**International Journal of Chemistry Studies**
**ISSN: 2581-348X**
**Impact Factor: RJIF 5.44**
**www.chemistryjournal.in**
**Volume 2; Issue 3; May 2018; Page No. 01-04**

# Degraded seeds cake polysaccharide structure from medicinal plant of *Madhuca longifolia* Linn. (Mahua)

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**Abstract**
Degraded seeds cake polysaccharide was isolated from *Madhuca longifolia* Linn. (Mahua) by partial acid hydrolysis and purified by fractional precipitation method then complete acid hydrolysis produced sugars as: D-glucose and L-rhamnose in 7:5 molar ratio on paper chromatogram. Degree of polymerization was found to be 17. Acid hydrolysis of methylated degraded polysaccharide yielded; 2, 3, 6-tri-O-methyl-D-glucose (7 moles) and 3, 4-di-O-methyl-L-rhamnose (5 moles). Periodate oxidation of degraded seeds polysaccharide consumed 2.30 moles of periodate and liberated 0.25 moles of formic acid per mole of anhydrohexose sugar unit. On the basis of above finding methylation and periodate oxidation results a tentative seeds cake polysaccharide structure of *Madhuca longifolia* Linn. has been assigned to the degraded polysaccharide and corresponded to the parent polysaccharide.

**Keywords:** degraded polysaccharide, *Madhuca longifolia* seeds

## Introduction
*Madhuca longifolia* Linn. Plant [1, 2] belongs to Sapotaceae family and known as *Mahua* or *Butternut tree*. It is a medium to large sized tree upto 17m in height and occurs in all over India, Nepal and Sri Lanka. Plants are used in Ayurvedic system of medicine. Seeds oil is used in skin diseases, rheumatism, cooking purposes, making soap and candles. Flowers are used for the precipitation of liquors. Wood is used for making furnitures, sports goods, musical instruments, ship building and railway sleepers. Stem bark is used for tonsil, leprosy, fever, scabies, skin diseases and snake bite. Leaves are applied as a poultice to relief azema. Its seeds cake contains a water soluble polysaccharide as D-glucose and L-rhamnose in the molar ratio of 7:4 as determined from hydrolysed product by column and paper chromatographic analysis. The preliminary investigation were carried out the nature of the constituent sugars [3] from seeds cake, methylation studies [4], periodate oxidation studies [5] with tentative polysaccharide structure and smith degradation studies [6] have already been reported. Present manuscript mainly deals with the isolation of degraded polysaccharide alongwith methylation and periodate oxidation studies and proposed for the detailed seeds cake *Madhuca longifolia* Linn. Degraded polysaccharide structure.

## Materials and Methods
Unless otherwise stated that all evaporations were carried out at 40-45$^0$C under reduced pressure. Optical rotations are in equilibrium values and all melting points are not experimentally corrected. Degree of polymerization was determined by Timell's method [7]. Paper chromatographic analysis were carried out by descending technique [8] on Whatman No. 1 and 3MM filter paper sheets, using upper phase of the following solvent mixture (v/v); (A) *n*-butanol-acetic acid-water (4:1:5) [9]; (B) *n*-butanol-ethanol-water (4:1:5) [10]; (C) benzene-ethanol-water (169:47:15) [11]; (D) butanone-ethyl acetate-water-ammonia (80:20:8:1) [12] and (R) *p*-anisidine phosphate [13] used as spray reagent for detecting the sugars.

### Isolation of degraded polysaccharide
Purified degraded polysaccharide (10 gm) was examined by heating with a sulphuric acid solution (1.5 N, 350 ml) [14] over a boiling water-bath at 100$^0$C for 72 hrs. The hydrolysate was cooled and neutralized with barium carbonate slurry then it concentrated to a thin syrup. The obtained syrup was poured in methanol solution when an insoluble barium salt of the degraded polysaccharide was precipitated out. The methanol solution containing neutral sugars was again concentrated to a syrup which revealed the presence of D-glucose and L-rhamnose. Degraded polysaccharide was obtained in the form of an amorphous powder, yield (7.8 gm), had $[\alpha]_D^{29}$ +75$^0$C ($H_2O$). The degree of polymerization was found to be 17 as determined by the Timell's method [7].

### Acid hydrolysis of degraded polysaccharide
Degraded seeds cake polysaccharide (2 gm) was hydrolysed [14] by heating with sulphuric acid (1 N, 40 ml) over boiling water-bath for 12 hrs. The course of hydrolysis being followed by iodometrically titration [15], consumed 7.6 moles of iodine, as shown in Table-1.

**Table 1:** Variation of iodine consumption during acid hydrolysis of degraded polysaccharide.

| S. No. | Time (hrs) | Volume of iodine solution mixed with hydrolysate (ml) | Volume of Sod. Thiosulphate required for excess iodine (ml) | Iodine consumed (ml) |
|---|---|---|---|---|
| 1. | 0 | 20 | 33.2 | 0.0 |
| 2. | 1 | 20 | 32.1 | 1.1 |
| 3. | 3 | 20 | 29.6 | 1.8 |
| 4. | 5 | 20 | 28.3 | 4.9 |
| 5. | 7 | 20 | 26.7 | 6.5 |
| 6. | 9 | 20 | 25.6 | 7.6 |
| 7. | 10 | 20 | 25.6 | 7.6 |
| 8. | 12 | 20 | 25.6 | 7.6 |

The hydrolysate was neutralized with barium carbonate slurry, filtered and filtrate concentrated to a syrup. Paper chromatographic analysis of the syrup on Whatman No. 1 filter paper sheet gave D-glucose and L-rhamnose sugars in solvent mixture (A) using spray reagent (R) for detecting the sugars.

**Quantitative estimation of degraded polysaccharide**

The degraded seeds cake polysaccharide from *Madhuca longifolia* Linn. was quantitatively separated[10] by paper chromatographic analysis on Whatman No. 3 MM filter paper sheet with solvent mixture (B) and different sugar zones as revealed by central and marginal guide strips were cut out with the help of guide spots and eluted with water according to the Dent's method [16]. These sugars were estimated by alkaline hypoiodite method [17] and obtained D-glucose and L-rhamnose sugars were present in the molar ratio of 7:5 moles respectively.

**Methylation of degraded polysaccharide**

Degraded seeds cake polysaccharide (10 gm) was methylated by dissolving in water (50 ml), dimethyl sulphate solution (75 ml) and sodium hydroxide solution (45 %, 200 ml) was added drop wise with mechanical stirring for 8 hrs by Hakomari's method [18]. The above procedure repeated by three successive treatments to obtained a partially methylated degraded polysaccharide. This methylated compound was again remethylated by dimethyl sulphoxide method [19] in an ice-bath. This reaction mixture after worked up as earlier was extracted with chloroform in a liquid-liquid extractor at pH 8.0 to remove the degraded non-acidic sugars. The aqueous solution was then acidified at pH 3.5 and again extracted with chloroform. The chloroform extracts on concentration yielded a residue (6.8 gm) of partially methylated degraded polysaccharide, Found, $-OCH_3$, 40.2% which showing a slight absorption band at 3500-3600 $cm^{-1}$ in IR-Spectra (KBr) [20].

The above partially methylated degraded polysaccharide was again methylated by Purdie's reagent as methyl iodide, methyl alcohol, silver oxide for three times to furnish a fully methylated product, yield (5.6 gm), Found : $-OCH_3$, 39.9$^0$C, $[\alpha]_D^{26}$ +78.0$^0$C ($CHCl_3$). Its IR-Spectra did not show any peaks for hydroxyl group at 3500-3600 $cm^{-1}$ region absorption band.

**Fractionation of methylated degraded polysaccharide**

Methylated degraded polysaccharide (1.5 gm) was successively extracted with 50ml of petroleum ether (40-60$^0$C) and chloroform mixture (50 ml) containing the increasing proportion of the lalter solvent. The methylated degraded residue at every stage was gently refluxed with the solvent mixture over a boiling water-bath at 100$^0$C for 2 hrs. The following five methylated degraded sugar fractions were obtained out of then 1-3 are oily liquids and 4-5 as a crispy solids and the physical constants of each fractions are given in Table-2.

**Table 2:** Fractionation of methylated degraded polysaccharide**.**

| Fr. No. | State | Solvent Composition (%) | | Yield (gm) | $-OCH_3$ (%) | $[\alpha]_D^{26}$ ($CHCl_3$) |
|---|---|---|---|---|---|---|
| | | Pet. Ether (4060$^0$C) | Chloroform | | | |
| 1. | Oily Liquid | 100 | 00 | 0.0420 | - | - |
| 2. | Oily Liquid | 90 | 10 | 0.0740 | - | - |
| 3. | Oily Liquid | 80 | 20 | 0.0860 | - | - |
| 4. | Crispy Solid | 75 | 25 | 0.6420 | 40.2 | +52$^0$ |
| 5. | Crispy Solid | 70 | 30 | 0.4242 | 31.8 | +19$^0$ |

**Characterization of methylated degraded polysaccharide**

The methylated degraded product from Table-2 (Fr. 4-5) was treated with sulphuric acid (72%, 20 ml)[21] on water-bath for 1 hr. The reaction content was diluted with water to make up a sulphuric acid concentration (12%) and left for overnight at room temperature. The obtained hydrolysate was neutralized with barium carbonate slurry, filtered and filtrate concentrated to a thin syrup. Paper chromatographic examination of the syrup on Whatman No. 1 filter paper sheet using solvent mixture (C) and used (R) as a spray reagent to revealed the presence of two methylated degraded sugars as shown in Table-3.

**Table 3:** Hydrolysis product of methylated degraded polysaccharide.

| Fr. No. | *Rg* value on solvent (D) | *Rf* value in solvent (A) | Probable methylated sugars |
|---|---|---|---|
| 1. | 0.86 | 0.56 | 2,3,6-tri-O-methyl-D-glucose |
| 2. | 0.72 | 0.64 | 3,4-di-O-methyl-L-rhamnose |

**Separation and identification of methylated degraded polysaccharide:**

Methylated degraded polysaccharides were separated by paper chromatography on Whatman No. 3 MM filter paper sheet in solvent mixture (B) and corresponding methyl sugar strips were cut out with the help of guide spots and eluted with water according to the Dent's method [16]. The different methyl sugar fractions were isolated in the form of syrup, which were dissolved in water and decolourised with animal charcoal then it evaporated upto syrup. The eluted methyl sugar components were concentrated to furnish two methyl sugars fractions which were identified as follows

**Fraction-I: 2, 3, 6-tri-O-methyl-D-glucose**

The methyl product (350 mg) in solvent mixture (A) gave a single spot on paper chromatogram, had $[\alpha]_D$ [26] $+52^0$C ($CHCl_3$), *R*f 0.56 in solvent (A) and *Rg* 0.86 in solvent (D). On demethylation [22], the product showed the presence of D-glucose, Found: $-OCH_3$, 40.2%, calculated for $C_9H_{18}O_6$ required, $-OCH_3$, 41.6%. The sugars was identified as 2,3,6-tri-O-methyl-D-glucose by preparing its lactone derivative as 2,3,6-tri-O-methyl-D-glucono-γ-lactone having m.p. & mixed m.p. 145-146$^0$C.

**Fraction-II: 3, 4-di-O-methyl-L-rhamnose**

The syrupy product (520 mg) in solvent mixture (A) gave a single spot on paper chromatogram, had $[\alpha]_D^{26}$ $+19^0$C ($CHCl_3$), *R*f 0.64 in solvent (A) and *R*g 0.72 in solvent (D). Demethylation [22] of the product showed the presence of L-rhamnose, Found : $-OCH_3$, 31.8%, calculated for $C_9H_{16}O_5$ required, $-OCH_3$, 32.2%. This sugar was identified as 3,4-di-O-methyl-L-rhamnose by preparing its lactone derivative as 3,4-di-O-methyl-L-rhamno lactone, having m.p. & mixed m.p. 76-78$^0$C.

**Quantitative estimation of methylated degraded polysaccharide**

The methylated degraded sugar mixture was separated by paper chromatographic examination on Whatman No. 3 MM filter paper sheet and each methyl sugar fractions were quantitatively estimated by alkaline hypoiodite method [17]. Methyl sugars were identified as 2,3,6-tri-O-methyl-D-glucose and 3,4-di-O-methyl-L-rhamnose were present in the molar ratio of 7:5.

**Periodate oxidation of degraded polysaccharide**

Periodate oxidation [23] of the barium salt of degraded polysaccharide was deionised with Duolite C-25 ($H^+$) and Duolite A-45 ($OH^-$) and eluate evaporated to dryness. It (150 mg) was dissolved in water (75 ml) and oxidized with sodium metaperiodate (0.25 M, 200 ml) in dark at 4.5$^0$C for 150 hrs. The reaction was carried out at different time intervals and results are shown in Table-4. In periodate oxidation it consumed 3.20 moles of periodate and liberated 0.25 moles of formic acid per mole of anhydrohexose sugar unit after 150 hrs.

**Table 4:** Periodate oxidation of degraded polysaccharide

| S No. | Sugar Unit | Time (hrs) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 20 | 40 | 60 | 80 | 100 | 120 | 130 | 140 | 150 |
| 1. | Periodate consumption of anhydrohexose sugar unit (moles/mole) | 1.12 | 1.50 | 1.85 | 2.12 | 2.20 | 2.25 | 2.30 | 2.30 | 2.30 |
| 2. | Formic acid liberation of anhydrohexose sugar unit (moles/mole) | 0.07 | 0.12 | 0.15 | 0.19 | 0.22 | 0.24 | 0.25 | 0.25 | 0.25 |

Periodate oxidised degraded polysaccharide product was hydrolysed with sulphuric acid (1 N) and neutralized with barium carbonate slurry, filtered and obtained filtrate was concentrate to a thin syrup. This syrupy product on paper chromatographic examination in solvent mixture (B) and used (R) as spray reagent which did not show any undegraded sugars corresponding to D-glucose and L-rhamnose.

**Results and Discussion**

*Madhuca longifolia* Linn. seeds cake degraded polysaccharide was isolated with partial acid hydrolysis, afforded D-glucose and L-rhamnose in 7:5 molar ratio. The degree of polymerization of degraded polysaccharide showed that the 12 anhydrohexose sugar units was composed of degraded polysaccharide structure. Acid hydrolysis of methylated degraded polysaccharide gave 2,3,6-tri-O-methyl-D-glucose and 3,4-di-O-methyl-L-rhamnose in 7:5 molar ratio. The polymer chain length of the degraded polymer is less (D.P. 15) and the ratio of D-glucose unit of the main chain is not negligible in comparison to the L-rhamnose units. This is also confirmed by the presence of (1→2)-α-type linkages between D-glucose and L-rhamnose and (1→4)-α-type linkages in D-glucose and D-glucose sugar units in the main polymer chain. This suggests that the degraded polysaccharide is represented by a chain of D-glucose and L-rhamnose unit in the main chain. After every 4 repeating units the D-glucose is present as a non-reducing terminal unit in the degraded polysaccharide. The D-glucose and L-rhamnose are linked through (1→2)-α-type position with an average of 12 hexose residues after every repeating unit. The specific rotations of methyl derivatives showed that the inter glycosidic linkages are of (1→2)-α-type. Above finding results a tentative degraded polysaccharide structure of *Madhuca longifolia* Linn. seeds cake polysaccharide may be proposed as shown in Figure-1.

The periodate oxidation studies of the degraded polysaccharide with sodium metaperiodate it consumed 2.30 moles of oxidant with simultaneous liberation of 0.25 moles of formic acid per mole of anhydrohexose sugar unit. In

accordance with the previous data and on the analogy with the structure of degraded polysaccharide, the parentpolysaccharide is composed of the anhydrohexose sugar units with D-glucose and L-rhamnose in 7:4 molar ratio are present in the main polymer chain. The proposed parent seeds cake polysaccharide structure of *Madhuca longifolia* Linn. after methylation and periodate oxidation studies as shown in Figure-2.

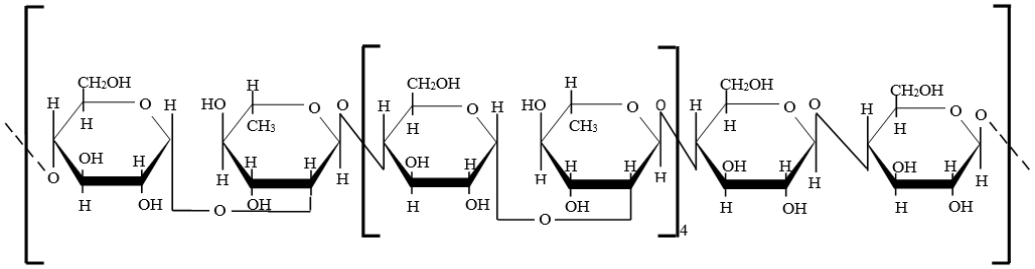

**Fig 1:** Structure of seeds cake degraded polysaccharide from *Madhuca longifolia* Linn. plant

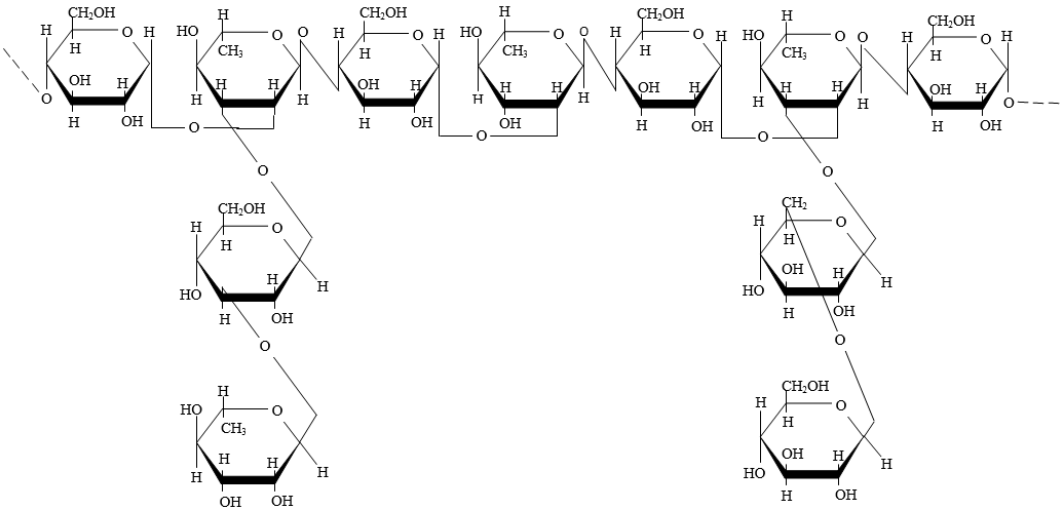

**Fig 2:** Seeds cake polysaccharide structure of *Madhuca longifolia* Linn. plant**.**

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