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Smith degradation method used for the determination of polyalcohols from periodate oxidised seed polysaccharide of *Quercus incana* Roxb. Plant

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Abstract
Alkali soluble seeds polysaccharide was extracted from *Quercus incana* Roxb. on acid hydrolysis with sulphuric acid and obtained hydrolysate on paper chromatographic analysis led to separation of D-galactose and D-mannose in 1:5 molar ratio. Purified seeds polysaccharide was reduced after periodate oxidation with sodium borohydride and sulphuric acid by Smith degradation method. The obtained hydrolysate produced polyalcohols as glycerol and erythritol in 1:6 molar ratio by paper chromatography. The derivatives of polyalcohols were produced from seeds polysaccharide as glycerol-tri-O-*p*-nitrobenzoate and tetra-O-tosyl-erythritol. The absorbance of polyalcohols were recorded in photoelectrocolorimeter at 480mµ for glycerol and erythritol.

Keywords: Polyalcohols, glycerol, erythritol, *Quercus incana* Roxb. seeds polysaccharide

Introduction
Quercus incana Roxb. Plant [1] belong to Fagaceae family and commonly called as *Banj* or *Phalat,* is a medium to large size tree upto 25m in height and 3m girth. It occurs in Himalayan region of Northern India, Japan, Nepal, U.S.A. and Europe. Plant is medically used in the Indigenous system of medicine for the treatment of diarrhoea, asthma and other human diseases. Ripe seeds are greedily eaten by birds [2] and beasts even before they ripen and their survival is difficult. Wood is used for making furniture, building and agricultural implements and for tool handles. Bark is extensively employed for tanning purposes. Leaves are also used as cattle fodder in hills. Seeds yielded alkali soluble polysaccharide as D-galactose and D-mannose in 1:5 molar ratio [3] on paper chromatogram. In our earlier communications, the nature of seeds polysaccharide [3], methylation studies [4], periodate oxidation studies for the confirmation of seeds polysaccharide structure [5] and structure elucidation of oligosaccharides [6] have already been studied. Present manuscript mainly deals with the determination of polyalcohols from reduction of periodate oxidised seeds polysaccharide by Smith degradation method [7] for the confirmation of proposed alkali soluble seeds polysaccharide structure of *Quercus incana* Roxb. plant. Recently the polyalcohols from seeds polysaccharide were determined from *Wrightia tinctoria* R.Br. (Roxb.) [8], *Withania somnifera* Dunal [9], *Cassia hirsuta* Linn [10], etc.

Materials and Methods
Separation of polyalcohol products
The polyalcohols obtained from alkali soluble *Quercus incana* Roxb. seeds polysaccharide were separated from periodate oxidised hydrolysed compounds by descending technique of paper chromatographic analysis [11] on Whatman No. 3 MM filter paper sheet. The following upper layer of the solvent mixture (v/v) were used as: (A) *n*-butanol-ethanol-water (4:1:5) [12] and (B) ethyl acetate-pyridine-water (2:1:5) [13] for the identification of polyalcohols. The spray reagent (R) acetonical silver nitrate alcoholic sodium hydroxide [14] was applied for the detection of polyalcohols. All evaporation were carried out under reduced pressure (45-50^{0}C) and srupy product yielded glycerol and erythritol on paper chromatogram.

Identification of polyalcohols by Smith degradation of periodate oxidised seeds polysaccharide
Purified alkali soluble seeds polysaccharide (1.5gm) was oxidised [15] with sodium metaperiodate (0.125, 30ml) in dark for 72 hrs in refrigerator. The obtained periodate oxidised compound was treated with ethylene glycol (5 ml) to decompose the excess of periodate and the solution was dialysed against running water for 48 hrs then concentrated to a thin syrup (30 ml). The resulting solution was reduced [16] by mechanical stirring with sodium borohydride (2 gm) at room temperature for 24 hrs. The excess sodium borohydride was acidified with glacial acetic acid (5 ml) and content was dialysed against running water then the solution was evaporated to dryness. The obtained residue was distilled with methyl alcohol to remove the borate ions as methyl borate. The borate free reduced product was again dialysed against running water for 48 hrs to remove the complete inorganic ions. It was concentrated to a thin syrup and further hydrolysed with sulphuric acid (1N, 10 ml) for 12 hrs on boiling water-bath. The hydrolysed product was neutralized with barium carbonate slurry with the help of mechanical stirrer then the reaction mixture left for 24 hrs. It was filtered off and obtained filtrate was deionised by Amberlite ion-exchange resins [17], IR-120 (H^+) and IR-45 (OH^-) then concentrated to a thin syrup.

Characterization of polyalcohols
The hydrolysed product of periodate oxidised alkali soluble *Quercus incana* Roxb. seeds polysaccharide was resolved into its components by descending technique of paper chromatographic separation method on Whatman No. 3 MM filter paper sheets. The solvent mixture (A) and used (R) as

spray reagent to relevant the presence of two spots of polyalcohols corresponding to the glycerol and erythritol. The component sugar strips were cut out with the help of guide spots corresponding to the authentic sample of polyalcohols. It was eluted with water according to the Dent's method [18], after evaporation of syrup which were characterized and identified as glycerol and erythritol as shown in Figure-1.

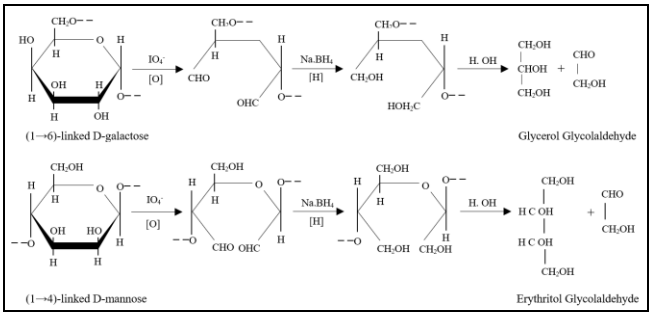

Fig 1: Glycerol

Sugar syrup (250 mg) was dissolved in ethanol (50 ml) and it decolourised with aqueous solution of animal charcoal (50 ml) for 24 hrs then filtered off. The filtrate was concentrated to syrup and it moved a single spot on paper chromatogram corresponding to the authentic sample of glycerol. The derivative was prepared by dissolving the residue (240 mg) in pyridine (5 ml) and *p*-nitrobenzoyl chloride (3 gm) then the content was heated for 1 hr at 70-75^{0}C. The reaction mixture was poured into ice-cold solution of sodium bicarbonate to obtain a precipitate which was filtered off. The filtrate gave crystals of glycerol-tri-O-*p*-nitrobenzoate derivative were obtained on cooling the reaction mixture, which were separated by filtration. It on recrystallization with acetone, had m.p. and mixed m.p. 186-187^{0}C, Lit. m.p. 186-188^{0}C [19].

Fraction-II: Erythritol

Sugar syrup (320 mg) was treated with aqueous solution of animal charcoal (50 ml) for 24 hrs, then filtered and filtrate concentrated to a syrup. It moved a single spot on paper chromatogram corresponding to the authentic sample of erythritol. It was again dissolved in ethanol (5 ml), on cooling the crystals of erythritol was obtained after recrystallization with ethanol then filtrated off. It had m.p. and mixed m.p. 118-119^{0}C, Lit. m.p. 117-118^{0}C [19], 120-122^{0}C [20] and 121^{0}C [21].

Derivative of erythritol syrup (250 mg) was prepared by dissolving it in anhydrous pyridine (5 ml) and *p*-toluene sulphonyl chloride (1.5 gm) at room temperature for 24 hrs. The content was poured into ice-cold water (50 ml) to crystallised out the needle shaped derivative of erythritol. The crystals were washed with water followed by ethanol were dried in air. On recrystallization with acetone and ethanol mixture gave tetra-O-tosyl-erythritol, had m.p. and mixed m.p. 166-167^{0}C, Lit. m.p. 166-168^{0}C [20].

Quantitative estimation of polyalcohols

Polyalcohols obtained from alkali soluble seeds polysaccharide of *Quercus incana* Roxb. were quantitatively estimated by chromotropic acid method [22]. The respective polyalcohols were separated by descending technique of paper chromatographic examination [11] on Whatman No. 3 MM filter paper sheet in upper phase of solvent mixture (B) and used (R) as spray reagent. Polyalcohols components were cut out with the help of guide spots and eluted with water according to the Dent's method [18], producing glycerol and erythritol in 1:6 molar ratio. The colour intensity and absorbance were read at 480 mµ in photoelectrocolorimeter and results are given in Table-1.

Table 1: Absorbance of polyalcohols from *Quercus incana* Roxb. seeds polysaccharide.

S. No.	Amount in micrograms		Klett reading (Absorbance at 480 mµ)	
	Glycerol	Erythritol	Glycerol	Erythritol
1.	2.0	2.0	26	17
2.	4.0	4.0	49	35
3.	6.0	6.0	70	53
4.	8.0	8.0	96	72
5.	10.0	10.0	112	90

Results and Discussion

Quercus incana Roxb. seeds yielded a alkali soluble seeds polysaccharide by usual manner as D-galactose and D-mannose in 1:5 molar ratio on paper chromatogram. Periodate oxidised seeds polysaccharide was reduced with sodium borohydride and sulphuric acid by Smith degradation method. It yielded polyalcohols as glycerol and erythritol in 1:6 molar ratio by paper chromatographic analysis. The large proportion of erythritol was released by acid hydrolysis of polyalcohols, produced by sodium borohydride serves as evidence that the main polymer linkages are of (1→4)-β-type with D-galactopyranose and D-mannopyranose units. The ratio of erythritol to the amount of glycerol was obtained due to the presence of D-galactose at the non-reducing end with (1→6)-α-type linkages in the main polymer chain of the polysaccharide structure. It indicated two branching point on the average of sixty hexose unit in the main polymer chain six hexose unit are in side chain in polysaccharide structure as shown un Figure-2. Derivative of glycerol was obtained by usual manner as glycerol tri-O-*p*-nitrobenzoate while erythritol as tetra-O-tosyl-erythritol. The absorbance of polyalcohols was recorded in photoelectrocolorimeter at 480 mµ for glycerol and erythritol.

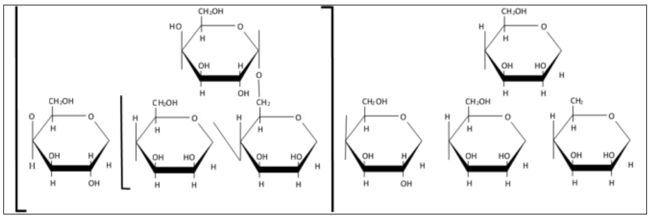

Fig 2: Alkali soluble seeds polysaccharide structure of *Quercus incana* Roxb. plant

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