# Analysis of the main constituents of Changshu tablet and its spasmolysis effect against contraction induced by acetylcholine in the rat-isolated intestinal smooth muscle

**Ying An[1, 2], Guangming Song[2], Dailin Liu[1, 2]*, Tianyi Mao[1], Yanmin Zhao[1, 2] and Wenli Xie[2]**
[1]Tianjin Key Laboratory for Prevention and Control of Occupational and Environmental Hazard, Tianjin, PR China
[2]Logistics College of Chinese People's Armed Police Forces, Tianjin, PR China

**Abstract**: The Changshu tablet (CST), one kind of Chinese patent medicine with astringent to the intestine and relieving diarrhea, was made by the root of *Rose odorata* Sweet var. gigantean (Coll.et Hemsl.) Rehd.et Wils. Although CST has a long history of clinical application, but the research of its chemical composition is less. So the objective of this study was to investigate the main constituents and preliminarily research its effect of the contraction of isolated intestine in vitro. The contents of total polyphenols (126.23mg/g) and total triterpenoids (132.75mg/g) in CST were determined by ultraviolet spectrophotometry. Procyanidin B3, epigallo catechin, catechin, epicatechin, (-)-fisetinidol-(4α, 8)-(-)-catechin, (4α, 8)-(-)-fisetinidol-(-)-epicatechins and (+)-guibourtinidol-(4β, 8)-epicatechin were identified and determined by high performance liquid chromatography and their contents were distributed from 0.04mg/g to 1.46 mg/g. CST showed significant inhibitory effect against acetylcholine-induced contraction on the rat-isolated intestinal smooth muscle with a dose-dependent manner from 0.06 to 0.6mg/mL.. The maxim inhibition rates of CST on duodenum, jejunum, ileum and colon were 65.70±3.47%, 79.74±1.27%, 58.90±1.87% and 45.75±2.21% respectively. These results indicated that CST has a spasmolytic role in gastrointestinal motility which was probably mediated through inhibition of muscarinic receptors. All these findings promote the improvement of the quality control standard of CST and provide pharmacological foundation for clinical application of CST in gastrointestinal tract.

**Keyword**: Changshu tablet (CST), polyphenols, triterpenoids, intestinal smooth muscle, acetylcholine.

## INTRODUCTION

Changshu tablet (CST), a Chinese patent medicine with astringent to the intestine and relieving diarrhea, was made by the root of *Rose odorata Sweet var. gigantean* (Coll.et Hemsl.) Rehd. et Wils. The root of *Rose odorata Sweet var. gigantean* (Coll.et Hemsl.) Rehd.et Wils also called "Gu-Gong-Guo", is a common folk medicine of Yi People only distributed in southwest of China with a long history of indigenous use for treatment of enteritis and dysentery caused by large intestine damp and heat accumulation in traditional Chinese medicine theory (Wu, 2006). Previous chemical studies of our group have led to the isolation of some phenolic acids and triterpenoids and confirmed that polyphenols and triterpenoids were the main ingredients (Zhu, 2008; Ma, 2010). So our group has already studied the control of the active ingredient content of *Rose odorata Sweet var. gigantean* (Coll.et Hemsl.) Rehd. et Wils (Liu, *et al*., 2010a).

CST was processed using the extract of the roots of *Rose odorata Sweet var. gigantean* as the main raw material and used in the clinical treatment of diarrhea, enteritis, colitis and other gastrointestinal diseases (Zhu, *et al*., 2010). Its clinical effect is remarkable, especially for children diarrhea, its effect is more obvious. But the scientific study of CST that accumulates its effective parts or active ingredients was less. The insufficiency is that the quality control of CST is very simple, just only Yunnan pharmaceutical standards drafted in 1974 (Yunnan Provincial Health Bureau, 1974) and the improved State Drug Administration trial standards of CST (State Food and Drug Administration, 2002).

In order to clarify the material basis of CST and to explore the mechanism of its effect on gastrointestinal function, in this paper, we established the determination methods of contents of total polyphenols and total triterpenes, and identified seven monomer composition and labeled with its content using HPLC, which further promote the improvement of the quality control standards of CST. At the same time, the effects of CST against acetylcholine-induced contraction on the rat-isolated intestinal smooth muscle were observed with a dose-dependent manner ranging from 0.04 to 0.4mg/mL which further confirmed that CST has a spasmolytic role in gastrointestinal motility.

## MATERIALS AND METHODS

### *Material of CST*

Changshu tablet was provided by Yunnan Xiongye Pharmaceutical Co., Ltd. with a voucher number Z20025848. The voucher specimen was deposited at the Department of Natural Medicinal Chemistry in Logistics College of Chinese People's Armed Police Forces.

**Corresponding author:* e-mail: dailinlch@163.com

***Preparation for sample of CST***

The CST was grinded through a 100 mesh screen, then accurately weighed 50g powder, placed into a 50ml volumetric flask, dispersed with an appropriate amount of distilled water under ultrasonic 15min and then added distilled water to scale to obtain the sample solution (1mg/ml) for UV determination. 10mg of CST powders were accurately weighed, dissolved with DMSO to obtain 10mg/ml solution, and then filtrated with0.45μm micro porous membrane and collected the continued filtrate for HPLC detection. The sample for UV detection were further dispersed with distilled water to get six samples with different concentrations for pharmacological tests and thus ensuring the final concentration of the test were 0.06, 0.12, 0.24, 0.36, 0.48, 0.60mg/mL, respectively.

***Drugs and reagents***

Chromatographic-grade methanol, acetonitrile and analytically pure DMSO were purchased from Tianjin Concord Reagent Co., Ltd. The tested standard including gallic acid, ursolic acid, catechin, epigallocatechin and epicatechin were collected from Tianjin Chroma-standard Co., Ltd.. Other standards including (-)-fisetinidol-(4$\alpha$, 8)-(-)-catechin, (4$\alpha$,8)-(-)-fisetinidol-(-)-epicatechins and (+)-guibourtinidol-(4$\beta$,8)-epicatechin were made by our laboratory. The purity of each standard was higher than 95%. Acetylcholine chloride was from Shanghai Ziyi Reagent Factory (Shanghai, China). Potassium chloride, sodium chloride, calcium chloride, magnesium chloride, Sodium dihydrogen phosphate, Sodium bicarbonate, Sodium and glucose were purchased from Tianjin Chemical Reagent (Tianjin, China). Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh on the day of experiment.

Tyrode's salts solution (composition in mM): $CaCl_2$ 1.8, NaCl 136.9, $MgCl_2$ 1.1, KCl 2.7, $NaH_2PO_4$ 0.4, $NaHCO_3$ 11.9, and glucose 5.6.

***Animals***

Male Sprague Dawley rats (260±10g) (License No.SCXK (Jun)-2012-0004) were provided by the Laboratory Animal Center of Health Science, Peking University, Beijing, China. All the animals were housed in clean aluminium cages placed in a well-ventilated house conditions (temperature 23±2$^{o}$C, photoperiod 12h natural light, 12h dark and humidity 60±5%). Housed in poly-carbonate cages (4 or 5 animals per cage) with wood chips for bedding, the rats were allowed to feed and drink water but prior to experiment they were kept fasting for 24 hours. This study was carried out in accordance with the Regulation for the Administration of Affairs Concerning Experimental Animals (State Council of China, 1988).

***Quantitative determination of secondary plant metabolites***

The detected secondary metabolites of polyphenols and triterpenoids were quantified as described.

***Total polyphenols***

The contents of total phenolic were detected based on the method according to the literature (Liu, *et al*., 2012). Briefly, 1mL of the CST solution was added to 10mL distilled water and 2.5mL Folin-Ciocalteu phenol reagent in a 25mL volumetric flask. The mixture was left undisturbed at room temperature for 5 min after which 29% sodium carbonate was added to volume. The resulting complex was then read spectrophotometrically at 760nm after incubation for 2 h at room temperature. gallic acid was used as a standard for the calibration curve. The total polyphenols was calibrated using the linear equation based on the calibration curve. The contents of the total polyphenols were expressed as mg gallic acid equivalent/g dry weight.

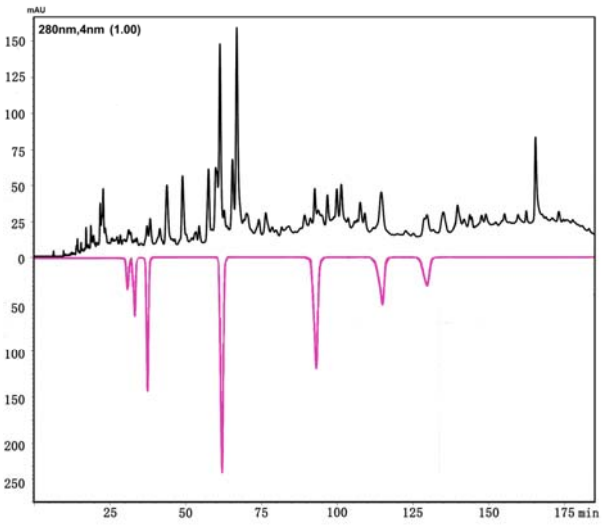


**Fig. 1**: HPLC Chromatogram of CST

***Total triterpenoids***

The content of total triterpenoids in CST was determined as described previously (Ragazzi and Veronese, 1973) with a slight modification. 0.2mL sample solution was put in a volumetric flask (10mL) and heated to evaporation in a water-bath, then added and mixed with 400μL 5% (W/V) vanillin-acetic solution and 1.4mL perchloric acid, incubated at 60°C for 25min. After being cooled in ice water bath, the reacted solution was diluted to 10mL with Methanol. The absorbance was determined at the maximum absorption at 550 nm against blank using a spectrophotometer. The blank consisted of all reagents and solvents without sample solution. The content was determined using the standard ursolic acid calibration curve and expressed as mg ursolic acid equivalent / g dry weight (DM).

***HPLC analysis of CST***

The phenolic ingredients in sample of CST were analyzed by HPLC-PAD. In detail, the HPLC system employed a Shimadzu (Shimadzu Corporation, Kyoto, Japan) LC-20A

binary HPLC pump separation module with an auto-injector and a Shimadzu SPD-M20A photodiode array detector. Separation was performed with a COSMOSIL 5C18-MS-Ⅱ(4.6mm ×250mm, 5μm) at 30°C with a gradient elution solution A, composed of formic acid–water solution (0.1% formic acid), and solution B, comprising acetonitrile and formic acid–water solution (0.1% formic acid) (6:4; v/v), which were delivered at a flow rate of 0.7mL/min as follows: 0 min, 96% (A); 9 min, 90% (A); 25 min, 90% (A); 40 min, 87% (A); 60 min, 85% (A); 75 min, 85% (A); 100 min, 81% (A); 130 min, 80% (A); 180 min, 70% (A); 195 min, 72%(A); 200 min, 90%(A)and 205 min, 0% (A). The UV spectra of every quantified peak were recorded between 190 and 600 nm. Phenolic compounds were detected in their maximum absorption wavelength, respectively.

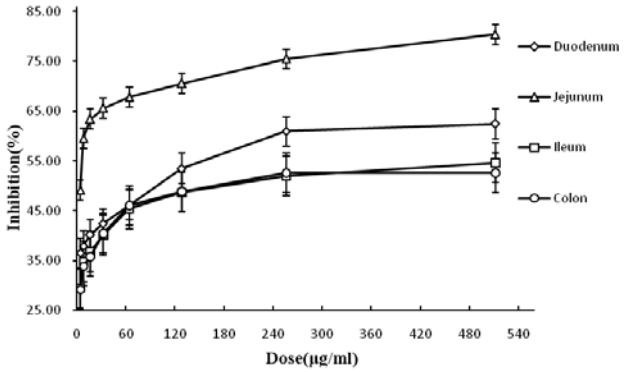


**Fig. 2**: Effects of different concentrations of CSP on the intestinal smooth muscle contraction of rat-isolated Duodenum, Jejunum, Ileum, Colon.

### *Effects of CST on the contraction of isolated intestinal smooth muscle*

To evaluate the effect of CST on the contraction of intestine, experiments were conducted using *in vitro* intestinal tissue from rat using previously described (Guo, *et al*., 2014). Rats fasted overnight for 24h were sacrificed by a blow on the back of the head and cervical dislocation. The intestine segments of rats were moved from duodenum, jejunum, ileum and colon in lengths of 2 cm and put in 0°C Tyrode's salts solution. Then the isolated intestinal segments were carefully washed with saline and put in the 25mL tissue organ bath containing Tyrode's salts solution, continually aerated with carbogen gas (95% $O_2$ and 5% $CO_2$) and maintained at 37±1°C. Intestinal segments were mounted under an initial load of 1.5g and started to exhibit uniform spontaneous rhythmic contractions after 20 min of equilibration in Tyrode's salts solution. Then the effects of CST on the spontaneous contraction were tested through adding the different concentrations of sample (0.06, 0.12, 0.24, 0.36, 0.48, 0.60mg/mL) into organ bath. At the same time, the effects of CST (0.06, 0.12, 0.24, 0.36, 0.48, 0.60mg/mL) on the longitudinal muscle contractions induced by pretreatment of intestinal smooth muscle strips with acetylcholine i.e. ($10^{-5}$M) were investigated. The experimental results were recorded on a computerized data processing system through an isometric force transducer (BiologyBL-410, China).

## STATISTICAL ANALYSIS

All experimental results were expressed as mean ± SD. Statistical significance between groups was analyzed using ANOVA followed by Dunnett's test and $p<0.05$ was considered to be statistically significant. Tests were performed using SPSS 19.0 system.

## RESULTS

### *Quantitative determination of secondary plant metabolites*

Preliminary quantitative determinations of CST revealed the presence of polyphenols and triterpenoids and showed the standard curve equations of gallic acid and ursolic acid were y=0.0194x-0.0697 ($R^2$=0.9993) and y= 0.1416x-0.039 ($R^2$=0.9988). The abundance of the secondary metabolites was in the order, triterpenoids ℘ polyphenols and their contents were 132.75 mg/g and 126.23 mg/g, respectively (table 1). The total concentration of polyphenols and triterpenoids was about 25.8% in the CST.

**Table 1**: Determination of polyphenols and triterpenoids in CST

| Contents (mg/g) | Polyphenols | Triterpenoids |
|---|---|---|
| | 126.23 | 132.75 |

In the present work, the polyphenols constituents of CST were isolated by HPLC (fig. 1.) and some peaks were firstly identified according to the retention times of standards. As shown in table 2, CST contained procyanidin B3 (1.04 mg/g) followed by epigallo catechin (0.49 mg/g), catechin (1.46 mg/g), epicatechin (0.03 mg/g), (-)-fisetinidol-(4$\alpha$,8)-(-)-catechin (0.71 mg/g), (4$\alpha$,8)-(-)-fisetinidol-(-)-epicatechins (0.04 mg/g) and (+)-guibourtinidol-(4$\beta$,8)-epicatechin (0.89 mg/g).

### *Effects of CST on isolated intestinal smooth muscle contractions*

CST concentration-dependently exhibited a spasmolysis effect on the rat intestinal smooth muscle *in vitro*, which can explain for its pharmacological effect. As shown in fig. 2 CST caused dose-dependent relaxations of spontaneously contraction rat duodenum, jejunum, ileum and colon with $EC_{50}$ value of 77.09, 19.99, 195 and 205 mg/mL and the maximum suppression ratios of duodenum, jejunum, ileum and colon are 62.30±0.85%, 69.41±1.50%, 53.46±1.17% and 52.32±1.05%.

**Table 2**: Standard curve equation and the contents of some compounds in CST

| Reference Name | Standard curve equation | $R^2$ | Content(mg/g) |
|---|---|---|---|
| Procyanidin B3 | y = 296220x - 4964.7 | 0.9996 | 1.04 |
| Epigallo catechin | y = 248534x - 5730.3 | 0.9999 | 0.49 |
| Catechin | y = 475346x + 69114 | 0.9994 | 1.46 |
| Epicatechin | y = 806963x + 273547 | 0.9996 | 0.03 |
| (-)-fisetinidol-(4α,8)-(-)-catechin | y = 403500x + 168992 | 0.9995 | 0.71 |
| (4α,8)-(-)-fisetinidol- (-)-epicatechins | y = 1,187,747x + 62,281 | 0.9996 | 0.04 |
| (+)-guibourtinidol-(4β,8)-epicatechin | y = 65786x + 611304 | 0.9994 | 0.89 |

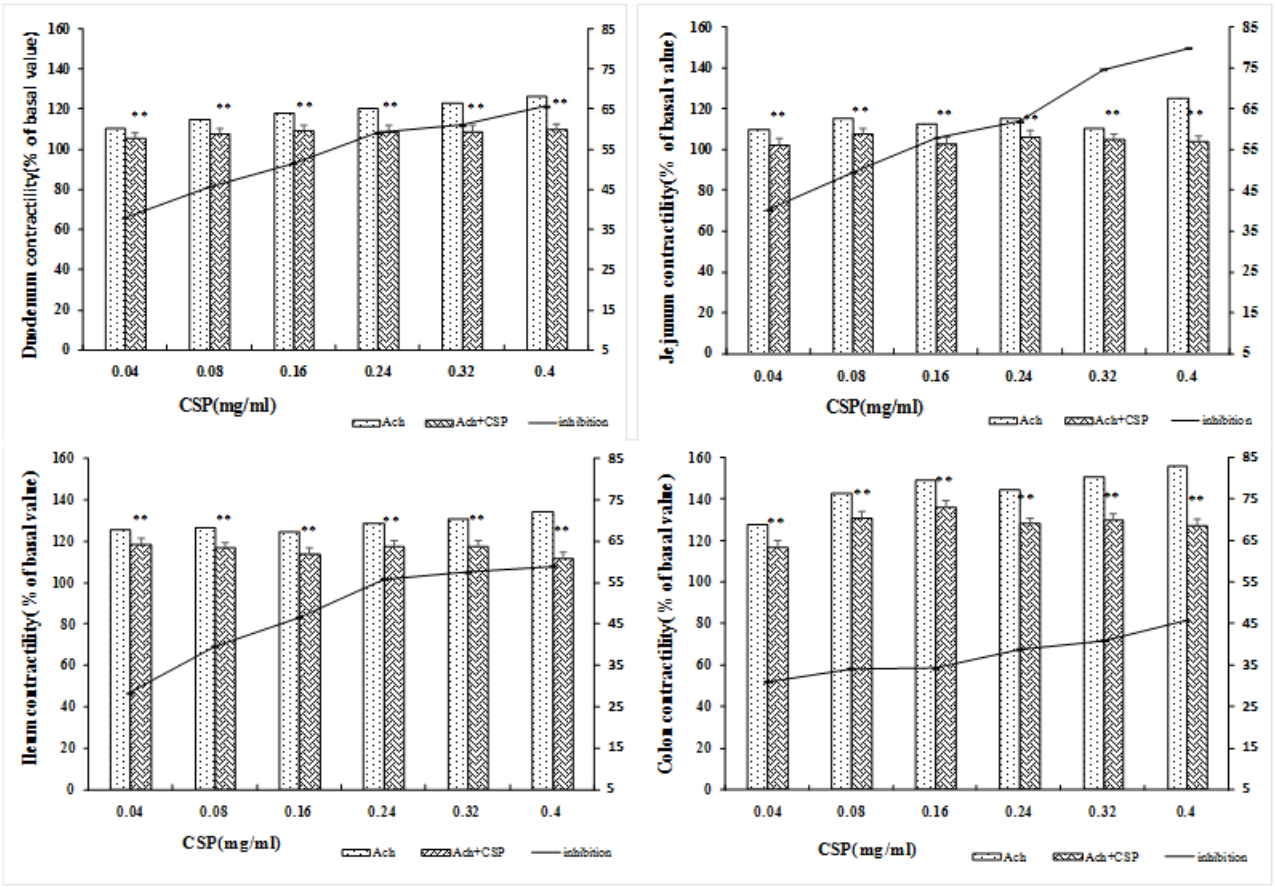


**Fig. 3**: Effects of different concentrations of CST on Ach-induced contraction of rat-isolated Duodenum, Jejunum, Ileum, Colon. The contraction induced by Ach in the absence of the extract acted as control. Results are mean ± SE, n=5. Significantly different from control. **p<0.01

Application of acetylcholine (Ach) to bathing medium of the isolated rat intestinal smooth muscle, at increasing doses greatly increased the contractions of the intestinal tissue. CST reduced the contractions induced by Ach ($10^{-5}$M) in dose-dependent (0.06~0.6mg/mL) manner. The contractility of duodenum, jejunum, ileum and colon are 65.70±3.47%, 79.74±1.27%, 58.90±1.87% and 45.75± 2.21%, on which CST has significant effect. CST has most obvious effect on the jejunum, duodenum followed by, and the worst effect on the colon (fig. 3.). Jejunum was the most sensitive part in which CST plays efficacy.

## DISCUSSION

People that customarily use plant (s) or plant-derived preparations consider the herbs to be effective against several diseases, including diarrhoea, without any scientific basis to substantiate or refute the potency/efficacy of such plants (Umer *et al*., 2013). Changshu tablet (CST) was one kind of selling finished drug in "Yi", made by the root of *Rose odorata Sweet var. gigantean* (Coll.et Hemsl.) Rehd.et Wils, which is astringent to the intestine and relieve diarrhea. In

traditional Chinese medicine theory, it can treat enteritis and dysentery caused by large intestine damp and heat accumulation. But there are not any scientific study that accumulates its effective parts or active ingredients at all. Therefore, in this study, an investigation into some of the secondary plant metabolites of CST as well as its spasmolysis effect as an antidiarrhoeal agent was carried out in chemically induced intestinal models *in vitro*.

From previous reports it was found that many triterpenoids have a good anti-inflammatory effect (Cheng and Xiong, 2007), some of them have been applied to the clinical, such as betulinic acid, ursolic acid and so on, while the natural phenolic acids such as tea polyphenols showed the remarkable activity of anti-diarrhea and repairing the role of intestinal mucosa (Oz *et al*., 2013). Some triterpenoids were isolated and determined from the roots of *Rose odorata Sweet var. gigantean* in the previously study of our group (Liu *et al*., 2010b) and they are mainly the derivatives of ursolic acid. But the research reports of phenolic acids are rare. So in this paper, seven polyphenols were firstly identified and detected from CST by comparison with the standard and they are the derivatives of cathine or its dimer. The investigation of the secondary plant metabolites indicated that triterpenoids and polyphenols are two main ingredients and may be the main effective constituents in CST.

At same time, we firstly explored the pharmacological effects of CST in the rat-isolated intestinal smooth muscle. CST dose-dependently showed a spasmolysis effect on the rat intestinal smooth muscle (duodenum, jejunum, ileum and colon) *in vitro*, significantly inhibited the contractions of the tissue induced by Ach and played the more sensitively effect in Jejunum. These results indicated that CST has a spasmolytic role in gastrointestinal motility which was probably mediated through inhibition of muscarinic receptors. All these findings provide pharmacological foundation for clinical application of CST in gastrointestinal tract.

At present, the polyphenols determined from CST mainly belongs to procyanidin. The previous reports (Namkung *et al*., 2010) indicated that procyanidin in green tea shows inhibition of the smooth muscle contraction in mouse ileum and colon. The structures of polyphenols in CST are different from these of procyanidin in green tea, but the difference is only in the number and location of hydroxyl and the basic skeleton is the same, so these polyphenols may also be the active ingredient of CST in the inhibition of intestinal smooth muscle contraction. Of course, there are some other active ingredients in CST need to be further developed.

## CONCLUSION

In conclusion, CST dose-dependently exhibited a spasmolysis effect and reduced the contractions induced by Ach on the isolated rat intestinal smooth muscle, which can explain for its pharmacological effect. Quantitative determination showed that polyphenols and triterpenoids were two main active ingredients and seven phenolic acids were identified and determined, which indicated polyphenols of CST contained many kinds of procyanindin that may be the active constituents of the inhibition of smooth muscle contraction. The clinical application of CST as "Yi" traditional medicine for the treatment of diarrhea is explained and supported by the pharmacological experimental results described in this paper.

## ACKNOWLEDGEMENT

We thank the Foundation (10JCYBJC14600) by Tianjin Natural Science Foundation of China for financial support. The work also supported by the Scientific Research Innovation Team Foundation of the Logistics University of CPAPF (WHTD201304).

## REFERENCES

Cheng XH and Xiong YX (2007). Advances in studies on pharmacological activities of pentacyclic triterpenoid saponins. *Chin Tradit Herbal Drugs*, **38**: 792-795.

Guo HM, Zhang JZ and Gao WY (2014). Anti-diarrhoeal activity of methanol extract of Santalum album L. in mice and gastrointestinal effect on the contraction of isolated jejunum in rats. *J Ethnopharmacol*, **154**: 704-710.

Liu DL, Ma R, Zhu SH, Bai SHF and Chen H (2010a). Method for identifying radix rosae gigantea extract. China Patent. ZL 2010105638823.9. Available at (http://www.pss-system.gov.cn/sipopublicsearch/ search/search/show View List. shtml)

Liu DL, Zhu SH, Ma R, Zhang JZ, Li HY and Chen H (2010b). Triterpenoids from the roots of Rosa odorata var. gigantea. *CJNM*, **8**: 12-15.

Liu ZHY, Bai SHF, Yu Q, Liu DL and Chen H (2012). Determination of total tannin in Changshu tablet by ultraviolet spectrophotometry. *Acta Academiae Medicinae PAPF*, **21**: 1-4.

Ma R (2010). Study on the bioactive constituents of the roots of Gugongguo. and its quality standard. Master's degree thesis of Shenyang Pharmacetuical University (ed.). Shenyang, China.

Namkung W, Thiagarajah JR, Phuan PW and Verkman AS (2010). Inhibition of $Ca2^{+}$-activated $Cl^{-}$ channels by gallotannins as a possible molecular basis for health benefits of red wine and green tea. *FASEB J*, **24**: 4178-4186.

Oz HS, Chen T and Villiers de WJ (2013). Green Tea Polyphenols and Sulfasalazine have Parallel Anti-Inflammatory Properties in Colitis Models. *Front Immunol.*, **31**: 132-137.

Ragazzi E and Veronese G (1973). Quantitative analysis of phenolics compounds after thin layer chromatographic separation. *J. Chromatogr. A*, **77**: 369-375.

State Food and drug administration (2002). National standard for traditional Chinese medicines assembly. (National standard upgraded from local standards of Chinese patent medicine, Department of internal medicine: the part of the spleen and stomach). *In*: State Food and drug administration (ed.). Beijing, China.

Umer S, Alemu T and Nigatu K (2013). Antidiarrhoeal and antimicrobial activity of Calpurniaaurea leaf extract. BMC Complementary and Alternative Medicine, **13**:21-22.

Wu ZHY (2006). The flora of Yunnan in China. In Science publisher (ed.). Beijing, China.

Yunnan Provincial Health Bureau (1974). Yunnan pharmaceutical standards. (Yunnan Q / WS 269-1974)

Zhu SH (2008). The bioactive constituents of the roots of Gugongguo. Master's degree thesis of Shenyang Pharmacetuical University (ed.). Shenyang, China.

Zhu SH, Liu DL, Wang SHP, Qiu F and Chen H (2010). Adsorption and desorption of total tannins from root of Rosa odorata var. gigantea by macro porous resin. *Chin Tradit Herbal Drugs,* **41**: 896-900.