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ISOLATION & CHARACTERIZATION OF SUGAR FROM SEED POLYSACCHARIDE OF WRIGHTIA TINCTORIA

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ABSTRACT

Aim of this study was to identify and characterize the sugars from seed polysaccharide of. Wrightia Tinctoria R.Br (Roxb) 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, anti dandruff & antisporiasis & there is no seasonal change in protein levels or composition of seed polysaccharide. The sugars isolated from seed Polysaccharide has not been reported in this species. The seed yield a deep red semi drying oil (yield-30.5% $d^{24}_{0.995}$) with fatty acid composition linoleic -38.8%, oleic -34.0%; myristic 0.1%, palmetic-8.7%, stearic -18.2% and arachidic-5.81%. The unsaponifiable matter (1.42%) consist mostly of sitosterol. The pods without seed contained beta sitosterol; alfa amyryns, ursolic and oleonic acids.

It 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.

Keywords: ISOLATION, SUGAR, SEED, POLYSACCHARIDE, WRIGHTIA TINCTORIA R. BR., (ROXB.)

INTRODUCTION

The seed of Wrightia Tinctoria R.Br (Roxb.) belongs to family Apocyanaceae. 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, cycloeucalenol were isolated identified by fractionated of methanol extract of the seed pods of wrightia tinctoria was saponified and non saponifiable matter was fractionated with methanol gave a colourless substances oleanolic acid.⁵The five flavonoid 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 dyeing 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).

ISOLATION, PURIFICATION OF SEED POLYSACCHARIDE AND PREPARATION OF TEST ORGANISM SUSPENSION

The Polysaccharide was isolated from seed by extraction with cold distilled 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 OF SUGAR BY PAPER CHROMATOGRAPHY

Paper chromatography examination of hydrolysate were carried out by descending technique on whatmann no. 1 filter paper sheet. Solvent mixture used for the detection of sugar was n-Butanol-Ethanol-H₂O (4:1:5), spray reagent was Silver nitrate-sodium hydroxide. Revealed the presence of D-galactose (Rf-0.18); D-mannose (Rf-0.24)

RESOLUTION OF SUGAR MIXTURE BY CELLULOSE COLUMN CHROMATOGRAPHY

The thin syrup of hydrolysate was resolved into its component by cellulose column chromatography using cellulose powder. A column was prepared in a glass tube with (55 x 2) fitted with glass stopper at the bottom from whatmann standard chromatographic cellulose powder (25 gm). The medium sized slurry of

cellulose powder (25 gm) was prepared in n-butanol half saturated with water by blending in sumit blender for 3 minutes. Homogeneity of column was tested by running methyl red dye and the same column was again washed with eluting solvent to remove the dye.

The obtained fractions were examined by paper chromatography on whatmann no. 1 filter paper sheet in solvent n-Butanol-Ethanol-H₂O (4:1:5) and fraction of individual sugar were combined and evaporated to dryness and found to contain the sugar

S.no.	Fraction No.	Sugar present
1	01-42	No Sugar
2	43-61	D-Mannose only
3	62-84	Mixture of Dmannose & Dgalactose
4	85-109	D-Galactose only
5	110-onwards	No sugar

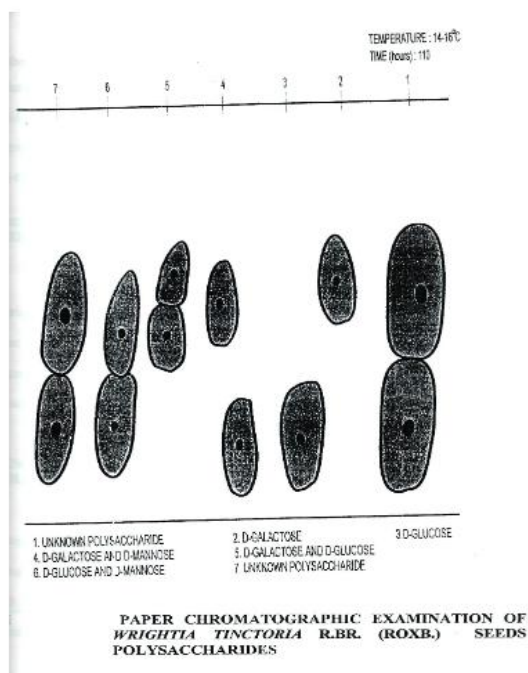
CHARACTERIZATION OF SUGARS

The monosaccharide were characterized and identified as follows

- I D-Galactose, had m.p and mixed m.p 165°-167°c, $[\alpha]^{25}_D +86.9^{\circ}C(0.5 H_2O)$
- II D-mannose, had m.p and mixed m.p. 131-133°c, $[\alpha]^{25}_D +12.6^{\circ}C(0.5 H_2O)$

IDENTIFICATION OF SUGAR FROM WRIGHTIA TINCTORIA R.Br (Roxb.) SEED POLYSACCHARIDE

S.No.	Sugars	Melting point & Mixed Melting Point	$[\alpha]^{25}_D$ H ₂ O	Phenyl Hydrazones Derivative
1	D-Galactose	165-167 ^u c	+86.9 ^u c	169-170 ^u c
2	D-Mannose	131-133 ^u c	+12.6 ^u c	194-195 ^u c



QUANTITATIVE ESTIMATION OF SUGARS BY PAPER CHROMATOGRAPHY

The purified seed polysaccharide (460 mg) of *Wrightia tinctoria* R.Br. (Roxb.) was quantitatively estimated by heating with sulphuric acid(1N,10 ml) in a sealed tube for 24 hrs on boiling water bath 100 °c and processed as usual ,filtered and filtrate was neutralized with barium carbonate slurry then again filtered and filtrate concentrated to a syrup. The hydrolysate was resolved in to its component by paper chromatography on whatmann no. 3MM paper sheet, in solvent mixture n-Butanol-

Ethanol-H₂O (4:1:5) & p-Anisidine hydrochloride reagent. The molar ratio of D-galactose and D-mannose was in the purified seed polysaccharide was found to be 1:3 moles respectively.

RESULT & DISCUSSION

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 we have led to the conformation of sugar D-galactose & D-mannose. Hydrazine derivatives of sugars which were prepared their melting point and optical rotation further supports the conformation of sugars.

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