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Research Article

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Water soluble seeds polysaccharide extracted from medicinal plant of *Withania somnifera* Dunal (*Ashwagandha*)

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ABSTRACT

Withania somnifera Dunal seeds yielded a water soluble polysaccharide as D-glucose and D-mannose in 1 : 3 molar ratio by acid hydrolysis method. The individual monosaccharides were identified by GLC, TLC, paper chromatography and separated by column chromatography. It consumed 1.30 moles of iodine by iodometrically. Its IR spectral data (KBr) indicated that the α-type linkages in D-glucopyranose units at non-reducing ends while β-type linkages in the backbone or main polymer chain with D-glucose and D-mannose units. It was identified by their melting points, preparation of derivatives, nature of the constituent sugars and preliminary analysis of polysaccharides.

Keywords : Polysaccharide, D-glucose and D-mannose, *Withania somnifera* Dunal seeds.

INTRODUCTION

Withania somnifera Dunal plant[**1]** belongs to the family – Solanaceae is commonly called as *Ashwagandha*, *Winter cherry*, *Indian ginseng*. It is an evergreen shrub about 30 – 150 cm in height. It occurs in Pakistan, African and Asian Tropics, Europe, Bangladesh, Thailand, Sri Lanka and Northern India particularly in Garhwal region. It is used in Ayurvedic system of medicine, for antioxidant, anticancer, anti-inflammatory, leucoderma, etc. Roots, leaves and bark has a potential role in the cancer therapy for growth inhibitory of human tumor cell lines: anticarcinogenic activity, abcetic sarcoma in skin carcinoma in rats, anti-granuloma, anti-oxidative and chemoprotective activity. Roots and leaves are used in tonic, abortifacient, abtringent, nervine, mental problem improvement and also used in arthritis, depression, chronic diseases, infertility, memory loss, breathing difficulties and hormonal imbalance. Medically, *Ashwagandha* plant have been used back over 3000 – 4000 years to the Bleaching Rishi Punarvasu Ariya. It has been described in scared test of Ayurvedic include Charaka and Bushruta Samhita, where it is widely extolled as babies tonic and embalancing the reproductive function of both men and women. Alkaloids extracted from the roots exhibits hypotensive, bradicardiac and respiratory stimulant activity in dogs. Its roots has a great demand in drug and in herbal drug industry and its extract used in uterine construction and recommended in child birth in difficult cases. Seeds powder are used for coagulating milk. *Ashwagandha* is given to old people and pregnant women as a nutrient of health restorative. Root extract of plant show antitumors and radio sensitizing effects in animal models. Seeds of fruits are diuretic and can be used as a substitute for rennet to curdle milk.

Present manuscript mainly deals with the extraction and isolation of the crushed seeds of *Ashwagandha* with water and precipitation of polysaccharide with ethanol. It was then purified by complexation method, ion exchange resins and ultra centrifugation method in authentic form. Its preliminary analysis like nature of linkages and constituent sugars then it hydrolysed with sulphuric acid and hydrolysate gave monosaccharides by GLC, TLC, paper and column chromatography.

EXPERIMENTAL SSECTION

Isolation and purification of polysaccharide :

Seeds (500 gm) of *Withania somnifera* Dunal were collected from F.R.I. Dehradun (Uttarakhand), then washed the seeds with water, dried and crushed to a greyish powder. Powdered seeds (250 gm) was dissolved in distilled water (800 ml)[**2]** for 24 hrs. and content was stirred thoroughly by mechanical stirrer then the viscous solution was filtered through muslin cloth, then again filtered by Sharple's Super Centrifuge[**3]** to remove all finely suspended particles. Filtrate was precipitated with ethanol (2 litre) to precipitated out all the polysaccharide in light brown form. Precipitate of polysaccharide was filtered through sintered funnel (G-3) under suction and dried in vacuum at 60ºC after washing with acetone and pet ether. It was obtained as a crude greyish powder (21.6 gm), having sulphated ash 1.8% and optical rotation, $[a]^{25}_{D}$ + 30.4ºC (H_2O) .

Crude polysaccharide (8 gm) was again purified by redissolving in water (500 ml) then the content was filtered and filtrate treated with 20% ethanol to precipitated out higher molecular weight polysaccharide, were removed by ultracentrifugation method[**4]**. Colloidal polysaccharide was treated with chloroform (50 ml) to precipitated out protein as impurities in gel form were collected at water-chloroform interface[**5]**, removed by filtration. It was further purified by bariam complex formation method and copper complexation with Fehling's solution[**6]**. The resulting centrifugate was treated with 40% & 60% ethanol concentration to precipitated out whole polysaccharide then triturated with absolute alcohol and pet. ether and dried over calcium chloride under vacuo at 60ºC. These two polysaccharide fraction when subjected to its IR-spectra (KBr)[**7]** showed the identical homogeneous spectrogram, yield (7.26 gm).

Preliminary analysis of polysaccharide :

Purified *Withania somnifera* Dunal seeds polysaccharide was obtained in the form of greyish amorphous powder. It had sulphated ash (0.862%), $[\square]_D^{25}$ + 29.2ºC (H_2O) for 40% and sulphated ash 0.624%, $[\alpha]_D^{25}$ + 29.6ºC (H_2O) for 60%. These two polysaccharide fractions showed identical homogenous spectrogram and did not reduce the Fehling's solution. After analysis it did not showed the presence of nitrogen, sulphur, halogens, acetyl groups, uronic acid and methoxyl groups percentages[**8]** but pentosans, pentoses and furfural[**9]** were present in 1.18%, 0.92% and 0.84%.

Nature and identification of sugars :

Purified seeds polysaccharide (2.6 gm) was hydrolysed[**10]** with sulphuric acid (72%, 10 ml) overnight at room temperature. The slurry was cooled and diluted with water (116 ml) to make up a normal solution with respect to H_2SO_4. Solution was refluxed in boiling water-bath at 100ºC, when the hydrolysis was completed by iodometrically[**11]**. Rate of hydrolysis of polysaccharide with H_2SO_4 (72%) followed by H_2SO_4 (1N) after definite interval of times with hydrolysate (2 ml) then added iodine solution (0.1N, 10 ml) and sodium hydroxide solution (0.1N, 15ml). It was acidified with sulphuric acid (2N, 25 ml) and excess iodine was titrated against sodium thiosulphate solution (0.1 N), using phenolphthalene as an indicator. It consumed 1.30 moles of iodine by iodometrically after 34 hrs.

Hydrolysate was neutralized ($BaCO_3$), while barium sulphate and unreacted barium carbonate were removed by filtration and residue washed with water. Resulting filtrate was passed through regenerated Amberlite IR-120 (H^+) and IR-45 (OH^-) ion exchange resins[**12]** then concentrated to syrup.

Resolution and identification of sugars by paper and column chromatography :

Hydrolysate was identified by descending technique of paper chromatography[**13]** on Whatmann No. 1 filter paper sheet, using solvent mixture (v/v) for the detection of sugars as : (A) *n*-butanol, ethanol, water (4:1:5, upper phase[**14]** and (R) *p*-anisidine phosphate reagent[**15]** used as spray reagent to revealed the presence of D-glucose (*Rf* 0.18) and D-mannose (*Rf* 0.24).

Sugar syrup (3 ml) was resolved into its components by column chromatography[**16]** with eluting solvent as *n*-butanol half saturated with water[**17]**. Column of cellulose powder (25 gm) was prepared in a glass column (55.2 cm) fitted with glass stopper. Solvent was allowed to percolate down using a constant head reservoir arrangement and the eluates were collected in 10 ml portion. Fractions of eluates were examined by paper chromatography on Whatman No. 1 filter paper using solvent mixture (A) as irrigant and appropriate sugars fractions were combined and evaporated to dryness and found to contain the sugars fraction and results are shown in Table – 1.

Table 1 : Resolution of sugar mixture by column chromatography

S.No.	Fraction No.	Sugar present
1	01 – 38	No sugar
2	39 – 81	D-mannose only
3	82 – 98	Mixture of D-mannose & D-glucose
4	99 – 114	D-glucose only
5	115 – onwards	No sugar

Characterization of sugars :

Appropriate sugar fractions of eluate containing single pure sugar were combined and identified as : D-glucose, had m.p. & mixed m.p. 147 – 148ºC, $[\alpha]_D^{30}$ + 12.8ºC (H_2O) while D-mannose, had m.p. & mixed m.p. 131 – 132ºC, $[\alpha]_D^{30}$ + 53.4ºC (H_2O).

Derivative was prepared with aqueous solution of D-mannose (25 ml), acetic acid (1 ml) and phenyl hydrazine (1 ml) in conical flask. Flask was kept in boiling water-bath for 30 minute and then shaken periodically, yellow precipitate of D-mannose phenyl hydrazone[**18]** was obtained, had m.p. & mixed m.p. 194 – 196ºC. Derivative of D-glucose (25 ml) was prepared with glacial acetic acid (1 ml), phenyl hydrazine (1 ml) and content was heated for 30 minute on boiling water-bath. After cooling a bulky yellow precipitate of D-glucose osazone[**19]** was obtained after recrystallisation with ethanol having m.p. & mixed m.p. 202 – 204ºC.

Quantitative estimation of sugars :

Purified seeds polysaccharide (500 ml) was quantitatively estimated[**20]** by sulphuric acid (1N, 8 ml) in a sealed tube on water-bath at 100ºC for 30 hrs. It was filtered and filtrate neutralized ($BaCO_3$), filtered and obtained filtrate concentrated to a thin syrup. Hydrolysate was separated on Whatman No. 3 MM filter paper sheet in solvent mixture (A) and used (R) as spray reagent. The areas of corresponding single sugar strips components were cut out with the help of guide spots and sugars were eluted with water according to the Dent's method[**21]**. The eluted sugars were estimated by phenol sulphuric acid method[**22]**. The molar ratio of D-glucose and D-mannose in the purified seeds polysaccharide was found to be 1 : 3 moles.

RESULTS AND DISCUSSION

Withania somnifera Dunal seeds yielded a water soluble polysaccharide having D-glucose and D-mannose in the molar ratio of 1:3 by paper and column chromatographic analysis. It consumed 1.30 moles of iodine by iodometrically. Since the rotation of parent polysaccharide is a low positive and anomeric linkages is predominantly of β-type possibly with few α-type linkages. Nature of linkages were also confirmed by IR-spectra (KBr)[**23]** and absorption bands were recorded at 814 cm^{-1} and 874 cm^{-1} region. It indicated α-type linkages in D-glucose at non-reducing ends while β-type linkages in D-gluocse and D-mannose in main polymer chain[**24]** of *Ashwagandha* seeds polysaccharide. Derivative of seeds polysaccharide was prepared by usual manner as D-mannose phenyl hydrazone and D-glucose osazone. Polysaccharides are commercially used in sugar, pastry, ice-cream, textile, pudding, industries. It is also used in the air pollution to minimize the air pollutant in the environment.

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