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Full Length Research Paper

Flavonoids from mulberry leaves by microwave-assisted extract and anti-fatigue activity

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Flavonoids from mulberry leaves (FML) were extracted using microwave-assisted extraction method (MAE). Several correlation factors were studied to optimise conditions at laboratory scale. The research results indicated that the optimal extraction parameters are as follows: ethanol concentration is 60%, material/solvent ratio is 1:15, microwave irradiation power is 560 W and irradiation time is 5 min. FML were tested in mice weight-loaded swimming model. Both high-dose and middle- dose of FML could obviously prolong the swimming endurance time, and had active effects on the serum blood urea nitrogen (BUN), hepatic glycogen and blood lactic acid levels in mice. The above results suggest that appropriate dose of FML possessed an anti-fatigue activity in mice.

Key words: Flavonoids, mulberry leaves, microwave-assisted extract, anti-fatigue.

INTRODUCTION

Morus alba L. (mulberry), a woody plant belonging to the genus Morus, family Moraceae, is a widely distributed plant in China, whose leaves, root bark and branches have long been used in Chinese medicine to treat fever, protect the liver, improve eyesight, facilitate discharge of urine and lower blood pressure (Chen and Li, 2007; Jia et al., 1999). The mulberry leaves are rich in flavonoids, alkaloids and polysaccharides components which are known as the most potently major active compounds by chemical constituent investigations (Wang et al., 2008). Among those, the flavonoids in mulberry leaves were contained rutin, quercetin, isoquercitrin and quercetin 3-(6-malonylglucoside) (Lee et al., 2007) . The health benefits of flavonoids are well known and are displayed as a remarkable range of biochemical and pharmaco-logical properties that may significantly affect the function of various mammalian cells (Middleton et al., 2000). The anti-inflammatory, antioxidant, antithrombotic and anticar-cinogenic effects of flavonoids are some of the properties that have been under consideration in view of therapeu-tical purposes for several human diseases (Snijman et al., 2007).

Flavonoids are thought to be one of the most critical constituents in mulberry that have therapeutic activity. But the anti- fatigue effects of flavonoids from mulberry leaves (FML) haven't been reported.

Conventional methods for the extraction of natural products from plant material, eg Soxhlet, liquid- liquid, and solid-liquid extractions are characterized by the con-sumption of large volumes of solvent and energy, lengthy extraction procedures, and the potentially deleterious degradation of labile compounds (Kerem et al., 2005). In recent years, new extraction techniques have been developed to reduce the volume of solvent needed for extraction (or to eliminate its use entirely), to reduce extraction and extract clean- up times, and to improve the reproducibility of compound recovery. These recent ex-traction techniques include accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), solid-phase microextraction (SPME), extraction with supercritical or subcritical water, and microwave-assisted extraction (MAE) (Barnabas et al., 1994; Buchholz and Pawliszyn, 1994; Hawthorne and Miller, 1994; Heemken et al., 1997; Llompart et al., 1997; Tian et al., 2008; Mandal et al., 2007). Most of these methods have similar pros and cons with regard to solvent volume, extraction time and extrac-tion efficiency. MAE is a process of using microwave energy to heat solvents and specific molecules in contact with a sample to enhance the extraction of soluble components contained therein. Its main advantages are reduced solvent volume and time consumption, and increased sample throughput (Shu et al., 2003). This method has been employed for the extraction of environ-

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mental matrices and natural products such as essential oils from plant materials (Chen and Spiro, 1995; Tu and Bi, 2005), glycyrrhizic from licorice root (Pan et al., 2000), taxans from Taxus biomass (Mattina et al., 1997), and azadirachtin-related limonoids from neem (Dai et al., 1999).

In the present study, the objectives were to develop a microwave-based method for the extraction of flavonoids from mulberry leaves (FML), and to determine whether the MAE extract exhibits anti-fatigue activity.

MATERIALS AND METHODS

Plant material

Mulberry leaves were collected during the summer season in Shandong Province, China. Identification of plant was verified by associate professor Jianlin wang, Dezhou University. Fresh, intact, viridity Mulberry leaves was picked to shade dried as experimental material.

Microwave assisted extraction (MAE)

Experiments were carried out in a domestic microwave oven (Set power: 280, 420, 560 and 700 W, Galanz Co., Guangdong, China). The shade dried Mulberry leaves was crushed in an electrical grinder and then powdered. Out of this powder, 100 g was mixed with ethanol solvents in suitable ratio. The suspension was radiated in microwave oven at regular intervals (one minute radiation and two minutes off) to keep temperature not rise above 100°C (Quan et al., 2006) . The extract was obtained which was filtrated by using the filter and was evaporated by using a rotary evaporator (RE52AA, Yalong Biochemical Instrument Co., Shanghai, China) under re-duced pressure at 40°C to get the flavonoids. It was stored at (0 - 4°C) until used.

Determination of total flavoniods content

The contents of flavonoids were determined by the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric assay and by reference to Rutin, and wavelenth in spectrophotometer (V-5100, Beijing Chenxiyong-chuang Science and Technology Co., Beijing, China) was set at 510 nm (Xu, 2007; Wang et al., 2004).

Selection of animals

Male mice of original Kun- ming strain (Dezhou University, Shandong, China), weighing 20 ± 2 g, which were housed in colony cages (eight mice per cage) at an ambient temperature of 25 ± 2°C with 12 h light and 12 h dark cycle. The mice were fed normal diets purchased commercially from vendors. The normal diets consists of corn starch 40%, bran 20%, soybean oil meal 20%, fish flour 14%, corn oil 2.0%, mineral mix 3.5%, salt 0.4%,Vitamin mix 0.1%. The animals were allowed to acclimatize to the laboratory environment for 1 week and then randomly divided into four groups equally based on body weight as given below:

i) Control group (CG) - mice administered water daily for 28 days;
ii) Low-dose group (LG) - mice administered FML (5 mg/kg) daily for 28 days;
iii) Middle-dose group (MG) - mice administered FML (10 mg/kg) daily for 28 days;
iv) High-dose group (HG) - mice administered FML (20 mg/kg) daily for 28 days.

All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee and had prior approval.

Anti-fatigue activity

The anti-fatigue activity of FML was evaluated by the weight-loaded swimming model in mice. The model was a reliable measure of anti-fatigue treatment as established in both laboratory animals and humans (Orlans, 1987; Lapvetelainen et al., 1997). Changes in the body weight of mice during initial, intermediate and terminal stages of the test, Swimming endurance time and corresponding biochemical parameters including blood lactate, serum blood urea nitrogen(BUN) and hepatic glycogen were observed (Technical Standards for Testing and Assessment of Health Food, Ministry of Health, PR China, 2003).

Swimming test was applied to the mice at the end of the 28 days. Mice were fasted for 12 h (over night) and FML was administered orally .The size of swimming pool was designed as 50 cm (length) ×50 cm (width) ×40 cm (depth), filled with fresh water at 30 ± 1°C.

Eight male mice were taken out from each group to make swimming endurance exercise. A lead block (5% of body weight) was loaded on the tail root of the mouse. The end point of the swimming endurance was taken as when the mouse remained at the bottom for more than 10 s (Shi et al., 2004; Xie et al., 2004; Li and Wen, 2006; Zhang et al., 2008; Qian et al., 2008). Swimming endurance time for each animal was recorded.

Eight male mice were taken out from each group for blood lactate analyses. A lead block (2% of body weight) was loaded on the tail root of the mouse. 20 µL of blood were collected from the veins of the tails of mice after the last administration of FML. Another 20 µL of blood samples were collected immediately after mice had been swimming for 30 min.

The levels of blood lactate were determined using a commercial diagnostic kit obtained from Jiancheng Diagnostic Systems (Nanjing, China).

Eight male mice were taken out from each group for hepatic glycogen and serum blood urea nitrogen (BUN) analyses. The mice made swimming exercise for 90 min without a load. After an hour's resting, the mice were killed to collect liver and plasma samples. The levels of hepatic glycogen and serum BUN were determined using commercial diagnostic kit obtained from Jiancheng Diagnostic Systems (Nanjing, China).

Statistical analysis

Data were assessed using SPSS program (version 15.0, SPSS Inc., Chicago, IL, USA). P values of < 0.05 between mean values were considered statistically significant.

RESULTS AND DISCUSSION

Effect of ethanol concentration on extraction yield of FML

Ethanol was employed in experiment because it is non-toxic and inexpensive solvent. Ethanol concentration is an important factor affecting extraction yield of flavonoids (Lan et al., 2007; Su et al., 2007), which was set at 20, 40, 60, 80 and 100%, respectively. Figure 1 shows the result of flavonoids extracted from leaves. When ethanol concentration increased from 20 - 60%, extraction yield

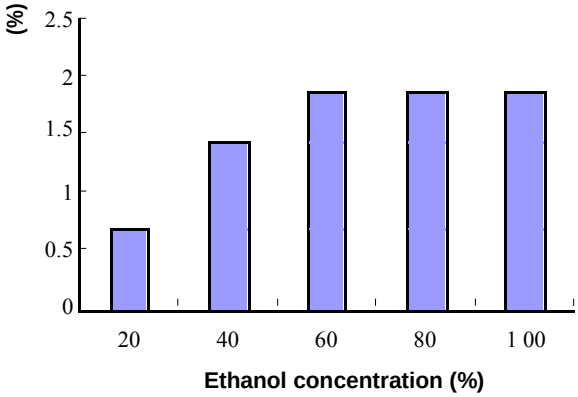

Figure 1. E ect of ethanol concentration on extraction yield of FML.

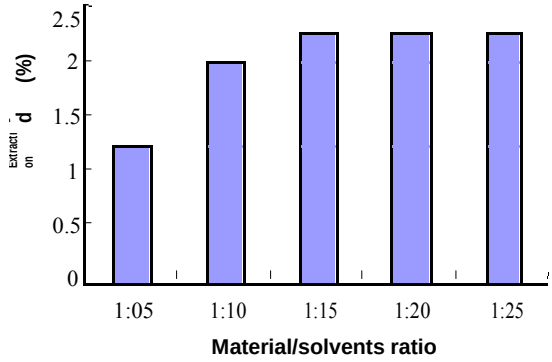

Figure 2. E ect of material/solvents ratio on extraction yield of FML.

increased significantly. Further increase of ethanol concentration 80% was only affected in slightly increase of extraction yield. In addition, when ethanol concentration increased from 80 - 100 %, however, extraction yield slightly decreased. It was in accordance with existed studies (Chen et al., 2008). Besides, the color of extracts deepened gradually when the volume of ethanol percentage was higher than 60%, which indicated that more undesirable compounds, primary chlorophyll, were also extracted. Therefore, it was decided to use ethanol 60% as extraction sovent in the following experiments.

Effect of material/solvents ratio on extraction yield of FML

Material/solvents ratio was another factor affecting extraction yield of flavonoids (Tang et al., 2005; Fu et al., 2008), which was set at 1:5, 1:10, 1:15, 1:20 and 1:25 respectively. As shown in Figure 2, extraction yield quickly increased with the increase of material/solvent ratio from 1:5 - 1:15, afterward, extraction yield slightly increased with the increase of material/solvent ratio from

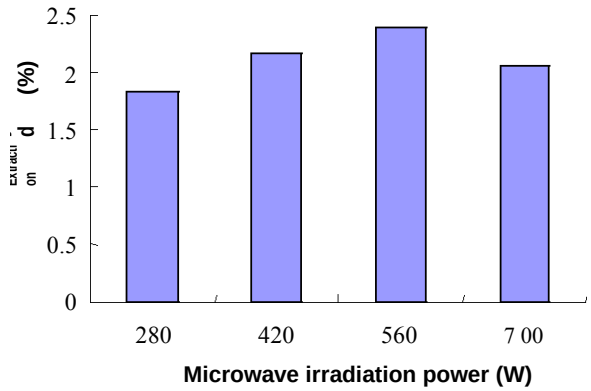

Figure 3. Effect of Microwave irradiation power on extraction yield of FML.

1:15 - 1:25. This is due to the fact that the increasing material/solvents ratio could decrease solution concentration difference inside and outside plant cells, which consequently prompted diffusion rate of solute particles and made more flavonoids molecules enter solution (Li et al., 2003). So using high ratio of material/ solvent could help achieve high extraction yield, however large amount of solvent resulted flavonoids content in the extract was unnecessary low. Therefore, material/solvent ratio of 1:15 was sufficient for the experiment.

Effect of microwave irradiation power and time on extraction yield of FML

Microwave irradiation power and time were another main parameter in the extraction procedure (Zhang et al., 2005). Extractions at different irradiation powers, such as 280, 420, 560 and 700W for 5 min, were tested. Results are listed in Figure 3. It was found that 560W is the optimal irradiation powder. Microwave irradiation time were investigated during 1, 2, 5, 10 min in 560 W, and the results is represented in Figure 4. Extraction yield was rapidly increased in the first 5 minutes of extraction. Further irradiation was only slightly increased extraction yield. Hence it was unnecessary to carry experiments with more than 5 min irradiation; 5 min is the optimal irradiation time.

Anti-fatigue activity of FML

Fatigue is the symptom, which indicated that the health is about or already subjected to harm (Zhang et al., 2008). Many reports have indicated that flavonoids can provide energy immediately during exercise and were helpful on extensive exercise (Cao et al., 2003; Yu et al., 2008). However, there is no information in detail concerning the anti-fatigue effect of FML.

In this study, the body weights of mice increased during

Table 1. Effects of FML on body weight of mice (mean ± SD, n = 24).

Group	Body weight (g)		
	Initial	Intermediate	Terminal
CG	19.45 ± 0.24	26.12 ± 0.45	30.04 ± 0.36
LG	20.04 ± 0.35	25.87 ± 0.39	30.54 ± 0.52
MG	20.42 ± 0.17	26.25 ± 0.51	29.77 ± 0.63
HG	19.76 ± 0.22	25.59 ± 0.34	30.16 ± 0.44

Table 2. Effect of FML on blood lactate, serum BUN and hepatic glycogen of mice (mean ± SD, n = 8).

Groups	blood lactate (mmol/L)		Serum BUN (mmol/L)	Hepatic glycogen (mg/g)
	Before swimming	After swimming		
CG	4.24 ± 0.51	11.23 ± 1.54	9.86 ± 1.13	7.54 ± 2.87
LG	4.53 ± 0.27	10.89 ± 1.14	9.34 ± 0.78	14.27 ± 3.62
MG	4.21 ± 0.65	8.15 ± 1.48	8.41 ± 1.06	18.46 ± 3.18
HG	4.50 ± 0.33	8.86 ± 1.74	8.13 ± 0.98	17.54 ± 4.02

*P < 0.05 as compared with CG.

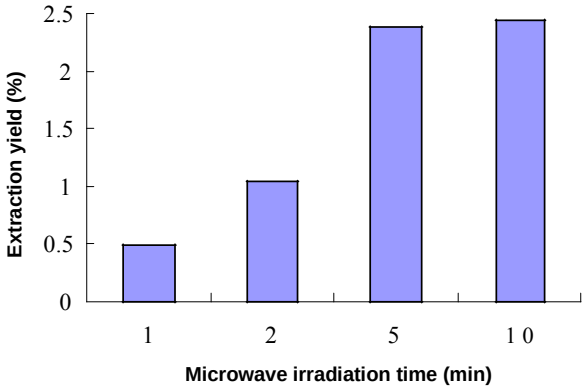

Figure 4. Effect of Microwave irradiation time on extraction yield of FML.

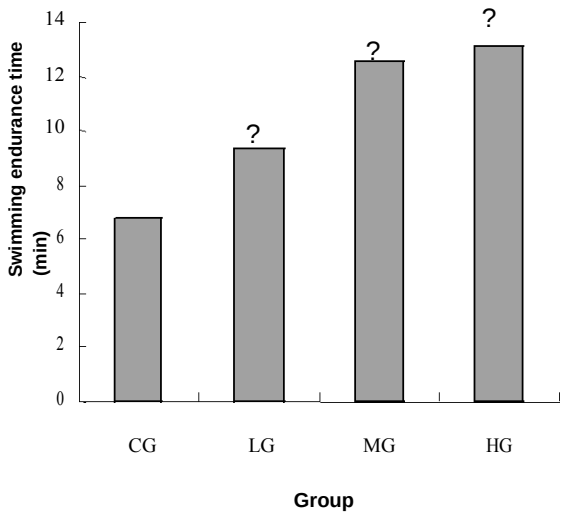

Figure 5. Effects of FML on swimming endurance time of mice (mean ± SD, n = 8).
*P < 0.05 as compared with CG.

the experimental period (28 days) is shown in Table 1. There was no significant difference in the body weights of mice in the three FML groups compared with CG during initial, intermediate and terminal stages in the experiment (p > 0.05).

Swimming endurance time of mice is shown in Figure 5. The average swimming time of mice of the treated groups were all remarkably longer than that of CG (P < 0.05). These results indicated that FML had significant effects on the endurance of mice in the experiment.

The levels of blood lactate, serum blood urea nitrogen (BUN) and hepatic glycogen of mice are shown in Table 2. These results revealed that treated groups reduced the serum BUN and blood lactate levels in mice compared with CG, and increased the hepatic glycogen level. The three biochemical parameters of MG and HG had significant difference (p < 0.05). Hepatic glycogen level of LG had significant difference (p < 0.05), but the other two biochemical parameters had the adverse results (p > 0.05).

Both high-dose and middle-dose of FML could obviously prolong the swimming endurance time, and had active effects on the serum BUN, hepatic glycogen and blood lactic acid levels in mice. It was considered that appropriate dose of FML possessed an anti-fatigue activity in mice.

Conclusions

Conditions of MAE method for extracting flavonoids from

mulberry leaves were studied and established. It can be concluded that the optimal extraction parameters are as followings: ethanol concentration is 60%, material/solvent ratio is 1:15, microwave irradiation power is 560 W and irradiation time is 5 min. Meanwhile, our results suggest that flavonoids from mulberry leaves (FML) had significant anti-fatigue effects. However further studies to clarify the detailed mechanisms involved in the anti-fatigue properties of FML are necessary.

REFERENCES

Barnabas IJ, Dean JR, Owen SP (1994). Supercritical-fluid extraction of analytes from environmental samples - a review. Analyst. 119: 2381-2394.

Buchholz KD, Pawliszyn J (1994). Optimization of solid-phase microextraction conditions for determination of phenols. Analytical Chemistry. 66: 160-167.

Cao WG, Liu ZQ, Shao Y, Tao YD (2003). A progress in pharmacological research of flavonoids. Acta Botanica Boreali-occidentalia Sinica. 23: 2241-2247.

Chen JJ, Li XG (2007). Hypolipidemic effect of flavonoids from mulberry leaves in triton WR-1339 induced hyperlipidemic mice. Asia Pacific J. .Clinical. Nutrition. 16: 290-294.

Chen SS, Spiro M (1995). Kinetics of microwave extraction of rosemary leaves in hexane, ethanol and a hexane + ethanol mixture. Flavour and Fragrance J. 10: 101-112.

Chen YH, Wang JP, Jiang HQ (2008). Optimization of Extraction Technology of Total Flavonoids from Mulberry Leaves by Orthogonal Design. Food and Drug. 3: 17-18.

Dai JM, Yaylayan VA, Raghavan GSV, Pare JR, Dai JM (1999). Extraction and colorimetric determination of azadirachtin-related limonoids in neem seed kernel. J. Agric. Food. Chem. 47: 3738-3742.

Fu WL, Yang LG, Sun GJ (2008). Microwave-assisted extraction of flavonoids from chrysanthemum. Food. Res. Dev. 29: 1-3.

Hawthorne SB, Miller DJ (1994). Direct comparison of Soxhlet and low-temperature and high-temperature supercritical CO2 extraction efficiencies of organics from environmental solids. Analytical Chem. 66: 4005-4012.

Heemken OP, Theobald N, Wenclawiak BW (1997). Comparison of ASE and SFE with Soxhlet, sonication, and methanolic saponification extractions for the determination of organic micropollutants in marine particulate matter. Analytical Chem. 69: 2171-2180.

Jia ZS, Tang MC, Wu JM (1999). The determination of flavonoid contents in mulverry and their scavenging effects on superoxide radicals. Food. Chem. 64: 555-559.

Kerem Z, German-Shashoua H, Yarden O (2005). Microwave-assisted extraction of bioactive saponins from chickpea (Cicer arietinum L). J. Sci. Food. Agric. 85: 406-412.

Lan DLXP (2007). Study on the Extracting Condition of the Total Flavonoid from Ipomoea batatas Lam leaves. Studies of trace elements and health. 1: 47-48.

Lapvetelainen T, Tiihonen A, Koskela P, Nevalainen T, Lindblom J, Kiraly K, Halonen P, Helminen HJ (1997). Training a large number of laboratory mice using running wheels and analyzing running behavior by use of a computer-assisted system, Lab. Anim. Sci. 4: 172-179.

Lee CY, Sim SM, Cheng HM (2007). Systemic absorption of antioxidants from mulberry (Morus alba L) leaf extracts using an in situ rat intestinal preparation. Nutr. Res. (N. Y., NY, U. S.) .27: 492-497.

Li MJ, You JY, Liu ZY, Zhang HQ (2003). Microwave-assisted extraction of flavonoids from flos sophorae. J. Guang Xi Normal University. 21: 103-104.

Li SF, Wen HB (2006). The Functionality of Hydrolysised Corn Protein Peptide-Study of Anti Fatigue. Food Sci. 27: 245-247.

Llompart MP, Lorenzo RA, Cela RF, Li K, Belanger JMR, Pare JRJ (1997). Evaluation of supercritical fluid extraction, microwave-assisted extraction and sonication in the determination of some phenolic compounds from various soil matrices. J. Chromatogr. 774: 243-251.

Mandal V, Mohan Y, Hemalatha S (2007). Microwave Assisted Extraction - An Innovative and Promising Extraction Tool for Medicinal Plant Research. Pharmacog. Rev. 1: 9-17.

Mattina MJI, Berger WAI , Denson CL (1997). Microwave-assisted extraction of taxanes from Taxus biomass. J. Agric. Food. Chem. 45: 4691-4692.

Middleton E, Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacol. ThRev. 52: 673-751.

Orlans FB (1987). Case studies of ethical dilemmas, Lab. Anim. Sci. Special Issue, pp. 59-64.

Pan X, Liu H, Jia G, Shu YY (2000). Microwave-assisted extraction of glycyrrhizic acid from licorice root. Biochem. Eng. J. 5: 173-177.

Qian LN, Chen P, Li XL, Xiao YY (2008). Study on the antifatigue function of total saponins from Panax japonicus. Chinese J. Hospital Pharm. 28: 1238-1240.

Quan PT, Hang TV, Ha NH, De NX (2006). Microwave-assisted extraction of polyphenols from fresh tea shoot. Sci. Technol. Dev. 9: 69-75.

Shi YL, Xin XL, Yang L, Cai DH (2004). Effect of Lentinan on Anti-Exhaustion and Liver Protection of Exhausted Mice. J. Jilin Agric. University. 26: 301-304.

Shu YY, Ko MY, Chang YS (2003). Microwave-assisted extraction of ginsenosides from ginseng root. Microchem. J. 74: 131-139.

Snijman PW, Swanevelder S, Joubert E (2007). The antimutagenic activity of the major flavonoids of rooibos (Aspalathus linearis): Some dose–response effects on mutagen activation–flavonoid interactions. Mutation Res. 631: 111-123.

Tang KW, Yi JM, Zhang YC (2005). Microwave-Assisted Process for Extraction of Flavonoid from Artemisia selengensis. Nat. Prod. Res.Dev. 17: 622-624.

Tian M, Yan HY, Row KH (2008). Extraction of Glycyrrhizic Acid and Glabridin from Licorice. Int. J. Mole. Sci. 9: 571-577.

Tu YS, Bi XL (2005). Application of Microwave - Assisted Extraction Technology to Study of Extraction of Traditional Chinese Medicine and Medicine from Natural Substances. World Science Technology-Modernization of Traditional Chinese Medicine. 3: 65-70.

Wang J, Wu FA, Zhao H, Liu L , Wu QS (2008). Isolation of flavonoids from mulberry (Morus alba L.) leaves with macroporous resins, African J. Biotechnol. 7: 2147-2155.

Wang QA, Zhou B, Shan Y. (2004). Progress on antioxidant activation and extracting technology of flavonoids. Chem. Product. Technol. 11: 29-33.

Xie W, Yang YL, Zheng HY (2004). Experiment Research Into the Influence of EGb on the Anti-Movement-Fatigue Ability of Small Mice. J.l Shandong. Physical. Education. Institute. 20: 51-53.

Xu G (2007). Studies on the extracting and antioxidant activities of flavonoids in sweet potatoes. J. Food. Sci. Biotechnol. 26: 22-27.

Yu FR, Feng ST, Xie MR (2008). Anti-fatigue effect of cynomorium songaricum flavone on old rats. Chinese J. Rehabilitation. Theory Pract. 14: 1141-1143.

Zhang F, Chen B, Xiao S, Yao SZ (2005). Optimization and comparison of different extraction techniques for sanguinarine and chelerythrine in fruits of Macleaya cordata (Willd) R. Br.. Separation Purification Technol. 42: 283-290.

Zhang GY, Zhao HJ, Xin ZJ, Qu JL, Wang BG, Wang H (2008). Effect of maple syrup on fatigue resistance in mice. J. Xi an Jiaotong University (Medical Sci.). 29: 644-646.