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Research Article

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Characteristics of Pu'er tea compounded with the Chinese traditional food materials and its evaluation on cellular immune function *in vitro*

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ABSTRACT

Pu'er tea served as main ingredient in conjunction with extracts of chrysanthemum, bamboo leaves and tremella was developed to be pie-shaped compound Pu'er tea, compound Pu'er tea bags and instant compound Pu'er tea. The sensory evaluation of the compound Pu'er tea and its feedstock were carried out,and their quality and texture chemicals such as tea polyphenols, free amino acid , caffeine water-soluble saccharides, crude fiber and total ash was assayed. The hydroxyl radical scavenging rates of the compound pu'er tea reached levels of 47-70%.The splenocyte treated with instant compound Pu'er tea had significantly lower splenocyte proliferative responding to ConA ($P < 0.01$), which did not yet perform in a dose-dependent manner.

Keywords: compound Pu'er Tea, splenocyte proliferation, sensory evaluation, quality and texture chemicals

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INTRODUCTION

Part of the Camellia (Theaceae) family, Pu'er tea is a large leaf species of tea originating from Yunnan Province, China. It is well-known for its velvety taste, rufous color and fragrant smell [1,2]. It has thus become increasingly popular as a natural beverage. Following pile-fermentation and other key processes, the finished product can also provide significant physiological benefits. Numerous studies have shown that Pu'er tea can be highly beneficial in the prevention of cardiovascular disease[3-5]. The Pu'er tea industry in China has thus become increasingly successful in recent years with signs of unprecedented market growth.

In China, chrysanthemum flowers and bamboo leaves are widely used as everyday cooking ingredients and as elements of traditional Chinese medicinal materials. Both are listed under food and medicine in the official records of the Department of National Medical and Health Management, China. Tremella and tea are both widely enjoyed by Chinese people in popular daily food and drinks.

In this study, Pu'er tea was served as main ingredient in conjunction with extracts of chrysanthemum, bamboo leaves and tremella, and developed to be novel compound products. The sensory characteristic, physicochemical properties, antioxidant effects and cellular immune function of the Pu'er tea compounds were also investigated. This study provided a new method especially for the development of low-grade pu'er tea and was very important to raise its economic value added.

EXPERIMENTAL SECTION

2.1 Materials and reagents

The Pu'er tea, chrysanthemum, bamboo leaves, tremella and other raw materials were produced in Dehong autonomous prefecture, Yunnan province ,China. Luminol was also acquired (≥98%, Suzhou Industrial Park Subfamily Chemical Reagent Co., LTD). Fetal calf serum (FCS) was provided by Hangzhou Sijiqing Corp. (China).All reagents were analytically pure.

2. 2 Processing methods of compound Pu'er tea

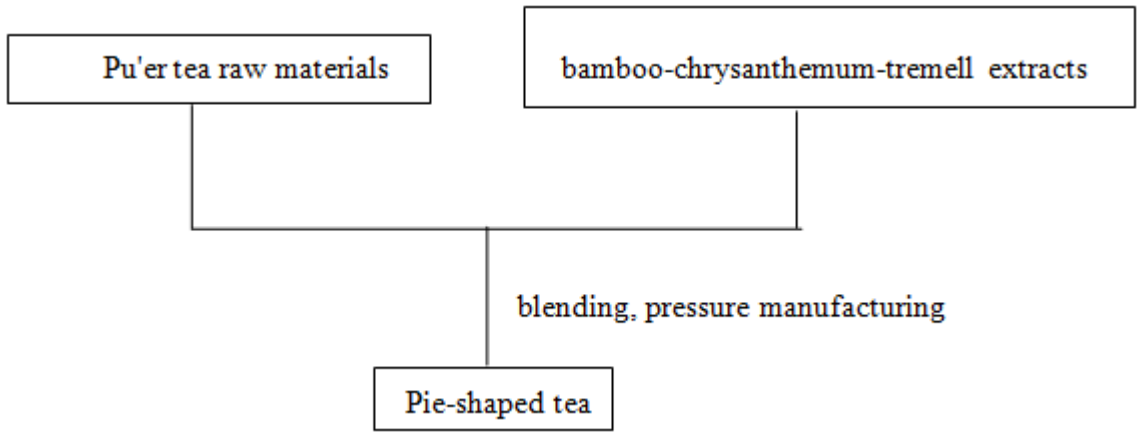
Pu'er tea was taken as the main ingredient; chrysanthemum, bamboo and tremella were taken as auxiliary materials. Based on the quality of the Pu'er tea, an orthogonal test was adopted to perform formulation and process optimization. The extracts of Pu'er tea, chrysanthemum, bamboo and tremella were prepared from the pharmaceutical company's GMP workshop for Chinese herbal medicine extraction.

Three forms of compound Pu'er tea were designed, i.e. pie-shaped tea, tea bags and instant tea. Their quality specifications were 5 g/bag, 2 g/bag and 0.5g/bag, respectively.

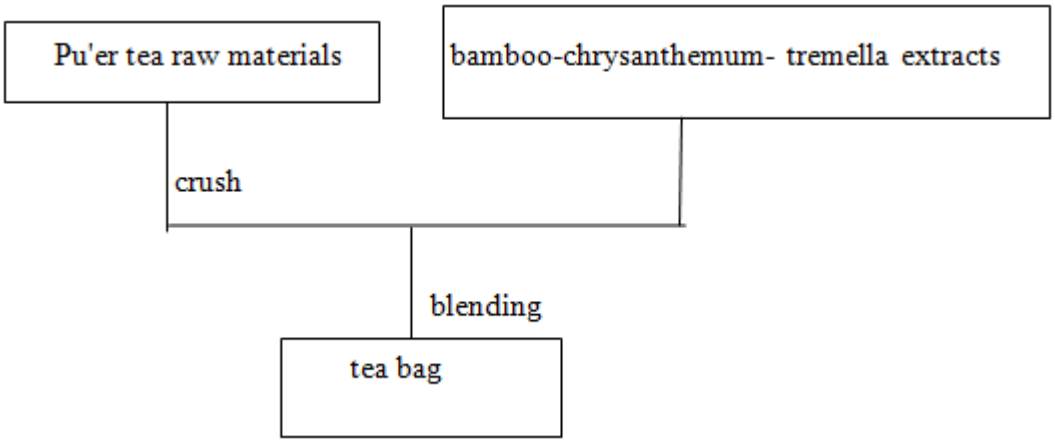
The processing of bamboo-chrysanthemum -tremella extracts:

chrysanthemum, bamboo and tremella→ sorting, rinse→ drying→ crush, extraction→ preliminary filtration→ fine filtration→ spray drying→ bamboo-chrysanthemum- tremella extracts

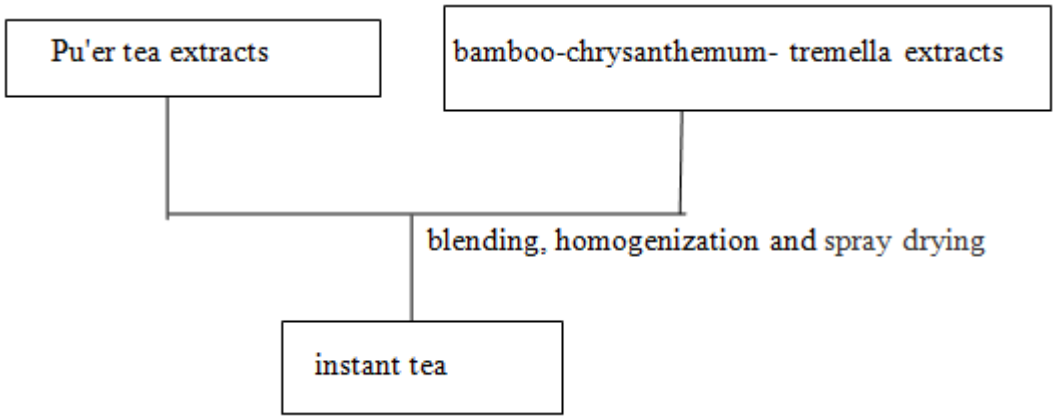
The processing of pie-shaped compound Pu'er tea:



The processing of compound Pu 'er tea bags:



The processing of instant compound Pu'er tea:
Pu'er tea extract → preliminary filtration → fine filtration → spray drying→ Pu'er tea extracts



2.3 Sensory evaluation of the compound Pu 'er tea and its feedstock
In accordance with the method on sensory evaluation of tea (GB/T23776-2009), the three varieties of compound Pu'er tea and its feedstock samples were professionally reviewed through a blind peer-review. 250 ml of boiling water was respectively added to 5 g of feedstock Pu'er tea and pie-shaped compound Pu'er tea, which were steeped for 5 minutes. 150 ml of boiling water was added to a compound Pu'er tea bag (2 g) which was steeped for 5 minutes. 150 ml of boiling water was respectively added to bamboo-chrysanthemum-tremella extracts (powder, 0.5g) and instant compound Pu'er tea (powder, 0.5g) which were steeped for 3 minutes. The aroma and taste quality of these tea samples were subsequently assessed.

2.4 Quality and texture chemicals in the compound Pu 'er tea and its feedstock
Polyphenols were detected in accordance with the national standard GB/T 8313-2008 method. Amino acids were also detected according to the GB/T 8314-2002 method. Water soluble polysaccharides were detected using the anthrone sulfuric acid method[6-8]. Caffeine was determined in accordance with the HPLC method of the national standard GB/T8312-2002. Water extracts were determined in accordance with the national standard GB/T8305-2002. The total nitrogen amount was determined through the Kjeldahl test. In addition, crude fiber was determined in accordance with the national standard GB/T8310-2002. The total ash content was determined according to the national standard GB/T8306-2002. Moisture content was determined according to the national standard GB/T8304-2002.

2.5 Hydroxyl radicals scavenging capacity of the compound Pu 'er tea
The scavenging capacity of hydroxyl radicals was determined through the flow injection chemiluminescence method[9-11]. The samples consisted of pie-shaped compound Pu'er tea, Compound Pu'er tea bags and instant tea compound Pu'er tea.

The weight of the samples was determined as 1.0 g (accurate to 0.0001 g). Then, 1:50 (m/v) boiling water was used in an extraction process for 15 min. The flask was shaken at 5 min intervals. Next, a suction filter was conducted while the liquid was hot. The filtrate was then diluted with distilled water to 100 ml after cooling to room

temperature. Around 100 μL of hydrogen peroxide (8.4%, v/v) was injected into the quantitative ring through a six-way valve. Then, the filtrate was mixed with the current-carrying phase of phosphate buffer solution (pH 7.4, 0.05 mol/L) containing Luminal (2. 0 x 10^{-4} mol/L), Fe^{2+}- EDTA (6.0 x 10^{-2} mol/L) and the sample. The mixture was subsequently taken into the photomultiplier tube. Chemiluminescence signals were then recorded. A control experiment was also performed using water to replace the test samples. The luminescent system (Fe^{2+} - H_2O_2 - Luminal system) was inhibited to assess the scavenging capacity. Meanwhile, a luminescent analysis was conducted using a IFFM-E flow injection chemiluminescence analyzer (Xi'an Ruimai Analysis Instrument Co., LTD.). The luminous inhibition rate (%) was also calculated using the following formula:

luminous inhibition rate (%) =(Fa-Fb)/Fa x100

Where Fa is the luminous intensity of control sample, and Fb is luminous intensity of the tested sample.

2.6 Splenocyte proliferation assay *in vitro*

2.6.1 Preparation of splenocytes suspension

Spleen of ICR mice was collected in Hank's balanced salt solution (HBSS, Sigma) under aseptic conditions. A homogeneous cell suspension was obtained from the minced spleen with its erythrocytes lysed by using ammonium chloride and then filtered through a steel mesh. The cells were washed twice with HBSS, collected and suspended with RPMI 1640 medium. When the survival ratio of splenocytes reached to above 95%, the concentration was diluted to 1×10^7cells /ml with the RPMI 1640 medium supplemented with 10% FCS, 12 mM HEPES (pH 7.1), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.05 mM 2-mercaptoethanol.

2.6.2 Splenocytes Proliferation

In the case of cell-based treatments, 100ul of Splenocytes were seeded into 96-well plates complemented with 50ul of ConA (final concentration of 2.5 μg/ml) .After the addition of 50ul of RPMI 1640 medium with the diverse concentrations of instant compound Pu'er tea (final concentration of 12.5， 25, 50, 100 and 200 μg/ml), a final volume of 200μl came into being.

The another treatment (100ul of Splenocytes,50ul of ConA and 50ul of RPMI 1640 medium without instant compound Pu'er tea) served as the normal control.

100ul of Splenocytes and 100ul of RPMI 1640 medium with the diverse concentrations of instant compound Pu 'er tea (final concentration of 12.5,25,50,100 and 200 μg/ml) were regarded as non-stimulated cultures.

The cells were incubated with 5% CO_2 at 37℃ in a humid atmosphere for 44 h， and then 50μl of MTT solution (2mg/ml) were added to each well, following by 4 h of incubation and the centrifugation of (1000rpm,10min). The supernate were discarded carefully by pipetting, 200μl of a DMSO working solution (192 μl DMSO with 8 μl 1 N HCl) was added to each well, and the absorbance at 570 nm was recorded in an ELISA reader after 15 min.

The stimulation index (SI) was calculated based on the following formula: SI = the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures.

2.6.3 Statistical analysis

All of the experimental data were analyzed by one-way ANOVA and Newman-Keul Multiple Comparison Test (Prism, GraphPad Software, San Diego, CA).

RESULTS

3.1 Sensory evaluation of compound Pu'er tea and its feedstock

The results of the sensory quality assessment of compound Pu'er tea and its raw materials are shown in Table 1. Bamboo-chrysanthemum-tremella extracts were shown to have a distinctively fresh and pungent taste, caramel aroma, deep brown-green color and soup-like texture. While in contrast, feedstock Pu'er tea had a typical aged aroma, alcoholic taste, red color and a thick soup-like texture.

The constructed compound of feedstock Pu'er tea with bamboo-chrysanthemum-tremella extracts showed obvious changes in taste and aroma. In contrast with the aged aroma of the original product, the samples had a fresh and pungent taste combined with a caramel aroma drawn from the bamboo-chrysanthemum-tremella extracts. The compound Pu'er tea bag had a pleasing aged aroma and sweet smell. Meanwhile, the instant compound Pu'er tea had a fresh and pungent taste with both caramel and aged aromas.The pie-shaped compound Pu'er tea had a caramel aroma, a touch of spiciness and a slight aged aroma. The compound Pu'er tea products were shown to maintain some

characteristics of taste and soup color of traditional Pu'er tea as well as embracing the smell of bamboo leaves, chrysanthemum and tremella.

3.2 Quality chemicals in compound Pu 'er tea and its feedstock

3.2.1 Tea polyphenols, free amino acid and caffeine

The polyphenol contents of pie-shaped compound Pu'er tea, compound Pu'er tea bags, feedstock Pu'er tea and bamboo-chrysanthemum-tremella extracts ranged between 7-11%. Instant compound tea reached 18%, which could be attributed to the concentration and enrichment in the extraction processes. Meanwhile, the polyphenol content of bamboo-chrysanthemum-tremella extracts could be ascribed to the flavonoid elements in bamboo leaves and chrysanthemum. Caffeine was not detected in bamboo-chrysanthemum-tremella extracts nevertheless 3-4% of caffeine existed in feedstock Pu ‘er tea, pie-shaped compound Pu'er tea and compound Pu'er tea bags. It reached levels of 6% in instant compound tea, which is roughly similar to levels in instant tea powder currently seen on the market. The contents of free amino acid in the three kinds of compound Pu'er tea were all about 0.5%, which were significantly lower than green tea, red tea and oolong tea[12]. This could be ascribed to the significant degradation of free amino acids especially for theanine during the pile fermentation process of Pu'er tea[13]**.**

3.2.2 Tea water-soluble saccharides

The contents of water-soluble saccharides in feedstock Pu'er tea and the compound Pu'er tea were around 3.0%, while higher levels were found in bamboo chrysanthemum- tremella extracts and instant compound Pu'er tea powder. The saccharides content of the former was largely as result of the tremella. However, the concentration and enrichment of the preparation processes were also contributory factors.

3.3 Texture chemicals in compound Pu'er tea and its feedstock

The texture chemicals in the compound Pu'er tea and its feedstock are shown in Table 3. The content of water extract in tea reflects the level of soluble matter, indicating the tea taste of thick or light. The content of water extract in the pie-shaped compound pu’er tea and the compound Pu'er tea bags were close to that of the feedstock Pu'er tea. The content of the total nitrogen and crude fiber reflected the tenderness of raw material to some extent; the former was positively related to the sample tenderness while the latter was negatively related. As shown in the results, there were no significant differences between the total nitrogen and crude fiber content of feedstock Pu'er tea and compound Pu'er tea. The total nitrogen content in bamboo-chrysanthemum-tremella extracts and instant compound Pu'er tea was largely a result of water-soluble proteins, which was lower than in the raw material, compound pu’er tea bags and pie-shaped compound tea; the latter also included water-insoluble protein.

The high levels of total ash content could be attributed to the crude old plant tissue. As a result of the enrichment in the extraction process, the ash content of instant compound Pu'er tea and bamboo-chrysanthemum-tremella extracts was significantly higher than in other kinds of compound Pu'er tea and feedstock Pu'er tea.

The ash content of the compound pu’er tea bags and pie-shaped compound pu’er tea showed similar levels and slightly higher than that of feedstock Pu'er tea, which could be attributed to the contribution from the ash content of bamboo-chrysanthemum-tremella extracts.

3.4 Hydroxyl radical scavenging capacity

The hydroxyl radical scavenging capacity can be accurately measured *in vitro* through the injection chemiluminescence method. The hydroxyl radical scavenging rates of the pie-shaped compound Pu’er tea, compound Pu'er tea bags and instant compound Pu'er tea reached levels of 48.82%, 47.38% and69.09%\, respectively. The strong scavenging capacity was attributed to the biochemical in Pu'er tea[12,13].

3.5 Effect of instant compound Pu'er tea on Splenocyte Proliferation

The effect of instant compound Pu'er tea on splenocyte proliferative responding to ConA stimulation are shown in Table 4. The splenocyte treated with instant compound Pu'er tea had significantly lower splenocyte proliferative responding to ConA than normal control group ($P < 0.01$), which did not perform in a dose-dependent manner. These results demonstrated that the instant compound Pu'er tea might have immunosuppression bioactivity.

The instant compound Pu'er tea, the compound Pu 'er tea bags and the pie-shaped compound Pu'er tea have the same material composition, differing only in product form. These varieties of compound Pu'er tea are probably suitable for the patients suffering from autoimmune diseases such as type I diabetes, rheumatoid, arthritis and lupus.Further research remains to be carried out.

DISCUSSION

Based on the theory of the Chinese traditional dietary therapy, Pu'er tea, chrysanthemum, bamboo leaves and tremella had been developed to be a compound product possessing a remarkable sensory characteristic of traditional Pu'er tea.

Pu'er tea has a significant bioactivity in hypolipidemic as well as tremella possessing hypoglycemic, antitumor and bamboo leaves having the prominent function in hypolipidemic and anti-fatigue [4,14-16]. Modern pharmacological studies have shown that chrysanthemums has the prevention of cardiovascular disease, antibacterial, anti-cancer, anti-aging activities, *et al.* [17-20].These compounded traditional food materials may have significantly multifaceted and synergistic effects.

In addition, the physicochemical property and sensory characteristics of raw materials and its products provided the guidance to quality control of the process. The research on the cellular immune function of compound Pu'er tea laid the foundation for its subsequent animal experiment on some activities such as immunoregulation, hypolipidemic, anti-aging *et al*. This study showed that the compound Pu'er tea had the potential to be health tea.

Table 1 the sensory evaluation of compound Pu'er tea and its feedstock

Index Samples	Shape	Aroma	Taste	Soup color
Feedstock Pu ‘er tea	tight, red brown, bearing gold vellus	thickly stale fiavour	thickly velvety, slight sweet	dark red ,bright
Bamboo-chrysanthemum-tremella extracts	brown-yellow powder	pungent, caramel aroma	slight pungent	dark green-brown, precipitate
Pie-shaped compound Pu 'er tea	small round pie-shaped, black brown	caramel aroma, Pungent, stale fiavour	thickly velvety, slight pungent	crimson
Compound Pu 'er tea bags	/	stale aroma, sweet incense	velvety, slight pungent, cleaning fiavour	crimson, bright
Instant compound Pu 'er tea	black-brown power	pungent as well as slight caramel aroma and slight stale fiavour	thickly velvety, sweet after taste	dark red, slight precipitate

Table 2 the quality chemicals of the compound Pu'er tea and its feedstock

	Feedstock Pu'er tea	Bamboo- chrysanthemum-tremella extracts	Instant compound Pu'er tea	Compound Pu er tea bags	Cake-type compound Pu'er tea
Polyphenols(%)	10.8	7.6	18.2	8.0	8.1
free amino acid (%)	0.5	0.4	0.6	0.5	0.4
water-soluble saccharides(%)	3.2	5.2	10.4	3.4	2.6
caffeine (%)	3.8	N.D.	6.5	3.3	3.0

N.D., not detected.

Table 3 the texture chemical composition of compound Pu 'er tea and its feedstock

	Feedstock Pu'er tea	Bamboo –chrysanthemum - tremella extracts	Instant compound Pu'er tea	Compound Pu'er tea bags	Pie-shaped compound Pu'er tea
water extract %)	39.7	/	/	33.4	39.1
total nitrogen(%)	5.1	2.9	3.7	5.0	4.9
crude fiber (%)	11.3	/	/	12.9	10.7
total ash(%)	7.4	24.2	20.2	9.2	8.9
moisture(%)	10.7	3.5	8.2	9.8	7.0

Table 4. Effect of instant compound Pu'er tea on ConA-stimulated splenocyte proliferation *in vitro*

Dosage (ug/ml) SI	12.5	25	50	100	200
instant compound Pu'er tea	1.36±0.05**	1.40±0.07**	1.33±0.06**	1.29±0.10**	1.30±0.06**
NC			1.81±0.17		

*Note：Compared with control group, * stand for P <0.05； ** stand for P <0.01. The treatment without instant compound Pu 'er tea) served as the normal control(NC).*

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REFERENCES

[1] JS Gong; WP Chen ;HJ Zhou;ZJ Dong; YF Zhang, *Journal of Tea Science*, **2007**,27,201~210

[2] DJ Yang ; LS Hwang, *J Chromatography A,*2**006**, 1119,277-284
[3] X Xu; M Yah ; Y Zhu, *Engineering in Life Sciences*, **2005**,**5**,382-386.
[4] KL Kuo;MS Weng; C Chiang, *J Agric Food Chem*., **2005**, 53, 480-489.
[5] HP Lv; JP Gu; Z Lin; L Guo; JF Tan ; QH Peng, *Journal of Tea Science*, **2007**,27, 8-18
[6] XQ Chen; Z Lin; Y Ye; JF Yin; YW Jiang ; HT Wan, *Carbohydrate Polymers*, **2010**, 82,28–33
[7] XQ Chen; Y Ye; H Cheng; YW Jiang ; YL Wu, *J Agric Food Chem*., **2009**,57, 5795–5798
[8] Singh R B, *J. Chem. Pharm. Res.*, **2012**, 4(8), 3765- 3768
[9] P Arulpriya ; P Lalitha, *J. Chem. Pharm. Res.,* **2012**, 4(2),1042-1047
[10] GJ Chandrashekar; M Gopal; D Vaigundan, *J. Chem. Pharm. Res.,***2011***,* 3(5), *340-347*
[11] AM AL-Mowali ; F M Al-Jabri1, *J. Chem. Pharm. Res.,* **2011**, 3(4),76-83
[12] XQ Chen; Y Ye; H Cheng; JF Yin ; C Sun, *Food research and development*, **2007**,28, 141-144
[13] GM She; XL Zhang; KK Chen; YJ Zhang ; CR Yang, *Acta Botanica Yunnanica* **2005**,27, 572-576
[14] GL Jie; Z Lin; LZ Zhang; HP lv; PM He ; BL Zhao, *J Agric Food Chem*., **2006**, 54,8058-8064.
[15] FF Chen ; DL Cai, *Journal of Chinese Integrative Medicine*,**2008**, 6, *862-866*
[16] Y Li; XL Liu; SP Pei; Y Shi ; DL Cai, *Chinese Journal of Clinical Nutrition*, **2005**,13,228-231
[17] J Mu; T Uehara; JZ Li; T Furuno, *Forestry Studies in China*, **2004**, 16,1-5
[18] MH Kweon; H J Hwang; H CSung, *J Agric Food Chem*., **2001**,49,4646 -4655
[19] QH Zhang; L Zhang, *Food and Drug*, **2007**, 9,60-63
[20] XQ Chen; YF Wang; YL Wu; BY Han; YJ Zhu; XL Tang ; QL Sun, *International Journal of Biological Macromolecules*, **2011**, 49,50–54