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Preliminary structural characterization and antioxidant activities of polysaccharides extracted from Hawk tea (*Litsea coreana* var. *lanuginosa*)

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ABSTRACT

Three polysaccharides were extracted from different leaf age Hawk teas (*Litsea coreana* var. *lanuginosa*) by hot water method. Preliminary structural characterization was conducted by physicochemical property, fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) analyses. Antioxidant activities against 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ferric reducing activity power (FRAP), hydroxyl radical and erythrocyte hemolysis were also evaluated. The physicochemical property analysis indicated significant differences in the three polysaccharides. The FTIR spectra revealed the general characteristic absorption peaks of the three polysaccharides. The SEM images demonstrated significant differences in the surface features of the different polysaccharides. The antioxidant activity assays revealed the obvious antioxidant activities of three polysaccharides, and the polysaccharides of Hawk primary leaf tea exhibited higher antioxidant activities than the other two polysaccharides. With current findings, the polysaccharides from Hawk primary leaf tea may have potential applications in food industries.

1. Introduction

Hawk tea (*Litsea coreana* var. *lanuginosa*), a medicinal and edible plant, is a herbal tea and one of the most popular traditional beverage in southwest of China. Hawk tea is produced from buds or leaves of *Litsea coreana* var. *lanuginose*. This arbor is widely distributed in the south of China. Most drinkers of this beverage live in the mountainous area of southwest China, and their population amounts to about 30 million. The infusion of Hawk tea is yellowish-red, with a slight camphor-aromatic smell, and it also has effects on detoxification and detumescence, benefit to eyesight, reduction of blood sugar and blood lipid (Ye, Liu, Zhang, Yang, & Wang, 2012). According to the maturity degree of the raw materials, Hawk tea can be divided into three types: Hawk bud tea (HB, made from the most tender shoots), Hawk primary leaf tea (HP, made from new leaves), and Hawk mature leaf tea (HM, made from mature leaves). The prices of three kinds of Hawk teas fluctuate dramatically because of their yields. The price of HB is about $50 per kilogram, while HP is about $20 per kilogram, the lowest price is HM which is just about $3 per kilogram.

Polysaccharides exist widely in plants. They are biopolymers comprised of monosaccharides linked together through glycosidic bonds. Their structures can be linear or contain branched side chains (Zong, Cao, & Wang, 2012). Many researches indicated that plant polysaccharides in general had strong antioxidant activities and could be explored as novel potential antioxidants (Wang & Luo, 2007; Yuan, Zhang, Fan, & Yang, 2008). Polysaccharides possess a wide range of biological properties, such as hematopoiesis (Sarker & Nahar, 2004), immunomodulatory activity (Yang, Jia, Meng, Wu, & Mei, 2006), anti-cancer (Li et al., 2010; Shang et al., 2003), and antioxidant activity (Yang et al., 2007).

To date, studies on Hawk tea mainly focus on their mineral elements (Yu & Gu, 2001), essential oil (Yu, Gu, & Ren, 2001) and flavonoids (Meng et al., 2012; Ye et al., 2012). No investigation has been carried out on polysaccharides of Hawk tea, and in this paper the structural characterization and antioxidant activities of polysaccharides from three types of Hawk teas are studied.

Polysaccharides from Hawk tea were extracted using hot water method. The structural characterization of the three polysaccharides was then conducted via physicochemical property, fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) analyses. Finally, the antioxidant activities of the three polysaccharides were estimated by 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ferric reducing activity power (FRAP), hydroxyl radicals and AAPH-induced erythrocyte hemolysis. The main aims

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of this research are to investigate the influence of polysaccharides from Hawk teas' different growth stages on its structures and antioxidant activities.

2. Materials and methods

2.1. Chemicals and reagents

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) and 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Ascorbic acid (Vc), 1,3,5-tri(2-pyridyl)-2,4,6-triazine (TPTZ) were purchased from the Sinopharm Chemical Reagent Co. (Beijing, China). All reagents were analytical grade, or were the highest grade available, and were used without further purification. Ultra-pure water was used throughout the experiments.

2.2. Plant materials

Three types of Hawk teas (*Litsea coreana* var. *lanuginose*) were purchased from local retail shops (Yucheng District, Sichuan Province, China). The samples were ground into fine powder using a powerful mill (FW177, Taisite Instrument Co., Ltd., Tianjin, China), and screened through a 40 mesh sieve. The materials were stored at room temperature in a desiccator until use.

2.3. Extraction

Dried Hawk tea powder was refluxed with anhydrous ethanol and petroleum ether for 4 h each to remove colored ingredients and small molecular impurities. The residues were dried at 50 °C for 24 h and extracted. The extractions were based on previously reported methods with some modifications (Wang & Luo, 2007). The hot water method that involved liquid/solid ratios of 1:10, a temperature 70 °C, and an extraction time of 8 h. After extraction, the supernatant and sediments were separated by vacuum filtration. The residues were re-extracted three times. All extraction solutions were condensed to about 100 mL. Ethanol (1000 mL, 100%, v/v) was slowly added to the condensed solution, which was left for 12 h at 4 °C. Precipitates were solubilized in deionized water, deproteinized by Sevag solution (chloroform:butyl alcohol, 4:1). The deproteinized solution was re-precipitated in anhydrous ethanol five times the solution volume. The precipitate was collected and successively washed with anhydrous ethanol. After drying, the polysaccharides were obtained and named HB, HP, HM according to leaf mature degree, respectively.

2.4. Physicochemical property analysis

The physical characteristics were analyzed by color and texture observation (Ge, Duan, Fang, Zhang, & Wang, 2009). The carbohydrate contents were determined by phenol-sulfuricacid colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The protein contents were measured by coomassie brilliant blue reaction (Bradford, 1976). The total polyphenol contents were estimated by Folin–Ciocalteu method (Lapornik, Prošek, & Golc Wondra, 2005).

2.5. FTIR analysis

The organic functional groups of the three polysaccharides were identified using a FTIR spectrophotometer (FTIR-8400S, Shimadzu Co., Japan) within 4000–400 cm^{-1} via the KBr pressed-disc method.

2.6. SEM analysis

The three polysaccharides were coated with a thin layer of gold under reduced pressure. They were then examined using a SEM system (JSM-7500F, JEOL, Japan) at a 5 kV acceleration voltage, as well as image magnifications of 1000× and 3000×.

2.7. Antioxidant activity analysis

2.7.1. DPPH radical scavenging activity

Radical scavenging activities by antioxidants in the Hawk tea extracts were evaluated by DPPH radicals (Razali, Razab, Junit, & Aziz, 2008). Polysaccharide samples were dissolved in distilled water to form sample solution in final concentrations of 0.25, 0.5, 0.75, 1 and 1.25 mg/mL, respectively. 0.5 mL of the diluted sample was mixed with 3 mL of 0.05 mmol/L DPPH methanol solution in a 5 mL cuvette. The mixtures were shaken vigorously and incubated in the dark for 20 min after that the reduction of DPPH• absorption was measured at 517 nm using the spectrophotometer (UV-1750, Shimadzu). The scavenging activity on DPPH radical was calculated by the following equation.

$$\text{DPPH radical scavenging activity } (\%) = \left[\frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}}\right] \times 100$$

where A_{DPPH} is the absorbance of the DPPH radical solution without sample and A_{sample} is the absorbance of the DPPH radical solution with tested samples.

2.7.2. Ferric reducing activity power

The FRAP assay was performed, as previously described by Vasco, Ruales, and Kamal-Eldin (2008) with some modifications. The fresh FRAP reagent was prepared before using, which contains 25 mL acetate buffer (300 mmol/L, pH 3.6), 2.5 mL TPTZ solutions (10 mmol/L in 40 mmol/L HCl) and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution (20 mmol/L). The reagent was warmed to 37 °C, then 500 μL was placed in a cuvette and the initiate absorbance was read. 20 μL of the sample solutions (0.25, 0.5, 0.75, 1 and 1.25 mg/mL) was added to the cuvette and the absorption value was determined at 593 nm. In this study, the reaction was followed until it reached the plateau. Values were calculated according to the calibration curve with aqueous solutions of $FeSO_4 \cdot 7H_2O$ in the range of 0–1800 μmol. The final results were expressed as the concentrations of $FeSO_4 \cdot 7H_2O$ with equivalent antioxidant activity.

2.7.3. Hydroxyl radical scavenging activity

Scavenging activity against hydroxyl radical was determined according to Fenton's reaction (Wu, Zhu, Zhang, Yang, & Zhou, 2012). The three polysaccharides were dissolved in water, yielding a series of sample solutions with different concentrations (0.25, 0.5, 0.75, 1 and 1.25 mg/mL). A mixture solution was prepared by mixing several solutions into 2 mL of PBS (pH 7.4) in the following order: 1 mL of water, 1 mL of 1,10-phenanthroline ethanol solution (0.75 mM), 1 mL of $FeSO_4$ (0.75 mM), and 1 mL of H_2O_2 (0.01%). The final mixture was incubated for 60 min at 37 °C, and was used as the blank solution. A similar procedure was used to prepare the control solution, wherein 1 mL of water instead of H_2O_2 was added. The absorbance of the blank (A_{blank}), control (A_{control}), and sample solutions (A_{sample}) was recorded at 510 nm. Hydroxyl radical scavenging activity was calculated using the following equation.

$$\text{Hydroxyl radical scavenging activity } (\%) = \left[\frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}}\right] \times 100$$

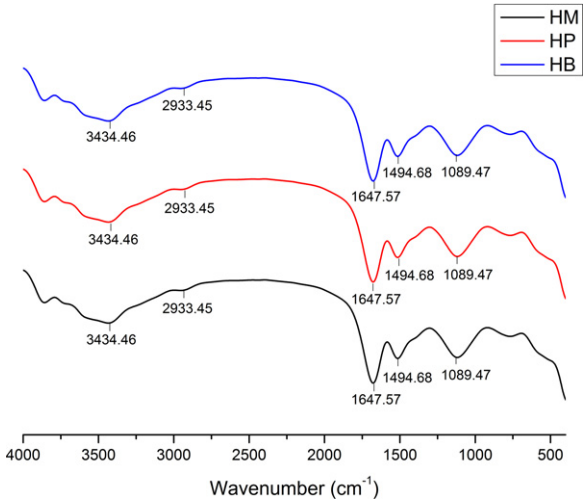

Fig. 1. Infrared spectra of polysaccharides: HM, HP and HB.

Table 1
Physical and chemical properties of HM, HP and HB.

Samples	HM	HP	HB
Color observation	Black	Brown	Brown
Texture	Tight	Loose	Loose
Carbohydrate (%)	80.09 ± 2.02^{a}	70.06 ± 1.15^{c}	76.85 ± 1.02^{b}
Protein (%)	2.96 ± 0.32^{a}	1.49 ± 0.19^{b}	nd
Total polyphenol (%)	2.31 ± 0.12^{b}	3.31 ± 0.21^{a}	1.76 ± 0.06^{c}

Each value is expressed as mean means ± standard deviation ($n = 3$). Means with different letters within a row are significantly different ($p < 0.05$); nd, not detected.

2.7.4. Erythrocyte hemolysis

Erythrocyte hemolysis was based on a method developed by Ng, Liu, and Wang (2000). Blood was isolated from human blood obtained from volunteers and collected in sodium citrate tubes. Erythrocytes were separated from plasma and the buffy coat was washed three times with 5 volumes of 0.15 mol/L NaCl. During the last washing, the erythrocytes were centrifuged at 2500 rpm for 10 min to obtain a constantly packed cell preparation. The reaction was initiated by mixing 0.25 mL of 200 mmol/L AAPH solutions to 0.25 mL of 10% erythrocyte suspension. Then, 0.25 mL of sample solution was added to the mixture. The reaction mixture was shaken gently while being incubated at 37 °C for 2 h. After incubation, the mixture was diluted with 4 volumes of 0.15 mol/L NaCl and centrifuged at 2500 rpm for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the mixture was treated with 4 volumes of distilled water to achieve complete hemolysis, and the absorbance B of the supernatant obtained after centrifugation

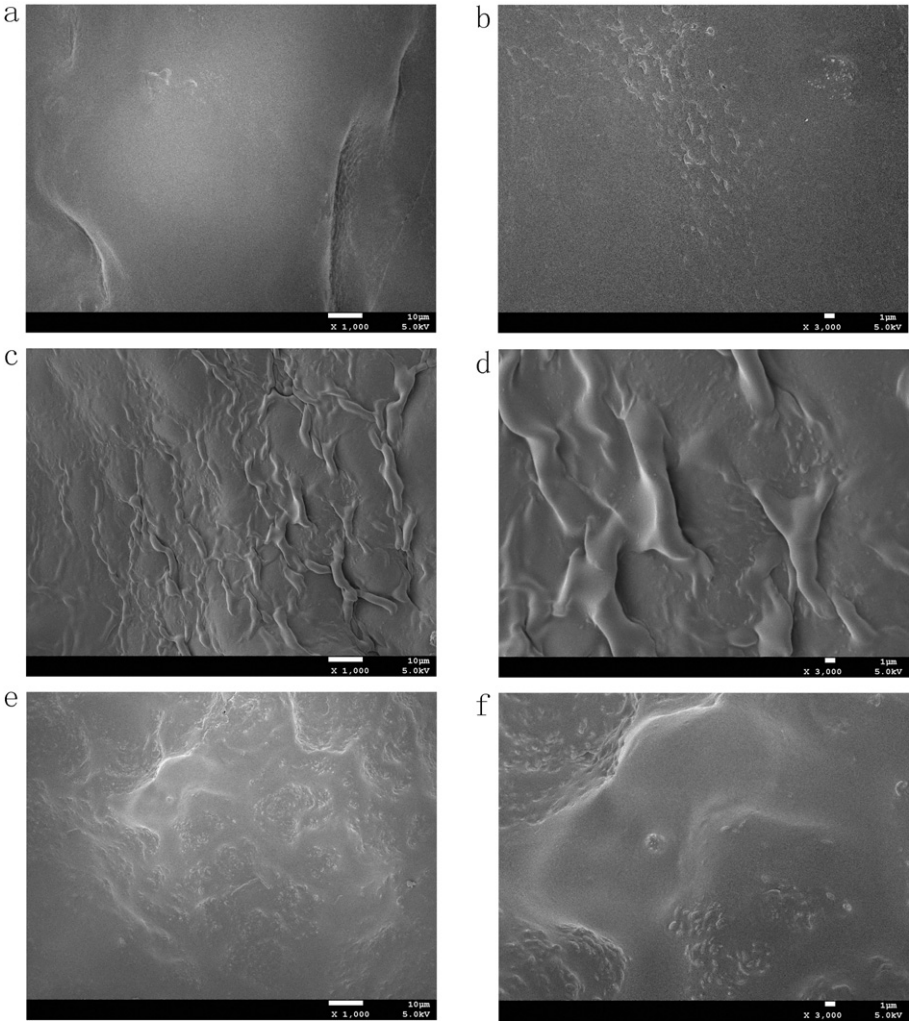

Fig. 2. Scanning electron micrographs of the three polysaccharides: (a) HM (1000×), (b) HM (3000×), (c) HP (1000×), (d) HP (3000×). (e) HB (1000×), (f) HB (3000×).

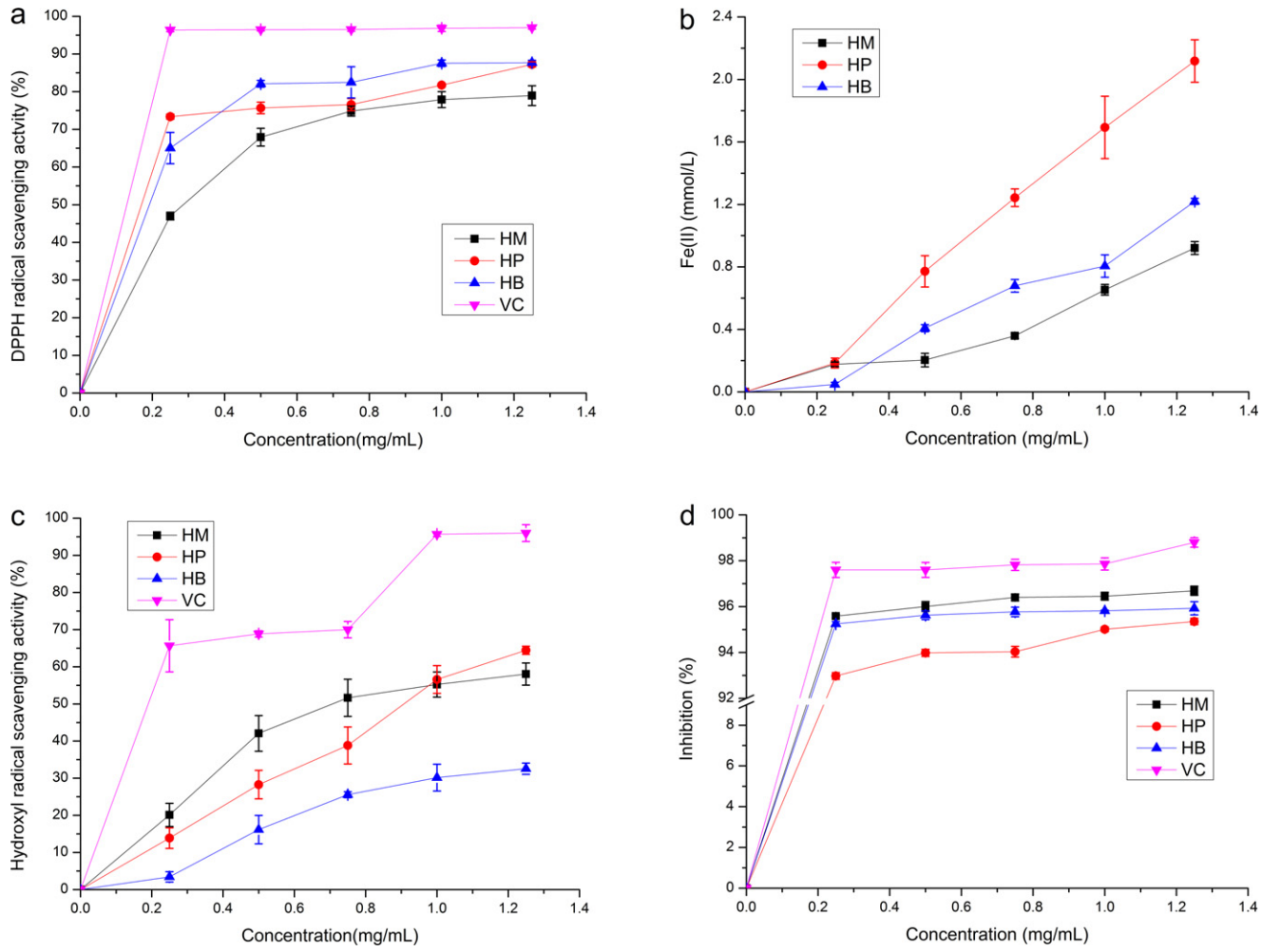

Fig. 3. (a) DPPH radical scavenging activities of HM, HP, HB and Vc. Each value is the mean ± SD of triplicate measurements. (b) Reducing power of HM, HP and HB. Each value is the mean ± SD of triplicate measurements. (c) Hydroxyl radical scavenging activity of HM, HP, HB and Vc. Each value is the mean ± SD of triplicate measurements. (d) AAPH radical induced hemolysis with HM, HP, HB and Vc. Each value is the mean ± SD of triplicate measurements.

was measured at 540 nm. The percentage hemolysis inhibition was calculated by the following equation.

$$\text{Hemolysis inhibition}\ (\%) = \left[\frac{B-A}{B}\right] \times 100$$

2.8. Statistical analyses

Experiments were carried out in triplicate, and data were reported as means ± standard deviation and evaluated by one-way analyses of variance (ANOVA). The p values were set at $p < 0.05$ to assess the statistically significant. All statistical analyses were using SPSS 19.

3. Results and discussion

3.1. Physicochemical property analysis

Table 1 lists the physicochemical properties of the three polysaccharides. The colors of the three polysaccharides extracted by hot water method had a few differences. HB and HP were both brown, whereas HM was black. The texture of the three polysaccharides also showed different characteristics. HB and HP were both loose, whereas HM was tight. HM showed significantly higher carbohydrate contents than HP, and HB ($p < 0.05$). Their values decrease in the order: HM > HP > HB. The protein contents in HM was higher than HP, but could not be detected in HB. HP showed significantly higher total polyphenol contents than HM, and HB ($p < 0.05$). These differences may be related to the age of their materials.

3.2. FTIR analysis

FTIR spectra of HM, HP and HB were shown in Fig. 1, there was a stretching vibration of O—H and saturated C—H at 3300–3500 and 2929–2989 cm^{-1}, respectively. The relatively strong absorption peak at around 1600–1650 cm^{-1} indicated the characteristic of C=O. The peaks at 950–1200 cm^{-1} suggested the presence of C—O—C and C—O—H link bonds. Characterization of three polysaccharides by FTIR analysis showed the typical absorption of polysaccharides.

In conclusion, no significant difference was seen in the FTIR of the three polysaccharides, and FTIR spectroscopy did not clearly distinguish the three polysaccharides from each other.

3.3. SEM analysis

The SEM images of the three polysaccharides are shown in Fig. 2. The results showed that the different material induced different physical changes. Fig. 2a and b showed that HM had a flat surface. Fig. 2c and d showed that HP had a rough surface with

characteristic large wrinkles. Fig. 2e and f showed that HB had a rough surface. In summary, the polysaccharides from Hawk tea extracted by hot water method were qualitatively identified by comparing their micrographs with those of the standards.

3.4. Antioxidant activity

3.4.1. DPPH radical scavenging activity

Fig. 3a showed the scavenging activities of HM, HP, HB and Vc against the DPPH radical. The scavenging effects of the three polysaccharides were remarkable at all tested concentrations, and well correlated with increased concentration up to 1.25 mg/mL. HM had the weakest activity ($p < 0.05$). At 1.25 mg/mL, the scavenging activities of HM, HP, HB and Vc were 79.0%, 87.3%, 87.7% and 97.0%, respectively.

3.4.2. Ferric reducing activity power

Fig. 3b showed that all polysaccharides possessed reducing power in the order of HP, HB and HM. The reducing power of the three polysaccharides was notable at all tested concentrations, and positively correlated with increased concentration up to 1.25 mg/mL. The FRAP values of HP, HB and HM were 2.1 mmol/L, 1.2 mmol/L and 0.9 mmol/L at the concentration of 1.25 mg/mL, respectively. Obviously, ferric reducing power of HP was significantly higher than that of HB and HM ($p < 0.05$).

3.4.3. Hydroxyl radical scavenging activity

Fig. 3c showed the scavenging activities of HM, HP, HB and Vc against the hydroxyl radical. The scavenging effects of the three polysaccharides were evident at all tested concentrations. HB had the weakest activity ($p < 0.05$). The three polysaccharides had weaker activities than Vc. At 1.25 mg/mL, the scavenging activities of HM, HP, HB and Vc were 58.1%, 64.4%, 32.6% and 96.0%, respectively.

3.4.4. Erythrocyte hemolysis

Fig. 3d showed the inhibition of HM, HP, HB and Vc against the erythrocyte hemolysis. HM had the strongest inhibition capacity ($p < 0.05$). At 1.25 mg/mL, the inhibition of HM, HP, HB and Vc was 96.7%, 95.3%, 95.9% and 98.8%, respectively. Obviously, although the three polysaccharides had strong inhibition against the erythrocyte hemolysis, Vc bested them all. These data on hemolysis assay indicated that polysaccharides could act as antioxidant to reduce the formation of free radical mediated by AAPH.

4. Conclusions

In the present study, three polysaccharides from different leaf age Hawk teas were extracted by hot water method. Structural characterizations were conducted using physicochemical property, FTIR, and SEM analyses. The physicochemical property indicated that different leaf age materials had different carbohydrate contents, protein contents and total polyphenol contents. FTIR results demonstrated that the characterization of three polysaccharides was similar. The SEM images revealed that different aging leaves also had different stereostructures. Moreover, significant antioxidant activities against DPPH, FRAP, hydroxyl radical and erythrocyte hemolysis were possessed by HB, HP and HM. They all showed significant antioxidant activities in a dose-dependent manner, HP showed significantly higher antioxidant activities than HM and HB. Hence, these polysaccharides are natural antioxidants, and may be potential for functional food ingredients.

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