Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2016.1733

59 (2017) 127–134
January

Extraction of natural coagulants from *Maerua subcordata* and *Moringa stenopetala* for use in turbid water treatment

Moa Megersa[a,b,*], Abebe Beyene[a], Argaw Ambelu[a], Ludwig Triest[b]

[a]*Department of Environmental Health Science and Technology, Jimma University, Jimma, P.O. Box 378, Ethiopia, Tel. +251913478127; Fax: +251471112040; emails: moa.megersa@ju.edu.et, moamegersa78@gmail.com (M. Megersa), abebebh2003@yahoo.com (A. Beyene), aambelu@yahoo.com (A. Ambelu)*

[b]*Plant Biology and Nature Management (APNA), Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Elsene, Brussels, Belgium, emails: moishmagi88@yahoo.com (M. Megersa), ltriest@vub.ac.be (L. Triest)*

Received 11 December 2015; Accepted 18 June 2016

ABSTRACT

Natural coagulants, especially those from *Moringa oleifera* Lam., have been employed since ancient times (200 B.C.) in the treatment of drinking water. The aim of this study was to extract natural coagulants from tubers of *Maerua subcordata* (Gilg.) DeWolf and seeds of *Moringa stenopetala* (Baker f.) Cufod, both native to Ethiopia, and to examine their potential utility in water treatment in the future. The coagulation activity of the extracts was measured in natural turbid river water and synthetic water made of a kaolin clay suspension. The turbidity removal efficiency was tested using a jar test and spectrophotometric-based assays. Proteins from the extracts were visualized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and gel staining. Relevant parameters affecting the effectiveness of coagulation (pH, extraction time, type/concentration of the extracting solvents, and storage duration/conditions) were also investigated. A wide range of abundant proteins, with molecular weights of 10, 15, 35, and 40 kDa, were found in the crude extracts. However, only the proteins with molecular weights of 10 and 15 kDa were resistant to prolonged heat at 95°C for 5 h. Interestingly, using synthetic turbid water, the tuber extracts from *M. subcordata* showed 90.5% coagulation activity within 3 h of contact, whereas the seed extracts from *M. stenopetala* showed only 83.5% coagulation activity. The removal efficiency of *M. subcordata* and *M. stenopetala* in turbid river water was 80% and 83%, respectively. Both the *M. subcordata* and *M. stenopetala* extracts showed the most efficient coagulation activity at pH = 8. Increasing the extraction time negatively affected the removal efficiency of the coagulation. In conclusion, coagulant proteins from these native species could provide a means of water treatment for communities living in rural areas, enabling access to adequate supplies of safe drinking water.

Keywords: Plant coagulant; Water treatment; Turbidity; Thermostability

1. Introduction

Coagulation-flocculation, followed by sedimentation and filtration, is the most widely used water treatment method. Aluminum, which has been linked to Alzheimer's disease, and iron salts are the most commonly used inorganic coagulants [1,2]. In urban areas, aluminum sulfate (alum) is generally employed as a coagulant. The aforementioned water treatment methods are often impractical for rural communities in developing countries because of the high cost of equipment and lack of chemical coagulants [3].

Currently, more than 663 million people worldwide are living without access to clean drinking water, mainly in rural areas of sub-Saharan Africa [4]. Therefore, the identification of cost-effective alternatives to conventional coagulants is necessary to combat potential adverse effects of current treatment methods and to provide rural communities with good-quality water.

*Corresponding author.

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For centuries, people have used natural coagulants for household water treatment, and some ethnic groups in developing countries still use different plant species in an attempt to purify turbid surface water [5,6]. Several studies have described the use of plant-based coagulants, mainly *Moringa oleifera*, in water treatment [7–9]. However, other plant species, such as *Phaseolus vulgaris*, *Jatropha curcas*, *Opuntia ficus indica*, *Zea mays*, *Prosopis juliflora*, and *Plantago ovata*, also display coagulation and flocculation capacities and proven turbidity removal potential [10–15].

Studies have reported the activity of several proteins in *M. oleifera* seeds in coagulation and flocculation [7,8]. According to one study, the active agent in aqueous extracts of *M. oleifera* seemed to be a dimeric cationic 13 kDa protein [7]. However, other studies reported that proteins, with a mass of about 6.5 kDa, were the active agents in aqueous and salt extracts of *M. oleifera* [8,16].

The identification of new environmental friendly plant extracts for use in water treatment would offer several advantages in terms of health and safety, availability, and costs [17]. People living in rural areas in Ethiopia use a number of plant species in water treatment. National surveys have been conducted to identify these plant species and determine their potential utility in turbid water treatment.

From those surveys, four candidate plants were identified: *Maerua subcordata*, *Moringa stenopetala*, *Sansevieria ehrenbergii*, and *Sansevieria forskaoliana* [18]. As only tubers of *M. subcordata* and seeds of *M. stenopetala* acted as natural coagulants, these were selected for further analyses.

To optimize the use of these two plant species in Ethiopia, this study aimed to determine the molecular weights of the protein(s) present in extracts from these plants, evaluate their protein content and thermostability, and optimize the dosage of coagulants, type and time of extraction, and pH, thereby improving the activity of these coagulants in the treatment of turbid water.

2. Materials and methods

2.1. Collection of plant materials

Fresh tubers of *M. subcordata* and dried seeds of *M. stenopetala* were obtained from Arbaminch and Konso, Ethiopia. *M. subcordata* is a shrub, which can reach a height of 2 m. Its leaves are blue-green, egg-shaped to rounded, and the shrub bears green-yellowish fruit (Fig. 1(a)). The roots of *M. subcordata* are swollen (Fig. 1(b)). Once the tubers are removed, remaining patches of tubers regenerate. As the removal of the tubers does not damage the plant in the long term, this is an ecologically sound means of obtaining a source of natural coagulants.

The genus *Moringa* is indigenous to several countries [19]. There are about 13 different known species of *Moringa*, of which *M. stenopetala* (Baker f.) Cufod is indigenous to Ethiopia (Fig. 1(c)). *M. stenopetala* is a small tree up to 12 m, with a many-branched crown. The leaves are tripinnate, with about five pairs of pinnae and the fruits are long reddish pods. Ethiopians often use the seeds of *M. stenopetala* (Fig. 1(d)) to treat turbid water.

2.2. Preparation of crude extracts

The husk from the seeds of *M. stenopetala* was first removed manually and good quality seeds were selected for extraction. The sun dried tubers and matured seeds were powdered using a mortar and pestle in the environmental

Fig. 1. (a) Shrub of *M. subcordata* with its fruits. (b) Tuber of *M. subcordata*. (c) *M. stenopetala* tree. (d) Seeds of *M. stenopetala* with husk.

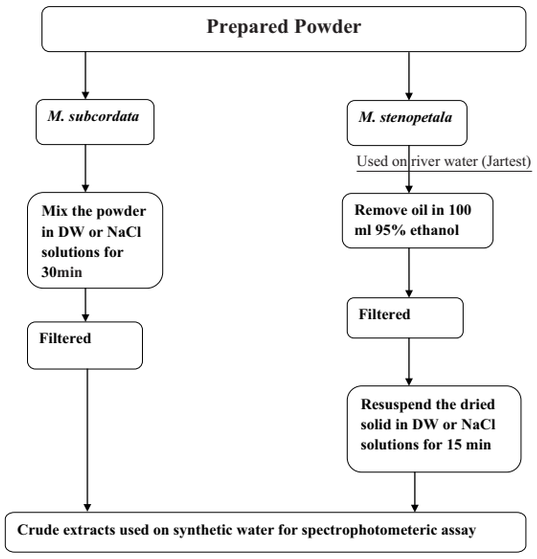

Fig. 2. Schema describing the processing of the extracts used in the coagulation experiments.

health science and technology laboratory of Jimma University, Ethiopia. An aliquot of the powder was then transported to the plant biology and nature management laboratory of Vrije Universiteit Brussel, Belgium for further analysis. The extraction was done following the protocols of Ghebremichael et al. [8]. The *M. stenopetala* powder was mixed in 100 mL of 95% ethanol for 30 min to remove any oil present. Then, the suspension was filtered through a Whatman No. 3 filter (GE Healthcare, UK) to obtain crude extracts. A more refined crude extract was obtained by centrifugation at 3000 rpm for 2 min (Fig. 2).

A 5% (w/v) solution of crude extracts was prepared from the powdered seeds and tubers in 0, 0.01, 0.1, 0.5, and 1 M sodium chloride (NaCl) using deionized water, as previously described by Madrona et al. [20]. The optimum extraction time for the seed powder from *M. stenopetala* was 15 min, whereas that of the *M. subcordata* tubers was 30 min.

2.3. Source of water samples

Surface water samples were collected from the Gibe River in Jimma, Ethiopia. Synthetic turbid water was prepared by adding 10 g of laboratory grade kaolin (Merck KGaA, Germany) to 1 L of deionized water. The suspension was stirred with a magnetic stirrer for about 1 h to achieve a uniform dispersion of the kaolin particles. It was then allowed to settle for 24 h for complete hydration of the kaolin [21]. After settling, the turbid-water supernatant was decanted and used as a stock solution.

2.4. Protein analysis

Protein quantification of the crude extracts was performed by Bradford analysis [22]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on 12% polyacrylamide Laemmli gels as described previously [23]. This experiment was conducted using a Mighty Small II SE 250 apparatus (Hoefer Scientific, USA).

2.5. Thermal tolerance of the coagulant proteins

Crude extracts prepared in deionized water were heated at 95°C for 3 and 5 h to check the stability or activation of the coagulant proteins in coagulation activity tests. After heating, the solution was cooled and filtered using Whatman filter paper No. 3. The clear supernatant was used in subsequent analyses. The protein content and profiles before and after the heat treatment were also compared by SDS-PAGE.

2.6. Other studied parameters

2.6.1. Extraction time

Crude proteins were extracted in deionized water, stirring for 15, 30, 45, and 60 min, and the removal efficiency of different amounts of coagulants was tested.

2.6.2. pH

To determine the effect of the pH on the coagulant activity of the proteins, considering the recommended pH of drinking water (between 6.5 and 8.5) set by the World Health Organization (WHO) [24], the initial pH of the kaolin suspension was adjusted to 5, 6, 7, 8, and 9 using sodium hydroxide or hydrochloric acid.

2.6.3. Storage duration and conditions

Stock solutions were stored at 4°C and room temperature (26°C) for 1, 2, and 3 months in opened and closed containers (funnel tube, 50 mL), and the coagulation activity of the proteins was determined.

2.7. Coagulation activity test

A jar tester (Thermotech Th-6001, Loctron Instruments Pvt. Ltd., India) and spectrophotometer (ThermoSpectronic Helios Epsilon, Thermoscientific, USA) were used in the coagulation activity tests of the turbidity removal efficiency of the powders and the extracts. The tuber powders of *M. subcordata* and seeds of *M. stenopetala* were employed in coagulation tests, using turbid river water collected from the Gibe River, with an initial turbidity of 24 NTU. The coagulation activity of powders was tested using jar tester which is a widely applied in coagulation tests [25]. Six 1-L beakers were filled with water and placed in the slots of the jar tester. Two beakers served as positive (aluminum sulfate solution) and negative (blank water) controls. The other four beakers were filled with different amounts of the coagulant (0.01 and 0.03 g L^{-1}). After agitating the suspension rapidly at 170 rpm for 2 min and slowly at 40 rpm for 20 min, the coagulation activity of the samples was measured at 30-min intervals for 6 h. The effects of the extraction time, pH, storage duration and conditions, and heat on coagulation activity of extracts were analyzed using semi-micro plastic cuvettes (Sigma-Aldrich, Germany) on a spectrophotometer. In these experiments, the initial turbidity of the kaolin suspension was

260 NTU. All the experiments were conducted in triplicates, and the means were calculated.

Residual turbidity was measured using a turbidimeter (Oakton T-100, Oakton USA), and the pH was measured using a pH meter (Ecoscan pH 5, Thermoscientific USA). The small assay coagulation method was used, as described earlier [8]. Different amounts of the coagulant solutions (2–20 µg) were added to 1 mL of clay suspension in a semi-micro plastic cuvette (Sigma-Aldrich) of 12.5 × 12.5 × 45 mm and instantly homogenized. The absorbance or optical density at 500 nm was measured at 30-min intervals for 90 or 180 min using a UV-Visible ThermoSpectronic spectrophotometer (Helios Epsilon). The coagulation activity of the tuber and seed powder extracts was calculated using Lee's equation [26], as follows:

$$CA = (RT\ blank - RT\ sample) / RT\ blank$$

where the CA is the coagulation activity, the RT blank is the residual turbidity of the control, and the RT sample is the residual turbidity with the coagulant.

3. Results and discussion

3.1. Effect of the coagulants on turbid natural river water

The turbidity removal efficiency of the powders from *M. subcordata* tubers and *M. stenopetala* seeds in river water was tested and compared with that of the widely used inorganic chemical alum. Alum showed excellent turbidity removal efficiency (83%). The optimum dosage was 0.01 g L^{-1} after 30 min of incubation. After 3 h of incubation, the removal efficiency of the *M. subcordata* tubers and *M. stenopetala* seeds was 62% at a dosage of 0.01 and 0.03 g L^{-1}, respectively. However, with an increase in the contact time, up to 6 h, the turbidity removal efficiency of both plant coagulants was nearly as effective as that of alum (Fig. 3). In a previous study, the turbidity removal potential of mustard species was reported to be 83% at a concentration of 20–30 µg mL^{-1} [17].

The removal efficiency of *M. oleifera* was 100% in a study in Malawi, with the optimum dosage being 250 mg L^{-1} when applied to shallow well water [13]. In a study where the residual turbidity met the guidelines set by the WHO [24], the optimum dosage of *Cactus latifaria* commonly called cactus was 10–20 mg L^{-1} [10]. The difference in the optimum doses of plant species could be due to the characteristics and type of water or the type and amount of active coagulant components present. Other than the variability among different plant species, the differences in efficiency could be due to intraspecific variations of seeds, with the dosage and coagulation performance of the seeds of plants varying according to the geographic regions where they are found [27]. Other reported plant species with proven coagulation activity in kaolin clay turbid water, silica suspensions, groundwater, and surface water are *P. juliflora*, *J. curcas*, *Parkinsonia aculeata*, and *Vigna unguiculata* [10,13,28–30]. These may also have potential as primary water treatment agents.

3.2. Activity of the coagulants in synthetic water

The performance of the crude extracts and alum in the synthetic turbid waters pointed to the turbidity removal efficiency of the coagulants. In the tests, 10–20 µg mL^{-1} of the crude extract proteins and 2 µg mL^{-1} of alum had the highest removal potential (Fig. 4). The results indicated that increasing the dosage of the coagulants also decreased the turbidity. A similar trend was reported in other studies of crude extracts of *Dolichos lablab* and *M. oleifera* [31,32].

3.3. Coagulation potential of the salt and water extracted fractions

Different molar concentrations of NaCl were used, in addition to deionized water, to study the effects of the types and concentrations of the extracting solvents on coagulation and flocculation processes. In the tests of *M. subordata* tubers and *M. stenopetala* seeds, the salt solution extraction was more efficient than the deionized water extraction in removing turbidity. At 0.5 M, using the salt solution extraction, the turbidity removal efficiency of *M. subordata* and *M. stenopetala* was 96% and 90.3%, respectively, whereas using the deionized water extraction; it was only 71% and 60%, respectively (Fig. 5).

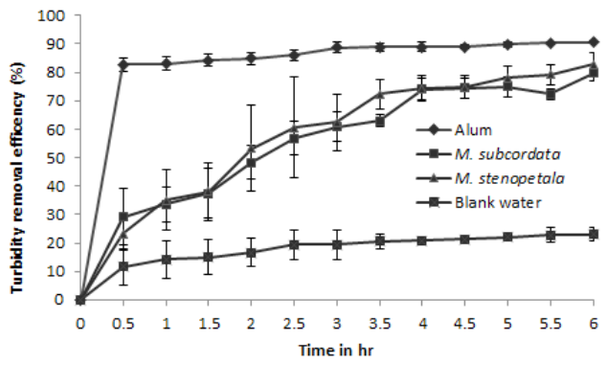

Fig. 3. The coagulation activity of the *M. subcordata* and *M. stenopetala* powders on river water samples.

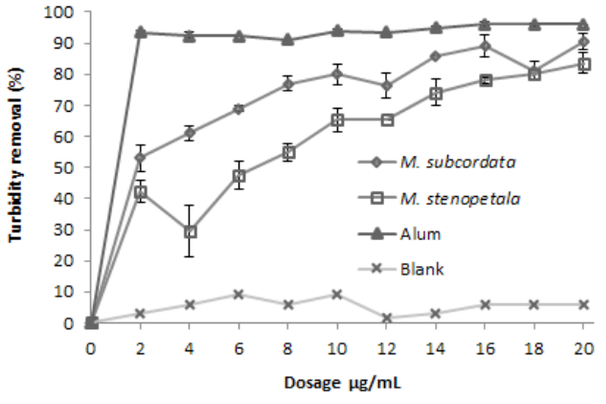

Fig. 4. Turbidity removal efficiency of plant extracts on synthetic water.

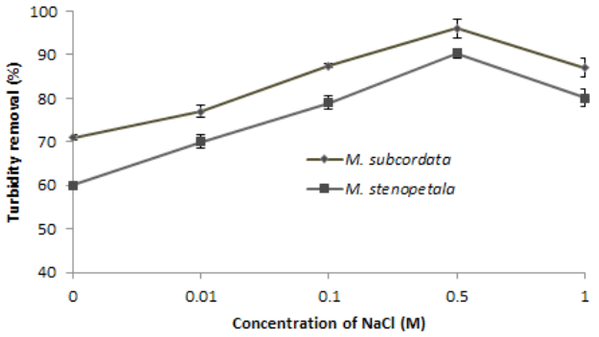

Fig. 5. The coagulation activities of the extracts using different concentrations of NaCl and deionized water.

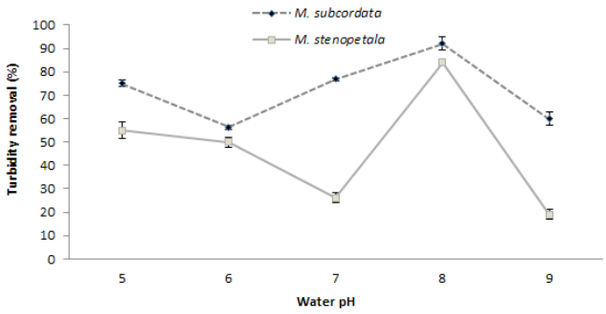

Fig. 6. Effect of the water pH on turbidity removal.

Similar findings were reported for seeds of *J. curcas* [29] and *M. oleifera* [33]. However, in another study, the salt concentrations did not affect the coagulation efficiency of *Vicia faba*, apart from increasing the amount of extracted compounds [34].

In the present study, with increased salt concentrations, the percentage of turbidity removal increased up to 0.5 M but then decreased at 1 M (Fig. 5). This may be explained by the so-called salt-in and salt-out effect, which controls the solubility of proteins in salt concentration solvents [29]. In the case of 0.5 M NaCl, more proteins are extracted and dissolved in extracting solvents; hence, increased turbidity removal efficiency can be expected [29]. However, above 0.5 M, the solubility of the proteins started to decrease and consequently decreased the coagulation and turbidity removal efficiency. The coagulation activity of the salt-extracted *M. oleifera* increased up to 1 M and then decreased at 3 M [35].

3.4. Effect of the extraction time on coagulation activity

Increasing the extraction time negatively affected the coagulation potential of both plant species. The greatest reduction in turbidity was observed after 30 min for *M. subcordata* and 15 min for *M. stenopetala* (Table 1). The decreased turbidity removal efficiency observed as a function of the increased extraction time may be due to the extraction of other organic matter (e.g., oil in the case of *M. stenopetala*), which may have subsequently hindered the coagulation and flocculation processes.

Table 1
Effect of extraction time on coagulation activity of crude extracts

Coagulant	*M. subordata*		*M. stenopetala*	
Time in minutes	Absorbance (OD_{500})	Turbidity removal (%)	Absorbance (OD_{500})	Turbidity removal (%)
15	0.68	57.5	0.56	65
30	0.54	66.2	0.80	50
45	0.80	50	1.04	35
60	0.88	45	1.08	32.5

O.D. measured at time 180 min.

3.5. Effect of the water pH on coagulation

The impact of pH on the turbidity removal efficiency of *M. subcordata* and *M. stenopetala* was studied in synthetic water, with the pH ranging from 5 to 9. The least reduction in turbidity removal was observed at an acidic pH for extracts of *M. subcoradata* and basic pH for extracts of *M. stenopetala* (Fig. 6). The optimum pH for the extracts of *M. subcordata* and *M. stenopetala* was pH = 8, with maximum turbidity reduction of 92% and 84%, respectively. At a pH of 9, the turbidity reduction efficiency decreased to 60% and 19% for *M. subcordata* and *M. stenopetala*, respectively. A similar optimum pH of 8 was previously reported for *M. oleifera* [36]. However, as shown elsewhere, the maximum turbidity removal efficiency of other natural coagulants occurred at various pH levels. The optimum pH for turbidity removal (maximum reduction of 80%) using crude extracts of *M. oleifera* seeds was 6.5 [15]. The turbidity removal efficiency of *J. curcas* was highest at an acidic pH of 3, with turbidity removal of 99% [29]. The most appropriate pH for extracts of the *Phaseolus vulgaris* (common bean), *Castanea sativa* (chestnut), *Quercus robur* (acorn), and *C. latifolia* was 10 [37,38]. Similarly, extracts of *M. oleifera* and *Opuntia* sp. performed well at a pH of 8 or above [6,39]. At a pH above 7, more negatively charged ions are present in kaolin suspensions, enhancing charge neutralization. In contrast, at a pH lower than 7, the kaolin particles are less negatively charged, causing increased repulsion effects between polyelectrolytes and particles, thereby reducing the coagulation efficiency [40]. The presumably paradoxical reports in the literature on the effect of pH on turbidity removal efficiency could be due to differences in the experimental conditions (type of water, extraction solvent type, etc.) and the specific coagulant used [41].

3.6. Effect of the storage duration and conditions on coagulation

After extracting the active components from the tubers of *M. subcordata* and seeds of *M. stenopetala*, the extracts were stored in both open and closed containers at 4°C and 26°C for up to 3 months. Storage for up to 3 months at 4°C resulted in no significant differences in the turbidity reduction potential. Interestingly, there was a slight increment (of about 8%) in the turbidity removal potential of the stock solutions stored in both the open and closed containers (Fig. 7). The increment in the turbidity reduction potential was probably due to organic acids produced by

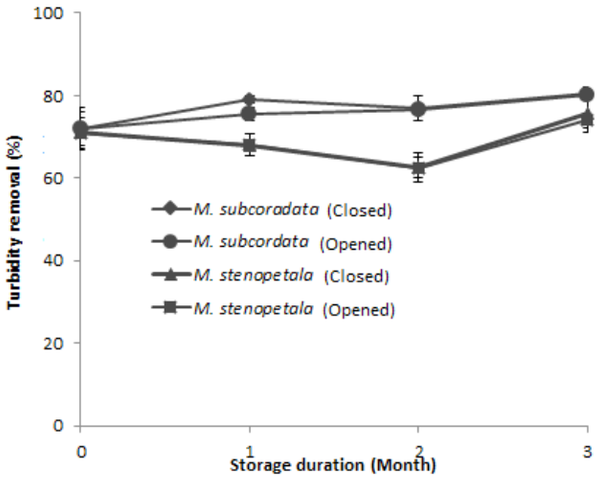

Fig. 7. Turbidity removal efficiency of stock solutions stored at 4°C.

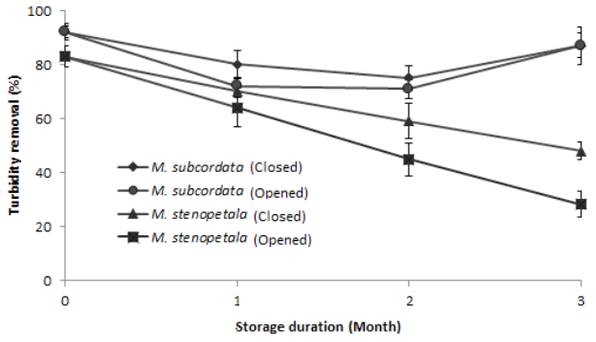

Fig. 8. Turbidity removal efficiency of stock solutions stored at room temperature (26°C).

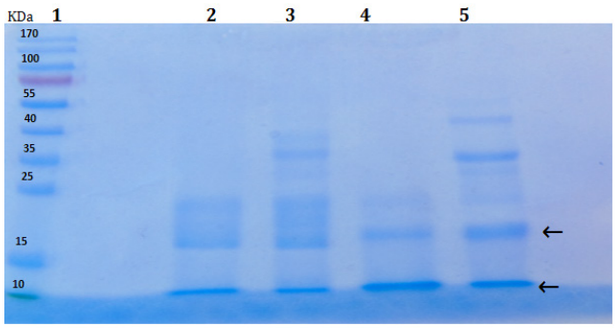

Fig. 9. Coomassie-stained SDS-PAGE displaying the proteins' profiles of the crude extracts before and after 5 h of heating at 95°C. Note: Lane 1 shows molecular weight markers; Lanes 2 and 3 are proteins from *M. subcordata* after and before heating, respectively; Lanes 4 and 5 are proteins of *M. stenopetala* after and before heating respectively. The arrows indicate heat resistance proteins.

the microbial decomposition of organic matter upon storage [42]. In contrast, in another study, stock solutions of *M. oleifera* stored at 3°C for more than 5 d lost their coagulation activity [43]. The discord between the findings of that study and the present one may be due to differences in the experimental set-ups, namely the type of water used, with the present study using synthetic water and the previous one using surface water, and the type of solution used for extraction.

Unlike stock solutions stored in a refrigerator, the coagulation efficiency of the crude protein extracts of *M. stenopetala* stored at room temperature (26°C) in both open and closed containers was greatly reduced (of about 60%) (Fig. 8). On the other hand, the turbidity removal efficiency of the extracts of *M. subcordata* increased slightly after 3 months of storage. This observed increment could be due to a reduction in the pH as result of organic acids produced by the decomposition of organic compounds, as described by Katayon et al. [44]. In a previous study, the turbidity removal ability of *M. oleifera* stock solutions kept at room temperature (28°C) for 3 d disappeared [35]. However, in another study, a reduction in turbidity removal efficiency was correlated with an increased storage time [44]. Furthermore, the coagulation efficiency potential of *M. oleifera* seeds stored for up to 2 months in open or closed containers was not significantly altered [33]. Although the results of the present study point to a reduction in the turbidity removal efficiency upon storage of the crude extracts, the coagulation potential of none of the extracts disappeared completely.

3.7. Analysis of the protein profiles before and after heating

The protein quantification of the crude extracts showed that the protein content of the *M. stenopetala* powder was higher (1,039 µg mL^{-1}) than that of the *M. subcordata* powder (718 µg mL^{-1}) before heat treatment. However, after heating, the protein content of *M. stenopetala* decreased to 513 µg mL^{-1}, whereas that of *M. subcordata* exhibited a 22% reduction (587 µg mL^{-1}). A previous report of coagulant proteins from various mustard species found a 71% reduction in the protein content after heating at 95°C for 5 h [17]. Fig. 9 shows the results of the SDS-PAGE analysis of the protein profiles before and after the heat treatment.

The SDS-PAGE profiles of the *M. subcordata* and *M. stenopetala* extracts were similar. Interestingly, proteins with molecular weights of 40, 35, 15, and 10 kDa were the most abundant in both the *M. subcordata* and *M. stenopetala* extracts before the heat treatment. In both cases, after heating for 5 h, only proteins of 15 and 10 kDa were observed, with similar concentrations. This indicates that proteins of 40 and 35 kDa were likely denatured upon heating at 95°C. The presence of different molecular weight proteins, ranging from 6.5 to 30 kDa, in *M. oleifera* before heating has been reported [20,45]. For instance, a cationic dimeric protein with a molecular weight of 12–14 kDa was identified as a coagulant protein [7]. A 6.5-kDa coagulant protein extracted from *M. oleifera* was found to be resistant to extreme heating [8]. The proteins of *M. subcordata* and *M. stenopetala* identified herein, with proteins smaller than 30 kDa detected, may represent potential coagulation factors, as their molecular weights are within the range of other coagulating proteins.

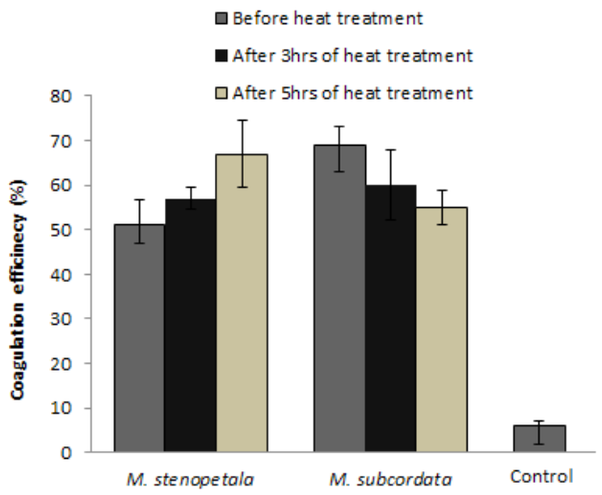

Fig. 10. Coagulation efficiency of the crude extracts before and after heat treatment measured after 90 min of settling time.

3.8. Turbidity removal activity of the coagulant proteins before and after heat treatment

High temperature is among a number of physical factors that denature proteins. However, in the present study, the coagulant proteins of both plant species were resistant to heat treatment and remained active after 3 and 5 h of boiling. Surprisingly, the turbidity removal efficiency of the *M. stenopetala* seed extracts increased slightly in response to heat treatment, showing a 16% gain in activity. On the other hand, heating had a negative effect on the tubers extracts of *M. subcordata*, although they retained 80% of their coagulation activity after 5 h (Fig. 10).

The enhanced coagulation activity detected after heat treatment of *M. stenopetala* was probably due to the removal of oil upon heating, as oil hinders coagulation activity and consequently yield poorer turbidity removal. Enhanced activity of coagulant proteins after heating was reported for extracts of red maize (*Zea mays*) [12] and mustard (*Brassica* sp.) [17], with a 25%–30% efficiency gain upon heating. The thermo-tolerance of the crude extracts observed in the present study and elsewhere suggests that they may be easy to extract and purify on an industrial scale. Furthermore, they may be practical in developing countries, with different climatic conditions [8,12,46].

4. Conclusions

Coagulant proteins were detected in both the plant species analyzed in the current study. The molecular weights of the putative coagulant factors were within the range previously reported for coagulant proteins. The coagulant proteins were stable after heat treatment at 95°C, and the coagulation efficiency of *M. stenopetala* increased after heating. The pH influenced coagulation, with better coagulation recorded at a pH of 8. Extraction using different concentrations of salt confirmed the coagulation potential of both plant species. Remarkably, both plant species preserved their coagulation efficiency when stored in both open and closed containers at 4°C for up to 3 months. However, the coagulation efficiency of extracts of *M. stenopetala* deteriorated upon storage at room temperature (26°C). The findings suggest that both plant species may be useful in water treatment and that they may help to ensure a supply of safe drinking water to rural communities in developing countries.

Acknowledgments

The authors are grateful to the Erasmus Mundus Action 2, Caribu scholarship of the Vrije Universiteit Brussel; the International Foundation for Science (IFS, W/5406-1); the Ministry of Water, Irrigation, and Energy (Ethiopia); and Jimma University for financial and logistic support. Professor Gustavo Gutierrez also deserves gratitude for laboratory facilities and improving English of the manuscript.

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