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Periodate oxidation method used for the confirmation of seeds polysaccharide structure of *Withania somnifera* Dunal plant

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ABSTRACT

Plant of *Withania somnifera* Dunal belongs to family – Solanaceae and called as Ashwagandha. Plants are medically used in Ayurvedic system of medicine for the treatment of antioxidant, anticancer, therapeutic, anti-inflammatory, leucoderma, antitumor, etc. Plants had a potential role in cancer therapy in the growth inhibitory of human tumor cells, anticarcinogenic activity, skin carcinoma, antioxidative and chemo-protective activity in humans. Leaves and roots are also used in memory loss, depression, infertility and in babies tonic. Seeds yielded a water soluble sugars as D-glucose and D-mannose in 1:3 molar ratio by GLC, TLC, column and paper chromatography. Periodate oxidation was carried by sodium metaperiodate as oxidant and periodate consumption and formic acid liberation was determined from seeds polysaccharide. It consumed 3.12 moles of periodate with simultaneous liberation of 1.36 moles of formic acid per equivalent moles of each anhydrohexose sugar units after 60hrs. Presence of (1→4)- β -type and (1→6)- α -type linkages which were obtained from methylation results are also confirmed by periodate oxidation studies. Formic acid is to be originating from the reducing as well as non-reducing terminal unit of D-glucose unit. It is concluded from the above facts that the probably one branch occurs 8 sugar hexoses repeating unit in the seed polysaccharide structure of *Withania somnifera* Dunal (Ashwagandha) which were obtained from methylation results was confirmed by periodate oxidation technique.

Keywords: Periodate oxidation, periodate consumption, formic acid liberation of *Withania somnifera* seeds polysaccharide.

INTRODUCTION

Withania somnifera Dunal^[1] plant belongs to Solanaceae family and commonly called as Ashwagandha. It is an evergreen plant upto 30-170cm in height. It occurs in India, Pakistan, Sri Lanka, Nepal, Myanmar, Thailand, Bangladesh, Europe, Java, Malaysia, African & Asian Tropics. It is widely distributed in Himalayan tract from Punjab to Nepal. Medically plant was also used in the treatment of dysentery and other painful infection of the intestine canal. Roots are used as a remedy for painful affection of the vowels. Leaves extracted as Withaferin-A alkaloid which are used in anticancer and antioxidant activity. Roots, leaves and seeds extracts are also used for the treatment of the intestinal disorder, diarrhea, dysentery, fever and cholera, etc. Plant had a unique alkaloid that have remarkable medicine properties, Withaferin-A is one of the important Withanolids alkaloids which isolated form leaves and roots. Isolation, purification and preliminary analysis^[2], methylation^[3] and determination of polyalcohols by Smith degradation technique^[4] of water soluble seed polysaccharide have already been studied for the identification of polysaccharide structure. Present manuscript mainly deals with periodate oxidation studies from the

medicinal plant of *Withania somnifera* Dunal seeds polysaccharide. Sugars was extracted from the water soluble crushed seeds which were identified as D-glucose and D-mannose in the molar ratio of 1:3 moles from hydrolyzed product by GLC, TLC, column and paper chromatographic analysis.

Periodate oxidation reaction in the carbohydrate chemistry was first determined by Malaparade^[5] and Fluery & Lange^[6] have given the periodic acid for the oxidation of glycol groups while Perlin^[7] given Lead Tetra Acetate and periodic acid showed that the glycol groups undergo cyclic ester formation with oxidation and reaction is to be dialdehyde type. Sarkar^[8] have used the periodate oxidation to determine the seeds polysaccharide structure. Periodate oxidation studies of the seeds polysaccharide of *Nyctanthes arbor-tristis* Linn.^[9], *Pongamia pinnata* Linn.^[10], *Abrus precatorius* Linn.^[11], *Rauwolfia serpentina* Benth.^[12] have already been studied by various Scientist for the confirmation of polysaccharide structure which was obtained after methylation studies of polysaccharide.

MATERIALS AND METHODS

Purified seeds polysaccharide of *Withania somnifera* Dunal (0.190gm) was oxidized with distilled water (100ml) then added an aqueous solution of sodium metaperiodate^[13] (0.25 M, 25ml) and volume of reaction mixture was made upto 250ml with water. The reaction flask was kept in Refrigerator at 4-8°C for 60hrs. Aliquot (5ml) was pipetted out from the reaction flask for the determination of periodate consumption and formic acid liberation which were estimated at various time intervals. For periodate consumption^[6], the reaction mixture (5ml) was taken in a conical flask then added sodium bicarbonate solution (2 ml), sodium arsenite solution (0.01 N, 25 ml) and potassium iodide solution (20%, 2 ml). Reaction mixture was shaken for 1 hr and added iodine solution (0.01 N, 5 ml), using starch as an indicator. The excess iodine was titrated against sodium thiosulphate solution (0.1 N). A blank titration was also carried out in a similar way. The difference between blank and experiment gives the periodate consumption of 3.12 moles of periodate after 60 hrs (Table-1).

Formic acid liberation^[14, 15, 16] was determined by taken the aliquot (5 ml) in a conical flask then added ethylene glycol (10 ml) to destroy the excess of periodate ions present in the reaction mixture for 30 min. The formic acid liberation was estimated by titration against sodium hydroxide solution (0.12 N), using methyl red dye as an indicator. A blank titration was also carried out in a similar way for the estimation of formic acid. It liberated 1.36 moles of formic acid per mole of anhydrohexose sugar units after 60 hrs. Results of periodate consumption and formic liberation of *Withania somnifera* Dunal seeds polysaccharide are given in Table-1.

Table-1: Periodate oxidation of *Withania somnifera* Dunal seeds polysaccharide

Sr. No.	Sugar present	Time (hrs)						
		10	20	30	40	50	55	60
1.	Periodate consumption of anhydrohexose sugar unit (moles/mole)	1.16	1.74	2.12	2.56	2.84	3.12	3.12
2.	Formic acid liberation of anhydrohexose sugar unit (moles/mole)	0.32	0.94	1.08	1.16	1.28	1.36	1.36

RESULTS AND DISCUSSION

Withania somnifera Dunal (*Ashwagandha*) seeds yielded a water soluble polysaccharide as D-glucose and D-mannose in the molar ration 1:3 moles by GLC, TLC, Column and Paper chromatographic analysis. For periodate oxidation the purified seeds polysaccharide was oxidized with sodium metaperiodate with usual manner. It consumed 3.12 moles of periodate and liberated 1.36 moles of formic acid per equivalent for each anhydrohexose sugar unit of the polymer after 60 hrs. Presence of (1→4)-β-type and (1→6)-α-type linkages obtained after methylation results are also confirmed by the periodate oxidation results. Polysaccharide were containing free hydroxyl groups resulting in the consumption of periodate ions during periodate oxidation reaction. Formic acid is to be originating from the reducing as well as non-reducing terminal unit of the D-glucose unit. It is concluded from the above fact that probably one branching point occurs 8 sugar hexoses repeating unit in the seed polysaccharide structure of *Withania somnifera* Dunal (*Ashwagandha*) which were obtained from methylation results was also confirmed by the periodate oxidation results as shown in Figure-1.

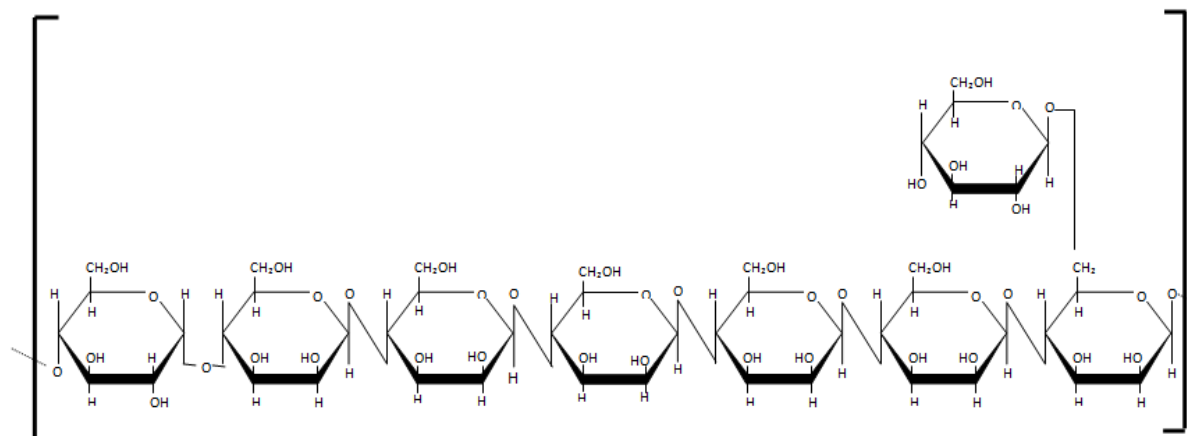


Figure- 1: Polysaccharide structure of *Withania somnifera* Dunal seeds polysaccharide

CONCLUSION

Withania somnifera Dunal (*Ashwagandha*) seeds yielded a water soluble sugars extract as D-glucose and D-mannose in the molar ratio 1:3. After methylation studies of seeds polysaccharide yields methyl sugars which are medically used in Ayurvedic system of medicine for the treatment of Cancer, Therapeutic, Dysentery, Diarrhoea, Leucoderma, etc. Periodate oxidation of purified seeds polysaccharide with usual manner consumed 3.12 moles of periodate and liberated 1.36 moles of formic acid per mole of anhydrohexose sugar units. The polysaccharide structure of *Withania somnifera* Dunal (*Ashwagandha*) obtained after methylation studies were confirmed by the periodate oxidation results. Seeds polysaccharide may also be commercially used in the Sugar, Pastry, Ice-cream, Bakery, Industry and seed alkaloids used in cough syrup, nervous disorder, anti oxidant, abortifacient and sedative. It has also be used in rheumatism, leprosy and arthritis.

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