# PURIFICATION AND PHYSICOCHEMICAL CHARACTERIZATION OF LECTIN FROM THE SEEDS OF *Treculia Africana* Decne

**BY**

**ADENIRAN, OLUKEMI ADETUTU**
**B.Sc (Hons) (Babcock)**

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**ABSTRACT**

The purification and characterization of the lectin from the seeds of *Treculia africana* Decne were carried out in order to determine some of its physicochemical properties as well as evaluate its toxicity in mice so as to understand the phenomenon of food intolerance caused by lectins.

The protein in *Treculia africana* seeds was extracted by stirring the powdered seeds in phosphate buffered saline for 3 hours, kept overnight, and followed by centrifugation at 3000 rpm. The purification of the hemagglutinating protein was by ion-exchange chromatography on a DEAE-cellulose column followed by gel filtration on a Sephadex G-100 column. The apparent and subunit molecular weight were determined by gel filtration and SDS-polyacrylamide gel electrophoresis respectively. Detection of covalently bound carbohydrate was done by staining the gels after electrophoresis with periodic acid Schiff s reagent. The specificities of the crude extract and the purified protein were defined with human blood groups and sugars. The effects of temperature and pH on the hemagglutinating activity were determined by incubating aliquots of the protein at different temperature and pH values. The metal ions ($Cu^{2+}$, $Fe^{2+}$, $Cr^{2+}$, $Ni^{2+}$. $Zn^{2+}$, $Co^{2+}$, $mg^{2+}$, $Mn^{2+}$ and $Ca^{2+}$) concentrations were determined by Atomic Absorption Spectroscopy. The effect of chelating agent (ethylenediaminetetra acetic acid EDTA) on the metal ions was investigated by dialyzing the protein against 10 mM EDTA and assaying for hemagglutination. Amino acid composition was analyzed with the Technicon sequential multi-sample amino acid analyzer. A 15-day acute toxicity of the lectin on mice was evaluated using standard procedures. Histopathological study was performed on some organs (brain, kidney, liver, lung, spleen and testis) of mice given intraperitoneal doses of the lectin.

The results revealed that phosphate buffered saline extract of the seeds of *Treculia africana* agglutinated human red blood cells

non-specifically. The hemagglutinating activity was inhibited by mannose with minimum inhibitory concentration of 0.8 mM and slightly enhanced by sorbose, dulcitol, glucose, and sorbitol. The purified lectin showed a single band in both denaturing and non-denaturing polyacrylamide gel electrophoresis. The subunit molecular weight as determined by SDS-PAGE and the native molecular weight as determined by gel filtration were 22,000 and 41,000 Daltons respectively. Amino acid composition analysis revealed that the protein contained 155 residues per subunit and was characterized by a high content of arginine, glutamic acid, aspartic acid, proline, cysteine, tyrosine and phenylalanine. Treatment of the protein with chelating agent (EDTA) had no inhibitory effect on the hemaglutinating activity. Analyses of metal ion contents revealed that the protein contained 42.13 mg/ml $Cu^{2+}$, 3.30 mg/ml $Fe^{3+}$, 21.01 mg/ml $Mg^{2+}$, 9.24. mg/ml $Mn^{2+}$ and 3.85 mg/ml $Ca^{2+}$. The protein showed maximum activity over the pH range 3 – 7 and is relatively thermostable up to 50°C. Periodic acid Schiff's reagent staining showed that the protein was non-glycosylated. Although, the lectin was toxic with $LD_{50}$ of 47.211 µg/g body weight of mice causing death instantaneously, there was no visible damaging effect on the organs of the mice following histopathological studies.

In conclusion, the study revealed that seeds of *Treculia africana* contained a lectin that was non-specific in its hemagglutinating property. The lectin exhibited properties and acute toxicity characteristic of ribosome-inactivating proteins (RIPS) with no damaging effect on the organs of the tested animals.