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Comparative Study of the Physiochemical and Coagulating Properties of *Moringa Oleifera* (Linn) Seeds and Stem for Use in Water Purification

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ABSTRACT: *Lack of access to clean and safe water is prevalent within many rural communities in developing countries in Africa, and the need for household water treatment is enormous. Moringa oleifera plant is a tropical plant known for its nutritional and medicinal properties. Moringa seeds have been widely used in traditional water treatment practices across many countries of Africa and the world. This study examines the physicochemical properties of the stem and the seed of the moringa plant to define its suitability for use in household water treatment. The moringa oleifera seeds and stems were pulverized into powdered forms and divided into two parts. The first parts were left in their raw state, and the second parts were calcined in a kiln at 800^{o}C. The raw and calcined moringa seed and stem samples were subjected to phytochemical analysis and XRF characterization. Aqueous extracts of the raw and calcined moringa seeds and stems were used in laboratory-based coagulation tests; coagulation efficiencies were determined using turbidity reduction in treated water over time. The results show similar elemental constituents in the raw and calcined moringa seed and stem. The necessary phytochemicals present were higher in the moringa seed samples than in the stem samples, both raw and calcined states. The coagulation tests indicated some coagulation efficiency in both raw and calcined samples, with turbidity reduction of up to 25% in 90 minutes. This study revealed that the moringa stems compare favourably with the moringa seeds in their raw or calcined forms as a bio-coagulant in water treatment.*

KEYWORDS: bio-coagulation-flocculation, household water treatment, moringa oleifera, phytochemicals, water supply, turbidity removal,

INTRODUCTION

Waterborne diseases are the most prevalent cause of death, especially among children under the age of five, in nations where access to safe water is lacking [1], [2]. This puts to the fore the need to both improve access (availability and quantity) to water and ensure the quality of water at the point of consumption. The need for increased access to improved water quality at the point of

consumption has resulted in a need to explore point-of-use water treatment options worldwide, especially amongst populations that are in dire need of access to safe drinking water [3].

The World Health Organization (WHO) encourages the use of Household Water Treatment Systems (HWTS) for effective treatment of drinking water to reduce incidences of water-borne diseases [4]. Household water treatment systems are a range of devices or methods employed for the purposes of purifying water in the home, mostly at the point of use. They have been proven to have the potential to significantly impact the health of users [5], especially in locations with low access to community-wide piped water systems.

The safety of drinking water from any source, whether open or private, centralized or household-based, state or community-wide, is subjective because drinking water can only be considered safe if it is free of contamination at the point of consumption This is due to the fact that contamination can occur during collection, transportation and storage of water even when it has been collected from an improved source [6], [7]. The challenge, therefore, is to ensure the safety of water at the point of consumption and at the most affordable cost and in the most user-acceptable manner, to ensure sustainability.

To achieve the 6th target of the Sustainable Development Goals, which is focused on improving universal access to water, it has become expedient to research alternative household treatment methods that would simply and effectively treat water at low cost while holding an appeal that would encourage sustained use among users in Nigeria [3]. The application of *Moringa oleifera (Linn.)* seeds as a bio-coagulant and disinfection agent in water purification have been in practice [8],[9],[10]. In recent times more focus has been on the use of chemicals as disinfectants for effective water treatment [11]. However, dissatisfaction centred on the taste and smell of water treated with chemical disinfectants has remained an issue that has greatly affected the uptake and widespread use of these treatment methods [4]. It is therefore desirable to explore the appropriate use of natural and organic materials with disinfecting abilities in household water treatment. This study, therefore, sought to assess the physiochemical properties of *moringa* seeds and stem with a view to finding applications for it in a ceramic-based water purification technology that would proffer a lower cost, sustainable solution to water treatment needs at household levels, thereby increasing access to improved drinking water in Nigeria.

Historical use of *Moringa* in water treatment

Moringa oleifera (Linn.) is a natural plant whose seeds are said to contain bio-coagulate compounds which can be used for water clarification and purification [11]. Previous studies confirmed *Moringa oleifera* seeds to be highly effective in the removal of suspended particulate matter in water. They are said to have both coagulant and antimicrobial properties [9], [12]. From ancient times, the use of *Moringa oleifera* seed for some form of water treatment has been a traditional practice, especially in rural areas. In ancient Egypt, the practice was to rub the surface of their clay water pots with dried *moringa* seeds with a view to purifying the water stored in them by killing bacteria in the water and clarifying the water through coagulation and flocculation [8].

Moringa oleifera seed has been found effective in water treatment, with up to 97 - 98% reduction of turbidity, and 45 - 80% reduction in total coliform values in groundwater and wastewater samples [12]. Past researchers have also declared it as non-toxic to humans and have no evidence of chronic or acute effects, especially when used for water treatment [10]. However, another study [8], suggests that using dried *moringa oleifera* seed powder alone may not be ideal in water purification, as surviving bacteria in the water may feed on the residual organic matter, thereby growing more colonies and making the water unsafe for drinking. The study [8] therefore suggested using the powdered *moringa* seed in a sand medium to provide a surface for the adsorption of bacteria from water.

In this research, a comparative study of the physiochemical properties of the *moringa oleifera* tree stem and seeds was carried out to assess the suitability of the plant stem as a bio-coagulant since these properties in the seed is already known but the feasibility of getting useful quantity from the seed for mass production of water purification devices is low. The coagulating capability of the *Moringa* stem as compared to the seed, as well as the phytochemicals and elements naturally present in both parts, were studied.

MATERIALS AND METHOD

Research Materials

Moringa Oleifera (Linn.) seeds and stems were used as organic components in this research because the *moringa* seeds have been reported to exhibit coagulating and antimicrobial properties in water [13]. However, these properties have not been determined to exist in the *moringa* stems; and this was investigated in this study to determine its suitability as an antimicrobial agent when mixed in the clay body.

Methods

Materials processing methods

The materials used in the study were prepared from *Moringa oleifera* plant parts. The stem and mature seeds of the plant were sourced and collected. *Moringa* plant stems were cut into logs, collected, washed, and sun-dried for between 5 – 7 days. The dried logs were shaved into dust and tailings using a circular saw. The wood shavings and tailings were subjected to further reduction of particle size using a grinding mill and, sieved using a 180 µm sieve on a sieve shaker. Matured seeds of the *Moringa Oleifera* plant were de-podded, air-dried and ground using the domestic grinder. The powdered seeds were sieved to about 300 µm particle size. Finer particle sieving was very difficult to achieve with this material because of its oily nature. A part of the powdered *Moringa* stem and seed was calcined in ceramic vessels to about 800^{o}C.

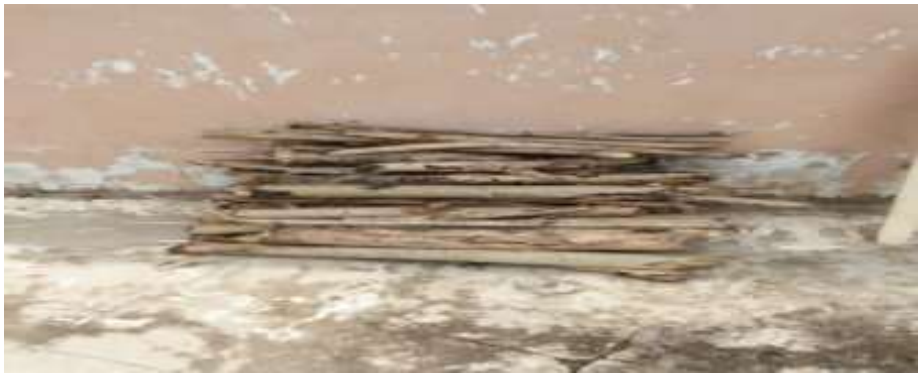

Figure 1: Washed *Moringa* stems

Figure 2: *Moringa* seed pods

Phytochemical analysis of the materials

Both raw and calcined (ashed) *moringa* stem and seed powder were analysed at the Laboratory to determine the phytochemical constituents of the materials. The preliminary test, the qualitative analysis, was to determine the phytochemicals present in each of the materials. While the quantitative analysis puts a value on the phytochemicals present. These analyses helped the researcher compare both parts of the plant via the composition of plant chemicals present in each, both as freshly dried samples and calcined/ashed samples; to distinguish the inherent properties available in both parts of the *moringa* plant.

Phytochemical analytical procedures

The procedures involved in carrying out the phytochemical analysis are outlined as follows;

The total phenol content of the sample was determined by the method as described in Singleton et al., wherein 0.20 millilitre (mL) of the plant extract was mixed with 2.5 mL of 10% Folin ciocalteau's reagent and 2.0 mL of 7.5% sodium carbonate solution. The mixture was subsequently incubated at 450^{o}C for 40 minutes and the absorbance of the coloured mixture was read at 700 nm using a UV visible spectrophotometer (Jenway 6300/6305). Garlic acid was used as standard

phenol. [14]. The total flavonoid content was determined using a colorimeter assay developed by Bao et al [15]. The alkaloid content of the sample was determined by the method described by Harborne [16]. 5.0 grams (g) of the sample extract was reacted with 200 mL of 10% solution of acetic acid in ethanol in a 250 mL beaker. This was covered with aluminium foil and left to stand for 4 hours. This was later filtered and the extract was concentrated in a water bath. Concentrated Ammonium hydroxide was added drop-wise to complete the precipitation process. The resulting solution was allowed to settle and the precipitate was collected and washed with distilled water. The residue was then weighed as the total alkaloid content. The Tannin content was determined using Van-Buren and Robinson's [17] method, wherein 500 milligrams (mg) of the sample was weighed into a plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and the volume made up to the mark. 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.10M $FeCl_3$ in 0.10 N HCl and 0.008 M potassium ferrocyanide. The absorbance was then measured at 120 nm. The determination of total saponin content involved weighing 20.0 grams (g) of the sample into a 250 mL flask and 100 mL of 20% aqueous ethanol was added. The mixture was heated over a water bath for 4 hours with constant shaking. The mixture was filtered and the residue was re-extracted with a further 200 mL of 20% ethanol. The concentrate was recovered and the ether layer was discarded. 60 mL of n-butanol was added and the solution was washed with 5% aqueous sodium chloride solution. The sample was then evaporated till dry in a water bath and the drying was completed in the oven, based on the method described in Nguyen et al [18]. The cyanogenic glycoside concentration of plant tissue was determined by hydrolysis of cyanogenic glycosides and trapping the resultant HCN in a NaOH well [19]. Determination of terpenoid content followed the procedure described in Sofowora. 0.5g of finely ground sample was weighed into a 50-millilitre conical flask. 20 millilitres of a chloroform-methanol mixture in a ratio of 2:1 was added and the mixture was shaken thoroughly and then allowed to stand for 15 minutes at room temperature. The suspension was centrifuged at 3000 rpm (Yescom 800-1 electric centrifuge, model 35CEN001-800-09), after which the supernatant was discarded. The precipitate was re-washed with 20 mL chloroform-methanol solution in the ratio (2:1) and then re-centrifuged. The latter precipitate was dissolved in 40 mL of 10% SDS solution. 1mL of 0.01 M ferric chloride was added and allowed to stand for 30 min before taking the ABS @ 510 nm. The STD Terpenoid (alpha-terpineol) concentration ranged from 0 – 5 mg/mL from the stock solution [20].

Characterization of the materials

Material characterization was carried out on the processed *moringa* oleifera plant materials to determine their elemental constitution. The equipment used for the characterization was the EDXRF SKYRAY instrument (Model: EDX3600B) which is an X-ray fluorescence spectrometer. The powdered *Moringa* samples of fine homogeneous particle size were pelletized. The Sample testing was started by Initialization (calibration) using pure silver standard. Then the appropriate working curve was selected according to the sample, which is all ore (natural) samples in this study. The test was run, and the result was presented in Microsoft Excel format.

Coagulation tests

These tests were carried out to help determine the efficacy of *Moringa* in expediting the coagulation and flocculation of particles in water. The jar test procedure was mirrored in this set of tests for coagulating efficiency. The *Moringa* samples extracts for the coagulation tests were prepared by adding 25 mL of distilled water to 2.5g of the raw and calcined *moringa* parts samples, which were then swirled lightly and left for 24 hours. The extraction process was completed by straining the extract through a Whatman filter paper (110 mm) to give a 100 mg/mL stock solution. Twenty millilitres (20 mL) of the stock (sample extract) was then introduced into 500 mL of water in a beaker and the jar test procedure was used in carrying out the coagulation tests.

Figure 3: Coagulation test set-up

Figure 4: Sampling for turbidity

The Coagulating efficiency of *Moringa* in this study was determined by measuring the reduction in turbidity as a water quality parameter of choice. Turbidity, which describes the concentration of suspended particles in water, arises from the presence of very finely divided solids. The existence of turbidity in water will affect its acceptability to consumers. It may also interfere with the treatability of water as there is a risk that pathogenic organisms could be shielded by the particles and hence escape the action of the disinfectant [21].

The turbidity of the treated water samples in this study was measured over time as compared to that of the raw water sample. The coagulating efficiency was therefore calculated as; the difference between the initial turbidity in the raw water and that of the treated water at a given time, divided by the initial turbidity multiplied by 100. A digital spectrometer (Hach DR890) was used to measure turbidity in Nephelometric Turbidity Units (NTU).

Determination of Turbidity

The Nephelometric method was used in determining the turbidity of samples. This method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity (APHA 2130B). The procedure involved measuring the water sample into a 25 mL calibrated cuvette to be determined in the digital spectrometer (Hach DR890). The distilled water was also measured in the other cuvette which was used as a standard reference to know and compare the degree of the dissolved solute present in the water sample. The absorbance of the sample was read at the wavelength of 750 nm and the reading was recorded. This was repeated for the raw and treated water samples.

RESULTS

Mineral characterization of *Moringa oleifera* Linn seeds and stem

The elements present in the seeds and stem of the *Moringa oleifera* plant tested are presented in Tables 1 and 2, and compared in Figure 5. The XRF results show that the *Moringa* seeds analyzed constituted mainly of the elements Sulphur (57%) and Potassium (16%). While the stems comprised more Potassium (49%) and Sulphur (23%).

Table 1: Mineral composition of *Moringa oleifera* Linn seeds

Sample Name	***Moringa* seed**		
Element	**Intensity**	**Content**	**% Content**
Al	0.0018	0.5535	2.9570
Si	0.0038	0.2894	1.5461
P	0.0161	0.7658	4.0912
S	0.0872	10.6775	57.0428
K	0.0369	3.0043	16.0500
Ca	0.0149	0.2971	1.5872
Fe	0.0014	0.2129	1.1374
Sn	0.0060	1.1289	6.0310
Sb	0.0078	0.9743	5.2050
		18.7184	100

Table 2: Mineral composition of *Moringa oleifera* Linn stem

Sample Name	***Moringa* Stem**		
Element	**Intensity**	**Content**	**% Content**
Al	0.0034	1.0734	4.3218
Si	0.0071	0.6308	2.5398
P	0.0144	0.6856	2.7604
S	0.0499	5.5919	22.5148
K	0.1504	12.2635	49.3767
Ca	0.0326	1.9705	7.9339
Fe	0.0032	0.3872	1.5590
Sn	0.0045	0.8414	3.3877
Sb	0.0059	0.7330	2.9513
		24.8366	100

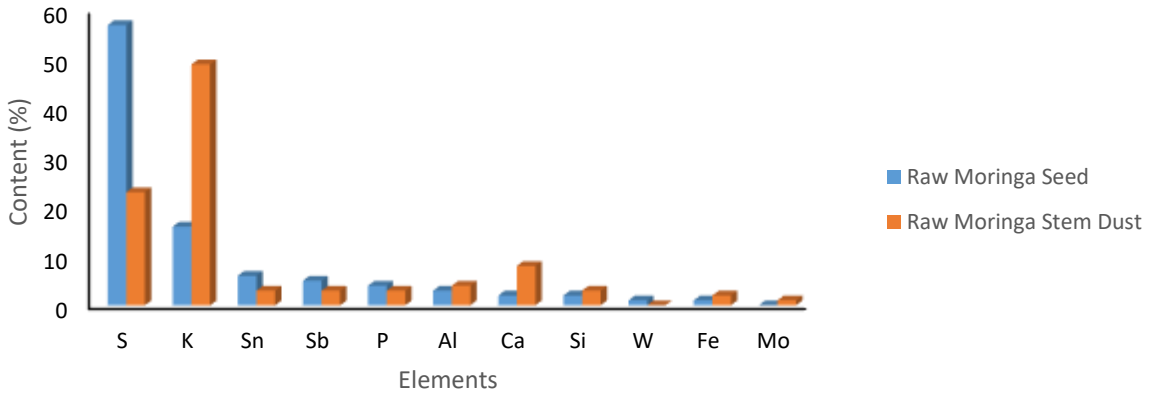

Figure 5: Comparing the elemental constitution of *Moringa oleifera* seeds and stem

Phytochemical characteristics of *Moringa oleifera Linn* plant parts

The qualitative and quantitative values of plant chemicals present in the seeds and stem of the *Moringa oleifera* plant tested are presented Tables 3 and 4. The results show that all the phytochemicals detected to be present in the *Moringa* seeds were also present in the stem except for alkaloids and glycosides. The quantitative values of the plant chemicals in the stem was shown to be less than that in the seeds.

Table 3: Qualitative Phytochemical constitution of raw *Moringa oleifera Linn* seeds and stem

Parameter	Seed sample	Stem sample
Saponins	-ve	-ve
Tannins	+ve	+ve
Phenols	+ve	+ve
Anthraquinones	-ve	-ve
Alkaloids	+ve	-ve
Phytates	+ve	+ve
Oxalates	+ve	+ve
Flavonoids	+ve	+ve
Terpenoids	-ve	-ve
Steroids	-ve	±ve *
Glycosides	+ve	-ve

Key*: +*ve *means positive, implying that the phytochemical is present; -*ve *means negative, implying that the phytochemical is absent*
***** *(± implies the parameter is slightly present/ very insignificant)*

Table 4: Quantitative Phytochemical constituents of raw *Moringa oleifera Linn* seeds and stem

Parameter	Seed sample	Stem sample
Tannins (mg/g)	30.208	8.854
Phenols (%)	31.800	9.258
Alkaloids (%)	43.073	N.A.
Phytates (mg/g)	53.560	12.800
Oxalates (mg/g)	6.933	0.900
Flavonoids (%)	14.650	4.487
Glycosides (mg/kg)	28.835	N.A.

Key: N.A. – Not Applicable (not detected)

Tables 5 and 6 present the results of both qualitative and quantitative analysis of the calcined *Moringa* plant samples. All the plant chemicals present in both raw and calcined *Moringa* seeds and stems are compared in Figures 6 and 7.

The results indicate that some of the phytochemicals present in the raw samples of the *Moringa* plant seeds and stems were burnt off upon calcination, leaving only phytates present in the seed samples and oxalates and flavonoids present in both seed and stem samples.

The quantitative analysis of the phytochemical constituents of calcined *Moringa oleifera* Linn seed and stem showed a reduction in all phytochemicals with heat treatment of the samples at 800^{o}C except in the flavonoids which increased in both seed and stem samples by 25% and 43.35% respectively. The oxalates were also shown to have reduced to almost insignificant quantities in the calcined samples.

Table 5: Qualitative phytochemical constituents of calcined *Moringa oleifera Linn* seeds and stem

Parameter	Seed sample	Stem sample
Saponins	-ve	-ve
Tannins	-ve	-ve
Phenols	-ve	-ve
Anthraquinones	-ve	-ve
Alkaloids	-ve	-ve
Phytates	+ve	-ve
Oxalates	+ve	+ve
Flavonoids	±ve *	±ve *
Terpenoids	-ve	-ve
Steroids	-ve	-ve
Glycosides	-ve	-ve

Key: +ve *means positive, implying that the phytochemical is present;* -ve *means negative, implying that the phytochemical is absent*
***** *(± implies the parameter is slightly present/ very insignificant)*

Table 6: Quantitative Phytochemical constituents of calcined *Moringa oleifera Linn* seed and stem

Parameter	Seed sample	Stem sample
Tannins (mg/g)	N.A.	N.A.
Phenols (%)	N.A.	N.A.
Alkaloids (%)	N.A.	N.A.
Phytates (mg/g)	12.096	N.A.
Oxalates (mg/g)	0.572	0.345
Flavonoids (%)	19.483	7.921
Glycosides (mg/kg)	N.A.	N.A.

Key: N.A. – Not Applicable (not detected)

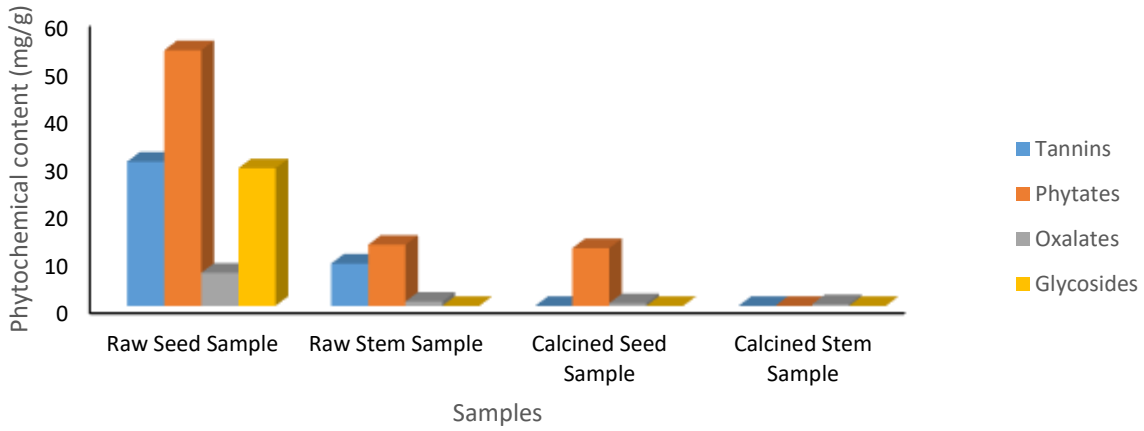

Figure 6: Comparing some phytochemical contents of raw and calcined *moringa* seeds and stem

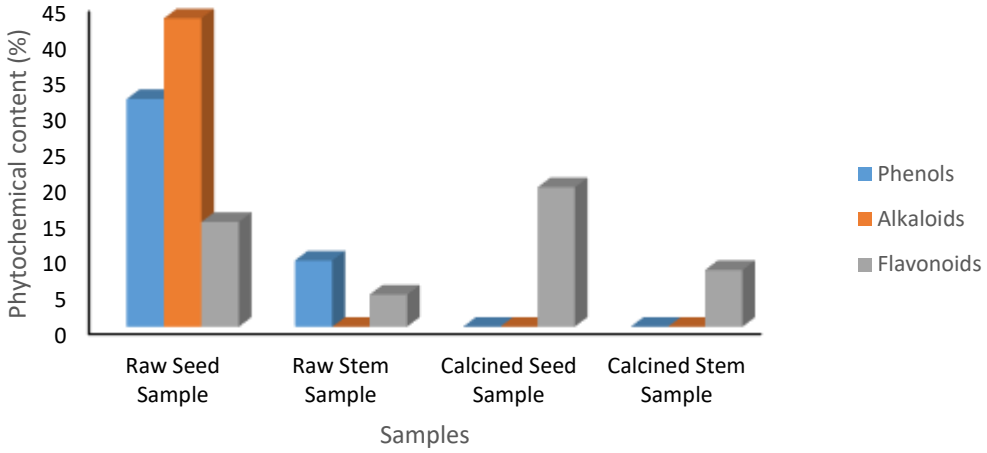

Figure 7: Comparing other phytochemical contents of raw and calcined *Moringa* seeds and stem

Water treatment activity (Coagulation) of *Moringa oleifera Linn* plant parts

Raw *Moringa oleifera* seeds and stem, and calcined *Moringa oleifera* seeds and stem samples were tested to assess the coagulating ability of the *Moringa* plant parts if any. The test results show that the raw and calcined *Moringa* seed and stem samples exhibited some coagulating ability, which tended to increase with treatment time; as shown in Figure 8.

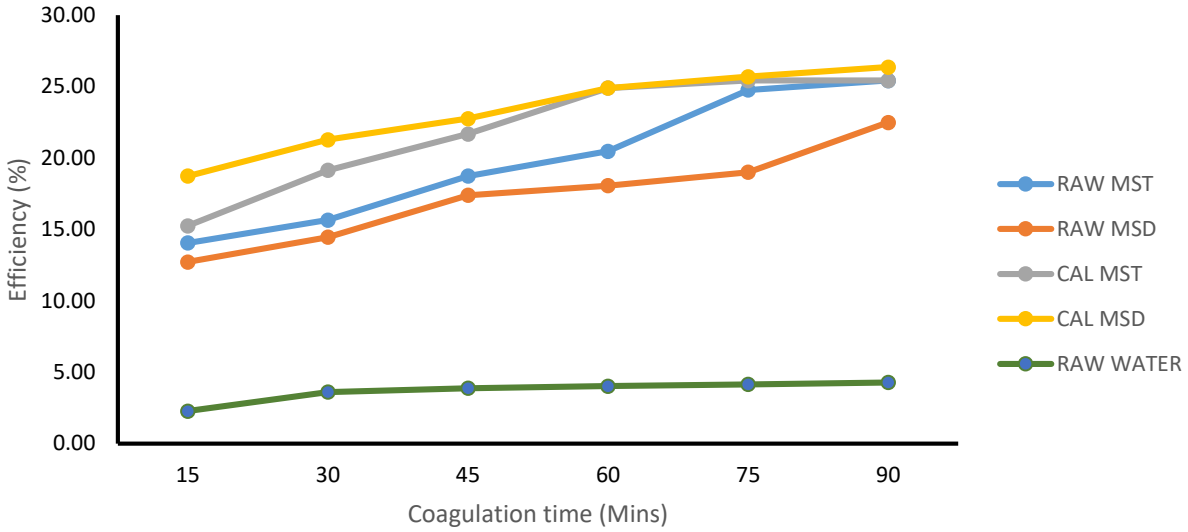

Figure 8: Time-dependent coagulating efficiencies of raw and calcined *moringa* seeds and stem

DISCUSSION

Mineral characterization of *Moringa oleifera* Linn seeds and stem

Elemental characterization of the *Moringa oleifera* seeds and stem indicated that the elemental constitution of both materials is mainly sulphur (S) and potassium (K). The aluminium content in both parts of the *Moringa oleifera* plant as detected in the XRF analysis ranges from 3 to 4%. The popular chemical coagulant, Alum, is known as an aluminium sulphate material, which can be either potassium (potash alum) or sodium-based (soda alum) [22]. Therefore, this research postulates that the presence of the elements, potassium, aluminium and sulphur in both the seed and stem of the *Moringa* plant suggests good coagulating and flocculating potentials in both parts of the plant.

Phytochemical characteristics of *Moringa oleifera* Linn plant parts

Both raw and calcined samples of the selected *Moringa oleifera* plant seeds and stem dust were analyzed for their phytochemical constitution. This was necessary to help understand the effect of heat treatment, if any, on these significant values. The information was important because this study sought to explore the feasibility of the use of the *Moringa* plant parts in a ceramic composite for water treatment. When mixed with the clay, the composed ceramic body will be likewise subjected to firing at similar temperatures, so it was essential to understand what transformations might occur in the *Moringa*.

The phytochemical test on both raw and calcined samples of the *Moringa oleifera* stem and seed showed that all the samples had oxalates present. This phytochemical is known to have chelating properties that enable coagulation by binding tightly to divalent cations [23]. The basis of chelation

is that the chelating molecule holds two or more binding groups in close proximity to the ligand, so the binding becomes cooperative [24],[25]. Therefore the results indicate that even when fired into the ceramic at 800ºC, *Moringa oleifera* is likely to retain its ability to cause coagulation to occur in water.

Interestingly, the results indicated that the flavonoid content of both *Moringa oleifera* seeds and stem increased, by about 25% and 43% respectively, with heat treatment at 800ºC. Further research is recommended to test this possibility. The calcination of samples in a kiln/furnace at these temperatures has not been researched widely. A probable explanation may be that, with the reduction in or elimination of other constituent phytochemicals during calcination, the flavonoid content may appear increased when in essence it may not have; it had only become the major phytochemical present in the absence of others in the calcined sample. This explanation is however anecdotal and subject to further research.

Flavonoids are plant chemicals that are known to add antimicrobial property [24]. The observed presence of this phytochemical in the calcined samples tested indicates that when added and fired into the ceramic, the *moringa* parts studied can aid the deactivation of microbes in treated water, giving an added advantage.

Water treatment activity (Coagulation) of *Moringa oleifera Linn* plant parts

The calcined samples showed greater turbidity removal efficiencies within the first 30 minutes of treatment, while the best reduction for the raw samples of both parts was achieved between the 30-45 minutes treatment time frame. The raw samples were slower to achieve turbidity reduction because they tended to form cloudy suspension on stirring in water especially with the seed samples. In comparison with the calcined samples, the raw samples were shown to rapidly increase turbidity removal between 60-75 minutes for raw *Moringa* dust powder and 75-90 minutes for raw *Moringa* seed powder samples. The seed powder was observed to be oily which had the ability to coat the inner surface of the sample vial, this could have also influenced the readings of the spectrometer. An average turbidity reduction of about 25% was achieved within a 90-minute frame, as shown in Figure 8.

CONCLUSION

Summary of findings

1. *Moringa oleifera* Linn. stem was observed to have good coagulating properties just as well as the seeds both in raw or calcined forms.
2. Coagulation efficiency of the *moringa oleifera* plant parts studied was also shown to improve over time of contact with the water during treatment, especially within the first 60 minutes.
3. Both parts of the *moringa* studied were shown to have increased flavonoid contents with heat treatment. However, in both the raw and calcined forms, the seeds recorded higher levels of the plant chemical.

4. The *moringa* stem showed zero alkaloid content while the seeds recorded above 40% content of the plant chemical.
5. The *moringa* seeds were shown to have almost 8 times more oxalate content (6.933mg/g) than the stem which recorded a very low content of oxalate (0.9mg/g). Oxalates add chelating properties which could promote coagulation.
6. The XRF analysis showed high concentrations of elemental Potassium and Suphur in both parts of the *moringa* oleifera plant studied. While the seed contained above 50% Sulpur and 16% Potassium, the Stem recorded almost 50% Potassium and 22% Sulphur in content. Both parts recorded low elemental aluminium (2-4%).
7. It can also be deduced from the findings of this study that, de-oiling or ashing of *moringa oleifera* seeds may make them more applicable for use as a coagulating agent in water treatment to overcome the problems of growth of organic matter. That is because the ash has been shown in this research, to have some coagulation properties.

Conclusion

The suitability of *moringa oleifera* (Linn.) stem as a biocoagulant in a ceramic media was tested in this study since it is a more feasible material to collect in such bulk quantities useful for mass production. The *Moringa Oleifera* stem parts were also shown to reduce turbidity in water, proving to be a good coagulant for water treatment. This has a great implication for sustainability, such that the *moringa* stem can be utilized for water purification while the very nutritious seeds are left to be used exclusively for medicinal and nutritional purposes.

Declarations

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Authors' contributions

All of the listed Authors are members of the Research Team and significantly contributed towards the success of the research and in the writing and editing of this manuscript. EAE, DJA and IAA carried out the lab experiments, EAE and DJA prepared and edited the manuscript, IAA, IBK and TLA reviewed and edited the manuscript.

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