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# Structure of a polysaccharide from *Gastrodia elata* Bl., and oligosaccharides prepared thereof with anti-pancreatic cancer cell growth activities

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ABSTRACT

To discover anti-pancreatic cancer cells growth inhibitor, a new water-soluble glucan, WTMA, was isolated from the rhizome of *Gastrodia elata* Bl. The mean molecular weight of the polysaccharide was estimated to be $7.0 \times 10^5$ Da by high performance gel permeation chromatography (HPGPC). The structure of WTMA was characterized by GC, GC–MS, NMR and MALDI-TOF. WTMA was deduced as an α-(1 → 4)-glucan with α-(1 → 4) linked branches attached to O-6 at branch points. Bioactivity tests *in vitro* indicated that WTMA and its partial acid hydrolysis-derived mixture product, WTMA-AD-O, could significantly inhibit the proliferation of pancreatic cancer cells. However, WTMA-DE, the degraded polysaccharide without branch chain by isoamylase, did not inhibit the proliferation of pancreatic cancer cells. Thus side chain on WTMA was deduced to be required for the anti-cancer cell activity. Further study demonstrated that WTMA and all of its hydrolysis products had no inhibition effect on liver LO2 cells.



## 1. Introduction

The incidence of pancreatic cancer varies among populations, being the fourth cause of cancer death in United States in 2010 (Jemal, Siegel, Xu, & Ward, 2010). Patients suffer severe pain, nausea and vomiting, anorexia, weight loss and weakness as the disease progresses. Less than 10% of patients could survive a year after diagnosis (Burris & Storniolo, 1997). Although a lot of clinical trials have been implemented, patients with this disease still have fatal prognosis (Nitsche et al., 2008). Higher clinical benefit and survival were reported by treatment with gemcitabine than with 5-fluorouracil. However, it still could not meet the clinical requirements (Burris & Storniolo, 1997). Interestingly, more and more evidences indicated that nature products derived from bioresource might be alternative drug candidate for anti-pancreatic cancer new drug development.

A variety of polysaccharides from plant or fungi have been found to have antitumor activities with low toxicity, such as lentinan (on the market) from *Lentinus edodes* (Sasaki & Takasuka, 1976; Surenjav, Zhang, Xu, Zhang, & Zeng, 2006), grifolan from *Grifola frondosa* (Cui et al., 2007; Iino, Ohno, Suzuki, Miyazaki, & Yadomae, 1985; Kato et al., 1983; Shih, Chou, Chen, Wu, & Hsieh, 2008) and TMP70S-1 from *Taxus yunnanensis* (Yin et al., 2010). However, few of them can directly inhibit tumor cells growth. The rhizome of *Gastrodia elata* Bl. has been used as a traditional herbal medicine in Asian countries, such as Korea, China and Japan. For centuries, it has been used as an anticonvulsant, analgesic and sedative agent for the medical treatment of headaches, epilepsy, dizziness, rheumatism, neuralgia, paralysis, hypertension and other neuralgic disorders (Qiu, Tang, Tong, Ding, & Zuo, 2007). Qiu, Yang, Pei, Zhang, and Ding (2010) reported structures of several polysaccharides from *G. elata*, They demonstrated that WSS25, a sulfation product derived from the polysaccharide WGEW, blocked angiogenesis to inhibit xenografted hepatocarcinoma cells growth *in vivo*. This suggested that other polysaccharides in this herbal medicine might have direct antitumor bioactivity.

To screen potent anti-pancreatic cancer cell growth inhibitor, one homogeneous polysaccharide, WTMA, was isolated from *G. elata*. In the present study we reported the structures of WTMA and its degradation products by chemical and enzymatic methods and their anti-cancer cell growth bioactivities.

## 2. Materials and methods

### 2.1. Materials

The dried rhizomes of *G. elata* (5.0 kg) were purchased from Yunnan province in China. DEAE-cellulose 32 was from Whatman Co. Ltd. Standard saccharides, sodium borohydride and iodomethane were from Fluka. Dimethy sulfoxide (DMSO) was from E. Merk.

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Other reagents were of analytical grade unless otherwise claimed. Human pancreatic cancer cell line (PANC-1) and human hepatocyte cell line LO2 were supplied by the Cell Library in Shanghai Institute for Biological Sciences, Chinese Academy of Sciences.

### 2.2. General methods

Optical rotations were determined with a Perkin–Elmer 241 M digital polarimeter. HPGPC was performed with an Agilent 1200 instrument, including a G1311A Qudra pump, a G1315D RI detector, a G1362A dual λ absorbance detector and GPC software. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 NMR spectrometer. Gas chromatography (GC) was performed with a Shimadzu-14B apparatus equipped with a 3% OV-225/AW-DMCS-Chromosorb W column (2.5 m × 3 mm). Gas chromatography–mass spectrometry (GC–MS) was performed with a Shimadzu QP-5050A apparatus equipped with a DB-1 capillary column (0.25 mm × 30 m). Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry were recorded on a Thermo instrument (Thermo, U.S.A.) and conducted in positive ion mode. The operating parameters were optimized as follows: sheath gas, $5.25 \times 10^6$ Pa; auxiliary gas, $1.75 \times 10^6$ Pa; spray voltage, 4 kV; capillary temperature, 375 °C; capillary voltage 35 V; tube lens voltage, 250 V and mass range 0–2000 m/z or 0–4000 m/z. Mass spectrometry data were collected through Xcalibur software.

### 2.3. Isolation and purification of polysaccharide

The dried rhizomes of *G. elata* (5.0 kg), defatted with 95% EtOH for 7 days, were extracted with boiling water for four times, 4 h for each time. The aqueous extract was concentrated and treated with 15% trichloroacetic acid at 4 °C to remove protein. After centrifugation and neutralization, the supernatant was extensively dialyzed (molecular weight cut-off 3000 Da). Three volumes of 95% EtOH were added to the concentrated retentate to precipitate the crude polysaccharide TM (260 g, yield 5.20%). 6 g of TM was fractionated on a column of DEAE-cellulose (50 cm × 5 cm, $Cl^-$ form), eluted stepwise with distilled water and 0.1 M NaCl solutions and monitored with a phenol-sulfuric acid method. The fraction eluted with 0.1 M NaCl (1.2 g, yield 1.04%) was further separated on a column of DEAE-cellulose (50 cm × 5 cm, $Cl^-$ form), which was equilibrated with water and eluted with 0.1 M NaCl, giving WTMA (0.8 g, yield 0.69%).

### 2.4. Gel-filtration chromatography

Size exclusion chromatography on column (100 cm × 2.6 cm) of Bio-Gel P-2 (Bio-Rad, U.S.A.) was employed to purify the oligosaccharides and eluted by distilled water, followed by detection using Shodex RI-102 detector.

### 2.5. Homogeneity and molecular weight

The homogeneity and molecular weight of polysaccharides were estimated by HPGPC with series-connected Ultrahydrogel™ 2000 and Ultrahydrogel™ 500 columns, while that of oligosaccharide was detected by TSK-Gel 2500 $PW_{XL}$ column. The columns were equilibrated and eluted with mobile phase containing 0.4 g/L $KH_2PO_4$ and 7.32 g/L $K_2HPO_4$ at a flow rate of 0.5 mL/min. For molecular weight estimation, the columns were calibrated by standard T-series Dextrans (T-700, 580, 300, 110, 80, 70, 40, 9.3 and 4, Pharmacia). The columns temperature was kept at 30.0 ± 0.1 °C. All samples were prepared as 0.2% (w/v) solution, and 20 μL of solution was analyzed in each run (Xu, Dong, Qiu, Cong, & Ding, 2010).

### 2.6. Monosaccharide composition analysis

The polysaccharide sample (3 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 2 mL) at 110 °C for 2 h in a sealed test tube. The acid was removed under reduced pressure by repeated evaporation with MeOH, and then the hydrolysate was converted into the alditol acetate, followed by GC analysis (Bao, Liu, Fang, & Li, 2001).

### 2.7. Partial acid hydrolysis

WTMA (1.0 g) was hydrolyzed in 1 M TFA at 100 °C for 1 h. The hydrolysate solution was evaporated with MeOH to dry product followed by dialyzing stepwise using dialysis bags with different molecular weight cut-off (MWCO), ranging from 3000, 2000, to 1000. The retentate in MWCO 3000 dialysis bag was named as WTMA-AD-I. The concentrated dialysate was designated as WTMA-AD-O, which was further dialyzed with MWCO 2000 dialysis bag, giving the retentate as O-1 and dialysate as WTMA-AD-O-O. WTMA-AD-O-O was then separated into two parts with MWCO 1000 dialysis bag to obtain O-2 and O-3 as the retentate and dialysate, respectively. All the fractions were lyophilized and subjected to methylation analysis.

### 2.8. Linkage analysis

The vacuum-dried polysaccharide (10 mg) was methylated three times as described by Needs and Selvendran (1993) with minor modification. Briefly, the polysaccharide was weighed precisely and dissolved in 3.0 mL DMSO. 200 mg of powdered NaOH was added into the polysaccharide solution followed by mixing with an ultrasonic bath for 10 min. After incubation for 10 min at room temperature with stirring, 1 mL iodomethane was added into the polysaccharide mixture slowly. The reaction mixture was kept in darkness for 1 h before 2.0 mL distilled water was added. The methylated polysaccharides were extracted with 2 mL chloroform for three times and dried at depressed pressure on a rotary evaporator. The completeness of methylation was confirmed by the disappearance of the hydroxyl absorption in IR spectrum (Nujol). The methylated alditol acetates were prepared and analyzed by GC–MS (Dong, Jia, & Fang, 2006).

The vacuum-dried oligosaccharide (5 mg) was methylated twice using the procedure as described above.

### 2.9. Enzymatic degradation

WTMA (188 mg) was dissolved in 5 mL phosphate buffered saline (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L$Na_2HPO_4$ and 0.24 g/L $KH_2PO_4$, pH 7.4), and digested for 24 h at 37 °C with 15 μL isoamylase (1000 U, I5284, Sigma, Germany). The enzymatic reaction was terminated by heating the solution at 100 °C for 20 min, then centrifuged and dialyzed. The retentate was lyophilized to obtain WTMA-DE (87 mg) followed by homogeneity analysis by HPGPC and methylation analysis (Wang et al., 2010).

### 2.10. NMR analysis and MALDI-TOF analysis

The polysaccharide sample (30 mg) was deuterium-exchanged and dissolved in 0.5 mL of $D_2O$ (99.8% D). The $^{13}C$ spectrum was measured at room temperature, with acetone as the internal standard at 31.50 ppm. All chemical shifts were referenced to $Me_4Si$.

For MALDI-TOF analysis, the saccharide sample was dissolved in 50% methanol solution. Mass spectrum was recorded using 2,5-dihydroxybenzoic acid (10 mg/mL) as matrix. 2 μL of the methanol solution fraction was mixed with 2 μL of the matrix solution. A 2 μL portion of this solution was applied on a stainless steel sample plate and allowed to dry under vacuum followed by MS analysis.

**Table 1**
Linkage analysis of WTMA and its degraded polymers and oligomers by LC–MS analysis.

| Methylation sugars | Linkages | Molar ratio (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | WTMA | WTMA-AD-I | WTMA-AD-O | O-1 | O-2 | O-3 | WTMA-DE |
| 2,3,4,6-Me4-Glc*p* | Terminal | 5.87 | 23.83 | 48.97 | 34.43 | 33.6 | 72.7 | 6.13 |
| 2,3,6-Me3-Glc*p* | 1,4-Glc*p* | 88.1 | 62.5 | 47.12 | 54.92 | 57.03 | 21.64 | 93.87 |
| 2,3,4-Me3-Glc*p* | 1,6-Glc*p* | – | 6.21 | 1.79 | 5.5 | 5.14 | 4.11 | – |
| 2,3-Me2-Glc*p* | 1,4,6-Glc*p* | 6.03 | 7.39 | 2.09 | 5.15 | 4.23 | 1.55 | – |

Linkage analysis of WTMA and its degraded polymers and oligomers by LC–MS analysis.

### 2.11. Cell culture

Human pancreatic cancer cell line PANC-1 and human hepatocyte cell line LO2 were both maintained in DMEM (Gibco BRL, U.S.A.) medium containing 10% FBS (v/v), 2 mM L-glutamine and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) (Gibco BRL, U.S.A.). Cells were cultured in a humidified incubator at 37 °C with 5% $CO_2$.

### 2.12. Cell proliferation and toxicity test

PANC-1 cell and LO2 cell were employed for the cell proliferation and toxicity measurement, respectively. Both PANC-1 and LO2 cells were seeded and cultured for 24 h before any treatment with saccharide compounds. A colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method was used for measuring the proliferation for adherent tumor cells. $0.5 \times 10^4$ of PANC-1 cells were grown in DMEM medium containing tested samples at different concentrations. The number of living PANC-1 cells at the end of the 72 h incubation was determined colorimetrically based on the tetrazolium salt MTT as described by Mosmann (1983). All results were expressed as the ratio of inhibition ($\xi$) of tumor cells proliferation and calculated by following equation:

$$\xi = \left[\frac{A - B}{A}\right] \times 100\% \quad (1)$$

where "*A*" and "*B*" are the mean of viable tumor cells of the control and experimental samples, respectively (Zhang, Li, Xu, & Zeng, 2005).

The procedure for toxicity test was the same as that of the above experiments described except LO2 cell line was used in the experiment.

## 3. Results and discussion

### 3.1. Isolation, homogeneity and molecular weight

WTMA (0.8 g, yield 0.69%) was isolated by anion-exchange chromatography on a DEAE-cellulose column. The crude polysaccharide extracted from *G. elata* with hot water was eluted with 0.1 M NaCl and purified by repeating elution with 0.1 M NaCl. The specific rotation $[\alpha]_D^{25}$ of this polysaccharide was +382° (*c* 0.05, $H_2O$). The homogeneity of WTMA was estimated by HPGPC in which showed one symmetrical peak. The mean molecular weight of WTMA was estimated to be $7.0 \times 10^5$ Da by comparing with the standards of different molecular weight.

### 3.2. Composition analysis of WTMA

WTMA showed no absorption at 280 nm and no nitrogen by element analysis, which indicated that it was free of protein. After complete hydrolysis with 2 M TFA, the results of thin layer chromatography (TLC) showed that WTMA did not contain uronic acid. This was confirmed by $^{13}C$ NMR spectrum of WTMA in which no signal of COOH group appeared. Monosaccharide analysis indicated that WTMA consisted of glucose only. All the above evidences suggested that WTMA was a glucan.

### 3.3. Hydrolysis and homogeneity

To obtain oligosaccharides or polysaccharides with lower molecular weight from WTMA, partial acid hydrolysis of the polysaccharide was performed. WTMA (1.0 g) was partially hydrolyzed with 1 M TFA followed by dialysis to obtain WTMA-AD-I (retenate, 180 mg) and WTMA-AD-O (dialysate, 640 mg). WTMA-AD-O was further separated into three fractions, O-1 (10 mg), O-2 (10 mg) and O-3 (460 mg), through different MWCO dialysis bags.

To find out the relationship linked side chain and bioactivity, enzyme hydrolysis was carried out. WTMA (188 mg) was hydrolyzed with isoamylase to achieve WTMA-DE (87 mg).

The homogeneity of WTMA-AD-I, WTMA-AD-O and WTMA-DE were estimated by HPGPC. WTMA-AD-I and WTMA-DE were shown as homogeneous polysaccharides with molecular weight of $7.0 \times 10^3$ Da and $3.0 \times 10^3$ Da, respectively, while WTMA-AD-O was heterogeneous mixture.

### 3.4. Linkage analysis of WTMA and its degradation products

In order to determine the linkage types, all saccharides were subjected to methylation analysis. The results of methylation analysis were shown in Table 1. The linkages of WTMA consisted of terminal Glc (T-Glc), 1,4- and 1,4,6-linked Glc, in the molar ratio of 1:15:1, which was similar to that of WGEW polysaccharide demonstrated by Qiu et al. (2007). However, the molar ratio of the three glycosyl residues of WGEW was 1:16:1. In addition, the discrepancy in molecular weight was significant different when the same method for the molecular weight estimation was employed. The molecular weight of WGEW is $2.8 \times 10^5$ Da, while that of WTMA is $7.0 \times 10^5$ Da. This indicated that polysaccharide from the same species of medicinal herb distributed in different places might had different structures and properties.

Linkages of all the hydrolysis products were also shown in Table 1. The results showed that the branches of WTMA were still remained after acid hydrolysis. However, all the acid degradation products contained 1,6-linked Glc while there is no such residue in WTMA (Table 1). This suggested that 1,6-linked glucosyl residue was from the products after acid hydrolysis. There might be a part of glucose residues removed by acid hydrolysis from the main chain which was linked to the position at O-4 of the original 1,4,6-linked glucosyl residue at the branching points. Methylation analysis of WTMA-DE implied that the side chains were all removed from WTMA by isoamylase.

### 3.5. MALDI-TOF and $^{13}C$ NMR analysis

As a mixture, WTMA-AD-O was further purified by Bio-Gel P-2 column. The result showed that WTMA-AD-O at least contained seven fractions (Fig. 1). Fraction I was out of exclusion as impurity

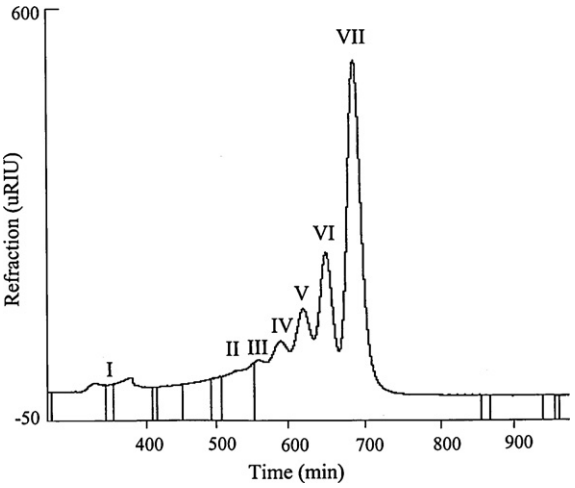


**Fig. 1.** Bio-Gel P-2 size exclusion chromatography of WTMA-AD-O.

**Table 2**
MALDI-TOF mass spectra of fraction II–VII.

| Fraction in Fig. 1 | Nominal mass (m/z) | Type | Ion composition |
|---|---|---|---|
| II (a in Supplementary Data) | 851 | $[M+Na]^+$ | $Glc_5$ |
| | 1013 | $[M+Na]^+$ | $Glc_6$ |
| | 1175 | $[M+Na]^+$ | $Glc_7$ |
| III (b in Supplementary Data) | 689 | $[M+Na]^+$ | $Glc_4$ |
| | 851 | $[M+Na]^+$ | $Glc_5$ |
| | 1013 | $[M+Na]^+$ | $Glc_6$ |
| | 1517 | $[M+H_2O+Na]^+$ | $Glc_9$ |
| | 1679 | $[M+H_2O+Na]^+$ | $Glc_{10}$ |
| IV (c in Supplementary Data) | 527 | $[M+Na]^+$ | $Glc_3$ |
| | 689 | $[M+Na]^+$ | $Glc_4$ |
| | 1355 | $[M+H_2O+Na]^+$ | $Glc_8$ |
| V (d in Supplementary Data) | 527 | $[M+Na]^+$ | $Glc_3$ |
| | 689 | $[M+Na]^+$ | $Glc_4$ |
| | 1031 | $[M+Na]^+$ | $Glc_6$ |
| VI (e in Supplementary Data) | 365 | $[M+Na]^+$ | $Glc_2$ |
| | 527 | $[M+Na]^+$ | $Glc_3$ |
| | 707 | $[M+H_2O+Na]^+$ | $Glc_4$ |
| VII (f in Supplementary Data) | 365 | $[M+Na]^+$ | $Glc_2$ |
| | 707 | $[M+H_2O+Na]^+$ | $Glc_4$ |
| | 851 | $[M+Na]^+$ | $Glc_5$ |

mixture on Bio-Gel P-2 column. Other fractions from II to VII were subjected to MALDI-TOF analysis to achieve mass spectrogram from a to f (Supplementary Data), respectively. The cationized pseudomolecular ions of fractions from II to VII were summarized in Table 2. As showed in Table 2 and Supplementary Data, the mass spectrum of each fraction indicated the presence of nine oligosaccharides having cationized pseudomolecular ions at m/z 1679 $[M+H_2O+Na]^+$, 1517 $[M+H_2O+Na]^+$, 1355 $[M+H_2O+Na]^+$, 1175 $[M+Na]^+$, 1013 $[M+Na]^+$, 851 $[M+Na]^+$, 689 $[M+Na]^+$, 527 $[M+Na]^+$, 365 $[M+Na]^+$, which corresponded to deca-, nona-, octa-, hepta-, hexa-, penta-, tetra-, tri-, disaccharide, respectively. The data suggested that there should be a lot of disaccharide as shown in fraction VII in the mixture. This is based on the fact that its retention time was same or similar as that of standard disaccharide maltose shown in Bio-Gel P-2 column (data not shown). Mass spectrogram of fraction VII (f in Supplementary Data) confirmed the conclusion.

The $^{13}C$ NMR spectrum of WTMA was shown in Fig. 2. The anomeric carbon signal of Glc residues at 100.922 ppm indicated that this polysaccharide adopted $\alpha$ configuration. The resonance at 78.000 ppm corresponded to C-4 of 1,4-linked glucose. The signal at 61.676 ppm was attributed to the C-6 of l,4-linked glucose. The signals at 74.570 ppm, 72.771 ppm and 72.407 ppm were originated from C-3, C-2 and C-5, respectively (Qiu et al., 2007).

Considering all the data, WTMA was proposed to have a repeating structure as below:

$$\alpha\text{-D-Glc}p\text{-}(1[\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1]_x$$
$$\downarrow$$
$$6$$
$$\text{-}\{[\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1]_y \rightarrow 4\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow\}_n$$
$$x + y = 15$$

### 3.6. Anti-cancer cells growth activity and toxicity

To test the bioactivities of WTMA and its degradation products, PANC-1 cells were treated with the glucans for 72 h followed by MTT assay. The results of PANC-1 cells growth inhibition by the glucans were summarized in Fig. 3. After the degradation, WTMA-AD-O contained a lot of disaccharides (Fig. 1, VII). To make sure whether disaccharide degraded from WTMA also had the anti-pancreatic cancer cells growth activity, maltose was separately included in the anti-cancer cells growth tests. As showed in Fig. 3A, except WTMA-DE and maltose, WTMA, WTMA-AD-I and WTMA-AD-O all could significantly inhibit the growth of PANC-1 cells. Among the glycans, WTMA-AD-O was the most potent PANC-1 cells growth inhibitor. Obviously maltose did not inhibit the growth of PANC-1 cells. This suggested that the side chain of the glycan might be required for the anti-cancer cells activity. Besides, after comparing the bioactivities of WTMA, WTMAE-AD-I, WTMA-AD-O, it seemed that the molecular size influences the bioactivity. Basically, when the molecular size of glycans increased, the anti-cancer cells growth activities were significantly weakened.

WTMA-AD-O was a glycan mixture in which at least contained some oligosaccharides, as detected by Shodex RI-102 detector. Generally speaking, one component might posses more potent activity than other components in the mixture. In order to obtain the most effective fraction, WTMA-AD-O was separated into three parts, albeit still heterogeneous fractions. Interestingly, none of the fractions had more potent anti-cancer cells growth bioactivity than that of the mixture when the same dose was used (Fig. 3B). The sum of inhibition of O-1, O-2 and O-3 was almost equal as that of the inhibition of WTMA-AD-O. Therefore, it was concluded that the anti-cancer cells growth activity of WTMA-AD-O was the results from the synergistic effects of the components in the carbohydrate mixture.

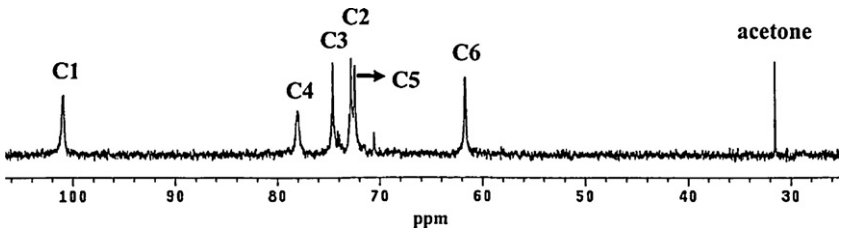


**Fig. 2.** $^{13}C$ NMR spectra of WTMA.

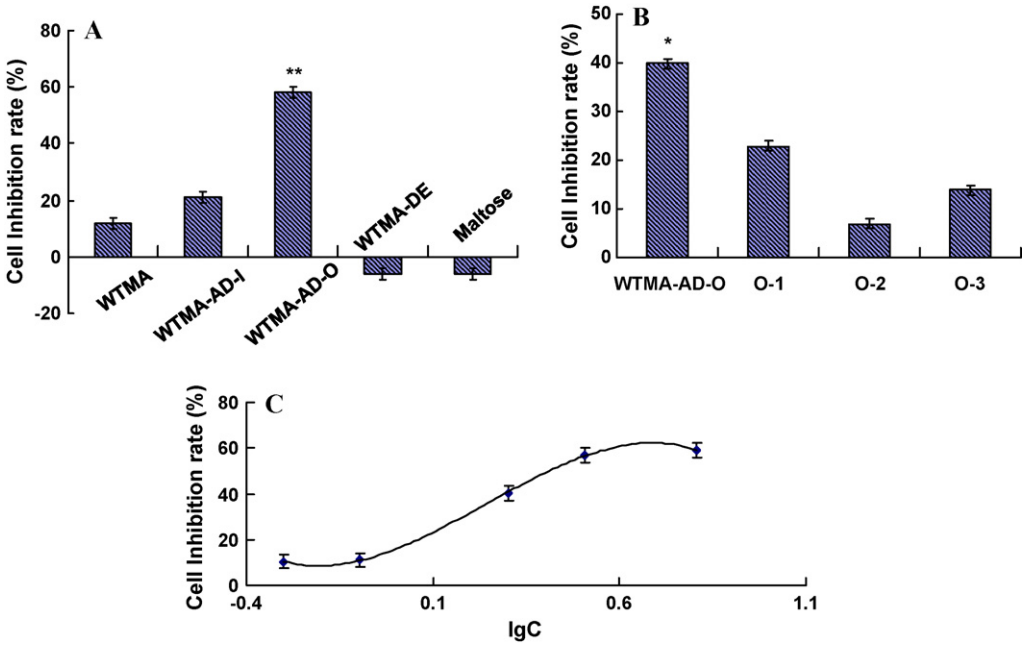


**Fig. 3.** PANC-1 cells were treated with polysaccharide WTMA and its gradation products for 72 h followed by cell viability assay using MTT method. (A) The PANC-1 cells growth inhibition rates are shown after the cells were treated with samples at concentration of 4 mg/mL. (B) The inhibition rates are demonstrated after PNAC-1 cells were incubated in the presence of WTMA degradation products at concentration of 2 mg/mg. (C) Raw data and fitted dose–response curves to estimated $IC_{50}$ on the dose* = log (dose). The unit of concentration was mg/mL. Two independent experiments were carried out in triplicates. Error bars represent standard error of triplicate data. Statistical comparison was made using Student's *t*-test. $^{**}p < 0.01$ vs blank group; $^{*}p < 0.05$ vs blank group.

Tests of WTMA-AD-O against PANC-1 cells at different concentration were also carried out to obtain an *in vitro* 50% inhibitory concentration ($IC_{50}$). The result in Fig. 3C showed that $IC_{50}$ was 2.6 mg/ml (Lyles, Poindexter, Evans, Brown, & Cooper, 2008).

To understand whether the inhibition effects caused by WTMA and its degradation products on PANC-1 cells were due to the cytotoxicity or not, hepatocyte LO2 cells were treated by WTMA, WTMA-AD-1, WTMA-AD-O, and WTMA-DE at the concentration of 8 mg/ml, respectively. The results showed that none of the samples tested had significant toxicity on PANC-1 cells (Fig. 4). Polysaccharide WTMA however, might slightly promote the proliferation of the cells.

WTMA possesses structure of amylopectin. Like some polysaccharide, amylopectin may activate immune system. For example, Ryoyama, Kidachi, Yamaguchi, Kajiura, and Takata (2004) demonstrated that partially degraded amylopectin (molecular weight is $1.1 \times 10^5$ Da) significantly stimulated macrophages-like cells, leading to increase product of nitric oxide and tumor necrosis factor-α. Raveendran et al. isolated an α-1,4-glucan (RR1, with molecular weight of $5.5 \times 10^5$ Da) from *Tinospora cordifolia*. The polysaccharide could activate the immune system through the activation of macrophages that occurred through TLR6 signaling, NF-κB translocation and cytokine production (Nair, Melnick, Ramachandran, Escalon, & Ramachandran, 2006). However to our knowledge, it seems that there is no evidence to date about any amylopectin which could impair pancreatic cancer cell growth. In this study we show that WTMA as an amylopectin and its degraded products may directly inhibit PANC-1 cell growth. This suggests that WTMA may be a mother compound which could be employed to be modified to achieve more potent drug candidate. Indeed, Qiu et al. (2010) found one sulfated amylopectin which inhibited tumor cells growth by blocking angiogenesis. Interestingly, except having the bioactivities on immune system and cancer cells, amylopectin may induce nonreversible insulin resistance in rats (Wiseman, Hiqqins, Denyer, & Miller, 1995). This suggests that amylopectin is not only an energy resource, but also could function as a bioactive compound in biological system.

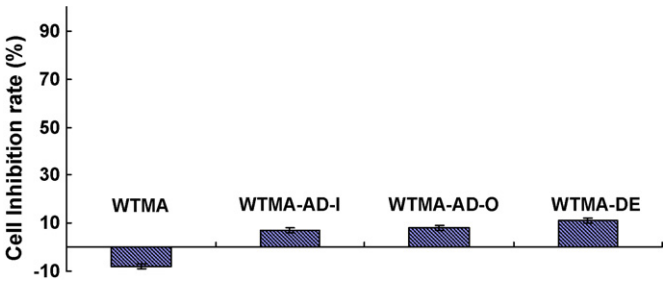


**Fig. 4.** Toxicities of WTMA and its degradation products to LO2 at the concentration of 8 mg/mL. Samples were added to the media after cells were maintained in the fresh medium for 24 h. Cell viability was evaluated by MTT assay after 72 h. Two independent experiments were performed in triplicates. Error bars represent standard error of triplicate data. Statistical comparison was made using Student's *t*-test.

## 4. Conclusion

In this report, one homogeneous polysaccharide, WTMA, was isolated from *G. elata*. Structure study showed that WTMA was an α-(1 → 4) glucan with α-(1 → 4) glucosyl branches attached to O-6 of branching points. Anti-cancer activity assay indicated that the polysaccharide could inhibit the growth of PANC-1 cell. Its acid hydrolysis derived products, WTMA-AD-I and WTMA-AD-O, demonstrated more potent anti-cancer cell growth activities. WTMA-DE, its enzymatic degradation-derived polysaccharide however, showed no effect on PANC-1 cells growth. This suggested that O-6 attached branch was necessary for this activity.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.06.029.

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