Screening and evaluation of natural coagulants for water treatment

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Abstract In developing countries many people are forced to drink turbid water and as a consequence many children are dying related to water borne diseases. Hence there is a need for inexpensive and easy methods to purify drinking water. The objective of this research is to screen different plant seeds to find a primary natural coagulant able to reduce the turbidity of the drinking water. The protein from 21 different seeds was extracted with water and salt solution and coagulation properties in synthetic clay solution were studied. The crude extract of Moringa oleifera (MO) showed the same coagulation activity as that of alum. The protein from red bean, sugar maize and red maize were promising in having coagulation activity, compared with a known coagulant protein from MO. These seeds were selected and purified to homogeneity with fast flow Sepharose ion exchange chromatography and the properties of the purified proteins were characterized. The characteristics of these coagulant proteins are different from MO protein based on molecular weight and ionic determinations. The coagulant proteins were temperature tolerant and can withstand temperature of around 85 °C and maintain the coagulation activity. The seeds identified as a coagulant source could be used as an alternative to chemicals for drinking water clarification.

Keywords Cereals; coagulant protein; fruit seeds; pulses; water treatment

Introduction

The treatment of water to render it fit for human consumption has become a problem of central importance, both in developing and developed countries. In developing countries, the quality of drinking water is often insufficient and hazardous to health. In developed countries, water purification processes use chemicals, despite the fact that their safety for health during long-term exposure and impact on the environment remain under question (Miller et al., 1984; Martyn et al., 1989; Biosvert et al., 1997; Nalm et al., 1998). As a result, it is desirable to find sustainable alternatives that are harmless to human health and to the environment.

Water treatment usually comprises water clarification and disinfection. The turbidity of water often results from the presence of negatively charged particles in a colloidal structure, the clarification of which requires acceleration of the sedimentation rate. For this purpose, positively charged agents are used to form complexes with negative charges of the colloid, in a process called coagulation. In developed countries, salts of aluminium and other metals are often used (Biosvert et al., 1997; Nalm et al., 1998) despite the concern that they may induce Alzheimer’s and other disease (Miller et al., 1984; Martyn et al., 1989). Recently, there have been many reports of the possible link between high levels of residual aluminium and several medical disorders and this initiated a global interest in the search for a substitute coagulant that will be safer for health.

Naturally occurring alternatives to currently used coagulants and disinfectant have been considered, including cultivated plants. Of particular interest are the seeds of a
tropical tree, *Moringa oleifera* (MO), as they contain an active coagulating protein traditionally used for the clarification of drinking water in the rural areas of Sudan and Malawi (Okuda *et al.*, 2001a, b).

In recent years, many processed natural coagulants have been discovered and studied in India and Africa. The coagulation effect of MO seed on turbid water has been reported by a number of researchers (Jahn, 1988; Sutherland *et al.*, 1990; Muyibi and Evison, 1996; Nkhata, 2001). It has also been indicated that MO seed has anti-microbial effect (Olsen, 1987; Broin *et al.*, 2002). Although it has high coagulation activity, it is effective only for highly turbid water. Another disadvantage with MO extract for water treatment is that it increases the concentration of dissolved organic carbon (DOC) in the water because DOC is regarded as a source of odor, color, taste and a precursor of disinfection by products in drinking water treatment. It would be better to remove the organic content from the seed extracts in order to use them in water treatment. Therefore we have developed a simple purification method for MO protein (Babcock and Singer, 1979; Bull and Kopfler, 1991; Ndabigengesere and Narasiah, 1998; Kebreab *et al.*, 2005). When using natural coagulants from MO seeds, the area needed for plantations and the method for harvesting and processing the seeds must also be considered. Hence it is important to find out the alternatives of using low cost and locally available natural material for water treatment. Hence the screening of natural coagulants from other sources would increase interest in finding a suitable coagulant for drinking water treatment. The present study focused on the screening of natural coagulants from fruit seeds, nuts, cereals and pulses. The coagulant activity was compared with MO extract; comparing the salt and water extracts; effect of temperature and characterization of coagulant protein. The alternatives to treat drinking water with environment friendly substitute to commonly used coagulant agents are discussed.

**Materials and methods**

**Plant materials**

The seed sugar maize, Indian maize and red maize were purchased from the botanical garden store. Fruits like yellow passion fruit, purple passion fruit, lychee, longan, tamarind, durina and pulses such as red bean, linus, cowpea, horse bean, black gram, white pea, elephant bean and green gram were purchased from a supermarket. Nuts such as sunflower, walnut, peanut, hazelnut were purchased from a pet store. The moringa seeds used as a positive control were purchased from Senegal.

**Water sample**

The water sample for the coagulation activity tests was prepared as 1% kaolin suspension using tap water. The suspension was stirred for 30 min and settled for 24 h.

**Coagulant extraction**

The initial step is the screening of different seeds for active coagulant proteins. The seeds were removed from the dried pods or from fruits, ground to fine powder. Oil was first extracted from the powder with 95% ethanol. Then a 5% (w/v) solution was prepared from the dried cake solids using distilled water or 0.5 mol L$^{-1}$ sodium chloride (NaCl). The solution was filtered through 0.45 μm fiberglass filter. The filtrate is termed crude water extract (CWE) and crude salt extract (CSE).

**Coagulation activity**

The coagulation activity test was carried out as described by Kebreab *et al.*, 2005 using an assay of 1 mL sample volume. Crude extracts (10 μL) were added to high turbidity clay suspension (250–300 NTU) in a semi-micro plastic cuvette (10 × 4 × 45 mm, Sarsted
Aktiengesellschaft & Co, Germany) and mixed instantly. This was allowed to settle for 1 h and thereafter absorbance was measured at 500 nm using a UV-Visible spectrophotometer (Cary 50 Bio). The activity was also measured in samples diluted 3, 9 and 27 times.

Desalting
Salt extract samples contains NaCl which may disturb purification and SDS-PAGE separation. The salt extracts were desalted before purification and characterization using a PD-10 column. The column was rinsed and equilibrated with milli-Q water. One millilitre of sample was loaded on to the column. The fractions (1 mL) were collected and the absorbance at 280 nm was measured.

Purification and characterization
Samples were selected based on the activity test for the purification of the active component. The selected samples were purified using a high trap CM FF 1 mL column cation exchanger in Äkta explorer (Pharmacia Biotech) as described by Kebreab et al., 2005. A flow rate of 2 mL min⁻¹ was applied and 1 mL samples were collected with an automatic fraction collector. The column was equilibrated with 10 mmol L⁻¹ ammonium acetate buffer (pH 6.7). The bound proteins were eluted from the column with a gradient of NaCl (0.1 – 1 mol L⁻¹). Coagulation activity was measured for each fraction and the active fractions were collected for further analysis.

Native PAGE was carried out according to Hultmark et al. (1983) using a Mini-PROTEAN 2 apparatus (Bio-Rad). After electrophoresis, the 0.5 cm gel pieces were cut horizontally and the protein was eluted into either milli-Q water or 50 mmol L⁻¹ phosphate buffer. The coagulation activity of each fraction was measured. Protein content was estimated by the dye-binding method (Bradford, 1976) with bovine serum albumin as a standard. The protein profile and the molecular mass of the partially purified protein were determined by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were stained with Coomassie brilliant blue and silver staining method depending on the concentration of the protein loaded on the gel. Thermal resistance of the coagulant protein was studied by incubating crude extracts at temperatures of 85°C and 95°C for 30 min or 60 min. The samples were removed and centrifuged at 10,000 rpm for 10 min and tested for coagulation activity.

Results and discussion
The screening of coagulation activity was performed in fruits, cereals and pulses. This screening was initially performed with 21 different seed extracts and compared with the known coagulant protein from MO. The steps involved in coagulant protein extraction and characterization are given in Figure 1.

Figure 1 Steps involved in coagulant protein extraction and characterization. Crude water extract (CWE); crude salt extract (CSE); crude buffer extract (CBE)

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Coagulation activity

The crude water extracts (CWE) and crude salt extracts (CSE) were examined for coagulation activity. It was observed that walnut, yellow passion fruit and purple passion fruit have coagulant activity by reduction in turbidity. Yellow passion fruit and sugar maize had similar coagulation activity to MO (Figure 2a). The nuts have lower activity compared to fruit seeds except walnut where the CSE had higher activity than purple passion fruit. Different maize varieties showed similar activities and are comparable to MO. The percentage of coagulation activity of green gram and black gram were lower than that of MO (Figure 2b). Moreover, comparing salt and water extracts, it is clear that all the salt extracts had more coagulation activity than water extract. The possibility of having high coagulation activity could be explained by the fact that salt is thought to associate with opposite charged groups in the protein. Removal of the salt or diluting the sample to a lower concentration will usually result in the recovery of a native form of protein.

Purification and characterization

Based on the activity tests in crude extracts and with dilutions, red bean, sugar maize and red maize was selected for further characterization of the active protein. The desalting method affects the coagulation activity and dilutes the sample. Hence in the following sections the extract was made directly with 10 mmol L\(^{-1}\) ammonium acetate buffer (Crude Buffer Extract, CBE) which is not affecting the activity and gives similar activity as salt extract. The advantage of using ammonium acetate buffer is that the buffer strength for purification of the active component need not to be adjusted.

The procedure for purifying MO coagulant protein was used in order to study the similarity of the coagulant proteins from the selected seed extracts. The red bean crude extract was bound to the cation exchange matrix and the bound protein was eluted with increasing concentration of NaCl gradient. The unbound fractions had lower activity compared to bound fractions. Based on SDS-PAGE, 2 major bands were observed with different molar concentrations of NaCl. Results from this experiment show that it’s possible to purify the coagulant protein from red bean following a similar method to that used for MO. The purification of sugar maize and red maize protein with the same method was not successful. All the active proteins were in the unbound fraction. The possible reason could be that even though the protein has similar coagulation activity, the nature or the structure of the protein might not be same.
The coagulation activity on native gel fractions showed the ionic properties of the coagulant protein. The ionic charge of the active coagulant protein in red bean is positive, which was also confirmed by purification. The active component from sugar maize was observed on the middle region on acidic gel. In contradiction, the active component from red maize stayed on the top on the acidic gel. This is also very well shown based on determination of molecular mass on SDS-PAGE. The MO has a molecular mass of 6 kDa whereas the sugar maize and red maize has higher molecular weight proteins. Even though red bean has similar properties to MO protein, the molecular mass is higher than MO protein. Three bands were seen in all the active fractions. A thick band with a molecular weight approximately 67 kDa, and 2 narrow bands with a molecular weight around 43 kDa. Further study is needed in order to completely characterize the coagulant proteins from these seed extracts.

Activity test in dilutions
This study was performed to determine the optimal sample concentration of the seed extracts. Since the CSE showed higher activity irrespective of the samples, the following experiments were done on CSE. The percentages of coagulation activity in the different samples were observed after diluting the samples. Results for MO crude extract show that its percentage of coagulation activity was not affected until samples were diluted 9 times (Figure 3). Red bean and green gram, showed an increase in percentage of coagulation activity compared to the initial activity. Red bean showed more or less similar activity up to 27 times diluted samples whereas green gram showed a decrease in activity of the 27 times diluted samples. However, the values are lower than obtained for MO. It is clearly seen that MO crude extract works in 9 times diluted samples whereas purified protein showed good coagulation activity even at 27 times diluted sample (data not shown). The concentration of the coagulant protein increased 3 fold in the purified samples indicating that purification would be rather helpful to concentrate the coagulant protein. From these experiments it was obvious that some of the extracts were having higher activity in diluted samples which would allow the use of low amount of coagulant in water treatment and thereby reduce the material cost.

Temperature influence
The extracts which showed activities were further tested on the influence of temperature in the coagulation activity. From Figure 4 it is shown that the coagulation activity of red
bean increases from the initial activity by 30% after incubating at 85°C for 30 min and 1 hr. When incubating at 95°C it also showed an increased activity compared to initial activity. On the other hand, sugar maize and red maize was negatively affected by heating at 85°C and 95°C. The activity was reduced by 5% from the initial activity. One possible reason could be activation of some enzymes present in the extract which causes the degradation of protein into several peptides, leaving the active sites free to act on substrates. This provides us with the possibility of using natural coagulants at any temperatures and temperature could even increase the coagulation properties of the extracts. Hence it would be possible to use the natural coagulant at very different climate conditions.

Conclusion
Coagulants play an important role in the treatment of drinking water and wastewater and sludge disposal. From this study we have found that red bean, sugar maize and red maize have coagulation activity. The coagulation activity was comparable to that of MO. The salt extracts showed higher activity than water extracts. The coagulation activity is not affected by a temperature of 85°C and up to boiling. It is possible to purify the active coagulant protein from red bean similar to that of MO. The sugar maize and red maize coagulant proteins have different chemical properties as shown by purification and acidic gels. This implies that coagulant proteins from different seeds have variations in protein characteristics. Without purifying coagulant protein it is possible to use crude extracts in water treatment. Red bean coagulant had increased activity at higher temperature and dilutions. The present study provides an alternative to Moringa seed extracts in the primary water treatment. It is possible to use a very low concentration of the natural coagulant thereby reducing the treatment cost. It could be advantageous to use natural coagulant as an environmentally friendly alternative in drinking water treatment.

References


