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Research Article

Development, Function Evaluation and Functional Constituent Determination of an Anti-alcoholic Beverage

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Abstract: An anti-alcoholic beverage was developed from traditional plant materials (Kudzu root, flower of kudzuvine, hovenia dulciis Thunb, licorice root) in this study. Orthogonal experimental design was applied to determine the optimal beverage formulation. The sensory qualities, microbial load, physicochemical properties, anti-alcoholic function and the functional constituents of the beverage were also evaluated. The animal experiment results showed that by giving the beverage 30 min before alcohol-drinking, the number of drunken rats decreased significantly by 20% ($p<0.05$) and the maintaining time of drunken rats was shortened significantly by 52.82% ($p<0.05$) compared with control. And the maintaining time could be shortened extremely significantly by about 32.70% ($p<0.01$) compared to control group by giving the beverage after rats getting drunk. Total flavonoid content (293.2 mg/L) and 6 flavonoids were determined qualitatively and quantitatively, puerarin (65.486 mg/L), tectoridin (24.866 mg/L) and myricetin (5.015 mg/L) were detected, which explained the anti-alcoholic function of the beverage.

Keywords: Anti-alcoholism, beverage, function evaluation, functional constituent

INTRODUCTION

Continuous development of new functional foods is the response of science and industry to the increased consumer awareness regarding health and the role of foods for improving quality of life (Bland and Medcalf, 1994; Blades, 2000; Verschuren, 2002; Angelov *et al*., 2006). In recent years, as people's consumption of alcoholic beverages increasing significantly, anti-alcoholic products, as a new type of functional food have drawn much attention because of their wide social and economic significance. It has been estimated that about 40% of Chinese population (about 500 million Chinese people), 74% of Russian population (about 111 million Russians) and 36% of American population (about 109 million Americans) drink alcoholic beverages on a regular basis and about 13.5% of Chinese population, 30% of Russian population and 6% of American population may be practicing unsafe drinking and/or suffering from alcohol-related diseases. Although moderate alcohol drinking is considered beneficial to human body, yet acute heavy alcohol drinking or chronic alcohol drinking might cause a number of adverse reactions and a large quantity of diseases, including mental disorder, polyneuropathy, cardiomyopathy, hypertension, haemorrhagic stroke, gastritis, liver cirrhosis and fibrosis, acute and chronic pancreatitis (Boffetta *et al*., 2006) and cancers (Boffetta and Hashibe, 2006), sometimes, might even cause death through respiratory and circulatory failure. Therefore, practices on relieving alcoholism after unsafe alcohol drinking has aroused much more attention lately.

Kudzu root, flower of kudzuvine and hovenia dulciis Thunb are traditional plant materials which were reported to have anti-alcoholic, anti-arrhythmic and hepatoprotective effects (Song *et al*., 1999; Niiho *et al*., 1989; Keung and Vallee, 1998; Lin and Li, 1998). Licorice root was found to have the effects of removing potential toxic substances and reconciling different medicine components. In ancient China, they have always been used together to counteract the problems associated with alcohol drinking, nowadays, they are very popular in the development of various anti-alcoholic products, the form of which was mainly focused on tablets, capsules and granule, of which the disadvantages are obvious because of the high cost, limited product forms, low consumer's acceptance and unpleasant taste and flavor etc. Thus, the advantages of developing anti-alcoholic beverage with them are distinct not only for its lower cost, more pleasant sensory experience, but also for its beverage form, which is an excellent medium for the addition of nutraceutical compoments for enrichment of the diet (Kuhn, 1998), besides, a beverage could also be

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consumed along with a typical meal, which could ehrich the nutritional value of the meal without altering a consumer's dietary habits (Temelli *et al*., 2004).

In the present study, Kudzu root, flower of kudzuvine, hovenia dulciis Thunb and licorice root were used to develop a 100% natural anti-alcoholic beverage. Orthogonal experimental design was applied to determine the optimal plant material formulation and beverage formulation. The sensory qualities, microbial load, physicochemical properties and the function of preventing drunkenness and relieving alcoholism of the beverage were evaluated and the qualitative and quantitative determination of the functional constituents of the beverage were also carried out, which might enrich the functional food market and provide a substantial basis for the further research on anti-alcoholic products development.

MATERIALS AND METHODS

Reagents and materials: Plant materials (Kudzu root, flower of kudzuvine, hovenia dulciis Thunb and licorice root) were purchased from a local herbal market in Beijing, China. Black bee honey was provided by Northeast Black Bee Development Co., Ltd., Heilongjiang, China. Pure water is obtained by filtering with 0.45 μm filtration membrane after the distilled water being purified with the Millipore purifier. Sucrose and citric acid were food grade and were obtained from Beijing Guangdahengyi Reagents Corporation. Chinese liquor (Red Star Erguotou brand, 52°) was purchased from a local supermarket, Beijing, China.

Preparation of anti-alcoholic beverage: Clean and free of mildew plant materials (Kudzu root, flower of kudzuvine, hovenia dulciis Thunb and licorice root) were selected as experimental materials. After removing the impurities, the raw materials were washed in tap water, drained for 5 min and then oven dried in an electro-thermostatic blast oven to 7% moisture individually. The dried materials were cooled for 10 min, each was milled into powder with a stainless steel Chinese herbal medicine mill and sieved through 60 mesh sieve. Kudzu root (10, 12, 14 g), flower of kudzuvine (4, 5, 6 g), hovenia dulciis Thunb (9, 10, 11 g) and licorice root (3, 5, 7 g) each was weighed accurately, mixed up according to the orthogonal experimental design and the extraction solution was prepared according to the method of Yi *et al*. (2004) with minor modifications. Material mixtures were cooked with ten times the amount of pure water at 70°C for 2 h twice to get the extraction solutions. The optimal extraction solution was determined by *in vitro* anti-alcoholism test according to the method of Zhang *et al*. (2008) with minor modifications. 20 mL of each extraction solution was blended with Chinese liquor at the ratio of 1:1 (V/V) in a 50 mL measuring cylinder. Control is the mixture of 20 mL pure water and Chinese liquor at the same ratio (V/V). After 5 min' standing, alcohol meter and thermometer were used to record the alcohol degrees of each blended solutions and the alcohol-relieving percentage could be counted accordingly.

Different concentrations of the optimal extraction solution (40, 50, 60%) was used to prepare beverage samples, black bee honey (5, 10, 15%), sucrose (2, 3, 4%) and citric acid (0.15, 0.2, 0.25%) were used to adjust the taste and flavor of the beverage by orthogonal experimental design, after centrifugation, beverage samples were pasteurized at 85°C for 30 min. the sterilized beverage samples were stored in 4°C refrigerator for further analysis.

Sensory evaluation: Sensory evaluation was carried out to determine the optimal beverage formulation according to the method of Verzera *et al*. (2008) with minor modifications. 20 judges were recruited from students and staffs of the institute. Candidates were submitted to preliminary tests to determine their sensory performance on basic tastes and flavor related to the beverage. A list of attributes was selected according to the frequency (%) of the terms used in beverage's sensory analysis sessions. The final set consisted of three attributes, separately referring to taste (four-point scale), outer appearance and color (three-point scale), flavor (three-point scale). All the judges were familiarized with the scoring scale and the sensory attributes to be evaluated during the training sessions. All evaluations were conducted from 10:00-12:00 a. m. in the morning in a sensory evaluation laboratory under normal lighting. 30 mL of each beverage sample was served at 23±1°C (room temperature) in glasses randomly labeled with a three-digit code and covered to prevent volatile loss. Each judge evaluated 9 beverage samples and was provided with water for mouth rinsing between different tests.

Physicochemical and microbiological analysis: Soluble solids were measured using a portable digital refractometer according to the determination method from Chinese National Standard (GB 12143.1-88). Titratable acidity (citric acid) was determined by titrating 10 mL beverage with 0.1 M NaOH to the phenolphthalein end point. pH was determined with a pH meter. Essential metals and toxic heavy metals (Pb, Cd, Hg, As) were determined with ICP-MS after beverage sample was diluted with 5% HNO_3 10 times. Total aerobic bacterial count was determined on pour plates containing nutrient agar with 2, 3, 5-Triphenyltetrazolium Chloride (TTC) indicator. The plates were incubated for 48 h at 37°C according to Chinese National Standard Method for aerobic bacterial count in food (GB 4789.2-94). Enterobacteriaceae count was determined on violet red bile glucose agar (VRVGA). Staphylococcus count was determined on

Mannitol Salt Agar (MSA). And mold counts were made on OCPDA according to the method of Kramer and Twing (1970).

Animals and experimental design:
Animals: Male Balb/c rats (inbred lines, 18-20 g) used in the studies were purchased from Vital River Laboratory Aminal Technology Co. Ltd. (Beijing, China), whose core animal groups were all from Charles River Laboratories in USA. The animal certificate No. is SCXK (J) 2012-0001. All animals were housed and acclimated in a specific pathogen free (SPF) laboratory conditions (22-25°C, 55-60% relative humidity and an automatic 12 h light-dark cycle) for 3 days before experiments were performed. Rats were allowed free access to food and drinking water during acclimation process.

Screening test of optimal gavage dose: After being deprived of food and drinking water for 12 h before each experiment, 48 Balb/c rats were randomly divided into 4 groups (12 rats per group) and treated beforehand: Control group (CG): rats were given sterile saline of the same quantity. Low dose group (LDG): rats were given 15.0 mg/g body weight beverage sample. Medium Dose Group (MDG): rats were given 25.0 mg/g body weight beverage sample. High Dose Group (HDG): rats were given 35.0 mg/g body weight beverage sample. 30 min after the above treatments, 0.15 mL per 10 g of body weight Chinese liquor (52°) were given to rats from each group. Disappearance of righting reflex was taken as the drunkenness indicator. The number of drunken rats, tolerance time and maintaining time were recorded to screen the optimal gavage dose of beverage sample.

Experiment of preventing drunkenness: After being deprived of food and drinking water for 12 h before experiments, 40 Balb/c rats were randomly divided into 2 groups (20 rats per group) and treated beforehand: Control Group (CG): Rats were given 35.0 mg/g body weight sterile saline. Beverage group (BG): rats were given 35.0 mg/g body weight beverage sample. 30 min later, 0.15 mL per 10 g body weight Chinese liquor (52°) were given to rats from each group. Disappearance of righting reflex was taken as the drunkenness indicator. The number of drunken rats, disappearance time of righting reflex and recovery time of righting reflex were recorded. The tolerance time and the maintaining time were calculated afterwards.

Tolerance time = Disappearance time of righting reflex-alcohol drinking time
Maintaining time = Recovery time of righting reflex-disappearance time of righting reflex

Experiment of relieving alcoholism: After being deprived of food and drinking water for 12 h before experiments, 40 Balb/c rats were randomly divided into 2 groups (20 rats per group): CG and BG. Rats from both groups were given 0.15 mL per 10 g body weight Chinese liquor (52°) first. After rats getting drunk (i.e., the disappearance of righting reflex), rats in CG were given 35.0 mg/g body weight sterile saline, rats in BG were given 35.0 mg/g body weight beverage sample. The maintaining time was recorded.

Acute toxicity test: After being fasted for 8 h before each experiment, 40 Balb/c rats were randomly divided into 2 groups (20 rats per group, half male and half female): Control Group (CG): rats were given sterile saline of the same quantity. Beverage Group (BG): rats were given o.4 mL/20 g body weight beverage sample each time, treated twice a day, which brought the maximum administration dosage to 40 g per Kg body weight. The toxicologic performance and body weight of the rats were recorded at regular intervals during the 14 days' observation.

Qualitative and quantitative determination of flavonoids:
Standards: Puerarin (CAS No. 3681-99-0) and tectoridin (CAS No.611-40-5) were purchased from Stru Chem Co. Ltd (Jiangsu, China). Quercetin (A0083), myricetin (A0048), kaempferol (A0129) and apigenin (A0113) were purchased from Beijing Century Ocote Biotechnology Co. Ltd. The purities of the 6 analytes were up to 98%. All standards were dissolved in 80% methanol to a concentration of 1 mg/mL and were stored in darkness at -20°C. All the standard solutions proved to be stable for over 3 months. The solvent of methanol and acetonitrile labeled as HPLC grade were purchased from Fisher Scientic (USA).

Preparation of samples: The diluted beverage samples ($V_{beverage}$:$V_{purified\ water}$ = 1:4) were filtered through a 0.45 μm filter for organic solvents (Xiboshi TPV1347 PVDF filter, Tianjin Fuji Science and Technology Co., Ltd. China) prior to the total flavonoid determination and the injection of 10 μL to HPLC analysis.

Determination of total flavonoids: Total flavonoids were determined according to the method of Lv *et al*. (2009) with minor modifications. 10 mg tectoridin was dissolved in 80% methanol solution to make a 0.2 mg/mL standard stock solution. 6 different volumes (0, 1, 2, 3, 4, 5 mL) of standard stock solution were separately put into 50 mL volumetric flasks and bring to volume by 80% methanol solution. After mixing well, absorbances (A) of standard solutions were determined at 265 nm to establish the calibration curve of flavonoid. To determine the total flavonoids of the beverage samples, the diluted beverage samples were scanned at 265 nm and the sample absorbance (A_1) could be obtained.

High-performance liquid chromatography: Chromatographic separations were performed on a Waters Symmetry C18 column (250×4.6 mm, 5 μm)

(Waters, USA). The column was placed in a column oven set at 25°C. The HPLC system consisted of Agilent (Agilent Technologies, USA) 1200 series pumping system, Agilent 1200 automatic injector furnished with a 100 μL loop, Agilent 1200 DAD detector set at 265 nm and 360 nm and system-provided chromatography data station software. Two solvents were used with a constant flow rate of 0.8 mL/min. Solvent A consisted of 100% acetonitrile, solvent B included 1% acetic acid solution. Both solvents used were of HPLC grade. The elution program was performed according to the method of Yao *et al*. (2010) with minor modifications: 0-35 min, 85-50% B; 35-36 min, 50-85% B; 36-40 min, 85% B.

Statistical treatment: Treatments in the experiment were repeated three times and samples were analyzed three times. Means and standard errors were analyzed using SPSS 19.0 software. Results were expressed as mean value±standard error. Analysis of Variance (ANOVA) was used to compare means to determine the levels of statistical significance ($p>0.05$, no significant difference; $0.01<p<0.05$, significant difference; $p<0.01$, extremely significant difference).

RESULTS AND DISCUSSION

Although the mechanism is not fully understood, flavonoids have been paid much attention in the anti-alcoholism effects of Kudzu root, flower of kudzuvine and hovenia dulciis Thunb. In previous research work, plant flavonoids were usually extracted by using organic solvents such as methanol, ethyl acetate, etc (Xiao *et al*., 2008; Shon *et al*., 2004). However, organic solvents always causes concerns for consumers because of their high toxic potential and solvent residue problems, especially nowadays, with food and pharmaceutical industries being challenged to detect and eliminate adulterants from foods and food ingredients including functional foods, dietary supplements, drugs and excipients (Griffiths *et al*.,

Table 1: The results of orthogonal test $L_9(3^4)$

Factors/Test numbers	A	B	C	D	Alcohol- relieving percentage (%)
1	1	1	1	1	34.62
2	1	2	2	2	32.21
3	1	3	3	3	34.43
4	2	1	2	3	36.56
5	2	2	3	1	40.25
6	2	3	1	2	48.65
7	3	1	3	2	36.54
8	3	2	1	3	40.27
9	3	3	2	1	36.60
K1	101.26	107.72	123.54	111.47	
K2	125.46	112.73	105.37	117.4	
K3	113.41	119.68	111.22	111.26	
m1	33.753	35.907	41.180	37.157	
m2	41.820	37.577	35.123	39.133	
m3	37.803	39.893	37.073	37.087	
R	8.067	3.986	6.057	2.046	
Excellent levels	A2	B3	C1	D2	
Factors priority	A>C>B>D	A>C>B>D	A>C>B>D	A>C>B>D	

Table 2: The results of orthogonal test $L_9(3^4)$

Factors/Test numbers	A	B	C	D	Sensory evaluation scores
1	1	1	1	1	7.9
2	1	2	2	2	8.4
3	1	3	3	3	7.8
4	2	1	2	3	8.1
5	2	2	3	1	9.3
6	2	3	1	2	8.5
7	3	1	3	2	7.6
8	3	2	1	3	8.0
9	3	3	2	1	7.5
K1	24.1	23.6	24.4	24.7	
K2	25.9	25.7	24	24.5	
K3	23.1	23.8	24.7	23.9	
m1	8.033	7.867	8.133	8.233	
m2	8.633	8.567	8.000	8.167	
m3	7.700	7.933	8.233	7.967	
R	0.933	0.700	0.233	0.266	
Excellent levels	A2	B2	C3	D1	
Factors priority	A>B>D>C	A>B>D>C	A>B>D>C	A>B>D>C	

2009), water as a "green" solvent has aroused much more interest in bioactive substances extraction because of their lower cost, free of toxicity and solvent residue, higher extraction efficiency and environmental compatibility.

Orthogonal experimental design is an experimental design method for multifactor and multilevel, it has always been used to optimize and determine the experimental conditions with fewer numbers of experiments according to the orthogonality of the multiple factors and levels. In this study, in vitro alcohol-relieving percentage was used to determine the optimal plant material formulation. After implementing the 9 experimental trials based on the L_9 (3^4) design, results were listed in Table 1. The results showed that the priority of the factors that affected the in vitro alcohol-relieving function of the extraction solution samples were A>C>B>D, the best level of each plant material was A_2, B_3, C_1 and D_2, separately, the optimal formulation was $A_2B_3C_1D_2$, which indicated that 12 g kudzu root, 6 g flower of kudzuvine, 9 g hovenia dulciis Thunb and 5 g licorice root had the strongest in vitro alcohol-relieving function.

The usages of extraction solution, honey, sucrose and citric acid were all important factors that might influence the sensory evaluation results and the final acceptability of the beverage. In this study, the optimization of beverage formulation was based on the sensory evaluation scores of different beverage samples, the results of L_9 (3^4) orthogonal design was listed in Table 2. The results showed that the priority of the factors that affected the sensory evaluation results were A>B>D>C. The best level of each component is A_2, B_2, C_3 and D_1, respectively and the optimal beverage formulation was $A_2B_2C_3D_1$, which indicated that beverage with 50% herb extraction solution, 10% honey, 4% sucrose and 0.15% citric acid had the best sensory evaluation result and the best sensory experience to consumers.

The physicochemical properties of the beverage were presented in Table 3. The results showed that the beverage contained 9% soluble solids, 0.10-0.15 g/100 mL titratable acid, had pH 4.0, which indicated that the physicochemical properties of the beverage could meet the quality requirement of liquid beverages.

Human body requires both metallic and non-metallic elements for healthy growth and development with certain permissible limits. The determination of these elements in beverage is thus of outermost important task (AL-Oud, 2003). To date, various studies have indicated the potential health implications of essential metals and toxic heavy metals in foods (AL-Oud, 2003). In this study, 9 essential metal elements and 4 toxic heavy metals in the beverage were analyzed with ICP-MS and the metal contents were listed in Table 3. The results showed that of all the essential metal elements in the beverage, the major constituents are K (854.743 mg/kg), Ca (286.011 mg/kg) and Mg (73.466 mg/kg), the minors are Na (9.132 mg/kg), Fe (2.860 mg/kg), Mn (1.368 mg/kg), Cu (0.185 mg/kg) and Zn (0.973 mg/kg) and the minimum was Sn (0.002 mg/kg), which indicated that the beverage could act as a beneficial supplement for nutrient metal elements in diet. Of all the toxic heavy metals detected in the beverage, the contents of Pb, Cd and As were separately 0.011 mg/kg, 0.001 mg/kg and 0.008 mg/kg and the content of Hg were too low to be detected using the available analytical technology, which was in accordance with the Limit Standard of Heavy Metals in Food (EC, No 629/2008) (Pb≤0.05 mg/kg, Cd≤0.05 mg/kg and Hg≤0.1 mg/kg) and also a good sign which indicated that the beverage was totally safe regarding to toxic heavy metals.

Table 3: Physicochemical and microbiological properties of the beverage

Parameter	Content in beverage
Soluble solids	9%
Titratable acid	0.10-0.15 g/100 mL
pH	4.0
Ca	286.011 mg/kg
Mg	73.466 mg/kg
K	854.741 mg/kg
Na	9.132 mg/kg
Fe	2.860 mg/kg
Mn	1.363 mg/kg
Cu	0.185 mg/kg
Zn	0.973 mg/kg
Sn	0.002 mg/kg
Pb	0.011 mg/kg
Cd	0.001 mg/kg
As	0.008 mg/kg
Hg	ND
Total aerobic bacteria	≤100 cfu/mL
Enterobacteriaceae	≤30 cfu/mL
Staphylococcus	0
Mold	0

Previous research pointed out that the varieties and contents of microorganisms were important causes of food safety issues, especially Enterobacteriaceae and Staphylococcus (Akubor, 2005). In this study, total aerobic bacteria, Enterobacteriaceae, Staphylococcus and molds were selected as microorganisms to be evaluated and the microbial load of the beverage was presented in Table 3. Results showed that total aerobic bacteria counts and Enterobacteriaceae counts were ≤100 cfu/mL and ≤30 cfu/mL, respectively, which were in accordance with the acceptable limits for foods by the International Commission on Microbiological Specifications for Foods (ICMSF, 1978) and Staphylococcus and molds were not detected. The low total aerobic bacterial counts indicated a satisfactory handling of the preparation of the beverage (Akubor, 2005). The absence of Staphylococcus and molds and the low number of Enterobacteriaceae suggested that there was no potential hazard of food spoilage and poisoning associated with beverage (Akubor, 2005).

The results for the screening test of optimal gavage dose of the beverage were presented in Table 4. The

Table 4: Results for screening test of optimal gavage dose (x±s)

Group	Gavage dose (g/kg)	Total number of rats	Number of drunken rats	Number of undrunken rats	Percentage of drunken rats (%)	Tolerance time (min)	Maintaining time (min)
CG		12	10	2	83%	41.8±24.5	140.4±46.7
LDG	15.0	12	7	5	53%	39.7±26.8	128.4±48.6
MDG	25.0	12	8	4	67%	43.3±17.2	86.9±54.5
HDG	35.0	12	5	8	42%	41.5±8.4	122.3±39.3

Table 5: Results for experiment of preventing drunkenness (x±s)

Group	Gavage dose (g/kg)	Total number of rats	Number of drunken rats	Tolerance time (min)	Maintaining time (min)
CG	35.0	20	12	41.8±24.5	443.4±74.5
BG	35.0	20	8	41.2±21.4	209.7±76.8*

*p<0.05

Table 6: Results for experiment of relieving alcoholism (x±s)

Group	Gavage dose (g/kg)	Total number of rats	Maintaining time (min)
CG	35.0	20	419.8±30.2
BG	35.0	20	282.9±47.7**

** p<0.01

results showed that when rats were given beverage samples of different doses, HDG had the minimum number of drunken rats compared with CG, which indicated that 35.0 mg/g body weight was the optimal gavage dose of the beverage. The tolerance time and maintaining time were not affected significantly by the gavage dose ($p<0.05$) in this experiment, which might because of the individual differences among different rats.

The results for the experiment of preventing drunkenness were presented in Table 5. The results showed that when 0.15 mL per 10 g body weight Chinese liquor (52°) was given to rats, 60% rats from CG got drunk, the average tolerance time was about 40 min and the average maintaining time was about 440 min. While the results for BG were totally different, for which the number of drunken rats decreased significantly by 20% ($p<0.05$) and the maintaining time of drunken rats was shortened significantly by 52.82% ($p<0.05$), which indicated that the beverage had significant function of preventing rats from getting drunk.

The results for the experiment of relieving alcoholism were presented in Table 6. The results showed that when rats from both groups got drunk after 0.15 mL/10 g body weight Chinese liquor (52°) was given, the average maintaining time of drunken rats from CG which was given 35.0 mg/g body weight sterile saline afterwards was about 420 min, while the average maintaining time of drunken rats from BG which was given 35.0 mg/g body weight beverage sample afterwards was about 280 min. The results indicated that the beverage could shorten the maintaining time by about 32.70% ($p<0.01$) compared to the CG, which demonstrated that the beverage had extremely significant function of relieving alcoholism after rats got drunk.

Besides, the toxicity of the beverage was also evaluated in a single-dose acute toxicity test and the body weight of the rats was recorded during the 14 days' observation (Fig. 1). The results showed that a dose up to 40 g/kg body weight was well tolerated by rats, no death or toxic side effects appeared during the 14 days' observation and no significant changes were observed in rats' body weight of BG compared to CG ($p<0.05$), which was far more than the dose limit (5 g/kg body weight) for actual non-toxic functional foods in China and demonstrated that the beverage was totally safe and free of toxicity.

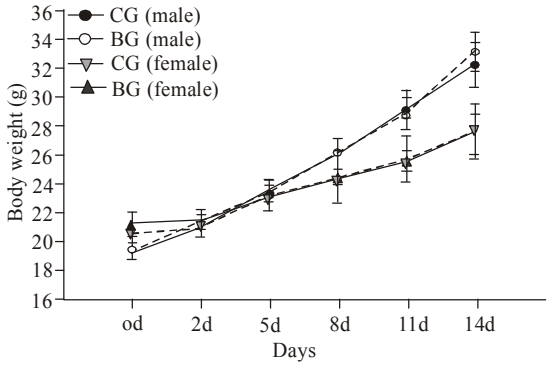

Fig. 1: Rats' body weight in acute toxicity test of the beverage

Kudzu root, flower of kudzuvine and hovenia dulciis Thunb are traditional plant materials which have been used widely for the treatment of alcoholism in ancient China. Their anti-alcoholism effects have also been documented in Compendium of Materia Medica and Thousand Golden Prescriptions. Recent studies showed that flavonoids in them are of great importance to lower blood alcohol concentration (Niiho *et al*., 1989), protect liver cell injury induced by alcohol (Keung and Vallee, 1998) and restrain central nervous disorders (Lin and Li, 1998). In this study, the qualitative and quantitative determination of flavonoids components and the total flavonoids determination were carried out separately.

The maximal absorbance wavelengths (λ_{max}) of the 6 analytes were analysed by scanning between 200 and

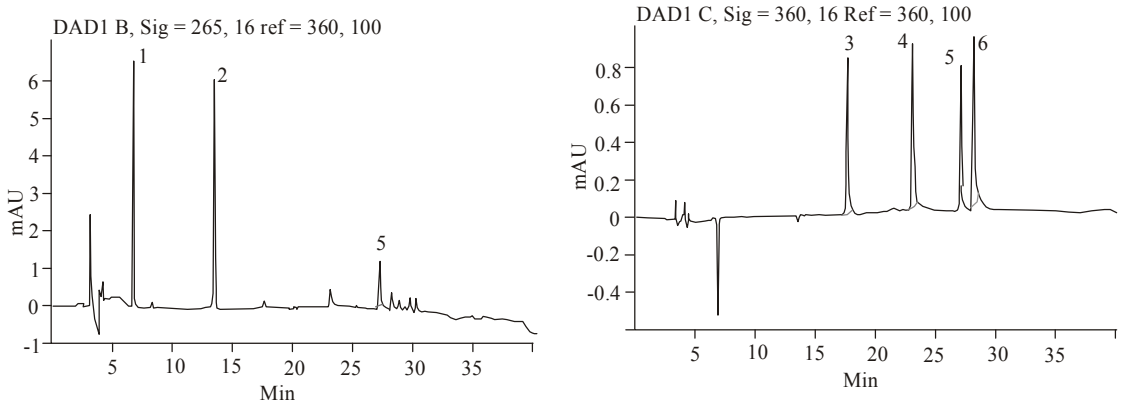

Fig. 2: Chromatogram of 6 flavonoids standards; (1): tectoridin; (2): puerarin; (3): myricetin; (4): quercetin; (5): apigenin; (6): kaempferol

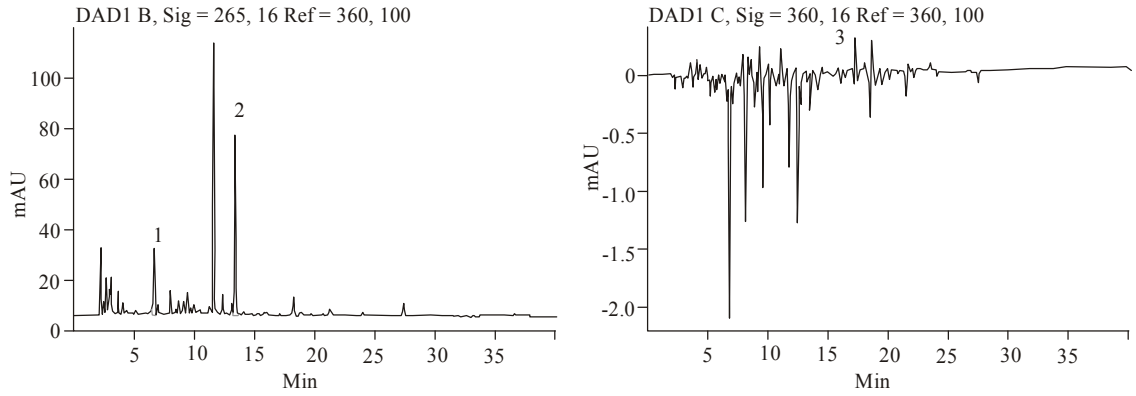

Fig. 3: Flavonoids compounds in beverage sample; (1): tectoridin; (2): puerarin; (3): myricetin

Table 7: Quantitative results of flavonoids by HPLC

Compound	Retention time (min)	Linear range (mg/L)	Calibration curve	Correlation coefficient (R)	Contents (mg/L)
Tectoridin	6.634	1-50	Y = 41.7795154X+7.6269668	0.99992	24.866
Puerarin	13.305	1-50	Y = 46.2650482X+7.7562813	0.99997	65.486
Myricetin	17.384	1-50	Y = 9.72930133X-2.0922879	0.99995	5.015

400 nm on the Agilent 1200 DAD detector in order to obtain the optimal detection wavelengths applied for the chromatography separation. Spectra chromatography showed that the absorbance peaks of the 6 analytes were different, puerarin, tectoridin and apigenin had the best or better absorbance peak at 265 nm, myricetin, kaempferol and quercetin had the best or better absorbance peak at 360 nm, therefore, the DAD detector was set at 265 nm and 360 nm in this method.

According to the chromatographic separation method described above, the chromatogram of the 6 standards was shown in Fig. 2 and the flavonoids compounds detected in beverage samples were shown in Fig. 3. To quantify the flavonoids compounds in the beverage, the calibration curve of them was established by injecting 5 different concentrations (1, 2, 5, 10, 50 mg/L) of the standard mixtures (Table 7), other results, such as retention time, linear range and correlation coefficient (R) were also listed in Table 7. The results showed that by the above mentioned HPLC method, the 6 flavonoids standards could be separated successfully, the major constituents of flavonoids in the beverage are puerarin (65.486 mg/L) and tectoridin (24.866 mg/L), the minor is myricetin (5.015 mg/L) and a good linear relationship could be observed between their peak area (Y) and concentration (X) in the range of 1-50 mg/L, while the other three flavonoids (quercetin, kaempferol, apigenin) were not detected in the beverage. And according to the above mentioned UV spectrophotometry method, the total flavonoids content in the beverage is 293.2 mg/L.

Puerarin, tectoridin, myricetin, quercetin, kaempferol and apigenin are all important flavonoids in nature. In recent years, their effects on anti-alcoholism and alcohol induced liver injuries have aroused much more interest because of their multiple physiological functions (antioxidant, antitumoral, anti-lipid peroxidation, etc.) (Gronbaek *et al*., 1995; Knekt *et al*.,

1997). Studies showed that puerarin from Kudzu root had the function of inhibiting the oxidative stress induced by acute alcoholism and protecting the acute alcoholic liver injury (Zhao *et al*., 2010), tectoridin from the flower of kudzuvine could protect against ethanol-induced liver steatosis mainly through ameliorating mitochondrial function (Xiong *et al*., 2010) and multiple mechanism interplay might explain the protective effect of quercetin, kaempferol and apigenin on liver injury induced by ethanol in rats (Chen, 2010; Tsai, 2010). In this study, we detected puerarin and tectoridin, which was in accordance with the previous results (Zhao *et al*., 2010; Xiong *et al*., 2010) and could explain the function of preventing drunkenness and relieving alcoholism of the beverage accordingly. Myricetin was also detected, which has great antioxidant function to protect liver tissues, where above 90% of the ethanol absorbed was metabolized. But quercetin, kaempferol and apigenin were not detected in the beverage, which was different from previous conclusions (Chen, 2010; Tsai, 2010), this might be related to the varieties and territory of the plant materials, the climate that the plants grows in and the processing method of the beverage.

CONCLUSION

In this study, an acceptable anti-alcoholic beverage was prepared from traditional plant materials. It could not only satisfy consumers' needs, but also enrich the market of functional foods with alternative anti-alcoholic products. The beverage was found to be microbiologically and toxicologically safe, physicochemically in line with the requirements of liquid beverage and could act as a beneficial supplement for nutrient metal elements. Kudzu root played the most important role as for the alcohol-relieving function of the plant material formulation, extraction solution usage contributed the most when it came to optimal beverage formulation. The beverage was confirmed to have anti-alcoholic functions by animal experiments, which could not only prevent rats from getting drunk, but also help to relieve alcoholism after rats got drunk. Puerarin, tectoridin and myricetin were determined to be the potential functional constituents of the beverage, but the anti-alcoholic mechanism of the beverage still needed to be studied further.

REFERENCES

Akubor, P.I., 2005. Production and quality evaluation of a no fermented beverage prepared from dehydrated plantain pulp. Eur. Food Res. Technol., 220: 152-155.

AL-Oud, S.S., 2003. Heavy metal contents in tea and herb leaves. Pak. J. Biol. Sci., 6(3): 208-212.

Angelov, A., V. Gotcheva, R. Kuncheva and T. Hristozova, 2006. Development of a new oat-based probiotic drink. Int. J. Food Microbiol., 112: 75-80.

Blades, M., 2000. Functional foods or nutraceuticals. Nutr. Food Sci., 30(2): 73-75.

Bland, J.S. and D.G. Medcalf, 1994. Future Prospects for Functional Food. In: Goldberg, I. (Ed.), Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals. Chapman and Hall, New York, pp: 537-551.

Boffetta, P. and M. Hashibe, 2006. Alcohol and cancer. Lancet Oncol., 7: 149-156.

Boffetta, P., M. Hashibe, C.L. Vecchia, W. Zatonski and J. Rehm, 2006. The burden of cancer attributable to alcohol drinking. Int. J. Cancer, 119: 884-887.

Chen, X., 2010. Protective effects of quercetin on liver injury induced by ethanol. Pharmacogn. Mag., 6(22): 135-141.

Griffiths, J.C., D.R. Abernethy, S. Schuber and R.L. Williams, 2009. Functional food ingredient quality: Opportunities to improve public health by compendial standardization. J. Funct. Food., 1: 128-130.

Gronbaek, M., A. Deis, T.A. Sorensen, U. Becker, P. Schnohr and G. Jensen, 1995. Motality associated with moderate intakes of wine, beer or spirits. Brit. Med. J., 310: 1165.

ICMSF, 1978. International Commission on Microbiological Specifications for Foods. I. Microorganisms in foods: Their significance and method of enumeration. 2nd Edn., University of Toronto Press, Toronto.

Keung, W.M. and B.L. Vallee, 1998. Kudzu root: An ancient Chinese source of modern antidipsortropic agents. Phytochemistry, 47(4): 499-506.

Knekt, P., R. Jarvinen, R. Seppanen, M. Heliovaara, L. Teppo, E. Pukkala and A. Aromaa, 1997. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am. J. Epidemiol., 146: 223-230.

Kramer, A. and B.A. Twing, 1970. Quality Control for the Food Industry. 3rd Edn., AVI, Westport, pp: 155-1205.

Kuhn, M.E., 1998. Functional food overdose? Food Process., 59: 21-30.

Lin, R.C. and T.K. Li, 1998. Effects of is flavones on alcohol pharmacokinetics and alcohol-drinking behavior in rats. Am. J. Clin. Nutr., 68(Suppl): 1512s-1515s.

Lv, G.H., J. Zhang and L. Li, 2009. Development of pueraria lobata beverage and determination of total flavonoids. Food Res. Dev., 30(8): 62-65.

Niiho, Y., T. Yamazaki, Y. Nakajima, H. Itoh, T. Takeshita and J. Kinjo, 1989. Pharmacological studies on Puerariae Flos. I. The effects of Puerariae Flos on alcoholic metabolism and spontaneous movement in mice. Yakuga. Zasshi, 109(6): 424-431.

Shon, M.Y., S.D. Choi, G.G. Kahng, S.H. Nam and N.J. Sung, 2004. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. Food Chem. Toxicol., 42: 659-666.

Song, H.P., J. Guan and Y.M. He, 1999. General situation of the pharmaceutical study and clinical application of pueraria. Shanghai J. Tradit. Chinese Med., 4: 47-49.

Temelli, F., C. Bansema and K. Stobbe, 2004. Development of an orange-flavored barley β-glucan beverage with added whey protein isolate. J. Food Sci., 69(7): 237-242.

Tsai, J.C., 2010. Hepatoprotective effect of scoparia dulcis on carbon tetrachloride induced acute liver injury in mice. Am. J. Chinese Med., 38(4): 761-775.

Verschuren, P.M., 2002. Summary report-functional foods: Scientific and global perspectives. Brit. J. Nutr., 88(2): 125-130.

Verzera, A., M. Ziino, A. Scacco, C.M. Lanza, A. Mazzaglia, V. Romeo and C. Condurso, 2008. Volatile compound and sensory analysis for the characterization of an Italian white wine from "Inzolia" grapes. Food Anal. Method., 1(2): 144-151.

Xiao, W.H., L.J. Han and B. Shi, 2008. Microwave-assisted extraction of flavonoids from Radix Astragali. Sep. Purif. Technol., 62(3): 614-618.

Xiong, Y., Y.Q. Yang, J. Yang, H.Y. Chai, Y. Li, J. Yang, Z.M. Jia and Z.R. Wang, 2010. Tectoridin, an isoflavone glycoside from the flower of pueraria lobata, prevents acute ethanol-induced liver steatosis in mice. Toxicology, 276: 64-72.

Yao, M.C., W.T. Liao, Y.M. Yuan and Y. Tang, 2010. Study on extraction process of isoflavone from Flos Puerariae Lobata. Chinese Wild Plant Resour., 29(6): 33-37.

Yi, J.P., X. Li and X. Wen, 2004. Development of herbal anti-alcoholism beverage and study on its anti-alcoholism effects. Food Res. Dev., 25(3): 112-114.

Zhang, Z., H.Y. Niu and H.L. Zhu, 2008. Study on alcohol detoxication beverage. Liquor Making, 35(2): 93-95.

Zhao, M., Y.Q. Du, L. Yuan and N.N. Wang, 2010. Protective effect of puerarin on acute alcoholic liver injury. Am. J. Chinese Med., 38(2): 241-249.