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Gum polysaccharide structure from *Moringa oleifera* Lam. plant gum by methylation technique

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ABSTRACT

Gum polysaccharide has been extracted from Moringa oleifera Lam. plant gum (Moringaceae) with water as L-arabinose and D-galactose in 1:4 molar ratio with traces of L-fructose. The purified gum polysaccharide was completely methylated by Hakomari and Purdie's method which afforded methyl sugars as : 2,3,4,6-tetra-O-methyl-D-galactose; 2,3,4-tri-O-methyl-D-galactose; 2,4-di-O-methyl-D-galactose and 2,2-di-O-methyl-L-arabinose were found in the molar ratio of 1:1:2:1 and also present uronic acid as : 2,3,4-tri-O-methyl-D-glucuronic acid (1 mole).

Key words: Methylation, methyl sugars, gum polysaccharide structure, *Moringa oleifera*

INTRODUCTION

Moringa oleifera Lam. plant[1] belongs to Moringaceae family and commonly called as *Sainjna* upto 10m in height. It occurs in Himalayan region of Northern to Southern India, Thailand, Pakistan, Africa, Sri Lanka, Afghanistan, Cambodia, Nepal, Indonesia, Mexico, Central of Southern America, Philippines, etc. Leaves, gum, seeds, bark, flowers and fruits are used in indigenous system of medicine for the treatment of cardio vascular disease, inflammation, gastrointestinal and haematological disorders. Gum used for the dental infection, astringent and blood pressure. Tender pods are used as vegetable and pickles. Leaves are rich in Vitamin A and C, β-Carotene, protein, calcium and potassium and used in scurby, catarrhal infections and good source of natural antioxidant. Seeds are considered antipyretic, acrid, bitter and seed oils 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 leaves alkaloid 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 and have specific protein fractions for skin and hair cure. Seeds peptide are also used to protects the human skin aging with dual activity as antipollution and conditioning of hairs. Seed extracts a globally acceptable innovative solution for hair cure. The gum contains a water soluble polysaccharide[2] as L-arabinose and D-galactose in 1:4 molar ratio with traces of L-fructose. The present manuscript mainly deals with the methylation studies of purified gum polysaccharide alongwith a proposed gum polysaccharide structure from *Moringa oleifera* Lam. plant.,

MATERIALS AND METHODS

Unless otherwise stated that the all evaporations were carried out under reduced pressure and melting points are uncorrected. The obtained mixture of methyl sugars were carried out by descending technique of paper chromatography[3] on Whatman No. 1 and 3 MM filter paper sheets. The upper phase of the following solvent

mixture (v/v) were used for the identification of *Moringa oleifera* Lam. gum methyl sugars (v/v) : (S_1) *n*-butanol, ethanol, water (4:1:5)[4] and (S_2) *n*-butanol, acetic acid, water (4:1:5)[4]. The (R) *p*-anisidine phosphate[5] was used as spray reagent for the identification of methyl sugars.

Methylation of gum polysaccharide :

Purified gum polysaccharide (15 gm) was methylated by Hakomari's method[6] for three times with distilled water (50 ml), dimethyl sulphate (175 ml) and sodium hydroxide solution (30 %, 400 ml) in an atmospheric nitrogen at 5-8^{0}C. Reaction mixture was further stirred for 12 hrs with the help of mechanical stirrer then neutralized with H_2SO_4 (5 N) and obtained precipitate of sodium sulphate was filtered off and it was further extracted with methanol. The methanolic extract and filtrate were combined and concentrated to gave partly methylated product (8 gm), Found : -OCH_3, 39 %. It was further remethylated four times by Purdie's reagent[7] with methanol (20 ml), methyl iodine (30 ml) and silver oxide (14 gm) which gave fully methylated product (6 gm), Found : -OCH_3, 42.56 %, $[\alpha]_D^{25}$ + 39.6^{0}C (H_2O).

Hydrolysis of methylated gum polysaccharide :

The methylated gum polysaccharide (5 gm) was refluxed with methanolic hydrogen chloride (5 %, 15 ml) then evaporated it to dryness on water-bath. It was saponified with barium hydroxide solution (0.03 N, 10 ml) for 3 hrs at 60^{0}C. The excess of barium hydroxide was removed by passing through CO_2 in the solution. The saponified product after being worked up was thoroughly extracted with dry ether to gave an ether soluble fraction (A), yield (2.004 gm) and other ether insoluble fraction (B), yield (0.3846 gm).

Examination of ether soluble fraction (A) :

The ether soluble syrup (2.142 gm) was hydrolysed with HCl (1 N, 50 ml) for 12 hrs on boiling water-bath. The hydrolysate was cooled and neutralized with barium carbonate slurry then evaporated to a syrup. It was resolved into four components on Whatman No. 3 MM filter paper sheet using solvent mixture (S_1). Paper strips corresponding to the individual methyl sugars were eluted with water according to the Dent's method[8]. The eluted sugar solutions were concentrated separately to furnish four methyl sugar fractions which were identified and characterized as follows :

Fraction-I : 2,3,4,6-tetra-O-methyl-D-galactose :

Methyl sugar syrup (0.3148 gm), had *R*f value 0.86 in solvent mixture (S_1), $[\alpha]_D^{25}$ + 112^{0}C (H_2O), Found : -OCH_3, 51.92 %, calculated for tetra-O-methyl-D-galactose, $C_{10}H_{20}O_6$, 52.5 %. It was converted into 2,3,4,6-tetra-O-methyl-N-Phenyl-D-galactosyl amine derivative having m.p. 190-192^{0}C, Lit. m.p. 192-193^{0}C[9].

Fraction-II : 2,3,4-tri-O-methyl-D-galactose :

Sugar syrup (0.3514 gm) showed a single spot as D-galactose (*R*f values 0.63) in solvent mixture (S_1) on paper chromatogram. It had $[\alpha]_D^{25}$ + 92^{0}C (H_2O), Found : -OCH_3, 39.6%, calculated for $C_9H_{18}O_6$ requires –OCH_3, 41.5 %. Derivative was prepared by usual manner as 2,3,4-tri-O-methyl-N-phenyl-D-galactosyl amine, having m.p. 163-164^{0}C, Lit. m.p. 164-165^{0}C[10].

Fraction-III : 2,4-di-O-methyl-D-galactose :

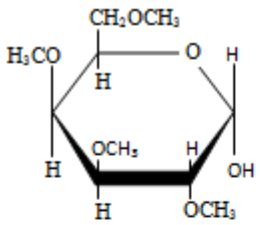
Methyl sugar syrup (0.5892 gm) gave a single spot parallel to D-galactose (*R*f value 0.41) on paper chromatogram in solvent mixture (S_1). It had $[\alpha]_D^{25}$ + 81.6^{0}C (H_2O), Found : -OCH_3, 29.2 %. The aniline derivative was prepared by the refluxing the syrup (0.36 gm) with aniline (0.134 gm) and ethanol (8 ml) for 2 hrs. The ethanolic solution was concentrated when the crystals of aniline derivative was separated out. This upon recrystallisation with ethanol provided crystals of 2,4-di-O-methyl-N-phenyl-D-galactosyl amine had m.p. 206-207^{0}C, Lit. m.p. 207-208^{0}C[10].

Fraction-IV : 2,3-di-O-methyl-L-arabinose :

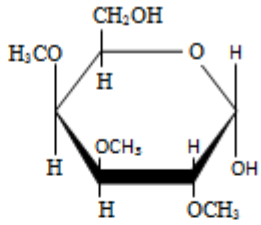
Methylated sugar syrup (0.2816 gm) gave a single spot parallel to L-arabinose (*R*f values 0.64) in solvent mixture (S_1) on paper chromatogram. It had $[\alpha]_D^{25}$ + 98^{0}C (H_2O), Found : -OCH_3, 34.62 %, calculated for $C_7H_{14}O_5$, 34.8 %. The sugar was converted to 2,3-di-O-methyl-N-phenyl-L-arabinosyl amine derivative, had m.p. 138-139^{0}C, Lit. m.p. 139-140^{0}C[11].

Examination of ether insoluble fraction (B) :

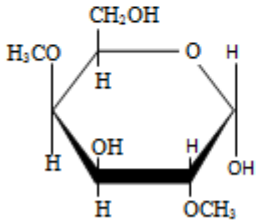
The methyl sugar syrup (0.3872 gm) on paper chromatographic analysis in solvent mixture (S_2) and used (R) as spray reagent to revealed the presence of a single spot (*R*f value 0.82), Found : -OCH_3, 38.6 %, calculated for tri methyl uronic acid, $C_9H_{18}O_7$, 39.4 %. The uronic acid was identified as (V) : 2,3,4-tri-O-methyl-D-glucuronic acid by preparing derivative as 2,3,4-tri-O-methyl-D-glucopyranoside uronamide had m.p. 182-183^{0}C[10].



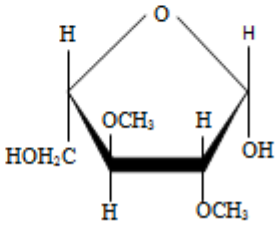
(I) 2,3,4,6-tetra-O-methyl-D-galactose.



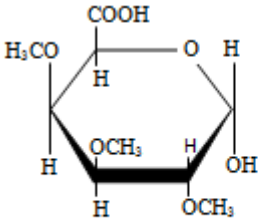
(II) 2,3,4-tri-O-methyl-D-galactose



(III) 2,4-di-O-methyl-D-galactose.



(IV) 2,3-di-O-methyl-L-arabinose.



(V) 2,3,4-tri-O-methyl-D-glucuronic acid.

Figure-I : Structure of methylated *Moringa oleifera* Lam. gum polysaccharide

RESULTS AND DISCUSSION

Moringa oleifera Lam. gum polysaccharide was completely methylated by Hakomari's method and Purdie's method. The methylated gum polysaccharide was hydrolysed with methanolic hydrogen chloride and obtained hydrolysate after saponification was separated in the ether soluble fraction (A) and ether insoluble fraction (B). The ether soluble fraction (A) methylated sugars were fractionated in paper chromatographic analysis on Whatman No. 3 MM filter paper sheet to revealed the presence of methyl sugar components as shown in Figure-1. The obtained methyl sugars were characterized and identified as : (1) 2,3,4,6-tetra-O-methyl-D-galactose, (II) 2,3,4-tri-O-methyl-D-galactose, (III) 2,4-di-O-methyl-D-galctose and (IV) 2,3-di-O-methyl-L-arabinose in 1:1:2:1 molar ratio. The ether insoluble fraction (B) methylated uronic acid was characterised and identified as : 2,3,4-tri-O-methyl-D-glucuronic acid (1 mole).

The isolation of 2,3,4,6-tetra-O-methyl-D-galactose sugars from the cleavage fragment suggested that the side chain of methoxyl group (R) is terminated by D-galactopyranose moiety. According to the gum polysaccharide structure of *Moringa oleifera* Lam. as shown in Figure-2, there should be 5 moles of 2,4-di-O-methyl-D-galactose where as in actual practice 6 moles have been obtained. The slight excess over the theoritical yield of di-methyl-D-galactose is difficult to explain unless and until. It is assumed that the partial demethylation of the tri-methyl-D-galactose has taken place. It may be pointed out here that in the structure of the gum polysaccharide, the L-fructose unit are not induced, although the sugar was obtained in the trace amount by the hydrolysis of the gum polysaccharide because the hydrolysis of methylated gum did not furnish any methylated sugars of L-fructose.

A tentative polysaccharide structure suggested for the gum polysaccharide of *Moringa oleifera* Lam. (Figure-2) clearly indicated that the gum is highly branched in the nature and linkages contains (1→6)-β-type, (1→3)-β-type and (1→5)-α-type linkages in the gum polysaccharide structure of *Moringa oleifera* Lam. plant.

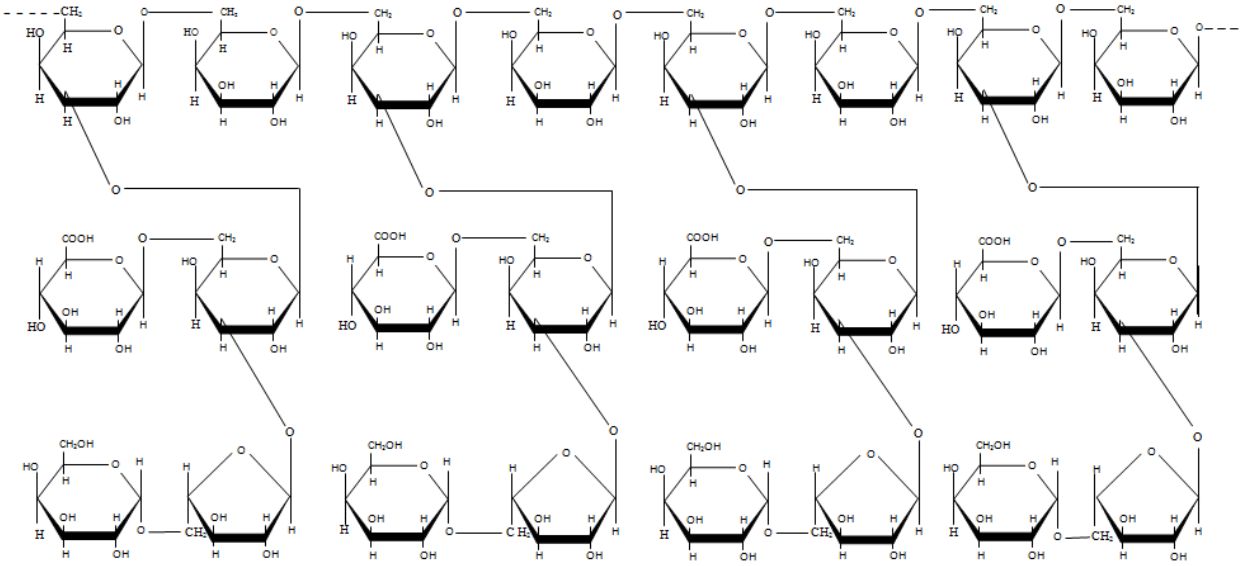


Figure-2: Polysaccharide structure of *Moringa oleifera* Lam. gum polysaccharide

REFERENCES

[1] Sastri BN, *The Wealth of India, Raw Materials*, Publication & Information Directorate, CSIR, New Delhi, India, **1962,** VI (L-M), 425-429.
[2] Pal Ashish, Singh RB, *Advances in Applied Science Research*, Pelagia Research Library, **2014,** 5 (6), 01-03.
[3] Partridge SM, *Nature (London)*, **1946,** 158, 270-274.
[4] Partridge SM, Westall RG, *Biochem. J.*, **1948,** 42, 238-240.
[5] Mukherjee S, Srivastava HC, *Nature (London)*, **1952,** 169, 330-332.
[6] Hakomari S, *J. Biochem. (Tokyo)*, **1964,** 55, 205-208.
[7] Purdie T, Irvine JC, *J. Chem. Soc.*, **1903,** 83, 1021-1024.
[8] Dent CE, *Biochem. J.*, **1947,** 41, 240-242.
[9] Smith F, *J. Chem. Soc.*, **1940,** 1035-1038.
[10] Bose S, Gupta K,C, *Indian J. Chem.*, **1964,** 2, 156-158.
[11] Hirst EL, Jones JKN, *J. Chem. Soc.*, **1948,** 120-124.