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Structural and Phytochemical Investigation of Water Soluble Polysaccharide Isolated From Seeds of *Cassia Angustifolia*

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Abstract – Cassia angustifolia Vahl (family: Caesalpiniaceae), commonly known as "sanaai," is a fast growing and spreading Indian shrub of which seeds, pods and leaves are extensively used for pharmaceutical applications. The seeds have been found to be an alternative source of commercial gums and are used as an anthelmintic, digestive, and to treat piles, skin diseases, and abdominal troubles. The current investigation found that a water-soluble galactomannan consisting of D-galactose and D-mannose in the molar ratio 3: 4 has been isolated from the seeds of Cassia angustifolia. Hydrolysis of the methylated polysaccharide resulted in three methylated sugars: (i) 2, 3-di-O-methyl-D-mannose, (ii) 2, 3, 4-tri-O-methyl-D-galactose, and (iii) 2, 3, 4, 6-tetra O-methyl-D-galactose in the molar ratio 2: 1: 2 Periodate oxidation of the polysaccharide indicated 61% end group while methylation gave 60 %. Reduction of the periodate oxidized polysaccharide and subsequent hydrolysis revealed the presence of (1→ 4) and (1→6) glycosidic bonds. Thus, the main chain of the galactomannan was found to consist of (1→4)-linked mannoypyranosyl units having 3-glycosidic bonds while (1→6)-linked α-glycosidically bonded galactopyranosyl units form the branching points.

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1. INTRODUCTION:

Cassia angustifolia (family: Caesalpiniaceae), found in the market by another name "tinnevelly" is a variably branched erect shrub, commercially cultivated in southern India. The plants of the genera Cassia generally possess considerable medicinal value and are also a good source of mucilages. Owing to the high medicinal value and increasing industrial demand of plant mucilages, we were prompted to undertake a structural study of the polysaccharides obtained from the seeds of *Cassia angustifolia*.

Seed galactomannans are important agrochemicals used in various industries worldwide. The growing industrial utility of these gums or modified derivatives in the field of paper, textile, petroleum recovery and pharmaceutical industries, has resulted in an impetus in India for intensified research on new sources having varying galactose–mannose ratios and fine structures. They are non-toxic, inexpensive. Our research studies show that *Cassia angustifolia* seed gum possesses the potential to become a new source of commercial gum

Keeping this in view, the structural studies of the seed gum has been carried out and is presented in the present note. This is the first report on the structural elucidation of a galactomannan isolated from the seed endosperm of *C. angustifolia*,

The seeds are brown in colour with medium sized (2.5–3.5×1.4–2.0×3.5–4.5 mm; wt. of 100 seeds ~2.55g). They contain endosperm, crude protein, pentosan, total ash, water-soluble gum.

Typically, the seeds have been found three components seed coat, endosperm and germ to be a potential source of commercial gums as they contain about 50-60% of the endosperm, which are easily separable by mechanical means. The crude gum was purified by the usual procedure of precipitation followed by crystallization with polar protic and nonpolar aprotic solvent and by complexation with Fehling's solution. Constituent sugars were identified by GC–MS and their m/z values have been found identical with literature. The ratio of constituent sugars was determined by HPLC and GLC

Fig.: ***Cassia angustifolia*** **seeds**

Fig: Cassia angustifolia **seed extracts**

1. Experimental:

A. Isolation and precipitation:

The collected seeds of *Cassia angustifolia* were roasted, and the endosperm was separated from the germ and hull by grinders and sieves and finally powdering to 100 mesh sizes. Powdered form of seeds was dissolved in 1% of acetic acid solution and precipitated by slow addition of ethyl alcohol under continuous stirring. Dissolution and re-precipitation of this polysaccharide was done 5 to 6 times until a precipitate was obtained.

B. Purification:

The desiccated polysaccharide was re-dissolved in water and shaken well with chloroform, the denatured protein in the form of gel which collected at the water/chloroform interface was removed. The procedure was repeated four times to get the polysaccharide free from protein. It was further purified by complexation with Fehling's solution. The complex was centrifuged, washed with dilute Fehling's solution, and decomposed with 1.5 molar HCl. The polysaccharide was regenerated by pouring the solution into an excess of ethanol under stirring. Finally, the pure polysaccharide was collected dried over fused calcium chloride under reduced pressure at 45°C.

C. Homogenity:

The homogeneity of galactomannan was approved by the following methods.

a) Fractional precipitation:

The polysaccharide (10 g) was dissolved in distilled water (800 ml) and the solution was precipitated first with 200 ml of ethanol to give fraction (a), then with 700 ml of ethanol to give fraction (b), and finally with 1500 ml of ethanol to give fraction (c). Fractions (a), (b), and (c). The results obtained such as ratio of the building sugars and optical rotation was identical to that of original polysaccharides showed presence of galactose and mannose in the ratio 3:4.

b) Zone Electrophoresis:

The polysaccharide (1 g) was subjected to conventional zone electrophoretic separation in 0.05M sodium tetra borate solution for 8 h at 360 volts and 12.5 mA. A plot of the absorbance against segment numbers showed only one sharp peak, indicating the presence of only one polysaccharide.

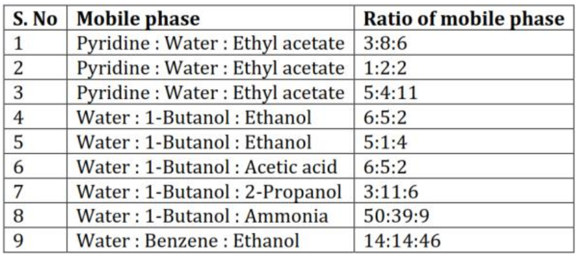
c) Acetylation and Deacetylation:

For the acetylation of polysaccharides used acetic anhydride and sodium acetate. The acetylated polysaccharides optical rotation shows $[\alpha]_D^{30}$: + 57° in chloroform. Deacetylation regenerated a material with $[\alpha]_D^{25}$: + 60° in water, confirming the homogeneity of the galactomannan.

d) Paper Chromatographic studies in different mobile phase:

Paper electrophoresis and paper and thin layer traditional techniques used to study monosaccharide composition and to separate very small oligosaccharides. The hydrolyzed polysaccharide was neutralized; filtered and concentrated the filtrate under reduced pressure up till a syrupy form and it was studied in the mobile phase no. 3, 5 and 7. The result in all the mobile phase was identical which showed the polysaccharide to be homogeneous.

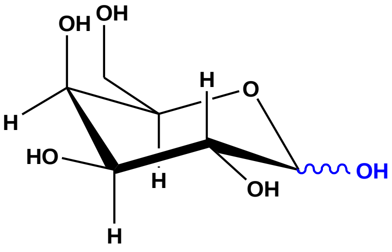
The following mobile phase used for chromatographic analysis.



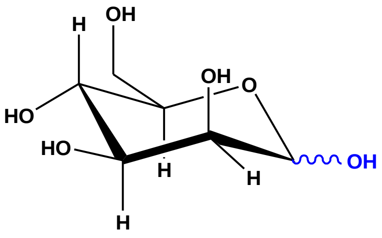
S. No	Mobile phase	Ratio of mobile phase
1	Pyridine : Water : Ethyl acetate	3:8:6
2	Pyridine : Water : Ethyl acetate	1:2:2
3	Pyridine : Water : Ethyl acetate	5:4:11
4	Water : 1-Butanol : Ethanol	6:5:2
5	Water : 1-Butanol : Ethanol	5:1:4
6	Water : 1-Butanol : Acetic acid	6:5:2
7	Water : 1-Butanol : 2-Propanol	3:11:6
8	Water : 1-Butanol : Ammonia	50:39:9
9	Water : Benzene : Ethanol	14:14:46

D. Hydrolysis:

Complete hydrolysis of the polysaccharide (1 g) by 2N sulphuric acid for 30 hrs. at 100°C yielded, D-galactose and D-mannose which were separated by paper chromatography using mobile phase No.7 Water : 1-Butanol : 2-Propanol (3:11:6) and which, on quantification (sodium metaperiodate method) [13], gave the molar ratio 2:4. These were identified by co-chromatography with authentic samples, m. p. and preparation of their derivatives: D-galactose, m. p. 164° C $[\alpha]_D^{30}$: + 80° (water): D-galactose phenylhydrazone, m. p. 153° C and D-mannose, m. p. 131° C, $[\alpha]_D^{30}$: + 14° (water): D-mannose phenylhydrazone, m. p. 196° C.



D-galactose



D-mannose

E. Graded hydrolysis:

The polysaccharide (300mg) on hydrolysis with 0.05N sulphuric acid for 4 hrs. 100°C and paper chloromatography of their hydrolysate after 5, 15, 30, 45, 60, 90 min indicated the early release of D-galactose followed by D-mannose

F. Methylation:

For methylation analysis, the gum was suspended in freshly distilled Me_2SO and extensively stirred for 22 hrs. at RT, followed by mild sonication for 20 min and stirring for another 5 h. The partially dispersed product was methylated according to the Hakomori method. The product was again subjected to Hakomori methylation, followed by two successive Purdie methylations. The completely methylated polysaccharide showed no IR–OH absorption (3600–3400 cm−1). The methylated polysaccharide was boiled under reflux with HCOOH. The resulting methylated sugars were converted to the corresponding alditol acetate derivatives and analyzed by GLC and GC–MS

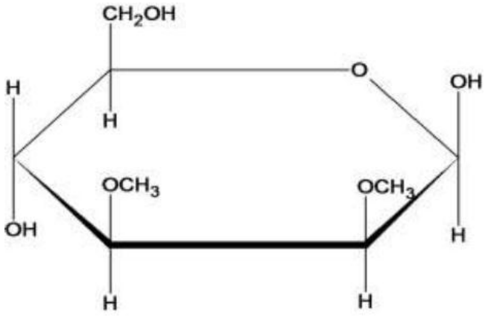
G. Structural studied of polysaccharide:

After completing the hydrolysis of polysaccharide with 2N sulphuric acid followed by paper chromatography studied, it was showed the presence of two sugar residues D- galactose and D-mannose. The sugar was identified by their melting point, specific optical rotation of their derivatives and co-chromatography. By the quantitative estimation of monosaccharide components by periodate oxidation taking D-ribose as reference sugar showed galactose and mannose present in the molar ratio of 2: 4 respectively.

The following methylated sugars were isolated and these were quantified by the hypoiodite method in the molar ratio 2: 1 : 2.

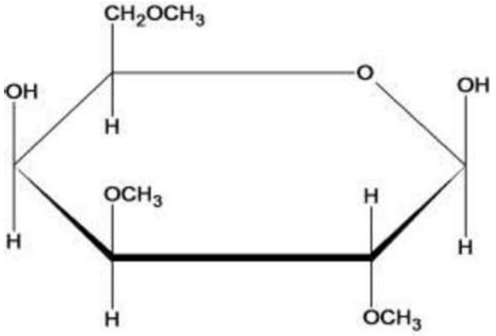
I. 2, 3-Di-O-methyl-D-mannose

R_{TMG} 0.53, m.p. 107-108° C, $[\alpha]_D^{25}$: -16° (water), lit. [19], m. p. 108° C, $[\alpha]_D^{25}$: -15.8° (water); the anilide had m.p. 137° C, lit. m.p. 138° C.



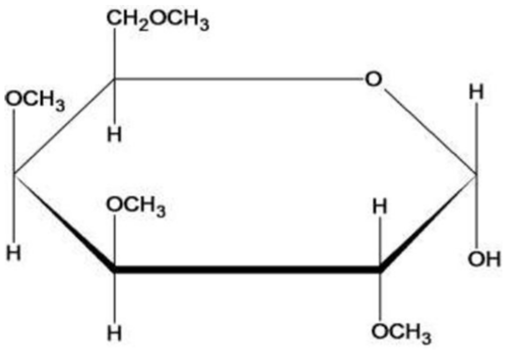
II. 2,3, 6-tri-O-methyl-D-galactose

R_{TMG} 0.65, m. p. 85-86°C, $[\alpha]_D^{25}$: + 15.4° (water), lit. [20] m. p. 85° C $[\alpha]_D^{25}$: + 15.4° (water).



III. 2, 3, 4, 6-tetra-O-methyl-D-galactose

R_{TMG} 0.87 $[\alpha]_D^{32}$: + 120.3° (water), m.p. 73° C, lit. [21] m.p. 74° C, $[\alpha]_D^{32}$ :: + 121° (water), the anilide had m. p. 193° C, $[\alpha]_D^{32}$: + 44° (acetone), lit. (21) m. p. 194° C $[\alpha]_D^{32}$: + 45° (acetone).



H. Periodate oxidation:

The polysaccharide (90 mg) was dissolved in water (25 ml) and the solution was cooled to 0 °C. A cold solution of sodium metaperiodate (0.15 mole, 30 ml) was added to the solution and volume was made up to 100 ml. The reaction was conducted at 4 °C and the amounts of periodate consumed and formic acid liberated, were estimated at different time intervals. The periodate oxidation was completed in 165 hrs.

I. Partial Acidic hydrolysis:

The seed of the polysaccharide was subjected to partial hydrolysis with 0.1 N sulphuric acids. After the paper chromatographic examination of hydrolysate, it was showed the separation on preparative scale given five oligosaccharides, which identification as below.

a. Epimelibiose: 6-O-α-D-galactosyl-D-mannose, m. p. 200° C, $[\alpha]_D^{32}$: + 122° (water), Lit.[22] m.p. 201-202° C, $[\alpha]_D^{32}$: 121-124° (water). Acid hydrolysis gave galactose and mannose in equal proportion. Methylation and subsequent hydrolysis gave 2, 3, 4, 6-tetra-Omethyl-D-galactose and 2, 3, 4-tri-O-methyl-D-mannose. It was not hydrolysed by emulsin showing the absence of β-linkage

b. Galactobiosylmannose: 6-O-α-D-galactosyl (1→6)-O-α-D-galactosyl-D-mannose, m.p. 123°C, $[\alpha]_D^{25}$:+ 120° (water), lit. [23] m. p. 124° C, $[\alpha]_D^{25}$: + 119° (water). Acid hydrolysis gave D-galactose and D-mannose in the molar ratio 2: 1. Methylation and its hydrolysis gave 2, 3, 4-tri-O-methyl-D-mannose, 2, 3, 4-tri-O-methyl-D-galactose, and 2, 3, 4, 6-tetra-O-methyl-Dgalactose. It was not cleaved by emulsin indicating the absence of β-linkage

c. Mannobiose, 4-O-β-D-mannosyl-D-mannose, m. p. 204° C, $[\alpha]_D^{25}$: -10.1° (water), lit. [24] m. p. 202-203° C, $[\alpha]_D^{25}$: -5.2° to 8.2° (water). Its phenyl hydrazone had m. p. 203° C, lit. m. p. 203°-206°C. Acid hydrolysis gave D-mannose only. Methylation and its hydrolysis yielded 2, 3, 4, 6-tetra-O-methyl-D-mannose and 2, 3, 6-tri-O-methyl-D-mannose. It was cleaved by emulsin showing the presence of β-linkage.

d. Mannotriose : 4-O-β-D-mannosyl-(1→4)-O-β-D-mannosyl-D-mannose, m.p.211°-213°C, $[\alpha]_D^{25}$: -14°(water), lit. [25] m. p. 214°-215°C, $[\alpha]_D^{25}$: -15° to -26° (water). Acid hydrolysis indicated the presence of D-mannose only whereas partial acid fission afforded mannose and mannobiose. It was hydrolysed by emulsin showing the presence of β-linkage. Methylation and subsequent hydrolysis afforded 2, 3, 4, 6-tetra-O-methyl-D-mannose and 2, 3, 6-tri-O-methyl-D-mannose.

e. Galactobiose/Swietenose : 6-O-α-D-galactosyl-D-galactose, m.p. 129° C, $[\alpha]_D^{25}$: +143° (water), lit.[23]. m.p. 130° C, $[\alpha]_D^{25}$: +149° (water). Complete hydrolysis by M sulphuric acid gave D-galactose only. Methylation and its hydrolysis resulted in 2, 3, 4, 6-tetra-O-methyl-D-galactose and 2, 3, 4-tri-O-methyl-D-galactose. It was not cleaved by emulsin indicating the presence of a bond.

Complete hydrolysis by 2N sulphuric acid gave D-galactose only. Methylation and its hydrolysis resulted in 2, 3, 4, 6-tetra-O-methyl-D-galactose and 2, 3, 4-tri-O-methyl-D-galactose.It was not cleaved by emulsin indicating the presence of α bond.

2. CONCLUSION:

The plants of the genera cassia generally possess considerable medicinal value and are also a good source of mucilages. Owing to the high medicinal value and increasing industrial demand of plant mucilages, we were prompted to undertake a structural study of the polysaccharides obtained from the seeds of Cassia angustifolia. The current investigation found that a water-soluble galactomannan consisting of D-galactose and D-mannose in the molar ratio 2 : 4 has been isolated from the seeds of Cassia angustifolia. Hydrolysis of the methylated polysaccharide resulted in three methylated sugars: (a) 2, 3-di-O-methyl-D-mannose, (b) 2, 3, 4-tri-O-methyl-D-galactose, and (c) 2, 3, 4, 6-tetra O-methyl-D-galactose in the molar ratio 2: 1: 2.

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