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Oligosaccharides structure by chemical hydrolysis from seeds polysaccharide of *Withania somnifera* Dunal (*Ashwagandha*) plant

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ABSTRACT

Chemical partial hydrolysis of water soluble Withania somnifera Dunal seeds polysaccharide gave a mixture of two disaccharides and one trisaccharide as : (I) 6-O-α-D-glucopyranosyl-(1→6)-O-α-D-mannopyranose; (II) 4-O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranose and (III) 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-mannopyranose.

Keywords: Oligosaccharides, *Withania somnifera* Dunal seeds polysaccharide

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INTRODUCTION

Withania somnifera Dunal (*Ashwagandha*) plant[1] belongs to family- Solanaceae is an evergreen herb about 30-170 cm in height. Plants are commonly used in Ayurvedic system of medicine for the treatment in anti-cancer, anti-oxidant, diarrhoea, dysentery, cholera, colic, fever, cancer therapy, anti bacterial, anti tumour, anti stress, anti depressant, anti inflammatory, etc. The water soluble seeds extract as glucomannan was found to be composed of D-glucose and D-mannose in the molar ratio of 1:3. Nature of the constituent sugars[2], methylation[3], periodate oxidation[4] and Smith degradation[5] studies of the seeds glucomannan revealed that the D-glucopyranose units occupy the terminal position in the main chain which consists of D-mannopyranose unit at the main polymer chain and D-glucopyranose at the non-reducing end position. Present manuscript mainly deals with the isolation, structure elucidation of the oligosaccharides obtained from the *Withania somnifera* Dunal seeds glucomannan by chemical hydrolysis. For identification of oligosaccharides, the column chromatography was carried out with chemical (partial acid) hydrolysis over charcoal-celite column[6] and characterisation of sugars by paper chromatographic analysis[7] on Whatman No. 3 MM filter paper afforded two disaccharides and one trisaccharide. These oligosaccharides were characterised by its optical rotation, formation of crystalline derivatives (disaccharides only), degree of polymerization[8], reduction with sodium borohydride, complete acid hydrolysis and periodate oxidation studies[9]. Oligosaccharide were characterized and identified as follows : (I) 6-O-α-D-glucopyranosyl-(1→6)-O-α-D-mannopyranose; (II) 4-O-β-D-glucopyranosyl-(1→4)-O-β-mannopyranose and (III) 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-mannopyranose.

MATERIALS AND METHODS

Separation of oligosaccharides from the hydrolysed compound of *Withania somnifera* Dunal water soluble seeds polysaccharide was carried out by descending techniques on Whatman No. 3 MM filter paper sheet by paper chromatography[7]. The upper phase pf following solvent mixture (v/v) were used for the detection of

monosaccharide and oligosaccharides as : (A) *n*-butanol-acetic acid-water (4:1:5)[10], (B) ethyl acetate-acetic acid-water (9:2:2)[11] and (C) ethyl acetate-pyridine water (10:4:3)[12]. The spray reagent (R) *p*-anisidine phosphate[13] was used for the detection of monosaccharide and oligosaccharides from the hydrolysed compound of polysaccharide. All evaporation of oligosaccharides was carried out under reduced pressure at 40-50^{0}C. The optical rotation values were recorded after equilibrium and melting points are uncontrolled. The *R* gal and *R* glu refer to the rate of movement of sugars relative to D-galactose and D-glucose respectively. Oligosaccharides mixtures were separated on charcoal-celite column (1:1, w/w) using followed by 2:5, 5.0, 7.5 and 10.0% (v/v) with aqueous ethanol as eluants. These eluants were further separated by paper chromatography on Whatman No. 3 MM filter paper sheet. Degree of polymerization (DP) was determined by Timell's method[8] and Deionisation was done with Amberlite ion-exchange resins[14], IR-45 (OH^-) and IR-120 (H^+).

Partial acid hydrolysis of oligosaccharides :

After a series of trial experiments the following method was carried out for the partial acid hydrolysis[15] to obtain the oligosaccharides. Polysaccharide (18 gm) was hydrolysed with sulphuric acid (1.5 N, 500 ml) for 24 hrs at 45^{0}C in refrigerator then the content was heated for 50 min. over boiling water-bath. The obtained hydrolysate was cooled, filtered and neutralized with barium carbonate slurry. It was filtered and filtrate concentrated to a small volume about 30 ml. Ethanol about 500 ml was added with the help of mechanical stirrer when the degraded polysaccharides was precipitated out as white coarse powder after filtration then dried. The paper chromatographic analysis[7] of the hydrolysate after concentration of ethanolic extract showed the presence of D-glucose and D-mannose and a member of oligosaccharides.

Degraded polysaccharide was again hydrolysed by keeping it in sulphuric acid (1.5 N, 600 ml) at room temperature for 72 hrs. It was heated on boiling water bath for 1 hr followed by cooling it in the same bath for 30 min then concentrated to a syrup. The syrup was deionised with Amberlite ion exchange resin, IR-45 (OH^-) and IR-120 (H^-).

Separation of oligosaccharides :

The oligosaccharides were separated by chromatographic adsorption method on charcoal-celite (1:1, w/w) glass column (60 × 2.5 cm) using the graded elution method[16]. Column was eluted with water (2 litres) under 7 lbs/sq. inch pressure to remove the monosaccharides then successively with 3 litres each of 2.5, 5.0, 7.5 and 10.0 % aqueous ethanol (v/v) as eluant. Oligosaccharides fraction (100 ml) was concentrated and then examined by paper chromatographic analysis on Whatman No. 3 MM filter paper sheet in solvent mixture (A) and used (R) as spray reagent. The corresponding sugar strips of oligosaccharides[17] were cut out with the help of guide spots and eluated with water according to the Dent's method[18] then filter concentrated to a syrup. It is observed that each fraction was not a single component but a mixture of 3 oligosaccharides. It led to the isolation of oligosaccharides were identified as: 2 disaccharides[17] and one trisaccharide[17] which were characterized as follows :

I : 6-O-α-D-glucopyranosyl-(1→6)-O-α-D-mannopyranose :

Syrup (280 mg) had *R* gal 0.67 in solvent (B) and *R* glu 0.48 in solvent (C), optical rotation $[\alpha]_D^{24}$ +35.0^{0}C→ +20.0^{0}C (H_2O), Lit $[\alpha]_D$ +34.5^{0}C → + 19.9^{0}C (H_2O)[19] and having m.p. 184-186^{0}C. Acid hydrolysis with sulphuric acid (1 N) by usual manner to obtained the hydrolysate which was paper chromatographically examined to show the presence of D-glucose and D-mannose only as determined by phenol sulphuric acid method[20]. Degree of polymerization was formed to be 1.84 indicating that this oligosaccharide was a disaccharide. Derivative of oligosaccharide was prepared by usual manner as phenyl osazone having m.p. 176-178^{0}C Lit. 175-1770C[21], periodate oxidation[22] of oligosaccharide (50 mg) was carried out with sodium metaperiodate (0.25 M, 10 ml) at 4-8^{0}C in refrigerator for 55 hrs. It consumed 5.61 moles of periodate with the simultaneous liberation of 2.84 moles of formic acid per mole of disaccharide after 55 hrs and results are given Table-1.

Table-1 : Periodate oxidation of oligosaccharide-I

S. No.	Sugar Content	Time (hrs)						
		10	20	30	40	45	50	55
1.	Periodate consumption of disaccharide - I (moles/mole)	2.82	3.97	5.26	5.58	5.61	5.61	5.61
2.	Formic acid liberation of disaccharide - I (moles/mole)	1.26	1.60	1.98	2.38	2.84	2.84	2.84

II : 4-O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranose :

Sugar syrup (290 mg) had *R* gal 0.63 in solvent (B) and *R* glu 0.54 in solvent (C), optical rotation $[\alpha]_D^{24}$ +15.8^{0}C (H_2O) and had m.p. 194-196^{0}C. The DP was found to be 1.89 which indicating that the oligosaccharide was a

disaccharide. Oligosaccharide was hydrolysed with sulphuric acid (1 N) and subsequent paper chromatography of hydrolyzate indicated that the presence of monosaccharide, D-glucose and D-mannose are in equal amount[21]. Phenyl hydrazone derivative[22] of disaccharide (50 mg) was prepared by usual manner having m.p. 193-195^{0}C, Lit. m.p. 192-1940C[23]. Periodate oxidation of oligosaccharide (60 mg) by usual manner consumed 6.12 moles of periodate and liberated 3.64 moles of formic acid per mole of disaccharide after 55 hrs and result are shown in Table-2.

Table-2: Periodate oxidation of Oligosaccharide-II

S. No.	Sugar Content	Time (hrs)					
		10	20	30	40	50	55
1.	Periodate consumption of disaccharide - II (moles/mole)	3.20	4.98	5.71	6.12	6.12	6.12
2.	Formic acid liberation of disaccharide - II (moles/mole)	1.92	3.10	3.54	3.64	3.64	3.64

III : 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose:

Sugar syrup (200 mg) had *R* gal 0.32 in solvent (B) and *R* glu 0.20 in solvent (C), optical rotation $[\alpha]_D^{24}$ - 21^{0}C (H_2O), Lit. $[\alpha]_D$ - 22^{0}C (H_2O), having m.p. 167-169^{0}C. Lit. m.p. 169.50C[22]. Degree of polymerization was found to be 3.12 indicating that the oligosaccharide was a trisaccharide. Methylation studies of trisaccharide by Hakomari's method[25] by paper chromatography gave 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-mannose which showed (1→4)-β-type linkages by emulsion. On periodate oxidation studies of the trisaccharide by usual manner consumed 6.24 moles of periodate and liberated 3.62 moles of formic acid per moles of trisaccharide after 55 hrs and results are shown Table-3.

Table-3: Periodate oxidation of oligosaccharide-III

S.No.	Sugar Content	Time (hrs)						
		10	20	30	40	45	50	55
1.	Periodate consumption of trisaccharide - III (moles/mole)	3.22	5.14	5.82	5.98	6.24	6.24	6.24
2.	Formic acid liberation of trisaccharide - III (moles/mole)	1.28	2.94	3.36	3.58	3.62	3.62	3.62

RESULTS AND DISCUSSION

Water soluble seeds polysaccharide from *Withania somnifera* Dunal (*Ashwagandha*) obtained as D-glucose and D-mannose in 1:3 molar ratio by paper chromatographic analysis of hydrolysed compound by usual manner. Present manuscript mainly deals with the chemical hydrolysis of purified seeds polysaccharide with sulphuric acid (1 N) followed by charcoal-celite column chromatographic analysis and paper chromatography of the hydrolysate afforded two disaccharides and one trisaccharide as major components. Oligosaccharides were purified separately and characterized by their optical rotation, melting points, formation of crystalline derivatives (disaccharides only), degree of polymerization (DP), complete acid hydrolysis and periodate oxidation studies. The oligosaccharides were identified as : (I) 6-O-α-D-glucopyranosyl-(1→6)-O-α-D-mannopyranose; (II) 4-O-β-D-glucopyranosyl-(1→4)-O-β-D-mannopyranose and (III) 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-mannopyranose.

Isolation of oligosaccharide (II) and (III) clearly indicates that the main chain or backbone of the glucomannan polymer contains D-glucopyranose and D-mannopyranose units which are joined through (1→4)-β-type linkages. Formation of oligosaccharide (I) suggests that the branches at main chain consists of non-reducing end with D-glucopyranose residues which are glycosidically attached through (1→6)-α-type linkages with D-mannopyranose. Further the trisaccharide (III) was also confirms that the D-mannopyranose units at the main chain by (1→4)-β-type linkages. The earlier proposed polysaccharide structure of *Withania somnifera* Dunal seeds polysaccharide (Figure-1) obtained after methylation, periodate oxidation results is favoured by the above oligosaccharide results.

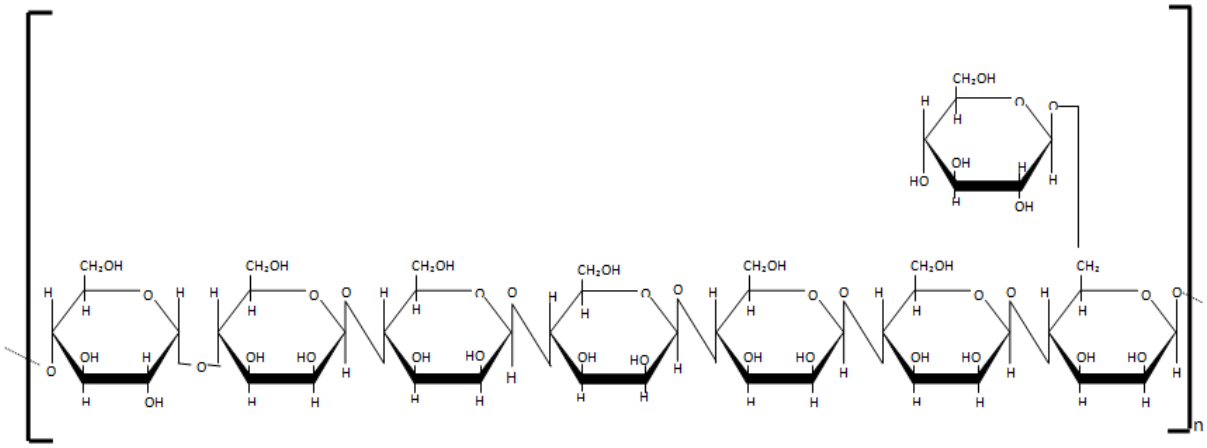

Figure- 1: Polysaccharide structure of *Withania somnifera* Dunal seeds polysaccharide

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