

Original Article

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ISOLATION OF GLYCEROL, ERYTHRITOL AND THRITOL FROM AQUEOUS EXTRACT OF SEED OF WRIGHTIA TINCTORIA R.BR (ROXB.)

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ABSTRACT

Isolation & characterization of polyalcohol from the seed polysaccharide is one of the most complicated & time consuming operation in carbohydrate chemistry. Aim of this study was to identify and characterize the polyalcohol isolated from seed of Wrightia tinctoria R.Br (Roxb). This plant belongs to a family Apocynaceae and is a small deciduous tree growing in several part of India. Wrightia tinctoria is a medicinally important plant & its extracts shows antimicrobial, antidandruff & antisporiasis & there is no seasonal change in protein levels or composition of seed polysaccharide. The unsaponifiable matter (1.42%) consist mostly of sitosterol. The pods without seed contained beta sitosterol; alfa amyrrins, ursolic and oleonic acids. Paper chromatography analysis of hydrolysate on whatmann no. 3 filter paper sheets revealed the presence of glycerol, erythritol & thritol.

It also showed the presence of D-galactose, D-mannose & 3 disaccharide & one trisaccharide. Each Oligosaccharide fraction was separated on whatmann no. 3mm filter paper sheets and then purified, it obtained 5 oligosaccharide out of them 3 disaccharide & one trisaccharide. The methanol extract showed broad spectrum of antimicrobial activity against Escherichia coli, Klebsiella pneumoniae, Aspergillus niger and Trichoderma viridae. The petroleum ether extract showed moderate antimicrobial activity against Bacillus subtilis and Fusarium moniliforme.

Keywords: ISOLATION, GLYCEROL, ERYTHRITOL, THRITOL, AQUEOUS EXTRACT, SEED, WRIGHTIA TINCTORIA R.BR (ROXB.)

INTRODUCTION

The seed of *Wrightia tinctoria* R.Br (Roxb.) belongs to family Apocynaceae .It occurs in particularly in Dehradun, Coastal forest of Coramandel, Mysore, konkan A.P, U.P, Bihar & many parts of India. It is known as Indrajau in Hindi. Plants are generally up to 1.8m tall & often under 60 cm in grits, sometimes up to 7.5 high. The wrightial a new terpene and other phytoconstituents such as cycloartenone, cycloeucaleanol were isolated identified by fractionated of methanol extract of the seed pods of wrightia tinctoria was saponified and non saponifiable matter was fractionated with metahanol gave a colourless substances oleanolic acid.⁵ The five flavonid compounds Indigotin, Indirubin, tryptanthrin, isatin and rutin were isolated and identified from leaves⁶. Flowers are used as vegetables, they are slightly bitter. Leaves are a source of blue indigo called Mysore Pala indigo-0.33-0.50% and have total nitrogen -2% & calcium oxide 3.8% .Seeds are used as an adjuvant to dying material .The seeds , leaves & roots have been shown to contain an indigo-yielding glycosides .

MATERIAL & METHOD

PLANT MATERIAL

The seed of *Wrightia tinctoria* R.Br (Roxb.) belongs to the family –Apocynaceae were collected in the month of September & February from Forest Research Institute Dehradun (Uttarakhand). Strains, including fungi and bacteria were obtained from National Chemical Laboratories (NCL).*Escherichia coli* NCIM 2110, *Staphylococcus aureus* NCIM 2110,*Staphylococcus aureus* NCIM 2079,*Bacillus subtilis* NCIM 2250, *Klebsiella pneumoniae* NCIM 2719 were used as test organism.

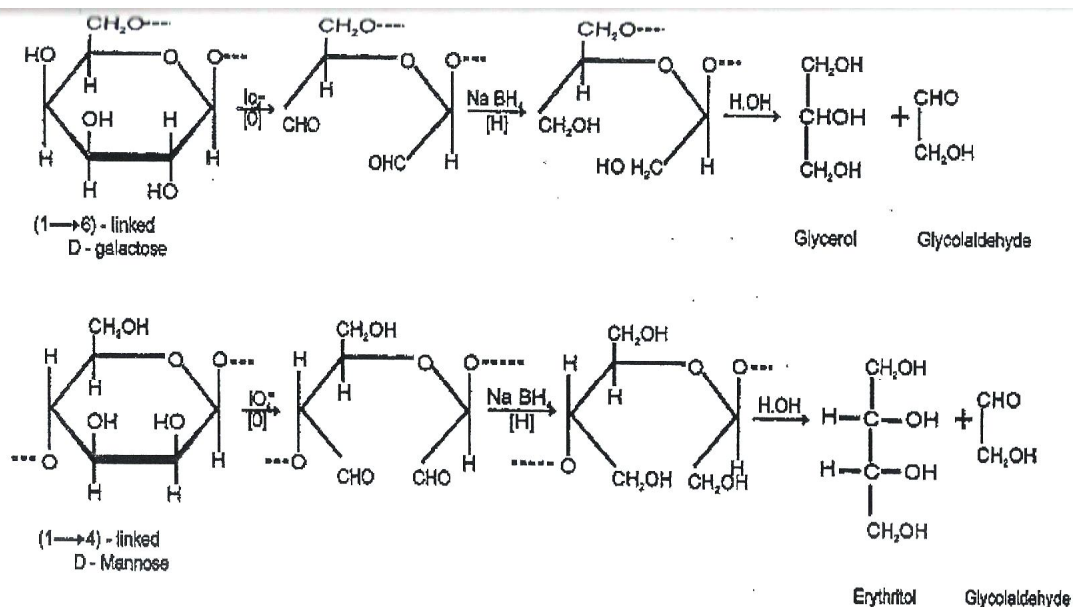
ISOLATION, PURIFICATION OF SEED POLYSACCHARIDE

The Polysaccharide was isolated from seed by extraction with cold distill water & precipitated with C₂H₅OH. The crude polysaccharide was obtained as grayish amorphous powder had sulphated ash 1.60%, optical rotation $[\alpha]_D^{25} +31.2^\circ$ C (H₂O).The crude seed polysaccharide was purified by redissolving in H₂O & fractionation with ethanol to different concentration (20-60%) . The fractions obtained from the 40% & 60 % C₂H₅OH. Concentration were then titrated with absolute alcohol, acetone & ether (3 to 4 times) & then dried over calcium chloride under vacuum at 60°C. These two fractions (40 &60%) of polysaccharide showed the identical homogeneous spectrogram in IR spectrum (KBr), $[\alpha]_D^{25} + 30^\circ$ C (H₂O) sulphated ash 0.82%.

IDENTIFICATION AND CHARACTERIZATION OF POLYALCOHOL

The purified *Wrightia tinctoria* R.Br(Roxb.) seeds polysaccharide (1.5 gm) was oxidized with sodium metaperiodate (0.125 M,30 ml) in dark for 72 hrs at 4-8 °C in refrigerator. The reaction mixture was further reduced with NaBH₄ (1 gm 0 at R.T. for 24 hrs. Excess period ate was removed by ethylene glycol (5 ml) to decompose excess period ate ion. The excess of NaBH₄ was decomposed by dil. CH₃COOH solution was again dialyzed against running water for 24 hrs and then concentrated to syrup. The syrup was hydrolyzed with sulphuric acid.(1N ,100 ml) at 100°C for 12 hrs. The paper chromatographic examination of syrup in solvent mixture n-Butanol-ethanol-water (4:1:5, upper layer) revealed the presence of glycerol, Erythritol, Thritol. The and an unidentified spot

having R_f value more than D-galactose and D-mannose units.



SMITH DEGRADATION OF SUGAR UNITS OF *WRIGHTIA TINCTORIA* R.BR. (ROXB.) SEEDS POLYSACCHARIDE

SMITH DEGRADATION STUDY OF PERIODATE OXIDISED SEED POLYSACCHARIDE

Peroxidised seed polysaccharide was degraded by Smith degradation method. It was then reduced by NaBH_4 & hydrolyzed with H_2SO_4 (1 N) & then hydrolysate was neutralized with BaCO_3 slurry, filtered & filtrate was deionized by Amberlite Ion Exchange resins. The paper chromatographic analysis of hydrolysate on whatmann no. 3MM filter paper sheets revealed the presence of Glycerol, Erythritol, and Thritol. The obtained polyalcohol fraction were purified, identified & estimated as follows.

- (I) Glycerol (1.10 mole) glycerol-tri-o-p-nitrobenzoate, M.P & Mixed Melting Point $187-189^\circ\text{C}$
- (II) Erythritol (4.85 mole) tetra-o-tosyl-erythritol M.P & Mixed Melting Point $165-167^\circ\text{C}$.
- (III) Thritol (0.009 mole & traces) it moved as a single spot on Paper chromatography

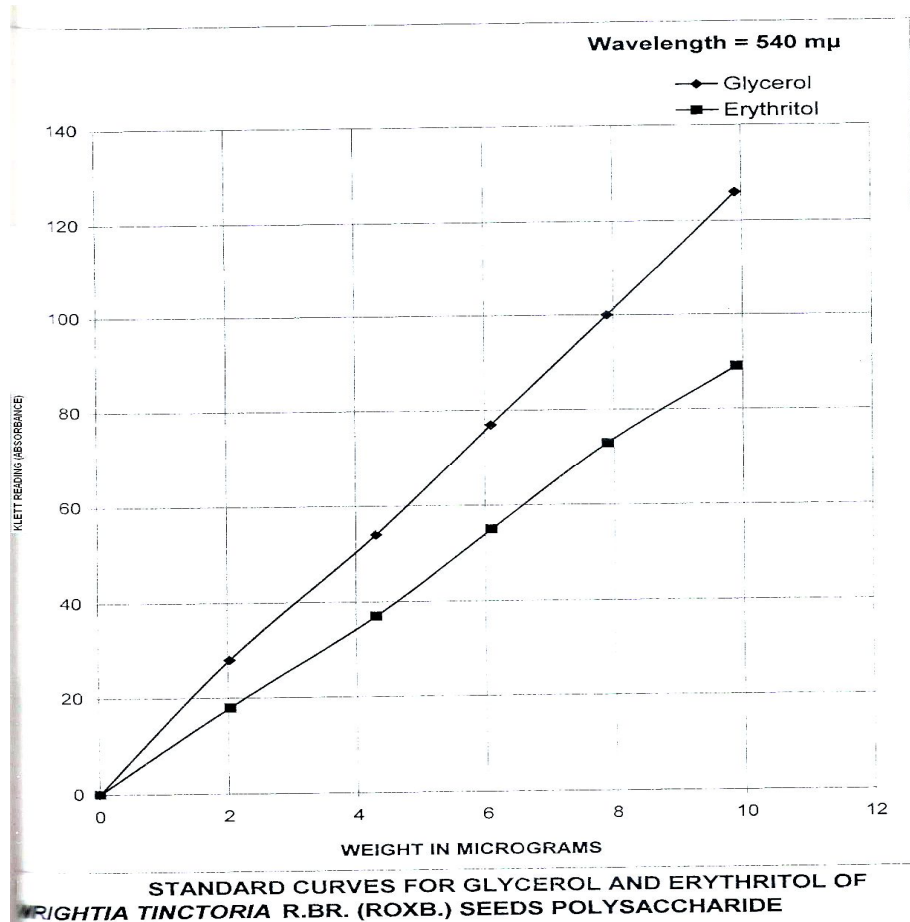
corresponding to thritol & spot is visible in UV light.

QUANTITATIVE ESTIMATION OF PERIODATE DEGRADED PRODUCT (POLYALCOHOLS)

The polyalcohols of Periodate oxidized *Wrightia tinctoria* R.BR (Roxb.) seed polysaccharide was quantitatively estimated by chromotropic acid method on Whatmann. No. 3MM filter paper seeds by descending technique of paper chromatography in solvent mixture n-Butanol acetic acid water (4:1:5 upper layer). A known amount of glycerol was dissolved in water (210 ml), aliquots (1,2,3,4 and 5 ml) were taken in 5 volumetric flask (100 ml) and these were diluted with water (20 ml). To the solution after acidification with sulphuric acid (1N, 1ml) and sodium metaperiodate (0.1 N) was added and shaken well. Volume of each flask was make up to 100 ml with water after 15 min. mixed thoroughly Aliquot (1 ml) was pippered out

from each flask in test tube triplicate and chromotropic acid reagent 10 ml was added with constant mechanical stirring and test tubes were heated in water bath in dark for 45

min. Test tubes were then cooled in water and the intensity of colours were read at 540 m μ . in photoelectrocolorimeter



Erythritol was also dissolved in water (200 ml) and procedure as above thrice time. A standard curves for glycerol and erythritol was plotted in above figure.

ABSORBANCE FOR GLYCEROL & ERYTHRITOL AT DIFFERENT CONCENTRATION

S.No.	Amount in Micrograms	Klett Reading (Absorbance) at 540 m μ
1	2.0	18
2	4.0	37
3	6.0	55
4	8.0	73
5	10.0	89

The mixture of polyalcohol's was separated on whatmann no. 3 MM filter paper sheets by paper chromatography and different polyalcohol's component were cut out with the help of guide spots and eluted with water. The glycerol and erythritol were estimated by periodate chromotropic acid method and their molar ratio are given in below mention table.

MOLAR RATIO OF PERIODATE OXIDISED PRODUCTS OF *WRIGHTIA TINCTORIA* R.BR (ROXB.) SEEDS POLYSACCHARIDE

Fr. No.	Components Polyalcohol	Weight obtained	Molar ratio
1	Glycerol	380mg	1.20
2	Erythritol	850mg	4.95
3	Thritol	50mg	0.009

RESULT & DISCUSSION

Per iodate oxidation studies showed the liberation of 0.1452 moles of formic acid for each an hydro hexose unit with consumption of 1.848 moles of periodate for each an hydro hexose unit of polymer after 75 hrs at 4-8°C in refrigerator. The Oxogalactomanan was degraded by smith degradation method & it on reduction with sodium boro hydride gave a mixture of glycerol, Erythritol, Thritol. The molar ratio of polyalcohol was found to be 1.20:4.95:0.009. The molar ratio of erythritol to that of amount of glycerol indicate one branching point on average after every 8 hexose unit main chain. Purified seed Polysaccharide upon partial acid hydrolysis followed by charcoal celite column chromatography & paper chromatography separation on whatmann no. 3MM filter paper sheet obtained hydrolysate afforded 3-disaccharide & one trisaccharide in authentic form. So on the basis of the above studies i.e. Periodate Oxidation, Smith Degradation, Optical Rotation & Partial acid hydrolysis we led to the conformation of the polyalcohol i.e. Glycerol

,Erythritol, Thritol in Wrightia tinctoria R.Br(Roxb.)

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