

Determination and Isolation of Ginsenosides from Araliaceous Plants by Using Eastern Blotting Fingerprinting

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Abstract

In the eastern blotting method, a new fashion to separate the ginsenoside molecule into two functional parts using a simple and well-known chemical reaction was developed. In principle, the sugar moieties were oxidized by NaIO₄ to form dialdehydes, which reacted with amino groups of the protein and covalently bound to the adsorbent PVDF membrane. The MAb bound to the adjycon part of the ginsenoside molecule for immunostaining. Double staining of eastern blotting for ginsenosides using anti-ginsenoside -Rb₁ and -Rg₁ MAbs promoted complete identification of ginsenosides in *Panax* species. As an application, we analyzed several Araliaceous plants by eastern blotting and enzyme-linked immunosorbent assay (ELISA) using anti-ginsenoside Rb₁ MAb leading to the investigation of ginsenoside Rb₁ from *Kalopanax pictus* and *Acanthopanax koreanum*. On the other hand, by immunoassay-guided fractionation and chromatography separation on the methanol extract of American ginseng, two new minor ginsenosides were isolated. As another application, identification of two known ginsenosides was achieved from the *P. japonicas* extract using eastern blotting and immunoaffinity column combined with anti-ginsenoside Rb₁ MAb.

Keywords: Araliaceae; *Acanthopanax koreanum*; *Kalopanax pictus*; *Panax quinquefolium*; Ginsenoside; Ginsenoside Rb₁; Monoclonal antibody; Eastern blotting

Introduction

Ginseng saponins, commonly called ginsenosides, are the principal components in Panax species and have been occurred in several other Araliaceae plants. According to different aglycones, ginsenosides can be classified into three types: the 20 (S)-protopanaxadiol type such as ginsenosides Rb1, Rc, Rb2, and Rd, the 20 (S)-protopanaxatriol type such as ginsenosides $\mathrm{Rg}_{\scriptscriptstyle 1}$ and Re, and the oleanolic acid type including ginsenoside Ro and polyacetyleneginsenoside Ro, respectively [1]. These compounds have been widely investigated for their effects on disturbances of the central nervous system, hypothermia and tumor metastasis, and for their antioxidant, antidiabetes, antiaging and radioprotective activities [2]. Especially, ginsenoside Rb₁, one of major ginsenosides, exhibited remarkable effects on the central nervous system [2,3] and drug-induced memory impairment [4]. In addition, the regulation of ChAT, NGF and trkA mRNA expression by ginsenoside Rb, in the rat brain was observed [3]. Therefore, ginsenosides including ginsenoside Rb, have played as important resources in the development of new drugs [5].

For qualitative and quantitative analyses of ginsenosides, thin layer chromatography (TLC) [6], high performance liquid chromatography (HPLC) [7,8], and liquid chromatography-mass spectrometry (LC-MS) [9] have been routinely used. Recently, an enzyme-linked immunosorbent assay (ELISA) system has been opened for natural product analysis as the most promising methodology. We have developed the preparation of monoclonal antibodies (MAbs) against ginsenosides Rb₁ [10], -Rg₁ [11], and -Re [12]. Furthermore, we set up the ELISA using individual MAbs and applied for the quantitative analysis of ginsenosides [13,14]. On the other hand, regarding immunostaining we succeeded to immunostain the steroidal alkaloid glycosides using anti-solamargine MAb [15] in the first stage, then established the immunostaining methods for ginsenosides-Rb, and -Rg, [13]. According to development of new staining method for glycosides, we named this methodology as eastern blotting for immunostaining of glycyrrhizin [16].

Eastern blotting fingerprint for ginsenosides

The ginsenoside Rb_1 , Rc, Rd, Rg_1 mixture was applied to TLC

plates and developed with n-BuOH-EtOAc-H₂O (15:1:4, v/v/v). One TLC plate developed was sprayed and stained with H₂SO₄. Another TLC plate developed was blotted on the PVDF membrane by heating at around 120°C for short period, the PVDF membrane was treated with NaIO₄ solution to release aldehyde groups in sugar moieties, then reacted with proteins such as bovine serum albumin (BSA) resulting in ginsenoside-BSA conjugates which can fix on the PVDF membrane. On the other hand, an aglycone and a part of sugar moiety as an antigen can be stained by MAb like western blotting. Therefore, it became evident that ginsenosides having small cross-reactivities for anti-ginsenosides Rb, and anti-ginsenoside Rg, MAbs could be stained in the case of eastern blotting for ginsenosides, suggesting that the specific reactivity of the sugar moiety in the ginsenoside molecule against MAb might be modified by NaIO₄ treatment of the ginsenoside on the membrane, resulting that other ginsenosides having protopanaxatriol as an aglycone such as ginsenosides Re, Rf and Rg, can be detectable by eastern blotting using anti-ginsenoside Rg, MAb although 3.3% of cross-reactivity for ginsenoside Re [13] (Figure 1). Likewise, ginsenosides possessing protopaxadiol as an aglycone like ginsenoside Rc, Rd and Rb, can be stained using anti-ginsenoside Rb, MAb [17] (Figure 2). This finding is important for the surveys of saponins having two types of aglycone like ginsenosides.

Analysis of ginsenoside by eastern blotting fingerprinting

Determination of ginsenoside Rb₁ from Acanthopanax koreanum Nakai: Acanthopanax koreanum (Araliaceae), which is a perennial shrub and distributed in Northeast Asia, has been used as a tonic and for treatment of rheumatism, allergies, hepatitis, and diabetes [18,19].

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Lanes 1, 2, 3, 4, 5 and 6 indicate ginsenoside Rb, Rc, Rd, Re, Rg and chikusetsusaponin IV, respectively. Lanes I, II, III, IV, V and VI are white ginseng, red ginseng, fibrous ginseng, *P. notoginseseng*, *P. quinquefolium* and *P. japonicus*, respectively.

Figure 1: Fingerprinting of ginsenoside standards and ginsenosides in various ginseng by H_2SO_4 (left) and eastern blotting (right) using anti-ginsenoside Rg₁ monoclonal antibody.



ginseng, fibrous ginseng, *P. notoginseseng*, *P. quinquefolium* and *P. japonicus*, respectively.

Figure 2: Fingerprinting of ginsenosides in various ginseng by $\rm H_2SO_4$ (left) and eastern blotting (right) using anti-ginsenoside Rb, monoclonal antibody.

Bioactive constituents of *A. koreanum* have been reported including several lignans, diterpenes [20,21], and lupane-type triterpene glycosides, which are considered as major constituents [22,23]. Since we have established the combination of ELISA and eastern blotting methods using anti-ginsenoside Rb_1 MAb for the identification of ginsenoside Rb_1 in *Panax* species and traditional Chinese medicines [14] as a high sensitive and rapid method, the finding and determination of ginsenoside Rb_1 in *A. koreanum* of Araliaceae family by using ELISA and eastern blotting monitoring will be reviewed in this section.

Figure 3 showed the double eastern blotting staining (A) and the H_2SO_4 staining (B) profiles of ginsenoside standards (right) and crude extracts of *A. koreanum* (left) using anti-ginsenoside Rb_1 and anti-ginsenoside Rg_1 MAbs. Although H_2SO_4 staining detected clearly all standard ginsenosides without color differences, the TLC profile of *A. koreanum* crude extract revealed complicated fingerprinting patterns indicating that ginsenosides are ambiguously determined. From double eastern blotting we confirmed that no ginsenoside having protopanaxatriol as an aglycone was contained in the *A. koreanum* crude extract because no purple spot appeared. On the other hand, blue spots were detected meaning that protopanaxadiol type ginsenosides are contained in *A. koreanum*. It is clear that Rf value on TLC reflects the sugar number in general. From these evidences it is easily suggested that *A. koreanum* contains small amount of ginsenoside Rb₁ and a more

polar ginsenoside which cannot be longer analyzed due to its trace amount [24].

We analyzed *A. koreanum* leaves crude extract by competitive ELISA using anti-ginsenoside Rb_1 MAb in order to confirm the existence and concentration of ginsenoside Rb_1 resulting in 0.000016% dry wt. of ginsenoside Rb_1 . The roots and stems were also analyzed separately by the same manner finding concentrations of 0.000039% and 0.000014% dry wt., respectively. The concentrations of ginsenoside Rb_1 in the sample is extremely low, therefore, it has been suggested that chromatographic purification and analyses of ginsenoside Rb_1 have been unaffordable to date. To our knowledge, this is the first evidence of ginsenoside Rb_1 in *Acanthopanax* species [24]. The results further support potential and promising application of MAb such as eastern blotting and ELISA for surveying ginsenoside sources.

Determination of ginsenosides from Panax japonicas: P. japonicas, which is wildly distributed in Japan and China, is morphologically different from the other Panax species. Regarding its constituents, Yahara et al. reported that no ginsenoside Rb, was found and isolated several dammarane-type saponins structurally related to ginsenoside like chikusetusaponin I~VI and oleanane-type saponins named as chikusetsusaponins as major components [25]. In addition, Morita et al. examined the varieties of P. japonicus by chemical analysis of saponins [26]. From these results, the concentration of ginsenoside Rb, might be at trace levels. However, higher concentration compared with previous reports was determined by ELISA [13], although relatively half the concentration of ginsenoside Rb, was detected by HPLC analysis compared with ELISA. In order to confirm these differences, immunoaffinity column chromatography was employed for immunoaffinity concentration of ginsenoside Rb₁. The crude root extract of P. japonicus was subjected to the immunoaffinity column and first washed with the washing solvent (PBS; fraction 1) and then with elution solvent (AcOH buffer and 20% MeOH (fraction 2) [14].

Figure 4 shows the H_2SO_4 staining (A) and the eastern blotting (B) profiles of the two fractions separated by the immunoaffinity



Purple spots are protopanaxtriol saponins and blue spots are protopanaxdiol saponins.

Figure 3: Fingerprinting of ginsenoside standards and crude extract of *Acanthopanax koreanum* by H_2SO_4 (right) and eastern blotting (left) using antiginsenoside Rb₁ and Rg₁ monoclonal antibodies.



Figure 4: Purification and determination of ginsenosides of *P. japonicas* by immunoaffinity column and eastern blotting.



Figure 5: Identified compound 1 and 2 by using immunoaffinity column and eastern blotting and their structures together with ginsenosides Rb₁, Rc and Rd in *Panax japonicas*.

column. Fraction 1 eluted with the washing solvent showed two spots in the eastern blotting profile. A clear unknown spot appeared in fraction 2 eluted with the elution solvent. However, ginsenoside Rb_1 was not detected by eastern blotting although it was determined by TLC as indicated in (Figure 4). The unknown compound is proposed to resemble molecular structure and a similar cross-reaction with ginsenoside Rb_1 , and likely a ginseng saponin having an aglycone of panaxadiol. Moreover, this compound might have the same sugar fragments in the molecule, but possess an additional sugar moiety compared with ginsenodise Rb_1 , as indicated by their R_j values. These evidences revealed that an unknown compound contained in fraction 2 is chikusetsusaponin VI comparing with the authentic sample directly (Figure 5).

The eastern blotting fingerprinting of fraction 1 indicated that an unknown ginsenoside has protopanaxadiol as an aglycone and three sugar components compared to the R_f value of ginsenoside Rc. Therefore, we determined that the unknown compound contained in fraction 1

is chikusetsusaponin III compared with the authentic sample (Figure 5). From this investigation we confirmed that the saponins having protopanaxdiol as an aglycone can be separated by the immunoaffinity column conjugated with anti-ginsenoside Rb_1 MAb. Moreover, we concluded that the combination of immunoaffinity concentration and ELISA is suitable for samples containing lower concentrations of ginsenoside Rb_1 since the immunoaffinity column can concentrate the ginsenoside Rb_1 concentration [14].

Isolation of ginsenosides by using eastern blotting fingerprinting

Isolation of ginsenoside Rb₁ from Kalopanax pictus Nakai: A number of Araliaceae species, including Panax species, have been used as tonics in Asian folk medicine. On the basis of phytochemical study, Araliaceous plants have been documented to contain similar constituents such as ginsenoside, depending on their chemotaxonomical classification. Various analytical methods have been used to analyze ginsenosides, and of them, ELISA appears to be the most promising methodology, considerably. This section reviewed the distribution of ginsenosides in Araliaceous species and isolation of ginsenoside Rb, from the bark of Kalopanax pictus using ELISA and eastern blotting monitoring. Since the bark of K. pictus (Figure 6 line 13) indicated a positive band, the crude extract was analyzed by competitive ELISA using anti-ginsenoside Rb, MAb, resulting in 0.0009% dry wt. of ginsenoside Rb1. The crude extract was subjected to repeated silica gel column chromatography guided with eastern blotting using anti-ginsenoside Rb, MAb to afford compound 1. Compound 1 was consistent with that of ginsenoside Rb, by eastern blotting, and the physical and spectroscopic data (1H- and 13C-NMR) of compound 1 resembled to those of authentic ginsenoside Rb₁. Further analyses of the stem bark and leaves by competitive ELISA revealed that a higher concentration of ginsenoside Rb, was occurred in the leaves (0.0037% dr. wt.) than in the bark (0.0009% dr. wt.) [27]. To our knowledge, this is the first isolation of ginsenoside Rb, from K. pictus although the isolation of various oleanane saponins was reported [28,29]. These findings suggested that K. pictus might be a new resource of ginsenoside Rb,, and moreover the immunoaffinity column conjugated with antiginsenoside Rb, MAb [13] is readily applicable for purification of ginsenoside Rb, in the final stage of the separation process.

Isolation of new ginsenosides from *Panax quenquefolium:* American ginseng (*Panax quinquefolium* L.), which is mainly cultivated in the USA, Canada and China, has been widely used as a tonic and functional foods in various forms such as decoction, powder, tea, capsule, etc. like Asian ginseng (*P. ginseng*, CA. Meyer). These conventional ginseng products have been reported to have a wide array of pharmacological and physiological actions including antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, antistress, and antifatigue, respectively [30-33]. The dammarane-type saponins (ginsenosides) are the major active constituents in American ginseng and with the development of modern chromatography, there are more than 30 ginsenosides such as ginsenosides Rb₁, Rb₂, Rg₁, Rc, Rd, and Re reported [34,35]. However, in comparison with a number of researches on Asian ginseng, the study on American ginseng and its constituents is much less extensive.

Figure 7 showed the double eastern blotting staining (A) and H_2SO_4 staining (B) profiles of crude extracts of *Panax* species including American ginseng (line 5) using anti-ginsenoside Rb_1 and anti-ginsenoside Rg_1 MAbs. In general, since H_2SO_4 staining detected all compounds, the TLC profile stained by this reagent showed complicated fingerprinting patterns so that ginsenosides are



ambiguously indicated. On the contrary, eastern blotting obviously and selectively revealed positive spots regarding ginsenosides. The crude extracts of American ginseng clearly indicated ginsenosides Rg₁, Re, Rd, Rc and Rb₁ as major ginsenosides. Besides, there have minor spots with the similar color indicating lower R_r value than ginsenoside Rc and ginsenoside Rb, (Figure 7-A-line 5) in respect to ginsenoside Rb, (blue) or ginsenoside Rg, (purple), suggesting more polar ginsenosides. Accordingly, two minor dammarane-type saponins, namely quinquenosides Ja (1) and Jb (2), were isolated from the American ginseng extract for the first time by immunoassayguided fractionation and chromatography separation (Figure 8). Their structures were elucidated as 6-O-[α -L-rhamnopyranosyl(1 \rightarrow 2)- β -D-glucopyranosyl]-20-O-[β -D-glucopyranosyl(1 \rightarrow 4)- β -Dglucopyranosyl]- 3β , 6α , 12β , 20β -tetrahydroxydammar-24-ene glucopyranosyl($1 \rightarrow 2$)- β -D-glucopyranosyl]-20and 3-O-[β-D-O-{[α -L-arabinofuranosyl(1 \rightarrow 6)- β -D-glucopyranosyl](1 \rightarrow 6)- β -D glucopyranosyl}- 3β ,12 β ,20 β -trihydroxydammar-24-ene (2) on the basis of chemical and spectroscopic methods [36]. The results further supported potential and promising application of MAb such as eastern blotting for surveying new ginsenoside sources.

Conclusions

It is well known that ginsenosides possesses wide pharmacological activities, and one of them, ginsenoside Rg₃ is now an anti-cancer drug in China suggesting that the other ginsenosides may have the possibility of drug development. However, since such ginsenosides can be supplied from ginsengs resulting in high cost performance of ginsenosides, a new supplement system of ginsenosides is needed. From these aims in this review the eastern blotting using anti-ginsenoside Rb, and/or anti-ginsenoside Rg1 MAbs was applied to analyze the distribution of ginsenosides in Araliaceous plants, like A. koreanum and K. pictus. From A. koreanum we determined ginsenoside Rb, by ELISA and eastern blotting using anti-ginsenoside Rb, MAb. P. quinquefolium were tested, and the structure elucidations of two new ginsenosides from P. quinquefolium were succeeded. The combination of eastern blotting and immunoaffinity column conjugated with anti-ginsenoside Rb, MAb makes it evident to separate ginsenosides having small crossreactivity with anti-ginsenoside Rb, MAb. In the case of P. japonicus we



Lanes 1, 2, 3, 4, 5, and 6 are white ginseng, red ginseng, fibrous ginseng, *P. notoginseseng*, *P. quinquefolium* and *P. japonicus*, respectively. Compound 1 and 2 are new ginsenosides isolated from *P. quinquefolium*

Figure 7: Staining of ginsenosides in Panax species by eastern blotting (A) and $\rm H_2SO_4$ (B).



used the immunoaffinity column conjugated with anti-ginsenoside Rb_1 and separated ginsenosides having the same aglycone with ginsenoside Rb_1 , and finally determined two ginsenosides. This biotechnological system has a great possibility to widely apply for quick separation of ginsenosides. In fact we succeeded to separate all solasodine glycosides in *Solanum khasianum* fruits by once immunoaffinity column conjugated with anti-solamargine MAb [37]. The results further

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supported potential and promising application of MAb such as eastern blotting for surveying pharmacologically active resources.

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References

- Liu CX, Xiao PG (1990) Recent advances on ginseng research in China. J Ethnopharmacol 36: 27-38.
- 2. Chang YS, Wu CR, Ho YL, Hsieh MT (1998) In Advances in Ginseng Research, Huh H (eds) The Korean Society of Ginseng: Seoul 289-299.
- Saito H (1989) In Recent Advances in Ginseng Studies, Shibata S, Otsuka Y, Saito H (eds) Hirokawa Publishing Company: Tokyo 99-111.
- Xie JT, Aung HH, Wu JA, Attele AS, Yuan CS (2002) Effects of American ginseng berry extract on blood glucose levels in ob/ob mice. Am J Chin Med 30: 187-194.
- Attele AS, Wu JA, Yuan CS (1999) Ginseng pharmacology: multiple constituents and multiple action. Biochem Pharmacol 58: 1685-1693.
- Kitagawa I, Taniyama T, Shibuya H, Noda T, Yoshikawa M (1987) Chemical studies on crude drug processing. V. On the constituents of ginseng radix rubra (2): comparison of the constituents of white ginseng and red ginseng prepared from the same *Panax ginseng* root. Yakugaku Zasshi 107: 495-505.
- Chan TWD, But PPH, Cheng SW, Kwok IMY, Lau FW, et al. (2000) Differentiation and authentication of *Panax ginseng*, *Panax quinquefolius*, and ginseng products by using HPLC/MS. Anal Chem 72: 1281-1287.
- Yamaguchi H, Kasai R, Matsuura H, Tanaka O, Fuwa T (1988) Highperformance liquid chromatography analysis of acidic saponins of ginseng and related plants. Chem Pharm Bull 36: 3468-3473.
- Wang X, Sakuma T, Asafu-Adjaya E, Shiu GK (1999) Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS. Anal Chem 71: 1579-1584.
- Tanaka H, Fukuda N, Shoyama Y (1999) Formation of monoclonal antibody against a major ginseng component, ginsenoside Rb, and its characterization. Cytotechnology 29: 115-120.
- Fukuda N, Tanaka H, Shoyama Y (2000) Formation of monoclonal antibody against a major ginseng component, ginsenoside Rg, and its characterization. Monoclonal antibody for a ginseng saponin. Cytotechnology 34: 197-204.
- Morinaga O, Takuhiro U, Yuan CS, Tanaka H, Shoyama Y (2010) Evaluation of a new eastern blotting technique for the analysis of ginsenoside Re in American ginseng berry pulp extracts. Fitoterapia 81: 284-288.
- Fukuda N, Tanaka H, Shoyama Y (2000) Application of ELISA, Western blotting and immunoaffinity concentration for survey of ginsenosides in crude drugs of *Panax* species and traditional Chinese herbal medicines. Analyst 125: 1425-1429.
- Tanaka H, Kukuda N, Shoyama Y (2007) Eastern blotting and immunoaffinity concentration using monoclonal antibody for ginseng saponins in the field of traditional Chinese medicines. J Agric Food Chem 55: 3783-3787.
- Tanaka H, Putalun W, Tsuzaki C, Shoyama Y (1997) A simple determination of steroidal alkaloid glycosides by thin-laer chromatography immunostaining using monoclonal antibody against solamargine. FEBS Lett 404: 279-282.
- 16. Shan S, Tanaka H, Shoyama Y (2001) Enzyme-linked immunosorbent assay for glycyrrhizin using anti-glycyrrhizin monoclonal antibody and an eastern blotting technique for glucuronides of glycyrrhetic acid. Anal Chem 73: 5784-5790.

 Tanaka H, Fukuda N, Shoyama Y (2006) Identification and differentiation of Panax species using rapid ELISA and eastern blotting. Phytochem Anal 17: 46-55.

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- 18. Lee YN (1996) New Flora of Korea. nhbs 1237.
- 19. Richard ES (1980) Medicinal plants of East and Southeast Asia: Attributed properties. 34: 361
- 20. Cai XF, Shen G, Dat NT, Kang OH, Kim JA, et al. (2003) Inhibitory effect of TNF-α and IL-8 secretion by pimarane type dirterpenoids from *Acanthopanax koreanum*. Chem Pharm Bull 51: 605-607.
- Cai XF, Lee IS, Dat NT, Shen G, Kang JS, et al. (2004) Inhibitory lignans against NFAT transcription factor from *Acanthopanax koreanum*. Arch Pharm Res 27: 738-741.
- Choi HS, Kim HJ, Nam SG, Kim IS, Lee KT, et al. (2008) Lupane glycosides from the leaves of Acanthopanax koreanum. Chem Pharm Bull 56: 1613-1616.
- Nhiem NX, Tung NH, Kiem PV, Minh CV, Ding Y, et al. (2009) Lupane triterpene glycosides from leaves of *Acanthopanax koreanum* and their cytotoxic activity. Chem Pharm Bull 57: 986-989.
- 24. Tung NH, Morinaga O, Uto T, Isoda S, Shoyama Y (2013) Analysis of Ginsenoside Rb, from *Acanthopanax koreanum* by Eastern Blotting and ELISA Using Anti-ginsenoside Rb, Monoclonal Antibody. Nat Prod Sci, in press.
- Zou K, Zhu S, Meselhy MR, Tohda C, Cai S, et al. (2002) Dammarane-type Saponins from Panax japonicus and their neurite outgrowth activity in SK-N-SH cells. J Nat Prod 65: 1288-1292.
- Morita T, Tanaka O, Kohda H (1985) Saponin composition of rhizomes of *Panax japonicus* collected in South Kyushu, Japan and its significance in oriental traditional medicine. Chem Pharm Bull 33: 3852-3858.
- 27. Tanaka H, Fukuda N, Shoyama Y (2005) Isolation of ginsenoside Rb₁ from *Kalopanax pictus* by Eastern blotting using anti-ginsenoside Rb₁ monoclonal antibody. Phytother Res 19: 255-258.
- Yao HK, Duan JY, Li Y, Wang JH, Yin XX, et al. (1992) Studies on the chemical constituents of *Kalopanax septemlobus*. Zhong Yao Cai 34: 716-718.
- Shao CJ, Kasai R, Ohtani K, Xu JD, Tanaka O (1989) Saponins from leaves of Kalopanax septemlobus (Thunb) Koidz-Structures of kalopanax-saponins La, Lb and Lc. Chem Pharm Bull 37: 3251-3254.
- 30. Court WE (2000) Ginseng: the history of an insignificant plant. Pharm Hist (Lond) 30: 38-44.
- 31. Jin Y, Hofseth AB, Cui XL, Windust AJ, Poudyal D, et al. (2010) American ginseng suppresses colitis through p53-mediated apoptosis of inflammatory cells. Cancer Prev Res 3: 339-347.
- Li BH, Wang CZ, He TC, Yuan CS, Du W (2010) Antioxidants potentiate American ginseng-induced killing of colorectal cancer cells. Cancer Lett 289: 62-70.
- Yuan CS, Dey L (2001) Multiple effects of American ginseng in clinical medicine. Am J Chin Med 29: 567-569.
- 34. Qi LW, Wang CZ, Yuan CS (2011) Ginsenosides from American ginseng: Chemical and pharmacological diversity. Phytochemistry 72: 689-699.
- Yuan CS, Wang CZ, Wicks SM, Qi LW (2010) Chemical and pharmacological studies of saponins with a focus on American ginseng. J Ginseng Res 34: 160-167.
- Tung NH, Shoyama Y (2012) Eastern blotting analysis and isolation of two new dammarane-type saponins from American ginseng. Chem Pharm Bull 60: 1329-1333.
- Putalun W, Tanaka H, Shoyama Y (1999) Rapid separation of solasodine glycosides by an immunoaffinity column using anti-solamargine monoclonal antibody. Cytotechnology 31: 151-156.