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ORIGINAL ARTICLE

Isolation, Purification and Preliminary Analysis of Seed Polysaccharide from *Cassia glauca* Lam. Plant

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ABSTRACT

Cassia glauca Lam. seeds contain a water soluble polysaccharide as D-galactose and D-mannose in the molar ratio of 1:4 on hydrolysed compound by TLC, Column and Paper Chromatographic analysis. The rotation of parent polysaccharide is a low positive and linkage must be of β-type and α-type. Linkages has also been confirmed from IR-Spectra (KBr) which showed that the α-type linkages with D-galactopyranose and β-type linkages with D-mannopyranose units.

Key words: *Polysaccharides, Cassia glauca Lam., seeds polysaccharide*

INTRODUCTION

Cassia glauca Lam. plant (Chopra, *et al.,* 1956; Kirtikar and Basu, 1990) belongs to the family-Caesalpiniaceae, is about 10m in height. It is an evergreen shrubs and occurs all over India, Pakistan, Sri Lanka, Malaysia, China, South America, Australia and Tropical Asia. Bark and leaves are used in diabetes and gonorrhoea in folk medicine. It is seeds oil are used in indigenous system of medicine for the treatment of skin diseases and leucoderma. Present investigation mainly deals with the isolation, purification and preliminary analysis of seeds polysaccharide in authentic form and its properties then the nature of the constituent sugars. Linkages are also confirmed by IR-Spectra (KBr) at 817cm^{-1} and 874cm^{-1} absorption band. Recently the seeds polysaccharide were isolated from some medicinal plants like, *Cassia alata* Linn., *Withania Somnifera* Dunal, *Wrightia tinctoria* R.Br. (Roxb.), *Madhuca longifolia* Linn. (Singh, 2017b; Singh, 2012; Singh, 2013b and Singh, 2017a).

ISOLATION AND PURIFICATION OF SEEDS POLYSACCHARIDE

Seeds (250 gm) was washed with water, dried and cleaved at a low speed hand grinder into yellowish grey powder. Seeds powder (100 gm) were soaked in distilled water (800 ml) (Singh, 2013a) overnight then stirred with the help of mechanical stirrer for 12hrs. The resulting viscous solution was squeezed through muslin cloth to remove the insoluble matter present in the solution. The obtained solution was then centrifuged through Sharples Super Centrifuge (Shachmann, 1948) at 25,000 r.p.m. to remove the finely suspended particles and filtered. Centrifugate was then treated with ethanol (2L) to precipitate out the polysaccharide in light brown course powder. Precipitate of polysaccharide was filtered through sintered funnel (G-3) under suction and dried in vacuo at 60^{0}C after washing with acetone and pet. ether (40-60^{0}C). Grey solid mass of polysaccharide (8.24gm) was obtained and has sulphated ash 1.05% as determined by sulphated ash method (Singh, 1999) had $[\alpha]_D^{25}$ +29^{0}C (H_2O).

Crude seed polysaccharide (5gm) was purified with water (500ml) by mechanical stirring for 12hrs, filleted and treated with ethanol (20%). This precipitated out some impurities, which was allowed to settle down overnight and obtained centrifugate was further treated with ethanol (30%), to precipitated out higher mol. wt. polysaccharide which was removed by ultracentrifugation method (Wilkil, 1957). Colloidal solution was treated with chloroform to remove protein in gel form at water-chloroform interface (Shaub, 1965). It was further purified by addition of Fehling's solution and copper complex was precipitated out by copper complex

formation method (Hirst, 1945). The resulting centrifugate was treated with 40% and 60% ethanol concentration to precipitate out whole of polysaccharide. The polysaccharide was obtained by 40% and 60% ethanol concentration were triturated with absolute ethanol, acetone and pet. ether (40-60^{0}C) and dried over calcium chloride in vacuo at 60^{0}C. These two fraction of the seed polysaccharide, when subjected to IR-Spectra (KBr) (Rao, 1963) showed the identical homogenous spectrogram, yield (2.84gm), sulphated ash (0.524%), optical rotation $[\alpha]_D^{25}$ +28.2^{0}C (H_2O) for 40% and $[\alpha]_D^{25}$ +28.9^{0}C (H_2O) for 60% respectively.

Seeds polysaccharide was obtained in the form of grey amorphous powder which did not reduce Fehling's solution. Nitrogen, sulphur, halogens, acetyl group, uronic acid, methoxyl group were absent but pentosans (2.05%), pentoses (2.32%) and furfural (1.02%) were present (Neiderl and Neiderl, 1948; Anderson, 1959; Belcher and Godberst, 1949 and Brown and Zerban, 1941).

NATURE OF THE SEEDS POLYSACCHARIDE

Purified seeds polysaccharide (4gm) was hydrolysed (Hamilton and Partlow, 1950) with sulphuric acid (72%, 8ml) then it kept overnight at room temperature. The slurry was cooled in a freezing mixture and diluted with water (115ml) to make up a normal solution with respect to sulphuric acid (1N). The solution was refluxed on water-bath for 12hrs at 100^{0}C, when the hydrolysis which was followed iodometrically (Baker and Hulton, 1920) was found to the completed. The results are shown in Table-1, which showed the rate of hydrolysis of polysaccharide with sulphuric acid (72%) followed by sulphuric acid (1N).

Table 1: Course of acid hydrolysis of seeds polysaccharide of *Cassia glauca* Lam.

S. No.	Time (hrs)	Hypo required (ml)	Iodine consumed (calculated on the basis of hypo consumption) (ml)	Remarks
1.	00	4.20	0.00	Sulphuric acid (72%) at R.T.
2.	12	3.40	0.80	
3.	14	3.25	0.95	Sulphuric acid (1N) at 100^{0}C
4.	16	3.15	1.05	
5.	18	3.10	1.10	
6.	20	3.05	1.15	
7.	22	3.00	1.20	
8.	24	3.00	1.20	
9.	26	3.00	1.20	

The obtained hydrolysate was neutralized with barium carbonate slurry by keeping constant well stirred during neutralization and kept overnight at room temperature. The barium sulphate and unreacted barium carbonate were removed from the solution by filtration and residue was washed with water. The resulting solution was passed through regenerated Amberlite. IR-120 (H^+) and IR-45 (OH^-) ion exchange resins (Kanin, 1958), then concentrated to a thin syrup. Paper chromatographic analysis by descending technique (Partridge, 1946), using upper phase of the solvent mixture (v/v): (A) *n*-butanol-ethanol-water (4:1:5) (Partridge and Westall, 1948) and used (R) *p*-anisidine phosphate (Mukherjee and Srivastava, 1948) as spray reagent to revealed the presence of monosaccharide as: D-galactose and D-mannose sugars.

RESOLUTION OF SUGARS BY CELLULOSE COLUMN CHROMATOGRAPHY

Sugars mixture was resolved into its component by cellulose column chromatographic analysis (Kowkobany, 1950). The column was prepared by using Whatman standard chromatographic cellulose powder and *n*-butanol half saturated with water (Chalson, *et al.,* 1956). The obtained sugar fractions in column were examined by descending paper chromatographic analyses to contain the sugars are given in Table 2.

Table 2: Resolution of sugars fraction on cellulose column chromatography

S. No.	Fraction No.	Sugar Faction Present
1.	01-39	No sugar
2.	40-60	D-mannose only
3.	61-73	Mixture : D-mannose & D-galactose
4.	74-100	D-galactose only
5.	100-onward	No sugar

CHARACTERIZATION OF POLYSACCHARIDE

Appropriate sugar fractions of the eluate containing the single pure manosaccharide were combined together and concentrated and identified as D-galactose and D-mannose are as : D-galactose, m.p. & mixed m.p. 165-166^{0}C, $[\alpha]_D^{20}$ +81.0^{0}C (H_2O) and D-mannose, m.p. & mixed m.p. 129-130^{0}C, $[\alpha]_D^{20}$ +120.0^{0}C (H_2O).

The derivatives of D-galactose was prepared with aqueous solution of D-galactose (25ml) was taken in a conical flask and added glacial acetic acid (10 drops) and phenyl hydrazine (5 drops) then periodically shaken. The flask was fitted with cork loosely and kept it in the boiling water-bath for 10 min. at 100^{0}C. A bulky yellow precipitate of D-galactose phenylhydrazone phenylhydrazone (Refique and Smith, 1950) derivative was obtained having m.p. & mixed m.p. 172-173^{0}C. Derivative of D-mannose (25 ml) was also prepared in the above same method as D-mannose phenylhydrazone (Refique and Smith, 1950), having m.p. & mixed m.p. 195-197^{0}C.

QUANTITATIVE ESTIMATION OF POLYSACCHARIDE

In the quantitative estimation (Hirst and Jones, 1949) of purified seed polysaccharide (280 mg) was treated with sulphuric acid (1 N, 10 ml) at 100^{0}C in a sealed tube for 26hrs on water-bath as usual. The obtained hydrolysate was separated on Whatman No.1 MM filter paper sheet using solvent mixture (v/v) as: *n*-butanol-ethanol-water (4-1:5-upper phase). The areas of paper containing single sugar components were cut out with the help of guide spots and sugars fraction were eluted with water according to the Dent's method (Dent, 1947). The eluted monosaccharide sugars were estimated by periodate oxidation method (Hirst and Jones, 1949) for necessary correction the blank reading were also made. The molar ratio of D-galactose and D-mannose in the purified seeds polysaccharide was found to be 1:4 moles respectively.

Some information about the nature of linkages in the purified *Cassia glauca* Lam. seeds polysaccharide was obtained from the Infrared-Spectra (KBr). The absorption bands were recorded at 817 cm^{-1} and 874 cm^{-1} which showed α-type linkages in D-galactopyranose and β-type linkages in D-mannopyranose sugar units.

RESULTS AND DISCUSSION

The water soluble seeds polysaccharide of *Cassia glauca* Lam. yielded D-galactose and D-mannose in 1:4 molar ratio on hydrolysed compound by column and paper chromatographic analysis. It consumed 1.20 moles of iodine by iodometrically and molar ratio by periodate oxidation method. Since the rotation of parent polysaccharide is a low positive and linkages must be of β-type possible with few α-type linkages. Linkages have also been confirmed by IR-Spectra (KBr) (Bellamy, 1958) and absorbances were recorded at 817 cm^{-1} and 874 cm^{-1} region. It showed that D-galactopyranose have α-type linkages at non-reducing while D-mannopyranose and D-galactopyranose have β-type linkages (Barker, *et al.,* 1956) in the main polymer chain of seed polysaccharide.

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