Coagulation activity of Chitin Binding Protein to the Turbid Pond Water from Garden Candytuft Seeds

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Article

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Abstract

In advanced nations, reservoir water is frequently utilized for consumption or domestic use, and its turbidity is higher during wet seasons. It requires many artificial coagulants, which increases the cost of therapy. Finding natural alternatives, particularly those derived from plants, has become more relevant because of the negative health and environmental implications of traditional synthetic substances used in water clarification. In this study, a natural coagulant protein called chitin binding protein (CBP) purified from the seeds of *Iberis umbellata* L. was utilized to treat water and remove turbidity. To increase the effectiveness of turbidity removal, this study focuses on the extraction, isolation, and purification of natural coagulants from the seeds of *I. umbellata* L. At pH 7, the maximum turbidity reduction effectiveness was attained. The molecular weight of CBP was approximately 14 kDa, as revealed by SDS-PAGE and purified by spin column chromatography. The CBP of *I. umbellata* showed coagulation activity against synthetic clay solution and pond water. The coagulation results showed that CBP was 87% and Moringra extract showed 89% activity against the synthetic clay solution after 120 min. CBP from *I. umbellata showed* 83% and Mo extract 82% coagulation activity against the pond water. CBP from *I. umbellata* may be a viable natural coagulant for water treatment based on its coagulation performance against severely murky pond water.

INTRODUCTION

Clean water is necessary for all life forms but poor drinking water is major problem all over the world. Due to this reason the possibility of clean drinking water is globally studied in the developing countries[1]. In the developing countries potable water is expensive undertaking[2]. The quality of water is important for drinking and its cleaning is very important where water quality is poor and lacking of treatment methods. There are 80% of all diseases and illness is due to the polluted water and inefficiency of sanitation all over the world[3]. The underdeveloped centuries are facing deleterious effects of poor drinking water. Almost 90% of human diseases are caused by poor drinking and polluted water and increase the health problems of people in worldwide[4]. Water –borne diseases have to cause by the poor drinking water quality.

Environment can be protected from the wastewater contaminants because water and wastewater are contaminated with many solid particles. These solid particles cannot be easily settled down and it changes the color of water and makes the water turbid[5]; which must be marked using coagulants[6]. In this circumstances, turbidity of drinking water has been reduced through the coagulation process by using coagulants[7] earliest times in human history. In this way, water quality can be improved by lowering turbidity[8,9]. Natural, inorganic and synthetic organic polymers coagulants are being used in water treatment processes[6,10]. Plant based natural coagulants are used to remove the turbidity and have been used by various civilization and communities[11]. In developing countries, natural coagulant has been gained interest for the treatment of water and wastewater[12]. Many problems are created by used of inorganic or synthetic organic coagulants such as their high cost, health related issues and

environmental impacts[13]. Megersa and his colleagues[9] reported that inorganic coagulant such as aluminum chloride, aluminum sulfate and aluminum polychloride used in the coagulation or flocculation process and have the negative impact on human health and environment. Although, large volume of sludge have been produced by alum[14]. Furthermore, alum is not biodegradeable, reduced the pH of treated water, have carcinogenic properties and cause of Alzheimer's disease[14–18].

Seed extract of the plant show the natural coagulation activity that is similar to or even better than alum. Plant based natural coagulants are renewable source in water treatment because they possess positive properties such as widely available, biodegradable, environmentally friendly and non-hazardous[4]. The advantages using natural coagulants are cost effective and low sludge production. Different seed proteins have the natural coagulation activity to water treatment, in previous studies like *Strychnos potatorum* seed have coagulant proteins, Moringa seed possess the natural coagulant bioactive protein that have the coagulation activity and seed of *Brassica nigra* have napin protein which have shown coagulation activity against the turbid pond water[19–21].

Garden candytuft or globe candytuft (*Iberis umbellata* L), *Brassicaceae* family is an annual and herbaceous flowering plant of genus *Iberis.* It is naturally found in Europe and especially in the coasts region but also available in Northern America. The goal of this study was to analyze the coagulation capability of garden candytuft and partially purified naturally occurring coagulant CBP from *I. umbellata* L. seeds, which exhibited coagulation activity as opposed to a manufactured clay mixture and turbid pond water.

MATERIALS AND METHODOLOGY

CBP protein from seeds of *I. umbellata* L. was purified and identified through gel electrophoresis, dialysis and spectroscopic techniques

Collection and extraction of protein from plant seeds

Seeds of *I. umbellata* L. were taken from a local market of Lahore, Pakistan. Plant specimens were identified and confirmed by a senior taxonomist. CBP from *I. umbellate* seeds were purified by described [22] with minor modification. Seeds (0.7 g) of *I. umbellata* L. were ground by pestle and morter to a powder form. The seeds powder was used for the preparation of crude extract. Extraction was performed in 14 ml of 0.05 M phosphate buffer, pH 7.0 for four hours at room temperature. After extraction, the extract was centrifuged at 12000 rpm for 20 minutes. The supernatant was filtered by using the Whatman filter paper (pore size 8 µm) and pellet was discarded. The clear crude extract five ml was used to precipitation of proteins through ammonium sulfate precipitation. The proteins were precipitated by stepwise slow addition of solid ammonium sulfate from the concentration of 30%, 50%, and 70% with constant stirring. The precipitated proteins were separated at low centrifugation speed of 5000 rpm for 5 min. The supernatant was removed and the resulting pellet was re-dissolved in 100µl of the same

phosphate buffer and dialyzed (Spectra/Por™, MWCO 3.5 kDa) thoroughly in 5L of 0.05 mM phosphate buffer, pH 7.0.

Purification of Coagulant Proteins

The partial purified proteins from ammonium sulphate precipitation were further purified by spin column chromatography as described[23]. The supernatant of 70% ammonium sulfate saturation constant was filtered in the < 30 kDa spin column, and centrifuged at 4000 rpm for 25 min. Finally, the filtrate was collected and tested for coagulation. The molecular weight of coagulation protein falls under 18–14 kDa.

Protein profile and quantification

The protein content was visualized by using 12% SDS-PAGE[24]. The gel was stained with Coomassie Brilliant Blue R-250 (CBB R-250) dye to visualize the protein bands. All protein quantifications for the crude extract or purified CBP were performed by using Bradford reagent[25] at wavelength of 595 nm (Biochrom Libra S60 Double Beam).

Preparation of kaolin clay solution

For the assessment of coagulation activity, turbid water was prepared as a 1% kaolin solution by using tap water. The prepared clay solution was stirred for 120 min and settled for 24 hours to allow complete hydration of the particle. The upper turbid layer of clay solution was separated and used for coagulation activity test.

Coagulation activity test

The coagulation activity test was carried out with kaolin synthetic clay solution as described by the Ghebremichael method[26]. The *I. umbellata* L. crude extract 0.01 ml was used with 1 ml of clay solution and the initial turbidity was observed at wavelength of 500 nm (A500) by using the spectrophotometer. The solution was allowed to settle for 30 minutes and thereafter A500 was measured again. The percentage of coagulation activity was calculated using the following formula: [(t0-t)/t0)*100], where t0 is the A500 measured instantly after the sample has been homogenized and t is the A500 measured after 30, 60, 90 and 120 min. Different concentrations of CBP protein were used for coagulation activity. Kaolin clay solution was used as a negative control and *I. umbellata* seed extract was used as positive control in the experiment.

Effect of pH on coagulation activity of CBP:

Effect of pH was investigated during the coagulation process, the factor pH effect on the surface charge of coagulant and plays the role in the stabilization process. Coagulation activity of CBP was measured against pond water, clay solution, and crude extract of *I. umbellata*, and Mo crude extract in different pH i.e. (3.0, 5.0, 7.0, 10.0, and 12.0). In this experiment 0.07 mg/ml dose of CBP was used to reveal the coagulation activity.

Coagulation activity of CBP against turbid pond water

The coagulation activity of CBP was measured against turbid pond water. For this purpose, turbid pond water was collected from a pond, near University of Okara, Pakistan. For coagulation activity of *I. umbellata* L. seed extract and CBP protein 0.055 mg was added in one ml turbid pond water separatly. Initial absorbance was measured at t = 0 and at every 30 min time interval (A500) up to 3 h. Turbid pond water had an initial absorbance of around 1.5 NTU (Nephelometric Turbidity Units) and served as the control in the whole experiment.

RESULTS AND DISCUSSION

The aqueous extract was being used as coagulant material. The aqueous extract contains the macromolecules such as lipids, proteins and carbohydrates. The water-soluble coagulant proteins were isolated and characterized from an aqueous crude extract of plant materials for purpose of purifying dirking water. The objective of this experiment is to isolate the protein from the seed of *I. umbellata* and to test its coagulation potential.

Extraction and isolation of CBP protein

The crude extracts of *I. umbellate* seeds exhibited the water soluble proteins with molecular weight fall under 14–70 kDa at 0.05 M, pH 7 as represent in lane 1 in Fig. 1. Chitin Binding Protein (CBP) was purified by the combination of ammonium sulfate precipitation and spin column chromatography from the crude extract of *I. umbellate* seeds. It is reported that a chitin-binding protein, Iu-CBP with molecular weight about 14kDa, belong the 2S belong to the 2S albumin seed storage family from seed extract. CBP be seen in 50 and 70% (w/v) pellets of $(NH4)_2SO_4$ saturation constant with impure form as indicate in lane 3 and 4[22]. In the final analysis, an optimized combination of ammonium sulfate precipitation along with spin column chromatographic steps. Ultimately, an optimized combination of ammonium sulfate precipitation along with chromatographic steps came up with > 95% pure CBP solution from seeds of *I. umbellate*, as viewed by SDS-PAGE analysis. Highly pure solution of CBP with 14 kDa molecular weight was obtained through spin column chromatography as exhibit in lane 5, Fig. 1. In 2012 some researchers purified a novel chitin binding protein from seed extract of *Moringa oleifera*, which exhibited to control the different pathogen plant diseases[27]. Total protein content of I. umbellate seed to be 110 mg/g was estimated by using Bradford and purification steps revealed in purification of 2.2 fold with 5% yield from one gram of *I. umbellate* seed powder exhibit in Table 1.

Table 1
Purification steps of Chitin Binding protein from one gram of seed powder.

Purification steps	Total Protein (mg)	Purification (times)	Recovery (%)
Crude	110	1	100
Ammonium sulfate fractionation (70%)	60	1.8	54
Spin column	50	2.2	45

Coagulation activity of seed extract and CBP:

Seed crude extract of *I. umbellate* and chitin binding protein exhibited the coagulation activity that changed the turbid water into a solid or semi-solid form. *I. umbellate* seed crude extract and chitin binding protein be seen approximately 85 and 87% of coagulation activity respectively after 120 min as contrasted to 89% *M. oleifera* (Mo) extract; whereas kaolin solution alone revealed 30% of activity. The time kinetics of the coagulation activity of *I. umbellata* crude extract and chitin binding protein in contrast with Mo and kaolin as represented in Fig. 2. While 70% pellets of $(NH_4)_2SO_4$ saturation constant showed the 85% coagulation activity. In one study reported the chitin binding protein isoform 3 – 1 (Mo-CBP3-1).from *M. oleifera* and this protein have antibacterial, thermostable, and water clarifying characteristics[28]. However, at different concentration range from 10–300 µg/ml.

I. umbellate crude extract and highly pure chitin binding protein was used for coagulation activity. Maximum coagulation activity was observed at the concentration between 0.075-0.1 mg/ml of chitin binding protein and protein revealed the low coagulation activity after 0.1 mg/ ml concentration as exhibited in Fig. 3 because flocculation formation become slow after 0.1 mg/ ml. It is reported that natural coagulant polymers will cover the whole surface of the colloidal particles[21]. Therefore, the protein has no charge to bind and stick with suspended particles and further flocculation process does not take palace. In 2021, Udayakumar reported that due to nature of colloidal particles become stable and could not to bind further with the natural coagulant[29,30]. Coagulation activity of natural coagulant depends on the turbidity of water, extraction and isolation method and ion present in the water sample[26]. In this study a natural coagulant chitin binding protein revealed a coagulant characteristic against the kaolin clay solution prepared with tap water.

Coagulation activity of CBP at different pH values

CBP revealed the maximum turbidity removal at pH 12 and 10 respectively against pond water as a represent in Fig. 4, while at pH 3 and 5 showed low coagulation activity against pond water. CBP showed the maximum coagulation activity i.e. 81% and 80% against turbid pond water at pH 12 and 10 respectively, while CBP revealed the 74% and 68% coagulation activity against the clay solution at pH 12 and 10 respectively. *I. umbellata* crude extract and Mo crude showed the 81%, 81% and 82% coagulation activity against the pond water at pH 7.0, 12 and 10 respectively, while *I. umbellata* crude extract and Mo crude observed low coagulation activity at pH 3 and 5 i.e. 49% and 68% respectively. Similar results reported[31] Okuda et al. in 2011 from the *Moringa oleifera.* Natural coagulants isolated from chestnut and acorn revealed the turbidity removal[32]. Zhang and his coworkers in 2006[33] reported that cactus showed a coagulant in water treatment

Coagulation activity of Chitin binding protein on turbid pond water

The competence of CBP was checked against the pond water and compared with Mo aqueous seed extract for coagulation activity. In this activity pond water was used as a control. Both Mo aqueous seed extract and CBP revealed the coagulation activity against the collected pond water.

Figure 5 exhibit that CBP is good natural coagulant and have the approximately 83% than Mo 82% coagulation activity against the pond water. However, Mo extract revealed the 89% coagulation activity against the synthetic clay solution as compared to CBP of *I. umbellate* as represented in Fig. 2. This result showed that are variability in the competencies of natural coagulant proteins according to the nature of turbid water, nature of synthetic clay solution.

The molecular weight of chitin binding protein from *I. umbellate* around 14 kDa reported[22] in his research work and he reported that this protein have 100% sequence homology with the chitin-binding protein of *Moringa oleifera* (MoCBP3-1) (UniProt ID: W5S2D2). In 2001 Okuda and his coworkers described that the coagulation activity depends on the extraction methods and nature of selected seeds[31]. Similarly, different ranges of molecular weight 12 and 6.5 kDa proteins from *Moringa oleifera* revealed the coagulation activity against the pond water[34].

This study result revealed that CBP of *I. umbellata* is responsible for the coagulation activity against turbid pond water.

CONCLUSIONS

The objective of the study is to work out the active natural coagulant chitin binding protein for coagulation with respect of *I. umbellata* seeds. It was purified by spin column chromatography with a molecular weight of 14 kDa, as revealed on SDS-PAGE. Chitin binding protein was utilized to treat water and remove turbidity. Maximum turbidity reduction was observed at pH 7. Chitin binding protein showed 87 and 83% coagulation activity against the synthetic clay solution and pond water respectively. This simple and cheap alternative for water treatment is feasible and environment -friendly in the present era of climate change and harsh environmental conditions.

Abbreviations

Coagulation binding protein (CBP),

Declarations

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Conflict of interest:

All authors declare no conflict of interest

Authors Contributions:

[US] idea formulation and design the experiment. [AR] added valuable suggestions during experiment and manuscript improvement. [BK] contributed various inputs during the manuscript preparation and supervision. [AAS], [MI] performed data analysis and design the manuscript. Validation, funding contributed by [AA]. [BS], [SS]involved in drafting article, revision, arranged the figures, tables and manuscript description. [MKG] approved the final version to be published. All the authors read and approved the finalized manuscript for publication.

Ethics approval and consent to participate

Notapplicable

Consent for publication

All authors give permission to the publisher to publish this research work.

Availability of data and materials

All data is presented in this manuscript. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no known competing financial interest.

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Figures

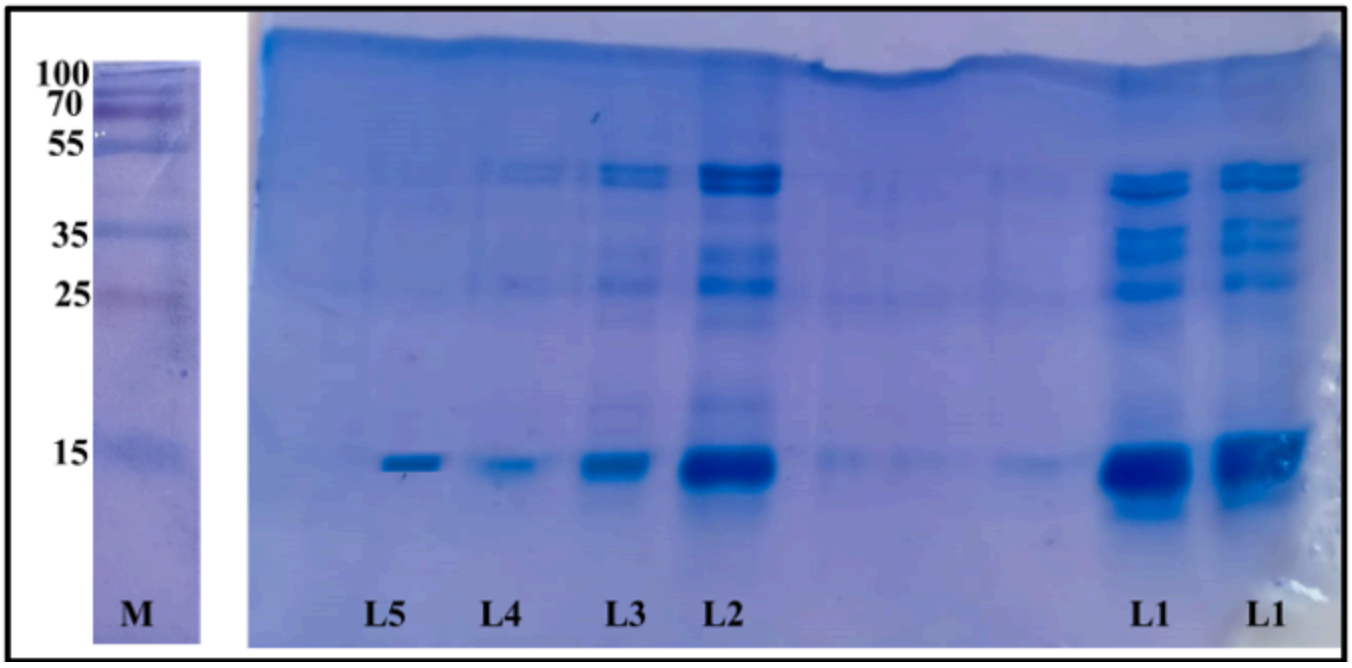

Figure 1

Original gel of extraction and purification of chitin-binding protein from the seed extract of *I. umbellate.* 12% SDS-PAGE reveal of *I. umbellata* purified protein comprising lane M for molecular weight standards, lane L1 for crude extract, lane L2 for 30% pellet of $(NH4)_2SO_4$ saturation constant, lane L3 and lane L4 for 50 % and 70% pellets of $(NH_4)_2SO_4$ saturation constant, respectively, and lane L5 for highly pure chitin-binding protein from spin column chromatography.

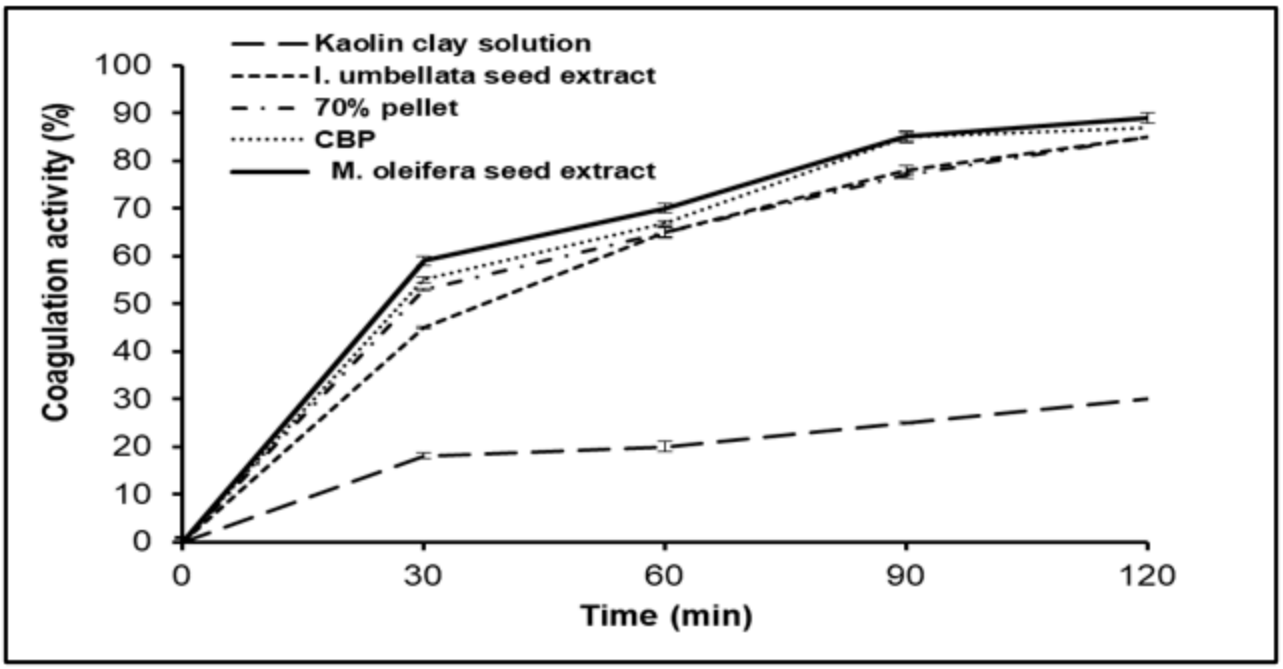

Figure 2

Time kinetics of coagulation activity using crude extracts of *I. umbellata* and Moringa seeds at pH 7 settling time was up to 120 min. Clay was used as a negative control

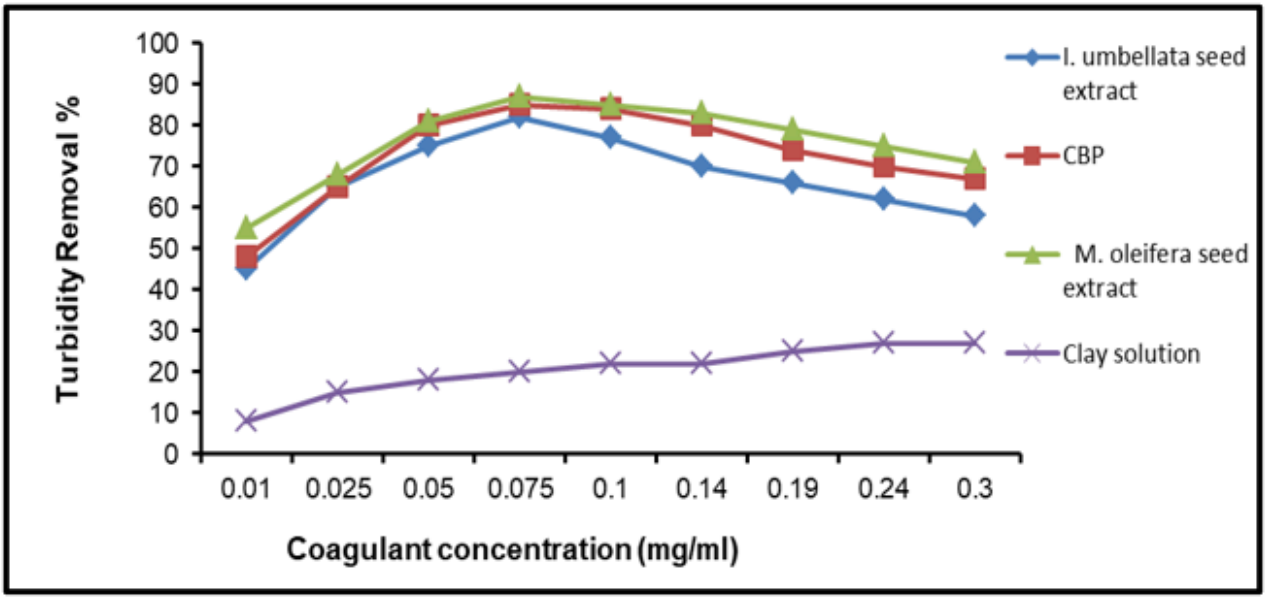

Figure 3

Coagulation activity of the chitin binding protein from *I. umbellata* seeds in turbidity meter was measured at pH 7. Clay and Mo extract worked for as a positive and negative control respectively, dosages of 0.01 to 0.3 mg/ml of chitin binding protein was used.

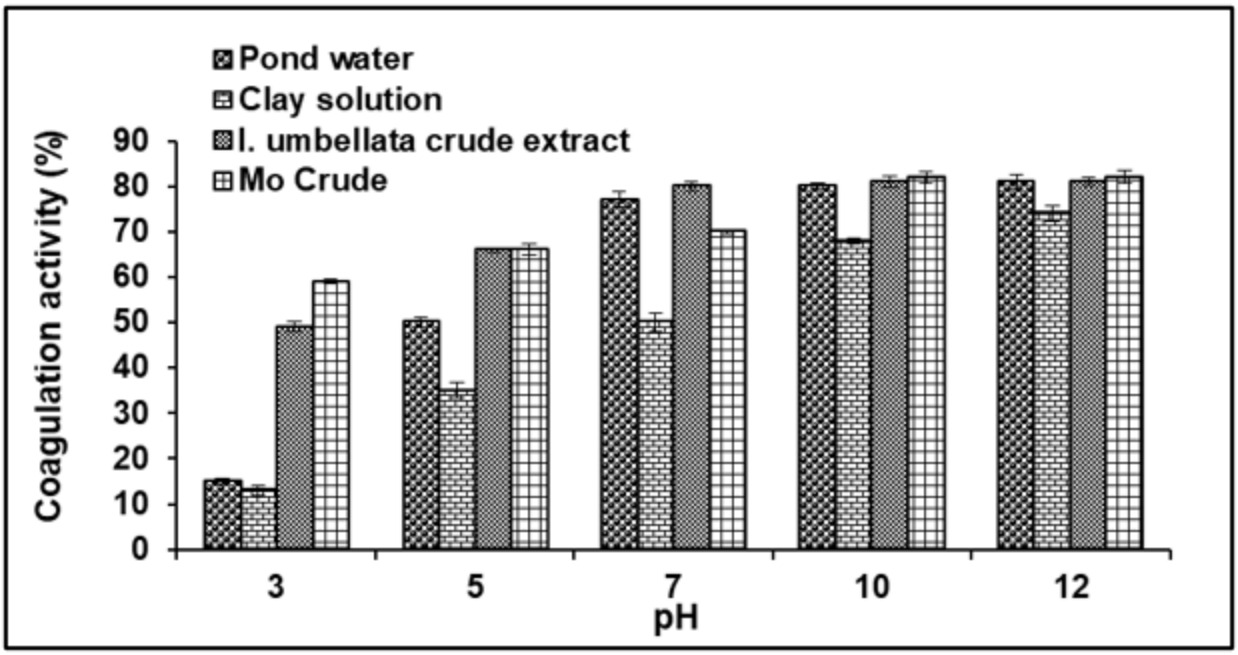

Figure 4

Coagulation activity of CBP, Moringa seeds extract and crude extract of *I. umbellata* at different pH values (3, 5, 7, 10 and 12) against pond water and clay solution.

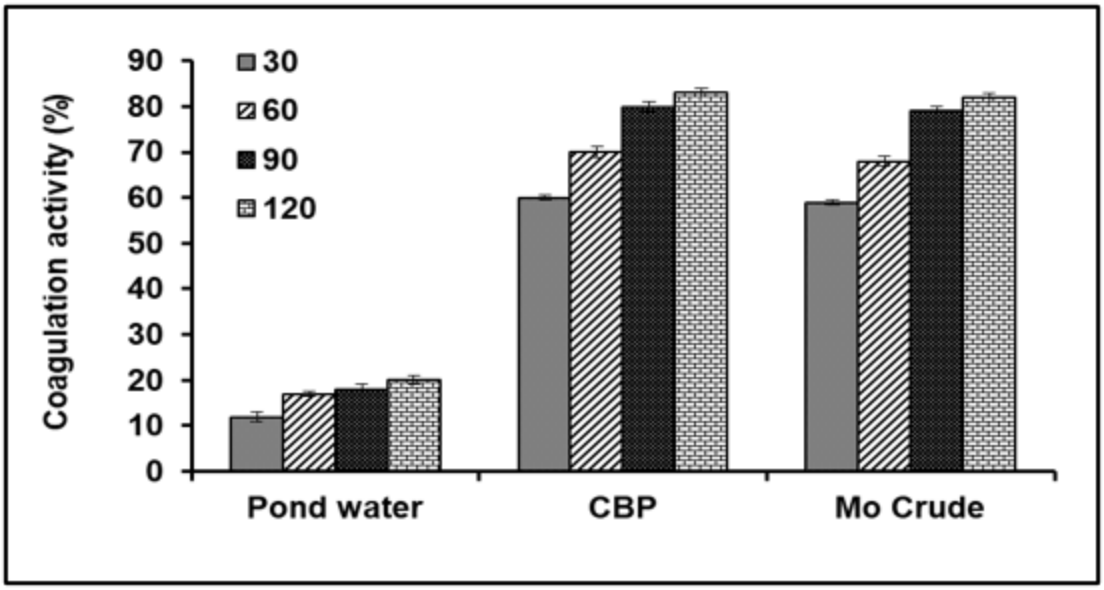

Figure 5

Coagulation activity of CBP and Moringa seeds extract prepared with 100 mM phosphate buffer (pH 7.5) against turbid pond water. Samples for measurement of turbidity are taken after 30, 60, 90 and 120 min.

Supplementary Files

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