with Milli-Q purified water (18.2 MΩ). All of glassware and plastic ware was washed and kept for 48 h in 10% (v/v) nitric acid and then rinsed several times with ultrapure water before use.

Apparatus. An Agilent technologied model 1260 liquid chromatograph (Agilent Technologies Co., USA) fitted with a diode array detector (DAD), online degasser, quatpump, and column heater was used for analysis of saponins. Milli-Q purified water was obtained from a Milli-Q purified water apparatus (Millipore Co., USA). Other equipments were used, including a Mettler Toledo 320-S pH-meter (Mettler Toledo Co., China) with a combined electrode, an RE-52 rotator evaporator (Ya Rong Biochemical Instrument Factory, China), an SHA-B temperature-consistent oscillating water bath (Guo Hua Co., China), an 86C ULT ultralow-temperature freezer (Thermo Electron Co., USA), and a ROTINA 420B high-speed centrifuge (Hettich Co., Germany).

HPLC Analytical Conditions. Because C18 columns were usually used for saponin separation,[29−31] an Agilent ZORBAX Eclipse XDB-C_{18} column (150 mm × 4.6 mm, 5 μm particle size) was used. The column was thermostated at 30 °C. Solvent gradient elution was performed as follows: initial mobile phase acetonitrile/water (20:80, v/v), reaching acetonitrile/water (35:65, v/v) in 15 min, maintaining this concentration for 10 min, and reconditioning for 10 min with the initial condition. The injection volume was 20 μL, and the flow rate was set at 1 mL/min. The UV-DAD detection was performed at the wavelength of 203 nm.

Extraction of Triterpene Saponins in San-Chi and PZH. Ten grams of San-Chi or PZH was thoroughly ground in an agate mortar. Sample powder (0.25 g) (PZH or San-Chi) was weighed and dissolved in 25 mL of ethanol. Ultrasound-assisted extraction was used for the extraction of saponins for 30 min.[32−34] Then the above samples were filtered through 0.45 μm nylon filters. The filtrates were used for the analysis of triterpene saponins by HPLC.

Digestion of San-Chi and PZH and Absorption of Triterpene Saponins in Bionic Gastrointestinal Tract. According to our previously published method,[23,27] 2.40 g of PZH or San-Chi powder was digested in a bionic mouth, stomach, and intestine at 37 °C on gentle oscillation, as shown in Figure 2. All chymes were filtered with a 0.45 μm membrane.

Egg-derived lecithin (0.25 g) was dissolved in chloroform and then transferred into a rotatory evaporator to evaporate chloroform. Twenty-five milliliters of chyme was mixed with liposome to form a homogeneous liposome suspension, frozen at −71 °C in a superlow freezer for 30 min, and then thawed at 37 °C. The freeze−thaw process was repeated five times to promote triterpene saponin distribution in the liposome−water system. AMLS could be separated by 0.22 μm membrane. AMLS was dissolved by 25 mL of ethanol and sonicated for 30 min at room temperature.

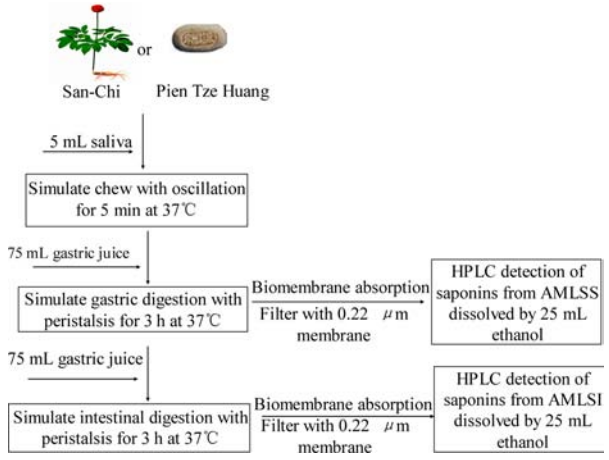

Figure 2. Schematic diagram of oral and gastrointestinal digestion.

Then the sample was filtered through a 0.22 μm filter. The filtrate was used for the analysis of AMLS by HPLC. AMLS in the chyme from gastrointestinal digestion of San-Chi or PZH was calculated as

$$C_{AMLS} = \frac{C_{chyme} \times V_{chyme}}{M}$$

where C_{chyme} is the concentration of AMLS in chyme (mg/mL), V_{chyme} is the volume of chyme (mL), and M is the mass of PZH or San-Chi (g).

RESULTS AND DISCUSSION

Method Validation. An external standard method was utilized for the quantitative determination of triterpene saponins, and a linear correlation between the concentrations of N-R1, G-Rb1, and G-Rd1 and their corresponding peak areas could be observed. The equations of the calibration curves, correlation coefficient, linear range, limit of detections, and relative standard deviation are available in Table 1. The method described was applicable for the determination of low levels (μg/mL) of triterpene saponins in San-Chi, PZH, and their chymes.

Triterpene Saponin Contents in San-Chi and PZH. N-R1, G-Rb1, and G-Rd1 were the main species of saponins in

Table 1. Calibration Curve, Relative Standard Deviation (RSD), and Detection Limits (LOD) for N-R1, G-Rb1, and G-Rd1 (n = 3)

					RSD (%)	
saponin	calibration curve	r	linear range (μg/mL)	LOD (μg/mL)	intraday	interday
N-R1	$Y = 2.39X - 0.28$	1.000	10−200	3.04	0.86−2.5	1.2−2.1
G-Rb1	$Y = 5.22X - 50.33$	0.999	10−400	2.93	0.42−1.3	0.88−2.2
G-Rd1	$Y = 5.95X - 4.13$	0.999	10−200	2.87	0.53−2.4	1.0−1.9

San-Chi and PZH; these triterpene saponins could be extracted by ethanol and detected, as shown in Figure 3. Satisfactory

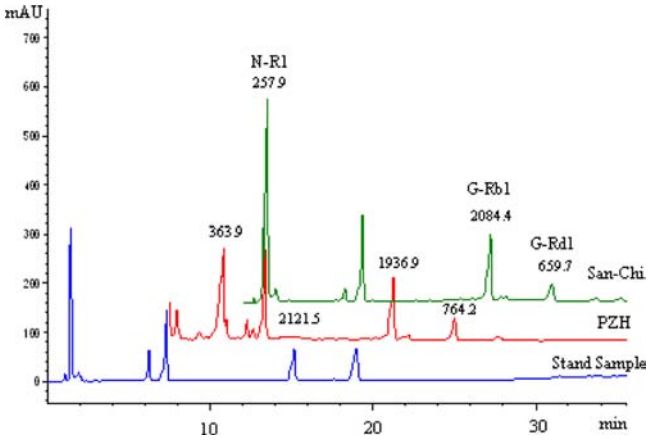

Figure 3. HPLC chromatograms of triterpene saponins in San-Chi, PZH, and stand sample.

separation of the three triterpene saponins was obtained in 20 min. The contents of N-R1, G-Rb1, and G-Rd1 in edible plants were 11.08, 40.90, and 11.16 mg/g for San-Chi and 15.64, 38.07, 12.29 mg/g for PZH, respectively. After combination, the concentration of G-Rb1 was decreased 6.9%. However, the contents of N-R1, G-Rd1, and total triterpene saponins were increased 41.1, 10.9, and 7.5%, respectively, so, the contents of saponins were improved by herbal combination.

Effect of Gastrointestinal Digestion and Edible Plant Combination on Oral Bioavailability of Triterpene Saponins. After bionic digestion, the product was referred to as the chyme. After being digested in the stomach or intestine, three triterpene saponins from edible plants (San-Chi or PZH) could be released, enter into the gastrointestinal tract, and possibly be available for gastrointestinal absorption. Because of affinity biomembrane, AMLS in the chyme was the fractional species of triterpene saponins that could be absorbed by gastrointestinal biomembrane. AMLS of these three triterpene saponins could be found both in the stomach (denoted AMLSS) and in the intestine (denoted AMLSI). The oral bioavailability of saponins in edible plants was assessed by the ratio of AMLSI content to total saponins. The chromatograms of N-R1, G-Rb1, and G-Rd1 in San-Chi and PZH are shown in Figure 4.

Because of the characteristic of functional groups, including the carboxyl group on an anionic sugar, glucuronic acid, and galacturonic acid, these three triterpene saponins were sensitive to changes in the gastrointestinal pH value. This posed a formidable challenge for saponins because saponins must pass through the extremely acidic mammalian stomach before reaching the pH-neutral small intestine. These three saponins' bioavailabilities were influenced by bionic digestion model.

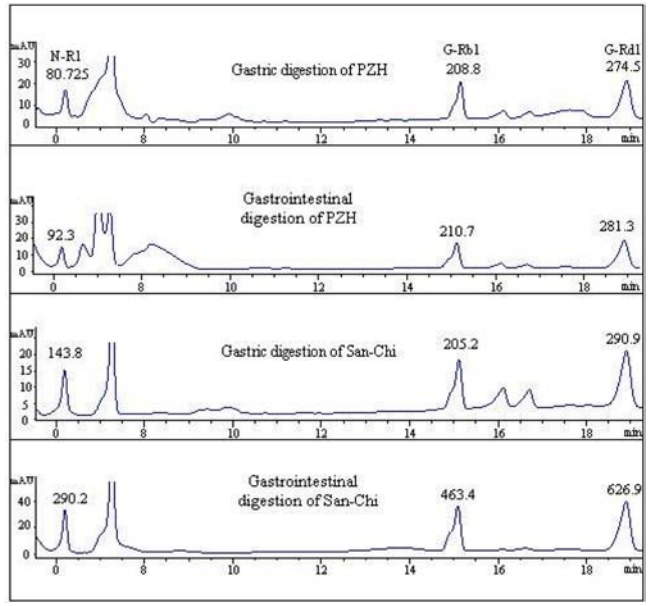

Figure 4. HPLC chromatograms of affinity-monolayer liposome saponin (AMLS) in the chyme from gastrointestinal digestion of San-Chi and PZH.

According to the peak areas of these three saponins in Figure 4, the contents of AMLSI were higher than those of AMLSS, so when San-Chi and PZH were digested in the stomach and then the intestine, the release of saponins from edible plants into the chyme was increased, and the increasing degree of San-Chi was more than that of PZH.

The oral bioavailabilities of saponins in San-Chi and PZH, including the contents of AMLSS, AMLSI, and the intestinal absorption ratio of N-R1, G-Rb1, and G-Rd1, are described in Tables 2 and 3. The intestinal absorption ratio was calculated by the ratio of AMLSI to the total content in San-Chi or PZH.

When San-Chi and PZH were digested from the stomach into the intestine, the contents of AMLSI were more than that of AMLSS, increasing by 4.66−4.96 times for San-Chi and 2.33−2.64 times for PZH, so the release of saponins in their chyme was increased. Because the small intestine is the primary absorption site for these saponins, AMLSI could be used for oral bioavailability assessment of saponins in edible plants. The intestinal absorption ratio (i.e., oral bioavailability ratio) of N-R1, G-Rb1, G-Rd1, and total saponins (i.e., three triterpene saponins) were 86.57, 18.56, 73.30, and 40.20% for San-Chi and 19.56, 10.11, 30.11, and 16.08% for PZH, respectively. The degree of oral bioavailability was described as follows: N-R1 > G-Rd1 ≫ G-Rb1 for San-Chi and G-Rd1 > N-R1 > G-Rb1 for PZH. The oral bioavailabilities of these three saponins in PZH were lower than those in San-Chi; that is, the oral bioavailabilities of saponins were depressed by edible plant combination and at the same time the overload of saponins could be avoided. According to the above results, the oral

Table 2. Contents of Affinity-Monolayer Liposome Saponins from Gastrointestinal Digestion Chyme of San-Chi (Milligrams per Gram) (n = 3)

	N-R1	G-Rb1	G-Rd1	total saponins
total content in San-Chi	11.08 ± 0.62	40.90 ± 3.67	11.16 ± 0.34	63.14
AMLSS in chyme	2.06 ± 0.15	1.63 ± 0.07	1.65 ± 0.26	5.34
AMLSI in chyme	9.61 ± 0.44	7.59 ± 0.27	8.18 ± 0.09	25.38
intestinal absorption ratio (%)	86.73	18.56	73.30	40.20

Table 3. Contents of Affinity-Monolayer Liposome Saponins from Gastrointestinal Digestion Chyme of PZH (Milligrams per Gram) (n = 3)

	N-R1	G-Rb1	G-Rd1	total saponins
total content in PZH	15.64 ± 0.16	38.07 ± 2.32	12.29 ± 0.24	66.00
AMLSS in chyme	1.16 ± 0.02	1.65 ± 0.08	1.56 ± 0.06	4.37
AMLSI in chyme	3.06 ± 0.05	3.85 ± 0.23	3.70 ± 0.14	10.61
intestinal absorption ratio (%)	19.56	10.11	30.11	16.08

bioavailability of saponins was controlled by saponin species, gastrointestinal digestion, and the combination of edible plants.

Safe Dosage of Triterpene Saponins and San-Chi. As a well-known traditional Chinese formula prescribed already in the Ming dynasty, PZH has been used safely for 458 years; no side effects have been reported. The dosage of PZH (1.8 g/day for adults and 0.9 g/day for children), including triterpene saponins in PZH, is safe for the human body. The safe dosage of saponins could be calculated by the product between the dosage of PZH and AMLSI from PZH. The safe dosage of San-Chi was proposed by the quotient between the safe dosage of saponins and AMLSI from San-Chi. The results of the safe dosage of three triterpene saponins (N-R1, G-Rb1, and G-Rd1) and San-Chi are shown in Table 4.

Table 4. Safe Dosage of Triterpene Saponins and San-Chi

			safe dosage of saponins (mg/day)		safe dosage of San-Chi (g/day)	
saponin	AMLSI from San-Chi (mg/g)	AMLSI from PZH (mg/g)	for children	for adult	for children	for adult
N-R1	9.61	3.06	2.75	5.51	0.29	0.57
G-Rb1	7.59	3.85	3.47	6.93	0.46	0.91
G-Rd1	8.18	3.70	3.33	6.66	0.41	0.81

The safe dosage of saponins was different for different species of saponins, and the safe dosages for adults were 2.0 times those for children. Because N-R1, G-Rb1, and G-Rd1 coexisted in the edible plants, the minimal safe dosage of San-Chi should be proposed for avoiding the overload of N-R1, G-Rb1, and G-Rd1 at the same time. When San-Chi is used singly, its dosage should be <0.29 g/day for children and <0.57 g/day for adults.

AUTHOR INFORMATION

Corresponding Author

*(S.-X.L.) E-mail: lishunxing@mnnu.edu.cn, shunxing_li@aliyun.com. Phone: +86 596 2591395. Fax: +86 596 2591395.

Funding

This work is supported by the National Natural Science Foundation of China (20775067 and 21175115, S.-X.L.), the Program for New Century Excellent Talents in University (NCET-11 0904, S.-X.L.), Outstanding Youth Science Foundation of Fujian Province, China (2010J06005, S.-X.L.), and the Science and Technology Committee of Fujian Province, China (2012Y0065, F.-Y.Z.).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Jenkins, K. J.; Atwal, A. S. Effects of dietary saponins on fecal bile acids and neutral sterols, and availability of vitamins A and E in the chick. *J. Nutr. Biochem.* **1994**, *5* (3), 134−137.

(2) Lee, K. K.; Kwong, W. H.; Chau, F. T.; Yew, D. T.; Chan, W. Y. Pien Tze Huang protects the liver against carbon tetrachloride-induced damage. *Pharmacol. Toxicol.* **2002**, *91*, 185−192.

(3) Zhuang, Q. C.; Hong, F.; Shen, A. L.; Zheng, L. P.; Zeng, J. W.; Lin, W.; Chen, Y. Q.; Sferra, T. J.; Hong, Z. F.; Peng, J. Pien Tze Huang inhibits tumor cell proliferation and promotes apoptosis via suppressing the STAT3 pathway in a colorectal cancer mouse model. *In. J. Oncol.* **2012**, *40*, 1569−1574.

(4) Karu, N.; Reifen, R.; Kerem, Z. Weight gain reduction in mice fed *Panax ginseng* saponin, apancreatic lipase inhibitor. *J. Agric. Food Chem.* **2007**, *55*, 2824−2828.

(5) Lai, Y. J.; Chen, J. P.; You, Y. J. Determination of four compounds in Pien Tze Huang by HPLC. *Chin. Pharm.* **2008**, *43*, 1096−1098.

(6) Kwon, J. H.; Bealanger, J. M.; Paré, J. J. Optimization of microwave-assisted extraction (MAP) for ginseng components by response surface methodology. *J. Agric. Food Chem.* **2003**, *51*, 1807−1810.

(7) Xie, X. S.; Yang, M.; Liu, H. C.; Zuo, C.; Li, H. J.; Fan, J. M. Ginsenoside Rg1, a major active component isolated from *Panax notoginseng*, restrains tubular epithelial to myofibroblast transition in vitro. *J. Ethnopharmacol.* **2009**, *122*, 35−41.

(8) Zhang, H. S.; Wang, S. Q. Notoginsenoside R1 from *Panax notoginseng* inhibits TNF-α-induced PAI-1 production in human aortic smooth muscle cells. *Vasc. Pharmacol.* **2006**, *44*, 224−230.

(9) Chen, W. X.; Wang, F.; Liu, Y. Y.; Zeng, Q. J.; Sun, K.; Xue, X.; Li, X.; Yang, J. Y.; An, L. H.; Hu, B. H. Effect of notoginsenoside R1 on hepatic microcirculation disturbance induced by gut ischemia and reperfusion. *World J. Gastroenterol.* **2008**, *14*, 29−37.

(10) Zhang, H. S.; Wang, S. Q. Notoginsenoside R1 inhibits TNF-α-induced fibronectin production in smooth muscle cells via the ROS/ERK pathway. *Free Radical Biol. Med.* **2006**, *40*, 1664−1674.

(11) Wina, E.; Muetzel, S.; Becker, K. The impact of saponins or saponin-containing plant materials on ruminant productions. *J. Agric. Food Chem.* **2005**, *53*, 8093−8105.

(12) George, A. J. Legal status and toxicity of saponins. *Food. Cosmet. Toxicol.* **1965**, *3*, 85−91.

(13) Kuo, P. C.; Lin, T. C.; Yang, C. W.; Lin, C. L.; Chen, G. F.; Huang, J. W. Bioactive saponin from tea seed pomace with inhibitory

effects against *Rhizoctonia solani*. *J. Agric. Food Chem.* **2010**, *58*, 8618−8622.
(14) Jane, H.; Monique, B.; Jean, D. Use of a simplified HPLC−UV analysis for soyasaponin B determination: study of saponin and isoflavone variability in soybean cultivars and soy-based health food products. *J. Agric. Food Chem.* **2005**, *53*, 3923−3930.
(15) Oleszek, W. Composition and quantitation of saponins in alfalfa (*Medicago sativa* L.) seedlings. *J. Agric. Food Chem.* **1998**, *46*, 960−962.
(16) Tang, H.; Ye, Z.; Ren, Y.; Zhu, Z.; Wu, Y. Investigation on the mechanism of ginsenoside Rg3 in treating murine primary mammary tumor. *Front. Med. China* **2009**, *3*, 421−425.
(17) Zhou, Y. Q.; Li, W. Z.; Chen, L. Y.; Ma, S. W.; Ping, L.; Yang, Z. L. Enhancement of intestinal absorption of akebia saponin D by borneol and probenecid in situ and in vitro. *Environ. Toxicol. Pharm.* **2010**, *29*, 229−234.
(18) Hur, S. J.; Lim, B. O.; Decker, E. A.; McClements, J. In vitro human digestion models for food applications. *Food Chem.* **2011**, *125*, 1−12.
(19) Versantvoort, C. H.; Oomen, A. G.; Van de Kamp, E.; Rompelberg, C. J.; Sips, A. J. M. Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* **2005**, *43*, 31−40.
(20) Xing, G. H.; Yang, Y.; Yan Chan, J. K.; Tao, S.; Wong, M. H. Bioaccessibility of polychlorinated biphenyls in different foods using an in vitro digestion method. *Environ. Pollut.* **2008**, *156*, 1218−1226.
(21) Fearn, R. A.; Hirst, B. H. Predicting oral drug absorption and hepatobiliary clearance: human intestinal and hepatic in vitro cell models. *Environ. Toxicol. Pharmacol.* **2006**, *21*, 168−169.
(22) Pixley, V. K.; Palacios-Rojas, N.; Glahn, P. R. The usefulness of iron bioavailability as a target trait for breeding maize (*Zea mays* L.) with enhanced nutritional value. *Field Crop Res.* **2011**, *123*, 153−160.
(23) Li, S. X.; Lin, L. X.; Zheng, F. Y.; Wang, Q. X. Metal bioavailability and risk assessment from edible brown algaLaminaria japonica, using biomimetic digestion and absorption system and determination by ICP-MS. *J. Agric. Food Chem.* **2011**, *59*, 822−828.
(24) Huh, D.; Matthews, B. D.; Mammoto, A.; Montoya-Zavala, M.; Hsin, H. Y.; Ingber, D. E. Reconstituting organ-level lung functions on a chip. *Science* **2010**, *328*, 1662−1668.
(25) Wang, C. J.; Zuo, Y. G.; Vinson, J. A.; Deng, Y. W. Absorption and excretion of cranberry-derived phenolics in humans. *Food Chem.* **2012**, *132*, 1420−1428.
(26) Zhang, K.; Zuo, Y. G. GC-MS determination of flavonoids and phenolic and benzoic acids in human plasma after consumption of cranberry juice. *J. Agric. Food Chem.* **2004**, *52*, 222−227.
(27) Li, S. X.; Chen, L. H.; Zheng, F. Y.; Li, Y. C. Effect of cp4-epsps gene on metal bioavailability in maize and soybean, using bionic gastrointestinal tract and determination by ICP-MS. *J. Agric. Food Chem.* **2013**, *61*, 1579−1584.
(28) Xiong, J.; Guo, J. X. The use of lipid-based formulations to increase the oral bioavailability of *Panax notoginseng* saponins following a single oral gavage to rats. *Drug Dev. Ind. Pharm.* **2008**, *34*, 65−72.
(29) Wang, Y.; Xu, C.; Wang, P.; Lin, X.; Yang, Y.; Li, D.; Li, H.; Wu, X.; Liu, H. Pharmacokinetic comparisons of different combinations of Shaoyao-Gancao-decoction in rats: simultaneous determination of ten active constituents by HPLC-MS/MS. *J. Chromatogr., B* **2013**, *932*, 76−87.
(30) Lin, J. T.; Chang, Y. Z.; Lu, M. P.; Yang, D. J. Simultaneous determination of furostanol, pennogenyl, and diosgenyl glycosides in Taiwanese Rhizoma Paridis (*Paris formosana* Hayata) by high-performance liquid chromatography with evaporative light scattering detection. *J. Agric. Food Chem.* **2011**, *59*, 1587−1593.
(31) Gómez-Caravaca, A. M.; Segura-Carretero, A.; Fernández-Gutiérrez, A.; Caboni, M. F. Simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quinoa* Willd) by a liquid chromatography-diode array detection-electrospray ionization time-of-flight mass spectrometry methodology. *J. Agric. Food Chem.* **2011**, *59*, 10815−10825.
(32) Wang, C. J.; Zuo, Y. G. Ultrasound-assisted hydrolysis and gas chromatography-mass spectrometric determination of phenolic compounds in cranberry products. *Food Chem.* **2011**, *128*, 562−568.
(33) Jiao, Y.; Zuo, Y. G. Ultrasonic extraction and HPLC determination of anthraquinones, aloe-emodine, emodine, rheine, chrysophanol, and physcione, in Radix Polygoni multiflori. *Phytochem. Anal.* **2009**, *20*, 272−278.
(34) Zuo, Y. G.; Zhang, L. L.; Wu, J. P.; Fritz, J. W.; Medeiros, S.; Rego, C. Ultrasonic extraction and capillary gas chromatography determination of nicotine in pharmaceutical formulations. *Anal. Chim. Acta* **2004**, *526*, 35−39.