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Research article

Efficacy of a natural coagulant protein from *Moringa oleifera* (Lam) seeds in treatment of Opa reservoir water, Ile-Ife, Nigeria

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ABSTRACT

Health related and environmental side effects associated with conventional chemical coagulants used in water treatment has prompted the search for natural alternatives, especially of plant origin. This study investigated the water coagulation activities of a purified protein from *Moringa oleifera* seeds on the water from Opa reservoir of Obafemi Awolowo University, Ile-Ife. *M. oleifera* coagulant protein (MoCP) was purified via ion exchange and gel filtration chromatography respectively. Subunit and native molecular weight as determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and gel filtration was 14.2 kDa and 30.3 kDa respectively. Modified jar test was used to investigate the coagulation activity of the purified protein in comparison to that of conventional chemical coagulant (aluminium sulphate). MoCP significantly reduced turbidity ($p < 0.05$) and organic load which contributed to about 58.18% reduction in total coliform of treated water. MoCP also elicited promising antimicrobial activity against bacterial isolates in the water from Opa reservoir.

1. Introduction

Adequate supply of water is of vital importance to human survival. Over-exploitation of this unique resources can result in scarcity (Van Loon and Van Lanen, 2013). Recent trends in development is consequentially diminishing availability of potable water due to upsurge in pollution as a result of booming economic activities, contributing to the annual death of more than 2 million children under age 5 from water-borne and pneumonia related disease (Muhammad et al., 2013; Gopal et al., 2018). Water shortage is more prevalent in rural communities of developing countries mostly due to inadequate water and waste treatment facilities (Kuta et al., 2014; Oludairo and Aiyedun, 2016; Israr et al., 2017). In addition, rapid population growth and low level of personal hygiene also contribute to this menace, which might result in water-borne pathologies (Isikwue and Chikezie, 2014; Thliza et al., 2015).

Quality of water meant for drinking purposes is determined in terms of physical, chemical and biological parameters and its monitoring is extremely important as chronic consumption of contaminated water has been known to accelerate the onset of many ailments (Mirzabeygi et al., 2017; Soleimani et al., 2018). Also, elevated concentrations of organic and suspended particles in water raise water turbidity, serving as media of transmitting pathogenic organisms, hence, turbidity removal is an important process in water treatment (Choubey et al., 2012). Removal of turbidity in water treatment is basically achieved via simple and cheap processes such as coagulation in most rural and developing communities (Raghuwanshi et al., 2002; Miller et al., 2008); coagulation is mostly achieved via addition of conventional chemicals such as aluminum and ferric salts (Teh and Wu, 2014; Rocha et al., 2019). Aside from generation of toxic large sludge volume by these chemical coagulants, aluminium originally has been implicated as possible trigger of neurological disorder (Sethi et al., 2008; Ribes et al., 2010; Walton, 2013; Farhaoui and Derraz, 2016; Carnacho et al., 2017). In addition, chlorination method used in many developing nations to disinfect water could result in several by-products with long-term harmful effects. In order to address these challenges, natural coagulants are being sourced from plants as they are usually neither toxic nor corrosive like their chemical counterparts (Hussain and Haydar, 2019; Iqbal et al., 2019; Megersa et al., 2019; Zaid et al., 2019).

Moringa oleifera Lam. belongs to the *Moringaceae* family, although considered a non-indigenous species, *M. oleifera* has found wide acceptance among various ethnics in Nigeria and many countries, and is being exploited for different uses such as food, medicine, fodder and recently in water purification (Aduro and Ebenso, 2019; Okuda and Ali, 2019; Saa

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et al., 2019). Water treated with *M. oleifera* crude seed extracts have low shelf life due to increase in organic loads, hence, isolation of the active coagulating agent becomes imperative (Ghebremichael et al., 2005; Baptista et al., 2017). This study therefore illuminates the physico-chemical properties of coagulant protein purified from *M. oleifera* seeds and the quality parameters of the water treated with the protein, with a view to investigating its potential in point-of-use water treatment.

2. Materials and methods

2.1. Reagents

β-mercaptoethanol, sodium dodecyl sulphate are products of BDH Chemical Limited, Poole, England. Acrylamide, N,N,N′,N′-tetamethyl ethylenediamine (TEMED), ammonium acetate, kaolin, bovine serum albumin (BSA), folin ciocalteau, egg albumin, α-chymotrypsinogen and low molecular weight protein markers for electrophoresis were purchased from Sigma Chemical Company, St Louiz, Mo, USA. Blue dextran, CM-sephadex C-50, sephadex G-75, Biogel P-100 are products of Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals and reagents used were of analytical grade.

2.2. Collection and identification of M. oleifera Lam seed

Mature fresh pods containing the seeds of *M. oleifera* Lam were obtained from a local farm in Ile-Ife, South-Western Nigeria. Seeds were identified and authenticated at the IFE HERBARIUM, Department of Botany, Obafemi Awolowo University, Ile-Ife where the plant with voucher specimen number IFE – 17627 was deposited.

2.3. Preparation of the crude extract of M. oleifera seed

M. oleifera seeds were removed from the pod, washed with distilled water, air-dried at room temperature and ground into fine powder using laboratory mortar and pestle. The deffated powdered sample was weighed and suspended in 10 mM ammonium acetate buffer, pH 6.8 in the ratio 1:10 (w/v). The mixture was stirred for 18 h on a magnetic stirrer and homogenate was kept overnight at 4 °C. Resultant mixture was then centrifuged at 3500 rpm for 20 min. The supernatant was collected and stored at -20 °C as the crude extract of *M. oleifera* seed.

2.4. Preparation of artificial turbid water

Artificial turbid water was prepared using the method of Ghebremichael et al. (2005). Typically, a kaolin clay suspension was prepared by adding 10 g of finely ground kaolin clay to 1 L of tap water. Resulting suspension was stirred for 30 min using magnetic stirrer at 200 rev min^{-1}. Resulting solution was allowed to settle for 24 h to enhance complete hydration of the clay particles and aliquot of the supernatant removed. Desired optical density of the supernatant was obtained by dilution with tap water and absorbance was read at 500nm.

2.5. Coagulation assay

Coagulation activity of the protein was assayed using a small volume of turbid water sample prepared from 1% (w/v) kaolin clay using the 1 ml cuvette test experiment of Ghebremichael et al. (2005) as described by Marobhe et al. (2007). Briefly, 2.7 ml of kaolin water with initial turbidity of about 450–500 NTU corresponding to optical density (OD_{500nm}) of 1.4–1.6 obtained by dilution was employed for this assay. Investigation of the active coagulant was done by continuous recording of optical density at 500nm (OD_{500nm}) to evaluate and to observe settling properties of the flocs generated. Aliquots (0.3 ml) of *M. oleifera* coagulant protein (MoCP), 5% (w/v) aluminum sulphate (positive control) and distilled water (negative control) were added to different 4 ml plastic cuvettes respectively. Resulting solutions were mixed for 3 min using a 1-ml pipette (HTL, Poland), and samples were allowed to settle for 1 h at room temperature. Aliquot of 900 μl from the top of the solution was transferred into a 1 ml plastic cuvette. Absorbance was measured at 500nm using a UV-Visible spectrophotometer (INESA) at time 0 (initial absorbance) and at 5 min intervals for 60 min. Reduction in absorbance relative to the negative control (prepared by adding distilled water to kaolin suspension) defines coagulation activity. Coagulation activity was calculated as follows:

$$\text{Coagulation activity} = \frac{Rt^* - Rt}{Rt^*} \text{ x } 100 \quad (1)$$

Where Rt* = Residual turbidity (OD_{500nm}) measured instantly at time 0

Rt = Residual turbidity (OD_{500nm}) measured at specific time intervals for 60 min.

2.6. Protein concentration determination

The protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin (1 mg/ml) as standard protein.

2.7. Purification of M. oleifera coagulant protein (MoCP)

2.7.1. Ion exchange chromatography on CM-sephadex C-50

CM-Sephadex C-50 (3 g) was swollen in 50 ml of 10 mM ammonium acetate buffer (pH 6.8) for 72 h and packed into column (1.5 × 20 cm). Approximately 27 mg (3 ml) of the crude extract was layered on the column and fractions (1 ml) were collected at a flow rate of 20 ml/h. Unbound fractions were eluted using the equilibration buffer (10 mM ammonium acetate buffer) while the bound proteins were eluted with the same buffer containing 0.6 M NaCl. The absorbance was read at 280nm and fractions assayed for coagulation activity after exhaustive dialysis against 10 mM ammonium acetate buffer and distilled water for 48 h. The active peak was pooled, lyophilized and stored at -20 °C.

2.7.2. Gel filtration chromatography on Sephadex G-75

Sephadex G-75 (15 g) was swollen in 250 ml of distilled water for 72 h and packed into a column (2.5 × 40 cm). The column was equilibrated with 10 mM ammonium acetate buffer pH 6.8. The CM active fraction (4 ml containing approximately 20 mg of protein) was layered on the column and fractions (4 ml) were collected at a flow rate of 30 ml/h. The absorbance was read at 280nm and the fractions assayed for coagulation activity. The fractions in the active peak were pooled, lyophilized and stored at -20 °C.

2.8. Characterization of MoCP

Subunit molecular weight of MoCP was determined by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) as described by Eghianruwa et al. (2011) using the following standard protein markers: Myosin (Mr 200,000), β-galactosidase (Mr 116,000), Phosphorylase b (Mr 97,000), Albumin (Mr 66,000), Glutamic Dehydrogenase (Mr 55,000), Ovalbumin (Mr 45,000), Glyceraldehyde-3-phosphate Dehydrogenase (Mr 36,000), Carbonic Anhydrase (Mr 29,000), Trypsinogen (Mr 24,000), Trypsin Inhibitor (Mr 20,000), α-lactalbumin (Mr 14,200) and Aprotinin (Mr 6,500). The native molecular weight was estimated by gel filtration column chromatography (70 × 1.5 cm) using Biogel P-100 matrix according to the method described by Akinyoola et al. (2016). A molecular calibration curve was prepared using the following protein markers, bovine serum albumin (Mr 66,000; 5 mg/ml), egg albumin (Mr 45,000; 5 mg/ml) and α-chymotrypsinogen (25,000; 5 mg/ml). Each of the standard protein (5 ml) was layered on the column and ran separately using 10 mM phosphate buffer, pH 7.2 as eluant at a flow rate of 10 ml/h. Fractions (5 ml)

were collected and the elution was monitored for each protein at 280nm. The void volume (Vo) of the column was determined using blue dextran, elution of which was monitored at 620nm.

The effect of temperature on coagulation activity of MoCP was carried out as described by Ghebremichael et al. (2005). Typically, aliquots (0.3 ml) of MoCP were incubated in a water bath (Cole Medical CU 600 model) at temperatures ranging from 40 to 100 °C for 1 h. The samples were removed from water bath, rapidly cooled on ice and assayed for coagulation activity. Results were expressed relative to the control kept at 25 °C.

The effect of pH on coagulation activity was determined by incubating aliquots (0.3 ml) of MoCP for 1 h with buffers of different pH values ranging from pH 3–12. The buffers used were 0.2 M sodium acetate buffer, pH 3–6; 0.2 M phosphate buffer, pH 7–8; and 0.2 M glycine-NaOH buffer, pH 9–12. The control was the coagulation activity of the protein in ammonium acetate buffer, pH 6.8. Results were expressed relative to the coagulation shown by control.

2.9. Water sampling and coagulation assay

Water sample was collected at the onset of rain season from Opa water reservoir located 07°27' N, 04°33' E within the Obafemi Awolowo University, Ile–Ife, Osun State, Nigeria, into new 10 L double-capped polyethylene bottles prewashed with doubly deionised distilled water, before influx of water into the aeration chamber of the reservoir. Different concentrations (20, 40, 60, and 80 mg/L) of MoCP and 5% (w/v) aluminum sulphate (alum) were added to glass jars containing 1 L of water samples respectively as described by Marobhe et al. (2007), keeping the first jar as blank (with no coagulant). Each jar was mixed for 3 min to disperse the coagulants which enhance particle collision and formation of larger stable flocs. The flocs were allowed to sediment for 1 h. Sampling bottles and covers were rinsed three times with the water to be sampled prior to collection and water samples were freshly collected (approximately 10 cm below water surface) at once for all analysis. All experiments were done in triplicates at room temperature.

2.10. Determination of water quality parameters

Water safety was investigated by measuring quality parameters of water treated with MoCP, 5% (w/v) aluminium sulphate (positive control) and distilled water (negative control) after sedimentation, using standard methods for examination of water and wastewater (Baird et al., 2012; Adams, 2017; Rice et al., 2017). Parameters analyzed were turbidity, pH, total dissolved solids (TDS), electrical conductivity, alkalinity, electrolytes (Ca^{2+} and Mg^{2+}), total hardness, nitrate, sulphate and oxygen parameters (chemical oxygen demand and biochemical oxygen demand). The three-tube procedure using lactose broth was used to detect the presence of coliform and to determine the most probable number (MPN) of coliform bacilli (Bakare et al., 2003).

2.11. Investigation of antibacterial activity of MoCP

2.11.1. Isolation and identification of bacterial isolates

Existing bacteria were isolated and identified from the raw water using standard microbiological techniques as described by Bergeys manual for determinative bacteriology (Buchanan and Gibbons, 1974). Typically, cell morphology (shape, arrangement and gram reaction) was used in differentiating the isolates. Primary identification of isolates was carried out by sub-culturing into Eosin Methylene Blue (EMB) agar, MacConkey agar, Triple Sugars Iron (TSI) agar for differentiating enteric bacilli and SIM agar medium for motility, indole and hydrogen sulfide (H_2S) gas production. Further confirmatory biochemical reactions in the identification process were carried out by employing various tests such as nitrate reduction, sugar fermentation, Methyl Red-Voges Proskauer (MRVP), catalase and citrate utilization.

2.11.2. Determination of antibacterial activity of MoCP

The agar well diffusion method was used to determine the antibacterial activity as described by Igbinosa et al. (2009). Briefly, test bacteria isolates were cultivated on nutrient broth at 37 °C for 24 h. The inoculum size was adjusted turbidimetrically to 0.5 McFarland turbidity standard at 600nm. Bacteria isolates were inoculated over the entire surface of freshly prepared Mueller Hinton Agar (MHA) in a 9 cm Petri plate. With the aid of a sterile cork borer, four wells of about 6 mm in diameter were bored on the plates equidistant from the center. Equal volume (50 µl) of MoCP (10 mg/ml, 20 mg/ml and 40 mg/ml) and standard drug (streptomycin 1 mg/ml) were dispensed into each well. Loaded plates were incubated at 37 °C for 48 h. A transparent ring around the agar plate signified antibacterial activity. Zone diameters (mm) around each of the wells were measured to the nearest mm.

2.12. Statistical analysis

Data obtained were expressed as mean ± standard deviation, analyzed statistically using one-way analysis of variance (ANOVA). Differences were considered statistically significant at the p-value of <0.05.

3. Results and discussion

3.1. Purification of MoCP

Results from our investigations showed that *M. oleifera* coagulant protein (MoCP) was purified to homogeneity via combination of ion exchange and gel filtration chromatography with a fold of about 5.27 (Table 1).

The elution profile of the ion exchange chromatography (IEX) of *M. oleifera* crude extract on CM-Sephadex C-50 column showed two protein peaks, only one of which (CM-2) exhibited coagulation activity using kaolin turbidity of 420 NTU after 60 min settling time (Figure 1). Gel filtration of the active peak CM-2 on Sephadex G-75 column resulted in two peaks (Figure 2), coagulation activity resided substantially in peak 2 (GF-2) which constituted the homogenous MoCP used for this study.

3.2. Molecular weight and stability of MoCP

Native molecular weight of MoCP as estimated by gel filtration chromatography was 30.3 kDa, while SDS – PAGE revealed a single band with subunit molecular weight of 14.2 kDa, suggesting a homodimeric structure for the coagulant protein (Figure 3). Previous studies have reported the active coagulating agent of *M. oleifera* seeds have molecular

Table 1. Summary of purification procedure.

Fraction	Total Protein (mg)	Total Activity (HU)	Specific Activity (HU/mg)	Percentage Yield	Purification fold
Crude Extract	276.50	0.7135	0.0026	100	1
CM Sephadex Peak 2 (CM-2)	55.53	0.6761	0.0111	20.10	4.26
Gel filtration Peak 1 (GF-1)	4.05	0.3697	0.0098	1.46	3.77
Gel filtration Peak 2 (GF-2)	48.30	0.6626	0.0137	17.47	5.27

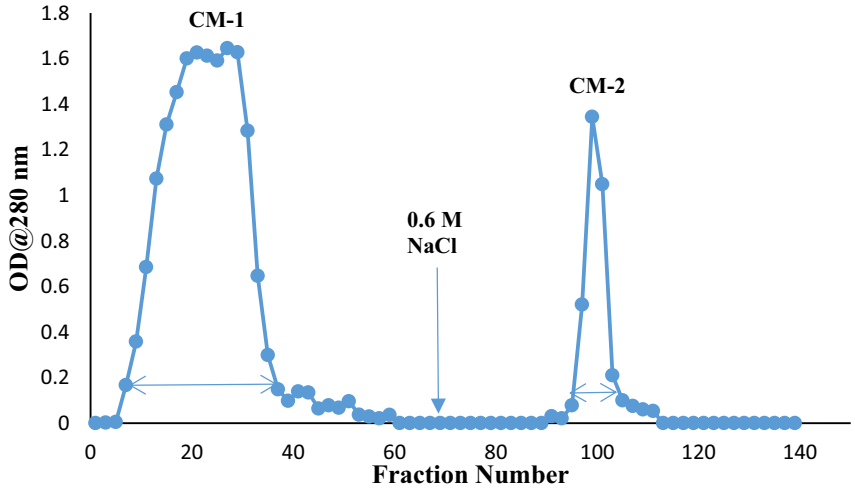

Figure 1. Ion exchange chromatography (IEX) of crude extract of *M. oleifera* seeds on CM-Sephadex C-50. Crude extract (approximately 27 mg) was layered on CM-Sephadex C-50 column (1.5 × 20 cm) previously equilibrated with 10 mM ammonium acetate buffer, pH 6.8. Fractions (1 ml) were collected at flow rate of 20 ml/h. Elution were monitored at 280nm. Two peaks were obtained (CM-1 and CM-2) with coagulation activity residing in peak CM-2.

weight ranging between 6.5 – 14 kDa (Gassenschmidt et al., 1995; Ghebremichael et al., 2005), in fact, Choudhary and Neogi (2017) reported that proteins of molecular weight less than 36 kDa isolated from *M. oleifera* seeds using 60–80% ammonium sulphate saturation exhibited a high turbidity removal against synthetic (kaolin) turbid water. Also, Santos et al. (2009) reported a 26.5 kDa coagulant lectin (cMoL) from *M. oleifera* seeds. These variations with respect to molecular weight might be due to environmental and ecological distribution of *M. oleifera* plant.

Several parameters contribute to the effectiveness of coagulants during water treatment processes, which include: temperature, coagulant dosage, pH, type and concentration of contaminants among others. The coagulation activity of MoCP was relatively stable at pH 3–6, slightly increased at pH 7 and remained stable at alkaline pH 8–12. MoCP was also found to be thermostable, as the coagulation activity was not affected at tested temperature range of 40–90 °C, maintaining a percentage turbidity removal of 78.85 ± 0.32% with kaolin suspension (1%), which declined to 46.39 ± 0.11% at 100 °C (Figure 4). Coagulant lectin (cMoL) isolated from *M. oleifera* seeds was also reported to be thermostable at 100 °C after 7 h heat treatment, while purified coagulant proteins from *Vigna unguiculata* and *Parkinsonia aculeata* seeds maintain activity of 92 and 94% respectively on heating at 80 °C for 2 h (Marobhe et al., 2007; Santos et al., 2009).

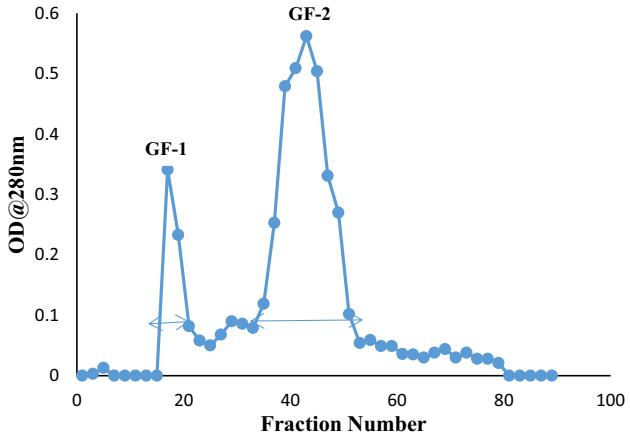

Figure 2. Gel filtration chromatography of fraction CM-2 (adsorbed peak) on sephadex G-75. Peak CM-2 (approximately 20 mg) was layered on sephadex G-75 column (2.5 × 40 cm) previously equilibrated with 10 mM ammonium acetate buffer, pH 6.8. Fractions (4 ml) were collected at flow rate of 30 ml/h. Elution were monitored at 280nm. Two peaks were obtained (GF-1 and GF-2) with coagulation activity residing more in peak GF-2.

3.3. Water quality parameters

Opa reservoir having a catchment area of approximately 116 km^2, extending in width from longitude 04°31' E to 04°39' E and in length from latitude 07°21'N to 7° 35'N was primarily constructed to supply potable water to the Obafemi Awolowo University community. However, due to

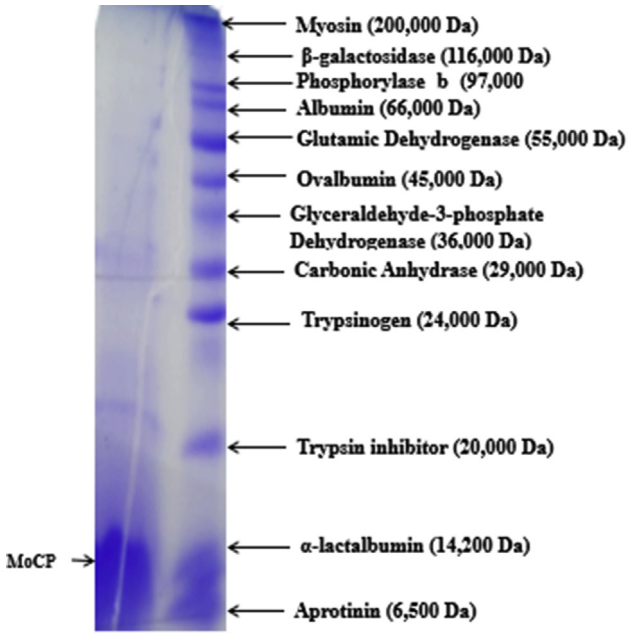

Figure 3. SDS-PAGE of *M. oleifera* Coagulant Protein (MoCP) (Lane 1), and molecular weight standards (Lane 2). 10 μg protein samples were loaded into each well and gel was stained with Coomassie blue. Protein markers used were; Myosin (Mr 200,000), β-galactosidase (Mr 116,000), Phosphorylase b (Mr 97,000), Albumin (Mr 66,000), Glutamic Dehydrogenase (Mr 55,000), Ovalbumin (Mr 45,000), Glyceraldehyde-3-phosphate Dehydrogenase (Mr 36,000), Carbonic Anhydrase (Mr 29,000), Trypsinogen (Mr 24,000), Trypsin Inhibitor (Mr 20,000), α-lactalbumin (Mr 14,200) and Aprotinin (Mr 6,500), MoCP (Mr 14,200).

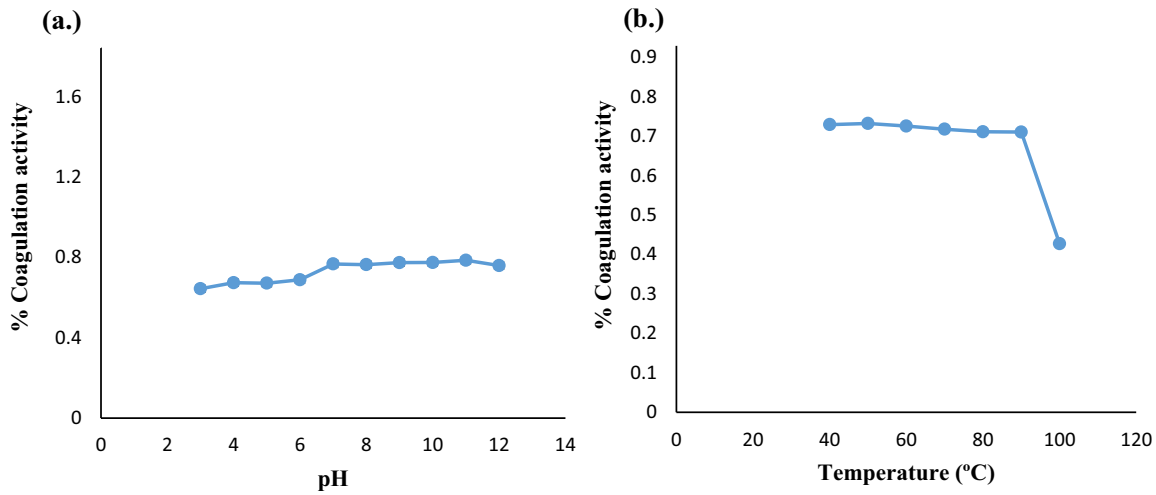

Figure 4. Effect of (a) pH and (b) temperature on coagulation activity of MoCP.

dredging activities over the years, it now has direct linkage to township drains (Adesakin et al., 2016). In effective water treatment processes, monitoring coagulant dosage is highly essential. Dosage at which highest degree of turbidity removal is achieved is termed optimum coagulant dosage (OCD), as dosages beyond OCD for specific water might increase water turbidity due to charge restabilization (Yuliastri et al., 2016). Optimum coagulant dosage of MoCP in the treatment of low turbid water (< 50 NTU) was 60 mg/L, culminating to significant decrease ($p < 0.05$) in residual water turbidity from 41.19 ± 1.5 NTU to 23.51 ± 1.03 NTU (Figure 5a). This resulted in average percentage turbidity removal of 40.77 ± 2.59% and 19.09 ± 1.62% respectively for MoCP and alum-treated water. Functional groups on the side chain of basic amino acids (arginine, lysine and histidine) are suggested to contribute to the water coagulation activity (Subramanium et al., 2011). Obtained result is in slight variance with the recommendation stipulated by Nigerian Standards for Drinking Water Quality and World Health Organization standards of less than 5 NTU (NSDQW, 2007; WHO, 2011) which might be influenced by relatively low turbidity level of water prior to treatment, as coagulants are reportedly more efficient in relatively highly turbid water (Asrafuzzaman et al., 2011). Increased sedimentation time might also improve residual turbidity removal of MoCP, as reported for *M. stenopetala* coagulant protein (Megersa et al., 2019).

Bicarbonate and carbonate (HCO_3^- and CO_3^{2-}) ions concentrations contributes largely to water alkalinity, which varies to a great extent due to differences in water geology. Although, alkalinity is not toxic to humans, however, ample concentration in water is usually associated with high dissolved solids and hardness (McNeely et al., 1979). MoCP significantly decrease ($p < 0.05$) the water alkalinity in a dose-dependent manner (Figure 5b), contributing to simultaneous removal of total hardness (Figure 5a). The result of this work is in agreement with previous studies where coagulation activities of purified protein from *Parkinsonia aculeata* seeds was documented (Marobhe and Gunno, 2013).

Mean hydrogen ion concentration (pH) of the raw water from Opa reservoir recorded in this study as at time of water collection was 6.95 ± 0.11, which falls within the recommended pH range 6.5–8.5 (WHO, 2017). Although, human health is not directly impaired by pH of drinking water, however, pH can indirectly affect ion solubility and pathogen survival. More so, acid and alkaline water can lead to corrosive and bitter taste (Shah et al., 2012; Khan et al., 2013: Radfard et al., 2019). There was no significant increase ($p > 0.05$) in the pH of water treated with MoCP (Figure 5b), in contrast to aluminium sulphate, which might result in the water being acidic as trivalent cation of aluminium could serve as Lewis acid (Theodoro et al., 2013).

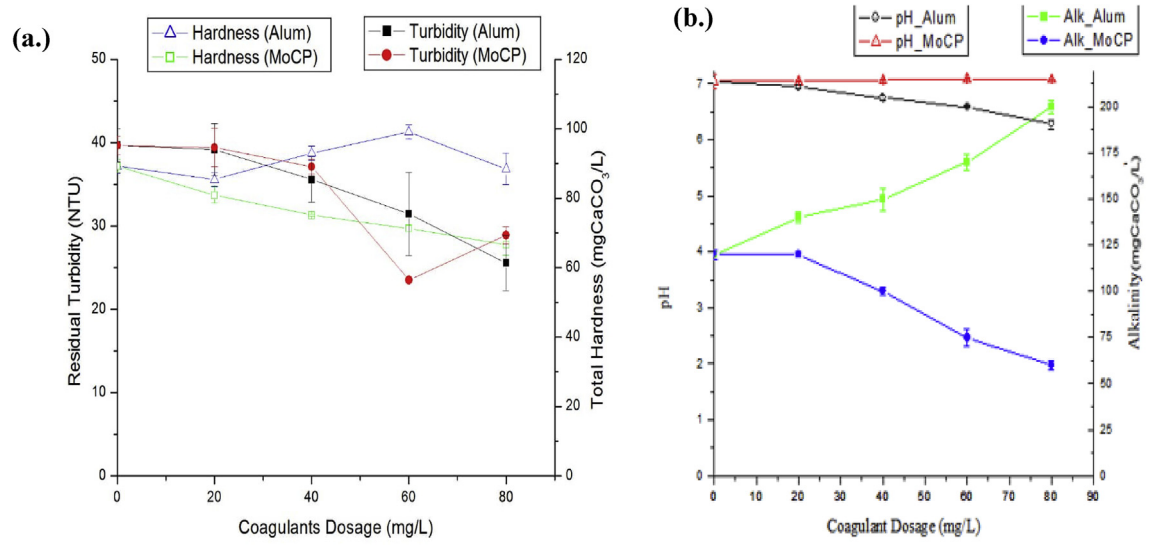

Figure 5. (a.) Effect of MoCP and Aluminium sulphate (Alum) on Residual turbidity and Total hardness of treated water. (b.) Effect of MoCP and Aluminium sulphate (Alum) on pH and Alkalinity of treated water.

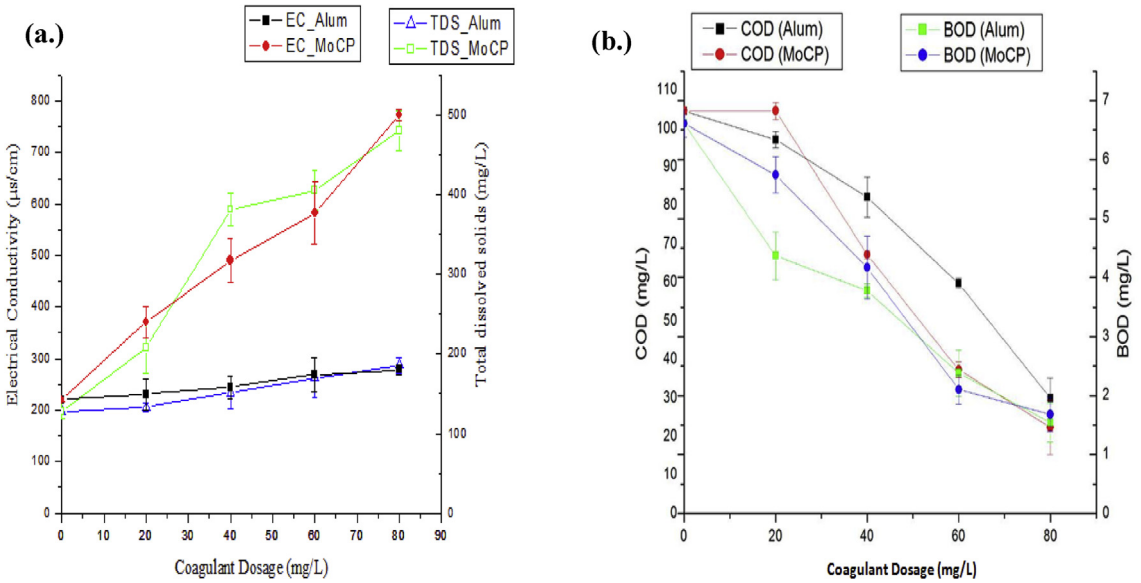

Figure 6. (a.) Effect of MoCP and Aluminium sulphate (Alum) on Electrical conductivity and Total dissolved solids of treated water. (b.) Effect of MoCP and Aluminium sulphate (Alum) on Organic loads measured as Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) of treated water.

Electrical conductivity (EC) and total dissolved solids (TDS) values of 219.5 ± 0.5 µS/cm and 128 ± 1 mg/L were recorded respectively in the raw Opa water. However, these concentrations significantly increased ($p < 0.05$) to 582 ± 1.0 µS/cm and 402.5 ± 2.5 mg/L respectively at 60 mg/L MoCP (Figure 6a), which might be due to negligible traces of chloride ion in treated water from residual sodium chloride used in purification process of the coagulant protein (Marobhe, 2013). The result obtained from this study was within the range specified for potable water with respective maximum permissible limits (MPLs) of 1000 µS/cm and 500 mg/L (NSDQW, 2007; Barstow, 2018).

During the course of high water pollution, bacteria present in polluted water breakdown and feed on complex organic matter in the water to simple compounds such as carbon dioxide and water, a process ultimately leading to bacteria multiplication. At 80 mg/L, biochemical oxygen demand concentration of MoCP-treated water decrease significantly ($p < 0.05$) from 6.61 ± 0.23 to 1.68 ± 0.29 mg/L corresponding to 74.6% reduction, which complies with drinking water quality standards of less than 2 mg/L (WHO, 2008). Water with BOD values of 3–5 mg/L is considered fairly clean, while concentration about 10 mg/L indicate water pollution (Oluyemi et al., 2010). In addition, 78.6% reduction in chemical oxygen demand (COD) of MoCP-treated water (Figure 6b) was also observed, a combination which suggested relatively high measure of water clarification.

Combined elevation of calcium and magnesium ions in water can cause or contribute to hardness (WHO, 2008). In the MoCP-treated water, there was no significant increase ($p > 0.05$) in calcium ion concentration in comparison to aluminium sulphate-treated water, however, this was not the same with magnesium ion concentration which decreased from 9.95 ± 1.3 mg/L to 4.44 ± 0.24 mg/L at 60 mg/L coagulant dose (Figure 7a), a concentration higher than the 0.2 mg/L specified (NSDQW, 2007), compared to 11.03 ± 0.31 mg/L in the positive control (aluminium sulphate).

Water palatability was also investigated via sulphate and nitrate concentrations as elevated nitrate and sulphate concentrations in

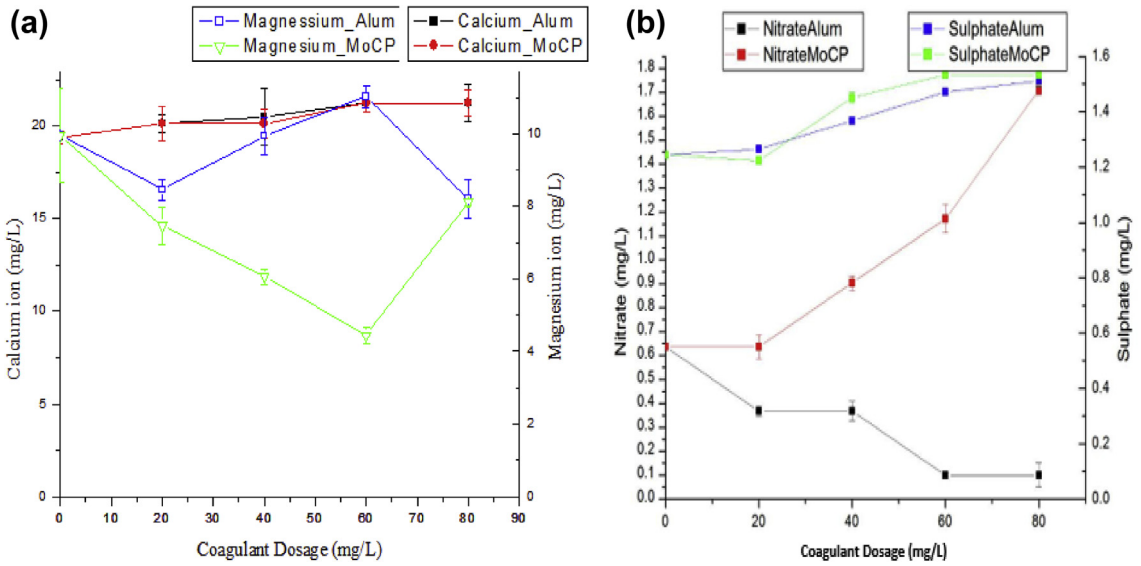

Figure 7. (a.) Effect of MoCP and Aluminium sulphate (Alum) on Cations (Ca^{2+} and Mg^{2+}) of treated water. (b.) Effect of *M. oleifera* coagulant protein (MoCP) and Aluminium sulphate (Alum) on Nitrate and Sulphate of raw water from Opa reservoir.

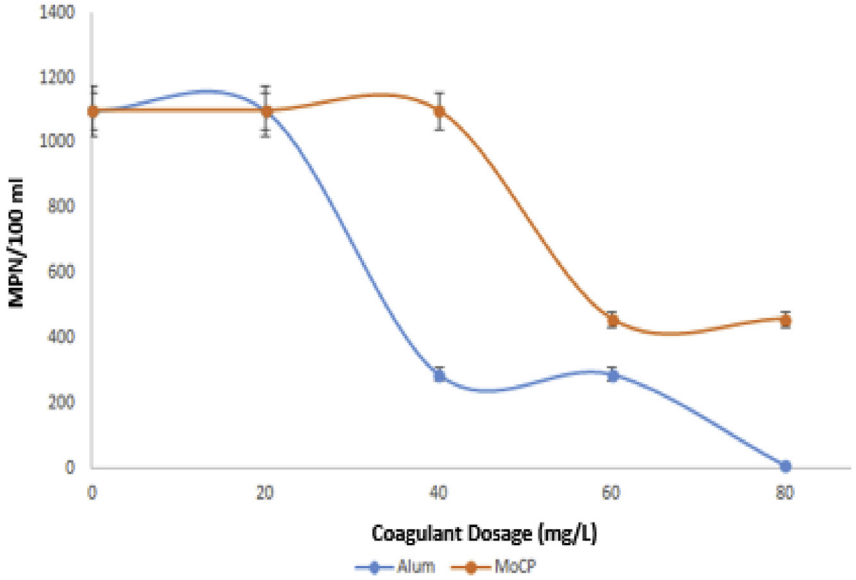

Figure 8. Effect of *M. oleifera* coagulant protein (MoCP) and Aluminium sulphate (Alum) on coliform bacilli count of raw water from Opa reservoir.

drinking water can cause methemoglobinemia in infants under 3 months and diarrhea respectively (NSDQW, 2007). Slight elevation in nitrate concentration of water treated with MoCP from 0.636 ± 0.01 mg/L to 1.708 ± 0.02 mg/L in comparison with aluminium sulphate (alum) was not surprising considering that plant-based coagulants might inevitably add natural seed nitrogen/nitrate to treated water. Nitrate level of up to 3 mg/L is generally believed to be safe for consumption, as elevated concentrations has been implicated in reduced fetal-maternal oxygen transfer which might contribute to abortion (Alhassan and Ujoh, 2012; Chetty and Prasad, 2016). However, there was no significant increase ($p > 0.05$) in sulphate concentration of treated water, as the concentration slightly increased from 1.2445 ± 0.05 mg/L to 1.5123 ± 0.02 mg/L and 1.5329 mg/L in water treated with aluminium sulphate and MoCP at 80 mg/L respectively (Figure 7b).

Poor sanitation contributes tremendously to the presence of coliform bacteria in surface and groundwater and consumption without prior treatment can cause serious water-borne diseases. MoCP reduced the total coliform count in treated water by 58.18% (Figure 8) at 60 mg/L. This might be due to entrapment of microorganisms by settling flocs. However, coliform reduction in aluminium sulphate-treated might be due to low pH, which could have resultant negative effect on microbial growth (Magaji et al., 2015).

3.4. Antibacterial activity of MoCP

Plant proteins with antimicrobial activities are ubiquitous in nature and usually target broad spectrum of microbial pathogens, with their biological activities majorly mediated by interaction with target membrane followed by membrane permeabilization and disruption (Izadpanah and Gallo, 2005; Goyal and Mattoo, 2014; Salas et al., 2015). *In vitro* antibacterial susceptibility by MoCP was determined against five bacterial strains; *Klebsiella edwardsii, Pseudomonas aeruginosa, Klebsiella ozoenae, Alcaligenes feacalis* and *Klebsiella pneumonia* isolated from the raw water, using streptomycin as positive control. Growth inhibition was higher in *Alcaligenes faecalis* at all tested concentrations after 24 h incubation, while growth inhibition in *Klebsiella edwardsii*, *Pseudomonas aeruginosa, Klebsiella ozoenae* and *Klebsiella pneumoniae* increased in a concentration and time-dependent manner after 48 h incubation period (Figure 9). However, at 40 mg/L MoCP, growth inhibition was more pronounced against all tested bacterial

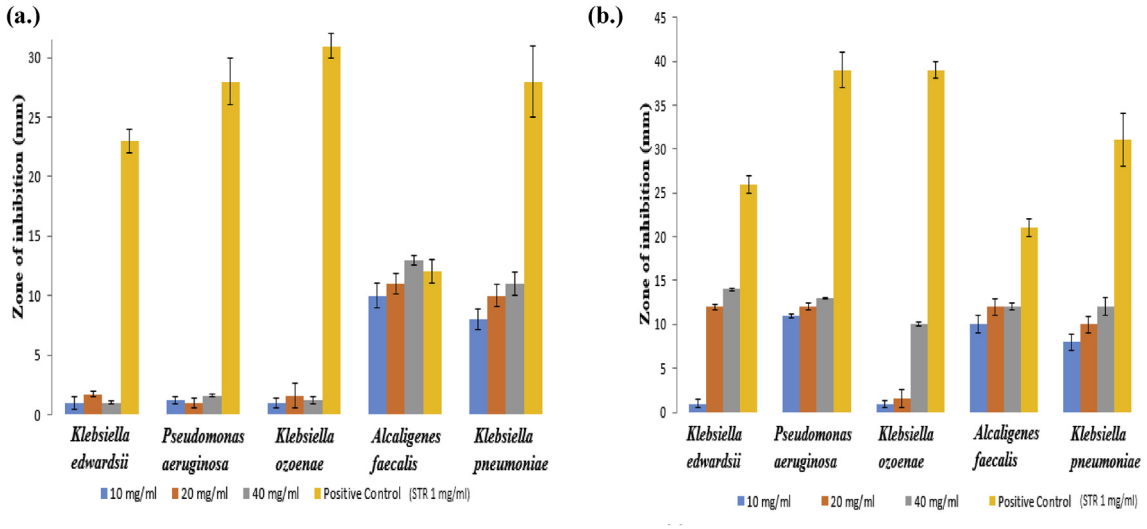

Figure 9. Antibacterial Activity of *M. oleifera* Coagulant Protein on Isolated Bacteria from Opa Reservoir (a.) After 24 h. (b.) After 48 h.

isolates. This result support earlier reports of antibacterial activity of coagulant proteins from *M. oleifera* seeds (Ghebremichael et al., 2005; Ferreira et al., 2011).

4. Conclusion

M. oleifera coagulant protein has shown biocoagulation activity, which is an exploitable property, since the quality parameters of water treated with the protein met most of the Nigerian Standards for Drinking Water Quality. The results of the antimicrobial activities also indicate that the biocoagulant has the potential to be used as a biodegradable active ingredient in water disinfection. However, despite all these benefits, natural coagulants should be effectively applied to the process of water coagulation and treatment in scale only after undergoing tests certifying their non-toxicity, biodegradability and viability. Further studies will still be required to understand the protein-microbe mechanisms of action to fully exploit the antimicrobial potentials of this unique biocoagulant.

Declarations

Author contribution statement

Adewole Scholes Taiwo: Performed the experiments; Analyzed and interpreted the data.

Kuku Adenike: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Okoya Aderonke: Conceived and designed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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