We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Quality Control by Immunoaffinity Concentration Using Monoclonal Antibody

Takuhiro Uto and Nguyen Huu Tung
Nagasaki International University
Japan

1. Introduction

The rapid and sensitive methods for separation and quantification of bioactive compounds are essential for quality control of the traditional Chinese medicine (TCM) and crude extracts. However, it seems that the current systems in use are not necessarily optimum approaches. For example, various methods for separation and quantification of glycyrrhizin (GC), the main active constituent in licorice (*Glycyrrhiza* spp.), have been used such as gas chromatography, high performance liquid chromatography (HPLC), and micellar trokinetic chromatography (Ong, 2002; Lay et al., 2004; Tan et al., 2002). Commercial purification of GC typically progressed through several steps, including crystallization, column chromatography, and liquid partitioning. However, these methods are far from satisfactory analytical purpose in terms of high sensitivity, reproducibility, large amounts of extraction solvents and time-consuming factors.

Owning to the rapid development of the molecular biosciences and their biotechnological applications, immunoassays using monoclonal antibodies (MAbs) against target molecules such as proteins, drugs and low-molecular-weight bioactive compounds have become one of the important tools by taking advantage of their specificity. Immunoassay by MAbs is used in a wide variety of analysis such as receptor binding assays, enzyme assays, and quantitative and qualitative analytical techniques both *in vivo* and *in vitro* studies.

Immunoaffinity purification is a highly specific technique that has the potential to be used for the one-step isolation of a target molecule from many complex mixtures like a cellular lysate. Immunoaffinity columns are conjugated with MAbs and work by specifically binding and removing the target molecules. Several immunoaffinity columns like a protein G affinity column for MAbs expressed by hybridoma and a metal chelate affinity column for single chain variable fragment (Fv) proteins expressed by *E. coli* have been commonly used for the purification of peptides and proteins. Application of an immunoaffinity column may decrease the amount of solvent used and the number of purification steps, shorten analysis time, and simplify sample analysis compared to traditional cleanup techniques. Although immunoaffinity purification against higher molecule analyte such as peptides and proteins are widely used in the research and commercial ways, there are too few cases of immunoaffinity purification targeting a small molecule compound. Fortunately, in our ongoing studies of naturally occurring bioactive compounds we have prepared many kinds of MAbs against terpenoids (Sakata et al., 1994; Xuan et al., 1999; Lu et al., 2003), alkaloids (Shoyama et al., 1996; Kim et al. 2004), plant saponins (Tanaka et al., 1999; Fukuda et al.,

2000b; Zhu et al., 2004; Shan et al., 2001; Ishiyama et al., 1996), and phenolics (Morinaga et al., 2000; Morinaga et al., 2001; Tanaka et al., 1996; Loungratana et al., 2004), and set up the enzyme-linked immunosorbent assay (ELISA) for quality control of the TCM. Furthermore, we have been establishing several affinity column with MAb against a kind of terpenoid, forskolin (Yanagihara et al., 1996), solasodine glycosides (Putalun et al., 1999), ginsenosides Rb1 (Fukuda et al., 2000c), and GC (Xu et al., 2007).

In this chapter, we introduce the new strategy to isolate and concentrate the target bioactive compounds from the crude extract by immunoaffinity column using individual MAb. We succeeded one-step purification of target compounds by MAb-conjugated immunoaffinity column, which leads to the knock-out (KO) extract which contains all components except an antigen molecule. The KO extract will be useful for the pharmacological investigation to reveal real effects of bioactive compound in the crude extract and the interaction between the target compound and other compounds.

2. One step concentration by immunoaffinity column conjugated with anti-ginsenoside Rb1 MAb

Ginseng, the crude drug of *Panax ginseng* root, has been used as a therapeutic herbal medicine in Asian countries and one of the most important components in TCM for thousands of years. The pharmacological and biological activities of ginseng have been reported to have anti-aging, anti-cancer, anti-inflammation, anti-diabetics, anti-stress, maintenance of homeostasis, and to affect on central nervous system and immune function (Gillis et al., 1997). Ginsenosides are believed to account for the pharmacological activities of ginseng. It is well known that the concentrations of ginsenosides vary in the ginseng root or the root extracts depending on the method of extraction, subsequent treatment, or even the season of its collection (Kitagawa et al., 1989; Tanaka et al., 1989). Recently, the individual ginsenosides in ginseng are purified by preparative HPLC. However, the repeated purification is required and may result in the decrease of the final yield. Therefore, the developed approaches for quality control are required in the field of TCM.

Ginsenosides are normally fractioned into two groups based on the types of aglycone, namely the protopanaxadiol group and the protopanaxatriol group. Within more than 30 different ginsenosides, ginsenoside Rb1 (G-Rb1), one of the protopanaxadiols, is considered the most important active factor (Washida & Kitanaka, 2003). G-Rb1 has various biological activities, including facilitating acquisition and retrieval of memory (Mook-Jung et al., 2001), scavenging free radicals (Lim et al., 1997), inhibition of calcium over-influx into neurons (Liu & Zhang, 1995), and preserving the structural integrity of the neurons (Jiang & Qian, 1995).

To control the quality of ginseng, the preparation of anti-G-Rb1 MAb, set up of ELISA, and a new immunostaining method named Eastern blotting were reported (Tanaka et al., 1999; Fukuda et al., 2000a). Herein, we describe an immunoaffinity column procedure for G-Rb1 and its application in single step isolation from crude extract of ginseng root.

2.1 Preparation of immunoaffinity column against G-Rb1

Purified anti-G-Rb1 MAb (10 mg) in diluted Affi-Gel Hz coupling buffer was dialyzed against the coupling buffer two times. The dialyzed anti-G-Rb1 MAb was treated by NaIO$_4$ to give dialdehyde group in sugar moiety which was coupled to Affi-Gel Hz hydrazide gel. The immunoaffinity gel was packed into plastic mini-column (Figure 1).
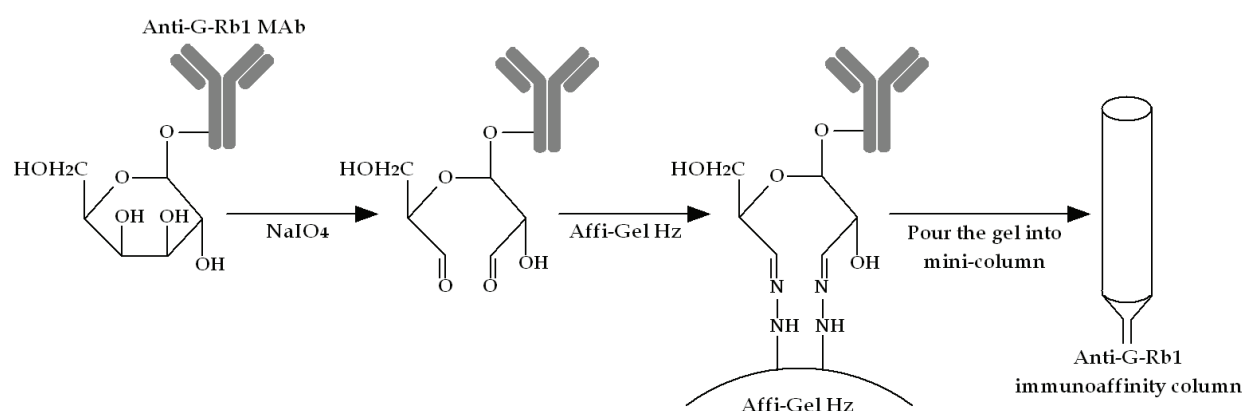
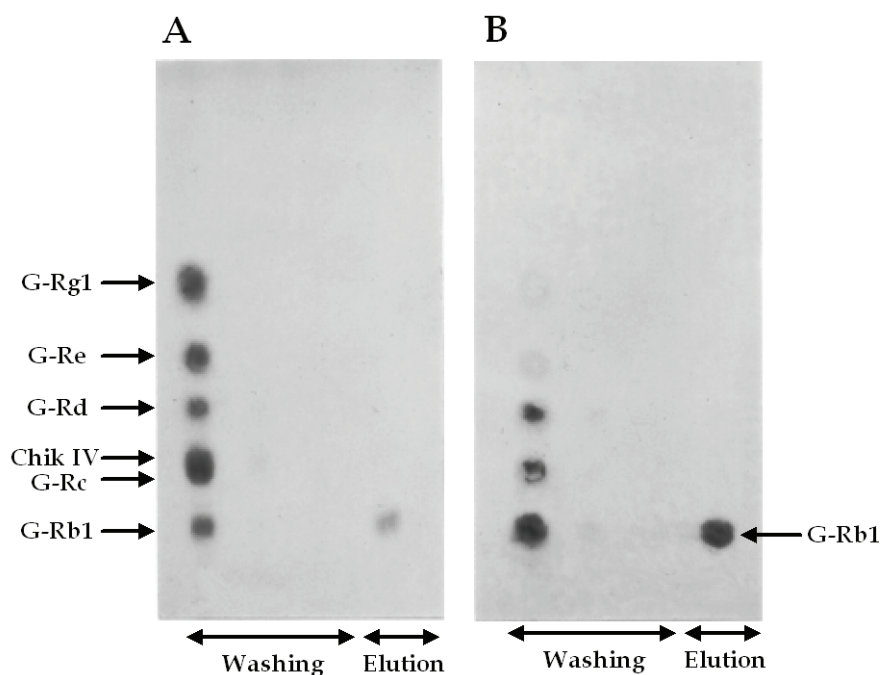Fig. 1. Scheme of preparation of anti-G-Rb1 immunoaffinity column

Fig. 2. TLC (A) and Eastern blotting (B) profiles for a standardized ginsenosides mixture. The TLC plate and Eastern blotting membrane were stained by H_2SO_4 and incubated with anti-G-Rb1 MAb, respectively

2.2 Optimal buffer systems for separation of G-Rb1

Firstly, to examine the optimal conditions of adsorption and elution, 400 µg of G-Rb1 was dissolved in phosphate buffer (PB) and loaded on anti-G-Rb1 affinity column. After washing with washing buffer (20 mM PB containing 0.5 M NaCl), various buffer solutions for elution were loaded on the column, and then the recovery efficiency was determined by ELISA. The G-Rb1 concentration was slightly increased by eluting with a 20 mM PB containing 0.5 M KSCN and 10 % MeOH. When the 20 mM PB was changed to 100 mM AcOH buffer (pH 4), the elution ability reached the optimal level. Since 20 % MeOH could elute G-Rb1 efficiently, higher MeOH concentration of over 20 % was ineffective. Thus, 100 mM AcOH buffer containing 0.5 M KSCN and 20 % MeOH could be used as an elution buffer in subsequent immunoaffinity chromatography.

2.3 One step purification of G-Rb1 from a standardized ginsenosides mixture by anti-G-Rb1 immunoaffinity column

To confirm the concentration of G-Rb1 by anti-G-Rb1 affinity column, a ginsenoside mixture containing 100 μg each of ginsenoside Rg1, Re, Rd, Rc, and chikusetsusaponin IV (chik IV; an oleanane-type saponin) were loaded on the column. Figure 2 shows the TLC and Eastern blotting profiles of ginsenosides and chik IV in washing and elution buffers flowed from the affinity column. When the column was washed with washing buffer (20 mM PB containing 0.5 M NaCl), ginsenoside Rg1, Re, Rd, Rc, chik IV, and overcharged G-Rb1 were detected. After these compounds were completely washed out by washing buffer, the G-Rb1 bound to anti-G-Rb1 MAb was eluted by elution buffer (100 mM AcOH buffer containing 0.5 M KSCN and 20 % MeOH).

2.4 One step purification of G-Rb1 from crude extract of P. ginseng roots by anti-G-Rb1 immunoaffinity column

A crude extract of *P. ginseng* roots (3.8 mg) was loaded onto anti-G-Rb1 immunoaffinity column. After washing with washing buffer (fractions 1-20), the column was eluted with elution buffer (fractions 21-40). As shown in Figure 3, the G-Rb1 concentration in the collected fractions was determined by ELISA. After loading of crude extract, overcharged G-Rb1 was detected in washing buffer of fractions 1-8. G-Rc, Rd, Re, and Rg1 were also detected in these fractions by Eastern blotting procedure (data not shown). A sharp peak of G-Rb1 was observed around fractions 21-24 of elution buffer. However, these eluted fractions were investigated by Eastern blotting, and the purified G-Rb1 in these fractions was still contaminated by a small amount of malonyl-G-Rb1, which having almost the same cross-reactivity with G-Rb1 (data not shown). Therefore, the mixture was treated with a mild alkaline solution (0.1 % KOH in MeOH) at room temperature to give pure G-Rb1. Overcharged G-Rb1 in washing solution (fractions 1-8) was repeatedly loaded and finally isolated in pure form. The anti-G-Rb1 MAb was stable during all procedures, and the immunoaffinity column showed almost no decrease in capacity (20 μg of G-Rb1/ml gel) after repeated use more than 10 times under same conditions.

After washing the column, each fraction of washing and elution were deionized and the solvent was lyophilized. Figure 4 demonstrated that TLC profile of the purification steps. Lane 1 and 2 were spotted the standard of ginsenosides (G-Rd, G-Rc, G-Rb1, G-Rg1, and G-Re). Lane A, B, and C were the crude extract, the washing fraction, and the eluted fraction, respectively. In the crude extract (lane A), all spots of ginsenosides were clearly detected. Interestingly, the washing fraction (lane B) contained all of the ginsenosides in the crude extract except G-Rb1. Furthermore, the spot of G-Rb1 was detected in the eluted fractions (lane C). These data strongly indicated that G-Rb1 molecule in the ginseng extract can be eliminated by an anti-G-Rb1 immunoaffinity column and the washing fractions was knock-out only by the antigen molecule, G-Rb1. Thus, we named the washing fractions a knock-out (KO) extract (Wang & Shoyama, 2006; Tanaka et al., 2007).

Since we succeeded the preparation of MAbs having a wide cross-reactivity like anti-solamargine MAb (Ishiyama et al., 1996), anti-saikosaponin a (Zhu et al., 2004) and ginsenoside-Re (Morinaga et al., 2006), the related total saponins can be removed by an immunoaffinity column conjugated with MAb in the case of solasodine glycosides, as reported previously (Putalun et al., 1999). The newly developed KO extract may be useful for the determination of real pharmacologically active principle in the TCMs.
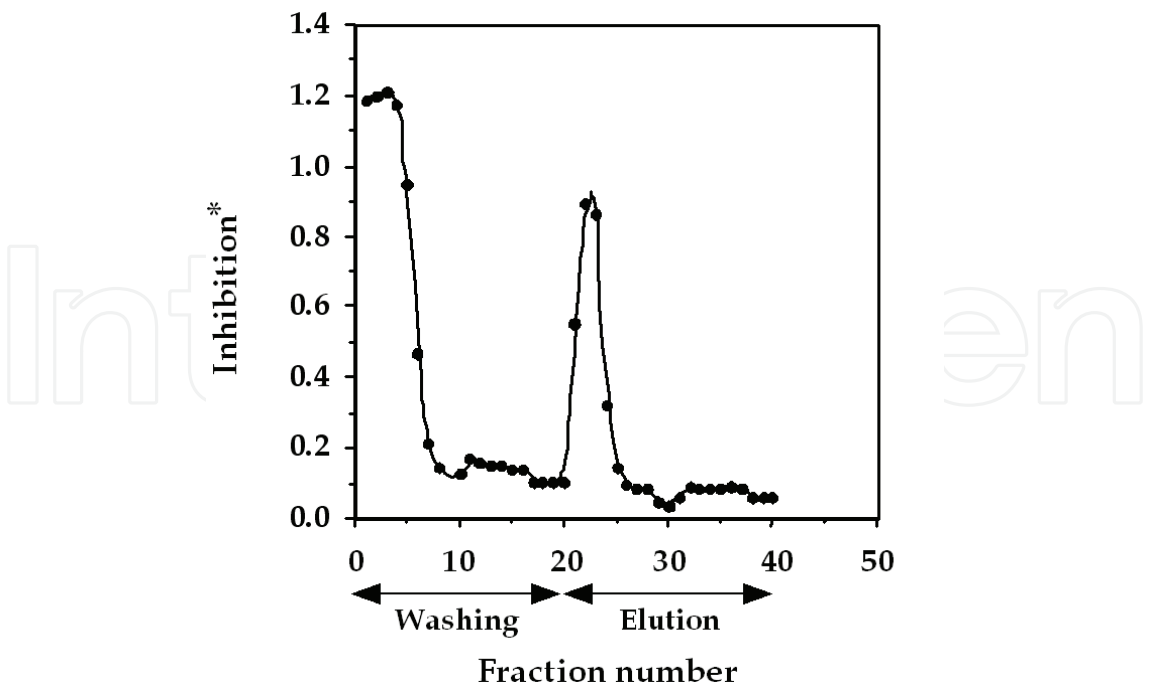
Fig. 3. Elution profile of *P. ginseng* crude extract by anti-G-Rb1 immunoaffinity column. G-Rb1 of each fraction was monitoring by ELISA using anti-G-Rb1 MAb. The volume of individual fraction was 2 mL. *Inhibition = $(A_0-A)/A_0$; A_0 is the absorbance in the absence of the test compounds. A is the absence in the presence the test compounds (Fukuda et al., 2000c)
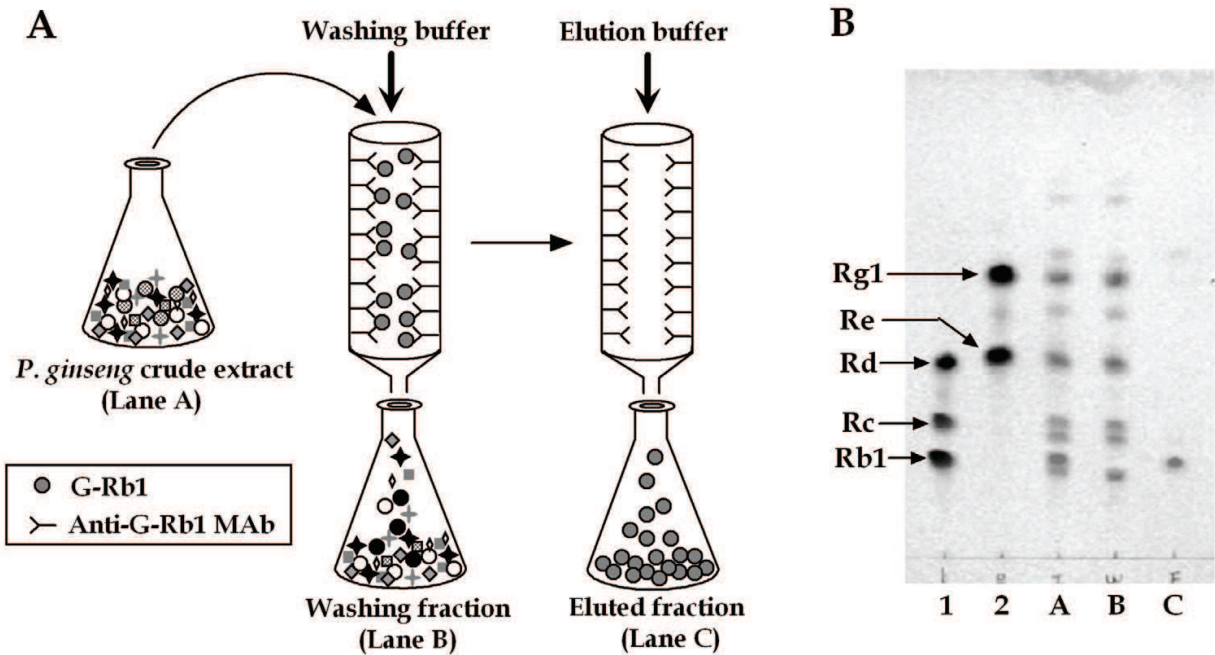

Fig. 4. Scheme of the preparation of G-Rb1 KO extract from *P. ginseng* crude extract using anti-G-Rb1 immunoaffinity column (A) and TLC profile of the separated fractions by the column (B). Lane 1 and 2 indicate the standard of ginsenosides (G-Rd, G-Rc, G-Rb1, G-Rg1, and G-Re). Lane A, B, and C were the crude extract, the washing fraction, and the eluted fraction, respectively (Fukuda et al., 2000c)

2.5 Isolation and determination of ginsenosides by anti-G-Rb1 immunoaffinity column

The anti-G-Rb1 immunoaffinity column could separate G-Rb1 from the crude extract of *P. ginseng.* In this case, malonyl-G-Rb1 was also eluted by elution buffer together with G-Rb1, because that this anti-G-Rb1 MAb has the cross-reactivity with malonyl-G-Rb1. Therefore, the pre-treatment of *P. ginseng* extract with a mild alkaline solution was needed to convert into pure G-Rb1.

Table 1 indicates the G-Rb1 concentration in several ginseng roots determined by ELISA by anti-G-Rb1 MAb and HPLC after pre-treatment under mildly alkaline condition. The fibrous ginseng made from the active growing part of *P. ginseng* showed the highest G-Rb1 concentration. White ginseng was prepared from *P. ginseng* simply by drying. The concentration of G-Rb1 was higher than that red ginseng, but lower compared with the other samples except Japanese ginseng. Red ginseng is prepared by steaming and drying the root of *P. ginseng.* Eastern blotting analysis indicated that the red ginseng contained few of malonyl-G-Rb1, which is a distinctive feature of it (data not shown). These data suggests that the steaming step for the preparation of red ginseng decreases the concentration of G-Rb1 by conversion into C-20 hydroxy free ginsenosides (Kitagawa et al., 1983). Among these tested samples, the lowest concentration of G-Rb1 was detected in Japanese ginseng (*P. japonicus*). Interestingly, the G-Rb1 concentration of *P. japonicus* was not correlated between ELISA and HPLC.

Sample	G-Rb1 content (µg/mg dry wt.)	
	ELISA	HPLc
White ginseng (*P. ginseng*)	5.49 ± 0.75	4.96 ± 0.05
Red ginseng	3.57 ± 0.62	3.93 ± 0.34
Fibrous ginseng	64.44 ± 3.64	69.75 ± 1.45
San-chi ginseng (*P. notoginseng*)	47.08 ± 3.34	42.39 ± 1.39
American ginseng (*P. quinquefolium*)	48.51 ± 1.79	47.96 ± 1.04
Japanese ginseng (*P. japonicus*)	1.37 ± 0.34	0.63 ± 0.06

Table 1. G-Rb1 content of various ginseng samples after pre-treatment with mildly alkaline condition

P. japonicus is distributed in Japan and China and it is morphologically different from the other *Panax* species. The phytochemical profiles indicated that no G-Rbl was detected in *P. japonicus*, and isolated oleanane-type saponins named as chikusetsusaponins and elucidated their structures (Yahara et al., 1977). Morita et al. examined the varieties of saponins in *P. japonicus* by chemical analysis (Morita et al., 1985). From these results, the concentration of G-Rb1 might be trace level in *P. japonicus*. However, as shown in Table 1, G-Rb1 was detected by ELISA in *P. japonicus* crude extract pre-treated with a mild alkaline solution and found higher concentrations compared with HPLC and previous reports (Morita et al., 1985). This data suggest that anti-G-Rb1 MAb using ELISA has the cross-reactivity with some unknown compounds.

To investigate the unknown compounds bound to anti-G-Rb1 MAb, the crude extract of *P. japonicus* was concentrated by immunoaffinity column using anti-G-Rb1 MAb. The crude root extract was loaded on the column and washed with the washing buffer, followed by the

elution buffer as indicated in Figure 4. Figure 5 shows the H_2SO_4 staining (A) and the Eastern blotting (B) profiles of the two fractions separated by the immunoaffinity column. Fractions eluted with the washing buffer (lane A-D) showed many spots, including ginsenosides and chikusetsusaponins, similar to the original extract of *P. japonicus*. After washing, the column was eluted by elution buffer (lane E), and then one spot was detected. Interestingly, Eastern blotting indicated two different spots on lane A (the washing fraction containing the overcharged compounds) and one spot on each lane B and E. These compounds bound with anti-G-Rb1 MAb have a dammarane saponin having protopanaxadiol as a framework.

Compound 1 detected in the washing fraction had an Rf value close to that of G-Rd, indicating that this compound has three sugar moieties in a molecule. Considering the previous study compound 1 is chikusetsusaponin III (chik III) has three sugars, one xylose and two glcoses in a molecule (Morita et al., 1985) (Figure 6). Finally, this compound was identified as chik III in a direct comparison with authentic sample. These data indicated that the specificity of anti-G-Rb1 MAb against chik III of immunoaffinity column is higher than that of Eastern blotting because the antigen (chik III) conjugated with carrier protein via sugar chain to fix on the membrane in case of Eastern blotting, so the structure of chik III was changed.

Another unknown spot, compound 2 appeared in fraction A of the overcharged washing fraction and fraction E of the eluted fraction. G-Rb1 was not detected by Eastern blotting although it was determined by TLC as indicated in Figure 5. It can be suggested that compound 2 has a similar molecular structure and cross-reactivity with G-Rb1, and seems to be related ginseng saponin having protopanaxadiol as an aglycone. Moreover, compound 2 is more polar than G-Rb1, indicating that it possess 5 sugar moiety compared with G-Rb1. Although compound 2 was treated with a mild alkaline solution, no change occurred. From these evidences compound 2 might be chikusetsusaponin III-20-*O*-gentiobiose (chikusetsusaponin VI; Figure 6) having 5 sugars in a molecule in good agreement with the R_f value previously reported (Kohda et al., 1991) and we confirmed that compound 2 is chik VI by the direct comparison with authentic sample (Fukuda et al., 2000a).
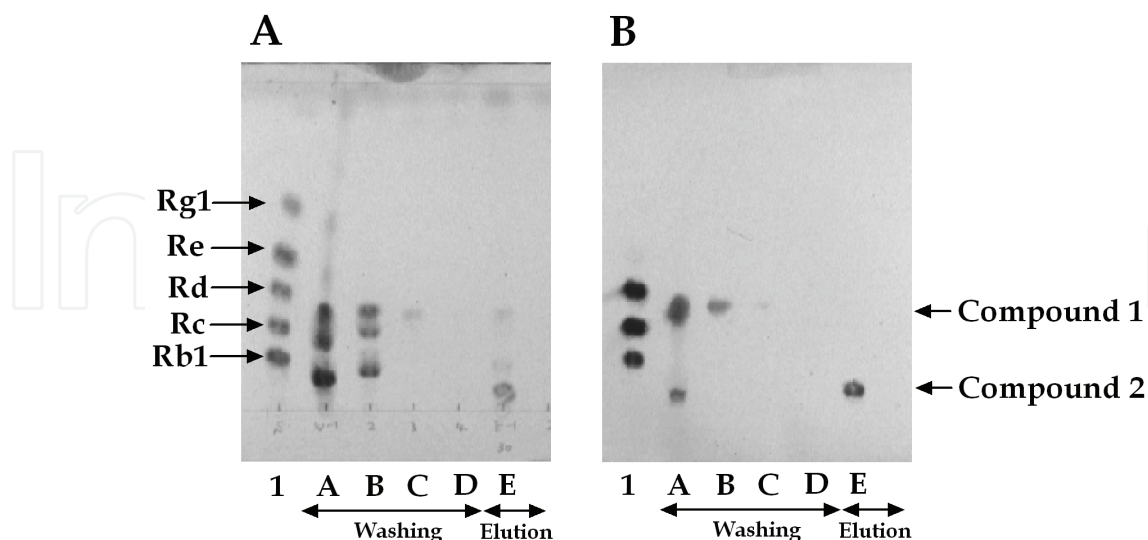

Fig. 5. TLC (A) and Eastern blotting (B) profiles of the separated fractions from *P. japonicus* crude extract by the anti-G-Rb1 immunoaffinity column. Lane 1 indicates the standard of ginsenosides (G-Rd, G-Rc, G-Rb1, G-Rg1, and G-Re). Lane A-D and Lane E were the washing fractions and the eluted fraction, respectively (Fukuda et al., 2000a)

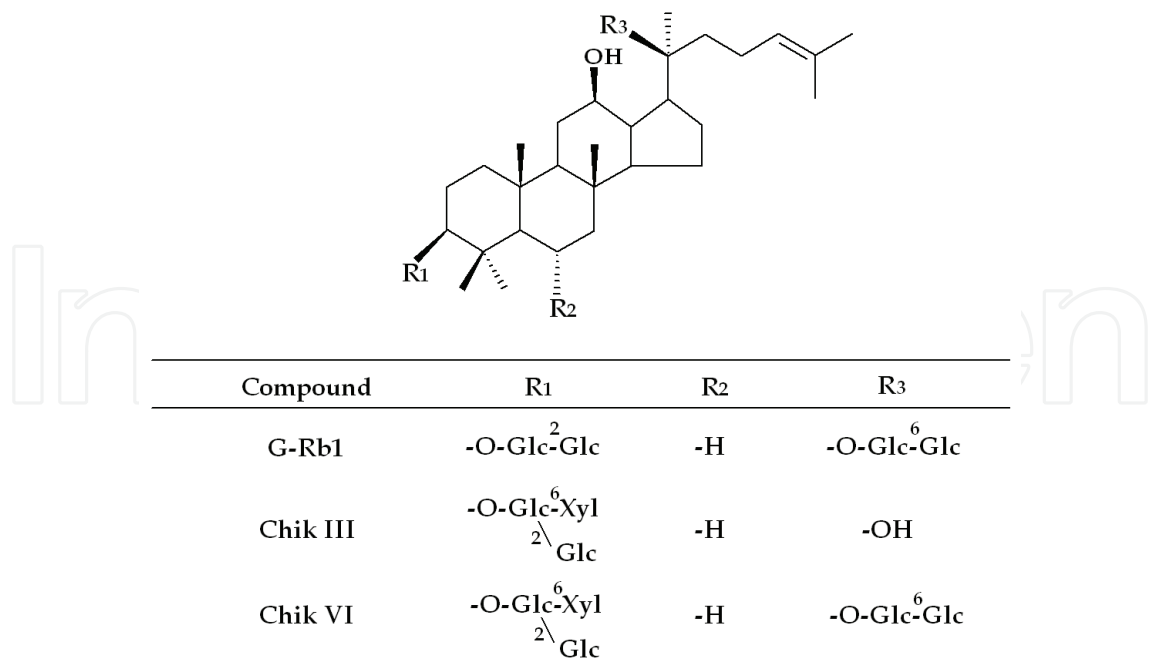| Compound	R1	R2	R3
G-Rb1	-O-Glc^2-Glc	-H	-O-Glc^6-Glc
Chik III	-O-Glc^6-Xyl $^2\backslash$Glc	-H	-OH
Chik VI	-O-Glc^6-Xyl $^2\backslash$Glc	-H	-O-Glc^6-Glc

Fig. 6. Chemical structures of G-Rb1 and chikusetsusaponins purified from *P. japonicus* by the anti-G-Rb1 immunoaffinity column

Taken together, *P. japonicus* root contains no G-Rb1 as previously reported (Yahara et al., 1977). However, the immunoaffinity column conjugated with anti-G-Rb1 MAb could concentrate some unknown structurally resemble compounds having cross-reactivity against anti-G-Rb1 MAb. Thus, this purification system will be applied to survey new compounds related to target compound of MAb. In a previous report, a immunoaffinity purification against solasodine glycosides purified all solasodine glycosides by one-step purification. In this case, all solasodine glycoside have almost same cross-reactivity against anti-solamargine MAb (Ishiyama et al., 1996).

3. Preparation of GC-knock out extract and its application for in vitro assay

Licorice (*Glycyrrhiza* spp.) is another important crude drug used in over 70 % of TCMs. It mainly consists of dried roots and stolons of *Glycyrrhizae uralensis*, *G. inflate* and *G. glabra*. Licorice is prescribed with other herbal medicines as an anti-tussive, an expectorant, and an anti-inflammatory agent (Asl & Hosseinzadeh, 2008). The pharmacological properties of licorice depend upon GC, which is considered to be its main active constituent (Figure 7). It has been reported that GC exhibits numerous pharmacological effects such as anti-inflammation, anti-ulcer, anti-cancer, and anti-viral activities (Asl et al., 2008; Wang & Nixon, 2001; Doll & Hill, 1962; Pompei et al., 1979). Clinically, GC has been used to treat patients with chronic hepatitis (Schalm et al., 1999; Coon & Ernst, 2004). Moreover, GC is used in food additives and cosmetics as a well-known natural sweetener (Kim & Kinghorn, 2002). The quality standardization of licorice is usually based on its GC content. Recently, the utilization volume of licorice is rapidly increasing, while the availability of wild licorice has declined to curb overexploitation of the natural resources (Yamamoto et al., 2003; Hayashi et al., 2003). For the promotion of effective cultivation of licorice as an additional and stable herbal resource, the rapid and easy measurement methods of the GC content are

required. Previously, we established anti-GC MAb and a fast and sensitive assay system using the MAb for the screening of GC concentration in large numbers of samples (Tanaka & Shoyama, 1998; Shan et al., 2001), the sensitive detection of GC by surface plasmon resonance-based immunosensor (Sakai et al., 2004), and the rapid detection by immunochromatographic assay (Putalun et al., 2005). The purpose of our recent studies is to purify and quantify GC from licorice crude extract by anti-GC-MAB immunoaffinity chromatography to expand the possibilities for pharmacological research and other applications.
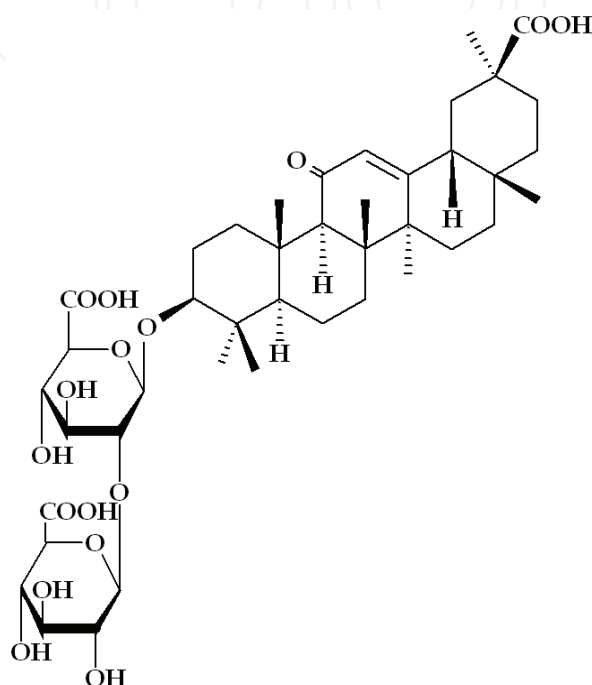
Fig. 7. Chemical structure of GC

3.1 Preparation of GC-KO extract by anti-GC immunoaffinity column

Previously, we have reported the preparation of anti-GC MAb, and its cross-reactivity of the anti-GC MAb against glycyrrhetic acid-3-O-glucuronide and glycyrrhetic acid were 0.585 % and 1.865 %, respectively, compared with GC (Tanaka & Shoyama, 1998; Shan et al., 2001). The other related compounds such as deoxycholic acid, ursolic acid, and oleanolic acid, were all less than 0.005 %. The purified 60 mg of the anti-GC MAb was coupled to 25 ml of an Affi-Gel Hz gel and used to prepare the immunoaffinity column.

Twelve milligrams of licorice crude extract containing 1275.0 µg of GC was dissolved in loading buffer (5 % MeOH), and then applied on the anti-GC-MAb immunoaffinity column. The column was washed with washing buffer (5 % MeOH) and then eluted with elution buffer (20 mM phosphate buffer containing 30 % MeOH). After separation, each fraction was deionized and the solvent was lyophilized. Table 2 indicates the GC concentration of washing and eluted fractions determined by ELISA. The washing and eluted fractions contain and 3.50 µg and 1269.26 µg of GC, respectively. These data indicate that the anti-GC column could eliminate 99.55 % of the loading GC. Thus, we named this washing fraction "GC-knock out (GC-KO) extract". The capacity of the anti-GC immunoaffinity column was effective to capture GC compared with the immunoaffinity columns for forskolin and

solasodine glycosides (Yanagihara et al., 1996; Putalun et al., 1999). This result may also infer that when the GC concentration is trace level or cannot be analyzed by even ELISA, this anti-GC immunoaffinity column can concentrate GC to the detectable level. The combination of the established anti-GC-MAb immunoaffinity column and ELISA provided a reliable and very high sensitive analysis for GC in different extracts of various medicinal herbs or other drugs.

	GC content (μg)	GC content rate (% of loading GC)
Loading sample	1275.0 (12 mg licorice extract)	100
Washing fraction	3.50	0.27
Eluted fraction	1269.26	99.55

Table 2. GC content of the loading licorice extract and the separated fractions by anti-GC immunoaffinity column

To investigate the profiles of GC-KO extract, we performed the TLC analysis and Eastern blotting. As shown in Figure 8A, several spots of GC and other compounds were detected in licorice extract (lane 2). However, although all other spots were clearly detected, the spot of GC was completely disappeared in GC-KO extract (lane 3). Furthermore, Eastern blotting by anti-GC MAb indicated that GC was detected in licorice extract (lane 2), but the spot of GC was disappeared in GC-KO extract (lane 3) (Figure 8B). Taken together, these data suggest that GC was specifically eliminated from licorice extract by anti-GC MAb immunoaffinity column.
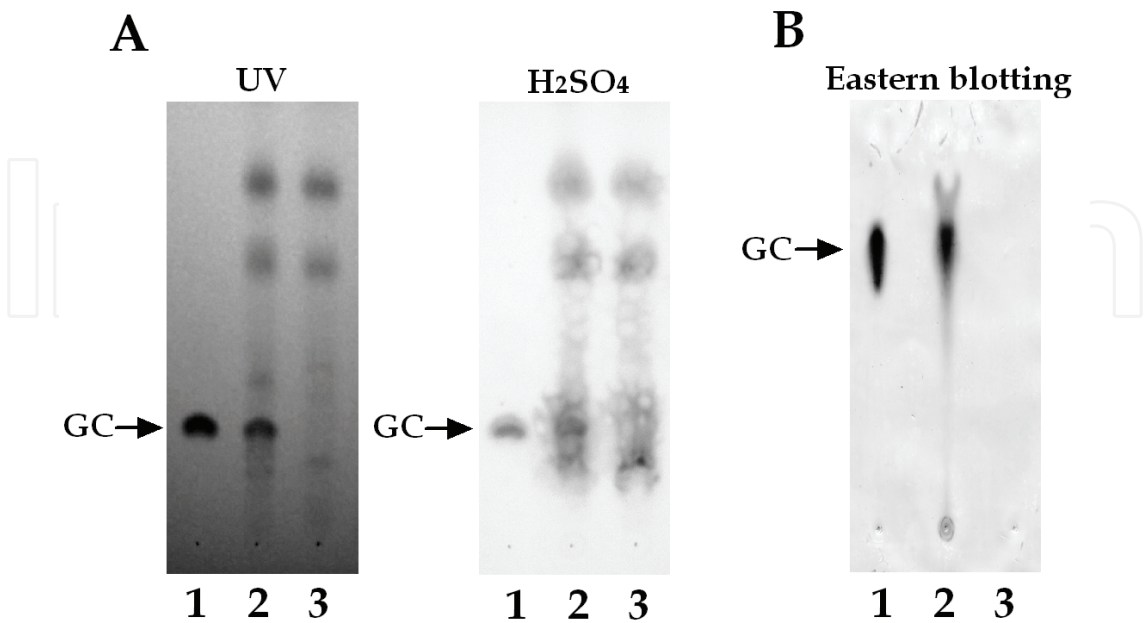

Fig. 8. TLC profiles (A) and Eastern blotting by anti-GC MAb (B) of licorice extract and GC-KO extract. Lane 1; GC strandard, Lane 2; licorice extract, Lane 3; GC-KO extract

3.2 The application of GC-KO extract prepared by anti-GC immunoaffinity column to in vitro assay

Inflammation leads to the up-regulation of a series of enzymes and signaling mediators in the affected tissue and cells. Nitric oxide (NO) is a highly reactive free radical involved in multiple physiological functions, such as vasodilatation, neurotransmission and inflammation (Moncada et al., 1991). During inflammatory process, a large amount of NO is produced by inducible nitric oxide synthase (iNOS) by inflammatory cytokines and/or bacterial lipopolysaccharide (LPS) in various cell types including macrophages (Nathan & Xie, 1994). Overproduced NO synthesized by iNOS triggers the pathogenesis of septic shock and organ destruction in certain inflammatory and autoimmune diseases (Naseem et al., 2005; Guzik et al., 2003; Abramson et al., 2001). Therefore, inhibiting NO production by blocking iNOS expression may be useful strategy to treat a variety of inflammatory diseases.

Initially, we examined whether licorice crude extract suppress NO release in LPS-treated mouse RAW264 macrophages. LPS evoked a dramatic increase of NO production, and this induction was blocked by treatment of licorice extract dose dependently (12.5-100 μg/mL) (data not shown). Next, we performed Western blot analyses and RT-PCR to investigate the effect of licorice extract on iNOS protein and mRNA expressions. LPS markedly up-regulated iNOS protein and mRNA level, and pretreatment of licorice extract reduced these up-regulations in the same concentration range as was used for NO production (data not shown).

At 100 μg/mL of licorice extract, iNOS protein and mRNA were completely suppressed. ELISA by using anti-GC MAb demonstrated that 100 μg of licorice extract contains 10.6 ± 0.618 μg of GC. To examine whether GC alone can reduce NO production and iNOS expression, we pretreated the cells with GC around 10.6 μg/ml before exposure to LPS. However, GC did not exert any significant influence on LPS-induced NO production and iNOS protein expression (data not shown).
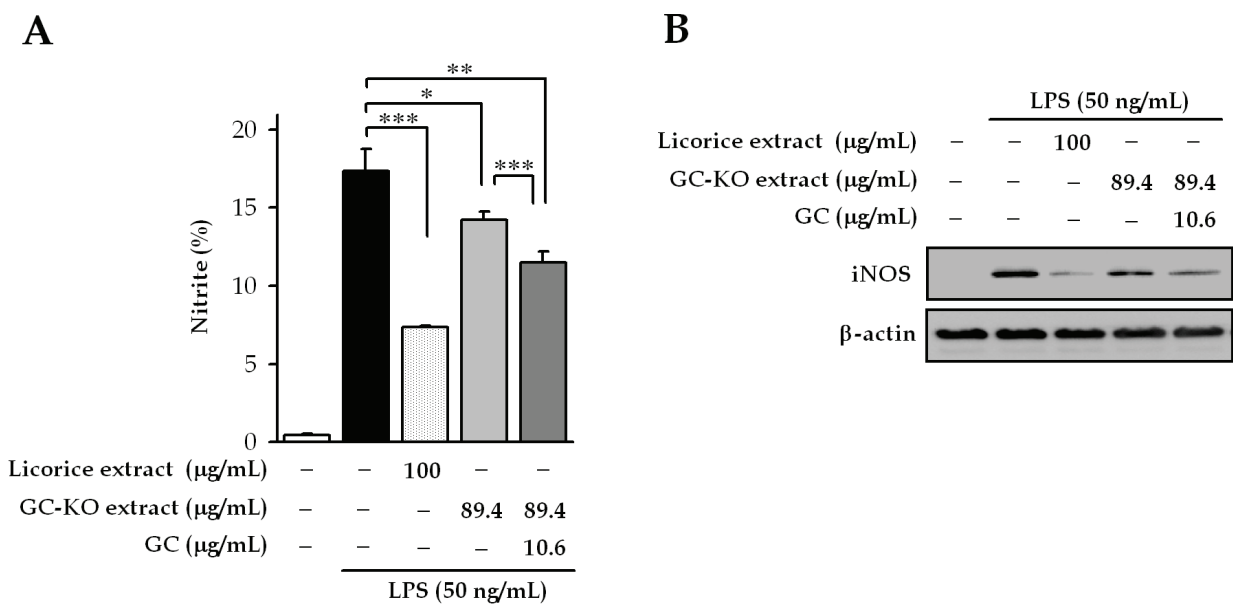

Fig. 9. Effect of licorice extract, GC-KO extract, and the co-treatment of GC-KO extract and GC on NO production (A) and iNOS protein expression (B) in LPS-treated RAW264 cells. Each bar represents the means ± S.D. from four separate experiments. *P<0.05, **P<0.01, *P<0.001 compared with LPS alone

To further elucidate the effect of GC on NO inhibition by licorice extract, we used GC-KO extract prepared by anti-GC immunoaffinity column. According to data of ELISA, 100 μg of licorice extract contains 10.6 μg of GC and 89.4 μg of the other compounds. Thus, the cells were treated with licorice extract (100 μg/mL), GC-KO extract (89.4 μg/mL), or combination of GC-KO extract (89.4 μg/mL) and GC (10.6 μg/mL). As shown in Figure 9A, although treatment of licorice extract lead to an inhibition of NO production as compared to LPS treatment [inhibition ratio (IR) = 57.7%], the inhibitory effect of GC-KO extract was decreased compared with licorice extract (IR = 17.8 %). Interestingly, the combination of GC-KO extract and GC significantly improved the inhibitory ability (IR = 33.5 %). Furthermore, we performed Western blotting to determine whether the combinational effect of GC-KO extract and GC was related to iNOS expression (Figure 9B). Inhibitory activity of iNOS expression by licorice extract was reduced in treatment of GC-KO extract. The addition of GC to GC-KO extract improved the inhibition. These data suggest that GC alone cannot suppress iNOS expression, but combinational inhibition of iNOS expression may occur when GC coexists with the other constituents contained in licorice extract.

In conclusion, this study demonstrate that KO extract prepared by anti-natural compound specific MAb-conjugated immunoaffinity column is a useful approach for determination of potential function of natural compound on *in vitro* and in *vivo* assays.

4. Conclusion

We describe the new strategy of one-step purification of target compounds from crude extract by anti-natural compound MAb-conjugated immunoaffinity column. The combination of the immunoaffinity column and ELISA by using MAb provided a reliable and high sensitivity analysis for target compound in various crude extracts and TCMs. Furthermore, by the use of the cross-reactivity of MAb, the affinity column can identify new unknown compounds related to target compound of MAb and determine their structures. Finally, we demonstrate that KO extract, which contains all components of crude extract except an antigen molecule, may be useful approach to determine the potential function of one principal compound on the crude extract or TCM by *in vitro* and *in vivo* assays.

5. Acknowledgment

6. References

Abramson, S.B.; Amin, A.R.; Clancy, R.M. & Attur, M. (2001) The role of nitric oxide in tissue destruction. *Best Pract. Res. Clin. Rheumatol.*, 15(5) 831-845

Asl, M.N. & Hosseinzadeh, H. (2008) Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. *Phytother. Res.*, 22(6) 709-724

Coon, J.T. & Ernst, E. (2004) Complementary and alternative therapies in the treatment of chronic hepatitis C: a systematic review. *J. Hepatol.*, 40(3) 491-500

Doll, R. & Hill, I.D. (1962) Triterpenoid liquorice compound in gastric and duodenal ulcer. *Lancet*, 2(7266) 1166-1167

Fukuda, N.; Tanaka, H. & Shoyama, Y. (2000a) Applications of ELISA, western blotting and immunoaffinity concentration for survey of ginsenosides in crude drugs of Panax species and traditional Chinese herbal medicines. *Analyst*, 125(8) 1425-1429

Fukuda, N.; Tanaka, H. & Shoyama, Y. (2000b) Formation of monoclonal antibody against a major ginseng component, ginsenoside Rg1 and its characterization. Monoclonal antibody for a ginseng saponin. *Cytotechnology*, 34(3) 197-204

Fukuda, N.; Tanaka, H. & Shoyama, H. (2000c) Isolation of the pharmacologically active saponin ginsenoside Rb1 from ginseng by immunoaffinity column chromatography. *J. Nat. Prod.*, 63(2) 283-285

Gillis, C.N. (1997) Panax ginseng pharmacology: a nitric oxide link? *Biochem. Pharmacol.*, 54(1) 1-8

Guzik, T.J.; Korbut, R. & Adamek-Guzik, T. (2003) Nitric oxide and superoxide in inflammation and immune regulation. *J. Physiol. Pharmacol.*, 54(4) 469-487

Hayashi, H.; Hattori, S. ; Inoue, K.; Khodzhimatov, O.; Ashurmetov, O.; Ito, M. & Honda, G. (2003) Field survey of *Glycyrrhiza* plants in Central Asia (3). Chemical characterization of *G. glabra* collected in Uzbekistan. *Chem. Pharm. Bull.*, 51(11) 1338-1340

Ishiyama, M.; Shoyama, Y.; Murakami, H. & Shinohara, H. (1996) Production of monoclonal antibodies and development of an ELISA for solamargine. *Cytotechnology*, 18(3) 153-158

Jiang, K.Y. & Qian, Z.N. (1995) Effects of Panax notoginseng saponins on posthypoxic cell damage of neurons *in vitro*. *Zhongguo Yao Li Xue Bao*, 16(5) 399–402

Kim, J.S.; Tanaka, H. & Shoyama, Y. (2004) Immunoquantitative analysis for berberine and its related compounds using monoclonal antibodies in herbal medicines. *Analyst*, 129(1) 87-91

Kim, N.C. & Kinghorn, A.D. (2002) Highly sweet compounds of plant origin. *Arch. Pharm. Res.*, 25(6) 725-746

Kitagawa, I.; Taniyama, T.; Yoshikawa, M.; Ikenishi, Y. & Nakagawa, Y. (1989) Chemical studies on crude drug processing. IV. Chemical structures of malonyl-ginsenosides Rb1, Rb2, Re and Rd isolated from the root of Panax Ginseng C.A. Meyer. *Chem. Pharm. Bull.*, 37(11) 2961–2970

Kitagawa, I.; Yoshikawa, M.; Yoshihara, M.; Hayashi, T. & Taniyama, T. (1983) Chemical studies of crude drugs (1). Constituents of Ginseng radix rubra. *Yakugaku Zassh*, 103(6) 612-622

Kohda, H.; Tanaka, S.; Yamaoka, Y. & Ohhara, Y. (1991) Saponins from Amaranthus hypochondriacus. *Chem. Pharm. Bull.*, 39(10) 2609-2612

Lay, H.L.; Chen, C.C. & Chiang, S.T. (2004) Simultaneous analysis of nine components in" Byi-Liang-Tang" preparation by high performance liquid chromatography. *J. Food Drug Anal.*, 12(2) 115-119

Lim, J.H.; Wen, T.C.; Matsuda, S.; Tanaka, J.; Maeda, N.; Peng, H.; Aburaya, J.; Ishihara, K. & Sakanaka, M. (1997) Protection of ischemic hippocampal neurons by ginsenoside Rb1, a main ingredient of ginseng root. *Neurosci. Res.*, 28(3) 191–200

Liu, M. & Zhang, J. (1995) Effects of ginsenoside Rb1 and Rg1 on synaptosomal free calcium level, ATPase and calmodulin in rat hippocampus. *Chin. Med. J. (Engl.),* 108(7) 544–547

Loungratana, P.; Tanaka, H.; & Shoyama, Y. (2004) Production of monoclonal antibody against ginkgolic acids in Ginkgo biloba Linn. *Am. J. Chin. Med.,* 32(2) 33-48

Lu, Z.; Morinaga, O.; Tanaka, H. & Shoyama, Y. (2003) A quantitative ELISA using monoclonal antibody to survey paeoniflorin and albiflorin in crude drugs and traditional Chinese herbal medicines. *Biol. Pharm. Bull.,* 26(6) 862-866

Moncada, S.; Palmer, R.M. & Higgs, E.A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.,* 43(2) 109-142

Mook-Jung, I.; Hong, H.S.; Boo, J.H.; Lee, K.H.; Yun, S.H.; Cheong, M.Y.; Joo, I.; Huh, K. & Jung, M.W. (2001) Ginsenoside Rb1 and Rg1 improve spatial learning and increase hippocampal synaptophysin level in mice. *J. Neurosci. Res.,* 63(6) 509-515

Morinaga, O.; Nakajima, S.; Tanaka, H. & Shoyama, Y. (2001) Production of monoclonal antibodies against a major purgative component, sennoside B, their characterization and use in ELISA. *Analyst,* 126(8) 1372-1376

Morinaga, O.; Tanaka, H. & Shoyama, Y. (2000) Production of monoclonal antibody against a major purgative component, sennoside A, its characterization and ELISA. *Analyst,* 125(8) 1109-1113

Morinaga, O.; Tanaka, H. & Shoyama, Y. (2006) Detection and quantification of ginsenoside Re in ginseng samples by a chromatographic immunostaining method using monoclonal antibody against ginsenoside Re. *J. Chromatography B,* 830(1) 100-104

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(9) 3852-3858

Nathan, C. & Xie, Q.W. (1994) Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.,* 269(19) 13725-13728

Naseem, K.M. (2005) The role of nitric oxide in cardiovascular diseases. *Mol. Aspects Med.,* 26(1-2) 33-65

Ong, E.S. (2002) Chemical assay of glycyrrhizin in medicinal plants by pressurized liquid extraction (PLE) with capillary zone electrophoresis (CZE). *J. Sep. Sci.,* 25(13) 825-831

Pompei, R.; Flore, O.; Marccialis, M.A.; Pani, A. & Loddo, B. (1979) Glycyrrhizic acid inhibits virus growth and inactivates virus particles. *Nature,* 281(5733) 689-690

Putalun, W.; Tanaka, H. & Yukihiro, S. (1999) Rapid separation of solasodine glycosides by an immunoaffinity column using anti-solamargine monoclonal antibody. *Cytotechnology,* 31(1-2) 151-156

Putalun, W.; Tanaka, H. & Shoyama, Y. (2005) Rapid detection of glycyrrhizin by immunochromatographic assay. *Phytochem. Anal.,* 16(5) 370-374

Sakai, T.; Shinahara, K.; Torimaru, A.; Tanaka, H.; Shoyama, Y. & Matsumoto, K. (2004) Sensitive detection of glycyrrhizin and evaluation of the affinity constants by a surface plasmon resonance-based immunosensor. *Anal. Sci.,* 20(2) 279-283

Sakata, R.; Shoyama, Y. & Murakami, H. (1994) Production of monoclonal antibodies and enzyme immunoassay for typical adenylate cyclase activator, Forskolin. *Cytotechnology,* 16(2) 101-108

Schalm, S.W.; Brouwer, J.T.; Bekkering, F.C. & van Rossum, T.G. (1999) New treatment strategies in non-responder patients with chronic hepatitis C. *J. Hepatol.*, 31(Suppl.1) 1184-1188

Shan, S.J.; 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(24) 5784-5790

Shoyama, Y.; Fukada, T. & Murakami, H. (1996) Production of monoclonal antibodies and ELISA for thebaine and codeine. *Cytotechnology*, 19(1) 55-61

Tan, T.W.; Huo, Q. & Ling, Q. (2002) Purification of glycyrrhizin from glycyrrhiza uralensis fisch with ethanol/phosphate aqueous two phase system. *Biochem. Lett.*, 24(17) 1417-1420

Tanaka, H.; Fukuda, N. & Shoyama, Y. (1999) Formation of monoclonal antibody against a major ginseng component, ginsenoside Rb1 and its characterization. *Cytotechnology*, 29(1) 115-120

Tanaka, H.; Fukuda, 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(10) 3783-3787

Tanaka, H.; Goto, Y. & Shoyama, Y. (1996) Monoclonal antibody based enzyme immunoassay for marihuana (cannabinoid) compounds. *Immunoassay*, 17(4) 321-342

Tanaka, H. & Shoyama, Y. (1998) Formation of a monoclonal antibody against glycyrrhizin and development of an ELISA. *Biol. Pharm. Bull.*, 21(12) 1391-1393

Tanaka, O. (1989) Saponin-composition of Panax species. In recent advances in ginseng studies. Shibata, S.; Ohtsuka, Y. & Saito, H., (Eds.), 43-47, Hirokawa Publishing, Tokyo, Japan

Wang, C.A. & Shoyama, Y. (2006). Herbal medicine: identification, analysis, and evaluation strategies, In: *Textbook of complementary and alternative medicine, second edition*, C.S. Yuan; E.J. Bieber & B.A. Bauer, (Ed.), 51-70, Informa Healthcare, ISBN 1-84214-297-6, London, UK

Wang, Z.Y. & Nixon, D.W. (2001) Licorice and cancer. *Nutr. Cancer*, 39(1) 1-11

Washida, D. & Kitanaka, S. (2003) Determination of polyacetylenes and ginsenosides in Panax species using high performance liquid chromatography. *Chem. Pharm. Bull.*, 51(11) 1314-1317

Xu, J.; Tanaka, H. & Shoyama, Y. (2007) One-step immunochromatographic separation and ELISA quantification of glycyrrhizin from traditional Chinese medicines. *J. Chromatog. B*, 850(1-2) 53-58

Xuan, L.; Tanaka, H. ; Xu, Y. & Shoyama, Y. (1999) Preparation of monoclonal antibody against crocin and its characterization. *Cytotechnology*, 29(1) 65-70

Yahara, S.; Kasai, R. & Tanaka, O. (1977) New dammarane type saponins of leaves of *Panax japonicus* C.A. Meyer. (1). Chikusetsusaponins L_5, L_{9a} and L_{10}. *Chem. Pharm. Bull.*, 25(8) 2041-2047

Yamamoto, Y.; Majima, T.; Saiki, I. & Tani, T. (2003) Pharmaceutical evaluation of *Glycyrrhiza uralensis* roots cultivated in eastern Nei-Meng-Gu of China. *Biol. Pharm. Bull.*, 26(8) 1144-1149

Yanagihara, H.; Sakata, R.; Minami, H.; Shoyama, Y. & Murakami, H. (1996) Immunoaffinity column chromatography against forskolin using an anti-forskolin monoclonal antibody and its application. *Anal. Chim. Acta,* 335(1-2) 63-70

Zhu, S.; Shimokawa, S.; Tanaka, H. & Shoyama, Y. (2004) Development of an assay system for saikosaponin a using anti-saikosaponin a monoclonal antibodies. *Biol. Pharm. Bull.,* 27(1) 66-71

Quality Control of Herbal Medicines and Related Areas

Edited by Prof. Yukihiro Shoyama

The authors of this thematic issue provide a comprehensive summary of most recent knowledge and references on quality control in wide fields. Quality control is essential for natural products like natural medicine and related food products. In this issue fifteen chapters have been included, discussing in detail various aspects of quality control. It will certainly prove useful not only for phytochemical researchers, but also many scientists working in numerous fields. Much effort has been invested by the contributors to share current information. Without their efforts and input 'Quality Control of Herbal Medicine and Related Areas' could not exist.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Takuhiro Uto and Nguyen Huu Tung (2011). Quality Control by Immunoaffinity Concentration Using Monoclonal Antibody, Quality Control of Herbal Medicines and Related Areas, Prof. Yukihiro Shoyama (Ed.), ISBN: 978-953-307-682-9, InTech, Available from: http://www.intechopen.com/books/quality-control-of-herbal-medicines-and-related-areas/quality-control-by-immunoaffinity-concentration-using-monoclonal-antibody

INTECH
open science | open minds