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Full Length Research Paper

Purification and anti-fatigue activity of flavonoids from corn silk

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Flavonoids are major active ingredient in corn silk (CS) and possess various pharmacological activities. In this study, microporous resin adsorption technology was used for obtaining purified flavonoids from corn silk (FCS) and its anti-fatigue activity was evaluated. Eight kinds of macroporous resins with different properties were tested through static adsorption and one macroporous resin labeled as AB-8 was selected. Some important parameters were optimized for most effective enrichment and preparative separation. As follows: 1. for adsorption: sample solution FCS concentration was 2.636 mg/ml; processing volume was 4 BV; flow rate was 2 BV/h; temperature was 25°C; 2. for desorption: elution solvent ethanol-water (90%, v/v) solution was 4BV; flow rate was 1.0 BV/h. Flavonoids with content of 63.15% was obtained with a flavonoid recovery of 94.27% in the purification process. The results showed that AB-8 resin revealed a good ability to purify FCS. Swimming exercise results indicated that FCS had anti-fatigue activity of mice by inhibiting the production of blood lactic acid, retarding the formation of BUN and increasing hepatic glycogen concentration.

Key words: Macroporous resins, purification, anti-fatigue activity, corn silk.

INTRODUCTION

The style of Zee mays is commonly known as corn silk (CS) and has been used for thousands of years as a folk medicine in many parts of the world for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis, diabetes mellitus and prostatitis (Grase et al., 1993; Velazquez et al., 2005; Ebrahimzadeh et al., 2008; Li and Yu, 2009). CS contains proteins, vitamins, carbohydrates, Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} salts, volatile oils and steroids such as sitosterol and stigmasterol, alkaloids, saponins, tanninsandflavonoids (Ebrahimzadeh et al., 2008). There are indications that utilization and acceptance tendency towards folk medicines to give relief and treat human ailments are globally very positive although there are side effects (Ramesh and Okigbo, 2008).

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Abbreviation: CS, corn silk; **FCS,** flavonoids from corn silk.

Flavonoids constitute a large group of secondary plant metabolites that are ubiquitous among higher plants. They are polyphenolic compounds which generally occur as glycosylated derivatives and have been shown to possess a range of biological activities (Rauha et al., 2000; Dufall et al., 2003). Also flavonoids are considered to be an active ingredient in CS (Wang and Li, 2004; Li and Yu, 2009). To date, several flavonoids, such as maysin, apigmaysin, 3□-methoxymaysine, ax-4″-OH-maysin, etc, have been isolated and identified from corn silk (Waiss et al., 1979; Elliger et al., 1980; Snook et al., 1995). The conventional method for separating flavonoids is normally carried out from the extracts by means of solid-liquid extraction or liquid-liquid extraction, followed by a column chromatography (Parejo et al., 2004; Sadhu et al., 2006), this separation process is not particularly effective regarding reagents, energy consumption and labor intensiveness. Alternatively, the adsorption-desorption process on macroporous resins is one of the more efficient methods with a moderate purification effect

and can be used for the recovery and concentration of plant secondary metabolites (Schieber et al., 2003; Kim et al., 2007). Macroporous resins are durable polar, non-polar or slightly hydrophilic polymers having high adsorption capacity with possible recovery of the adsorbed molecules, relatively low cost and easy regeneration (Liu et al., 2004). They are currently used for adsorption of flavonoids and other components extracted from many plants. For example, macroporous resin has been successfully applied for recovery of narirutin from water-extracts of *Citrus unshiu* peels, anthocyanins from grape pomace extracts, separation of flavonoids and glycyrrhizic acid from licorice extracts, adsorption and separation of naringin, etc (Kammerer et al., 2005; Fu et al., 2005; Fu et al., 2006). Therefore, the objectives of the present study were to investigate the adsorption and desorption properties of flavonoids from corn silk (FCS) on different macroporous resins, to develop an efficient method for the preparative separation of FCS with the resin and to determine whether the FCS exhibits anti-fatigue activity.

MATERIALS AND METHODS

Chemicals and materials

Ethanol, nitrite, aluminum nitrate and sodium hydroxide (all AC grade) were purchased from the local market and used without further purification. Standard samples of rutin were purchased from the Chengdu Mansite Pharmaceutical Co.,Ltd.(Chengdu, China). Diagnostic kits and reagents for measuring blood lactate, hepatic glycogen and blood urea nitrogen (BUN) were purchased from the Nanjing Jiancheng Bioengineering Co.,Ltd.(Nanjing, China).

Macroporous resins (AB-8, NKA-2, NKA-9, D3520, D4006, D4020, S-8 and X-5) were purchased from Chemical plant of Nankai University and Haiguang Chemical Co.,Ltd. (Tianjin, China). The adsorbent was pre-treated according to the factory instructions to remove the monomers and porogenic agents entering inside the pores during the synthesis process.

Corn silk was collected from the horticultural center of Kunming University of Science and Technology (Kunming, China). Corn silk was dried for 24 h by using a hot air oven at 60℃ and powdered and then was stored in an drying cabinet before the experiment.

Extraction of FCS

The extraction conditions of FCS was determined according to the known References and our pre-study (Yan and Li, 2007; Ren and Ding, 2007; Bo and Fu, 2009; Li and Yu, 2009) as following: The dried powder (10 g) was subjected to hot continuous extraction in Erlenmeyer flask with ethanol (ethanol concentration 60%, ratio of solvent to raw material 10), while the temperature of the water bath was 70℃ and was kept steady (within ± 1.0℃). After 1.5 h, the extract was filtrated by using the filter. The obtained filtrate was evaporated by using a rotary evaporator (RE-52A, Shanghai Yalong Co.,Ltd., Shanghai, China) under vacuum at 60℃ to get the crude flavonoids (Li and Yu, 2009). It was stored at 0 - 4℃ until used. The flavonoid content was measured using colour comparing method of $NaNO_2$-$Al(NO_3)_3$ and by reference to rutin (Huang et al., 2006; Chen et al., 2007). The UV-2300 spectrophotometer (Shanghai Tianmei Co.,Ltd., Shanghai, China) was used and wavelenth was set at 510 nm.

Static adsorption and desorption tests

In the static adsorption experiment, 1 g of resin and 20 ml of crude flavonoids solution (2.636 mg/ml) were introduced into an air-tight Erlenmeyer flask of 100 ml capacity, which was then shaken (60 r/min) in a water-bath shaker at 25℃ for 24h.

The desorption process was conducted as follows: After adsorption equilibrium was reached, the resin was first washed by deionized water and then desorbed with 80 ml 90% ethanol solution. The flask was shaken (60 r/min) in a water-bath shaker at 25℃ for 24 h.

The preliminary choice of resins was evaluated by their adsorption capacities and the ratios of adsorption and desorption.

The adsorption kinetic curves of FCS on the preliminarily selected AB-8, X-5 and NKA-9 resins were studied according to the method described above. The respective concentration of FCS in the sample solution after adsorption of a certain time was monitored at equal time intervals till equilibration.

Dynamic adsorption and desorption tests

Dynamic adsorption and desorption experiments were carried out in a glass column (1.2 × 30 cm) wet-packed with 5 g of the selected AB-8 resin. The bed volume (BV) of the resin was 20 ml. The flow rate of sample solution was 2 BV/h through the glass column. After reaching adsorptive equilibrium, the loading of the sample was stopped. The adsorbate-laden column was washed first by deionized water with 4 BV and then desorbed with ethanol-aqueous solution.

Calculation of adsorption capacity, ratios of adsorption and desorption

The following equations were used to quantify the capacity of adsorption as well as the ratios of adsorption and desorption (Fu et al., 2006; Yu et al., 2007; Zhang et al., 2009).

Adsorption capacity

$$Q_e = \frac{(C_0 - Ce) \times V_1}{W}$$

where Q_e is the adsorption capacity at adsorption equilibrium (mg/g resin); C_0 and C_e are the initial and equilibrium concentrations of FCS in the solutions, respectively (mg/ml); Vi is the volume of the initial sample solution (ml) and W is the weight of the dry resin (g)

Adsorption ratio

$$E(\%) = \frac{C_0 - Ce}{C_0} \times 100\%$$

Where E is the adsorption ratio (%); C_o and C_e are the same as above.

Desorption ratio

$$D(\%) = \frac{C_d \times V_d}{Q_e \times W} \times 100\%$$

Table 1. Results of adsorption capacities, adsorption and desorption ratios of different resins.

Varieties of resin	Adsorption capacity (mg/g)	Adsorption ratio (%)	Desorption ratio (%)
AB-8	16.46	45.39	93.46
NKA-2	9.27	21.34	71.42
NKA-9	13.78	35.38	75.37
D3520	8.96	20.47	84.29
D4006	7.14	15.66	84.03
D4020	5.61	11.91	7536
S-8	14.38	37.51	55.43
X-5	15.22	40.59	87.52

Where D is the desorption ratio (%); C_d is the concentration of FCS in the desorption solution (mg/ml); V_d is the volume of the desorption solution (ml); Q_e and W are the same as above.

Recovery yield

$$Y(\%) = \frac{C_d \times V_d}{(C_0 - C_a) \times V_p} \times 100\%$$

Where Y is the recovery yield of FCS (%); C_a is the concentration of FCS in the effluent liquid (μg/ ml); V_p is the processing volume of the sample solution (ml); C_0, C_d and V_d are the same as defined above.

Animals and grouping

Kunming mice were purchased from the Center of Laboratory Animal of Kunming University of Science and Technology (Kunming, China). The mice weighed between 18 and 22 g at the time of experiment. They were housed in standard cages 20 × 30 × 15 cm (5 mice/cage) under controlled conditions of temperature (23±1%), humidity (50±5%) and lighting (lights on from 08:00 to 20:00). They were provided a normal diet and water ad libitum. Sixty mice were randomized into 3 groups equally based on body weight after one week adoption: low-dose FCS treatment group (LFG), high-dose FCS treatment group (HFG) and control group (CG). The treatment groups were orally administered different doses of FCS (100 and 400 mg/kg) and the control group was received the same volume of distilled water for 14 consecutive days, respectively. The FCS solution used in treatment groups were prepared through dissolving FCS in distilled water.

Anti-fatigue activity

The anti-fatigue activity of FCS was evaluated by swimming exercise in mice (Ikeuchi et al., 2006). The swimming exercise was carried out in an acrylic plastic pool (65 × 50 × 50 cm), filled with 30 cm depth of water at 30±2%.

Ten mice were taken out from each group to make swimming exercise after being administrated with different dose of FCS for 14 days. Each mouse's tail was loaded with galvanized wire, which was 5% of its body weight. Mice were regarded as being exhausted when they were underwater for 8 s (Chi et al., 2008) and their swimming time was immediately recorded. Blood samples were collected from the veins on the tails of individual mice and the lactate and blood urea nitrogen (BUN) were determined by using a commercial diagnostic kit.

Ten mice from each group were forced to swim 30 min later after final administration of FCS. After swimming for 90 min, each mouse was anesthetized to death with high concentration aether in an acrylic plastic immobilizer and its liver was collected as soon as possible. The contents of hepatic glycogen were determined by using a commercial diagnostic kit.

The data were analyzed with SPSS 10.0 software. ANOVA was used to determine the effects of FCS on anti-fatigue. The values were expressed as mean ± SD. The test differences were considered statistically significant when a P value was less than 0.05.

RESULTS AND DISCUSSION

Results of static adsorption and desorption tests

Eight macroporous resins with different properties were tested through static adsorption and adsorption capacity, ratios of adsorption and desorption were used to evaluate their adsorbent efficiency. As shown in Table 1, the adsorption and desorption ratios of FCS on AB-8, NKA-9 and X-5 resins were considerably higher than those of other resins. Though some other resins also have high adsorption capacities, they are not easy to release the flavonoids. So AB-8, NKA-9 and X-5 resins were selected to further investigate their adsorption behavior towards FCS.

Adsorption kinetics curves were obtained for FCS on AB-8, NKA-9 and X-5 resins. As shown in Figure 1, for the three resins studied, the adsorption capacities increased with the extension of adsorption time. At the beginning, the adsorption capacities increased rapidly; after 5 h the adsorption capacities of AB-8 resin increased slowly and reached equilibrium at about 7 h; after 6 h the adsorption capacities of NKA-9 resin increased slowly and reached equilibrium at about 9 h; after 7 h the adsorption capacities of X-5 resin increased slowly and reached equilibrium at about 11 h. That indicated that AB-8 resin is fast adsorption resin. In addition, the adsorption capacity of AB-8 towards FCS was the highest at any time. Hence, AB-8 resin was selected as the most suitable resin for the preparative separation of FCS and was used in the further test.

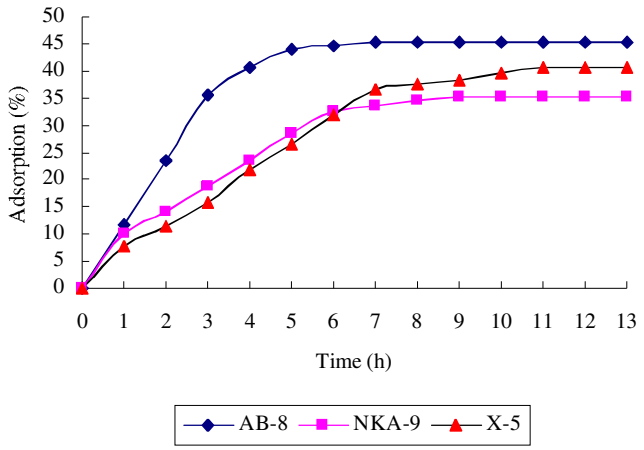
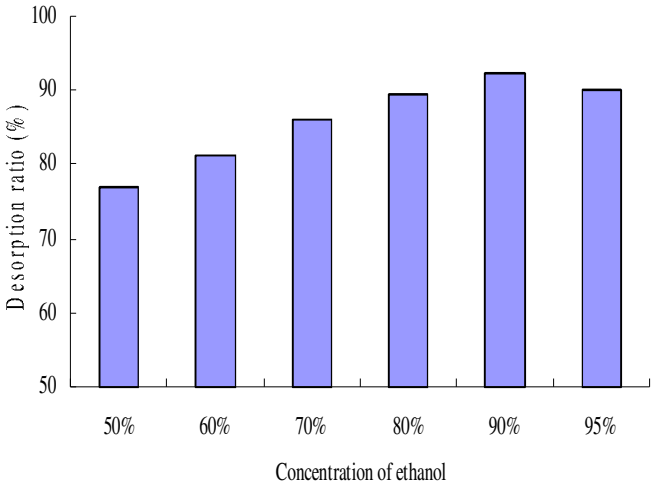


Figure 1. Adsorption kinetics curves for FCS on AB-8, NKA-9 and X-5 resins.



Figures 2. Effect of different ethanol-water solutions on the static desorption ratio of FCS on AB-8 resin.

The proper desorption solution was chosen according to the polarity of resins and the FCS solubility in the desorption solution. FCS dissolves easily in methanol, ethanol, acetone and other organic solvents (Zhang and Xu, 2007; Li, 2007; Ren and Ding, 2007; Li and Yu, 2009). For low cost and safety, ethanol-water was often chosen as desorption solution. Different concentrations of ethanol solutions, from 50 to 95% (v/v), were used to perform desorption test after adsorption equilibrium. As can be seen from Figure 2, at the beginning, the desorption ratio of FCS increase accordingly with increasing of ethanol concentration. The maximum desorption ratio was found to be 92.21%, when using ethanol at a concentration of 90%. Therefore, ethanol-water (90%, v/v) solution was considered as the appropriate desorption solution and was performed in the dynamic desorption process.

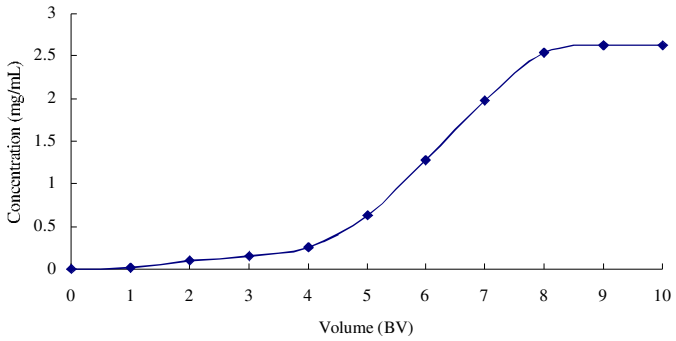


Figure 3. Dynamic leakage curves of FCS on column packed with AB-8 resin.

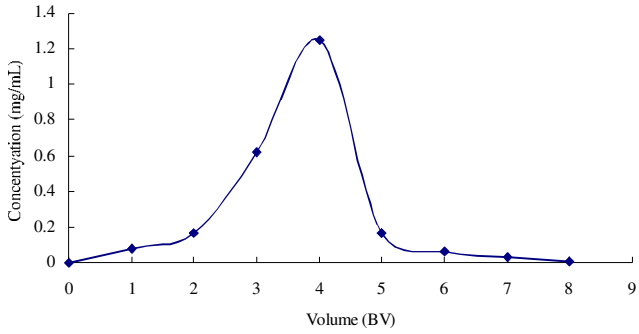


Figure 4. Dynamic desorption curves of FCS on column packed with AB-8 resin.

Results of dynamic adsorption and desorption tests

The solute leakage from the column at breakthrough point was due to the resin in the column approached to be saturated. The breakthrough point was defined as 10% of the eluent to inlet solution concentration. Therefore, it was important to construct the leakage curve in order to calculate the quantity of resins, the processing volume of sample solution and the proper sample flow rate. The dynamic leakage curves on AB-8 resin were obtained according to the volume of effluent liquid and the tested constituents' concentration. As shown in Figure 3, under this condition, the processing volume of sample solution on AB-8 resin was approximate 80 ml (4 BV) and the flow rate was 2 BV/h.

According to the results of breakthrough volume determined above, the 80 ml sample solution was fed on the column packed with 5 g AB-8 resin. After adsorption equilibrium the resin was first flushed with 80 ml of distilled water for removing the high polar components in the crude extraction of corn silk, such as polysaccharides and amino acids. And then the adsorbent was eluted with 90% ethanol aqueous. The dynamic desorption curves on AB-8 resin were obtained based on the volume of effluent and the concentration of solute therein. The flow rate in this test was 1.0 BV/h. As can be seen in Figure 4,

Table 2. Effect of FCS on blood biochemical parameters (mean ± SD, n = 10).

Group	Lactate (mmol/L)	Blood urea nitrogen (mmol/L)
CG	11.18±1.16	10.23±1.05
LFG	8.89±1.13*	8.27±0.94*
HFG	8.13±0.97*	7.71±0.86*

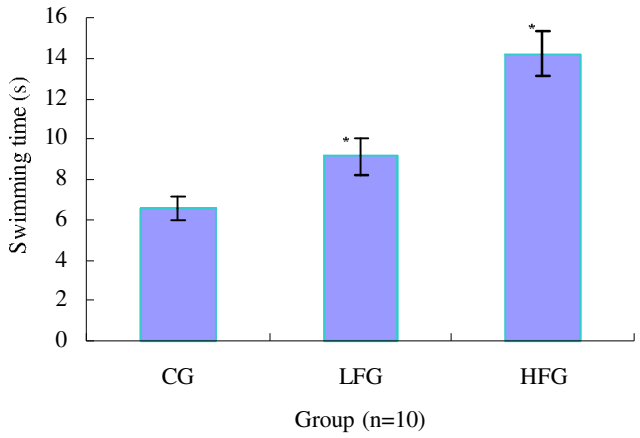


Figure 5. Effects of FCS on swimming time of the mice (mean ± SD, n=10).

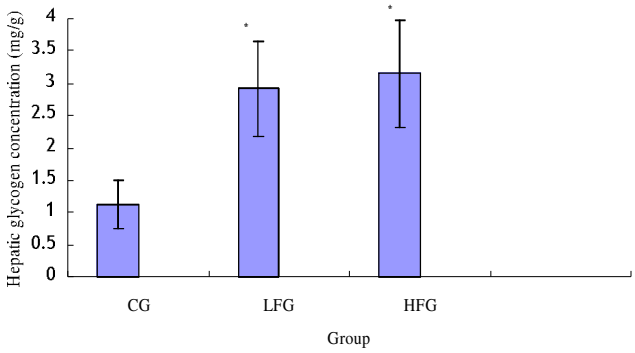


Figure 6. Effect of FCS on hepatic glycogen concentration.

adsorption solution of 80 ml (4 BV) could completely desorbed FCS from AB-8 resin.

The optimization of the adsorption and desorption conditions of AB-8 resin was as follows: 1. for adsorption: sample solution FCS concentration was 2.636 mg/ml; processing volume was 4 BV; flow rate was 2.0 BV/h; temperature was 25℃; 2. for desorption: elution solvent ethanol-water (90%, v/v) solution was 4 BV; flow rate was 1.0 BV/h. Flavonoids with content of 63.15 % was obtained with a flavonoids recovery of 94.27 % in the purification process.

Anti-fatigue activity

The anti-fatigue activity of FCS was investigated by using swimming exercise. As shown in Figure 5, swimming time of treatment groups were all remarkably longer than that of the control group ($P < 0.05$). The average swimming time of the LFG and HFG increased by 39.6 and 115.9%, respectively. These results indicated that FCS can elevate the exercise tolerance and had significant anti-fatigue activity of mice.

In order to clarify anti-fatigue activity of FCS mechanism, blood biochemical parameters were measured in the swimming-treated mice. The swimming exercise was known to induce blood biochemical changes (Moriura et al., 1996). Blood biochemical parameters are shown in Table 2. It was found that the blood lactate and blood urea nitrogen concentrations of treatment groups were significantly lower than that of the control group ($P<0.05$). The results suggest that FCS can inhibit the production of blood lactic acid during exercise and retard the formation of BUN after exercise.

It was known that endurance capacity of body markedly decreased if the energy was exhausted. As glycogen was the important resource of energy during exercise, the increasing of glycogen stored in liver is advantage to enhance the endurance of the exercise (Yu et al., 2008). Hepatic glycogen concentration are shown in Figure 6. It was found that the hepatic glycogen concentration of treatment groups were higher than that control group ($P < 0.05$), increasing rate were 260.71 and 281.25%, respectively. The results suggest that FCS can elevate hepatic glycogen concentration during exercise. However its detailed mechanism isn't clear. The possible reason is that FCS may increase the content of Hepatic glycogen of mice post-exercise by improving glycogen reserve, or by reducing the consumption of glycogen during exercise, or both. It still needs further studies.

Conclusions

In this study, micro porous resin adsorption technology were used for obtaining purified flavonoids from corn silk and it's anti-fatigue activity was evaluated. Among the eight resins investigated, AB-8 resin shows the best enrichment and purification efficiency. Some important parameters were optimized for most effective enrichment and preparative separation. As follows: 1. for adsorption: sample solution FCS concentration was 2.636 mg/ml;

processing volume was 4 BV; flow rate was 2.0 BV/h; temperature was 25°C; 2. for desorption: elution solvent ethanol-water (90%, v/v) solution was 4BV; flow rate was 1.0 BV/h. Flavonoids with content of 63.15% was obtained with a flavonoids recovery of 94.27% in the purification process. Compared to conventional separation methods of flavonoids, this adsorption method is superior because of its procedural simplicity, lower cost, high efficiency and it may provide scientific references for the large-scale FCS production. The present study demonstrates for the first time that FCS can elevate the exercise tolerance and have anti-fatigue activity of mice by inhibiting the production of blood lactic acid, retarding the formation of BUN and increasing hepatic glycogen concentration. However, further study is needed to elucidate the more exact mechanism of the effect of FCS on fatigue and/or exercise durability.

REFERENCES

Bo NN, Fu H (2009). The Extraction and Contented Termination of Flavonoids in Maize Silk. J. Capital Normal University (Natural Science Edition). 4: 44-47.

Chen D, Chen X, Liu X (2007). Determination of Total Flavones Content in Maize Style by Colorimetry. J. Maize Sci. 15: 246–248.

Chi AP, Chen JP, Wang ZZ, Xiong ZY, Li QX (2008). Morphological and structural characterization of a polysaccharide from Gynostemma pentaphyllum Makino and its anti-exercise fatigue activity. Carbohydr. Polymers, 74: 868–874.

Dufall K, Ngadjui B, Simeon K, Abegaz B, Croft K (2003). Antioxidant activity of prenylated flavonoids from the West African medicinal plant Dorstenia mannii. J. Ethnopharmacol. 87: 67–72.

Ebrahimzadeh MA, Pourmorad F, Hafezi S (2008). Antioxidant Activities of Iranian Corn Silk. Turkish J. Biol. 32: 43-49.

Fu BQ, Liu J, Li H, Li L, Lee Frank SC, Wang XR (2005). The application of macroporous resins in the separation of licorice flavonoids and glycyrrhizic acid. J. Chromatogr. A. 1089: 18-24.

Fu YJ, Zu YG, Efferth T, Zhang NJ, Liu XN, Kong Y (2006). Optimization of luteolin separation from pigeonpea [Cajanus Cajan(L.) Millsp.] leaves by macroporous resins. J. Chromatogr. A. 1137: 145-152.

Grase F, March JG, Ramis M, Costa-Bauza′ A (1993). The influence of Zea mays on urinary risk factors for kidney stones in rats. Phytother Res. 7: 146-149.

Huang Y, Long GJ, Jiang LH, Huang SY (2006). The Total Flavanone of Corn Silk Withdraws and Distinction. Lishizhen Med. Mater. Med. Res. 17: 1008-1009.

Ikeuchi M, Koyama T, Takahashi J, Yazawa K (2006). Effects of Astaxanthin Supplementation on Exercise-Induced Fatigue in Mice. Biol. Pharm. Bull. 29: 2106-2110.

Kammerer D, Kljusuric JG, Carle R, Schieber A (2005). Recovery of anthocyanins from grape pomace extracts (Vitis vinifera L. cv. Cabernet Mitos) using a polymeric adsorber resin. Eur. Food Res. Technol. 220: 431-433.

Kim MR, Kim WC, Lee DY, Kim CW (2007). Recovery of narirutin by adsorption on a non-ionic polar resin from a water-extract of Citrus unshiu peels. J. Food Engr. 78: 27-32.

Li DZ (2007). Study on the Extraction and Application of Flavonoids from Corn. J. Binzhou University, 3: 37-38.

Li FL, Yu L (2009). Flavonoids extraction from maize silk and its function on blood sugar control. China Food Additives. 94: 121-124.

Liu XM, Xiao GS, Chen WD, Xu YJ, Wu JJ (2004). Quantification and Purification of Mulberry Anthocyanins with Macroporous Resins. J. Biomed. Biotechnol. 5: 326-331.

Moriura T, Matsuda H, Kubo M (1996). Pharmacological study on Agkistrodon blomhoffii blomhoffii Boie. V. Anti-fatigue effect of the 50% ethanol extract in acute weight-loaded forced swimming-treated rats. Biol. Pharm. Bull. 19: 62-66.

Parejo I, Caprai E, Bastida J (2004). Investigation of Lepechinia graveolens for its antioxidant activity and phenolic composition. J. Ethnopharmacol. 94: 175-184.

Ramesh P, Okigbo RN (2008). Effects of plants and medicinal plant combinations as anti-infectives. Afr. J. Pharm. Pharmacol. 2: 130-135.

Rauha JP, Heinonen M, Hopia A, Kahkonen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P, Remes S (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiol. 56: 3–12.

Ren SC, Ding XL (2007). Study on Flavonoids Content in Different Corn Silk Varieties. J. Maize Sci. 15: 135-137.

Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M, Yesilada E (2006). Prostaglandin inhibitory and antioxidant components of Cistus laurifolius, a Turkish medicinal plant. J. Ethnopharmacol. 108: 371-378.

Schieber A , Hilt P, Petra S, Endress HU, Rentschler C, Carle R (2003). A new process for the combined recovery of pectin and phenolic compounds from apple pomace. Innov. Food Sci. Emerg. Technol. 4: 99-107.

Velazquez DVO, Xavier HS, Batista JEM, de Casro- Chaves C (2005). Zeamays L. Extracts modify glomerular function and potassium urinary excretion in conscious rats. Phytomedicine. hytomedicine. 12: 363-369.

Wang YPi, Li XG (2004). Progress in Study on Chemical Constituent and Pharmacological Activity of Corn Silk. Special Wild Economic Anim. Plant Res. 2: 42-46.

Yan Z, Li CY (2007). Study on Extraction Technique of Flavonoids from Green Corn Silk. Acta Agriculturae Jiangxi. 19: 108-109.

Yu B, Lu ZX, Bie XM, Lu FX, Huang XQ (2008). Scavenging and anti-fatigue activity of fermented defatted soybean peptides. Eur Food Res. Technol, 226:415–421.

Yu ZF, Wang M, Zhang JF (2007). Optimization of purifying technology of tartary buckwheat total flavonoids with macroporous resin. Trans. Chin. Soc. Agric. Eng. 23: 253-256.

Zhang HE, Xu DP (2007). Study on the Chemical Constituents of Flavones from Corn Silk. J. Chin. Med. Mater. 30: 164–166.

Zhang ZF, Liu Y, Luo P, Zhang H (2009). Separation and Purification of Two Flavone Glucuronides from Erigeron multiradiatus (Lindl.) Benth with Macroporous Resins. J. Biomed. Biotechnol. 875629: 1–8.