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Confirmation of seeds cake polysaccharide structure from *Madhuca longifolia* Linn. Plant by periodate oxidation studies

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Abstract
Periodate oxidation is one of the most important reaction in the structural study of the non-ionic seeds polysaccharide. Periodate oxidation was done using with sodium metaperiodate as oxidant. It consumed 3.55 moles of periodate with simultaneous liberation of 1.45 moles of formic acid per mole of anhydrohexose sugar unit after 165 hrs. The isolated water soluble seeds cake polysaccharide as D-glucose and D-mannose were present in 7:4 molar ratio. The proposed polysaccharide structure from *Madhuca longifolia* Linn. seeds have been elucidated with methylation results was confirmed by the periodate oxidation results.

Keywords: Periodate oxidation, *Madhuca longifolia* seeds polysaccharide

Introduction
Madhuca longifolia Linn. Plant [1, 2] belongs to the family- Sapotaceae and commonly known as *Mahua or Butternut tree.* It is a medium to large sized deciduous tree about 17m height and occurs in India, Nepal and Sri Lanka. Plant are used in Ayurveda system of Medicine. Seed oil is used in skin diseases, rheumatism, and headache and also used in the making of soap, cooking purposes and candle. Flowers are used for the preparation of distilled liquors. Wood is used for making furniture, sports goods, musical instruments, ship building and railway sleepers. Stem bark is used in chronic tonsillitis, leprosy, fever and snake bite as antidote, skin diseases. Powdered bake is used for the treatment of scabies. Leaves are applied as a poultice to relieve eczema. In our earlier investigation, the nature of sugar obtained from water soluble seed cake polysaccharide [3], methylation studies for polysaccharide structure [4]. Seeds cake contains a water soluble polysaccharide as D-glucose and L-rhamnose in 7:4 molar ratio from the hydrolysed compound by column and paper chromatographic analysis. Present manuscript mainly deals with the periodate oxidation study for the confirmation of seeds cake polysaccharide structure which was obtained from the methylation studies. The periodate oxidation reaction in the seeds polysaccharide was discovered by Malaprade[5], Fluery & Lange[6] have given periodic acid for the oxidation of glycol. Perlin[7] observed that the periodic acid and lead tetra acetate showed that the glycol groups undergoes cyclic ester formation with oxidants.

Experimental
Upon periodate oxidation [8] of the *Madhuca longifolia* Linn. Purified seeds cake polysaccharide (550 mg) was dissolved in water (50 ml) then added aqueous solution of sodium metaperiodate (0.5M, 30ml) then volume was made upto 250ml with water. The reaction mixture was kept in dark for 150hrs at 4-8^{0}C in refrigerator. Aliquot (5ml) was pipetted out from the reaction flask at different intervals of time then added sodium bicarbonate solution (0.1N, 5ml), sodium arsenite solution (0.01N, 25ml) and potassium iodide solution (0.01N, 5 ml). The reaction mixture was left for 2hrs and then added iodine solution (0.01N, 5ml). It was titrated against sodium thiosulphate solution (0.01N) using starch as an indicator. A blank titration was also carried out in a similar way. The difference between blank and experimental values gives the value of periodate consumption [6] of 3.55 moles per anhydrohexose sugar unit after 165hrs and results are shown in Table-1.
The formic acid liberation [9, 10, 11] from periodate oxidation studies was determined by taking the aliquot (5ml) from reaction mixture in a conical flask and added ethylene glycol (100ml) to

destroy the excess of periodate present in the reaction mixture for 1hr. The formic acid evolved was titrated against sodium hydroxide solution (0.01N) using methyl red dye as an indicator. A blank titration was also carried out in a similar way for the estimation of formic acid. It liberated 1.45 moles of formic acid per mole of anhydrohexose sugar units after 165 hrs and results are given in Table-1.

The periodate oxidized compound was again oxidised in a separate lot under conditions, similar to the above experiments was hydrolysed with sulphuric acid (1N) and worked upto a syrup. This hydrolysed sugar syrup on paper chromatographic examination gave D-glucose and L-rhamnose sugars.

Table 1: Periodate oxidation of *Madhuca longifolia* Linn. seeds cake polysaccharide.

S. No.	Sugar Unit	Time (hrs)									
		20	**55**	**75**	**95**	**110**	**125**	**135**	**150**	**160**	**165**
1.	Periodate consumption per anhydrohexose sugar unit (moles/mole)	1.40	2.50	2.90	3.10	3.25	3.35	3.45	3.55	3.55	3.55
2.	Formic acid liberation per anhydrohexose sugar unit (moles/mole)	0.40	0.75	0.95	1.10	1.25	1.35	1.40	1.45	1.45	1.45

Results and Discussion

Water soluble *Madhuca longifolia* Linn. Seeds cake polysaccharide yielded D-glucose and L-rhamnose in the molar ratio of 7:4. The purified seeds cake polysaccharide was oxidised with sodium metaperiodate by usual manner. It liberated 1.45 moles of formic acid per mole equivalent of polysaccharide with simultaneous consumption of 3.55 moles of periodate for anhydrohexose sugar units of the polymer after 165 hrs. The presence of (1→3)-α-type, (1→3)-β-type, (1→2)-α-type, (1→4)-α-type, (1→6)-β-type linkage are also confirmed by periodate oxidation results. Polysaccharide containing free hydroxyl groups resulted in the consumption of periodate ions during periodate oxidation reaction. It is concluded from the above facts that the probably there is two branching point from the repeating unit of the polysaccharide structure. The formic acid appears is to be originating from reducing as well as non-reducing terminal point unit at the seeds polysaccharide structure.

Periodate oxidation study showed the consumption of 3.55 moles of periodate ions per anhydrohexose sugar units as determined by volumetrically. The probable reaction by which the periodate oxidation of seeds polysaccharide occurs. Periodate oxidation reaction showed that the D-glucopyranose and L-rhamnopyranose units were containing adjacent free hydroxyl groups resulting in the consumption of periodate ions. It is concluded from the above facts that probably two branching points occurs eleven repeating units of the polysaccharide constituting the non-ionic polysaccharide. The periodate consumption indicates that on increasing the time from 150-165 hrs, the consumption of periodate moles become constant (3.55 moles) after 165 hrs. The formic acid appears is to be originating from reducing as well as non-reducing terminal units of the D-glucopyranose and L-rhamnopyranose.

From the above facts that, it may be concluded the terminal D-glucopyranose and L-rhamnopyranose units of the polysaccharide are not substituted. The amount of released formic acid become constant (1.45 moles). Seeds cake polysaccharide structure of *Madhuca longifolia* Linn. was obtained from methylation studies (Figure-1) was also confirmed by the periodate oxidation results.

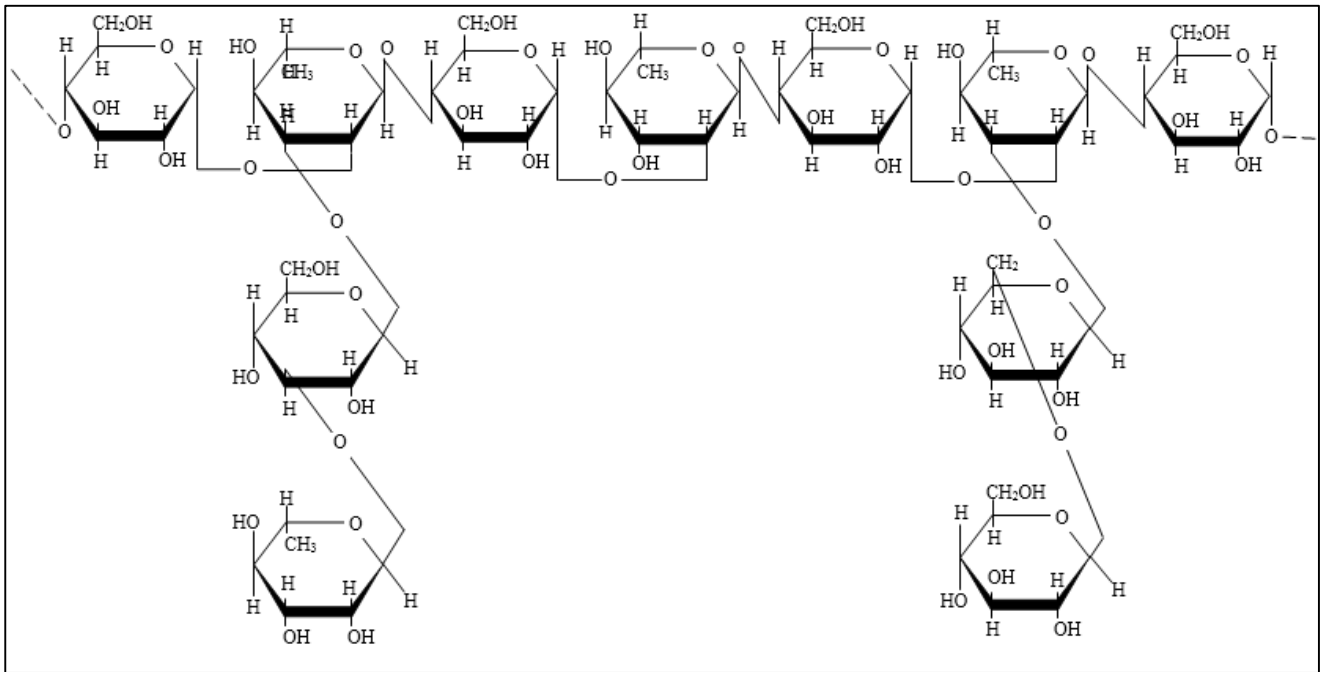

Fig 1: Seeds cake polysaccharide structure from *Madhuca longifolia* Linn. Plant

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