academicJournals

Vol. 7(22), pp. 1438-1447, 15 June, 2013
DOI 10.5897/AJPP12.170
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Analysis of the polysaccharides from *Urtica angustifolia* and their anti-fatigue activity

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Accepted 15 April, 2013

The crude polysaccharides (UA) were obtained from *Urtica angustifolia* by hot water extraction, 80% ethanol precipitation and deproteination by using sevage reagent in a yield of 1.6%. The polysaccharides were fractionated into six fractions. Their sugar compositions and structures were analyzed by high performance liquid chromatography (HPLC) and nuclear magnetic resonance spectrometer (NMR). The HPLC analysis results showed that UA contain two neutral polysaccharide fractions and four acidic fractions. The neutral fractions are mainly composed of glucose (Glc), and the acidic fractions are mainly composed of galacturonic acid (GalA). The NMR results showed that the neutral fractions are starch-like polysaccharides and the acidic fractions are rhamnogalacturonan (RG)-I domian containing pectin. The anti-fatigue activity of the polysaccharides was evaluated in mice and the results showed *U. angustifolia* polysaccharides increased the swimming time, and the average swimming time of the low doses (LD), middle doses (MD) and high doses (HD) group were increased by 85.4, 114.6 and 151.2%, respectively. The blood urea nitrogen content of the LD, MD and HD group was reduced by12.8, 13.6 and 19.8% compared with that of control group, respectively. The hepatic glycogen of every treated group was higher than that of control group, increasing rates were 87.0, 469.6, and 747.8%, respectively.

Key words: *Urtica angustifolia*, polysaccharide, anti-fatigue, structure analysis.

INTRODUCTION

Urtica is a perennial wild herb. It is rich in natural resources and has very strong vitality. *Urtica* is a kind of traditional medicine edible wild plant in China. Nettle (the same meaning with *Urtica*) is stated to possess hypoglycemic properties (Newall et al., 1996). Moreover, it has been shown that a preparation containing various plants extraction with nettle had antidiabetic activity (Petlevski et al., 2001). *Urtica angustifolia* belongs to the family of Urticeae, and is widely distributed in China. Moreover, nettle is known in folk medicine as hypotensive and antidiabetic (Ziyyat et al., 1997). Nettle plant roots and rhizomes can also treat high blood pressure, hand and foot numbness, diabetes, leprosy, weak labor wounds, indigestion, and constipation. It can also be used to treat polio sequelae and hernia pain etc. (Quan 1997).

At present, Europe, the United States and other developed countries have researched on the nettle, especially the *Urtica dioica*. In particular, the development of medicine in Germany has made remarkable achievements.

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They have had specific studies on different parts of the chemical composition of U. dioica and obtained that the active ingredients are sterols, flavonoids, organic acids, phenols, phenylpropanoids, plant proteins and polysaccharides (Kraus and Spiteller, 1990; Kavtaradze et al., 2001; Neugebauer et al., 1995; Zhang et al., 2011).

U. angustifolia is widely distributed in northwest regions of china. Its claimed benefits include anti-inflammatory, analgesic effect, anti-intoxication, spasmolysis and improving immune function, and also restrain hyperplasia of prostate (Bnouham et al., 2003; Schulze-Tanzil et al., 2002; Konrad et al., 2000). But the anti-fatigue effects of *U. angustifolia* and the dose-dependent relation have not been reported (Dong et al., 2007; Guo et al., 2006). The present study was designed to investigate the anti-fatigue effects of *U. angustifolia* and its effective dose.

MATERIALS AND METHODS

Plants

The roots of *U. Angustifolia* were cultivated and collected in October at Liuhe, Jilin, China. The diethylaminoethyl (DEAE)-cellulose and sepharose CL-6B were purchased from Sigma. All other chemicals were of analytical grade.

Animals

Swiss mice weighing 18 to 22 g were used. The animals were kept in a room at a controlled temperature of 22 ± 2°C with a 12 to 12 h light-dark cycle. The animals were treated according to the ethical guidelines of the Animal Center, College of Animal Science and Technology, Jilin University.

Experimental

Total carbohydrate content was determined by the phenol-sulfuric acid method (Dubois et al., 1956). The standards used in the assay were prepared with the ratio of the monosaccharide that constituted the polysaccharide to be tested, according to the sugar composition. Uronic acid contents were determined by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) using galacturonic acid as a standard. Gel permeation and anion-exchange chromatographies were monitored by assaying the total sugar and uronic acid contents. Protein content was determined by the Bradford assay using bovine serum albumin as the standard.

Extraction

The roots of *U. angustifolia* were dried at 50°C for three days before grinding. The ground material (900 g) were extracted with 8.0 L distilled water at 100°C for 2 h and filtered through four sheets of gauze (100 mesh). The solid material was extracted twice again under the same conditions. The filtrates were combined, centrifuged to remove water-insoluble materials, concentrated to 200 ml and precipitated by the addition of 4 volumes of 95% ethanol. After centrifuging and re-dissolving the precipitate by distilled water, the solution with Sevag reagent (1:4 n-butanol:chloroform, v/v, 40 ml) to remove proteins (Sevag et al., 1938). After precipitation by ethanol again and drying in vacuum, the deproteined polysaccharide fraction UA (14.4 g) was obtained. The procedure for the extraction and fractionation of *U. angustifolia* polysaccharides is shown in Figure 1.

Chromatography on DEAE-cellulose

Analytical chromatography on DEAE-cellulose

The UA (10 mg) was dissolved in distilled water (1 ml) and loaded on a DEAE-cellulose (Cl^-) column (1.5 × 14 cm) pre-equilibrated with distilled water. The column was eluted first with 40 ml distilled water at 1.0 ml/min and then with a linear gradient from 0.0 to 1.0 M NaCl within 400 ml. The eluate was collected at 4 ml per tube and assayed for total sugar and uronic acid contents.

Semi-preparative chromatography on DEAE-cellulose

The UA (9 g) was applied to a DEAE-cellulose column (8.8 cm × 25 cm) and eluted stepwise with distilled water and 0.5 M NaCl. The eluate was monitored by the phenol-sulfuric acid method. The fraction which was eluted by 0.5 M NaCl was dialyzed against distilled water and lyophilized after concentrating to obtain the fraction unbound fraction (UAN) (3.4 g) and bound fraction (UAA) (4.0 g). UAA (4.0 g) was re-dissolved in distilled water, loaded onto a column (3.8 cm × 27) of DEAE-cellulose and eluted stepwise with distilled water, 0.1, 0.3 and 0.5 M NaCl. The four fractions were dialyzed against distilled water and lyophilized after concentrating to obtain the fractions UAA-1 (1.0 g), UAA-2 (0.3 g), UAA-3 (1.7 g) and UAA-4 (0.9 g).

Gel permeation chromatography on sepharose CL-6B

Analytical chromatography on sepharose CL-6B

Each sample (5 to 10 mg) was dissolved in 0.15 M NaCl (1 ml), loaded onto a sepharose CL-6B column (1.5 × 90 cm) and eluted with 0.15 M NaCl at a flow rate of 0.15 ml/min. The eluate was collected at 3 ml per tube and assayed for total sugar and uronic acid contents.

Semi-preparative chromatography on sepharose CL-6B

UAA-3 (1 g) was applied to a preparative sepharose CL-6B column (3.0 × 90 cm) and eluted with 0.15 M NaCl at 0.5 ml/min. The eluate (10 ml per tube) was collected and assayed for total sugar and uronic acid contents. The appropriate fractions were combined, concentrated, dialyzed against distilled water and lyophilized to give UAA-3-1 (110 mg) and UAA-3-2 (540 mg).

High performance gel permeation chromatography

High performance gel permeation chromatography was carried out at 40°C using a TSK-gel G-3000PW_{XL} column (7.8 × 300 mm, TOSOH, Japan) connected to a Shimadzu HPLC system. The column was pre-calibrated with standard dextrans. Sample was eluted with 0.2 M NaCl at a flow rate of 0.6 ml/min and monitored using a refractive index RID-10A detector (Shimadzu, Tokyo, Japan). Sample concentration was 5 mg/ml and injection amount was 20 µl.

Sugar composition analysis

Polysaccharide samples (2 mg) were hydrolyzed first with anhydrous methanol containing 1 M HCl at 80°C for 16 h and then with 2 M trifluoroacetic acid (TFA) at 120°C for 1 h. The resulting

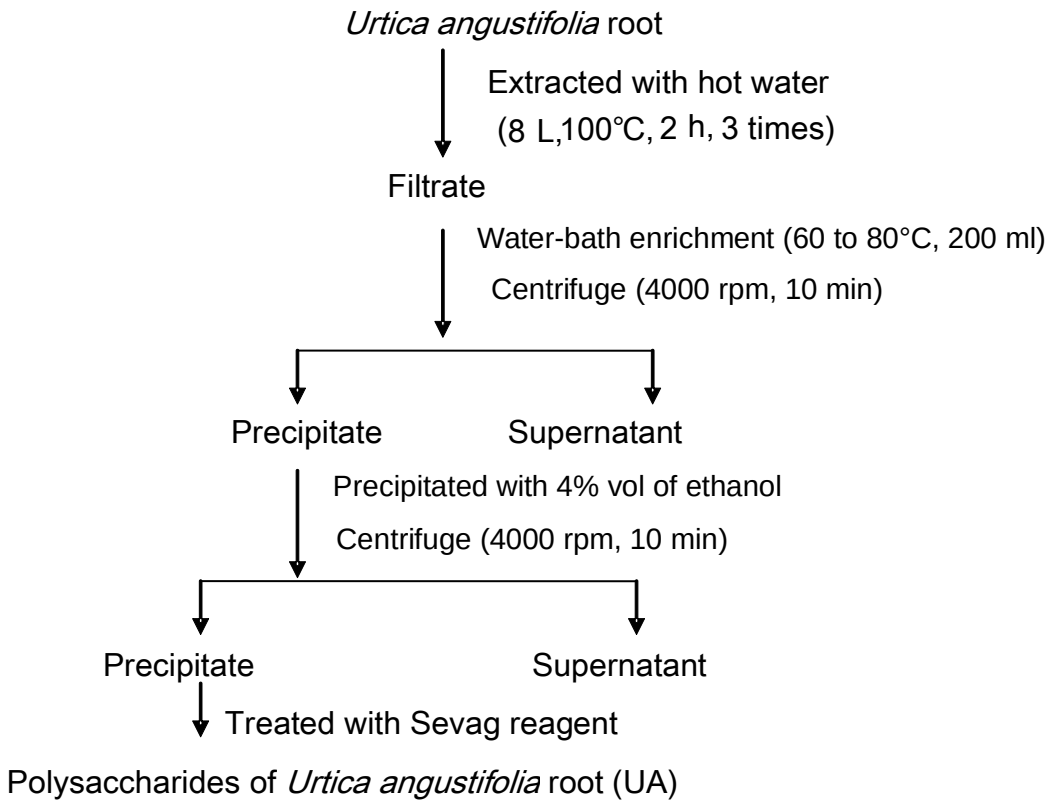

Figure 1. Fractionation scheme of polysaccharides from *Urtica angustifolia* root.

hydrolysates were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP) according to the method in the literature (Yang et al., 2005) and analyzed on a DIKMA Inertsil ODS-3 column (4.6 × 150 mm) connected to a Shimadzu HPLC system (LC-10ATvp pump and SPD-10AVD UV-VIS detector). The PMP derivative (20 µl) was injected, eluted with 82.0% phosphate buffer saline solution (PBS) (0.1 M, pH 7.0) and 18.0% acetonitrile (v/v) at a flow rate of 1.0 ml/min and monitored by ultra violet (UV) absorbance at 245 nm.

Nuclear magnetic resonance (NMR) spectra

The carbon nuclear magnetic resonance (^{13}C NMR) spectra were obtained on a Bruker AV600 spectrometer at 150 MHz. The samples (20 mg) were dissolved in D_2O (1 ml, 99.8%) with overnight stirring at room temperature. The spectra were recorded at 25°C after 57,000 scans (Shuqun and Robert, 1997).

Swimming endurance experiment

The mice were randomly divided into 4 groups, 10 mice per group. The collected crude polysaccharide was dissolved in water, respectively. Concentrations were 4, 8, and 12 g/ml. Mice were intra-gastric administrated for ten days, and the doses were 100, 200 and 300 mg/kg/d, respectively. Mice in the control group were given the quivalent volume of water. Ten days later, mice were swam with wire of 5% body weight tied their tails in the pool (length: 50 cm, width: 50 cm, depth: 40 cm) filled with 30 cm depth of water at 25°C ± 1.0°C. The anti-fatigue activity of *U. angustifolia* polysaccharide was evaluated by the weight loaded swimming model in mice. Mice were regarded as exhaustion when they stayed in the water for 8 s, and their swimming time was immediately recorded. The time of each group of mice was averaged and the data of the different groups was analyzed with F-test.

Effect of *U. angustifolia* polysaccharides on the blood urea nitrogen of mice

Mice were randomly divided into 4 groups, 10 mice per group. The crude polysaccharide collected in the present study was dissolved in a small amount of water, respectively. Mice were intra-gastric administrated for 10 days, and the doses were 100, 200 and 300 mg/kg/d, respectively. Mice in the control group were given the equivalent volume of water. Ten days later, mice were swam for 90 min,30 min later after final administration of *U. angustifolia* polysaccharides, mice were forced to swim without wire tied to their tails in the pool (length: 50 cm, width: 50 cm, depth: 40 cm) filled with 30 cm depth of water at 30°C. Blood eye samples were collected after mice have been resting for 60 min. After the blood was put in refrigerator for 3 h, concentrated (2000 rpm), serum urea nitrogen was determinated by automatic biochemical analyzer (Ding et al., 2008).

Increase ratio = (a - b) / b
Reduce ratio = (a - c) / c

Where a is the blood lactate concentration of mice after swimming immediately, b is the blood lactate concentration of mice before swimming and c is the blood lactate concentration of mice after resting for 20 min.

Effect of *U. angustifolia* polysaccharides on the hepatic glycogen of mice

To determine the hepatic glycogen of the mice 30 min later after final administration of *U. angustifolia* polysaccharides, each mouse was killed to get the liver, dried by the dry filter paper, checked at 100 mg weight, to it was added 8 ml Trichloroacetic acid (TCA), centrifuged at 3,000 rpm for 15 min, 4 ml 95% ethyl alcohol (Et-OH) added to the supernatant and finally erected at room temperature overnight. After total precipitation, it will was concentrated for 15 min at 3,000 rpm. The precipitation was erected for 10 min and 2 ml distilled water added to dissolve hepatic glycogen.

Statistical analysis

All the results were expressed as the means ± standard deviation (SD) in the tables, and differences between groups were determined by analysis of variance and Student's t-test. Probability value p less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Isolation of *U. angustifolia* polysaccharides

The water-soluble polysaccharides were extracted from the roots of *U. Angustifolia* with boiling water and deproteined by using the Sevag method. A crude polysaccharide fraction (UA) was obtained with a yield of 1.6 % (w/w). UA contained 44.6% total sugar, 12.7% uronic acid and less than 1% protein. Sugar composition analysis by HPLC indicated that UA consisted of glucose (Glc) (64.3%), galactose (Gal) (8.2%), galacturonic acid (GalA) (15.9%), glucuronic acid (GlcA) (1.3%), mannose (Man) (1.1%), rhamnose (Rha) (2.6%) and arabinose (Ara) (5.9%).

Fractionation by DEAE-cellulose chromatography

Preliminary separation on DEAE-cellulose column

Before fractionation on a semi-preparative scale, we analyzed UA using an analytical DEAE-cellulose column. The profile showed an unbound portion eluted with water and a bound portion eluted with a linear gradient of NaCl. It failed to separate all fractions in a single run of DEAE-cellulose chromatography with a 0.0 to 0.5 M NaCl linear gradient elution, because the abundant unbound neutral polysaccharides interfered with the fine separation of the acidic polysaccharides. Thus, we separated UA first into two fractions on a semi-preparative scale: an unbound fraction (UAN) by water elution with a recovery of 37.4% and a bound fraction (UAA) by 0.5 M NaCl elution, with a recovery of 44.6%. As expected, the unbound portion UAN did not contain uronic acid and was not able to be separated further by anion-exchange chromatography. The bound portion UAA contained uronic acid and could be fractionated continually by a second step of DEAE-cellulose chromatography (Figure 2).

The second run of the separation on DEAE-cellulose column

In the second run of DEAE-cellulose chromatography, the bound portion (UAA) was separated as follows. First, analytical DEAE-cellulose chromatography of UAA was carried out. The elution profile indicated that UAA still contained neutral polysaccharides and a large proportion of acidic polysaccharides. We decided to separate UAA on a preparative DEAE-cellulose column by elution with a stepwise gradient of NaCl. With these elution steps, UAA was separated into four fractions (Figure 2d): UAA-1 (25.9%), UAA-2 (6.4%), UAA-3 (42.0%) and UAA-4 (21.5%) corresponding to 0.0, 0.1, 0.3 and 0.5 M NaCl, respectively. Total sugar and uronic acid assays indicated that UAA-1 was a neutral polysaccharide fraction and UAA-2, UAA-3 and UAA-4 were acidic polysaccharide fractions (Figure 3).

Fractionation by sepharose CL-6B chromatography

All collected fractions from the DEAE-cellulose column were analyzed on a sepharose CL-6B column. As shown in Figure 2, UA gave a wide distribution almost from the void volume to the total volume. By the first separation on DEAE-cellulose, the degree of homogeneity of UAN and UAA was not significantly improved compared to the original UA fraction. After the second separation step, UAA-1 and UAA-2 had a similar distribution to that of the precursor (UAA), showing a polydispersed profile from chromatography on sepharose CL-6B. Thus, UAA-4 had a wide distribution on the column, while UAA-3 could be further fractionated on sepharose CL-6B. UAA-3 presented two major molecular populations, one rich in neutral sugars was eluted first and the other rich in uronic acid was eluted later (Figure 3). Therefore, UAA-3 was further fractionated on sepharose CL-6B to give two fractions UAA-3-1 (12.7%) and UAA-3-2(54.1%).

Homogeneity and molecular weight

The homogeneity of each fraction purified by the combinatorial procedure was analyzed by sepharose CL-6B chromatography and high performance gel permeation chromatography (HPGPC) on a TSK column. On the sepharose CL-6B column, UAA-3-1 and UAA-3-2 each showed a single and symmetrical narrow peak, and the distribution of total sugars was consistent with that of uronic acid (data not shown). The elution profiles from the TSK column showed a single and symmetrical narrow peak for each fraction. These results indicated that UAA-3-1 and UAA-3-2 were homogeneous fractions related to

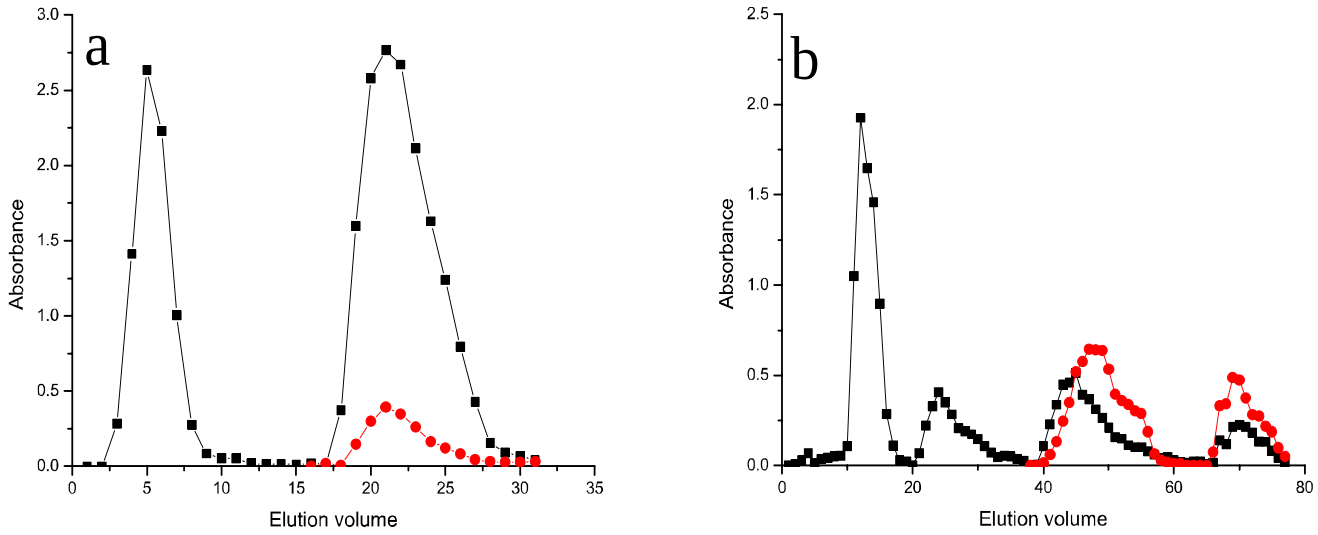

Figure 2. Elution profiles of UA and UAA on DEAE-cellulose column, eluted by a stepwise gradient of NaCl (a and b), respectively (total sugar, -■-; uronic acid, -●-)

molecular weight. The molecular weights were approximately deduced from the calibration curve of dextran standards by HPGPC to be 11 × 10^4 Da (UAA-3-1) and 1 × 10^4 Da (UAA-3-2).

Sugar composition of final fractions

The recoveries and sugar compositions of the collected fractions are listed in Table 1. It shows that there are three neutral fractions UAN, UAA-1 and UAA-2. UAN was mainly composed of glc (92.4%) and contained man, ara and gal as minor components. UAA-1 was only composed of glc (100%). UAA-2 was mainly composed of glc (78.0%) and contained man, ara and gal as minor components. The other four fractions were all acid fractions which showed that UAA-3 was mainly composed of galA, gal and ara, and contained man, glc, glcA and rha as minor components. The ratio of galA, gal to ara was 2.7: 1.2: 1.0. UAA-4 was mainly composed of galA, gal and ara, and contained man, glc, glcA and rha as as minor components. The ratio of galA, gal to ara was 4.3: 1.2: 1.0. UAA-3-1 was mainly composed of gal and ara, and contained man, glc, glcA, galA and rha as minor components. The ratio of gal to ara was 1.3: 1.0. UAA-3-2 was mainly composed of galA (72.1%) and contained man, glc, glcA, galA, ara and rha as minor components.

Features of collected fractions

To get more information about macromolecular features, the obtained fractions were tested with iodine to check for the presence of starch-like glucans. Moreover, NMR, periodate and Smith digestion were also used to characterize the structural features of these homogeneous fractions.

Macromolecular features of UAN and UAA-1

UAN and UAA-1 were two neutral fractions and generated a blue color when tested with iodine, which indicated the presence of starch-like glucans.

Macromolecular features of UAA-3-1 and UAA-3-2

UAA-3-1 contained gal and ara as the main sugars detected and glc, man, rha, galA and glcA as minor components, suggesting that these fractions might be composed of arabinans, galactans and/or arabinogalactans. The ratios of rha/galA determined for UAA-3-1 was 0.75, which is among the RG-I range from 0.05 to 1.0 defined by Schols and Voragen (1996). This suggested that it might contain RG-I domains. RG-I has been reported to be composed of α-(1,4)-linked D-galacturonic acid and α-(1,2)-linked L-rhamnose, which are alternatively combined with each other in the backbone; and some of the rhamnose residues contained side chains, such as arabinan, galactan and arabinogalactan at 4-O-rhamnose (McNeil et al.,1980). The arabinans, galactans and/or arabinogalactans might be associated with RG-I domains in non-covalent form or as side chains of RG-I.

The ^{13}C NMR spectra of UAA-3-1 resembled those of arabinogalactans-α reported in the literature (Gane, 1995; Wang et al., 2005). In the anomeric region, the signals at 108.2 and 106.8 ppm were assigned to α-Ara*f*, and the

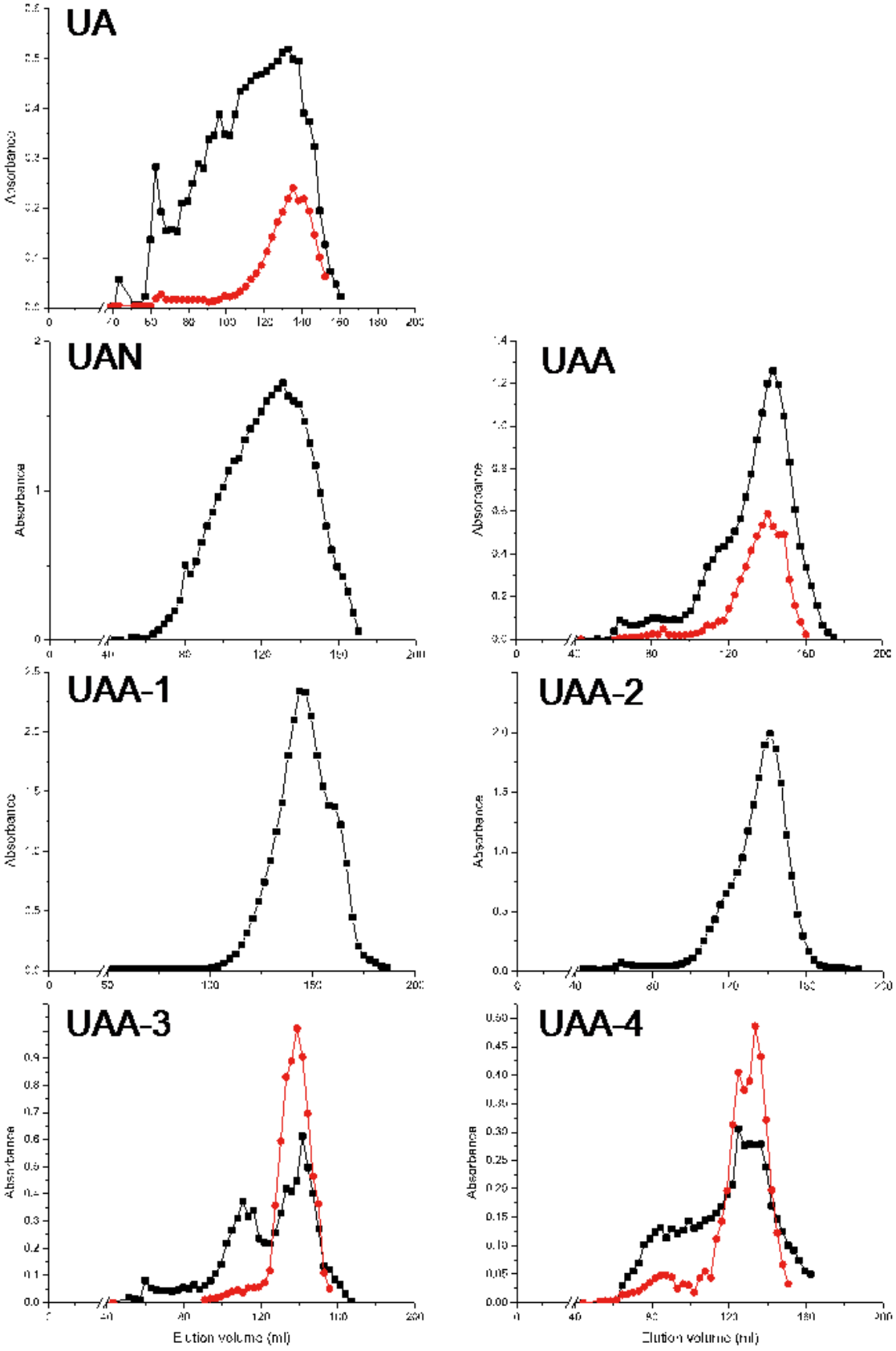

Figure 3. Sugar and uronic acid distribution of the different fractions on sepharose CL-6B (total sugar, -■-; uronic acid, -●-).

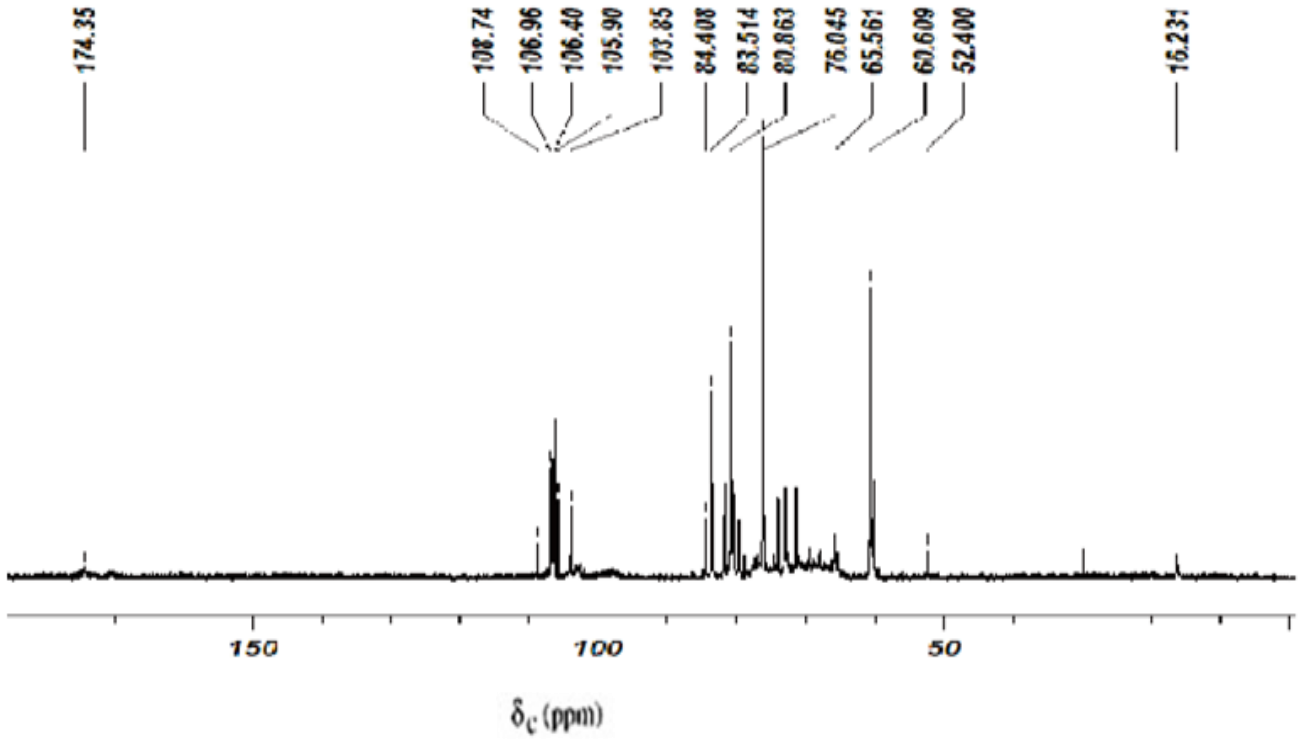

Figure 4. ^{13}C NMR spectra of UAA-3-1.

signals at 106.3 to 103.3 ppm were β-Gal*p*. Although the content of ara is less than that of gal in UAA-3-1, the signals for carbons of α-Ara*f* were obviously stronger than those of β-Gal*p*, which suggested that ara residues were in flexible side chains. Three anomeric carbon signals from ara residues of UAA-3-1 indicated that ara in this fraction might be present in two linkage forms, which usually exist in pectic arabinogalactan, including α-(1-5)-Ara*f* and non-reducing terminal α-Ara*f* (Polle et al., 2002). As seen in Figure 4, the multiple signals for C-1 of β-Gal*p* indicated that gal had more linkage forms. These results implied that UAA-3-1 had very complex structures similarly to other pectins. Ara and gal residues constituted hair regions containing type II arabinogalactan (Pérez et al., 2003), in which gal residues were in the backbone and ara residues were side chains or linked at non-reducing ends. Trace resonances at 19.2 and 172.6 ppm were assigned to C-6 in rha residues and C-6 in uronic acids, which was consistent with the detected sugar compositions (Figure 4).

UAA-3-2 contained high amounts of galA. The NMR spectra (Figure 5) of UAA-3-2 showed predominant resonances from carbons in free galactopyranosyl uronic acid residues in accordance with those previously observed in HG-rich pectins (Catoire et al., 1998). The signals for C-1 to C-6 of unesterified galA residues appeared at 97.8 (C-1), 67.0 (C-2), 67.7 (C-3), 76.8 (C-4), 70.2 (C-5) and 174.3 (C-6) ppm. The result showed that UAA-3-2 is HG-rich pectin, a linear α-(1, 4)-linked D-galacturonic acid. A small amount of neutral sugars that existed in these fractions might constitute RG-I domains linked to homogalacturonan (HG) domains in covalent or non-covalent forms. Pectin structure generally encompasses HG, RG-I and RG-II domains (Vidal et al., 2003; Yapo et al., 2007). Here, HG-rich pectins and RG-I-rich pectins were found in *U. Angustifolia* polysaccharides. However, RG-II domains were not detected by determining their diagnostic sugars (unpublished result). Perhaps the amount of RG-II domains was too low to be detected by the method used (Figure 5).

Swimming endurance experiment

The average time of loaded-weight swimming of mice of the treated groups were all remarkably longer than that of the control group. The average time of loaded-weight swimming of mice of the treated groups were all remarkably longer than that of the control group. The average swimming time of the LD, MD and HD group was increased by 85.4, 114.6 and 151.2%, respectively. These results indicated that *U. angustifolia* polysaccharides had significant effect on the anti-fatigue of the mice in the experiment (Table 2).

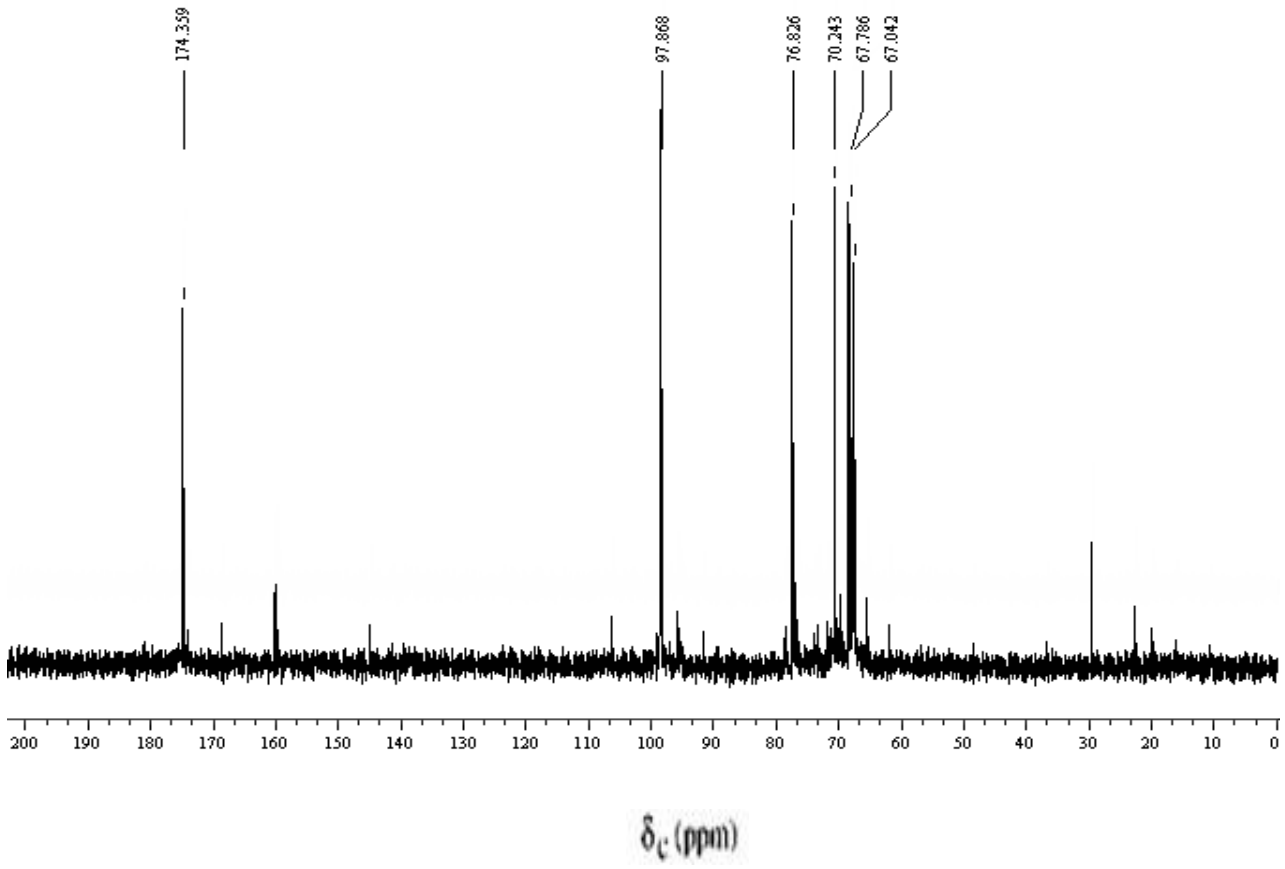

Figure 5. ^{13}C NMR spectra of UAA-3-2.

Blood urea nitrogen analyses

Blood urea nitrogen was the sensitive indicator to evaluate the body load bearing capacity. The blood urea nitrogen content of the LD, MD and HD group was reduced by 12.8, 13.6 and 19.8% compared with that of control group, respectively (Table 3). The results show that the blood urea nitrogen content of mice increased with the rising of the labor and exercise stress. However, the capacity to adapt to the load of mice deteriorated. The phenomenon is more significant when the blood urea nitrogen content is larger.

Hepatic glycogen analyses

Glycogen is an important energy source in the body movement which directly impact on the athletic ability, so improving glycogen reserves to improve speed, and endurance of great significance. The hepatic glycogen of every treated group was higher than that of control (CD) group, increasing rates were 87.0, 469.6, and 747.8%, respectively (Table 4). The results show that nettle root polysaccharide has a positive role to maintain glycogen content levels which can delay the appearance of fatigue.

Conclusion

The crude polysaccharides (UA) were obtained from *U. angustifolia* by using sevage reagent in a yield of 1.6%. The polysaccharides were fractionated into six fractions, using HPLC and NMR to analyze their sugar compositions and structures. The HPLC analysis results showed that UA contained two neutral polysaccharide fractions and four acidic fractions. The neutral fractions are mainly composed of glucose (Glc), and the acidic fractions are mainly composed of galacturonic acid (GalA). The NMR results showed that the neutral fractions are starch-like polysaccharides and the acidic fractions are rhamnogalacturonan (RG)-I domian containing pectin.

Fatigue is a complex process of physiological and biochemical changes, generally referring to recession network of physical and mental state leading to a decline in working ability and efficiency. The fatigue results are multi-faceted; lactic acid accumulation, glycogen lack and material consumption of energy are the major cause of fatigue. Nettle polysaccharide can replenish the energy. The length of swimming time may reflect the extent of exercise-induced fatigue, a strong indicator to evaluate the ability of anti-fatigue exercise. In this study, the length

Table 1. Yield and monosaccharide composition of collected fractions.

Fraction	Yield (g%)[a]	Monosaccharide composition (%)							Structure feature
		Gal	Glc	Ara	Rha	Man	GalA	GlcA	
UA	1.6	8.2	64.3	5.9	2.6	1.1	15.9	1.3	-
UAN	37.4	4.1	92.4	1.5	-	2.1	-	-	Starch-like glucans and arabinogalactans
UAA	44.6	14.7	30.6	10.8	4.6	1.8	35.3	2.2	-
UAA-1	25.9	-	100.0	-	-	-	-	-	Starch-like glucans
UAA-2	6.4	11.9	78.0	7.9	-	2.2	-	-	Starch-like glucans and arabinogalactans
UAA-3	42.0	20.5	4.8	17.0	5.8	1.4	45.8	3.6	-
UAA-4	21.5	14.2	9.6	12.0	8.9	0.8	51.9	9.6	-
UAA-3-1	12.7	41.9	4.8	31.5	7.0	2.3	9.3	4.8	AG-I containing RG-I domains
UAA-3-2	54.1	9.0	4.5	7.1	3.5	1.6	72.1	4.5	α-(1-4)-GalA backbone (HG)

[a]Yield in relation to fraction applied into column.

Table 2. Effect of the *U. angustifolia* polysaccharides on the body weight, swimming time of mice.

Group	Swimming time (s)	Percent of increase to control
Control doses (CD)	41±28	-
Low doses (LD)	76±40	85.4
Middle doses (MD)	88±47	114.6
High doses (HD)	103±80*	151.2

*p < 0.01(compared with CD).

Table 3. Effect of the *U. angustifolia* polysaccharides on the blood urea nitrogen of mice.

Group	Dose (mg/kg)	Blood uria nitrogen (BUN) (mmoL/L)	Percent of decrease to control
Control doses (CD)	-	10.59±0.61	-
Low doses (LD)	100	9.39±1.04*	12.8
Middle doses (MD)	200	9.32±1.65*	13.6
High doses (LD)	300	8.84±0.76**	19.8

*p < 0.05, **p < 0.01(compared with CD).

Table 4. Effect of *U.angustifoli* polysaccharides on the hepatic glycogen of mice.

Group	Dose (mg/kg)	Nitrogen content (mg/g)	Percent of increase to control
Control doses (CD)	-	0.23±0.075	-
Low doses (LD)	100	0.43±0.035	87
Middle doses (MD)	200	1.31±0.81**	496.6
High doses (HD)	300	1.95±0.478**	747.8

**p < 0.01(compared with CD).

of swimming time of nettle polysaccharide dose group was longer than the duration of swimming, playing anti-fatigue role.

Studies have shown that blood urea nitrogen levels of the body increases as the exercise load increase. Determining the changes of blood urea nitrogen in the movement is a simple and feasible method of knowing protein catabolism. From this experiment, blood urea nitrogen levels decreased as the dose increased, in which high-dose levels of blood urea nitrogen were significantly lower than the control group. This product can reduce the catabolism of protein and nitrogen-containing compounds, and enhance the body's ability to adapt to the exercise. The crude polysaccharides (UA)

obtained from *U. angustifolia* has a strong anti-fatigue effect. This indicated that the extractions can be efficient as a potent functional food.

REFERENCES

Bnouham M, Merhfour F, Ziyyat A, Mekhfi H, Aziz M, Legssyer A (2003). Antihyperglycemic activity of the aqueous extract of *Urtica dioica*. Fitoterapia 74:677-681

Blumenkrantz N, Asboe-Hansen G (1973). New method for quantitative determination of uronic acids. Anal. Biochem. 54:484-489.

Catoire L, Goldberg R., Pierron M., Morvan C., Herve´ Du Penhoat C (1998). An efficient procedure for studying pectin structure which combines limited depolymerization and C-^{13} NMR. Eur. Biophys. J. 27:127–136.

Dong YJ, ZhangHY, Guo XL, Li DF (2007). A new kind of health food resources- development and utilization of *Urtica angustifolia*. Sci. Technol. Food Ind. 12:213-215

Dubois M, Gilles KA, Hamilton JK, Rebers PA (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Ding XH, Gao Y, Chao H, Xia D (2008). Study on Anti-fatigue Effect of Polysaccharide from Moso Bamboo Leaves. Food Sci. 29(4):389-391.

Guo X L, Zhang H Y, Li D F (2006). A new food resources of development value - nettle. Food Technol. 3:58-60

Gane AM, Craik D, Munro SLA, Howlett GJ, Clarke AE, Bacic A (1995). Structural analysis of the carbohydrate moiety of arabinogalactan-proteins from stigmas and styles of *Nicotiana alata*. Carbohydr. Res. 277:67-85

Konrad L, Müller HH, Lenz C (2000). Antiproliferative effects on human prostate cancer cells by a stinging nettle root (*Urtica dioica*) extract. Planta Med. 66(1):44-47

Kraus R, Spiteller G (1990). Lignanglucoside aus Wurzeln von *Urtica dioica*. Liebigs Ann Chem. 12:1205-1213.

Kavtaradze NS, Alaniya MD, Aneli JN (2001). Chemical compounds of *Urtica dioica* growing in Georgia. Chem. Nat. Compd. 37(3):287.

McNeil M, Darvill AG, Albersheim P. (1980). Structure of plant cell walls. X. Rhamnogalacturonan I, a structurally complex pectic polysaccharide in the walls of suspension-cultured sycamore cells. Plant Physiol. 66:1128-1134.

Newall CA, Anderson LA, Phillipson JD (1996). Herbal medicines: a guide for health-care professionals. London: The pharmaceutical press. P 201.

Neugebauer W, Winterhalter P, Schreier P (1995). 3-Hydroxy-α-ionyl-β-D- glucopyranosides from stinging nettle (*Urtica dioica*) leaves. Nat. Prod. Lett. 6(3):177-180.

Polle AY, Ovodova RG, Shashkov AS, Ovodov YS (2002). Some structural features of pectic polysaccharide from tansy, *Tanacetum vulgare* L. Carbohydr. Polym. 49:337-344.

Pérez S, Rodríguez-Carvajal MA, Doco T (2003). A complex plant cell wall polysaccharide: rhamnogalacturonanII. A structure in quest of a function. Biochimie 85:109-121.

Petlevski R, Hadžija M, Slijepčević M, Juretić D (2001). Effect of antidiabetis herbal preparation on serum glucose and fructosamine in NOD mice. J. Ethnopharmacol. 75:2-3

Quan CL, Guo CL (2007). Research on hypoglycemic constituents of natural medicines. J. Liaoning Med. Univ. 28(6):86-88.

Schulze-Tanzil G, Souza PH, Behnke B (2002). Effects of the antirheumatic remedy Hox alpha-a new stinging nettle leaf extract on matrix metalloproteinases in human chondrocytes *in vitro*. Histol, Histopathol. 17(2):477-485

Sedmark JJ, Grossberg SE (1979). A rapid,sensitive,and versatile assay for protein using Coomassie brilliant blue G250. Anal. Biochem. 79:544-552.

Shuqun S, Robert C (1997). Structure of the capsular polysaccharide of Clostridium Perfringens Hobbs 10 determined by NMR spectroscopy. Carbohydr. Res. 305:65-72.

Schols HA, Voragen AGJ (1996). Complex pectins: Structure elucidation using enzymes. In: Visser J, Voragen AGJ (eds.), Pectins and Pectinases. Amsterdam: Elsevier Sci. BV. pp. 3-19.

Vidal S, Williams P, Doco T, Moutounet M, Pellerin P(2003). The polysaccharides of red wine: Total fractionation and characterization. Carbohydr. Polym. 54:439-447.

Wang XS, Liu L, Fang JN. (2005). Immunological activities and structure of pectin from *Centella asiatica*. Carbohydr. Polym 60:95-101.

Yapo BM Lerouge P, Thibault JF, Ralet MC (2007). Pectins from citrus peel cell walls contain homogalacturonans homogenous with respect to molar mass, rhamnogalacturonan I and rhamnogalacturonan II. Carbohydr. Polym. 69:426-435.

Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W (1997). Phytotherapy of hypertension and diabetes in oriental Morocco. J. Ethnopharmacol. 58(1):45-54.

Zhang HY, Yan XJ, Jiang Y, Han YS, Zhou YF (2011). The extraction, identification and quantification of hypoglycemic active ingredients from stinging nettle (*Urtica angustifolia*). Afr. J. Biotechnol. 10(46):9428-9437.