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Structure of aldobiouronic acid and glucuronic acid from *Moringa oleifera* Lam. degraded gum polysaccharide

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Abstract
Moringa oleifera Lam. water soluble gum polysaccharide on acid hydrolysis with sulphuric acid yielded L-arabinose and D-galactose in the molar ratio of 1:4 moles with traces of L-fucose. The components of aldobiouronic acid and glucuronic acid was obtained as methyl ester methyl glycoside for treatment of methanolic hydrogen chloride.

Keywords: aldobiouronic acid, glucuronic acid, *Moringa oleifera* degraded gum polysaccharide

Introduction
In recent years, biased ferrite material for microstrip antenna structures has attracted noticeable attention. Ferrite is one of the important magnetic materials which are used as in both types single and polycrystalline. Some novel characteristics of *Moringa oleifera* Lam. Plant [1] belongs to the family-Moringaceae and commonly called as *Sainjna* upto 10 m in height. It occurs in all over India, Pakistan, Thailand, Sri Lanka, Africa, Nepal, Afghanistan, Mexico, Philippines and America. Plants are used in indigenous system of medicine for the treatment of cardiovascular and gastrointestinal diseases. Gums are used for the treatment of dental infection and blood pressure. Green pods are used as pickles and vegetable purposes. Leaves are rich sources of Vitamin A & C, β-Carotene Protein, Calcium and Potassium contents which are used in scurby and natural antioxidant. Leaves extracts are used for piles, fevers, bronchitis, eyes, ear infection, antitumor and anticancer. Leaves alkaloids Niazi Mian has been proposed to be a potent chemopreventive agent in Carcinogenesis. Seeds are antipyretic, acrid, bitter and seeds oils are used in rheumatism. Seeds extract have also been found to be a effective on hepatic carcinogen metabolizing enzyme and antioxidant parameter and have specific protein fractions for skin and hair cure. Seeds peptide are also used to protects the human skin ageing with dual activity as antipollution and hair conditioning. Gum contains a water soluble polysaccharide [2] as L-arabinose and D-galactose in 1:4 molar ratio with traces of L-fucose by paper chromatographic analysis. Methylation studies were carried out for polysaccharide structure [3]. Degraded polysaccharide structure and periodate oxidation studies [4] for confirmation of gum polysaccharide structure after methylation studies. Present manuscript mainly deals with the graded hydrolysis of degraded gum polysaccharide to obtain the aldobiouronic acid and to study the properties and structure of aldobiouronic acid and glucuronic acid.

Materials and Methods
Moringa oleifera Lam. gum was collected from Forest Research Institute (FRI), Dehradun (Uttarakhand) in the form of clean nodules of yellowish orange colour which are practically free from dirt and bark. Aqueous solution of gum was precipitated with ethanol to form crude polysaccharide [2]. It was purified by precipitating with barium complex [5], filtration and acidification of the aqueous solution of gum with alcohol (Fraction-A). This product had sulphated ash, 0.28%, which was finally purified by cation exchange resin Duolite C-25 and anion exchange resin A-7 [6]. The structure of aldobiouronic acid and D-glucuronic acid was obtained by usual manner [7] from the treatment of graded hydrolysates of degraded gum polysaccharide (Fraction-B).

Results and Discussion
The aqueous solution of *Moringa oleifera* Lam. gum polysaccharide was acidic to undergo slow autohydrolysis [8] which was heated on water-bath. Hydrolysis was completed after 110 hrs of heating as indicated by iodometric titration, which consumed 8.6 moles of iodine by iodometrically. The hydrolysed solution after neutralisation with barium carbonate was filtered and filtrate concentrated to syrup that was exhaustively extracted with methanol. The methanolic extract were concentrated (Fraction-B) and examined by descending technique of paper chromatography [9] on Whatman No. 1 filter paper sheet in the upper phase of the solvent mixture (v/v), (s) *n*-butanol, ethanol, water (4:1:5) [10] and using (R) *p*-anisidine phosphate [11] as spray reagent for the detection of sugars and sugars spot corresponding to D-galactose, L-arabinose and traces of L-fucose were observed.

Autohydrolysis of gum polysaccharide
Moringa oleifera Lam. gum polysaccharide (35gm) was heated with distilled water (800 ml) on water-bath (110 hrs)

and course of hydrolysis was completed iodometrically by usual manner. Auto hydrolysate (2ml) was taken out in a conical flask then added iodine solution (0.1N, 20ml) and sodium hydroxide solution (0.1N, 30ml). The content was acidified with sulphuric acid (1N) and excess iodine was titrated against sodium thiosulphate solution (0.05N). Auto hydrolysate was cooled and neutralised with barium hydroxide solution, filtered and filtrate evaporated to brown syrup. This syrup was 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 extracted with petroleum ether then extract dried in vacuum yield (18gm).

Resolution of Fraction-A by column chromatography

Sugar fraction-A were resolved into its components by column chromatography [12] with cellulose powder, by using the solvent mixture *n*-butanol half saturated with water as eluate [13]. Glass column (14"×1") was washed with eluate and distilled water till the washing becomes colourless. Sugar mixture was added slowly to the column and allowed to absorb the cellulose. Fractions were collected in several test tubes and results are given in Table-1.

Table 1: Resolution of sugars fraction-A by column chromatography

Sr. No.	Fraction No.	*R*f values (S)	Wt. of sugars (gm)	Sugar Present
1.	12-21	-	-	No Sugar
2.	22-28	0.21	0.0090	L-fucose
3.	29-39	0.21 & 0.12	0.4792	L-fucose & L-arabinose
4.	40-61	0.12	0.9124	L-arabinose
5.	62-81	0.12 & 0.07	0.8428	L-arabinose & D-galactose
6.	82-104	0.07	1.4602	D-galactose
7.	105-above	-	-	No sugar

Each sugar fraction was examined by paper chromatography in solvent mixture (A) which showed the presence of L-arabinose and D-galactose with traces of L-fucose.

Graded hydrolysis of degraded gum polysaccharide (Fraction-B) to obtain aldobiouronic acid and glucuronic acid

Barium salt of degraded gum polysaccharide (Fraction-B, Ba-12.2%, 2.5gm) after autohydrolysis of gum was dissolved in sulphuric acid (0.1N, 50ml) and resulting solution was heated on water-bath for 36 hrs. This mixture was neutralized with barium carbonate, filtered and filtrate was evaporated to a syrup which was then extracted with boiling methanol. The methanolic extract after concentration to a syrup was examined by paper chromatography in solvent mixture (S). Subsequently the silver nitrate was used as a spray reagent, showed a strong spot corresponding to D-galactose was observed. It was separated by column chromatography. The methanol insoluble fraction was the barium salt of an aldobiouronic acid (C_{12} H_{19} $O_{12})_2$, corresponding to Ba 16.2%. The barium salt was treated with sulphuric acid (1N) on water-bath (24 hrs) at 100^0C. After hydrolysis, the solution was allowed to cool and then neutralised with barium carbonate then hydrolysate was concentrated under reduced pressure at 40-50^0C, which showed strong spot of D-galactose on paper chromatogram. The methanol insoluble barium salt (Ba28%), barium hexuronic $(C_6H_9O_7)_2$. Barium (Ba26.5%) was treated with sulphuric acid (1N) and filtering out the precipitated barium sulphate. It did not give a brick red colour when treated with basic lead acetate, showing that it was not glucuronic acid [14]. Hexuronic acid was examined by paper chromatography on Whatman No. 1 filter paper sheet in solvent mixture (S) and used (R) as spray reagent to gave two pink spots corresponding to glucuronic acid (strong) and glucurone (faint) spot.

Complete acid hydrolysis of gum polysaccharide

Purified gum polysaccharide (10gm) was hydrolysed [15] with sulphuric acid (1N, 200ml) on water-bath for 40 hrs at 100^0C. After definite intervals of time, hydrolysate (2ml) was taken out and mixed with iodine solution (0.1N, 20ml) in a conical flask. Sodium hydroxide solution (0.1N, 30ml) was then added to it and after keeping for 20 min., acidified with sulphuric acid (1N) and excess iodine was titrated against hypo solution (0.05N) which showed the consumption of iodine (36%). Hydrolysate was neutralized with barium carbonate, filtered and filtrate concentrated to a syrup. It was extracted with methanol and concentrated to syrup, which on paper chromatography in solvent mixture (S) and used (R) as spray reagent on Whatman No. 1 filter paper sheet gave L-arabinose, D-galactose and L-fucose. These sugars mixture were separated by column chromatography. The methanolic insoluble barium salt of uronic acid was deionised to free from Ba by passing through a column of cation exchange resin Duolite C-25 (H^+). Uronic acid was further purified by absorbing it in a column of anion exchange resin Duolite A-101 (OH^-) in acetate form and subsequently eluting the column with formic acid (0.05N). Eluate was concentrated to a syrup identified as D-glucuronic acid by its migration rate on paper chromatogram.

Quantitative hydrolysis of gum polysaccharide

The molar ratio of monosaccharides present in *Moringa oleifera* Lam. gum polysaccharide (0.512gm) was estimated by carrying out quantitative hydrolysis[16] with sulphuric acid (1.5N, 40ml) in a sealed tube for 40 hrs. The liberated sugars were separated by paper chromatography on Whatman No. 3MM filter paper sheet corresponding to different sugars, were eluted with water [17]. Sugars were then estimated by oxidising with sodium metaperiodate and titration the liberated formic acid with sodium hydroxide solution (CO_2

free). The results indicated that two major sugar spots L-arabinose and D-galactose were present in the molar ratio of 1:4 proportion.

Reduction of aldobiouronic acid with lithium aluminium hydride and hydrolysis of disaccharide

Barium salt (1gm) of aldobiouronic acid was refluxed with methanol hydrogen chloride (2%, 10ml) for 8hrs. Reaction mixture was cooled and neutralised with silver carbonate (10% sodium carbonate to 10% silver nitrate), filtering the silver carbonate precipitate and washed with distilled water and methanol then it left for 4hrs. Precipitated silver chloride and unreacted silver carbonate were removed by filtration, washed with methanol and combined filtrate evaporated under reduced pressure to dryness to give methyl glucoside of methyl aldobiouronate.

Reduction of methyl glycosides and methyl ester with lithium aluminium hydride

A portion of resulting methyl ester methyl glycoside (500mg) was dissolved in dry tetra hydro furan (500ml) and solution added drop wise [18] over a period of one hr to a stirred the suspension of Lithium Aluminium Hydride ($LiAlH_4$) in tetrahydrofuran (75ml), The reaction mixture was heated for 30min., cooled and excess Lithium Aluminium Hydride was decomposed by ethyl acetate and water, filtered and filtrate concentrated to a small volume. It was deionised with Duolite C-25 (H^+) cation and A-7 (OH^-) anion exchange resin. Solution was obtained free from salt after anion exchange column and concentrated under reduced pressure at 45-50^{0}C to give a disaccharide (0.3gm).

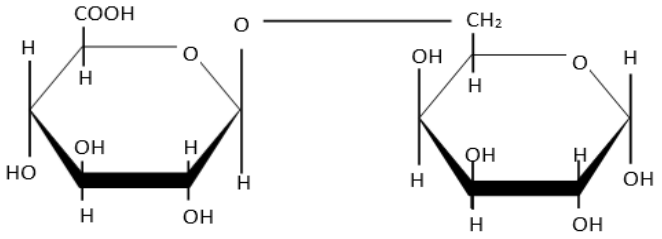

Fig 1: Structure of aldobiouronic acid of *Moringa oleifera* Lam. gum polysaccharide

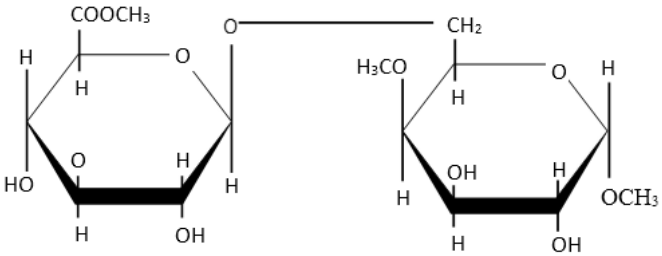

Fig 2: Structure of methyl ester methyl glycoside derivative of aldobiouronic acid of *Moringa oleifera* Lam. gum polysaccharide

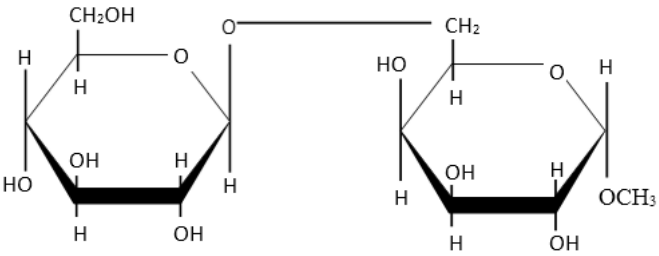

Fig 3: Structure of neutral disaccharide of *Moringa oleifera* Lam. gum polysaccharide

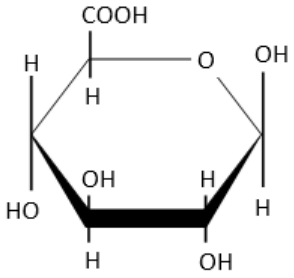

Fig 4: Structure of D-glucuronic acid of *Moringa oleifera* Lam. gum polysaccharide

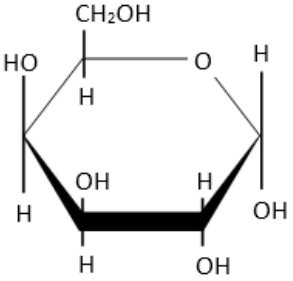

Fig 5: Structure of D-galactose of *Moringa oleifera* Lam. gum polysaccharide

Structure of aldobiouronic acid and glucuronic acid

Degraded *Moringa oleifera* Lam. gum polysaccharide (500mg) on hydrolysis with sulphuric acid (0.1N) which furnished an aldobiouronic acid along with D-galactose. Aldobiouronic acid was purified and homogeneity of sample estimated by paper chromatography when it showed a single spot (Rf 0.08). Pure aldobiouronic acid was found to carry one methoxyl group, when hydrolysed with sulphuric acid (1N) to furnished D-galactose and D-glucuronic acid. This showed that the aldobiouronic acid is built up of D-galactose and D-glucuronic acid moieties. Observation is further confirmed by the reduction of aldobiouronic acid with sodium borohydride to corresponding neutral disaccharide and its subsequent hydrolysis. Aldobiouronic acid (Figure-1) was first converted into methyl ester methyl glycosides (Figure-2) by treatment with methanolic hydrogen chloride, so as to protect the hemiacetal grouping of sodium borohydride. The reduction of

$COOCH_3$ group of uronic acid in methyl ester methyl glycosides derivative of aldobiouronic acid (Figure-2) was easily converted into CH_2OH group. Thus giving rise to neutral disaccharide (Figure-3) which was subsequently hydrolysed for the identification of its component sugar. Since the glycosidic linkages in aldobiouronic acid shows the resistance of hydrolysis. The methyl glycoside of neutral disaccharide obtained was then hydrolysed into the corresponding monosaccharides. Paper chromatography of hydrolysate gave two spots corresponding to D-glucuronic acid (Figure-4) and D-galactose (Figure-5). Since the D-galactose was obtained by hydrolysing the aldobiouronic acid itself, it follows that D-glucose was obtained as a reduction product of uronic acid component. Therefore the uronic acid moiety of aldobiouronic acid (Figure-1) was confirmed to be D-glucuronic acid (Figure-4).

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