JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1175–1180

Received March 25, 2007
Accepted May 9, 2007
DOI 10.1211/jpp.59.8.0017
ISSN 0022-3573

Separation and purification of saponins from Semen *Ziziphus jujuba* and their sedative and hypnotic effects

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Abstract

Semen *Ziziphus jujuba* (SZJ) has long been used in Chinese traditional medicine for the treatment of anxiety and insomnia because of its effect of decreasing monoaminergic system activity; saponins are thought to be the main bioactive factors. To investigate the sedative and hypnotic effects of saponins from SZJ, silica gel column chromatography and thin-layer chromatography were used to purify the crude extract of total saponins from SZJ. High-performance liquid chromatography showed that saponins from SZJ comprised two components, compounds I and II. Animal tests were conducted using these two compounds to compare their sedative and hypnotic effects. Results showed that both compounds had a significant effect ($P < 0.05$, $P < 0.01$) on walking time compared with that of the control group. Compound I had a significant effect on coordinated movement ($P < 0.05$). Both compounds prolonged the suprathreshold barbiturate induced sleeping time ($P < 0.05$). The number of sleeping animals increased by 30% and 20% for compounds I and II, respectively, under the subthreshold dose of sodium barbital.

Introduction

The seed of Semen *Ziziphus jujuba* (SZJ), local name suanzaoren, is used in Chinese traditional medicine, and in some other countries, to treat anxiety and insomnia (Siekwoo 1978; Peng et al 2000), by decreasing monoaminergic activity in the brain (Zhao et al 1995). In Chinese medicinal formulae, SJZ is often used as the principal drug because of its additional hypotensive, antihypoxia, antihyperlipidaemia, hypothermic and anxiolytic properties (Li et al 1999). In recent years, the search for new and bioactive minor components from SZJ has increased (Li et al 2005). Jujuboside A, a main glycoside of jujubogenin extracted from SZJ has been studied to determine its effects on brain in-vivo and in-vitro. It has been reported that jujuboside A has inhibitory effects on hippocampal formation, Glu-mediated excitatory signal pathway in hippocampus (Zhang et al 2003) and penicillin sodium induced hyperactivity. The neurophysiological inhibitory effects of jujuboside A and diazepam were more persistent than their Glu inhibitory effects (Lu et al 2005).

Compared with the therapeutic effects of established medicinal drugs, herbal products are generally more benign in terms of side-effects (Sardesai 2002) and cost of potential dependence (Foster et al 2004; Capasso & Sorrentino 2005; Wheatley 2005), although some herbal products such as Kava Kava are known to cause hepatotoxicity (Maddrey 2005) and are thus no better than synthetic drugs containing a single chemical entity. Unlike synthetic drugs that contain single chemical entities of known composition and specific therapeutic actions, herbal drugs are derived from plant extracts of mixed composition. It is often not known which are the active compounds and which are simply adjuvants (Wheatley 2005). For this reason, more and more studies have been conducted on the components of plant medicines and on their pharmacological analysis (Pieters & Vlietinck 2005; Soares et al 2005; Jung et al 2006). Drug discovery is substantially benefited by ethnopharmacological approaches (Carvalho-Freitas & Costa 2002). In the present study, preparations of saponins obtained from SZJ were investigated in order to evaluate their ability to induce sedative and hypnotic effects in experimental models.

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Materials and Methods

Separation and purification of saponins

Extraction of saponins

The isolation of saponins was modified from the method of Khan et al (1997). SZJ samples were shattered and screened with a 40 mesh. After evaporation at 60°C, samples were Soxhlet extracted in petroleum benzin, 1:10 (v/v), for 4 h at 60°C, and the extraction was repeated three times. After drying, levigation and screening with 40 mesh, the residues were inverse-flow extracted in 70% ethanol, 1:25 (v/v), for 3 h at 85°C, filtered and the alcohol solution evaporated to dryness with reduced pressure at 60°C, and then dissolved with water. After filtration and discarding of extraneous components, the alcohol phase was extracted by adding water-saturated *n*-butanol (1:1 v/v); the *n*-butanol phase was then treated with 1 M KOH. The *n*-butanol phase was evaporated to dryness under pressure and the raw saponins were obtained.

Silica gel column chromatography

Silica gel column chromatography, as previously described by various authors (Guil-Guerrero et al 2000; Kim et al 2001; Shibata et al 2004; Simekova & Berek 2005), was used for the fractionation of saponins. Silica gel (100 g; 100–200 mesh) for column chromatography was dried at 120°C for 1 h, and then dipped in 95% (v/v) ethanol for 24 h. Agitation was continued for 10 min and ethanol was evaporated in a rotary evaporator under vacuum at 60°C. This material was cooled and kept in the dark in a desiccator until required. A slurry of silica gel (800 mg) in H_2O (4 mL) was poured into a column (36 mm × 800 mm) previously half filled with H_2O. A slight flow of H_2O was allowed during packing. The H_2O level was lowered until it was 1 cm above the stationary phase. The packed height of the glass chromatography columns was lowered to between 7 and 9 cm from the top end. The above crude extracts (10 mL; 25 mg mL^{-1}) were applied onto the chromatography columns. The column was eluted with 60% ethanol (established after several assays) passed consecutively through the column, and the flow rate was 0.5 mL min^{-1}. The eluates were collected as fractions of every 10 mL.

Silica gel thin-layer chromatography (TLC)

TLC was conducted according to methods described previously (Higashida et al 2002; Taylor et al 2004; Zivkovic-Radovanovic & Vuckovic 2005) with some modifications. Silica gel G-75 (100–200 mesh) was impregnated with 0.5% carboxymethylcellulose sodium. A suspension was prepared from 75 g silica gel in 200 mL 0.5% carboxymethylcellulose sodium. After 15 min of vigorous shaking, the solvent was removed by volatilization under vacuum. The adsorbents were then suspended in distilled water (mass ratio 1:2) and applied to glass plates (10 mm × 20 mm). The layers (0.4 mm) were developed in the ascending direction for 40–120 min at room temperature (28 ± 2°C) with *n*-butanol/glacial acetic acid/water in a percentage ratio of 40:10:50) as mobile phase. Spots were detected by spraying the plates with freshly prepared 2% vanillin ethanol solution (2 g vanillin was dissolved in 98 mL ethanol containing 10% sulfuric acid). The reproducibility of RF values was ± 0.02.

HPLC analysis

HPLC (P680, Dionex) was done under the following conditions: Water's symmetry 300 C_{18} 5 μ column (3.9 mm × 300 mm); flow rate 1.0 mL min^{-1}; temperature 25°C; solvent system acetonitrile/water (30:70, v/v); diode array detector PDA-100 and evaporative light scattering detector (Alltech ELSD 2000ES), with detection at 210 nm (Taylor et al 2004).

Animal tests

With Ethical Committee approval, all experiments were performed using ICR mice (20 ± 2 g, 7–9 weeks of age) obtained from the Experimental Animal Centre of the Guangdong Province. A total of 60 mice were housed in groups of 10 in cages where the ambient temperature was 20–25°C and a 12-h light–dark cycle was in effect. Mice were allowed free access to food and water. All studies were conducted during the light cycle.

Mouse behaviour studies

Locomotor activity The sedative properties of saponins were compared using spontaneous activity as an index of sedation. At 45 min after dosing, each mouse was placed in a cage that was similar to their home cage, and was surrounded by an array of 16 photobeams 2.5 cm apart from side to side on the upper level (Model Opto-Varimex, Columbus Co., USA). The acrylic cage within the monitor measured approximately 39 cm in width, 41 cm in length and 30 cm in height. The analyser converted the patterns of broken beams into different measures of locomotor activity. After being kept in this cage for 5 min, a 5-min test was given, during which time the ambulation time was automatically recorded. At the same time, the times in open and closed arms were manually recorded.

Coordinated movement At 45 min after the last dosing, mice were tested for active avoidance retention (Cao et al 2005). A slippery glass plate (60 × 50 cm) was inclined at an angle of 42.5°; each mouse was placed on the upper end and the time taken for it to glide from the upper end to lower end was recorded. If the drugs had a sedative effect, the coordination of the mouse would be decreased and the gliding time would be shortened.

Mouse sleeping studies

Suprathreshold barbiturate induced sleeping time At 30 min after each group of animals was given saponin solutions and physiological saline, each group was administered pentobarbital sodium at dose of 50 mg kg^{-1} by intraperitoneal injection (the dose was determined after several assays; Jiang et al 2007). The sleep duration was measured based on the loss and regaining of the righting reflex.

Subthreshold barbiturate induced sleeping time The method was the same as above, but the subthreshold sleeping dose of 40 mg kg^{-1} pentobarbital sodium was used for intraperitoneal injection (determined after several assays; Jiang et al 2007).

The number of mice that fell asleep was observed and recorded.

Statistical analysis

The results are expressed as mean ± s.e.m. (Gilani et al 2000). Differences between groups at specified time points were analysed using one-way analysis of variance followed by Scheffe's test for group differences. Differences were regarded as significant at a value of $P < 0.05$.

Results

Preparation of saponins

The crude extract of saponins was analysed by HPLC (Figure 1) using the diode array detector PDA-100. There were several peaks between 2.0 min and 4.0 min, and a peak at 13.5 min, indicating that the crude extract included several components. The first step in the purification of the crude extract was silica gel column chromatography. With UV detection, three absorption peaks were observed at 2 h 48 min, 3 h 29 min and 7 h 18 min, suggesting that there were three components in the sample after column chromatography.

For the fraction collection, eluent did not pass through the UV detector. A sample of 1 mL was taken from each collecting duct, and 1 mL 2% vanillin solution and 2 mL concentrated sulfuric acid were added; resulting colouration was detected at 507 nm. Fraction I (tubes 11–26) and fraction II (tubes 29–35) were collected. Chloroform-concentrated sulfuric acid reaction was conducted and it was found that the sulfuric acid layer of fraction I had a green colour, whereas fraction II samples were colourless, indicating that fraction I included saponins and fraction II did not. Therefore, enough fraction I was collected for the next purification step.

Fraction I was concentrated to a residue using a rotary evaporator on 0.1 degree of vacuum at 50°C. The residue was prepared as a 15-mg mL^{-1} solution for the TLC analysis. Two spots, blue/green in colour, were detected (Figure 2). After spreading for 20 cm, there were still only two spots, indicating that there were two saponin compounds in the sample.

The silica gel spots were collected and immersed in 70% ethanol solution for 24 h, and then stirred for 30 min. The solution was centrifuged for 5 min at 20 000 *g*; supernatants were reduced under pressure and concentrated at 60°C, yielding concentrated solutions of compounds I and II. The process was repeated (about 300 runs of TLC) to collect enough samples for the animal tests.

A total of 2 mL of each of compounds I and II was filtered with 0.45-μm microporous filtering film and conserved for use. HPLC-ELSD was used to identify the purity of compounds I and II. The ELSD response does not depend on the optical characteristics of the samples, eliminating the common problems associated with other HPLC detectors. It can achieve a stable baseline with multi-solvent gradients for improved resolution and faster separations (Zhao et al 2006). ELSD is increasingly being used in liquid chromatography as a quasi-universal detector and has been successfully applied to the analysis of non-volatile compounds such as saponins (Ganzera et al 2004; Yoo et al 2006; Oleszek & Bialy 2006). Figure 1 shows the HPLC results of compounds I and II, respectively. For compound I, the retention time was 1–1.590 min and the peak height was 230.659 mV. The peak area % by HPLC of compound I was 100%. For compound II, the retention time was 1–1.599, peak height was 237.950 mV and the peak area % was 100%. These results suggest that compounds I and II were single compounds in the measurable range.

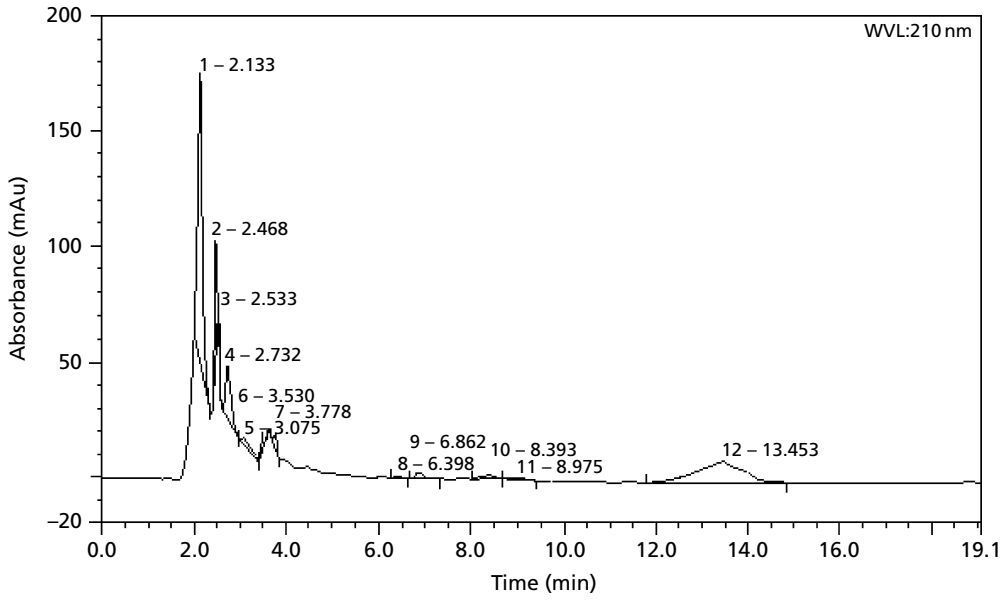

Figure 1 High-performance liquid chromatography of crude saponins extracted from Semen *Ziziphus jujuba*.

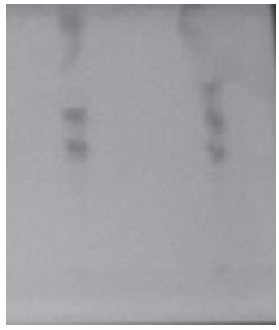

Figure 2 Thin-layer chromatography of saponins of fraction I.

Mouse behaviour tests

Spontaneous motility

A total of 10 mg mL^{-1} of each of compounds I and II was prepared. All compounds were given by oral gavage as suspensions. Compounds I and II were given at doses of 0.15 mL per 10 g per day, equivalent to 17 g kg^{-1} per day of crude drug. The control group was treated with a 0.9% NaCl solution under the same conditions. Administration was continued for 3 days, once a day. Mice were fasted 24 h before the last administration.

A total of 30 mice were subdivided into three groups (10 animals in each group). The data for the individual animals are presented in Table 1. Based on the results, it can be seen that the mean motility values of animals treated with compounds I and II were lower than that of the control group. The percentages for the reduction of motility in mice treated with compounds I and II compared with the control values were 69% and 43%, respectively.

Statistical analysis showed that the spontaneous motility values of the groups treated with compounds I and II were significantly different compared with those of the control group ($P < 0.01$ and $P < 0.05$, respectively), suggesting sedative effects of the two compounds.

At the same time as the recording of walking time, the arm elevating times of mice were also recorded (Table 1). Unlike the walking time test, the arm elevating times of the mice treated with compounds I and II were not significantly different compared with the control group.

Coordinated movement

After spontaneous motility tests, the same administrations were continued for 3 days and an active avoidance retention test, slipping time on the glass plate, was conducted (Table 1). Results clearly show that the slipping times of the control groups were longer than those of groups treated with compounds I and II. The percentage increases in time induced by compounds I and II were 52% and 42%, respectively. The coordinated movement ability of the groups treated with compound I was significantly different compared with the control group ($P < 0.05$); treatment with compound II did not show any differences compared with the control group ($P > 0.05$).

Table 1 Effects of compounds I and II on spontaneous activity, arm elevating times, coordinated movement and sleeping time induced by suprathreshold sodium pentobarbital

Animal number	Walking time (s)			Arm elevating times			Slipping time (s)			Sleeping time (min)		
	Control	Compound I	Compound II	Control	Compound I	Compound II	Control	Compound I	Compound II	Control	Compound I	Compound II
1	62	26.36	67.28	20	27	7	3.14	0.94	0.71	95	144	108
2	108.25	18.21	26.33	21	6	10	1.22	2.03	0.45	122	90	103
3	51.3	15.28	25.3	15	31	18	0.32	1.56	2.13	103	108	117
4	77.25	38.89	12.14	8	21	13	4.07	0.51	2.02	86	102	124
5	50.74	20.38	42.9	11	15	8	3.19	0.83	1.54	69	159	92
6	63.4	9.42	89.17	26	17	6	2.36	0.7	2.88	56	89	104
7	55.94	35.07	36.25	6	8	28	0.68	0.78	0.69	92	108	103
8	91.28	18.19	20.26	12	5	11	3.6	1.82	1.83	141	117	156
9	42.5	20.58	18.61	11	12	9	1.93	2.11	0.4	78	131	183
10	82.92	12.3	54.32	30	16	11	3.55	0.36	1.32	79	110	114
Mean ± s.e.m.	68.56 ± 6.58	21.47 ± 2.99**	39.26 ± 7.75*	16.00 ± 2.51	15.80 ± 2.74†	6.54 ± 2.07†	2.41 ± 0.42	1.16 ± 0.21*	1.40 ± 0.26†	92.10 ± 7.93	115.80 ± 7.17*	121.30 ± 8.87*

Results are expressed as mean ± s.e.m. of sleep times. Compounds I and II were given at doses of 0.15 mL per 10 g per day; sodium pentobarbital was given at an intraperitoneal dose of 50 mg kg^{-1}. The number of animals in each group was 10. *$P < 0.05$, **$P < 0.01$, †$P > 0.05$ (one-way analysis of variance followed by Scheffe's test).

Mouse sleeping studies

Suprathreshold barbiturate sleeping time

An additional 30 mice were subdivided into three groups (10 animals in each group). At 30 min after the last administration, the test was conducted. Data were obtained in seconds and submitted to statistical analysis, but in order to simplify the visualization, the results were transformed into minutes in Table 1 and the individual data for all animals are presented. It can be concluded from the results that the mean sleep duration of the control group was 92.10 min, while the sleep duration was prolonged by 25% and 32% in the groups that were administered compounds I and II, respectively. Statistical analysis showed that groups treated with compounds I and II produced a significant increase in the hypnotic effect induced by pentobarbital ($P < 0.05$).

Subthreshold barbiturate sleeping time

The enhanced sleeping time may be due to interference with barbiturate enzymatic metabolism (Carvalho-Freitas & Costa 2002). In order to exclude this situation, a subthreshold sodium barbiturate test is necessary. After suprathreshold barbiturate (50 mg kg^{-1}) induced sleeping tests, animals in each of the groups were continuously treated with the same doses of compounds I and II for 12 days. At 30 min after the last administration, subthreshold sodium barbiturate (40 mg kg^{-1}) was injected. At 15 min after injection of sodium barbiturate, the animals in which the loss and regaining of the righting reflex continued for 1 min or more were considered positive. The number of sleeping animals was recorded. The percentage of animals falling asleep in the control group and groups treated with compound I and compound II were 0%, 30% and 20%, respectively.

Discussion

SZJ is a well known medicinal plant in Southeast Asia and its aqueous extracts have been traditionally used in China for the treatment of anxiety and insomnia (Li et al 1999; Wang et al 2003). The ethanolic extracts of SZJ exhibit anxiolytic effects at low doses and sedative effects at higher doses (Peng et al 2000). The plant was recommended in the earliest medicine monograph, Shen Nong's Herbal known as Classic on the Herbal (2500 years ago), for anxiety and insomnia (Zhang 2004). Its mechanism of action is by decreasing monoaminergic system activity (Hsieh et al 1986) and inhibiting the Glu-mediated excitatory signal pathway in hippocampus (Zhang et al 2003).

In recent years, using a single type of compound, such as flavonoids, saponins, oils or alkaloids, extracted from SZJ, some sedative and hypnotic effects have been reported. Although some authors suggest that flavonoids, alkaloids and oils of SZJ could be the potential active components in sedation and hypnosis (Yuan et al 1987; Wu et al 1993; Guo et al 1996; Cheng et al 2000), another viewpoint is that spinosins could be the major component of SZJ contributing its action on insomnia (Siekwoo 1978; Guo et al 1998; Peng et al 2000). The extract of total saponins from SZJ was used in animal tests and the hypnotic activity in mouse and rat was shown (Guo et al 1996).

To investigate the sedative and hypnotic effects of saponins of SZJ, we used silica gel column chromatography and TLC to further purify the crude extract of total saponins from SZJ. Using HPLC analysis, two single components of saponins, compounds I and II, were identified. A side-by-side comparison between the sedative and hypnotic effects of the two compounds of saponins was conducted. The sedative activity of both compounds was apparent in multiple assays in mice. Except for the effect on arm elevating, the inhibition of locomotor activity by both compounds in mice, reduced ability to maintain balance on a slippery glass plate, elongation of sodium pentobarbital induced sleeping time, and increased number of sleeping animals under the subthreshold dose of sodium barbital all suggest a consistent sedative profile. The activity of compounds I and II in the locomotor activity and passive avoidance tests in mice, both widely used as measures of sedative effects, may have resulted from the sedative effects of the compounds. The suprathreshold and subthreshold barbiturate induced sleeping tests are the basic methods for testing the hypnotic action of drugs. Both compounds increased the sleeping time and number of sleeping mice, suggesting that they have hypnotic activity.

In this study, we intended to isolate and purify the saponins from SZJ and then test the activity of the purified compounds using animal tests. If the compounds were found to have sedative and hypnotic activity, the next step would be the identification of their structures to determine which one of the known saponins from SZJ they belong to. Preliminary GC-MS analysis showed that the molecular weights of compounds I and II were 712 and 840, respectively, evidently lower than jujuboside A (1120), B (940), D (1070) or E (1236). Thus, further structural identification to the two compounds is necessary. After that, we aim to submit the purified compounds I and II to extensive in-vivo pharmacological studies, including dose–response patterns (mouse behavioural studies, delayed non-match to sample, Vogel test, pentobarbital sleeping time test) and combined animal tests with the two compounds, to estimate their potential as drugs.

In conclusion, two single components of saponins were isolated and identified from SZJ. Behavioural and sleeping tests showed that both compounds had sedative and hypnotic effects in mice. When assessed over all of the assays, the sedative and hypnotic effects of compound I was a little higher than that of compound II. The structures of the two compounds need to be determined by a combination of spectroscopic and chemical methods, and extensive pharmacological studies should be conducted on the two compounds in the future.

References

Cao, X., Ming, L., Li, J., Huang, R. R., Ding, T. (2005) Sedative and hypnotic effects of the fermentative broth of Cordyceps Sisnensi. *Acta Universitatis Medicinalis Anhui* **40**: 314–316

Capasso, A., Sorrentino, L. (2005) Pharmacological studies on the sedative and hypnotic effect of Kava kava and Passiflora extracts combination. *Phytomedicine* **12**: 39–45

Carvalho-Freitas, M. I. R., Costa, M. (2002) Anxiolytic and sedative effects of extracts and essential oil from *Citrus aurantium* L. *Biol. Pharm. Bull.* **25**: 1629–1633

Cheng, G., Bai, Y. J., Zhao, Y. Y., Tao, J., Lin, Y., Tu, G. Z., Ma, L. B., Liao, N., Xu, X. J. (2000) Flavonoids from *Ziziphus jujuba* Mill var. spinosa. *Tetrahedron* **56**: 8915–8920

Foster, A. C., Pelleymounter, M. A., Cullen, M. J., Lewis, D., Joppa, M., Chen, T. K., Bozigian, H. P., Gross, R. S., Gogas, K. R. (2004) In vivo pharmacological characterization of indiplon, a novel pyrazolopyrimidine sedative-hypnotic. *J. Pharmacol. Exp. Ther.* **311**: 547–559

Ganzera, M., Stuppner, H., Khan, I. A. (2004) Simultaneous determination of saponins and isoflavones in soybean (Glycine max L.) by reversed-phase liquid chromatography with evaporative light-scattering and ultraviolet detection. *J. AOAC Int.* **87**: 1189–1194

Gilani, A. H., Aziz, N., Khan, M. A., Shaheen, F., Jabeen, Q., Siddiqui, B. S., Herzig, J. W. (2000) Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of Lavandula stoechas L. *J. Ethnopharmacol.* **71**: 161–167

Guil-Guerrero, J. L., Campra-Madrid, P., Belarbi, E. H. (2000) γ-Linolenic acid purification from seed oil sources by argentated silica gel chromatography column. *Process Biochem.* **36**: 341–354

Guo, S., Fan, X., Song, S., Zhao, Q. (1996) Study on the effect of total saponins in Semen Ziziphus jujuba on the central inhibition. *J. Chin. Med. Mater.* **4**: 166–168

Guo, S., Fan, X., He, J. (1998) Effects of total flavonoids in Semen Ziziphus jujuba on the central inhibition. *J. Chin. Med. Mater.* **11**: 578–580

Higashida, H., Hossain, K. Z., Takahagi, H., Noda, M. (2002) Measurement of adenylyl cyclase by separating cyclic AMP on silica gel thin-layer chromatography. *Anal. Biochem.* **308**: 106–111

Hsieh, M. T., Chen, H. C., Kao, H. C., Shibuya, T. (1986) Suanzaorentang, an anxiolytic Chinese medicine, affects the central adrenergic and serotonergic systems in rats. *Proc. Natl. Sci. Counc. Repub. China B* **10**: 263–268

Jiang, J. G., Huang, X. J., Chen, J., Lin, Q. S. (2007) Comparison of the sedative and hypnotic effects of flavonoids and polysaccharides extracted from Semen Ziziphus jujube. *Nat. Prod. Res.* **21**: 310–320

Jung, M., Park, M., Lee, H. C., Kang, Y. H., Kang, E. S., Kim, S. K. (2006) Antidiabetic agents from medicinal plants. *Curr. Med. Chem.* **13**: 1203–1218

Khan, I. A., Clark, A. M., McChesney, J. D. (1997) Antifungal activity of a new triterpenoid glycoside from *Pithecellobium racemosum* (M). *Pharm. Res.* **14**: 358–361

Kim, Y. H., Lee, Y. G., Choi, K. J., Uchida, K., Suzuki, Y. (2001) Transglycosylation to Ginseng saponins by cyclomaltodextrin glucanotransferases. *Biosci. Biotechnol. Biochem.* **65**: 875–883

Li, H., Li, P., Yu, G. (1999) Advance of research and prospect of development of Zizyphus jujuba var. spinosa (Bunge) Hu ex H F Chow. *Chin. Wild Plant Resour.* **18**: 15–19

Li, L. M., Liao, X., Peng, S. L., Ding, L. S. (2005) Chemical constituents from the seeds of Ziziphus jujuba var. spinosa (Bunge) Hu. *J. Intergr. Plant Biol.* **47**: 494–498

Lu, Y. J., Zhou, J., Zhang, S. M., Zhang, H. Y., Zheng, X. X. (2005) Inhibitory effects of jujuboside A on EEG and hippocampal glutamate in hyperactive rat. *J. Zhejiang Univ. Sci. B* **6**: 265–271

Maddrey, W. C. (2005) Drug-induced hepatotoxicity. *J. Clin. Gastroenterol.* **39**: S83–S89

Oleszek, W., Bialy, Z. (2006) Chromatographic determination of plant saponins – an update (2002–2005). *J. Chromatogr. A* **1112**: 78–91

Peng, W. H., Hsieh, M. T., Lee, Y. S., Lin, Y. C., Liao, J. (2000) Anxiolytic effect of seed of Ziziphus jujuba in mouse models of anxiety. *J. Ethnopharmacol.* **72**: 435–441

Pieters, L., Vlietinck, A. J. (2005) Bioguided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds. *J. Ethnopharmacol.* **100**: 57–60

Sardesai, V. M. (2002) Herbal medicines: poisons or potions? *J. Lab. Clin. Med.* **139**: 343–348

Shibata, S., Ishihara, C., Matsumoto, K. (2004) Improved separation method for highly purified lutein from chlorella powder using jet mill and flash column chromatography on silica gel. *J. Agric. Food Chem.* **52**: 6283–6286

Siekwoo, W. (1978) The structure of spinosin (2″-*O*-β-glucosyl-swertisin) from Zizyphus Vulgaris Var. Spinosns. *Phytochemistry* **18**: 353

Simekova, M., Berek, D. (2005) Studies on high-performance size-exclusion chromatography of synthetic polymers I. Volume of silica gel column packing pores reduced by retained macromolecules. *J. Chromatogr. A* **1084**: 167–172

Soares, A. M, Ticli, F. K., Marcussi, S., Lourenco, M. V., Januario, A. H., Sampaio, S. V., Giglio, J. R., Lomonte, B., Pereira, P. S. (2005) Medicinal plants with inhibitory properties against snake venoms. *Curr. Med. Chem.* **12**: 2625–2641

Taylor, W. G., Fields, P. G., Sutherland, D. H. (2004) Insecticidal components from field pea extracts: soyasaponins and lysolecithins. *J. Agric. Food Chem.* **52**: 7484–7490

Wang, X. S., Kapoor, V., Smythe, G. A. (2003) Extraction and chromatography-mass spectrometric analysis of the active principles from selected Chinese herbs and other medicinal plants. *Am. J. Chin. Med.* **31**: 927–944

Wheatley, D. (2005) Medicinal plants for insomnia: a review of their pharmacology, efficacy and tolerability. *J. Psychopharmacol.* **19**: 414–421

Wu, S. X., Zhang, J. X., Xu, T., Li, L. F., Zhao, S. Y., Lan, M. Y. (1993) Effects of seeds, leaves and fruits of Ziziphus Spinosa and Jujuboside A on central nervous system function. *Zhongguo Zhong Yao Za Zhi* **18**: 685–687, 703–704

Yoo, H. H., Kwon, S. W., Park, J. H. (2006) The cytotoxic saponin from heat-processed *Achyranthes fauriei* roots. *Biol. Pharm. Bull.* **29**: 1053–1055

Yuan, C., Wang, Z., Jiao, Y., Cao, A., Huo, Y., Cui, C. (1987) Studies on the sedative and hypnotic constituents of flavonoids in Semen Ziziphi Spinosae. *Zhongguo Zhong Yao Za Zhi* **12**: 34–36

Zhang, M., Ning, G. M., Shou, C. H,. Lu, Y. J., Hong, D. H., Zheng, X. X. (2003) Inhibitory effect of jujuboside A on glutamate-mediated excitatory signal pathway in hippocampus. *Planta Medica* **69**: 692–695

Zhang, Z. J. (2004) Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Sci.* **75**: 1659–1699

Zhao, J., Li, S. P., Yang, F. Q., Li, P., Wang, Y. T. (2006) Simultaneous determination of saponins and fatty acids in Ziziphus jujuba (Suanzaoren) by high performance liquid chromatography-evaporative light scattering detection and pressurized liquid extraction. *J. Chromatogr. A* **1108**: 188–194

Zhao, Q., Wang, Q., Huang, J. (1995) Effects of oil of zizyphus jujuba mill seeds on central nervous system of mice. *J. Xi'an Med. Univ.* **16**: 432–435

Zivkovic-Radovanovic, V., Vuckovic, G. (2005) Poly(ethylene glycol) as impregnator for silica gel in salting-out thin-layer chromatography of some Co(III) complexes. *Chromatographia* **62**: 91–97