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Degraded gum polysaccharide structure from *Moringa oleifera* Lam. gum polysaccharide by methylation studies

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Abstract
Moringa oleifera Lam. plant gum contain a water soluble gum polysaccharide as L-arabinose and D-galactose in 1:4 molar ratio with traces of L-fucose. The degraded gum polysaccharide was methylated by Brown, *et.al.* and Purdie's method which yielded degraded methyl sugars as : 2,3,6-tri-O-methyl-D-galactose; 2,3,4-tri-O-methyl-D-galactose; 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid in 1:6:2:3 molar ratio.

Keywords: Degraded methyl sugars, *Moringa oleifera* gum polysaccharide

1. Introduction
Moringa oleifera Lam. Plant [1] belongs to family- Moringaceae and commonly called as *Sainjna*, upto 10m in height. It occurs in all over India, Thailand, Pakistan, Africa, Sri Lanka, Indonesia, Nepal, Philippines, Mexico, America, etc. Gum of plant contains a water soluble polysaccharide [2] as L-arabinose and D-galactose in 1:4 molar ratio with traces of L-fucose. The present manuscript mainly deals with the methylation studies of degraded gum polysaccharide structure of *Moringa oleifera* Lam. plant.

2. Materials and Methods
Degraded methyl sugars of *Moringa oleifera* Lam. gum polysaccharide was separated and identified by descending technique of paper chromatography[3] on Whatman No. 1 & 3 MM filter paper sheet. The following upper phase of solvent mixture (v/v) were used for the detection of degraded methyl sugars as: (A) *n*-butanol-ethanol-water (4:1:5) [4] and (B) *n*-butanol-acetic acid-water (4:1:5) [4]. The spray reagent (R) *p*-anisidine phosphate [5] was used for the appearance of the degraded methyl sugars.

2.1 Isolation of the Barium salt of degraded gum polysaccharide
The degraded gum polysaccharide of *Moringa oleifera* Lam. gum (35gm) was prepared with distilled water (750ml) for 40hrs by stirring with mechanical stirrer. The obtained autohydrolysate [6] was cooled and neutralized with barium carbonate slurry when the Barium salt of the degraded gum polysaccharide was obtained as an amorphous powder. This product was freed from the adhering sugar impurities by boiling four times on water-bath with fresh methanol (100ml). The sample was then dried and analysed, yield (18gm), Ba 12.6%, equivalent weight from Barium percentage about 65.7%.

2.2 Methylation of degraded gum polysaccharide
The degraded gum polysaccharide (free from L-arabinose) was methylated extensively with dimethyl sulphate and sodium hydroxide [7]. The entire experiment was carried out in an atmosphere of Nitrogen. To a solution of Barium salt of the degraded gum polysaccharide (10gm) in distilled water (50ml) and dimethyl sulphate (120ml) was added slowly. A solution of sodium hydroxide (30%, 300ml) was added in such a manner that the whole addition took place in 7hrs. After stirring with mechanical stirrer for another 12hrs. The reaction product was neutralized with dil. sulphuric acid in cold. The precipitated sodium sulphate was filtered off and extracted with methanol. Filtrate and methanolic extract was combined and then concentrated to a thin syrup. This syrup was again methylated by dissolving in sodium hydroxide (30%, 300ml) and dimethyl sulphate (120ml).

The reaction product was extracted with chloroform in a liquid-liquid extractor at pH 8.0 to remove the neutral methylated sugars. The aqueous solution of methylated product was then acidified at pH 3.4 and extract exhaustively with chloroform and it on concentration left a residue (9.2gm). This residue was dissolved in methanol (25ml) and mixed with iodine solution (50ml). The mixture was refluxed gently and freshly prepared silver oxide (22gm) was added to it in small portion during 8hrs. The methylated product was filtered and residue extracted with hot methanol. The combined filtrate was concentrated and again methylated three times by Purdie's reagent [8] with methyl alcohol, methyl iodide and silver oxide. It did not reduce the methyl content, yield 7.80gm, $–OCH_3$, 40.6%.

2.3 Hydrolysis of fully methylated degraded gum polysaccharide

The fully methylated degraded gum polysaccharide (8gm) was hydrolysed [9] by dissolving in methanolic hydrogen chloride (6%, 120ml) and refluxed for 12hrs on water-bath. The reaction mixture was cooled and neutralized with freshly prepared silver carbonate (Ag_2CO_3), filtered and filtrate evaporated to a thin syrup. The resulting syrup was saponified with barium hydroxide (0.2N, 75ml) for 2hrs at 55 ^{0}C. The excess barium hydroxide was removed by passing through CO_2. The precipitated barium carbonate was removed by filtration and washed with distilled water. The combined filtrate was concentrated to a syrup which was again saponified with barium hydroxide and extracted with liquid-liquid extractor. The chloroform extract consisting of the methyl glycosides of neutral methylated degraded sugars was evaporated to a syrup (Fraction-A; yield 2.50gm). The aqueous solution left after chloroform extraction was acidified with dil. H_2SO_4 (Acidic to congo red) in cold and again extracted with chloroform. The chloroform extract was evaporated to a syrup to obtained methylated uronic acid moiety (Fraction-B; yield, 0.86gm).

2.4 Examination of neutral methylated sugars (Fraction-A)

Mixture of methyl glycosides of neutral methylated sugars from Fraction-A was hydrolysed [9] with hydrochloric acid (1N, 50ml) for 12hrs on boiling water-bath. The obtained hydrolysate was neutralized with silver carbonate, filtered and silver ions removed from the filtrate by passing through hydrogen sulphide gas then concentrated to a syrup, yield 2.6gm. It was resolved into three fractions by paper partition chromatography on Whatman No. 3 MM filter paper sheet using solvent mixture (A) and (R) used as spray reagent for the detection of methyl sugars. Paper strips corresponding to the individual methyl sugars were eluted with water according to the Dent's method [10]. The eluted methyl sugars were concentrated separately to furnished three fractions were characterized and identified as follows:

2.5 Fraction-I: 2,4-di-O-methyl-D-galactose

The sugar syrup (190mg) on paper chromatographic examination gave a single spot (*R*f 0.40) in solvent mixture (A). It had $[\alpha]_D^{29}$ +84 ^{0}C (H_2O), Found : $–OCH_3$, 29.6%, calculated for $C_8H_{16}O_6$, required, $–OCH_3$, 29.6%. Sugar (35mg) was refluxed for 2hrs with absolute alcohol and freshly distilled aniline (15mg). After the refluxing was over and alcoholic solution was concentrated when crystals of aniline derivative were separated out. This upon recrystallisation from ethanol furnished crystals of 2, 4-di-O-methyl-N-phenyl-D-galactosyl amine, had m.p. 205-206 ^{0}C, Lit. m.p. 206 ^{0}C [11].

A portion of methylated sugars (20mg) was oxidized[12] with sodium metaperiodate (0.2M, 2ml), distilled water (100ml) and sodium bicarbonate (1N, 2ml) and allowed to stand for 24hrs. Hydrochloric acid (1N, 2ml) and sodium arsenite (1.2N, 2ml) were then added to the reaction mixture, when the yellow colour of the solution had completely disappeared. The sodium acetate (1M, 2ml) and 2ml of dimedon reagent (5, 5–dimethyl dihydroresorcinol, 80ml per mole of 90% alcohol) was added. The resulting crystalline dimedon derivative of formaldehyde filtered out and dried, had m.p. 186-188 ^{0}C. This demonstrate that the hydroxyl group at C_6 position of sugar is unmethylated.

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

Sugar syrup (550mg) gave a single spot of D-galactose (*R*f 0.62) in solvent mixture (A), when examined on a paper chromatography. It had $[\alpha]_D^{30}$ +140 ^{0}C → +115 ^{0}C (H_2O), Found : $–OCH_3$, 39.8%, calculated for $C_9H_{18}O_6$ requires, $–OCH_3$, 41.6%. Derivative was prepared by usual manner as: 2, 3, 4-tri-O-methyl-N-phenyl-D-galactosylamine, m.p. 161-163 ^{0}C [13].

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

Sugar syrup (100 mg) gave a single spot parallel to D-galactose (*R*f 0.60) in solvent mixture (A) on paper chromatogram. It had $[\alpha]_D^{30}$ + 116 ^{0}C → +87 ^{0}C (H_2O), Found : $–OCH_3$, 40.14%, calculated for $C_9H_{18}O_6$ requires, $–OCH_3$, 41.6%. Derivative was prepared by usual manner as: 2,4,6-tri-O-methyl-N-phenyl-D-galactosyl amine, m.p. 176-178 ^{0}C [14].

2.8 Acidic methylated sugar (Fraction-B)

2.8.1 Fraction-IV: 2, 3, 4-tri-O-methyl-D-glucuronic acid

Methylated sugar fraction (B) was hydrolysed with HCl (1N, 20ml) for 20hrs on water-bath. Hydrolysate was cooled and neutralized with silver carbonate and worked up in usual manner to yield methylated uronic acid. It gave single spot (*R*f 0.82) insolvent mixture (B), Found:$–OCH_3$, 38.6%, calculated for $C_9H_{18}O_8$, requires, $–OCH_3$, 39.8%. Its amide derivative was prepared by usual manner and on recrystallisation with ethanol and petroleum ether mixture gave crystals of methyl, 2, 3, 4-tri-O-methyl-D-glucopyranoside uronamide, m.p. 183-184 ^{0}C, Lit. m.p. 182-183 ^{0}C [13].

3. Results and Discussion

Moringa oleifera Lam. degraded gum polysaccharide (eq. wt. 656) was methylated by Brown [7] and Purdie's [8] method. Methyl sugar mixture was resolved by partition paper chromatography on Whatman No. 3 MM filter paper sheet. The individual methyl sugars components (Figure-I) were characterized and identified as: 2,4,6-tri-O-methyl-D-galactose; 2,3,4-tri-methyl-D-galactose; 2,4-di-O-methyl-D-galactose and 2,3,4-tri-O-methyl-D-glucuronic acid were present in the molar ratio of 1:6:2:3 moles. The isolation of 4 cleavage fragments from the methylated degraded gum polysaccharide indicated its branched chain character and also demonstrated that all D-galactose and D-glucuronic acid units were of pyranose structure.

On the basis of above finding results, an average repeating unit composed of nine D-galactose residues and three D-glucuronic acid residues which can be built up for degraded gum polysaccharide structure. The isolation of 3 moles of 2,

3, 4-tri-O-methyl-D-glucuronic acid suggested that it formed a part of the aldobiouronic acid units and the constitution of which has already been settled (β-D-glucopyranosyl-uronic acid-D-galactose) in the repeating units of the degraded gum polysaccharide. The 3 aldobiouronic acid occurred as side chain and were linked the main chain of D-galactose through C_1 of their D-galactose moieties, which gave rise to 6 moles of 2, 3, 4-tri-O-methyl-D-galactose in the main chain after methylation and hydrolysis. The isolation of 2 moles of 2, 4-di-O-methyl-D-galactose as a hydrolysis product of the methylated degraded gum polysaccharide indicates that they may be regarded as the parts of the branching point and 2 aldobiouronic acid are linked to C_2 position. The absence of any tetra-methyl sugars in the methanolysis product indicated that the degraded *Moringa oleifera* Lam. gum polysaccharide showed the branching probably started from the non-reducing end of the main polymer chain. Considering all the above aspects (Figure-2) can be proposed as a tentative degraded polysaccharide structure for the repeating unit of the degraded *Moringa oleifera* Lam. gum polysaccharide which accommodates all the known types of linkages as (1→6)-β-type; (1→3)-β-type and (3→1)-α-type in the degraded gum polysaccharide structure.

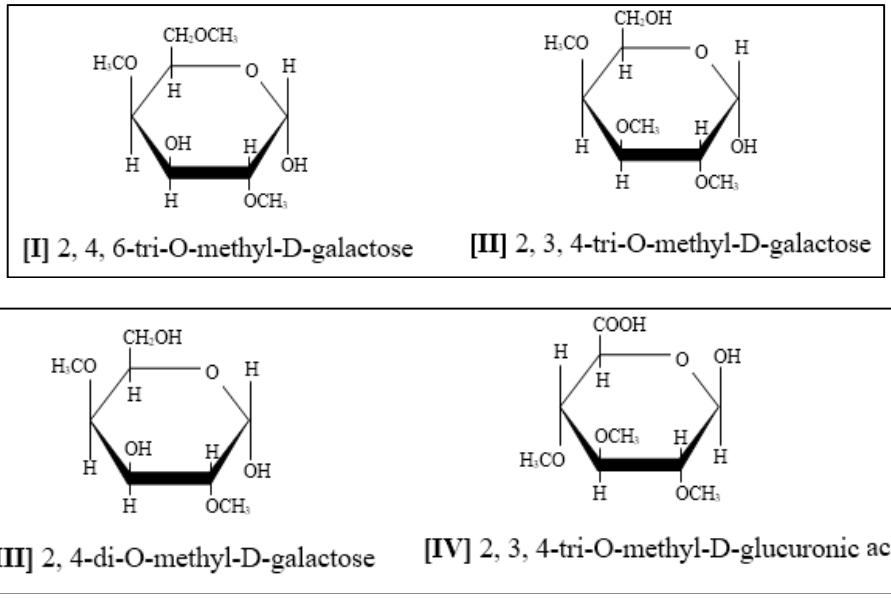

Fig 1: Structure of methylated degraded *Moringa oleifera* Lam. gum polysaccharid

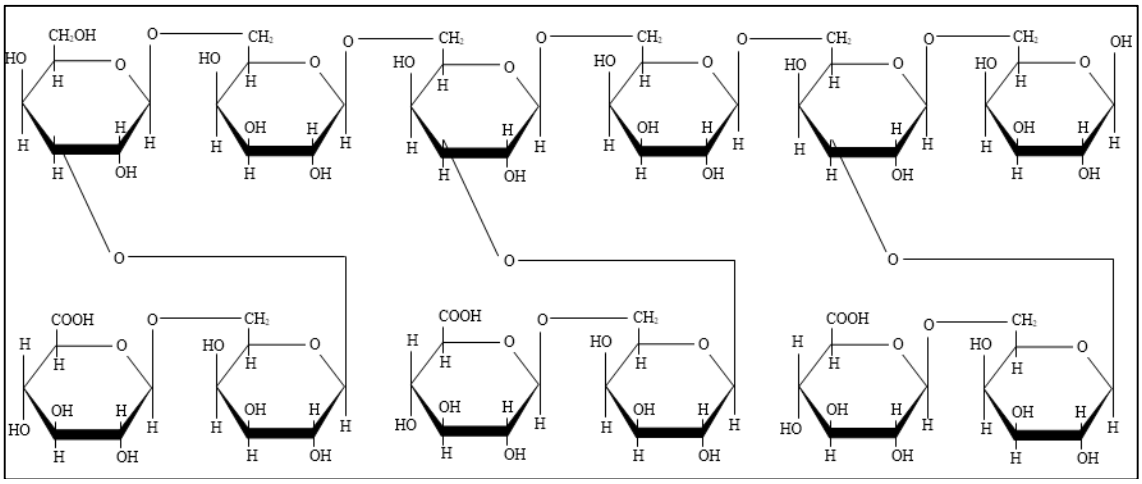

Fig 2: Structure of degraded *Moringa oleifera* Lam. gum polysaccharide

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