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Preparation, partial characterization and bioactivity of water-soluble polysaccharides from boat-fruited sterculia seeds

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Abstract

Crude water-soluble polysaccharides (SP) isolated from boat-fruited sterculia seeds by hot water extraction and ethanol precipitation were fractionated into a neutral polysaccharide (NSP) and an acidic one (ASP) by anion-exchange chromatography. The molecular weight, intrinsic viscosity and radius of gyration of NSP and ASP were determined by high performance size exclusion chromatography (HPSEC). NSP was rich in glucose (85.86%), with small amounts of galactose, arabinose and xylose. Whereas ASP consisted mainly of galacturonic acid (40.13%) along with rhamnose, arabinose, galactose, and small amounts of xylose and glucose, indicating a pectin-like polysaccharide which was confirmed by FT-IR spectra. Bioactivity of NSP and ASP was tested using ear edema induced by dimethylbenzene and cotton pellet-induced granuloma tissue in murine models. The results showed ASP possessed a potent dose-dependent anti-inflammatory activity. The results from the current study provided a scientific basis for the traditional use of this plant as a medical remedy for its anti-inflammation effects.

Keywords: Boat-fruited sterculia seed; Polysaccharides; Preparation; Characterization; Anti-inflammatory activity

1. Introduction

Boat-fruited sterculia seed (*Semen Sterculiae Lychnophorae*) is a tropical herb of the Sterculiaceae family, mainly distributed in Vietnam, Thailand, Malaysia, Indonesia, as well as South China (Wang et al., 2003). This plant is commonly used for the treatment of many diseases such as clearing phlegm (by "clearing heat from the lungs" as explained in Chinese medicine), relieving sore throat to restore the voice on the upper respiratory tract, and relaxing the bowels to relieve constipation (Xiao, 2002). It has been used for hundreds of years in traditional Chinese medicinal prescriptions because of its pharmacological effects.

In recent years, medicinal plant polysaccharides have been widely studied in order to understand their relations between physicochemical properties and biological activities (Inngjerdingen et al., 2005; Sun, Tang, Gu, & Li, 2005). Most of the polysaccharides isolated from medicinal plants, including pectic polysaccharides, not only are the main structural components of plant cell walls but also possess immunomodulatory, complement-modulating, anti-HSV (Herpes simplex virus), anti-inflammatory activities (Xu, Lee, Lee, White, & Blay, 1999; Capek & Hříbalová, 2004; Hokputsa et al., 2004; Popov, Popova, Ovodova, & Ovodov, 2005). However, the properties of water-soluble polysaccharides from boat-fruited sterculia seeds were rarely reported (Chen, Li, Shen, Peng, & Xu, 1994; Chen, Cao, & Song, 1996; Somboonpanyakul, Wang, Cui, Barbut, & Jantawat, 2006). The aqueous extracts from boat-fruited sterculia seeds were reported to exhibit potent

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anti-inflammatory property as shown by the inhibition of croton oil-induced acute inflammation in rats and antibacterial activity against the *Escherichia coli* and *Bacillus dysenteriae* (Du, Sun, Yu, Chen, & Wu, 1995; Yu & Zhu, 1997). In the present study, we report the preparation and partial characterization of water-soluble polysaccharides from boat-fruited sterculia seeds and investigate their potential anti-inflammatory bioactivity in murine models.

2. Materials and methods

2.1. Preparation and partial characterization of water-soluble polysaccharides from boat-fruited sterculia seeds

2.1.1. Plant material and chemicals

The boat-fruited sterculia seeds harvested in Vietnam were provided by Shanhe Pharmaceutical Co. Ltd. (Wuxi, China). Sepharose CL-6B was from Pharmacia (Amersham Bioscience, Uppsala, Sweden). All chemicals used were reagent grade unless otherwise specified.

2.1.2. Extraction and purification of water-soluble polysaccharides

Water-soluble polysaccharides were extracted and purified according to the schematic diagram in Fig. 1. Boat-fruited sterculia seeds were ground into powder (Particle size: 420 μm) using high speed disintegrator (Model SF-2000, Chinese traditional medicine machine works, Shanghai, China) and were defatted in a Soxhlet apparatus with petroleum ether (30–60 °C) for 5 h. The defatted residue was pre-extracted with 80% ethanol twice to remove some colored materials, oligosaccharides and some other low molecular weight compounds, and the organic solvent left in the residue was volatilized at room temperature, as described previously (Chen et al., 1996). The pretreated dry powder was extracted twice with deionized water (water to seed ratio (ml/g) at 75:1) under constant stirring for 2.5 h in a 65 °C water bath. The mixture was centrifuged (2000*g*, 20 min), then the supernatant was separated from insoluble mucilage with nylon cloth (Pore diameter: 38 μm). The extracts were precipitated by the addition of ethanol to a final concentration of 75% (v/v), and the precipitates were collected by centrifugation, washed with acetone, dissolved in deionized water and finally lyophilized. Dark reddish brown crude water-soluble polysaccharides (SP) were obtained.

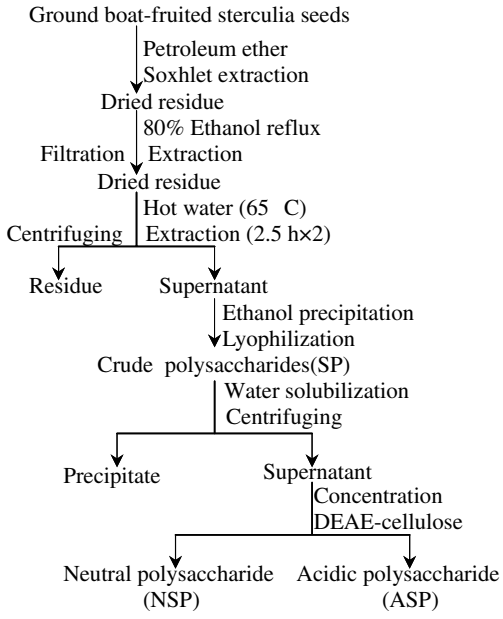

Fig. 1. Scheme of extraction and fractionation of water-soluble polysaccharides from boat-fruited sterculia seeds.

SP were dissolved in hot deionized water (50 °C) to a concentration of 1.5% (g/ml), centrifuged (10,000*g*, 15 min) to remove the insoluble substance, then the supernatant was decanted and concentrated. The concentrated SP were fractionated by anion-exchange chromatography on DEAE-Cellulose column (D 2.6 × 30 cm) equilibrated with 0.02 M acetic acid–sodium acetate buffer (pH 5.0). The column was first eluted with the same buffer at a flow rate of 2.0 ml/min followed by a linear gradient of NaCl concentration (0–2.0 M). Neutral polysaccharide (NSP) and acidic one (ASP) were collected with a fraction collector, concentrated using a rotary evaporator at 50 °C, dialyzed for 3 days and lyophilized. ASP was then loaded onto a Sepharose CL-6B gel column (D 1.6 × 100 cm) and eluted with phosphate buffer at a flow rate of 0.3 ml/min. The eluate obtained was pooled, concentrated, dialyzed and lyophilized. The eluting fractions were monitored for the presence of carbohydrate using phenol–sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and protein by Coomassie Brilliant Blue method (Bradford, 1976).

2.1.3. Analytical methods

Total neutral sugar content was determined by the reaction with phenol in the presence of sulfuric acid (Dubois et al., 1956) using glucose as a standard. Proteins in the solution were estimated by the method of binding of Coomassie Brilliant Blue G-250 to protein (Bradford, 1976) using bovine serum albumin as a standard. The total uronic acid content was colorimetrically determined by the *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid as a standard. The type of uronic acid was distinguished by zymohydrolysis of Driselase (Sigma–Aldrich) using a Dionex HPAEC system equipped with pulsed amperometric detection (Sunnyvale, CA) (Nakamura, Furuta, Maeda, Takao, & Nagamatsu, 2002). Moisture and ash were determined according to AOCS (1997) and AOAC (2005) methods (AOCS, Ba 2a-38; AOAC 942.05), while the protein content in the solid polysaccharide was determined using the Kjeldahl method with a conversion factor of 6.25 (AOCS Ba 4a-38).

2.1.4. Molecular weight and intrinsic viscosity measurement

The molecular weight, intrinsic viscosity and radius of gyration of the polysaccharides were determined using high performance size exclusion chromatography (HPSEC) (Wang, Wood, Cui, & Ross-Murphy, 2001; Wang, Wood, Huang, & Cui, 2003). The HPSEC system used consists of a Shimadzu SCL-10Avp pump unit and automatic injector (Shimadzu Scientific Instruments Inc., Columbia, Maryland 21046, USA), Viscotek Triple detectors (Viscotek Co., Houston, TX) composed of a refractive index detector (RI, Model 200), a viscometer (DP, Model 250) and a right angle laser light scattering detector (RALLS, Model 600), and two columns in series: a Shodex Ohpak KB-806M (Showa Denko K.K., Tokyo, Japan) and an Ultrahydrogel linear (Waters, Milford, CT, USA). The mobile phase was 100 mM $NaNO_3$ with a flow rate of 0.6 ml/min, and the injection volume of sample was 100 μl. Pullulans (P-82, JM Science, Inc., NY, USA) of known molecular weight and intrinsic viscosity were used as standards and d*n*/d*c* of 0.146 ml/g as a refractive index increment was used for polysaccharides solution. The polysaccharides were dissolved in 100 mM $NaNO_3$ (60 °C, 1 h), cooled and filtered through a 0.45 μm nylon filter prior to injection into the column. The weight average molecular weight, intrinsic viscosity and radius of gyration were calculated using the software TriSEC provided by Viscotek.

2.1.5. Monosaccharide composition of polysaccharides

The monosaccharide composition analysis was performed by gas chromatography (GC-14A, Shimadzu, Japan) (Erbing, Jansson, Widmalm, & Nimmich, 1995). The polysaccharides were first hydrolyzed using 2 M trifluoroacetic acid (121 °C, 1 h) into monosaccharides before derivatization as their alditol acetates, were separated by a capillary column (i.d. 0.53 mm × 30 m, SPB-5) at a temperature program of 150–190 °C (10 °C/min) to 240 °C (2 °C/min) and detected with a flame ionization detector (FID). Inositol was used as internal standard. The percentage of monosaccharides in the sample was calculated from the peak areas using response factors.

2.1.6. Infrared spectra of water-soluble polysaccharides

The IR spectra of the polysaccharides were determined using a Fourier transform infrared spectrophotometer (Nexus 5DXC FT-IR, Thermo Nicolet, America). The sample was ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into 1 mm pellets for FT-IR measurement in the frequency range of 4000–400 cm^{-1} (Mid infrared region).

2.2. Anti-inflammatory activities assessment of water-soluble polysaccharides

2.2.1. Animals

Male Kunming (KM) mice weighing 23–26 g and male Sprague Dawley (SD) rats weighing 100–120 g were used for the assessment of the anti-inflammatory activity. The animals were kept in polyethylene boxes at room temperature, housed under standard environmental conditions and fed with standard rodent diet and water ad libitum.

2.2.2. Acute anti-inflammatory activity

The acute anti-inflammatory activity was evaluated by dimethylbenzene-induced mice ear edema (Sosa et al., 2002; Yu, Song, Zhao, Bin, & Zhang, 2004). The mice were divided randomly into six groups (eight mice per group). NSP (200 mg/kg d) and ASP (50, 100 and 200 mg/kg d) were prepared with normal saline, and were administered to mice of the four test groups. Positive control group mice were treated orally with aspirin at the dose of 100 mg/kg d dissolved in saline. Normal group received the same amount of normal saline. Thirty microliters of dimethylbenzene was applied to the inner and outer surface of the right ear of each mouse after oral administration on the fifth day. The mice were killed an hour later, and ear discs from two ears were made using a cork borer with the diameter of 8 mm. The oedema response was measured as weight difference of between right and left ear discs and the acute anti-inflammatory activity was expressed as percentage of oedema reduction in treated mice with regard to normal mice.

2.2.3. Chronic anti-inflammatory activity

The chronic anti-inflammatory activity was assessed by the cotton pellet-induced hyperplasia of granuloma tissue method (Diniz, Garla, Schneedorf, & Carvalho, 2003). Cotton pellets weighing 20 mg were made with 5 mm (diameter) of dental cotton tampon. The pellets were sterilized, and impregnated with 0.4 ml of 5% ampicillin aqueous solution at the moment of implantation. After the animals were anesthetized by pentobarbital sodium, the pellets were subcutaneously introduced through abdominal skin incisions. Then the rats were separated stochastically into five groups: normal group (Oral administration of normal saline), positive control (100 mg/kg d aspirin), tested groups (50, 100 and 200 mg/kg d ASP), six rats per group. The administration was initiated 24 h later following the implantation of the pellets and continued until the 14th day. After oral administration on the 15th day the animals were first weighed, and an hour later were killed with overdose ether. The granulomas were removed and left to dry for 2 h in an 80 °C oven. The difference between the initial and the final weight was the weight of the granulomatous tissue sample produced. The chronic anti-inflammatory activity was estimated as percentage of granulomas reduction against animal weight in treated rats compared to normal control ones.

2.2.4. Statistical analysis

The results were expressed as mean ± standard deviations and a *t*-test was used to evaluate the significance of differences between the treated and control groups.

3. Results and discussion

3.1. Preparation of water-soluble polysaccharides

The yield of SP obtained was 15.2% (w/w), based on dried boat-fruited sterculia seeds. SP were further fractionated by ion-exchange chromatography on a DEAE-Cellulose column eluted with buffer and NaCl (0–2.0 M) linear gradient, and were separated into NSP and ASP (see Fig. 2). The recovery of the eluted polysaccharides was 80.4%. Size-exclusion chromatography on a Sepharose CL-6B column did not lead to further separation of ASP, and only a single symmetrical peak appeared.

Appearance, yield, moisture, ash, protein and total carbohydrate content of SP and its fractions obtained after the anion-exchange chromatography are given in Table 1. After solubilization, centrifuge and ion exchange chromatography, the color of the polysaccharides changed from dark brown (SP) to white (NSP) and slightly yellow (ASP). The protein content was reduced significantly whearas the total carbohydrate content was increased, as shown in Table 1.

3.2. Molecular weight and intrinsic viscosity

The chromatograms from the HPSEC analysis of NSP and ASP fractions are shown in Fig. 3. The molecular weight, intrinsic viscosity and radius of gyration of each fraction are summarized in Table 2. The weight average molecular weight (Mw) of ASP was 1,125,000 Da, more than double that of NSP (586,800 Da). Chemical analysis indicated that the ash content of ASP (5.05%) was also

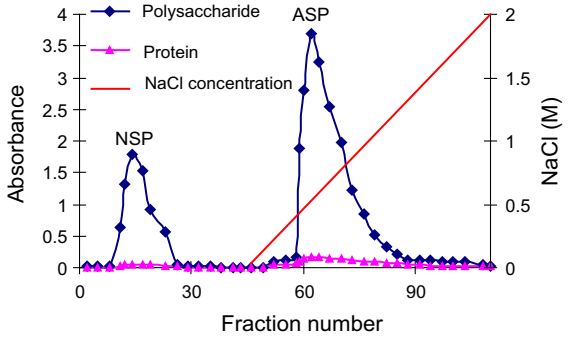

Fig. 2. Elution profile of water-soluble polysaccharides extracted from boat-fruited sterculia seeds on DEAE-Cellulose column.

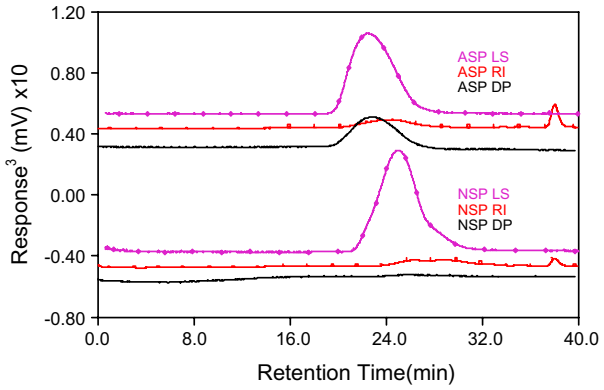

Fig. 3. High performance size exclusion chromatograms of fractions of water-soluble polysaccharides from boat-fruited sterculia seeds.

higher than that of the NSP (1.17%), indicating that the acidic polysaccharides probably are in the salt form. It is well known that the increase in Mw is concerned with some structural associations among polysaccharide molecules, for example divalent ions, such as Ca^{2+}, can interact with uronic acid from polysaccharide molecules to form supramolecular aggregates, or in the extreme case form gel networks via the egg box model (Braccini, Grasso, & Pérez, 1999). Molecular weight of ASP was also higher than that of water extracted polysaccharide obtained from the same species of boat-fruited sterculia seed (162,200 Da) (Chen et al., 1996), but it was lower than that of alkaline extracted gum from the different species [*Scaphium scaphigerum* (G. Don) Guib and Planch] (3,300,000 Da) (Somboonpanyakul et al., 2006). This may reflect the differences in growing region, plucking time and species of boat-fruited sterculia seed, as well as in the extraction approaches. The HPSEC method also provided the intrinsic viscosity [η] and radius of gyration (Rg) of NSP and ASP, which reflect the conformation of the polymers in the solvent system. The intrinsic viscosity values are in agreement with the results (NSP

Table 2
Molecular weight (Mw), intrinsic viscosity ([η]) and radius of gyration (Rg) of NSP and ASP fractions obtained by SP purification

Polysaccharide fraction	Molecular weight Mw (Da)	Intrinsic viscosity [η] (dl/g)	Radius of gyration Rg (nm)
NSP	586,800	0.129	12.42
ASP	1,125,000	2.242	42.46

Table 1
Chemical analysis of polysaccharide fractions obtained after separation of the crude extracts SP on the DEAE-Cellulose column

Polysaccharide fraction	Appearance	Yield wt.%	Moisture wt.%	Ash wt.%	Protein wt.%	Total carbohydrate wt.%
SP	Dark reddish brown powder	15.2 ± 0.61[a]	9.15 ± 0.43	4.47 ± 0.27	24.66 ± 0.61	61.17 ± 1.16
NSP	White fluffy	19.6 ± 0.69[b]	7.08 ± 0.73	1.17 ± 0.32	2.51 ± 0.43	89.06 ± 2.75
ASP	Slightly yellow fluffy	60.8 ± 1.08[b]	6.88 ± 0.86	5.05 ± 0.71	3.02 ± 0.51	86.38 ± 1.74

[a] Of the dry plant raw material.
[b] Based on the SP.

0.14 dl/g, ASP 2.04 dl/g) measured using an Ubbelohde Viscometer.

3.3. Monosaccharide composition and FT-IR spectroscopy of water-soluble polysaccharides

The uronic acids in SP and ASP were identified as galacturonic acid. The content of galacturonic acid and neutral sugars in SP, NSP and ASP are shown in Table 3. SP appeared as a heterogeneous mixture of polysaccharides consisted of neutral sugars (50.52%) and galacturonic acid (10.65%), as well as substantial amount of proteins (24.66%). The neutral sugars were composed mainly of glucose, rhamnose, arabinose, galactose and xylose. NSP was regarded as a neutral polysaccharide as it was only eluted out with HAc-NaAc buffer in the anion exchange chromatography (DEAE-Cellulose) and contained no acidic sugar. Glucose (85.86%) appeared to be the major monosaccharide, followed by small amounts of galactose, arabinose and xylose. ASP eluted with NaCl solution ranging from 0.2 to 1.0 M was acidic polysaccharides as galacturonic acid was found in the fraction and contained certain amount of protein (3.02%). ASP was rich in galacturonic acid (40.13%), rhamnose (11.36%), arabinose (17.46%) and galactose (15.70%), as well as xylose (0.63%) and glucose (0.35%) in a decreasing order of mol ratio: Rha:Ara:Gal:Xyl:Glu = 1.00:1.68:1.26:0.06:0.03, and the kinds of sugar are similar to pectic polysaccharide (Phillips & Williams, 2000). This sugar composition is somewhat different from the aqueous extracted polysaccharide reported by Chen et al. (1996) and alkaline extracted gum from Malva nut (Somboonpanyakul et al., 2006). But the major components of the three polysaccharides have a similar monosaccharide ratio of Rha:Ara:Gal = 1.00:1.68:1.26, 1.00:1.67: 1.01 and 1.00:1.10:1.00 respectively, i.e. a significantly high proportion of arabinose. Moreover, the uronic acid content in malva nut gum (6.40%) was much lower than that in ASP (Fig. 4).

The FT-IR spectra of the fractions of water-soluble polysaccharides are presented in Fig. 3. ASP showed IR absorbance band at 3394 cm^{-1} (Corresponding to the absorption due to stretching of the hydroxyl groups, –OH), 2937 and 1383 cm^{-1} (Attributed to the C–H stretching and bending vibrations), 1740 and 1254 cm^{-1} (The esterified carboxylic groups, –COOR), and 1622 and 1416 cm^{-1} (The carboxylate anions groups, $–COO^-$ asymmetric and symmetric stretching) (Gnanasambandam & Proctor, 2000; Singthong, Cui, Ningsanond, & Goff, 2004). Each particular polysaccharide has a specific band in the 1200–1000 cm^{-1} region (Kačuráková, Capeka, Sasinková, Wellner, & Ebringerová, 2000). This region is dominated by ring vibrations overlapped with stretching vibrations of (C–OH) side groups and the (C–O–C) glycosidic band vibration. ASP rich in uronic acid showed intense peaks at 1146, 1070, and 1035 cm^{-1} which are characteristic peaks of pectic polysaccharides (Coimbra, Barros, Rutledge, & Delgadillo, 1999). But NSP, which contains no uronic acid, exhibited a different spectral pattern from ASP: no 1740 and 1254 cm^{-1} absorbance and 1200–1000 cm^{-1} characteristic peaks, indicating lack the presence of GalA.

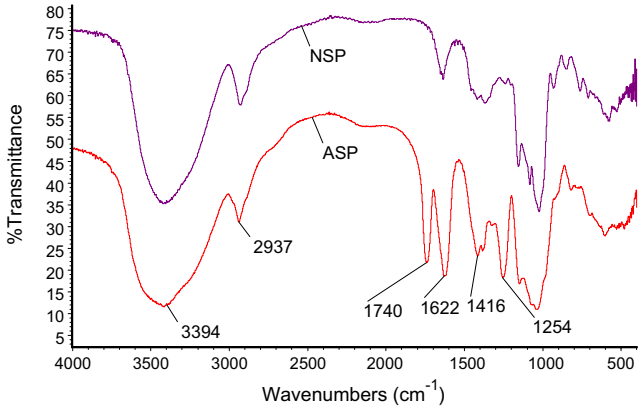

Fig. 4. Fourier transform infrared spectra of fractions of water-soluble polysaccharides from boat-fruited sterculia seeds. NSP, neutral polysaccharide; ASP, acidic polysaccharide.

3.4. Anti-inflammatory activities assessment of water-soluble polysaccharides

It was reported that many polysaccharides exhibited anti-inflammatory activity (Pereira, Silva, Pereira, & Parente, 2000; Pereira da Silva & Paz Parente, 2001; Pereira da Silva & Paz Parente, 2003; Popov et al., 2005). Acute anti-inflammatory activity of NSP and ASP was tested in a model of ear edema formation in mice induced by dimethylbenzene. The inhibition of ear edema induced by the stimulus was significantly different between the normal group and test ones ($P < .05$). From the inhibition rate,

Table 3
Galacturonic acid, neutral sugars and sugar composition of SP, NSP and ASP

Polysaccharide fraction	Gal A (%)[a]	Neutral sugars (%)[a]	Contents of the neutral sugar residues (%)[a]				
			Rha	Ara	Xyl	Glu	Gal
SP	10.65 ± 0.42	50.52 ± 1.36	10.00	7.88	0.77	25.65	5.04
NSP	n.d.[b]	89.06 ± 2.75	n.d.[b]	0.89	0.59	85.86	1.35
ASP	40.13 ± 1.77	46.25 ± 1.50	11.36	17.46	0.63	0.35	15.70

[a] Contents of galacturonic acid (GalA), neutral sugars and sugar residues are calculated as wt.%.
[b] Not detected.

NSP (3.21%) was shown to exhibit a very low or negligible anti-inflammatory activity whereas ASP presented a significant inhibitory effect, as shown in Table 4. As a result, NSP was not taken for further consideration following the experiment. The activity of ASP was dose-dependent at the range of low concentration studied and reached nearly maximum at the concentration 200 mg/kg d with the inhibition rate of 26.29%. At the same time, the inhibition rate of ASP (26.29%) on the inflammation is 85.36% that of aspirin (30.80%) (a good anti-inflammatory medicine) used in this study. This indicated that ASP could play a good inhibition role on the acute inflammation.

Chronic anti-inflammatory activity of ASP was estimated by a well-accepted model of hyperplasia of granuloma tissue in rats, and the results are presented in Table 5. Oral administration of ASP brought about a statistically significant inhibition in the chronic inflammation. The inhibition of granuloma tissue was significantly different between the normal group and the test ones ($P < .01$) and between the positive control and test groups ($P < .05$). The anti-inflammatory effect was enhanced by increasing concentrations of ASP. At the dose of 200 mg/kg d inhibition rate of ASP (28.38%) was 82% of aspirin at a dose of 100 mg/kg d (34.38%).

These results from the present study suggest that ASP from boat-fruited sterculia seeds exhibits good acute and chronic anti-inflammatory activities, and it may be a potential therapeutic agent for inflammatory disorder. Compared with other anti-inflammatory polysaccharides such as the pectic polysaccharide from *Comarum palustre*, acidic polysaccharide from the fruit bodies of *Auricularia* species and polysaccharides from cultured *Cordyceps militaris*, *Costus spicatus*, *Cyrtopodium cardiochilum*, *Centaurea cyanus* flower-heads and *Orbignya phalerata*, ASP belongs to polysaccharides with medium inhibition rate (Kiho, Sakai, Uka, Hara, & Tanaka, 1985; Garbacki et al., 1999; Pereira da Silva et al., 2001; Pereira da Silva et al., 2003; Yu et al., 2004; Popov et al., 2005; Barreto & Parente, 2006). Generally, an inflammatory stimulus makes the body release mediators producing the inflammation, such as prostaglandin, kinin and histamine. In fact the anti-inflammation is to block the cyclo-oxygenase or lipoxygenase pathways which synthesize the mediators (Choi & Koo, 2005). Moreover, the important aspect of the anti-inflammatory process is the leukocytes migration to the inflamed area to protect the body (Carvalho et al., 1999). Leukocyte adhesion represents one of the first steps in the anti-inflammatory response initiation and it is essential for accumulation of active immune cells at the sites of inflammation (Popov et al., 2005). Therefore, it is logical to study the possible relations between ASP and oxygenase or leukocytes, as well as the anti-inflammatory mechanism of ASP, in further investigations.

Table 4
Effects on ear edema induced by dimethylbenzene in mice

Group	Dose (mg/kg d)	Edema (X ± S, mg)	Edema rate (%)	Inhibition rate (%)
Normal control	0	18.69 ± 3.22	106.12 ± 4.73	0
Positive control	100	12.38 ± 2.12**	73.43 ± 5.13	30.80
NSP test group	200	16.83 ± 2.44*△	102.71 ± 3.39	3.21
ASP test group	50	15.32 ± 3.52*	91.51 ± 5.62	13.76
ASP test group	100	14.02 ± 2.82*△△	81.84 ± 4.55	22.88
ASP test group	200	13.96 ± 3.32**△	78.22 ± 6.01	26.29

* $P < .05$.
** $P < .01$ vs. the normal control.
△ $P < .05$.
△△ $P < .01$ vs. the positive control.

Table 5
Effects on hyperplasia of granuloma tissue induced by cotton-pellet in rats

Group	Dose (mg/kg d)	Granuloma/rat weight (X ± S, mg/100 g)	Inhibition rate (%)
Normal control	0	46.02 ± 6.01	0
Positive control	100	30.20 ± 5.82**	34.38
ASP test group	50	39.39 ± 8.02**△	14.42
ASP test group	100	34.03 ± 5.66**△	26.06
ASP test group	200	32.96 ± 6.58**△	28.38

** $P < .01$ vs. the normal control.
△ $P < .05$.

4. Conclusions

Water-soluble polysaccharides from boat-fruited sterculia seeds were obtained using hot water extraction and then fractionated into two fractions by DEAE-Cellulose anion exchange and gel-filtration chromatography. The molecular weight, intrinsic viscosity and radius of gyration of ASP were much higher than those of NSP, and their values were determined by HPSEC to be 1,125,000 and 586,800 Da, 2.242 and 0.129 dl/g, 42.46 and 12.42 nm, respectively. NSP was rich in glucose, and contained a little amount of galactose, arabinose and xylose. ASP was considered to be the major constituent for the water-soluble crude extracts and was comprised mainly of galacturonic acid (40.13%) along with rhamnose, arabinose, galactose, xylose and glucose in the molar ratio of: 1.00:1.68:1.26:0.06:0.03. The monosaccharide composition indicates a pectin-like polysaccharide which was further confirmed by FT-IR spectra to contain –COOR, $–COO^-$ and 1200–1000 cm^{-1} characteristic peaks of GalA. The data presented convincingly demonstrate that ASP exhibited relatively good acute and chronic anti-inflammatory activity by oral administration using in vivo murine models. The present study provided evidence that the acidic polysaccharides may be the important active ingredient in traditionally prepared remedies made from boat-fruited sterculia seeds.

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