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Nature of seeds polysaccharide obtained from medicinal plant of *Wrightia tinctoria* R.Br. (Roxb.)

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ABSTRACT

Polysaccharides was isolated from crushed seeds of Wrightia tinctoria R.Br. (Roxb.) with water and precipitation with ethanol to obtained in crude form as D-galactose and D-mannose in 1:3 molar ratio, as determined by alkaline hypoiodite method. Crude polysaccharide consumed 1.36 moles of iodine by iodometrically. The obtained monosaccharides were characterized by paper chromatograph on Whatman No. 1 and 3 MM filter paper sheet and identified by column chromatography using cellulose column. The nature of linkages are of (1→4)-β-type in D-galactopyranose and D-mannopyranose units at main polymer chain while (1→6)-α-type linkages in D-galactopyranose at non-reducing ends which were confirmed by IR-spectra (KBr). Sugars were identified by their melting points, sulphated ash and optical rotation. Derivatives were prepared by usual manner as D-galactose phenyl hydroazone, m.p. 169-171^{0}C, Lit mp. 170-171^{0}C and D-mannose phenyl hydrazone m.p. 194-195^{0}C, Lit m.p. 195-196^{0}C. Absorbance were recorded at 814 and 874 Cm^{-1} region by IR-spectra (KBr.).

Key words: Polysaccharide, D-galactose, D-mannose, *Wrightia tinctoria* seeds.

INTRODUCTION

Wrightia tinctoria R.Br. (Roxb.)[1] plant belongs to the family-Apocynaceae and commonly called as *Indrajau.* It is a medicinal deciduous plant upto 1.8-7.5 cm tall 60 cm in grith and 1200 m altitude in Tropical India. It occurs in Northern, Central India, Peninsular region, Rajasthan, M.P., U.P., Garhwal region, Deccan, Konkan, Western Ghats, Coromandel coast, A.P., Karnataka, Myanmar, Sri Lanka, Nepal, Malaysia and Australia. Medically the bark is used in alkaloidal content, antidysenteric, Hallorrhena drug and possess protolytic activity and amino acid. Seeds and bark are used in flatulence and billions infection while seeds are said to possess. Leaves are pungent and chewed for relief from tooth ache, approdisiac and anthelmentic properties. Alcoholic extract of leaves and roots are demonstrated on cats and possess hypotensive activity. Plant yielded 5% latex rubber and juice of fruits for coagulation milk. Flowers are used as vegetable while leaves are also eaten by cattle, sheep and goats. Flowers are a sources of blue dye indigo called as *Mysore Pala Indigo* and leaves are used as a wrappers for *Bidis.* Present investigation highlights the isolations, purification, preliminary analysis and nature of the constituent sugars of water soluble seeds polysaccharide from *Wrightia tinctoria* R.Br. (Roxb.) plant.

MATERIALS AND METHODS

Powdered seeds (250 gm) were soaked in 800 ml water[2] for 24 hrs, then stirred for 48 hrs by mechanical stirrer. Viscous solution was squeezed through muslin cloth to remove insoluble matter. Solution was then centrifuged through a Sharple's Super Centrifuge[3] to remove finely suspended matter. Centrifugate was precipitated with ethanol (2 litre) by mechanical stirrer to precipitated out the polysaccharide in light brown form. Precipitate of polysaccharide was filtered through a sintered funnel (G-3) under suction, then dried in vacuo at 60^{0}C after washing

with acetone and pet ether (40-60^0). Polysaccharide was obtained as a brownish crude powder (20.28 gm), had sulphated ash 1.60% and optical rotation $[\alpha]_D^{25}$+ 31.2^{0}C (H_2O).

Crude polysaccharide (8.18 gm) was redissolved in water (500 ml) with mechanical stirring for 12 hrs and content filtered then filtrate treated with ethyl alcohol in different stages. Centrifugate was first treated with 30% ethyl alcohol to precipitated out higher molecular weight polysaccharide which were removed by ultracentrifugation method[4]. Colloidal solution of polysaccharide was treated with chloroform to remove the protein in gel form at water-chloroform interface[5]. It was further purified by the addition of Fehling's solution and copper complex was precipitated out by copper complex formation method[6]. Centrifugate was then treated with ethyl alcohol 40% and 60% concentration to precipitated out whole of the polysaccharide, which were triturated with absolute alcohol, acetone and pet. ether (40-60^{0}C), filtered and residue dried over calcium chloride under vacuo at 60^{0}C. These two fractions of polysaccharides was subjected to IR-spectra (KBr)[7], showed an identical homogeneous spectrogram, yield (3.5 gm), sulphated ash 0.82%, $[\alpha]_D^{25}$+ 30^{0}C (H_2O).
Polysaccharide was obtained in light brown powder, had optical rotation $[\alpha]_D^{25}$+ 30.4^{0}C (H_2O) for 40%, sulphated ash (0.92%), while $[\alpha]_D^{25}$+ 30.8^{0}C (H_2O) for 60%, sulphated ash (0.892%). This indicates that these two polysaccharide fractions are in identical and homogeneous was confirmed by IR-Spectra (KBr). It did not reduce the Fehling's solutions, which showed the absence of nitrogen, halogens, sulphur, acetyl groups[8], uronic acid[9] and methoxyl group percentages[10]. The pentosans, pentoses and furfural[11] were present in 1.04%, 0.96% and 0.76% respectively.

Identification of seeds polysaccharide (4 gm) was hydrolysed[12] with sulphuric acid (72%, 10 ml) for 24 hrs. Slurry was cooled and diluted with distilled water (114 ml) to make up a normal solution (1N) with respect to sulphuric acid (72%), carebeing taken that the polysaccharide did not reprecipitated out. Solution was refluxed on boiling water-bath for 14 hrs at 100^{0}C, when the hydrolysate which followed iodometrically[13] was found to be complete. Rate of hydrolysis of polysaccharide with H_2SO_4 (72%) followed by H_2SO_4 (1N) after a definite intervals of time with hydrolysate (1 ml) then added iodine solution (0.1 N, 5 ml) and sodium hydroxide (0.1 N, 10 ml). It was acidified with sulphuric acid and excess iodine was titrated against sodium thiosulphate solution (0.1N) using phenolphthalein as an indicator and it consumed 1.36 moles of iodine after 32 hrs.

Hydrolysate was neutralised with barium carbonate slurry by keeping the contents well stirred during neutralization and kept overnight. Barium sulphate and unreacted barium carbonate were removed from the solution by filtration and residue washed with distilled water. Filtrate was deionised by passing through regenerated Amberlite ion-exchange resins[14], IR-120 (H^+) and IR-45 (OH^-) and concentrated to a thin syrup.

Paper chromatographic examination of the hydrolysate were carried out by descending techniques[15] on Whatman No. 1 filter paper sheet. Solvent mixture (v/v) were used for the detection of sugars as (A) *n*-butanol, ethanol, water (4:1:5, upper phase)[16] and (R) *p*-anisidine phosphate[17] used as a spray reagent to revealed the presence of D-galactose (R_f 0.18) and D-mannose (R_f 0.24).

Hydrolysate syrup was resolved into its components by column chromatography[18] using cellulose powder. A column of Whatman standard chromatographic cellulose powder (25 gm) was prepared in a glass tube (552 cm) fitted with glass. Glass tube was thoroughly cleaned, dried and thin even layer of glass wool was packed at bottom of column. Medium sized cellulose powder slurry (25 gm) was prepared by blending in Sumit blender with eluting solvent *n*-butanol half saturated with water[19] for 3 min. Cellulose was allowed to settle down to a height of 12" deep at bottom. Carebeing taken that the column did not run dry otherwise air bubbles would be formed and packing procedure had to be repeated. Column was washed with eluting solvent, till the washing becomes colourless. Homogeneity of column was tested by methyl red dye and column was again washed with the same eluting solvent to remove the dye completely.

Sugar syrup (2 ml) was introduced on the column with the help of a bent pipette and allowed to drain into the cellulose column by gravity. The eluting solvent (10 ml) was added to washed the sides of column above the cellulose (30 cm). Solvent was allowed to percolate down using a constant head reservoir arrangement and eluates were collected in 10 ml portions manually. The obtained fractions were examined by paper chromatography on Whatman No. 1 filter paper sheet in solvent mixture (A) and sugars fractions were combined and evaporated to dryness and found to contain the sugars fractions are given in Table-1.

Fractions of eluate containing single pure sugars were combined and concentrated to a syrup. Monosaccharides were characterised and identified as : D-galactose, m.p. & mixed m.p. 165-167^{0}C, $[\alpha]_D^{25}$+ 86.9 ^{0}C (H_2O) and D-mannose, m.p. & mixed m.p. 131-133^{0}C, $[\alpha]_D^{25}$+ 12.6^{0}C (H_2O). Derivative was prepared with solution of D-galactose (25 ml) was taken in a conical flask then added glacial acetic acid (10 drops) and phenyl hydrazine (5 drops) were mixed

with periodically shaken. The flask was kept in a boiling water-bath for 15 min., after cooling a bulky yellow precipitate of D-galactose phenyl hydrazone[20] was obtained. It was recystallised with ethanol, had m.p. & mixed m.p. 169-171 ^{0}C, Lit. m.p. 170-1710C[20]. Derivative of D-mannose (25 ml) was also prepared in the same manner. as D-mannose phenyl hydrazone had m.p. & mixed m.p. 194-195^{0}C, Lit. m.p. 195-1960C[20].

Table-1: Resolution of sugars mixture by column chromatography

Sr. No.	Fraction No.	Sugar Present
1.	01-42	No sugar
2.	43-61	D-mannose only
3.	62-84	D-mannose and D-galactose mixture
4.	85-109	D-galactose only
5.	110-Onwards	No sugar

In quantitative estimation[21] of sugars component were present in the purified seeds polysaccharide (560 mg) were estimated by heating with sulphuric acid (1N, 10ml) in a sealed tube for 24 hrs over boiling water-bath at 100^{0}C and filtered. Filtrate was neutralized with barium carbonate slurry, filtered and filtrate concentrated to a syrup. Hydrolysate was resolved into its components by paper chromatography on Whatman No. 3 MM filter paper sheet in solvent mixture (A) and used (R) as spray reagent. Areas of individual sugar components were cut out with the help of a guide spots and sugars were eluted with water according to Dent's method[22]. The eluted sugars were estimated by periodate oxidation method[21] with sodium metaperiodate solution (0.25 M), for necessary corrections, the blank reading were also made. Molar ratio of D-galactose and D-mannose in the purified seeds polysaccharide was found to be 1:3 moles.

RESULTS AND DISCUSSION

Wrightia tinctoria R.Br. (Roxb.) seeds yielded a water soluble polysaccharide as D-galactose and D-mannose in 1:3 molar ratio as determined by alkaline hypoiodite method, thus indicating that the polysaccharide is a galactomannan. It consumed 1.36 moles of iodine by iodometrically. Monosaccharides were characterised and identified by paper chromatography and column chromatography. Since the rotation of parent polysaccharide is a low positive and anomeric linkages are predominantly of β-type possibly with few α-type linkages. Some informations about the nature of linkages was obtained from its IR-spectra (KBr) were recorded on Perkin Elmer Model 137-B spectrophotometer[23]. The absorption bands were recorded at 814 cm^{-1} and 874 cm^{-1} region, which indicated α-type linkages in D-galactopyranose units at non-reducing end while β-type linkages in D-galactopyranose and D-mannopyranose units[24] in the main polymer chain of the *Wrightia tinctoria* R.Br. (Roxb.) seeds polysaccharide. Derivatives of monosaccharides were prepared by usual manner as D-galactose phenyl hydrozone and D-mannose phenyl hydrazone.

REFERENCES

[1] Chadha, Y. R. **(1976),** *The Wealth of India, Raw Materials,* Publication & Information Directorate, CSIR, New Delhi, India-*10 (Sp-W)*: 62-66.
[2] Singh, R. B. and Jindal, V. K., **(1988),** *Egyptian J. Chem., 31 (5)* : 615-620.
[3] Shachmann, H. K., **(1948),** *J. Physc. Chem., 52* :1034-1036.
[4] Wilkil, K. C. B., Jones, J. K. N., Excell, B. J. and Sompel, R. E., **(1957),** *Canadian Journal of Chemistry, 35* : 795-797.
[5] Staub, A. M., **(1965),** *Methods in Carbohydrate Chemistry, 5* : 5-7.
[6] Hirst, E. L. and Jones, J. K. N. and Walder, W.O., **(1945),** *J. Chem. Soc.*,1443-1945.
[7] Rao, C. N. R., **(1963),** *Chemical Application Infrared Spectroscopy,* Academic Press, New York.
[8] Neiderl, J. S. and Neiderl, V., **(1948),** *Method in Quantitative Analysis,* John Wiley & Sons, New York, 252-254.
[9] Anderson, D.M.W., **(1959),** *Talenta, 2* : 73-75.
[10] Belcher, R. and Godbert, A. L., **(1949),** *Semi-micro Quantitative Organic Analysis,* Ballatyne Press, London, 119-120.
[11] Brown, C. A. and Zerban, F. W., **(1941),** *Physical and Chemical Methods in Sugar Analysis,* John Wiley & Sons, New York, 904-906.
[12] Hamilton, J. K. and Partlow, E. G. V., **(1950),** *J. Amer. Chem. Soc., 80* : 4880-4882.
[13] Baker, J. L. and Hulton, H. F. E., **(1920),** *Biochem. J., 14* : 754-575.
[14] Kanin, R., **(1958),** *Ion Exchange Resins,* Champman and Hall, London, *2* : 106-108.
[15] Partridge, S. M., **(1946),** *Nature (London), 158* : 270-272.
[16] Partridge, S. M. and Westall, R. G., **(1948),** *Biochem. J., 42* : 238-240.
[17] Mukherjee, S. and Srivastava, H. C., **(1948),** *Nature* (London), *169* : 330-332.
[18] Kowkobany, G. N., **(1950),** *Advances in Carbohydrate Chemistry, 9* : 304-306.

[19] Chalson, A. J., Nunn, J. H. and Stephens, A. M., **(1956),** *J. Chem. Soc.*, 269-271.
[20] Rafique, C. M. and Smith, F., **(1950),** *J. Amer. Chem. Soc., 72* : 4634-4635.
[21] Hirst, E. L. and Jones, J. K. N., **(1949),** *J. Chem. Soc.* 1959-1963.
[22] Dent, C. E., **(1947),** *Biochem J., 41*: 240-242.
[23] Bellamy, L. J., **(1958),** *The Infrared Spectra of Complex Molecules*, Mathuen, London, *2* : 21-32.
[24] Barker, S. A., Bourne, S. J. and Whiffen, O. H., **(1956),** *Methods of Biochemical Analysis, 3* : 313-315.