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ORIGINAL ARTICLE

Identification of Polyalcohols from Periodate Oxidised Water Soluble Seeds Polysaccharide of *Erythrina indica* Lam. Plant by Smith Degradation Method

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ABSTRACT

Water soluble seeds polysaccharide was extracted from Erythrina indica Lam. on acid hydrolysis with sulphuric acid and obtained hydrolysate on paper chromatographic analysis led to separation of D-galactose and D-mannose in 2:3 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 1.98:3.86 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 was recorded in photoelectrocolorimeter at 540mμ for glycerol and erythritol.

Key words: *Polyalcohols, glycerol, erythritol, Erythrina indica Lam. seeds polysaccharide*

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INTRODUCTION

Erythrina indica Lam. plant (Drury, Colonel Heber, 1991 and Santapau, 1966) belong to Family-Papilionaceae and commonly called as *Indian coral tree* or *Pangara,* is a medium to large size tree upto 30 feet in height. It occurs in Himalayan region of Northern and Southern India, Malaysia and Peninsula. Plant is medically used in the Indigenous system of medicine for the treatment of diarrhoea, asthma and other human diseases. Wood is fairly light and used for making furniture, building and agricultural implements, sword sheath and for tool handles. Bark and leaves are used in the case of fever. Plant is often planted as a wind break and exposed to strong winds. It is cultivated as a support for various climbing plants such as Betel leaf, Grape wines, Pumpkin, Jasmine and climbing Rose. Among Hindu the tree is supposed to flowers in Indian garden and leaf with its three leaflets is said to represents the Hindu Trimurti representing middle Vishnu, right Brahma and left Shiva. Seeds yielded water soluble polysaccharide as D-galactose and D-mannose in 2:3 molar ratio on paper chromatogram. In our earlier communications, the nature of water soluble seeds polysaccharide (Singh, 1999), methylation studies (Singh, *et al*., 2000a) periodate oxidation studies for the confirmation of seeds polysaccharide structure (Singh, *et al*., 1998) to obtained the methyl sugars for the determination of proposed polysaccharide structure and structure elucidation of oligosaccharides (Singh, *et al*., 2000b) have already been studied. Present manuscript mainly deals with the identification and determination of polyalcohols from reduction of periodate oxidised seeds polysaccharide by Smith degradation method (Smith 1940) for the confirmation of proposed water soluble seeds polysaccharide structure of *Erythrina indica* Lam. plant.

Recently the polyalcohols from seeds polysaccharide were determined from *Wrightia tinctoria* R.Br. (Roxb.) (Singh, 2014), *Withania somnifera* Dunal(Singh, 2013), *Cassia hirsuta* Linn. (Singh, 2014), etc.

MATERIALS AND METHODS

SEPARATION OF POLYALCOHOL PRODUCTS:

The polyalcohols obtained from water soluble *Erythrina indica* Lam. seeds polysaccharide were separated from periodate oxidised hydrolysed compounds by descending technique of paper chromatographic analysis (Partridge, 1946) on Whatman No. 3 MM filter paper sheet. The following upper phase of the solvent mixture (v/v) were used as : (A) *n*-butanol-ethanol-water (4:1:5) (Partridge and Westall, 1948) and (B) ethyl acetate-pyridine-water (2:1:5) Jermyn M.A. and Isherwood F.A. (1949) for the identification of polyalcohols. The spray reagent (R) acetonical silver nitrate alcoholic sodium hydroxide (Trevelyan, et al., 1950) was applied for the detection of polyalcohols. All evaporation were carried out under reduced pressure (45-50^{0}C) and serupy product yielded glycerol and erythritol on paper chromatogram.

IDENTIFICATION OF POLYALCOHOLS:
(by Smith degradation from periodate oxidised seeds polysaccharide)

Purified water soluble seeds polysaccharide (1.5gm) was oxidised (Abdel, et al., 1952) with sodium metaperiodate (0.125, 30ml) in dark for 72 hrs in refrigerator at 4-8 0C. 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 (Morimoto, *et al.,* 1960) 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 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 (Adams and Halmes, 1955), IR-120 (H^+) and IR-45 (OH^-) then concentrated to a thin syrup.

CHARACTERIZATION OF POLYALCOHOLS:

The hydrolysed product of periodate oxidised water soluble *Erythrina indica* Lam. 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 revealed 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 (Dent, 1947), after evaporation of syrup which were characterized and identified as glycerol and erythritol as shown in Fig. 1.

FRACTION- I: GLYCEROL:

Sugar syrup (290 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 of glycerol was prepared by dissolving the residue (280 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. 189-190^{0}C, Lit. m.p. 190-191^{0}C (Unrau, 1961).

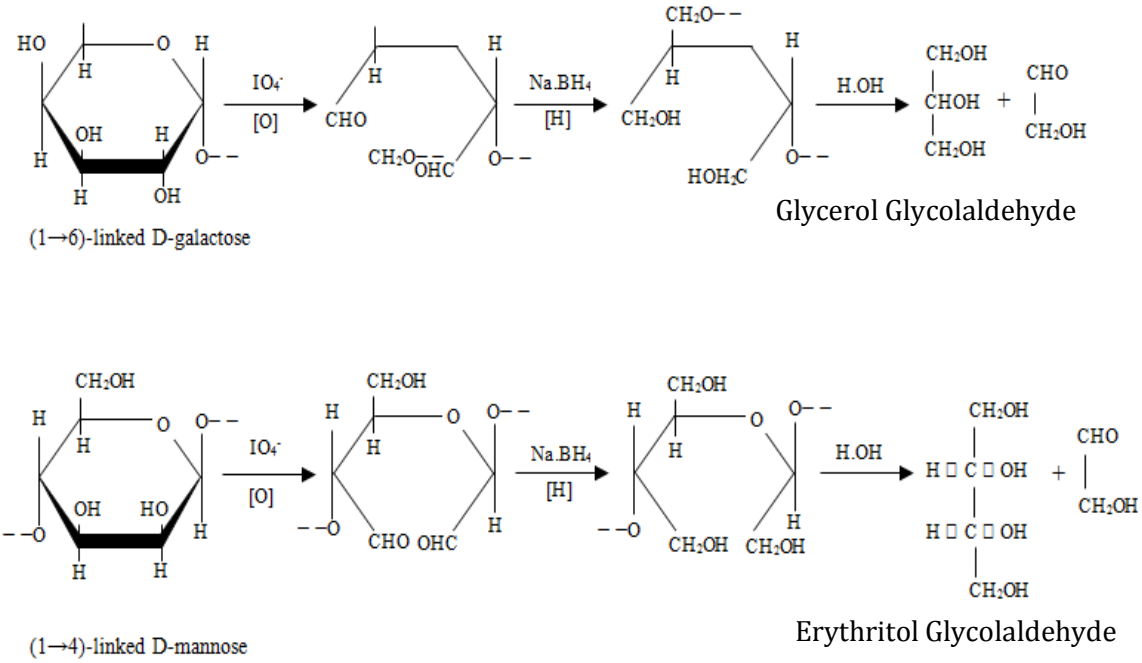

Fig. 1: Smith degradation of polyalcohols from *Erythrina indica* Lam. seeds polysaccharide

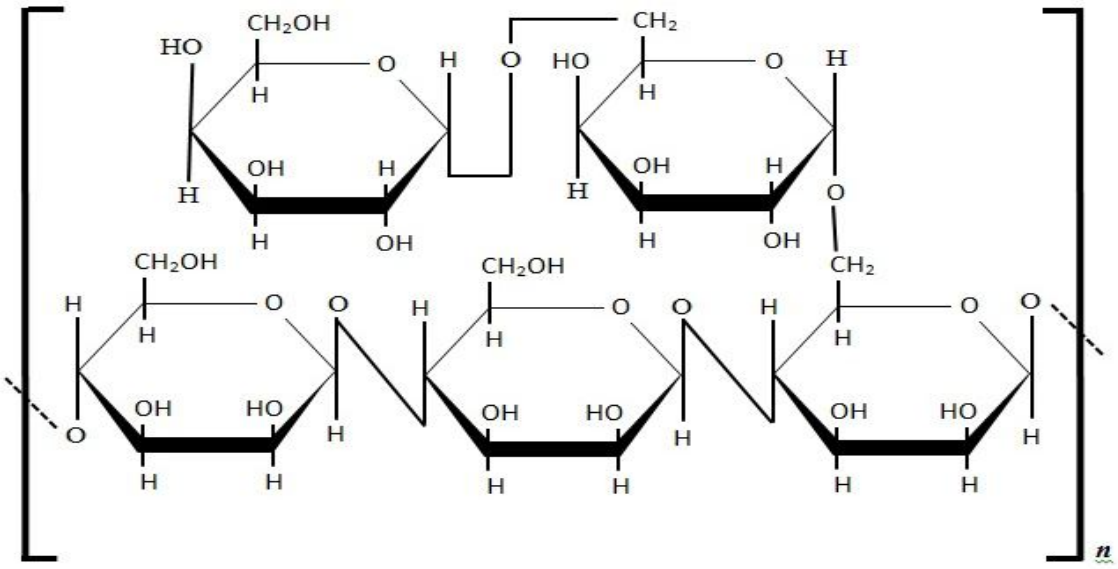

Fig. 2: Water soluble seeds polysaccharide structure of *Erythrina indica* Lam. plant

FRACTION- II: ERYTHRITOL:

Sugar syrup (650 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 (Hamilton and Smith, 1956), 120-122^{0}C (Richards, *et al.,* 1968) and 121^{0}C (Lambert and Neish, 1950).

Derivative of erythritol syrup (280 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 crystallise 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(Hamilton and Smith, 1956).

QUANTITATIVE ESTIMATION OF POLYALCOHOLS

Polyalcohols obtained from water soluble seeds polysaccharide of *Erythrina indica*_Lam. were quantitatively estimated by chromotropic acid method (Lambert and Neish, 1950). The respective polyalcohols were separated by descending technique of paper chromatographic examination (Partridge, 1946) 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 (Dent, 1947), producing glycerol and erythritol in 1.98:3.86 molar ratio. The colour intensity and absorbance were read at 540 mμ in photoelectrocolorimeter and results are given in Table 1.

Table 1: Absorbance of polyalcohols from *Erythrina indica* Lam. seeds polysaccharide

S. No.	Amount in micrograms		Klett reading (Absorbance at 540 mμ)	
	Glycerol	Erythritol	Glycerol	Erythritol
1.	2.0	2.0	27	17
2.	4.0	4.0	53	36
3.	6.0	6.0	76	54
4.	8.0	8.0	99	72
5.	10.0	10.0	114	88

RESULTS AND DISCUSSION

Erythrina indica Lam. seeds yielded a water soluble seeds polysaccharide by usual manner as D-galactose and D-mannose in 2:3 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 1.98:3.86 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 one branching point on the average of five hexoses unit in the main polymer chain two hexose unit are in side chain in polysaccharide structure as shown in 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 540 mμ for glycerol and erythritol. It indicated one branch point on the average of three hexoses units are in the backbone and two hexose units are in the non-reducing end for the support of the earlier proposed water soluble seeds polysaccharide structure of Erythrina indica Lam. plant as shown in Fig. 2.

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