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**STRUCTURAL STUDIES OF THE CAPSULAR  
POLYSACCHARIDE FROM *STREPTOCOCCUS PNEUMONIAE*  
TYPE 25F AND  
STUDIES ON GREEN TEA CONSTITUENTS**

A THESIS PRESENTED BY

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**Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 25F and studies on green tea constituents**

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**ABSTRACT**

The thesis consists of two parts, Part I and Part II. Part I deals with the structural studies of the capsular polysaccharide isolated from *Streptococcus pneumoniae* type 25F.

The composition and the mode of linkage of the glycosyl constituents present in the polysaccharide, and in a degraded polysaccharide obtained after treatment with 48% aqueous hydrogen fluoride, were determined using sugar and methylation analysis. Nuclear Magnetic Resonance (NMR) Spectroscopy of the degraded polysaccharide was employed to establish the presence of the following structural units in the repeating unit of the polysaccharide.



The sequence of the sugar residues in the repeating unit, however, could not be established using these methods. Attempts to cleave the polysaccharide using Smith degradation and triflic acid hydrolysis were not successful.

Part II of the thesis describes the extraction and separation of tea constituents in fresh tender tea shoots, using High Speed Countercurrent Chromatography (HSCCC). The ethyl acetate extracts from the tea clones TRI 2023, TRI 2025, TRI 2043, TRI 3079 and TRI 4006 were used for HSCCC separation, and the fractions obtained were analyzed by High Performance Liquid Chromatography (HPLC). Catechins present were identified by comparing the HPLC retention times with those of standard reference samples. It was found that the catechins epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), epicatechin (EG), two other catechins FLA1 and FLA2, and theobromine, were extracted with high purity after a single HSCCC run. The structure of the two catechins FLA1 and FLA2 were elucidated using NMR and Electron Impact Mass Spectroscopy (EI-MS) data. FLA1 was found to be catechin gallate (CG) and FLA2 was identified as epigallocatechin-3-5-di-*O*-gallate.

The antioxidant activity (AOA) of the catechins and some catechin extracts was compared with that of the antioxidants  $\alpha$ -tocopherol (Vitamin E) and Butylated Hydroxyanisole (BHA). The  $\beta$ -carotene bleaching method and the DPPH radical scavenging method were used for this determination. The AOA of EGCG, CG, epigallocatechin-3-5-di-*O*-gallate and catechin extracts containing a high proportion of EGCG was found to be comparable to that of Vitamin E and BHA. The high AOA of epigallocatechin-3-5-di-*O*-gallate has not been reported previously.