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Inhibition of α-glucosidase by polysaccharides from the fruit hull of *Camellia oleifera* Abel

Sheng Zhang, Xiang-Zhou Li*

College of Materials and Engineering, Central South University of Forestry and Technology, Changsha 410004, PR China

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ABSTRACT

We isolated and purified polysaccharides from the *Camellia oleifera* Abel. fruit hull and studied its hypoglycemic potential. Our results revealed six polysaccharides (CFPA-1–5 & CFPB) from the aqueous extract from the defatted *C. oleifera* fruit hull. Purified polysaccharides (purity >90%) were investigated for the inhibition of α-glucosidase activity in vitro. Two polysaccharides, CFPB and CFPA-3 were present in high concentration in the fruit hull and showed a dose-dependent inhibition of α-glucosidase activity, with IC_{50} concentrations of 11.80 and 10.95 μg/mL, respectively. This result suggests that polysaccharides (CFP) extracted from the fruit hull of *C. oleifera* may have potential as functional foods with featuring a hypoglycemic effect.

1. Introduction

Camellia oleifera Abel is a tea oil plant originated in China. The camellia oil has high nutrition value on account of oleic acid and linoleic acid (Ma, 2007). *C. oleifera* distributes mainly in hilly regions of Hunan, Jianxi and south western China. Polysaccharides, polyphenols and other saponin compounds have been identified in the seed and fruit hull of *C. oleifera*. Polysaccharides from various plants have many pharmacological activities, such as anti-tumor (Jin & Ning, 2012; Yoshikawa et al., 2004), immunomodulatory (Chen et al., 2012; Kim, Cho, Karnjanapratum, Shin, & You, 2011), antioxidant (Wang, Zhang, Zhang, & Li, 2008; Zhang et al., 2003), antiviral (Dong, Hayashi, Lee, & Hayashi, 2010; Pereda-Miranda, Kaatz, & Gibbons, 2006), anti-atherosclerosis (Kaji et al., 2002; Shekharam, Venkataraman, & Salimath, 1987), and hypoglycemia activities (Chen, Jin, & Tang, 2011; Kim et al., 2010). Recently, anti-tumor and antioxidative activities of polysaccharides from the *C. oleifera* fruit hull (CFP) have been reported (Jin & Ning, 2012; Jin, 2012; Shen, Kang, & Chen, 2010). TP (tea polysaccharide) has been shown to feature hypoglycemic effects (Quan, Yin, & Kazushi, 2007). It is likely that polysaccharides from the *C. oleifera* fruit hull have the same effect as that of tea (*Camellia sinensis*) because both plants belong to the same genus. However, to date, there is no direct evidence to support this hypothesis.

Type 2 diabetes mellitus (DM), known as non insulin-dependent DM, is a common disorder of glucose and fat metabolism that causes immense health care costs (Chan, Tsang, Lee, & Lee, 2007). Diabetes is characterized by high concentrations of blood sugar, which can cause serious complications in the kidneys, eyes and cardiovascular system. Therefore, the treatment of diabetes primarily focuses on reducing fluctuations in blood sugar, attenuating subsequent complications (Morrison, Shubina, & Turchin, 2011). α-Glucosidase is the most important element for absorption of glucose in the small intestine (Gray & Olefsky, 1982). Therefore, inhibition of α-glucosidase can significantly decrease the postprandial hyperglycemia after a mixed carbohydrate diet and can be a key strategy in the control of type 2 DM. Recently, α-glucosidase inhibitors have been reported to retard the absorption of glucose and improve post prandial hyperglycemia (Bhandari, Jong-Anurakkun, & Hong, 2008). Consequently, it would be meaningful to develop new glucosidase inhibitors from natural products (Tu & Li, 2010; Zhang, Lu, & Shen, 2007).

CCP (polysaccharides from the defatted *C. oleifera* seed cake) composed of arabinose, galactose, rhamnose, galacturonic acid, glucose, mannose and features a molecular mass between 3216 and 12,210 Daltons (Da). CCP has an appreciable α-glucosidase inhibitory effect in a concentration-dependent manner (Li, Zhang, & Li, 2012). This suggests that CCP can be considered as a potential candidate for the management of DM.

In the present study, we systematically extracted and purified CFP (polysaccharides from the fruit hull of *C. oleifera*). Effects of CFPs on α-glucosidase inhibition were determined in vitro. These results may promote further research on CFP as a functional food.

* Corresponding author. Tel.: +86 73185623208; fax: +86 73185623208.
E-mail addresses: gingshen123@126.com, rlxz@163.com (X.-Z. Li).

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2. Experimental

2.1. Materials and chemicals

1-Phenyl-3-methyl-5-pyrazolone was purchased from Acros Organics (New Jersey, USA). Dextrans of different molecular weights and acarbose were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The standard monosaccharides were purchased from the Sigma-Aldrich (St. Louis, MO, USA). Glucose assay kit was purchased Zhongsheng-Beikong Bioscience (Beijing, China). Polyamide was purchased from DEAE-52 and Sephadex G-100 was purchased from Rui-Da-Heng-Hui Science& Technology Development (Beijing, China). Sucrose was purchased from Kermiou Chemical Reagent (Tianjing, China). Phosphate buffer saline (PBS) was purchased from Genomapping Technology (Tianjing, China). Normal saline was purchased from Kelun Pharmaceutical (Hunan, China). Trifluoroacetic acid (TFA), methanol, and acetic acid, ethanol, acetic anhydride and all other chemicals and reagents were analytical grade.

2.2. Extraction and purification polysaccharide from Camellia oleifera Abel fruit hull

The fruit hull of *C. oleifera* was powdered with a pulverizer. The powder was extracted with petroleum ether at 70 °C for 2 h to remove lipids. After filtration, the residues were air-dried, then extracted with double-distilled water at 80 °C for 2 h, twice and filtered. Polyamide was used to remove the residuary protein according to the method of Meng and Zhang (2007). The combined filtrate was passed through polyamide column for deproteinization and the column was eluted with distilled water. The eluent was concentrated with reduced pressure and precipitated with ethanol (4 times the volume of aqueous extract) at 4 °C for 24 h. The precipitate was lyophilized, which yielded crude polysaccharides (CFP). The crude polysaccharides were then dissolved in double-distilled water and the solution was injected to a column of DEAE-52 and Sephadex G-100, respectively. The DEAE-52 column was eluted with distilled water, sodium chloride (0.1 mol/L, 0.2 mol/L, 0.5 mol/L) and 0.5 mol/L sodium hydrate solution. The Sephadex G-100 column was eluted with 0.2 mol/L sodium chloride. The carbohydrate contents of the eluent were determined by the phenol–sulfuric acid method. The eluents were consolidated and lyophilized.

2.3. Determination of molecular weight

The molecular mass of CFP was determined by the Gel Permeation Chromatography (GPC) technique (Wei & Fang, 1989) using a Waters HPLC apparatus (Waters 600, Waters, Milford, MA, USA) equipped with an Ultrahydrogel™ Linear column (300 mm × 7.8 mm, two series connection) and a model 2410 refractive index detector (RID). The column was eluted with 0.1 mol/L sodium acetate with a flow rate of 0.9 mL/min and calibrated with Dextran standards (*MW*: 180, 2500, 7100, 21,400, 133,800, and 200,000 Da).

2.4. Analysis of monosaccharide compositions

Aliquots of 100 μL CFPA-3 and CFPB (10 mg/mL, each) were hydrolyzed in a sealed glass tube with 4 mol/L TFA (100 μL) at 110 °C. The hydrolysate was dried by N_2, and dissolved by adding 50 μL of 0.3 mol/L NaOH in a tube. Then 0.5 mol/L PMP (1-phenyl-3-methyl-5-pyrazolone, 50 μL) was added to the solution and mixed thoroughly by vortexing. The tube was put into the oven at 70 °C for 100 min, and afterwards cooled to room temperature. Followed a neutralization process with 50 μL of 0.3 mol/L HCl, then 1 mL of deionized water was added, and chloroform was used to extract three times. The water-phase was separated and filtered for HPLC. A 20 μL aliquot was loaded onto a ZORBAX Eclipse XDB-C_{18} HPLC column (250 m × 4.6 mm i.d., 5 μm; Agilent 1100, Santa Clara, CA, USA). The mobile phase mixture rate was 17:83 (acetonitrile and water containing 0.1 mol/L PBS). Detector wavelength was 250 nm and flow rate was 1.0 mL/min.

2.5. Infrared spectra of DDP

The purified polysaccharide was ground with KBr powder and then pressed into pellets for the estimation of the infrared (IR) spectra between 4000 and 500 cm^{-1}. Spectra were recorded on a Perkin-Elmer Fourier Transform IR spectrophotometer (Perkin-Elmer Corp., Waltham, MA, USA).

2.6. SEM

Polysaccharides samples were first adhered on a conductive adhesive plate, and then free powder was purged by high purity nitrogen. Samples were treated with carbon coating prior to examination under scanning electron microscopy.

2.7. Assay for inhibition of α-glucosidase activity

The α-glucosidase inhibitory activity of CFPA-3 and CFPB were determined according to the chromogenic method described previously (Ma, 2007), with a slight modification. A mixture of sample solution (12.5 μL); 0.2 mol/L phosphate buffered saline solution (pH 6.8, 12.5 μL); α-glucosidase solution (12.5 μL); sucrose solution (12.5 μL) was incubated in 96 well plates at 37 °C for 20 min. After pre-incubation, phenol reagent solution (1500 μL) was added to each well at timed intervals. The reaction mixtures were incubated at 37 °C for 20 min. The absorption intensity at 505 nm wavelength, which is correlated to the concentration of glucose, was obtained on a Multiskan Ascent instrument (Thermo Co., Waltham, MA, USA). Acarbose was used as the positive control. The absorbance of the wells was measured at 505 nm and the inhibitory activity was calculated using the following formula:

$$\text{Inhibition}\,(\%) = \left(\frac{\Delta A_{\text{Control}} - \Delta A_{\text{Sample}}}{\Delta A_{\text{Control}}}\right) \times 100$$

$$\Delta A_{\text{Control}} = A_{\text{Control}} - A_{\text{Blank}}$$

$$\Delta A_{\text{Sample}} = A_{\text{Sample}} - A_{\text{Blank}}$$

The values expressed are means of three replicate determinations ± standard deviation. The statistical analysis was carried out by SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Polysaccharide isolation and purification

The total yield rate of crude polysaccharides was 0.97% by the boiling-water extraction method. The extraction procedure is summarized in Fig. 1. Six polysaccharide-enriched fractions, termed CFPA-1–5 and CFPB, were extracted from the fruit hull *of C. oleifera* Abel. The yield of CFPA-1, CFPA-2, CFPA-4 and CFPA-5 were too small from this process (fractions and yields shown in Table 1); therefore, CFP, CFPA-3 and CFPB were used in this study. The molecular mass of CFPA-3 and CFPB were calculated to be 186,019 and 378,824 Da, respectively, based on the calibration curve obtained with standard dextrans.

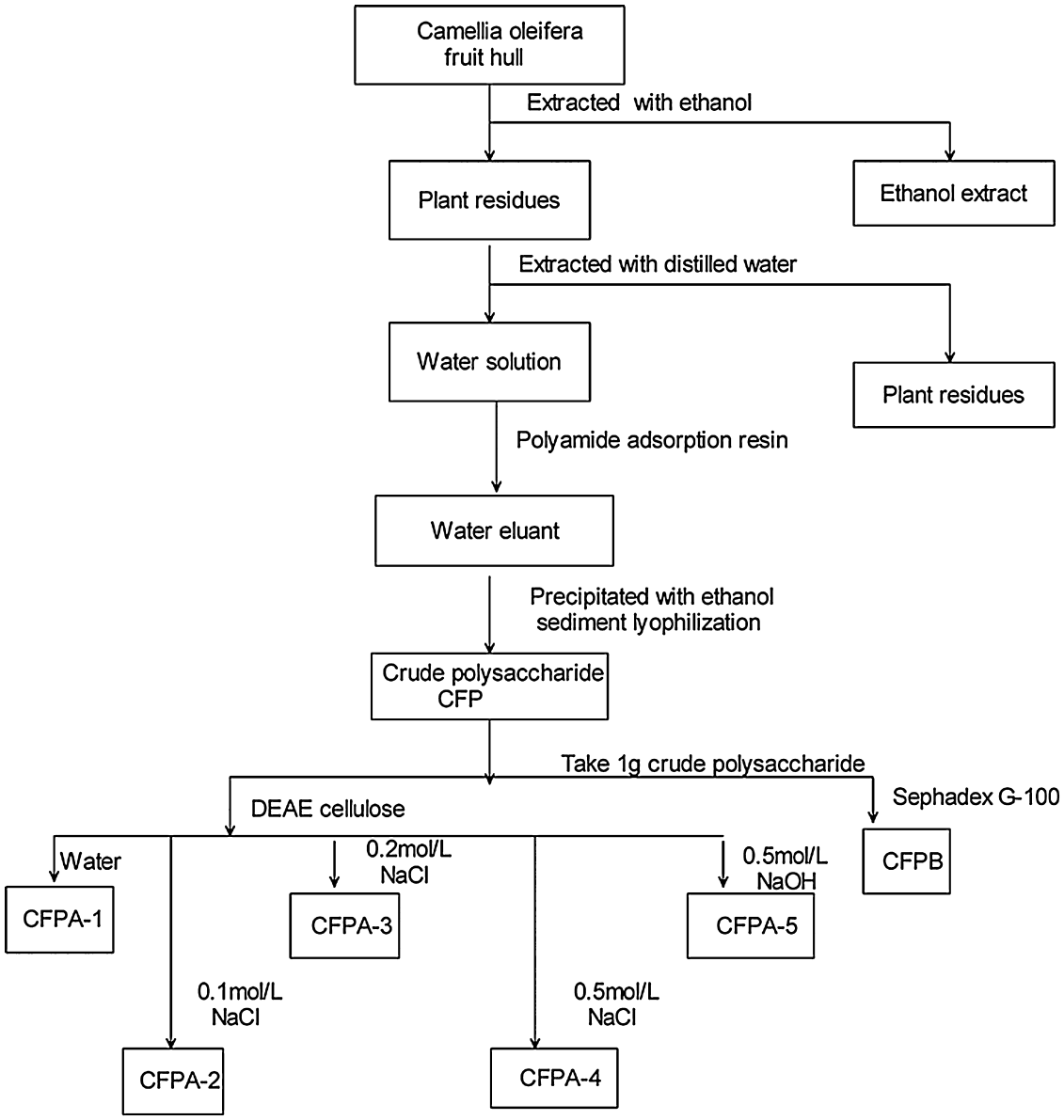

Fig. 1. Flow chart for extraction and fractionation of polysaccharides from the fruit hull of *Camellia oleifera* Abel.

Table 1
Fractions and yields of CFP.

Fraction	CFPA-1	CFPA-2	CFPA-3	CFPA-4	CFPA-5	CFPB
Yield (%)	<1	2.56	9.89	4.33	6.56	15.00

CFPA-1: fraction of polysaccharide from the fruit hull of *C. oleifera* which was eluted using a DEAE-52 column with distilled water; CFPA-2: polysaccharide eluted by DEAE-52 column with 0.1 mol/L sodium chloride; CFPA-3: eluted by 0.2 mol/L sodium chloride; CFPA-4: eluted by 0.5 mol/L sodium chloride and CFPA-5: eluted by 0.5 mol/L sodium hydrate solution. CFPB: polysaccharide eluted by sephadex G-100 column with 0.2 mol/L sodium chloride.

Table 2
Monosaccharide composition of CFPA-3 and CFPB.

	CFPA-3	CFPB
Glucuronic acid	0.80	1.01
Galacturonic acid	0.06	n.d.[a]
Galactosamine	4.16	3.27
Galactose	0.22	1.79
Xylose	n.d.	1.19
Arabinose	1.14	n.d.

[a] n.d.: not detectable.

3.2. Monosaccharide composition of CFP

Monosaccharide composition of CFP was analyzed by pre-column derivatization high performance liquid chromatography. The results shown in Table 2 indicate that galactosamine is the major monosaccharide backbone of CFP.

3.3. IR spectra of the CFP fractions

As shown in Fig. 2, the isolated CFP exhibited a significant, broad characteristic peak at around 3420 cm^{-1} for the hydroxyl group, as well as an amide bond band at around 2955 cm^{-1}. The polysaccharide also appeared to have a specific band between 1200 and 1080 cm^{-1}, which is dominated by ring vibrations

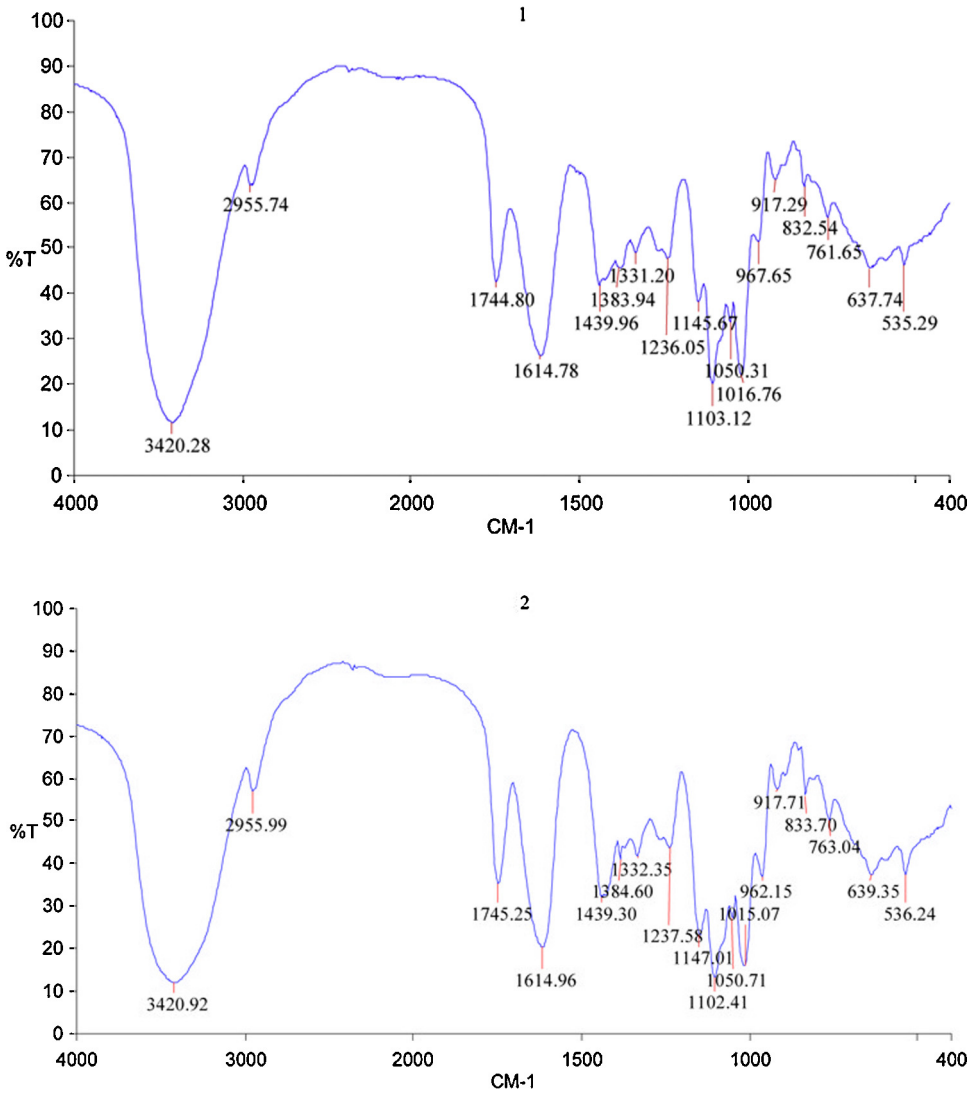

Fig. 2. (1) FT-IR spectrum of the polysaccharide CFPB. CFPB: Fraction of polysaccharide from the fruit hull of *C. oleifera* which was eluted by sephadex G-100 column with 0.2 mol/L sodium chloride. (2) FT-IR spectrum of the polysaccharide CFPA-3. CFPA-3: Fraction of polysaccharide from the fruit hull of *C. oleifera* which was eluted by DEAE-52 column with 0.2 mol/L sodium chloride.

overlapped with stretching vibrations of (C—OH) side groups and the (C—O—C) glycosidic band vibration. The two peaks toward 1745 and 1615 cm^{-1} in the IR spectra revealed the presence of a (COO) deprotonated carboxylic group. Positive specific rotation and characteristic absorption at around 832 cm^{-1} were identified in the IR spectrum, indicating the α-configuration of the sugar units. The characteristic IR absorptions further confirmed both of CFPA-3 and CFPB were acidic and amino-bond polysaccharides. But the IR spectra of CFPA-3 and CFPB were similar and no obvious differences could be observed.

3.4. SEM

As shown in Fig. 3, CFPA-3 featured a flaky surface characteristic by scanning electron microscopy (SEM). CFPA-3 had an uneven surface and a very small gap between the crystals indicating that the polysaccharide is not completely stable. CFB had an irregular branching shape, very similar to a fiber, which was possible due to the number of polysaccharide molecules or molecular groups forming these structures.

3.5. Results of α-glucosidase inhibition activities

The inhibitory effects of CFPs on α-glucosidase are shown in Fig. 4 and the IC_{50} is shown in Table 3. Both polysaccharides and controls (acarbose and TP) showed a dose-dependent inhibitory effect on α-glucosidase. Furthermore, the IC_{50} (50% inhibitory effect on α-glucosidase) of CFPB and CFPA-3 were 11.80 and 10.95 μg/mL, respectively. They were not different from each other ($p > 0.05$), but 6-fold higher than that of acarbose (2.09 μg/mL) and 2-fold higher than TP (4.30 μg/mL).

3.6. Discussion

The molecular mass of polysaccharides CFPA-3 and CFPB isolated from the fruit hull of *C. oleifera* was 186,019 and 378,824 Da, respectively, and was higher than CCPs (3216 and 12,210 Da) (Li et al., 2012) but smaller than TP ($10–10.5 \times 10^5$) (Zhou, Wang, & Wang, 2008). Furthermore, the monosaccharide composition of CCP, CFP and TP polysaccharides was different from each other (Li et al., 2012; Zhou et al., 2008). This may be due to the different species and the different parts of materials for isolation.

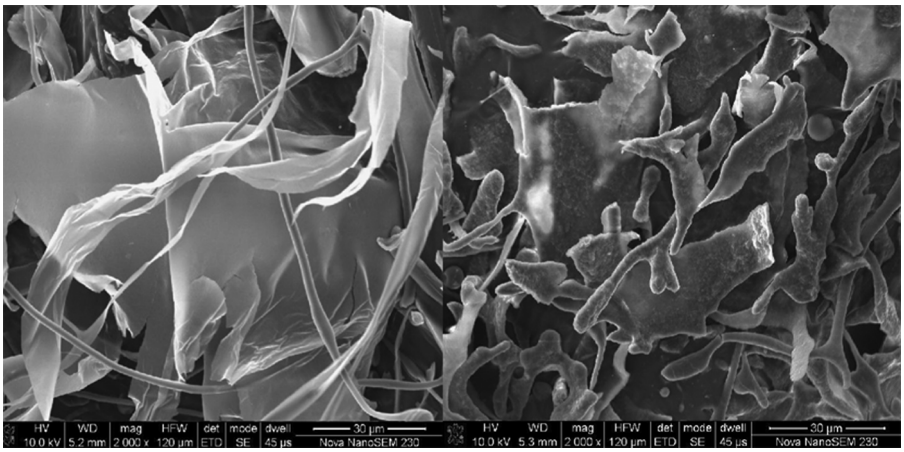

Fig. 3. The image of polysaccharides by SEM (left: CFPA-3; right: CFB).

Table 3
IC_{50} of inhibition of α-glucosidase activity (mean ± SD, $n = 3$).

Sample	Acarbose	TP	CFP	CFPA-3	CFPB
IC_{50} (μg/mL)	2.09 ± 0.31	4.30 ± 1.25	22.84 ± 1.04	10.95 ± 1.53	11.80 ± 0.24

When three kinds of polysaccharides were compared for inhibitory potency on α-glucosidase activity, we found that the IC_{50} of CFP (22.84 μg/mL) was significantly lower than CCP (630.0 μg/mL) (Li et al., 2012), but higher than TP (4.30 μg/mL). Therefore we concluded that the hyperglycemic potency of CFP was better than CCP but slightly lower than TP.

The structural features of polysaccharide from *C. oleifera* fruit hull were evaluated by the monosaccharide composition, molecular weight distribution, IR spectra, and molecular morphology. The molecular weight of polysaccharide was neither too high nor too low. High molecule weight indicates that polysaccharide may not easily pass through the cell membrane barrier into the organism and carry out functional activity. Low molecule weight indicates that saccharides may not form a polymeric structure to generate biological activity. The molecular weight of CFPs ranged from 10,000 to 1000,000 and were within the ranges for most active polysaccharide reported (Zhou et al., 2008).

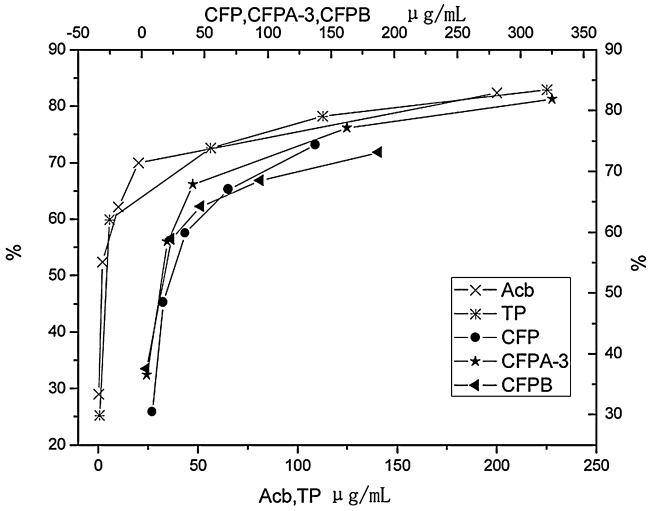

Fig. 4. Inhibitory effects of CFPA-3 and CFPB on α-glucosidase activity. Acb: acarbose; TP: tea polysaccharide; CFP: crud polysaccharides from the fruit hull of *C. oleifera*.

CFP structures of CFPs were determined to be galactosamine-based acidic polysaccharide as assessed by monosaccharide composition and infrared results. Tea polysaccharides with α-glucosidase inhibition activities are also a kind of acidic polysaccharide (Zhou et al., 2008). Tea and *C. oleifera* belong to the same genus, and both have similarities in chemical composition and activities. Therefore, acidic polysaccharides could be associated with α-glucosidase inhibition activity.

The biological activity of polysaccharide is not only relates to chemical composition but also its spatial configuration. For example, β-1, 3-D-glucan with triple helix is very important factor for immunoregulation activity (Nie & Ning, 2003). The results of CFP SEM image suggest that the specific molecular morphology might be involved with the inhibition of α-glucosidase. Our CFP results may lead to the development of effective anti-diabetes drugs via modification of the key structure of polysaccharide.

4. Conclusions

We evaluated the α-glucosidase inhibition activity of polysaccharides derived from the fruit hull of *C. oleifera* Abel. CFPs showed potent effects compared to tea polysaccharides. CFPB and CFPA-3 were present in high concentrations and inhibited α-glucosidase activity, with IC_{50} concentrations of 11.80 and 10.95 μg/mL, respectively. The molecular mass of CFPA-3 and CFPB were calculated to be 186,019 and 378,824 Da, respectively. Monosaccharide composition and IR spectra showed that CFPs are galactosamine based acidic polysaccharides and featured typical molecular morphology under SEM. We concluded that the CFP structure was related to α-glucosidase inhibition activity. Further animal and clinical studies are warranted to determine whether CFP may be a functional food to control blood glucose in diabetic patients.

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