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# Studies on a Water Soluble Polysaccharide from *Tinospora cordifolia*

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Polysaccharide from *T. cordifolia* was isolated, purified, hydrolyzed and trimethylsilylated; then subjected to GC-MS studies and found (%) arabinose 0.5, rhamnose 0.2, xylose 0.8, mannose 0.2, galactose 0.3 and glucose 98.0.

**Key words:** ***Tinospora cordifolia*, Menispermaceae, Polysaccharide, Trimethylsilylation.**

## INTRODUCTION

*Tinospora cordifolia* (Willd) Miers ex. Hook f & Thoms (Menispermaceae) is a succulent climbing shrub, indigenous and found distributed throughout most part of India. In spite of the fact that this plant is so extensively used in folk medicine and Ayurveda, the traditional Indian system of medicine, the pharmacological action of the active principle involved has not been worked out. The aqueous extract itself as well as tincture prepared from it are now official in the Indian Pharmacopoeia[1]. Clinical and pharmaceutical studies have revealed that an aqueous extract of the stem helps in reducing the blood sugar in alloxan induced hyperglycaemic rats and rabbits[2]. The aqueous extract was also found to be an effective remedy for different types of rheumatic afflictions[3]. Antipyretic, anti-inflammatory and immuno-stimulating activities of the aqueous extract have been reported[4–6]. As many of the immunostimulants are polysaccharides, the present work is on the study and characterisation of the polysaccharide from *T. cordifolia.*

## EXPERIMENTAL

**Isolation:** Standard procedure was followed for the isolation of the polysaccharide[7]. The shade-dried stem bark of *T. cordifolia* was treated with water at room temperature for 48 h with stirring. From the aqueous extract, the polysaccharide was precipitated by addition of 3 volumes of 95% ethanol. The solution was concentrated below 60°C with rotary flash evaporator under reduced pressure. The precipitate was collected by centrifugation at 20,000 rpm (15 min). The residue was then dissolved in water and dialyzed against distilled water. The collected polysaccharide was dried over fused calcium chloride under reduced

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pressure in a vacuum desiccator. The desiccated polysaccharide was redissolved in water. The associated proteins were removed by the method of Sevag[8]. The solution was shaken with chloroform in a separating funnel till it formed an emulsion/gel which remained in the water-chloroform interface, and was removed. To facilitate the denaturation, a pH 4–5 buffer (0.025 M phosphate buffer) was used instead of water, and small quantities of 1-butanol were added. Since this technique removed only small quantities of protein, it was repeated four times and significant losses of polysaccharide resulted. The clear aqueous layer was again treated with 95% ethanol to precipitate the polysaccharide, dialyzed against distilled water for 48 h in the cold; the solution in the dialysis bag was lyophilized to a colourless powder.

Homogeneity of the polysaccharide was ascertained by purification by gel filtration chromatography on Sephadex G-200. Elution of the polysaccharide fraction was monitored by the absorbance of the eluents at 490 nm using the phenol-sulphuric acid method. The purified sample showed apparent homogeneity by giving single band in zone electrophoresis experiment in polyacrylamide gel. The total carbohydrate content was determined by the phenol-sulphuric acid method using arabinose as standard. The percentage of carbohydrate in the sample was estimated as 76%.

Weight average molecular weight was determined by gel filtration studies on Sephadex G-200 using a series of dextrans of different molecular sizes as reference standards. From the data obtained, the weight average molecular weight was calculated by linear correlation between the logarithm of the molecular weights of the standards and ratios of their elution volumes to the void volume of the column[9]. The weight average molecular weight was calculated to be 40,000 D.

**Hydrolysis:** Complete hydrolysis of the homogeneous polysaccharide was done according to Kram and Franz[10] by 1 M $H_2SO_4$ for 6 h in sealed tube in a boiling water bath. Graded hydrolysis was carried out with 25 μM $H_2SO_4$ at 100°C. Sulphate ions and charged sugars were removed from the hydrolysate by passage through a column of Dowex 50-X4 200–400 mesh ($H^+$ form) coupled to a column of Dowex 1-X8 200–400 mesh (formate form)[11].

Thin layer chromatography and paper chromatography of the hydrolysate after 15, 30, 45, 60, 90 and 120 min indicated the early release of D-glucose followed by D-mannose. These were identified by co-chromatography with authentic samples. The following solvent mixtures were used:

(A) 1-Butanol-ethanol-water[12] 5 : 1 : 4
(B) 1-Butanol-2-propanol-water[13] 11 : 6 : 3
(C) Ethyl acetate-pyridine-water[14] 10 : 4 : 3
(D) Ethyl acetate-pyridine-water[15] 2 : 1 : 2
(E) 1-Butanol-ethanol-water[16] 31 : 11 : 8

Detection of sugar spots was achieved by aniline-diphenylamine-phosphoric acid[17], anthrone and alkaline silver nitrate[18].

**Trimethylsilylation:** 100 mg of freeze-dried polysaccharide was transferred to 13 × 100 mm test tube. 250 μL 1 M HCl in methanol was added and the resulting solution was heated at 80°C for 16 h. This converted the polysaccharide

into a mixture of methyl glycosides. The methanolic HCl was removed by adding 100 μL *t*-butyl alcohol and then evaporating with a stream of air at room temperature[19]. The methyl glycosides were silylated by using 5 mL of anhydrous pyridine (reagent grade pyridine dried over KOH pellets), 1 mL hexamethyldisilazane (HMDS) and 0.5 mL trimethylchlorosilane (TMCS), which was purchased conveniently in these proportions as Tri-Sil (Pierce Chemical Company, USA). The sample was heated to 80°C for 20 min and the silylating agent was gently evaporated at room temperature. The solution became cloudy on addition of trimethylchlorosilane owing to precipitation, presumably of ammonium chloride. No attempt was made to remove this, which in no way interfered with the subsequent gas chromatography. The derivative is redissolved in hexane (10 mL) and insoluble salts are allowed to settle. The supernatant was transferred to a clean test tube and carefully evaporated. The residue was dissolved in 100 μL hexane and 1 μL of this solution was analysed by GC-MS using an SP 2330 Supelco column with myo-inositol as internal standard.

## RESULTS AND DISCUSSION

The relative retention times of the products and their main fragments in the mass spectra are listed in Table-1. Response factor and peak area of the trimethylsilyl derivatives along with myo-inositol, used as internal standard, are given in Table-2. The mole per cent of glycosyl residues were then calculated by dividing the peak areas by the appropriate response factor and the resulting quotients were normalized to 100%[20]. Table-3 shows the mole per cent of glycosyl residues of polysaccharide from *T. cordifolia.*

TABLE-1
RETENTION TIMES ON GC AND MAIN FRAGMENTS IN MS OF TRIMETHYLSILYL DERIVATIVES OF *T. CORDIFOLIA*

| Sugar | Retention time (min) | Main fragments (m/z) |
|---|---|---|
| Arabinose | 11.27 | 59, 73, 133, 147, 204, 217 |
| | 11.50 | 59, 73, 133, 147, 204, 217 |
| Rhamnose | 12.00 | 73, 89, 117, 133, 147, 204, 217 |
| Xylose | 13.78 | 73, 89, 101, 116, 147, 191, 204, 217 |
| | 14.36 | 73, 89, 101, 116, 133, 147, 191, 204, 217 |
| Mannose | 18.03 | 73, 103, 117, 133, 147, 204, 217, 231 |
| Galactose | 19.37 | 73, 89, 103, 117, 133, 147, 204, 217, 242 |
| Glucose | 21.67 | 59, 73, 89, 103, 125, 133, 147, 204, 217 |
| | 22.62 | 59, 73, 89, 117, 129, 133, 147, 204, 217 |
| Myo-inositol | 30.06 | 73, 147, 217, 305, 318 |

TABLE-2
TRIMETHYLSILYL DERIVATIVES OF *T. CORDIFOLIA*

| RES (peak) | Response Factor | Peak area | wt (μg) | mmol CHO/ mg sample |
|---|---|---|---|---|
| Ara | | 813 | | |
| Ara | | 366 | | |
| | 0.476895324 | | 2.786565191 | 37.1294459 |
| Rha | | 802 | | |
| | 0.444900041 | | 2.031844134 | 24.74840601 |
| Xyl | | 1802 | | |
| Xyl | | 0.784 | | |
| | 0.669726642 | | 4.352205683 | 57.99074861 |
| Man | | 822 | | |
| | 0.62878024 | | 1.473504272 | 16.35409847 |
| Gal | | 1134 | | |
| | 0.74630763 | | 1.7126698 | 19.00854384 |
| Glc | | 16560 | | |
| Glc | | 113991 | | |
| | 0.864628336 | | 369.641042 | 4102.564284 |
| μg INOS = | 20 | 17744 | | |
| mg Sample = | 0.5 | | | |
| mg CHO = | 0.381997831 | | | |
| % CHO = | 76.39956621 | | | |

TABLE-3
COMPOSITION OF EQUILIBRIUM MIXTURES AS DETERMINED BY GAS CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES AND GLYCOSYL COMPOSITION OF POLYSACCHARIDE FROM *T. CORDIFOLIA*

| Sugar | α | β | γ | Mol (%) |
|---|---|---|---|---|
| Arabinose | 50.8 | 43.8 | 5.4 | 0.5 |
| Rhamnose | — | — | — | 0.2 |
| Xylose | 41.3 | 55.2 | 3.4 | 0.8 |
| Mannose | 72.0 | 28.0 | — | 0.2 |
| Galatose | 31.9 | 62.6 | 5.4 | 0.3 |
| Glucose | 39.8 | 60.2 | — | 98.0 |

The compositions of the equilibrium anomers calculated[21] on the basis of the retention times and relative areas of the peaks are given in Table-3. This agrees well with the known compositions of the equilibrium solutions established by optical rotations. It is generally agreed that aqueous equilibrium solutions of sugars consist entirely of α and β anomers with small amounts of γ sugars (probably free aldehydes or furanose modifications).

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