

Extraction of Galactomannan from Medicinal Plant Cassia Fistula

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Abstract- A water soluble galactomannan having D-Mannose [4-part] and D-Galactose [2-parts] was isolated from Cassia fistula. In cassia fistula Hydrolysis of methylated seed gum furnished three methylated sugars, 2, 3-di-O-methyl-D-mannose, 2, 3,6-tri-O-methyl-D-mannose and 2, 3,4,6-tetra-O-methyl-D-galactose was present in molar ratio 3:2:1. Partial acid catalysed hydrolysis of the seed gum gave five oligosaccharides epimelibiose, mannobiose, galactosyl mannobiose, mannotriose and Mannotetraose along with the component sugars. Periodate oxidation and methylation studies in cassia fistula showed 33.6% of end groups from the recent studies it is concluded that galactose unit in galactomannan only terminal positions.

Key words : Galactomannan, Cassia fistula, Polysaccharides, Extraction, Mannose, Galactose

I. INTRODUCTION

Cassia fistula, known as the golden shower tree and by other names, is a flowering plant in the family Fabaceae. Cassia is a genus of flowering plant in the legume family [1]. While many legume trees exude variety of gums. Industrial gums [2] [Polysaccharides and their Derivatives] consumed tremendous quantities by industries. Gum and mucilages are used in large scale of industries like paints [3], textiles, adhesives, ink [4] cosmetics, paper, pharmaceutical products, smoking products etc.

Cassia fistula is dispersed in deciduous and mixed monsoon forests throughout greater parts of India. It is a deciduous tree 6-9 m high trunk straight and bark smooth and pale grey when young, turns to rough and dark brown when get old. 5-12 cm long pairs, the pods are pendulous cylindrical almost straight. Seeds are generally ovate 8 mm long slightly less in width and 5 mm thick. The species is native to the Indian Subcontinent and adjacent regions of Southeast Asia. It is found in Mullai region of Sangam landscape. Cassia fistula is the national tree of Thailand, and cassia fistula flower is national flower of Thailand's. In India Cassia fistula is state flower of Kerala and having great importance amongst Malayali population. The potential of aqueous and isolated fraction from leaves of cassia fistula linn as antibacterial agent (5) Antimicrobial activity of Aragvadha (Cassia fistula linn.) is well known and use as a ayurvedic medicine for various diseases. (6). Traditional dye yielding plant like cassia fistula [7] having good importance found in Uttarakhand. Cassia fistula is a popular ornamental plant and having great importance in herbal medicine. Cassia fistula seed galactomannan (8) act as potential binding agent for pharmaceutical formulation.

II. MATERIALS AND METHODS

Polysaccharide was isolated from crushed seeds of cassia fistula which were decolorized and defeated by ethyl alcohol and petroleum ether and against treatment with 1% of acetic acid solution. For complete precipitation of the polysaccharide was extracted with 90% solution of ethyl alcohol. To purification of polysaccharide using three methods, repeated precipitation, Deproteinization, Complexation with Fehling's solution. After purification of polysaccharide was found a white amorphous material which was easily dispersed in water forming viscous solution at the room temperature. It was showed sulphated ash 0.12%. The methoxy and acetyl was negligible and found to be free from halogen, nitrogen and sulphur.

The homogeneity of the polysaccharide was checked by following methods:

A. FRACTIONAL PRECIPITATION

As per experimental data show different fraction and their Paper chromatography analysis [fraction I and fraction II], gave D-galactose and D-mannose in molar ratio 5:1 and both the fraction indicating homogeneous nature of polysaccharide.

B. ZONE- ELECTROPHORESIS [9]

Complete hydrolysis of the polysaccharide with 1 N sulphuric acid and then the polysaccharide was subjected to conventional zone electrophoresis on whattman no.-1 chromatography paper in borate buffer which pH - 9.2. The intensity of characteristic yellow orange colour developed in aqueous eluates of each segment was measured in Klett Summerson photo-electric colorimeter using filter no-44. The plot of absorbance against segment number showed only single and sharp spot in chromatogram which identified the polysaccharide to be homogeneous. For calculating the quantity of polysaccharide used the following formula.

$$M = 31.74 \times \text{absorbance} / 0.211$$

Where M is in microgram per ml of polysaccharide solution .absorbance was measured as following formula.

$$\text{Absorbance} = 2 \times \text{klett reading} / 1000$$

C. ACETYLATION AND DEACETYLATION

The acetylation of the polysaccharide was done by acetic anhydride and sodium acetate. The deacetylated polysaccharide had the same optical rotation as original polysaccharide, which further confirmed that the polysaccharide is homogeneous.

D. PAPER CHROMATOGRAPHIC EXAMINATION IN DIFFERENT MOBILE PHASE

The hydrolyzed polysaccharide was neutralized, filtered and concentrated the filtrate under reduced pressure up till a syrupy form. And it was studied that the selected mobile phase. The result in all the mobile phase was identical which showed the polysaccharide to be homogeneous.

Sugar identification was identified by chromatographic analysis. Paper Chromatographic analysis show RF and RG value of two spots corresponded to D-galactose and D-mannose. The identity of the sugars D-galactose and D-mannose was confirmed by the co-chromatography developed in different mobile phase. In column chromatographic analysis the hydrolysate polysaccharide was dissolved in the solution of water and methanol in 1: 1 and the solution absorbed over a cellulose column. Each fraction by paper chromatography compare with standard sample of D-galactose and D-mannose. Two fractions were obtained in crystalline form. Quantitative estimation of sugars after the total recovery of D-ribose result shows that the ratio of D -mannose and D-galactose in the seeds are 1:5. Graded hydrolysis result was showed that D-galactose was found to be liberated first and then D-mannose. D-galactose units are present at the periphery as end groups and D-mannose forms the basic chain of the polysaccharide. Methylation of the pure polysaccharide done by Haworth's [10] method by using dimethyl sulphate and sodium hydroxide then by Purdie's [11]. method using silver oxide and methyl iodide. Methylated product was as brownish masses. The hydrolysed was subjected to preparative P.C. [Solvent A] . The following products were isolated and characterised by $[\alpha]_D$ and mp of anilide/ hydride derivative. Hydrolysis of methylated polysaccharide material was dark brown colour. The methylated sugars were separated on whattman no.1 chromatography paper, by using mobile phase. The chromatography shows only, three spot after spraying with aniline hydrogen phthalate and drying at 115°C. The RTMG values were calculated in each case and compared it with reported values. methylated sugar 2, 3-di-O-methyl-D-mannose, 2, 3, 6-tri-O-methyl-D-mannose, 2, 3, 4, 6 - tetra - O -methyl-D-galactose as 3:2:1. The calculation of methylated sugar were done as methyl ether of the anhydrohexene units assuming 99.47% recovery of the D- galactose and end group analysis from methylation studies was found 33.6 . 2, 3 -di-O-methyl-D-mannose was obtained as syrup RTMG, in the mobile phase no. 5, 0.49, O-me: 29.9% calculated for dimethyl hexose, O-me: 29.88% $[\alpha]_D$ 27-16.7° [water] , melting point 192-193°C, $[\alpha]_D$ 27-64.5° [Chloroform] . 2, 3, 6-tri-O-methyl D-mannose was obtained as a syrup RTMG in the mobile phase no. 5 0.83, $[\alpha]_D$ 27 -14° [water] , melting point-131-133°C, $[\alpha]_D$ 26-19.4° [water] . 2, 3, 4, 6-tetra-O-methyl - D - galactose was obtained as a solid material with melting point 69° -71°C and, RTMG in mobile phase.no-5, 0.90, $[\alpha]_D$ 30 -122° [water] lit. These oligosaccharides were obtained and identified.

Epimelibiose : $[\alpha]_D$ -D-galactosyl [1-6] -D-mannopyranose] m.p. 202-204 °C, $\{\alpha\}_D$ 25+ 120.9° water

Acid hydrolysis gave galactose and mannose; it reducing R glu in solvent [A] was found 0.58 . [0.59] , and Rman. in mobile phaseno.-2,5,and 7 was 0.18,0.36 and 0.24 respectively. It reduced Fehling solution and tollen's reagent. [1→6] linkage between galactose and mannose unit .the disaccharide was not hydrolysed by emulsion indicating the presence of α glycoside linkage show high positive optical rotation.

Mannobiose [β -D-Mannopyranosyl [1→4] -D-Mannopyranose m.p.201-202° C , $\{\alpha\}_D$ 25- 11.9° C, m.p.-202-204° C $\{\alpha\}_D$ 25 -7 to 8.9° C. Acid hydrolysis showed the presence of mannose units only. Emulsion hydrolysis showed the presence of β -linkage between the mannose units which were also confirmed by the optical rotation of sugar osazone had m.p. 204 °C [203-206] .

Mannotriose [β -D- Mannopyranosyl] [1→4] - β -D- Mannopyranosyl [1→4] -D-mannopyranose, m.p.164-167 ° C , $\{\alpha\}_D$ 25 - 18.2 ° C. Rman in mobile phase no-2 was found 0.23 and Rglu in mobile phase no-3 was found 0.35.it reduced Fehling's solution tollan's reagent. Anhydrous hydrolysis indicated the presence of mannose units only and partial hydrolysis resulted in the formation of mannose ,mannobiose. Further emulsion hydrolysis suggested mannose units are linked through β -linkage.

Mannotetrose [α -D- mannopyranosyl] [1→4] - β -D- mannopyranosyl [1→4] - β -D- mannopyranosyl [1→4] -D-mannopyranose, m.p.233- 235 ° C , $\{\alpha\}_D$ 29 - 29.8° C. Rman in mobile phase no-2 was found 0.12 and Rglu in mobile phase no-3 was found 0.17.it reduced Fehling's solution tollan's reagent.

Galactosyl manobiose: [α - D-galactopyranosyl [1→6] - β - D -mannopyranosyl [1→4] -D-mannopyranose] experimental m.p.228-230 ° C $\{\alpha\}_D$ 25 - 92.8 ° C [water] [12] actual m.p. 227-229 °C $\{\alpha\}_D$ 25 - 93° C [water] .Rglu in mobile phase no-2, 0.31,and Rman , in mobile phase 6 & 7 0.19 and 0.09 respectively. and was found to be pure in solvent mixture.

Hydrolysis yielded galactose and mannose, its equivalent weight 265.0 corresponded to monohydrated trisaccharide. 3.0 moles of HCCOH methylation and subsequent hydrolysis gave 2, 3, 4, 6 –tetra-o-methyl-D-galactose, and 2, 3, 6- tri-o methyl-D- mannose. Hydrolysis with emulsin gave mannose and epimelibiose indicating one α and β linkage.

III. RESULTS AND DISCUSSION

The polysaccharide was extracted with 1% aqueous Acetic acid and precipitated with 4 volume of ethanol. This process was repeated until minimum ash content, 0.12% was obtained. Homogeneity of the polysaccharide was tested by fractional precipitation and zone – electrophoresis methods. The dry polysaccharide was soluble at room temperature and had negligible methoxy, acetyl and uronic acid contents. Complete hydrolysis yielded D- galactose and D- mannose in molar ratio 2:4 respectively. On graded hydrolysis with 0.05 M H₂SO₄ galactose was liberated first, followed by mannose.

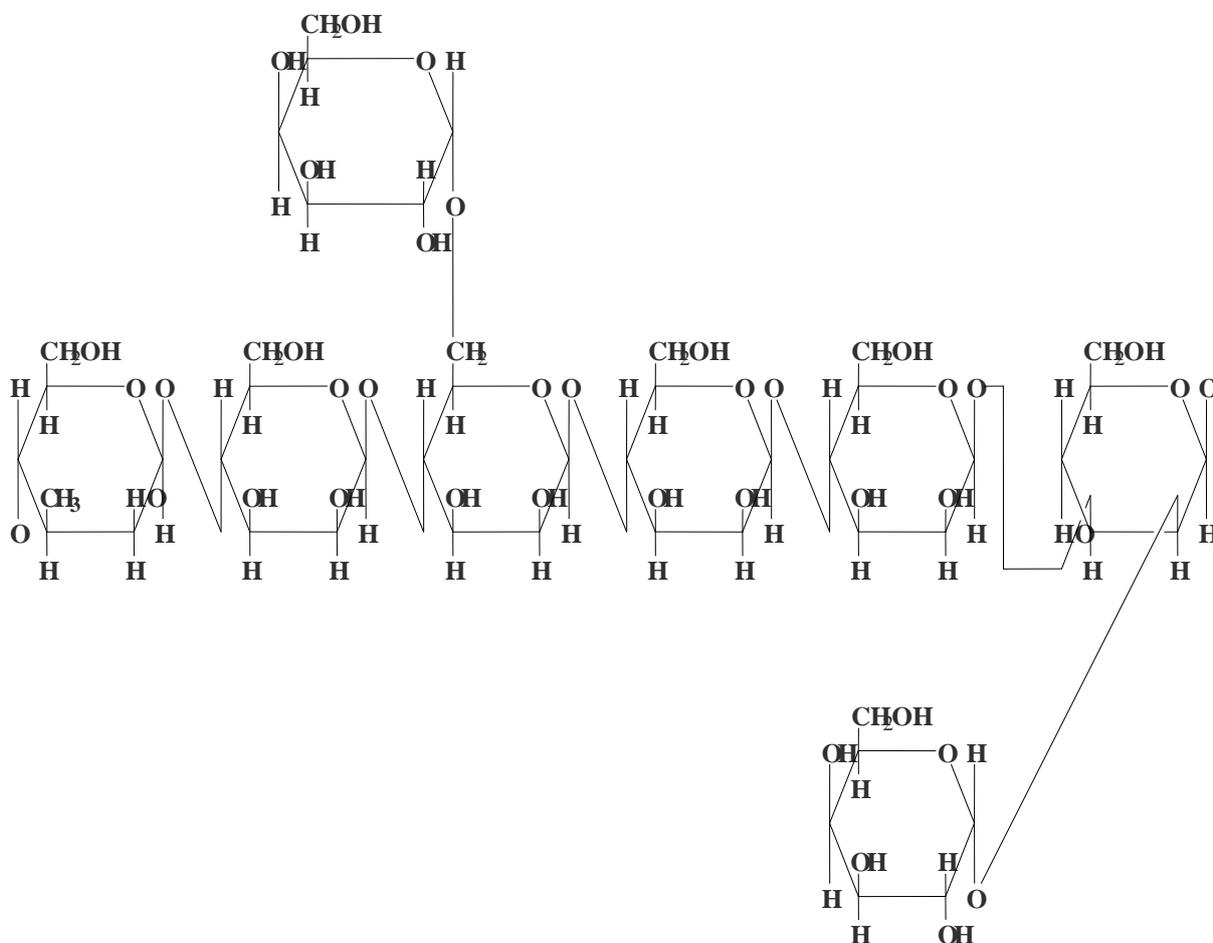
Completely methylated polysaccharide on hydrolysis gave 2,3,-di-O-methyl D- mannose, 2,3,6-tri-O-methyl-D-mannose and 2,3,4,6-tetra-O-methyl-D- galactose in the molar ratio 3:2:1.

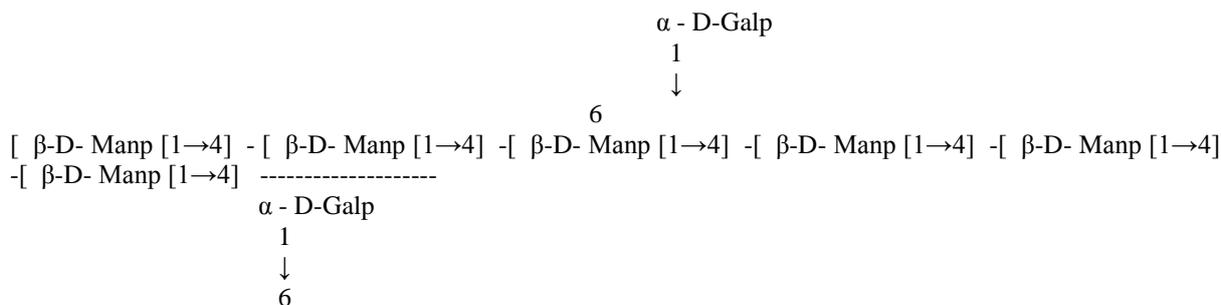
Analysis of the percentage of end groups by the periodate method was close agreement with the methylation studies. Partial acid hydrolysis of the polysaccharide with 0.5 M H₂SO₄ for 14 hours liberated the following oligosaccharides [i] Mannotriose [ii] lactose mannobiose , [iii] Epimelibiose and [iv] Mannobiose, [v] Mannotetrose

They were identified by PC and by the preparation of their derivatives as well as by periodate oxidation. It was found to give four oligosaccharides where two were homogeneous and two were heterogeneous, in addition to the two component mannoscharides D- galactose, and D-mannose.

The homogeneous oligosaccharides were found to have [1→4] linkage between mannose units but the heterogeneous members had [1→6] linkage between galactose and mannose units.

These structural studies of the polysaccharides and of different oligosaccharides obtained by partial acid hydrolysis suggest the following structure of the repeating units of polysaccharide.





The above structure contains eight units of monosaccharide, two galactose units and six mannose units. It explains the formation of oligosaccharides as well as, the percentage of end groups which are incomplete agreement with analytical data of the polysaccharide.

IV. CONCLUSION

This research work based on improves the yield and quality of polysaccharide. As per experimental data it was found improve yield of polysaccharide up to 5%. Purification method of polysaccharides such as repeated precipitation method slight change like dilution plus reprecipitation process was repeated to 8 times to give better yield. After the repeated precipitation Deproteinization process was repeated six time for obtained total rid of material. The deprotenized aqueous solution of the polysaccharides was added fehling's solution. It was formed the blue copper complex and washed again with water. In Description, melting point, optical rotation, paper chromatographic analysis, HPLC analysis of our experimental sample is nearly same as our reported sample.

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