

Nature of gum polysaccharide extracted from *Moringa oleifera* Lam. (*Sainjna*) plant

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ABSTRACT

Water soluble polysaccharide have been extracted from *Moringa oleifera* Lam. gum mucilage as L-arabinose and D-galactose in the molar ratio of 1:4 with traces of L-fucose. Hydrolysed gum polysaccharides were separated by paper chromatography and identified by column chromatography. It consumed 8.2 moles of iodine by iodometrically after 110 hrs. Nature of α and β -type linkages were confirmed by IR-Spectra (KBr). Sugars were identified by their specific rotations, melting points and preparation of their suitable crystalline derivatives.

Keywords: Gum polysaccharide, *Moringa oleifera* plant.

INTRODUCTION

Moringa oleifera Lam. plant^[1] belongs to the family Moringaceae and commonly called as *Sainjna*. It is a medium sized tree upto 10m in height. It occurs in Northern Himalayas to Southern India, Thailand, Pakistan, Sri Lanka, Africa, Afghanistan, Cambodia, Nepal, Indonesia, Mexico, Central & Southern America, Philippines, etc. Plant has many important medicinal values from leaves, roots, bark, gum, seeds, flowers and fruits for the treatment of various oilment in the indigenous system of medicine including the treatment of inflammation and infection diseases alongwith cardio vascular, gastrointestinal, hematalogical and hepatorenal disorders. Gum used for dental caries, astringent and in blood pressure. Tender pods are used as vegetable and also in pickled. Leaves are rich in Vitamin A and C, β -carotene, protein, calcium and potassium and are used in scurby and catarrhal infections and good source of natural antioxidant. Seeds are considered antipyretic, acrid, bitter and seed oil is applied in rheumatism. Leaf extract are used for the treatment of piles, fevers, bronchitis eyes and ear infections. Leaves have a potential source for antitumor and anticancer activities and Niazimian has been proposed to be a potent chemopreventive agent in chemical carcinogenesis. Seeds extract have also found to be effective on hepatic carcinogen metabolizing enzyme and antioxidant parameters. Seeds have specific protein fractions for skin and hair cure. Seed peptide are also used to protects the human skin aging with dual activity as antipollution and conditioning of hair. Seed extracts a globally acceptable innovative solution for hair care. The present manuscript mainly deals with the isolation, purification, preliminary analysis and nature of the constituent sugars from the *Moringa oleifera* Lam. gum.

MATERIALS AND METHODS

Isolation and purification of gum polysaccharide :

Polysaccharides were isolated from the collected *Moringa oleifera* Lam. gum (500gm) by dissolving it in distilled water^[2] (900ml) overnight at room temperature then filtered. Filtrate was precipitated out with ethanol (2 litre) in crude form, yield (310 gm), sulphated ash (3.6%). The crude gum polysaccharide (300gm) was further extracted in a soxhlet apparatus with hot ethanol (400 ml) for 10 hrs in order to remove the rasineous matter (50%). The deresinified crude gum (200gm) was grounded to a fine powder and was stirred (8hrs) with distilled water (900ml). The brown coloured solution was filtered and filtrate was acidified with hydrochloric acid and acidic solution was poured slowly into absolute alcohol (4litres) with continuous mechanical stirring. The precipitated gum

polysaccharide was collected and the purification process was 4 times repeated to obtain gum acid in the form of a slightly brown powder with a high ash content, found sulphated ash 0.76%. Final purification of gum polysaccharide was carried out by passing its aqueous solution through Amberlite Ion. Exchange resins IR-120 (H⁺) and IR-45 (OH⁻) to yield (130gm) of pure gum acid which was almost white in colour. It did not reduce Fehling's solution and showed negative tests for nitrogen, halogens, sulphur, acetyl groups and methoxyl contents.

Preliminary analysis of gum polysaccharide :

The equivalent weight of purified gum polysaccharide (300mg) was obtained by dissolving it in distilled water (50ml) and titrated against CO₂ free sodium hydroxide solution (0.06N) using phenolphthalein as an indicator. During titration the CO₂ free sodium hydroxide solution was protected from atmospheric CO₂ by fixing a soda lime guard tube at the upper end of the micro burette. The experiment was performed thrice and average equivalent weight of gum was found to be 1529. The specific rotation of gum polysaccharide was carried out by usual manner as $[\alpha]_D^{29} +28.6^{\circ}\text{C} (\text{H}_2\text{O})$. Preliminary analysis of gum polysaccharide was found to contain furfural^[3] (11.2%), pentosans (18.4%) and pentoses (22.6%).

Homogeneity of gum polysaccharide :

Homogeneity of gum polysaccharide (6gm) was carried out by fractional precipitation method^[4] with distilled water (50ml), solution was acidified by hydrochloric acid and poured in absolute alcohol (100ml) with continuous mechanical stirring. The precipitated gum sample-I, yield (3.5gm) and was filtered off. The filtrate was diluted with more ethanol (100ml) when further precipitation took place, filtered to form Sample-II. Yield (1.5gm) and filtrate diluted with ethanol (200ml) to obtain the resinous mass. Sample-I and II were washed with ethanol and petroleum ether (40-60^o) and dried in vacuum. The deresinified crude gum (2.5gm) was dissolved in water (25ml) and acidified with hydrochloric acid was poured into ethanol (80ml). The precipitated gum polysaccharide was filtered to obtain the gum acid in the form of slightly brown powder. It was again dissolved in water (20ml) and treated with freshly regenerated cation exchange resin Duolite C-25 and poured into absolute alcohol (100ml) when the gum acid was obtained in the form of white precipitate, Sample No. III, yield (1.10gm). It was filtered and the residue was washed with ethanol and petroleum ether and dried in vacuum.

Hydrolysis and nature of the constituent sugars :

All three gum polysaccharide samples (I, 0.4240gm, II, 0.520gm and III, 0.3140gm) were hydrolysed separately in a sealed tube with sulphuric acid (1.5N, 40ml) for 24hrs in a boiling water-bath. The obtained hydrolysate were transferred quantitatively to different beakers containing anhydrous L-rhamnose (0.0430gm; 0.0436gm and 0.0410gm) respectively and neutralized with barium carbonate and left for 20hrs. They were filtered and filtrate after concentration to a syrup were exhaustively extracted with methanol. The concentrated methanolic extract were examined by descending technique of paper chromatography^[5] on Whatman No. 1 and 3MM filter paper sheet using solvent mixture (v/v) : (A) *n*-butanol, ethanol, water (4:1:5, upper phase)^[6] and used (R)-*p*-anisidine phosphate^[7] as spray reagent when sugar spots corresponding to D-galactose, L-arabinose, L-fucose and L-rhamnose were observed.

Autohydrolysis of gum mucilage polysaccharide :

A solution of purified gum mucilage polysaccharide (50gm) was dissolved in distilled water (800ml) then heated on a water-bath for 72 hrs and course of hydrolysis being followed by iodometric titration^[8]. Autohydrolysate (2ml) was taken out in a stoppered conical flask then iodine solution (0.1N, 20ml) and sodium hydroxide solution (0.1N, 30ml) was added. The reaction mixture was acidified with sulphuric acid (1N) and excess iodine titrated against sodium thiosulphate solution (0.05N) and it consumed 8.2 moles of iodine after 110 hrs. The autohydrolysate was cooled and neutralized with barium hydroxide slurry, filtered and filtrate evaporated to a brown syrup. This syrup was extracted exhaustively with methanol and the extract was concentrated to a syrup (Fraction-A) which consisted of neutral sugars released on autohydrolysis. The solid amorphous residue (Fraction-B) consisting of Barium salt of degraded gum polysaccharide was with a petroleum ether (40-60^o) and dried in vacuum, yield (29gm).

Resolution of sugar fraction (A) into its components by cellulose column chromatography :

The sugar syrup of Fraction (A) were resolved into its pure constituents on a cellulose column chromatography^[9] using *n*-butanol half saturated with water as eluate^[10]. It furnished three crystalline sugars which were characterized and identified as L-arabinose, D-galactose and L-fucose. The column chromatographic resolution was carried out in a glass tube (14"×1") filled with a glass stopper. A thin layer of glass wool was packed at the bottom of the tube and cellulose powder was introduced with the help of glass tube. Column was washed with the *n*-butanol half saturated with water till the washing became colourless. Sugar mixture (Fraction-A) was added slowly to the column and allowed to absorb the cellulose. The column was then eluted with eluant and sugar fractions (15ml) were collected manually in several test tubes and obtained results are given in Table-I. Each sugar fraction was examined by paper chromatographic analysis on Whatman No.3 Mm filter paper sheets.

Table-I : Resolution of sugar mixture by cellulose column chromatography

Sr. No.	Fraction No.	Rf values in solvent (A)	Weight of sugar (gm)	Sugars Present
1.	01-22	-	-	No Sugar
2.	23-28	0.22	0.0182	L-fucose
3.	29-40	0.22 & 0.14	0.4894	L-fucose & L-arabinose
4.	41-63	0.14	0.9126	L-arabinose
5.	64-84	0.14 & 0.08	0.8628	L-arabinose & D-galactose
6.	85-108	0.08	1.4608	D-galactose
7.	109-onward	-	-	No Sugar

Characterization of Sugars :

Fraction of the eluate containing pure sugars were mixed then isolated sugars were identified by determining their specific rotation and preparing their suitable crystalline derivatives. The gum polysaccharides were characterized by column chromatography and was again identified by paper chromatography by usual manner as follows :

- i. L-arabinose, m.p. & mixed m.p. 152-154⁰C [α]_D²⁹ +102⁰C (H₂O), derivative *p*-nitro-N-phenyl-L-arabinosyl amine, m.p. 203-204⁰C Lit. m.p. 201-202⁰C.
- ii. D-galactose, m.p. & mixed m.p. 165-166⁰C, [α]_D²⁹ +82.4⁰C (H₂O), derivative, *p*-nitro-N-phenyl-D-galactosyl amine, m.p. 206-207⁰C, Lit. m.p. 205-206⁰C.
- iii. L-fucose, m.p. & mixed m.p. 114-116⁰C, [α]_D²⁹ -73⁰C (H₂O), derivative L-fucose methyl phenyl hydrazone, m.p. 175-176⁰C, Lit. m.p. 174-175⁰C.

Quantitative estimation of sugars :

In order to determine the quantitatively ratio of different constituent sugars obtained from *Moringa oleifera* Lam. gum hydrolysed syrup which were separated on Whatman No. 3 MM filter paper sheet by descending technique of paper chromatography using the solvent mixture (A) and used (R) as a spray reagent. The sugar strips were cut out with the help of guide spots then eluted with water according to the Dent's method^[11]. The eluted sugars were estimated by phenol sulphuric acid method^[12] and periodate oxidation method^[13]. It indicated L-arabinose and D-galactose in 1:4 molar ratio with traces of L-fucose.

RESULTS AND DISCUSSION

Moringa oleifera Lam. gum mucilage yielded a water soluble polysaccharide as L-arabinose and D-galactose in the molar ratio of 1:4 with traces of L-fucose from hydrolysed compound by descending technique of paper chromatography on Whatman No. 3 MM filter paper sheet. It consumed 8.2 moles of iodine by iodometrically after 110 hrs. The specific rotation of the parent gum polysaccharide is a low positive and linkages between L-arabinose and D-galactose must be of α -type possible with β -type. Some information about the nature of linkages in gum polysaccharide was obtained from its IR-spectroscopy^[14] (KBr) were recorded on a Perkin-Elmer Model-137-B Spectrophotometer. The sugars were characterized by column chromatography and identified by paper chromatographic analysis. Sugars were characterized and identified by their melting points, optical rotations and preparation of their suitable crystalline derivatives as: *p*-nitro-N-phenyl-L-arabinosyl amine, *p*-nitro-N-phenyl-D-galactosyl amine and L-fucose methyl phenyl hydrazone in traces.

REFERENCES

- [1] Sastri BN, *The Wealth of India, Raw Materials*, Publication and Information Directorate, CSIR, New Delhi, India, **1962**, VI (L-M), 425.
- [2] Singh RB, *Biosciences, Biotechnology Research Asia*, **2006**, 3 (2A), 385.
- [3] Brown CA, Zerban FW, *Physical and Chemical Method of Sugar Analysis*, John Wiley & Sons, New York, **1941**, 3, 904.
- [4] Heyne R, Whistler RL, *J. Amer. Chem. Soc.*, **1948**, 70, 2249.
- [5] Partridge SM, *Nature (London)*, **1946**, 158, 270.
- [6] Partridge SM, Westall RG, *Biochem. J.*, **1948**, 42, 238.
- [7] Mukherjee S, Srivastava HC, *Nature (London)*, **1952**, 169, 330.
- [8] Baker JL, Hulton HFE, *Biochem. J.*, **1920**, 14, 754.
- [9] Hough L, Jones JKN, Wadman WH, *J. Chem. Soc.*, **1949**, 2511.
- [10] Chalson AJ, Nunn JR, Stephen AM, *J. Chem. Soc.*, **1955**, 269.
- [11] Dent CE, *Biochem. J.*, **1947**, 28, 350.
- [12] Dubois M, Gills KA, Hamilton JK, Rebers RA, Smith F, *Anal. Chem.*, **1956**, 28, 56.
- [13] Hirst EL, Jones JKN, *J. Chem. Soc.*, **1949**, 1959.
- [14] Rao CNR, *Chemical Applications in IR-Spectroscopy*, **1903**.