Journal of Advances and Scholarly Researches in Allied Education
Vol. 18, Issue No. 3, April-2021, ISSN 2230-7540

Investigation and Chemical Study of Water-Soluble Polysaccharides Isolated from Seeds of *Cassia Alata*

Prabhu Balaji[1]* Dr. D. S. Singh[2]

[1] Research Scholar of University of Entrepreneurship & Technology, USA

[2] Research Guide

Abstract – Water soluble polysaccharides were extracted from seeds of Cassia alata. The galactomannan extraction was based on mechanical separation of the endosperm, water dissolution, centrifugation and precipitation with acetone and pet ether. In the current investigation found that water-soluble galactomannan consisting of D-galactose and D-mannose in the molar ratio 1:3. Methylation and hydrolysis of the galactomannan produced 2,3-di-0-methyl-D-mannose (1 mole), 2,3,6-tri-0-methyl-D-mannose (4 moles), and 2,3,4,6-tetra-0-methyl-D-galactose (2 moles). Partial acid catalysed hydrolysis of the seed gum gave four oligosaccharides epimelibiose, mannobiose, galactosyl mannobiose, and mannotriose. The characterized polysaccharides from the gum has the basic structure of galactomannans with a main chain of (1→4)-linked β-D-mannopyranosyl units to which single α-(1→6)-D-linked galactopyranosyl units.

----------X----------

1. INTRODUCTION:

Cassia alata Linn. Plant [1] belongs to the family-Caesalpiniaceae and commonly known as Dadmurdan and occurs in Garhwal region of Northern Himalayas. Plant is a large shrub and grown as an ornamental purpose. Cassia species have been well known for their laxative and purgative properties and for the treatment of skin diseases [2] there is now an increasing body of scientific evidence demonstrating that the plants possess many other beneficial properties [3]. Seeds are used in Ayurvedic system as medicine and leaves are used for the treatment of ringworm[4], skin diseases[5] and also for snakebite treatment. *Cassia alata* is also known as the Candle Bush and it is an important medicinal tree[6,7].

Present manuscript mainly deals with the isolation, purification, preliminary analysis and nature of the constituent of water-soluble sugars from seeds of *Cassia alata*.

The current investigation found that galactomannan consisting of D-galactose and D-mannose in the molar ratio 1:3 has been isolated from the seeds of *Cassia alata.* Hydrolysis of the methylated polysaccharide resulted in three methylated sugars: (a) 2,3-di-O-methyl-D-mannose, (b) 2,3,6-tri-O-methyl-D-mannose, and (c) 2,3,4,6-tetra-O-methyl-D-galactose in the molar ratio 1:4:2.

Fig.: *Cassia alata* seeds and plant

Fig.: *Cassia alata* seed extract

2. EXPERIMENTAL

A. Collection of plant material

Cassia alata pods were collected from the local area of Thane, Maharashtra (India) in the month of October–December. The seeds were manually separated dried and kept in a cool and dry place until further use.

B. Isolation of Polysaccharide Polysaccharide was isolated from the crushed seeds (300 gm) in water (1000 ml) [8] for 24 hrs. at room temperature then stirred for 48 hrs. with the help of mechanical stirrer.

Viscous solution was squeezed through muslin cloth to remove the insoluble matter. Filtrate was then centrifuged through a Sharple's super centrifuge [9] to remove finely suspended matter. Centrifugate was precipitated with ethanol (3 litre), by mechanical stirrer to precipitated out the whole polysaccharide in light brown form. Precipitate of polysaccharide was further filtered through a sintered funnel (G-3) under suction then dried in vacuo after washing with acetone and pet. Ether. Polysaccharide was obtained as a brownish crude powder and isolated polysaccharide was found with sulphated ash [10] (0.74%).

C. Purification of polysaccharide

Purification of galactomannans was done by following steps.

a) Repeated precipitation

In 1% acetic acid solution dissolved the crude polysaccharide and precipitated by adding the solution slow rate to the excess of ethyl alcohol under continues stirring dilution and re-precipitation process was repeated five times to obtain the total material. The precipitated polysaccharide was filtered, washed with ethanol and dried in air. White fibrous material was obtained

b) Deproteinization

After the repeated precipitation of the polysaccharide was deproteinated by shaking its aqueous solution with the chloroform up till to formed a milky gel at the water - chloroform interface. This process was repeated six times for obtain the total rid of the proteins.

c) Complexation with Fehling's solution

The deproteinized aqueous solution of the polysaccharides was added Fehling's solution, it was formed the blue copper complex and washed again with water. The complex was destroyed with 1.5 N hydrochloric acid solution. The polysaccharide was regenerated by adding slowly excess of ethyl alcohol under continues stirring. Dissolution and re-precipitation were repeated to obtained total materiel. The purified polysaccharide was a white, non-reducing amorphous material which was easily dispersed in water forming viscous solution at the room temperature. It was showed the optical rotation $[\alpha]_D^{25}$: - 40.5˚(water) and sulphated Ash 0.74% The methoxy and acetyl was negligible and found to be free from Halogen, nitrogen and sulphur.

D. Homogeneity of polysaccharide:

a) Fractional Precipitation

5.0 gm. of purified polysaccharide was dissolved in 500 ml of distilled water and by Adding 700 m1 of 90% of ethyl alcohol precipitate was obtained. The precipitated polysaccharide was filtered, washed with ethanol followed by ether and dried in vacuum oven it was fraction first. The filtrate was again treated with 700 ml of 90% ethyl alcohol filtered washed and dried as above it was fraction second separated in to fractions with 2N sulphuric acid. By paper chromatography analysis of the fraction first and fraction second, gave D-galactose and D-mannose in molar ratio 1:3 and both the fraction retained the original specific rotation $[\alpha]_D^{25}$: -40.6˚ (water) indicating homogeneous nature of polysaccharide.

b) 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 indicated that the polysaccharide is homogeneous.

c) Zone Electrophoresis

A part of polysaccharides was subjected to conventional zone electrophoresis [11] on Whatman no.-1 paper in borate buffer pH 9.2. The intensity of the characteristic yellow orange colour developed into the phenol-sulphuric acid regent, was measured in Klett-Summerson photoelectric colorimeter. A single sharp peak indicating the homogeneous nature of galactomannan by plot of absorbance against segment number.

d) Paper chromatographic examination in different mobile phase

900 mg of pure polysaccharide dissolved in 30 ml of 2N sulphuric acid reflux for 55 hrs. on water bath. The following mobile phase used for identification of the hydrolysate polysaccharide.

S. No.	Mobile Phase	Ratio of mobile phase
1	Water : 1-Butanol : Ethanol	6 : 5 : 1
2	Water : 1-Butanol : Acetic Acid	7 : 5 : 1
3	Water :1-Butanol : 2-Propanol	3 : 9 : 5

In all above three mobile phases the result was essentially the same showing homogeneity of the polysaccharide.

E. Sugars identification:

2.5 g of pure polysaccharides dissolved in 2N sulphuric acid and reflux for 40 hours on water bath and hydrolysate substance neutralized with barium carbonate, filtered, diluted and analyzed with chromatographically.

a) Paper chromatographic analysis[12]:

Prepared two sheets of Whatman no. -1 paper and a small quantity of hydrolysate polysaccharide was dissolved in water and this solution spots on the sheets. The paper was developed in mobile phase water: 1-butanol: ethanol (6:5:1) and water: 1-butanol: 2-propanol (3:9:5) Chromatogram were dried in air and sprayed with aniline hydrogen phthalate. Two spots were observed on the chromatogram after heating at 125°C. The RF and RG value of two spots corresponded to. D-galactose and D-mannose. Result as table no: 1.

Table No.-1

Sugar identification	Rf in mobile phase 3		RG in mobile phase 1	
	Observed	reported	observed	Reported
D-galactose	0.18	0.18	0.07	0.08
D-mannose	0.25	0.24	0.13	0.12

The identity of D-galactose and D-mannose was confirmed by co-chromatography with authentic samples of D-galactose and D-mannose when the chromatograms were developed in the mobile phase no.-3

b) Analysis with column chromatography:

The hydrolysate substance was fractionated by elution from cellulose column [13], small sample of hydrolysate dissolved in methanol –water mix solution and examine and on the column 2x25 cm. The column was left overnight for separation with mobile phase water: 1-butanol: ethanol (6:5:1) and fraction of elute were collected in tubes. Fractions were examined by paper chromatography with standard samples of D-galactose and D-mannose.

The fractions containing same sugars combined together, concentrated and recrystallized, so obtained two fractions examined as below.

Observation of first fraction:

The first fraction was solid, it was recrystallized from aqueous methanol had m.p.131°-132°C, $[\alpha]_D^{25}$: +12.6°(water), it formed the derivative D-mannose Phenylhydrazone and m.p. 195°-196°C (literature:199°-201°C), so this fraction was identified D-mannose.

Observation of second fraction

After recrystallization of second fraction from aqueous methanol found m.p. 165°- 166°C, $[\alpha]_D^{25}$: +78.6°(water) and derivative D-galactose phenyl hydrazone [14] m.p.168°-170°C (lit.m.p.170-171°C [12]), so above result identified that fraction to be D- galactose.

F. Quantitative estimation of sugars

Hydrolysate was resolved into its components by paper chromatography on Whatman No. 3 MM filter paper sheet in solvent mixture water: 1-butanol: ethanol (6:5:1) and used aniline hydrogen phthalate [12] as spray reagent. Areas of individual sugar components were cut out with the help of guide spots and eluted with water according to Dent's method [15]. The eluted sugars were estimated by periodate oxidation method [16] with sodium metaperiodate solution (0.3 M) for necessary corrections the blank reading was also made. Molar ratio of D-galactose and D-mannose in purified seeds polysaccharide was found to be 1:3 molar ratio

G. Graded hydrolysis of polysaccharide

400 mg of polysaccharide was dissolved in 20ml of 0.1N sulphuric acid hydrolysate was carried out at water bath for 8 hrs. The hydrolysate was taken out at the various intervals of time and examined chromatographically used mobile phase no.-3

At the time hydrolysis of the polysaccharide D-galactose obtained first and then D-mannose. From above it is clear that D-galactose units are present at the periphery as end group and D-mannose forms basic chain of the polysaccharide and it is attached to main chain by weaker bond.

H. Methylation:

Methylation of the pure polysaccharide done by Haworth's [17] method by using sodium hydroxide and dimethyl sulphate and then by Purdie's [18] method using silver oxide and methyl iodide. 10 g of pure polysaccharide taken in 500ml of round bottom flask and filtered with ground glass joint dissolved it in 150 ml of 10% sodium hydroxide solution under stirring. The mixed solution of (50% solution of NaOH) sodium hydroxide and dimethyl sulphate in

the 2:1 ratio also under stirring and maintaining the temperature 38 to 45°C. This process repeated and then the solution was concentrated with acetone under the reduce pressure and extracted for the removing of sodium sulphate, this extraction repeated four times for the complete methylation of the process. Final extraction done by chloroform and dried over anhydrous sodium sulphate,

The partly methylated product was 8.3 g and colour was whitish brown. This product was further methylated by the Purdie's method. The partly methylated polysaccharide was dissolved in moisture free methanol in round bottom flask.

The temperature of the reaction mixture maintaining at the 38 to 42°C C on the water bath. A calcium chloride tube was placed at the top of the condenser to prevent the entry of the moisture at the time of reaction. Add silver oxide 10 g and methyl iodide 12 ml by the addition in 10 hrs. Vise-versa of about similar quantity of methyl iodide and silver oxide. The contents were stirred continuously during the reaction after the completion of addition; reaction mixture was heated on a water bath under stirring for reflux using the calcium chloride guard tube and nitrogen gas. The total filtrate and extracts were evaporated under reduced pressure and resulting thick material was methylated two times under the same condition. The methylated product was obtained as brownish masses.

I. Hydrolysis of methylated polysaccharide:

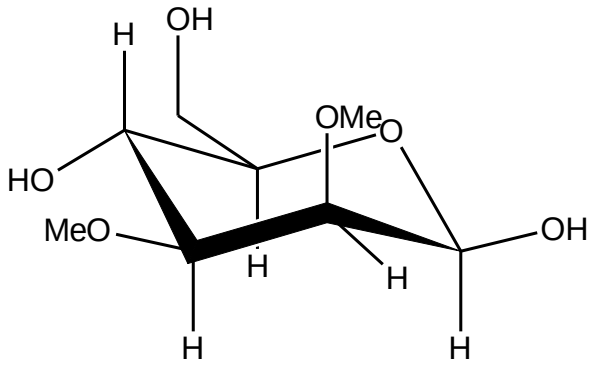
200 mg of methylated polysaccharide was dissolved in 30 ml of 85% of formic acid and the solution was reflux for 8 hours on the water bath. The solution was cooled and concentrated under reduced pressure to a thick from which acid was removed under vacuum and it dissolved in 20 ml of 2N sulphuric acid and hydrolyzed for 15 hours on the water bath, cooled and neutralized with barium carbonate and filtered with filter paper. The precipitate washed with distilled water. The total filtrates concentrated under reduced pressure yellowish brown colour thick material obtained.

J. Identification of methylated compounds

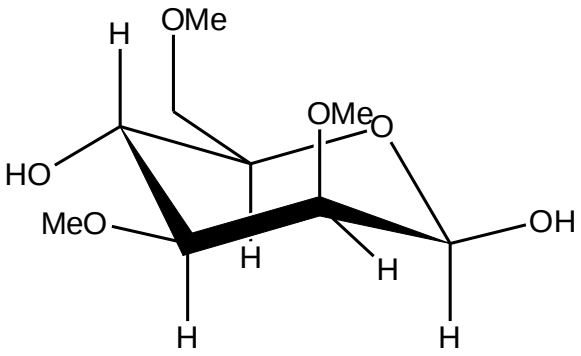
The methylated sugar was separated on Whatman no.1 chromatography paper, by using mobile phase water: 1-butanol: ethanol (6:5:1) and water: 1-butanol: 2-propanol (3:9:5). The chromatography shows only three spots after spraying aniline phthalate and drying at 110°C C. The R_{TMG} value were calculated in each case and compared those reported in given in table no.-2

Table No.-2

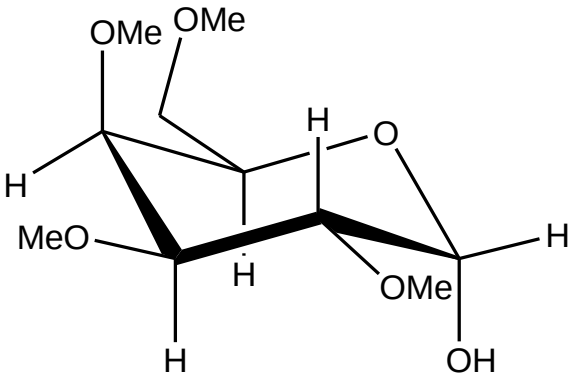
S. No.	Methylated sugars identity	Mobile phase no.-1		Mobile phase no.-3	
		R_{TMG} Found	R_{TMG} Reported	R_{TMG} Found	R_{TMG} Reported
1	2,3-di-O-methyl-D-mannose	0.57	0.59	0.65	0.66
2	2,3,6-tri-O-methyl-D-mannose	0.86	0.87	0.91	0.91
3	2,3,4,6-tetra-O-methyl-D-galactose	0.91	0.90	0.98	0.98



Structure No.-01



Structure No.-02



Structure No.-03

The methylated monosaccharide quantitative estimation done by the method of Hirst, Hough, and Jones indicated that the methylated sugars 2,3-di-O-methyl-D-mannose [structure No.-01], 2,3,6-tri-O-methyl-D-mannose [structure No.-02] and 2,3,4,6-tetra-O-methyl-D-galactose [structure No.-3] were present in molar ratio 1:4:2 respectively in the methylated galactomannan

K. Periodate oxidation:

The polysaccharide (100 mg) was dissolved in water (25 ml) and the solution was cooled to 0 °C. A cold solution of sodium metaperiodate (0.20 mole, 45 ml) was added to the solution and volume was made up to 100 ml. The reaction was conducted at 6 °C and the amounts of periodate consumed and formic acid

liberated, were estimated at different time intervals. The periodate oxidation was completed in 169 hrs.

L. Partial Acidic hydrolysis:

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 four oligosaccharides, which identification as below.

a. Epimelibiose: 6-O-α-D-galactosyl-D-mannose, m. p. 200° C, $[\alpha]_D^{32}$: + 122° (water), Lit.[19] 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, 6-tri-O-methyl-D-mannose. It was not hydrolysed by emulsin showing the absence of β-linkage

b. Galactosylmannose: 6-O-α-D-galactosyl (1→6)-O-α-D-galactosyl-D-mannose, m.p. 123°C, $[\alpha]_D^{25}$:+ 120° (water), lit. [20] 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, 6-tri-O-methyl-D-mannose, 2, 3, 6-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. [21] 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. [22] 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.

3. 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 alata*. The current investigation found that galactomannan consisting of D-galactose and D-mannose in the molar ratio 1:3 has been isolated from the seeds of *Cassia alata*. Hydrolysis of the methylated polysaccharide resulted in three methylated sugars: (a) 2,3-di-O-methyl-D-mannose, (b) 2,3,6-tri-O-methyl-D-mannose, and (c) 2,3,4,6-tetra-O-methyl-D-galactose in the molar ratio 1:4:2.

4. ACKNOWLEDGEMENTS

The authors are thankful to research guide Dr. D.S. Singh and Director of Dexo Chem Laboratories, Ankleshwar Gujarat for providing facilities and helpful discussions and direction of research work. The authors are also thankful to the director of *Indian Management Academy*, Ahmedabad, Gujarat for providing facilities and encouragement throughout the work.

5. REFERENCES:

1. Chopra, R.N; Nayar, S.L. and Chopra, I.C. (1956). Glossary of Indian Medicinal Plants, CSIR Pub., New Delhi (India), pp. 54-56.
2. Dalziel JM (1956). Useful Plants of West Tropical Africa. Crown Agents for Overseas Governments; pp. 179-183.
3. Kirtikar KR and Basu BD (1962). Indian Medicinal Plants; 2: pp. 856-860.
4. C. E. Seaforth (1962). Cassia, trop sci., 4, pp. 159.
5. K. R. Kirtikar and B. D. Basu (1975). Indian medicinal plant, Jayyed press Delhi, 2(2), pp. 30-45.
6. Umarao Singh, A. M. Wadhwani and B. M. Johri (1990). "Dictionary of economic plant in India", ICAR, New Delhi, pp. 44.
7. D. Ibrahim and H. Osman (1995). "Antimicrobial activity of Cassia Alata from Malaysia", J. Ethnopharmacol, 45 (3), pp. 151-156.
8. R. B. Singh and V. K. Jindal (1988). Water soluble polysaccharides from Cassia laevigataseeds: Isolation, purification and preliminary analysis, Egyptian Journal of Chemistry, 31 (5), pp. 615-620.
9. H. K. Schachmann (1948). Centrifugation of sugars by Sharple's super centrifuge method, J. Physc. Chem., 52, pp. 1034-1036.

10. R. B. Singh (1999). Water soluble polysaccharide from Garhwal region flora of Mucuna pruriens Bak. seeds, Oriental J. Chem., 15 (1), pp. 187-188.

11. A.B. foster (1957). "Zone electrophoresis of carbohydrates" Advances in carbohydrate Chemistry, 12, pp. 81-115.

12. S. M. Patridge (1946). Separation of sugars from Clover & Lucerne seeds by paper chromatography, Nature (London), 158, pp. 270-273.

13. G. N. Kowkobony (1950). Separation of monosaccharides by cellulose column chromatographic analysis, Advances in Carbohydrate Chemistry, 9, pp. 304-306.

14. C. M. Rafique and F. Smith (1950). The constitution of Guar gum, J. Amer. Chem. Soc., 72, pp. 4634-4635.

15. C. E. Dent (1947). Identification of sugars by paper chromatography, Biochem. J., 41, pp. 240-242.

16. E. L. Hirst and J. K. N. Jones (1946). Recent process in the chemistry of the seeds polysaccharide and plant gum, J. Chem. Soc., pp. 1959-1963

17. N, Haworth (1915). "A new method of preparing alkylated sugars" J. Chem. Soc., 107, pp.8-12.

18. T. Purdie, and J.C. Irvine (1903). "The alkylation of sugars" J. Chem. Soc., 83, pp. 1021-1026.

19. Bailey, R. W. (1965). Oligosaceharides, Vol. V, Pergamon Press, Oxford, PP. 97-101.

20. Grandchamp-Chaudun, A. De, Courtois, J. E., a. Dizet, P. Le (1960). Bull. Soc. Chim. Biol. 42, pp. 227-230.

21. Aspinall, G. 0., Rashbrook, R. B., Kessler, G. (1958). J. Chem. Soc. pp. 215-221.

22. Tyminski, A. & Timell, T. E. (1960). J. Am. Chem. Soc. 82, pp. 2823-2827.

Corresponding Author

Prabhu Balaji*

Research Scholar of University of Entrepreneurship & Technology, USA