Extraction of Galactomannan from Medicinal Plant Cassia Sophera

Manoj Singh, J.P. Singh*, Dr. Rajeev Dubey
Department of chemistry S.G.R.P.G. College Dobhi Jaunpur
V.B.S. Purvanchal University Jaunpur, 222149 [India]

E-mail: *manojsingh231982@yahoo.co.in*

Abstract- **A water soluble galactomannan having D-Mannose [4-part] and D-Galactose [3-parts] was isolated from Cassia Sophera. In cassia Sophera Hydrolysis of methylated seed gum furnished three methylated sugars, 2, 4-di-O-methyl-D-mannose, 2, 4,6-tri-O-methyl-D-mannose and 2, 3,4,6-tetra-O-methy-D-galactose was present in molar ratio 2:3:2. Partial acid catalysed hydrolysis of the seed gum gave four oligosaccharides epimelibiose, mannobiose, galactosyl mannobiose, mannotriose and along with the component sugars. Periodate oxidation and methylation studies in cassia Sophera showed 45% of end groups from the recent studies it is the concluded that galactose unit in galactomannan only terminal positions.**

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

I. INTRODUCTION

Cassia sophera have been described to be medicinal, great economic value and rich source of polysaccharides. It has been seen single –trunked trees that grow as high about 15m. to 20m., bark fairly smooth, branches slender. Cassia sophera having bushy habitat leaflets 6-9 in numbers and 3-6 cm. diameter. Flower yellow pink or white in colour, globose, globrous, and corolla about 3 cm. Long, each flower perfect and sub trended by palate. Seeds triangle shaped and gray compressed. A large proportion of the world's population depends on traditional medicine because of the scarcity and high costs of orthodox medicine [1]. Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents [2]. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases [3]. There is now an increasing body of scientific evidence demonstrating that the plants possess many other beneficial properties [4].The central analgesic [5] & anticonvulsant [6]. action of the seeds of Cassia sophera studied by Eddy's and Leimbach. Cassia sophera exhibit dose dependent increase in reducing power in turn suggests the antioxidant [7] & anticancer [8] potential of the plant. The formalin test is very useful method for not only assessing antinociceptive [9] drugs but also helping in the elucidation of the action mechanism. The hepatoprotective activity [10], [11] of medicinal plant Cassia Sophera is well known. Cassia sophera is a well known homeopathic medicine commonly known as Kasunda, is obtained from Cassia sophera Linn. used for diagnosis of bronchial asthma [12].

II. MATERIALS AND METHODS

Polysaccharide was isolated from crushed seeds of cassia Sophera 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 o.159% .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 3:4 and both the fraction indicating homogeneous nature of polysaccharide.

B. ZONE- ELECTROPHORESIS

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 - 9.3. 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.

$$M = \frac{31.74 \times \text{absorbance}}{0.24}$$

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

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

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 l: 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 4:3. 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 method by using dimethyl sulphate and sodium hydroxide then by Purdie's. 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 {α}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 105°C. The RTMG values were calculated in each case and compared it with reported values. methylated sugar 2, 4-di-O-methyl-D-mannose, 2, 4, 6–tri–O–methyl–D-mannose, 2, 3, 4, 6 – tetra – O –methyl–D-galactose as 2:3:2. The calculation of methylated sugar were done as methyl ether of the anhydrohexene units assuming 99.9% recovery of the D- galactose and end group analysis from methylation studies was found 45%.. 2, 4 -di-O-methyl-D-mannose was obtained as syrup RTMG, in the mobile phase no. 5, 0.56, O-me: 29.8% calculated for dimethyl hexose, O-me: 29.90% [α] D25-16.9˚ [water] , melting point 192-194˚C, [α] D27-65˚[Chloroform] . 2, 4, 6-tri-O-methyl D-mannose was obtained as a syrup RTMG in the mobile phase no. 5 0.84, [α] D27 -13° [water] , melting point-131-132°C, [α] D26-19.9˚[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, 0.89, [α] D30 -122˚ [water] lit. These oligosaccharides were obtained and identified.

Epimelibiose : [α-D-galactosyl [1-6] –D-mannopyranose] m.p. 200-202 ºC, { α }D26+ 120.5º water reported m.p. 202-203 ºC, { α }D26+ 120º water. Acid hydrolysis gave galactose and mannose; it reducing R glu in solvent [A] was found 0.58 . [0.58] , and Rman. in mobile phaseno.-2,5,and 7 was 0.17,0.37 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 reported m.p.203-204º C , { α }D25- 8 to - 9 º C, experimental m.p.-202-204º C { α }D25 -9º C. Acid hydrolysis showed the presence of mannose units only. Emulsin hydrolysis showed the presence of β-linkage between the mannose units which were also confirmed by the optical rotation of sugar osazone had experimental m.p. 202 - 204 º C [203-205] .

Mannotriose [β-D- Mannopyranosyl] [1→4] - β-D- Mannopyranosyl [1→4] -D-mannopyranose, experiential m.p.165- 167 º C , { α }D25 - 17 º C. Reported m.p.164- 166 º C , { α }D25 - 16 to -17 º 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.

Galactosyl manobiose: [α - D-galactopyranosyl [1→6] – β - D –mannopyranosyl [1→4] -D-mannopyranose] experimental m.p.226-228 º C, {α} D25 - 92 º C [water] [12] reported m.p. 227-230 ºC {α }D25 - 93º C [water] .Rglu in mobile phase no-2, 0.32,and Rman , in mobile phase 6 & 7 0.18 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 tri-saccharide. 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.

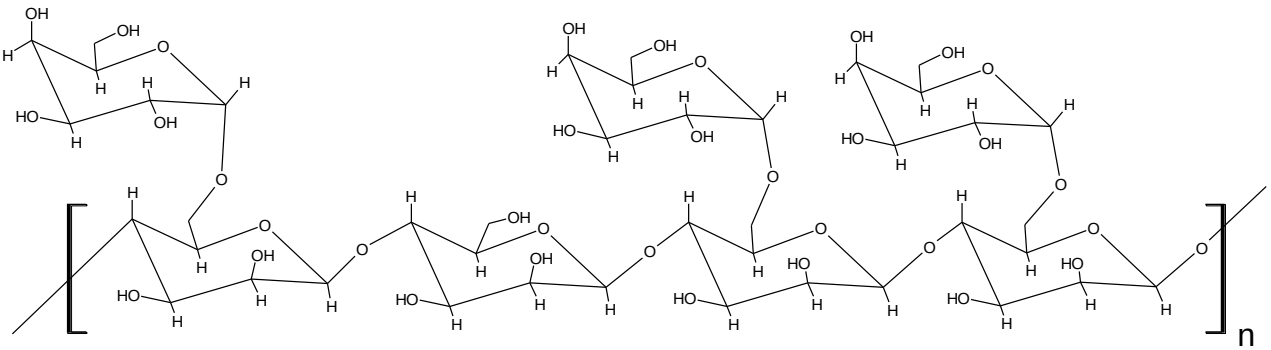
III. RESULTS AND DISCUSSION

The polysaccharide was extracted with 1% aqueous Acetic acid and precipitated with 4 volume of ethanol. This process was repeated until minimum ash content, 0.159% 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 3:4 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 2:3:2.

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] mannobiose , [iii] Epimelibiose and [iv] Mannobiose, 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 mannoschharides 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.



α-D-Galp α-D-Galp α-D-Galp

↕ ↕ ↕

6 6 6

|→4)-β-D-Manp (1→4)- β-D-Manp (1→4)- β-D-Manp (1→4)- β-D-Manp (1→|n

The above structure contains seven units of monosaccharide, three galactose units and four 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, NMR, Mass analysis of our experimental sample is nearly same as our reported sample.

REFERENCES

[1] Tagboto S and Townson S. Antiparasitic properties of medicinal plants and other naturally occurring products. Adv Parasitol. 50, 2005, pp.199-295.
[2] Evans WC. Trease and Evans Pharmacognosy. 2000, pp. 19-20.
[3] Newman DJ, Cragg GM and Snadder KM. Natural products as sources of new drugs over the period. J. Nat. Prod.2003, 66(7), pp 1022 -1037.
[4] Kirtikar KR and Basu BD. Indian Medicinal Plants. 2006, 2, pp 856-860.
[5] Bilal K, Naeem A and Inamuddin G.Pharmacological investigation of Cassia sophera Linn. Medical Journal of Islamic world academy of sciences.2005, 15, pp 105-109.
[6] Ahamad B, Naeem AK, Ghufran A, Inamuddin Pharmacological investigation of Cassia sophera. Medical Journal of Islamic World Academy of Sciences. 2005, 15(3), pp105–109.
[7] Atiqur Rahman, Minzanur Rahman M,Mominui Islam Sheik MD, Mashiar Rahman M, Shabah Mohammad Shadil and Alam MF. Free radical scavenging activity and phenolic content of Cassia Sophera. 2008, 7(10), pp 1591-1593.
[8]Longtin A, Laprise C, Legault J. Antioxidant, antiinflammatory and anticancer activities of methanolic ex tracts from Ledum groenlandicum. Journal of Ethnopharmacol. 2007, 111, pp 22–28.
[9] Dheeraj H, Vivek Kumar Ghosh, Manohar J and Atul M. In vitro antioxidant and invivo anti-inflammatory activity of Cassia sophera. 2010, 2(1), pp 113-120
[10] Arijit M, Sanjay Kumar K, Tanushree S, Rajalingam D and Tapan M. Evaluation of hepatoprotective effect of leaves of Cassia sophera. Evidence based complementary and alternativemedicine. 2012, 4(1), pp 1-5.
[11] Khan N., Gufran A., Hepotoprotective effect of seeds of cassia sophera Var.Purpurea, roxb. Against CCl4 induced hepatic damage in albino mice, Pharmacognosy mag. 2005, 11, pp 68-69.
[12] Morice AH; The diagnosis and management of chronic cough. European Respiratory Journal, 2004, 24(3), pp 481-492.