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Original Research Article

Evaluating the Water Treatment Potential of *Hibiscus sabdariffa* Extracts

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ABSTRACT

This study investigated the use of sabdariffa crude extract (SCE) in water treatment using jar test experiments. In the work reported here, varying coagulant doses and pH ranges were tested using synthetic turbid water. The results show that SCE achieved 94 and 85% turbidity removal in 200 and 50 NTU water samples respectively. Similarly, the heat-treated and stored SCE samples recorded 96% and 97% turbidity removal respectively. Additionally, SCE was found to be thermo-stable at various temperature tested which showed improved performance at the end of the treatment process. Furthermore, the pH of the final treated water remained largely unaffected due to buffer effect (i.e. buffer capacity of 0.029) of the protein. Therefore, Hibiscus sabdariffa seed could be a potential water treatment material in developing countries where access to clean drinking water is still a major challenge.

1. INTRODUCTION

Access to clean water is still a major challenge in many parts of the world despite the achievement recorded by the millennium development goal (MDGs) target to half the population without water by the year 2015 (WHO, 2000). It has been reported that more than 663 million people lack access to clean drinking water and a child dies every 15 s in developing countries due to lack of clean drinking water (WHO, 2015). In such areas, conventional water treatment is limited due to high cost of importing equipment and chemicals. However, to provide potable water to the rural people, several studies have shown the potential of naturally-occurring extracts in water treatment (Jahn et al., 1986, Ghebremichael et al., 2005, Choy et al., 2015). *Moringa oleifera* (MO) is the most studied natural extract in water treatment (Ndabigengesere and Narasiah, 1998). In their separate studies, Ndabigengesere and Narasiah (1996) and Diaz et al. (1999) reported the use of MO seed extract and *Cactus latifaria* in reducing turbidity in water which does not also affect the pH of the treated water. MO seed also possesses some remarkable disinfection properties against enteric bacteria (Ghebremichael et al., 2005). Similarly, other natural plants such as *Mustard seeds* and *Okra* seed extracts have been tested as potential water treatment materials, as coagulants and disinfectants (Bodlund et al., 2014, Jones and Bridgeman, 2016a). Although some of the natural extracts tested could compete favorably with

the inorganic chemicals used in water treatment, the main drawback is the addition of organic loads in the final water. However, this challenge was overcome by Okuda et al. (2001) and Ghebremichael et al. (2006) who separately isolated the active protein compounds in MO seed and observed improved adsorption capacity with no trace of released organic nutrients in the treated water. Water treated using natural extracts are presumed to be safe for human consumption because they are primary source of food.

Hibiscus sabdariffa is a shrub belonging to the family of *Malvaceae* plants. It is a primary source of food (vegetable) in many tropical countries of the world, including Nigeria. Rao (1996) performed proximate analysis of the whole mature seeds of two sabdariffa species and found the protein content to be between 18.8 and 22.3% with lipid content of between 19.1 and 22.8%. In Nigeria, a digested sabdariffa seed is used and given to augment low and poor milk production; poor let down and maternal mortality (Okasha et al., 2008). Phytochemical analysis of the seeds by Mungole and Chaturvedi (2011) who showed high concentrations of alkaloids, saponins, steroid ring, deoxy sugar, tannin, phenolic and flavonoids. These compounds are likely the main agents that make them viable in folk medicine. However, there are still some gaps in this area as little information are available regarding the use of *Hibiscus sabdariffa* seed in water treatment. This study aimed to close this gap by investigating the potential of *sabdariffa* seed as a coagulant in drinking water treatment.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of *Sabdariffa Seeds*

Hibiscus sabdariffa seeds were obtained in Hawul Local Government Area of Borno State, Nigeria. Good quality seed was purchased from a local market and the seeds were sorted, packaged and labeled appropriately and then transported to the Civil Engineering laboratory at the University of Birmingham, UK. Prior to the extraction of the extract, the seed was cleaned by washing with tap water to remove contaminants such as dust, damaged seeds and other debris. The seeds were then oven-dried at 60ºC for six hours before grinding.

2.2. Reagents used to Prepare the Extract

Analytical grade chemicals and reagents (sodium chloride, aluminum sulfate and hydrogen chloride) were obtained from Fisher Scientific, UK, and kaolinFluka-60609, from Sigma Aldrich, Germany). Deionized (DI) water was used to prepare all the suspensions and solutions in this study.

2.3. Extraction of Crude Extract in *Sabdariffa* Seed

Sabdariffa seeds were ground to fine powder using a laboratory Mill (Tema mill, Germany) for two minutes to obtain the desired powder following Jones and Bridgeman (2016a) and Jones (2017). The resulting seed powders were sieved through a set of stainless steel sieves (600 to 212µm). The powders retained on the 212 and 300µm were combined and used in the study. The crude seed extract (CSE) was prepared from the ground seed powders by adding 1.0 M NaCl solution to the seed powder to make 2% (w/v) suspension, i.e. 2g of the seed powder in 100 ml NaCl. The suspension was vigorously stirred using a magnetic stirrer for 15 minutes at room temperature (19±2ºC). The suspension was then centrifuged at 4000 rpm for 10 minutes using a Heraeus Megafuge 16 (Thermo Scientific, Germany). The suspension was decanted, and the residual solids were dried in an oven at 50ºC overnight. The weight of the dried solid material was measured to ascertain the amount of seed powder used in making the suspension. The decanted suspension was then filtered through a Whatman No. 42 filter paper and the filtrate termed sabdariffa crude extract. Additionally, protein concentration was estimated according to Bradford (1976) with standard bovine serum albumin (BSA), absorbance was measured at 595 nm using Ultrospec 2100 (UV/visible spectrophotometer).

2.4. Denaturation of SCE Stock Solution

The SCE extracts were heated at temperatures of 60, 97 and 140 ^{o}C for 6, 4 and 2 hrs respectively, using a hot plate. The heated samples were then centrifuged at 4500 rpm for 10 minutes and were filtered through a Whatman no. 42 filter paper. Similarly, crude extract stock solutions were stored for 1, 3, 7, 10 and 14 days to denature the extract (Jones and Bridgeman, 2016a). Standard procedures according to the American oil chemists' society (AOCS, 1990) and American society for testing and materials (ASTM) methods of analysis were adopted in the characterisation of the raw oil and the biodiesel produced.

2.5. Preparation of the Synthetic Turbid Water

Synthetic turbid water for the experiments was prepared by adding kaolin particles into tap water according to Jones and Bridgeman, (2016b). Forty grams (40g) of laboratory grade kaolin (Fluka and high grade, Sigma Aldrich) was added to 400ml of tap water and the suspension stirred for 30 minutes using a magnetic stirrer.

2.6. Coagulation and Flocculation Test

Jar tests were conducted using a standard apparatus comprising of six (6) number of one-liter beakers (Phipps and Bird, 7790-900B USA) according to Jones and Bridgeman, (2016a) to evaluate the optimum coagulant dose for the coagulation tests. For effective dispersion of the coagulant, the water was rapidly mixed at 200rpm for 1 min. The mixing speed was then reduced to 30rpm for a further 30 minutes to simulate the flocculation stage. All experiments were conducted in triplicate at room temperature of 19 ± 2 °C. Finally, buffer capacity (BC) of protein was calculated using Equation 1 according to Morr et al. (1973).

$$\mathrm{BC} = \frac{titrant\ (\frac{mg}{l})}{wt\ of\ protein} \times \Delta\mathrm{pH} \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. Coagulation Potential of the Extract

Jar test experiment was conducted to assess the quality of water treated using SCE in terms of turbidity removal. Figure 1 shows the performance of the extract in treating synthetic water with initial turbidity of 200 and 50 NTU respectively. The results show that the lowest residual turbidity was achieved with coagulant doses of 60 mg/l (6 ml of each 1 ml contains 10 mg/l dose of crude extract) in the 200 NTU, yielding turbidity removal efficiencies of 94%. It is noteworthy that the concentration of protein in the extracts was found to be 0.918 mg/ml in SCE as obtained from protein concentration analysis following (Bradford, 1976). With this concentration, the amount of protein used for coagulation to achieve these performances was 5.508 mg (0.918×6) with SCE. Additionally, it was observed that at coagulant dose higher than the optimum value, residual turbidity began to increase mainly due to re-stabilisation of the suspended particles in the water. Furthermore, Figure 1 shows the performance of SCE in water with initial turbidity of 50 NTU. As the turbidity of the water decreased from 200 to 50 NTU, optimum coagulant dose demand also decreased, indicating the dependence of coagulant dosing on particle concentration for efficient turbidity removal. The application of SCE in water treatment showed improved performance in 200 NTU water than in 50 NTU turbid water due to increased particle concentration. This finding is in agreement with previous study as reported by Muyibi and Evison (1995) and Katayon et al. (2004) who used MO extract to treat varying turbidity levels. Natural extracts consist of multiple molecules which exhibit high interaction with particles in water by adsorption and bridging action. Earlier studies have reported that, for effective flocculation activity to occur, the amount of polymer dosed should be proportionate to the particle concentration in order to make the collision efficiency $\alpha = 1$ (Birkner and Morgan, 1968; Gregory, 2005; Gregory and Barany, 2011).

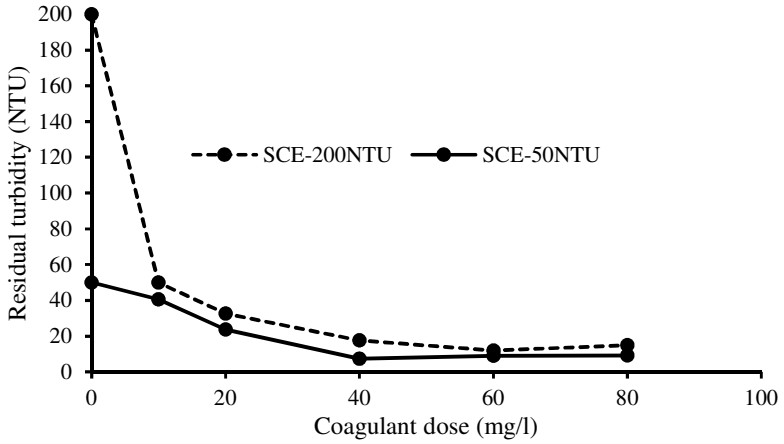

Figure 1: Coagulation performance of SCE on 200 and 50 NTU synthetic kaolin water

In the work reported here, a 40 mg/l (i.e. 4 ml, each 1 ml contains 10 mg/l dose of the extract) dose of SCE was used to achieve a final residual turbidity of less than 7 NTU from 50 NTU water sample. Turbidity removal performance was observed to be 85%. To achieve this performance by the extract, the amount of protein used for coagulation declined to 3.672 mg (0.918 mg of protein concentration/ml × 4) in SCE compared with 5.508 mg used in the medium turbidity (200 NTU) water sample. Similarly, Figure 1 shows that an attempt to increase the coagulant dose beyond the optimum dose in both 200 and 50 NTU water samples did not enhance turbidity removal efficiency, rather the turbidity of the water increased considerably. Naturally-occurring extracts such as MO have been shown to exhibit poor coagulation performance in low turbidity water (Ndabigengesere and Narasiah, 1998). The statistical significance of coagulant dose demand due to increased water turbidity reveals that the optimum dose that achieved the lowest residual turbidity were significantly ($p<0.05$) dependent on the initial turbidity. An increase in water turbidity from 50 to 200 NTU caused an increase in coagulant dose, with $R^2 = 0.9643$ in SCE. The optimum coagulant doses required to achieve successful turbidity removal with SCE were 40 and 60 mg/l for 50 and 200 NTU, corresponding to 3.672 and 5.508 mg in SCE protein respectively. Katayon et al. (2006) found the dependence of MO coagulant dose on the level of initial turbidity to be significant with an R^2 value equal to 0.985. As seen in this study, any further addition of coagulant dose above the optimum dosages did not result in improved efficiency, rather, the final residual turbidity significantly ($p<0.05$) increased mainly due to overdosing.

3.2. Effect of Coagulant Dose on pH of Treated Water

For decades, traditional inorganic coagulants have been used in water treatment to remove turbidity in raw water. Chemical coagulants such as aluminium sulphate (AS) [$Al_2(SO_4)_3.14H_2O$] and ferric sulfate [$Fe_2(SO_4)_3$] are employed within certain pH range for optimum performance, but their addition to colloids in water can affect the treated water pH (Kawamura, 1991). Studies have shown that the application of natural extracts in water treatment does not affect the pH of the water (Ndabigengesere et al., 1995, Ndabigengesere and Narasiah, 1998, Diaz et al., 1999, Zhang et al., 2006) and that there is no standard operational optimum pH (Ndabigengesere and Narasiah, 1996), known to date. Figure 2 presents the effect of coagulant addition on treated water pH during the jar tests experiments using AS and MO as previously reported by Jones and Bridgeman (2016a) and SCE by incrementally dosing 10−100 mg/l to 200 NTU water. The treated water pH remained unaltered after using SCE as a coagulant. Similarly, MO extract did not reduce the pH of the treated water as well. However, AS dosing to 40 mg/l produced an approximately linear reduction in pH from an initial value of pH 7.36 to pH 6 with 10 mg/l dose, then to pH 4.7 with 40 mg/l. It was followed by a reduced rate of pH change (pH 5 to less than pH 4.3 when coagulant dosed from 40 to

100 mg/l). The study observed a buffering effect of the protein between the amino groups (NH_2) and the carboxylic group – COOH – in the seed proteins. The buffer capacity (BC) was estimated to be 0.017 with MO and 0.029 in SCE samples. The minor change in final pH was most likely caused by the balancing of hydrogen ion and the raw water hydroxide ion (Katayon et al., 2004). While some previous studies have shown the most efficient coagulation pH using natural extracts to be above the neutral from pH 9 (Okuda et al., 2001, Zhang et al., 2006), this study observed the performance of SCE at approximately neutral pH.

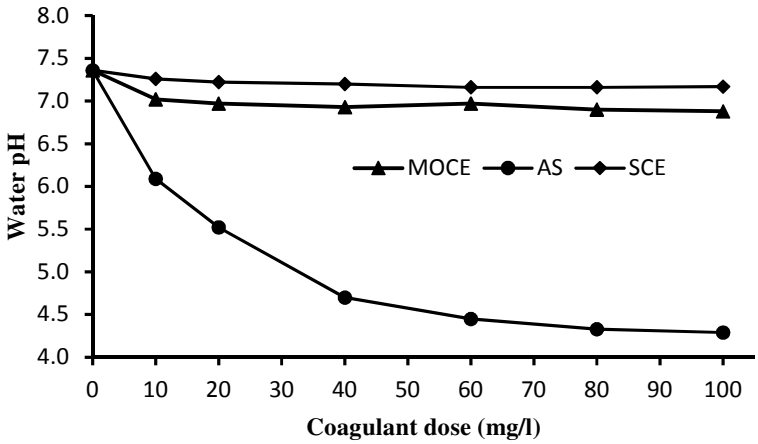

Figure 2: Effect of different natural coagulants addition and AS on water pH using 200 NTU

In addressing the effect of coagulant dose on pH depression, it was observed that the buffer effect of the various amino acid combined to oppose the change in pH. This is advantageous as it eliminates the cost of additives required to adjust treated water pH. Different pH values were employed in order to determine the optimum coagulation pH because of its importance in water treatment as it affects the stability of the protein. Figure 3 shows the evaluation of optimum coagulant dosages on 200 NTU water. Specifically, 60 mg/l of SCE was used on different pH range (4-9). SCE extracts achieved minimum residual turbidity at pH 4 (acidic level) and the maximum residual turbidity was found at pH 9 (alkaline level). The effect of pH adjustment in removing turbidity in water using extracts was seen to be significant ($p < 0.05$) at lower pH than alkaline pH levels. Turbidity removal efficiency was 98% for SCE at pH 4. However, it is not practically possible to treat water at such a low pH value because drinking water guideline stipulates pH of between 6.5 and 7.5 otherwise an additive must be introduced to bring the pH to an acceptable level. The work reported here observed that the high performance of SCE at pH 4 is an indication that an acidic environ improves proton binding affinity and the net surface charge decline to encourage double layer compression. Conversely, this result is not in agreement with other studies where natural extracts such as MO and *Cactus* were conducted (Diaz et al., 1999, Okuda et al., 2001, Zhang et al., 2006). In those studies, optimum pH was found from pH 8 and above. This glaring difference could be attributed to the nature and types of protein compounds in the seed. Additionally, the result shows that SCE extracts significantly ($p < 0.05$) achieved turbidity removal above 94% at pH 6. It is noteworthy that the treatment efficiency was reduced to 65% for SCE at pH 9. The percentage reduction in performance was also noted to be significant ($p < 0.05$), with highly reduced rate observed in SCE. The lowest reduced rate recorded in SCE samples was from 98% to 65%.

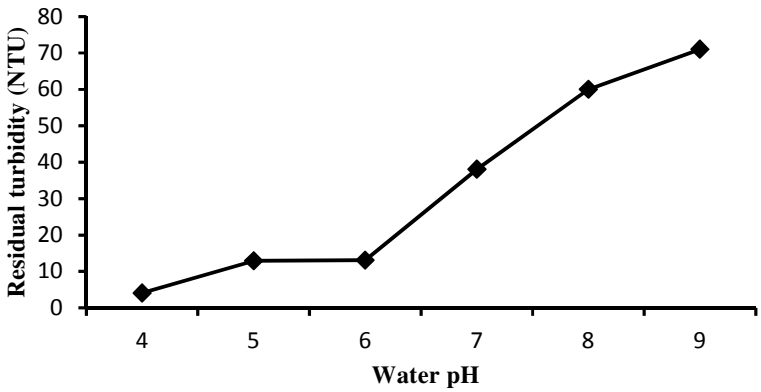

Figure 3: Performance of SCE as coagulant on different pH values on 200 NTU water

3.3. Effect of Temperature Variation on SCE Stock Solution

Table 1 presents the performance of SCE, which was heated to a range of temperatures. As in okra crude extract (OCE), SCE samples were heated to 60, 97 and 140 °C for 6, 4 and 2 hours respectively. The effect of temperature variation on SCE performance was evaluated in treating synthetic water with a turbidity of 200 NTU. The results show that with 10 mg/l dose, a notable reduction in turbidity was found across all the extracts. The non-treated sample achieved approximately 82% turbidity removal while the 60 and 97° C heated samples achieved 90% coagulation efficiency. Similarly, with 10 mg/l dose, the performance of the 140 °C heat-treated sample attained a turbidity removal efficiency of 70%. Further addition of coagulant resulted in increased performance until the optimum dose. The optimum coagulant dose to achieve the lowest residual turbidity was 80 mg/l; the native sample recorded 93% while the samples heated to 97 and 140 °C achieved 96 and 92% turbidity removal respectively. On the other hand, the performance of SCE heated to 60 ºC was 94% with 60 mg/l dose. In addition, the heat treatment largely denatured the seed protein by destroying both the secondary and tertiary structures, hence improved coagulation was witnessed because of the active adsorption ability of the primary structure. The maximum coagulant dose used in the study was 100 mg/l. With 100 mg/l dose, the performance of all the extracts deteriorated except that of the native sample which attained 90% compared with 82 and 89% attained with 97 and 140 ºC stock sample respectively.

Table 1: Effect of heat treatment on the performance of SCE on 200 NTU turbidity removals in water

Coagulant dose (mg/l)	% Reduction			
	Untreated	T=60°C 6 hrs	T=97°C 4 hrs	=140°C 2 hrs
10	81.7	89.5	89.9	69.5
20	83.8	89.9	93.3	78.9
40	91.3	90.9	94.0	84.5
60	91.5	93.7	94.3	88.6
80	92.5	93.0	96.4	92.0
100	90.2	91.0	82.4	88.9

3.4. Effect of Storage Duration on SCE Stock Solution

One crucial physical factor that can cause protein denaturation is storage time. Several proteins can be denatured and lose their stability within minutes upon storage. In order to understand the impact of protein

storage on coagulation activity of seed extract for people in low-income countries, the extracts were stored, and their subsequent coagulation potential evaluated. The effect of storage time on the performance of SCE stock solution was investigated as shown in Figure 4. A stock solution of the extracts was prepared and stored at room temperature of 19 ± 2 °C. Fresh SCE was prepared and then stored in a 200-ml open beaker at room temperature between 1 and 14 days' interval to observe its denaturation process. Performance evaluation was then conducted using 1, 3, 7, 10 and 14 days stored extracts. This is vital in developing countries, where electricity supply is a major challenge, and the cost of obtaining modern, temperature-controlled storage facilities is prohibitive. A test was investigated on the turbidity removal potential of SCE following storage for 1, 3, 7, 10 and 14 days respectively. Figure 4 shows that the performance of SCE as coagulant increased with storage duration to day 10, beyond which its efficiency deteriorated. Optimum performance was observed with 80 mg/l dose of SCE stored for 3 days. Turbidity reduction was approximately 97%, from 130 NTU to less than 4 NTU. At the end of the 7 and 10-day storage time in both cases, maximum turbidity removal was 89% using a coagulant dose of 60 mg/l from 130 NTU to approximately 14 NTU. However, with the 14-day storage, residual turbidity was 34 NTU at a coagulant dose of 20 mg/l, yielding only 74% removal efficiency.

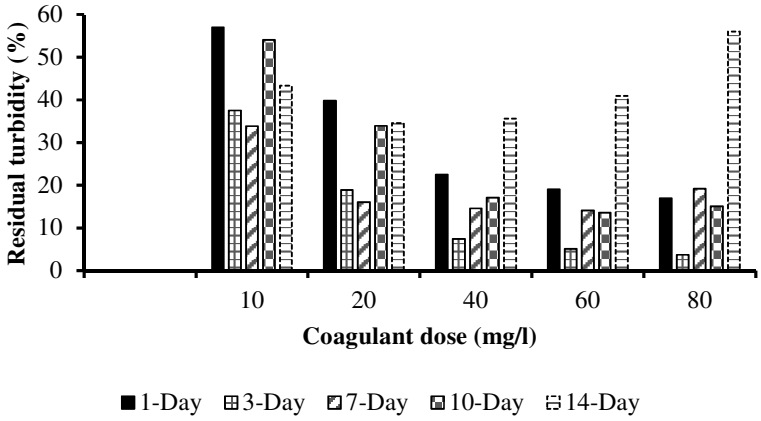

Figure 4: Effect of storage time on the performance of SCE in 130 NTU turbidity removal

4. CONCLUSION

The extract showed high turbidity removal performance in 200 NTU water sample than that of 50 NTU as a results of increased particle concentration. It is noteworthy that storing SCE could improve its turbidity removal performance to approximately 97%. Furthermore, because of its buffering potential, water treated using SCE does not require chemical additive to adjust the treated water pH as it remains unaltered after the treatment processes. It is hereby established that the protein in SCE is thermo-stable.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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