Purification of a coagulant protein from seeds of *Moringa concanensis*

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**ABSTRACT**

A coagulant protein from seeds of *Moringa concanensis* was isolated and purified using CM-Sepharose column chromatography. The column matrix was equilibrated with ammonium acetate buffer and maximum protein was eluted at 0.8 M NaCl. The molecular mass of the purified protein was identified as 14 kDa and its pI value was around 9.5. The purified coagulant protein retained 90% coagulation activity even after incubation at 90°C for 3 h. The purified protein does not release organic content to the water. This paper suggests that coagulant protein from *M. concanensis* can be used in drinking water treatment.

**Key words** | CM-Sepharose, coagulant protein, *Moringa concanensis*, purification, water treatment

**INTRODUCTION**

Water is the most essential resource for survival for a living being. In recent decades, due to development, water pollution has become a serious problem. Hence drinking water treatment, to render it fit for human consumption, is very important. Regular water purification processes use aluminium sulfate and synthetic polyelectrolytes as coagulants. They are expensive and may have negative effects on the environment (Kaggwa *et al.* 2001). Naturally occurring alternatives to conventionally used chemical agents are generally considered to be safe. Such agents include compounds found in plants. There are several reports regarding the use of a natural coagulant from the seeds of *Moringa oleifera* for water treatment (Okuda *et al.* 2001; Ghebremichael *et al.* 2005). In this paper an attempt has been made to isolate and purify the coagulants from seeds of *Moringa concanensis*.

*M. concanensis* (locally known as Kattumurungai) is a small tree with thick bark which is glabrous, except young parts and inflorescence, which belongs to the family Moringaceae. Leaves are bipinnate, pods are linear being 30–45 cm long, sharply three-angled. The appearance of the bark is a distinctive feature of *M. concanensis*, and its leaves and flowers are larger in size than *M. oleifera*. The seed fats of *M. concanensis* have been studied by various researchers for their chemical composition and characterization (Manzoor *et al.* 2007; Ravichandran *et al.* 2009). However, the coagulant protein of *M. concanensis* has not yet been reported. During the search for antimicrobial proteins from seeds of *M. concanensis*, the presence of coagulant protein was identified and this triggered this study of purification and characterization.

The specific objectives of this study include:
1. To purify the natural coagulant present in the seeds of *M. concanensis*.
2. To identify the molecular mass and pI, and to determine optimum temperature for coagulant activity.
3. To check the coagulation effectiveness of the purified protein in removing turbidity.

**MATERIALS AND METHODS**

**Extraction of coagulant protein**

Dried *M. concanensis* were collected from Puliansolai hills, Tamil Nadu, India during the months of April–May and stored at room temperature. The kernels were ground using a kitchen blender. Oil was extracted with 95% (v/v) ethanol
and separated by centrifugation and dried at room temperature. Samples (5% w/v) were prepared from the dried solids using 10 mM ammonium acetate buffer, pH 6.5, stirred for 30 min and filtered through Whatman paper No.3 and 0.45 μm fiberglass. The filtrate was termed as crude extract. Aqueous aluminium sulfate (alum) was prepared as a 5% (w/v) solution.

**Coagulation activity test**

A coagulation activity assay on a clay suspension was performed as described by Ghebremichael et al. (2005). Coagulant (10 μl) was added to 1,000 μl of clay suspension and mixed instantly. After 1 h settling time, absorbance was measured at 500 nm (UV-Visible Spectrophotometer, Shimadzu, Japan). A difference in OD500 between an alum treated (5% w/v solution) sample and a blank (clay suspension without coagulant) was defined as 10 coagulation activity units.

**Protein estimation**

Total protein content was determined by the dye-binding method of Bradford (1976) using bovine serum albumin, fraction V as a standard.

**Purification of a coagulant protein**

The crude extract was saturated with ammonium sulfate (80% w/v) and kept at 4 °C overnight, centrifuged at 5,000 G for 10 min. Precipitate was resuspended in 0.01 M ammonium acetate buffer pH 6.5 and dialyzed overnight against deionized water at 4 °C. The dialyzed sample was loaded on a CM-Sepharose column (bed 30 × 2.5 cm, bead size 45–165 μm, Sigma Chem. Co., USA), pre-equilibrated with 10 mM ammonium acetate buffer (pH 6.5) and proteins were eluted using the same buffer with a linear gradient of NaCl (0.2 to 1.5 M) as described earlier (Sathiyanabam & Balasubramanian 2000). Fractions were analyzed for protein content as well as coagulation activity. The coagulation active fractions were pooled.

**Determination of molecular mass and purity**

Purity and molecular mass were analyzed using 8% SDS-PAGE (Laemmli 1970) stained with silver stain using Blum et al.’s (1987) method. Molecular markers were from Genel Co., Bangalore, India.

**Isoelectric focusing (IEF)**

IEF of purified coagulant protein was done on 4% polyacrylamide gel according to the method of Hayes & Wellner (1969) using Biolyte (BioRad Co., USA) carrier ampholytes ranging from three to 10. After focusing, the gel was stained for proteins with coomassie brilliant blue, using the method of Malik & Berrie (1972). The last lane in the gel was cut into 1 cm pieces and incubated in sterile distilled water for 30 min and pH was measured using a pH meter.

**Estimation of optimum temperature for coagulant activity**

The purified protein was incubated at various temperatures (40–100 °C) for a maximum of 3 h and then analyzed for coagulation activity.

**Organic and nutrient content**

The amount of organic matter released from *M. concanensis* extract to the water was determined in terms of chemical oxygen demand (COD) using Booth’s (1985) method.

Phosphate and nitrate contents in the crude extract, non-adsorbed fractions and purified sample were determined by Booth’s (1985) method.

**Desalting**

To find out whether NaCl present in eluted fractions affect its use for drinking water treatment, they were desalted and analyzed for total dissolved solid (TDS) content.

**RESULTS**

**Protein content and coagulation activity**

Protein content of the crude extract (5% w/v) solution was estimated to be 2.7 mg/mL. Coagulation activity of crude extract was determined as 8.7 units.
CM-Sepharose chromatography

CM-Sepharose bound protein was eluted with 10 mM ammonium acetate buffer, pH 6.5 with a linear gradient of NaCl. Coagulant active fractions (27–33) were eluted at 0.8 M NaCl (Figure 1). Coagulation activity analysis of various fractions indicated that non-adsorbed as well as fractions eluted at 0.4 M NaCl do not contain coagulation activity, whereas proteins eluted at 0.8 M NaCl showed more coagulation activity.

Molecular mass and purity determination

Purified protein eluted at 0.8 M NaCl showed a single band with molecular mass at 14 kDa in SDS-PAGE stained with silver stain indicating its purity (Figure 2). Its pI value was observed to be 9.5.

Temperature optimum for coagulation activity

The purified protein incubated at various temperatures showed its optimum at 55 °C. However they were active even when they are kept at 90 °C for 3 h (Table 1). Nearly 90% of the coagulation activity was observed even when they were incubated up to 90 °C.

Organic content

The main concerns in using crude extract for water treatment are the release of organic matter (Okuda et al. 2001). The purified protein eluted at 0.8 M NaCl did not import...
organic load even at higher dosages than the required amount for maximum turbidity removal (Figure 3).

To check whether the purification step removes nutrient contents, nitrate and phosphate concentrations in various fractions were estimated. Crude extract contains phosphate and nitrate whereas the purified sample contained trace amounts (data not shown).

Since the increase in TDS can influence the use of coagulant protein, it is important to check whether the salt content (used during elution) would affect its use for drinking water treatment applications. At the optimum coagulation dosage, coagulation protein increased the TDS content of the water by less than 24 mg/L.

**DISCUSSION**

Coagulants play an important role in the treatment of drinking water, wastewater and sludge removal. A number of researchers have reported the presence of coagulant protein in seeds of *M. oleifera*, *Vigna unguiculata*, *Phaseolus vulgaris*, *Zea mays*, *Strychnos potatarum* and their use in water treatment (Okuda et al. 1999; Raghuwanshi et al. 2002; Ghebremichael et al. 2006; Sciban et al. 2006; Gunaratna et al. 2007; Yin 2010). In this study we have isolated the coagulant protein from the seeds of *M. concanensis*. To our knowledge, this is the first report on the presence and purification of coagulant protein from *M. concanensis*. The coagulant protein content present in *M. concanensis* (8.7 units) was higher than in *M. oleifera* (7.7 units) reported by Ghebremichael et al. (2006).

Crude protein extract passed through CM-Sepharose equilibrated with 10 mM ammonium acetate buffer pH 6.5 showed more adsorption of coagulant protein (Figure 1). The adsorbed proteins were eluted with increasing concentration of NaCl. Proteins eluted at 0.8 M NaCl (fractions 27–33) showed coagulation activity (Figure 1). Ghebremichael et al. 2006 reported that effective desorption of coagulant protein from a CM-Sepharose column were at 0.6 M NaCl. The purified coagulant active fractions run on SDS-PAGE showed a single band at 14 kDa (Figure 2). The pI value of the purified sample was identified as 9.5. The purified sample does not show any increase in COD (Figure 3) which indicated that there is no release of organic matter during incubation. The purified sample contains a negligible amount of phosphates and nitrates which indicate that the purification step removed the nutrient contents.

To check whether NaCl present in eluted fractions affect its use for drinking water treatment, eluted fractions were desalted and analyzed for TDS content. The desalted fractions showed no increase in TDS. The fractions containing NaCl increased TDS content of water by less than 24 mg/L. According to the World Health Organization (WHO), TDS content of drinking water is 1,000 mg/L (WHO 1996). Hence desalting is not necessary and it can be used directly after a purification step for drinking water treatment. The purification method must be scaled up to get a larger amount of coagulant protein from *M. concanensis*.

The purified coagulant protein from *M. concanensis* retained 90% coagulation activity even after incubation at 90°C for 3 h (Table 1). The high thermo stability of the *M. concanensis* coagulant protein makes it attractive as a flocculent and provides the possibility of using this natural coagulant at any temperature. Hence it could be possible to use the natural coagulant in different climate conditions.

Seeds of *M. concanensis* contain a high amount of water soluble and highly cationic proteins with water clarifying activity analogous to aluminium sulfate (alum). The provision of safe drinking water in many parts of the developing world heavily depends on alum which can be substituted for by this natural coagulant. The present study indicates that *M. concanensis* seed extracts can be used in primary water treatment. It could be advantageous to use...
a natural coagulant as an environmentally friendly alternative in drinking water treatment.

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