American Journal of Engineering Research (AJER)
e-ISSN: 2320-0847 p-ISSN : 2320-0936
Volume-8, Issue-8, pp-139-147
www.ajer.org
Research Paper Open Access

Effect of Particle size on Turbidity Removal using Aqueous Extracts of *Moringa oleifera* Seed

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ABSTRACT*: The study evaluated the effect of particle size on turbidity removal efficiency of aqueous extracts of Moringa oleifera seeds. Six sample sizes (300µm, 425µm, 600µm, 850µm, 1.18mm and 2.0mm) were selected. Preliminary phytochemical screening of the samples revealed the presence of phytochemcals such as tannins, phenols, flavonoids, alkaloids and saponins. The percentage yield of the extracts increased as the particle size decreased. The 2.0mm size sample showed the least yield of 13.33% while the 300µm size sample had the highest yield of 48.16%.The coagulation study was conducted using the jar test apparatus on a turbid surface water sample of an initial turbidity of 190 NTU obtained from the Ezu River in Anambra state, Nigeria. Among the particle sizes, the crude aqueous extracts of the 600µm size sample had the highest turbidity removal efficiency of 89.5% at optimum coagulant dosage of 0.8g/l. The extracts from 2.0mm and 300µm sample sizes showed the least removal efficiencies of 69.5%when compared to the other sample extracts at dosage of 0.8g/l. On analyzing for any significant difference between the turbidity removal efficiencies, it was established that particle size had significant effect ($p<0.05$) at 95% confidence limit. This study provides a new direction on the use of this coagulant in turbidity removal to avoid wastages and make available more natural coagulant material that will serve the growing populace of the developing nations.*
Keywords: *Coagulation, Moringa oleifera seed, Grain size, Turbidity removal, Extract yield, Phytochemicals.*

Date of Submission: 05-08-2019 Date of Acceptance: 20-08-2019

I. INTRODUCTION

In developing countries, potable water is scarce in nature and expensive to obtain especially by rural dwellers (Nwaiwu *et al*., 2012a). Consequently only naturally available sources such as streams, rivers, ponds and lakes are opted for. Water from these sources is turbid and contaminated, with undesirable impacts on the health of consumers, contributing to the death of 15 million children under the age of five per year (World Health Organization (WHO) and UNICEF, 2014).According to (Moa *et al*., 2014), improving physicochemical and microbiological qualities of drinking water at a household level is effective in preventing this trend. Use of synthetic chemicals practiced in most developing countries for water treatment has harmful effects with problems (Razz *et al*., 2014). Alternative treatment procedures using natural coagulants have become the objective of many investigations (Jones, 2017; Omodamiro *et al*., 2014).

Natural coagulants produce less sludge than inorganic coagulants such as alum (Blix, 2011). They are biodegradable, environmentally friendly and are expected to be non-toxic, therefore making it a potentially suitable substitute to artificial coagulants in addressing the challenges facing water supply in rural communities (Egbuikwem and Sangodoyin, 2013). In this regard, numerous plants products such as the extracts of plant leaves, roots, barks, seeds etc, have been studied for their coagulation properties (Alfred and Bridgeman, 2015; Fatehah *et al*., 2013; Vijayaraghavan *et al*., 2011), and plants seeds in particular have been reported to exhibit potentials to serve as bio-coagulants (Birima *et al*., 2013; Edogbanya *et al*., 2013; Lee, 2017; Shen *et al*., 2011; Ugonabo *et al*., 2016). These activities have been attributed to the presence of active agents such as flavonoids, alkaloids tannins, phenols and saponins which aidscoagulation (Muyibi *et al*., 2013; Subramanium *et al*., 2011).

The removal of suspended colloidal particles through the introduction of coagulants to overcome the forces of repulsion is the main aim of the coag-flocculation process (Andréa *et al*., 2014; Okolo *et al*., 2014).

Moringa oleifera is a tropical tree which belongs to *Moringaceae* family. Its origin is traced back to the Asian countries of India, Pakistan, Bangladesh, and Afghanistan (Abalaka *et al*., 2012), and has spread over to many countries of the South-Saharan African, South East Asia, Middle East, South America, Malaysia, Philippines, and Srilanka (Abalaka *et al*., 2012). The seeds are numerous and globular in the fruits. The fruits are elongated, and capable of flying with non-dehiscent berries (Mishra *et al*., 2011). For the past two decades, the various application of *Moringa oleifera* seeds as coagulants as well as coagulant aid have been well documented and the results revealed that the seeds shows high coagulation activity in turbid waters (Egbuikwem and Amori, 2017; Camachoa *et al*., 2015; Nwaiwu *et al*., 2012 b; Idris *et al*., 2016; Sasikala and Muthuraman, 2016).

Amidst the various studies conducted to test the coagulation potentials of *Moringa oleifera* seeds, there is relatively little literature on the effect of particle size on its coagulation potentials. Hence, the purpose of this work is to study the impact of particle size on the turbidity removal efficiency of aqueous extracts of *Moringa oleifera* seeds.

II. MATERIALS AND METHODS

2.1 Collection and Identification of seed samples

The *Moringa oleifera* seeds used in this study were bought from a local market popularly known as Mopo market, Mopo town, Yagba East Local Government Area of Kogi state, Nigeria. The seeds were transported by road to Awka, Anambra state, Nigeria, for studies.

The collected seed samples of *Moringa oleifera* were identified and authenticated by a Botanist (Dr. C. A. Ezeabara) at the Botany Department, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. The representative samples of the seeds were deposited at the herbarium of the department of Botany in cabinet number 3, shelf number 6.

2.2 Preparation of the seed samples

On successful identification of the collected samples, the *Moringa oleifera* seeds were de-shelled with the aid of a clean laboratory knife and air dried at room temperature for five days. The dried seed kernels of *Moringa oleifera* were divided into three equal parts and blended with a mechanical grinder (Excella marlex), a clean laboratory mortar and pestle (wooden type) and an electric blender (Tema mill, Germany) respectively into powder form. The blended samples were mixed together. The reason for adopting three methods of blending was to ensure representation of all possible particle sizes.

2.3 Sieve Analysis

Sieve analysis was conducted using a mechanical sieve arrangement. After grinding, the ground seeds were sieved out using a mechanical sieve to obtain the desired material particle sizes. Six sample sizes were selected for study.

The material that passed through 2.0mm sieve and was retained on 1.18mm sieve represented 2.0mm size sample. The material which passed through 1.18mm sieve and was retained on 850µm sieve represented 1.18mm size sample. The material that passed 850µm sieve and was retained on 600µm sieve represented 850µm size sample. The material that passed 600µm sieve and was retained on 425µm sieve represented 600µm size sample. The material that passed 425µmsieve and was retained on 300µm sieve represented 425µm size sample. The material that passed 300µm sieve and was retained on 150µm sieve represented 300µm size sample. The samples were packed separately in plastic containers and carefully labeled and kept at room temperature prior to extraction.

2.4 Phytochemical Analysis

Chemical tests were carried out on the samples for the quantitative determination of phytochemical constituents based on standard methods and procedures.

2.4.1 Alkaloid determination

The alkaloid content was determined gravimetrically. Five grams of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid (CH_3COOH) in ethanol was added and covered to stand for 4hours. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration using Whatman filter paper No. 4 (125 mm) and weighed.

2.4.2 Saponin determination

Saponin content was determined using the method described by Obadoni and Ochuko, (2001). Twenty grams (20 g) of each ground samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4hrs with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted further with 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath set at 90 °C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether ($(C_2H_5)_2O$) was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added, thereafter the mixture of n-butanol and extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath at 90°C. The samples were dried in an oven at 100 °C until a constant weight was obtained. The saponin content was calculated in percentage.

2.4.3 Tannin determination

Tannin content was determined going by the method described by Van-Burden and Robinson (1981). Five hundred milligrams of the sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark, after which 5 ml of the filtrate was pipetted into a test tube and mixed with 3 ml each of 0.1M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance of the solution was measured in a spectrophotometer at 120 nm wavelength after 10mins and recorded.

2.4.4 Flavonoid determination

Flavonoid content was determined using the method described by Boham and Kocipai (1994). Ten grams of the ground samples were extracted repeatedly with 300 ml of methanol: water (80:20). The resultant mixture was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed.

2.4.5 Determination of total phenols

Total phenols were determined using the method described by Obadoni and Ochuko (2001). For the extraction of phenolic component, 10g of the ground sample was extracted with 20ml of 70% acetone with continuous stirring for 2hrs after which the mixture was filtered. The fat free residue was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipette into a 50 ml volumetric flask, then 10 ml of distilled water was added. To this, 2 ml of ammonium hydroxide (NH_4OH) solution and 5 ml of concentrated amyl alcohol ($C_5H_{15}O$) was added. The samples were left to react for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths and recorded.

2.5 Extraction of seed samples

(i) Oil Extraction

Each of the selected sample size of the blended *Moringa oleifera* seed was defatted using a Soxhlet extractor according to the method described by Nwaiwu *et al*. (2012 a).

In the process, a known quantity of the samples (200g) was prepared, weighed and put into a thimble. The contents of the thimble were transferred to a Soxhlet extractor. 700ml of hexane was poured into a round bottomed flask of the Soxhlet extractor with boiling chips. The Soxhlet apparatus was then set for the extraction. After about an hour of extraction, the round bottom flask was heated in a water bath of the concentrator apparatus. The resulting mixture was filtered to remove debris. The floating oil was collected.

(ii) Solvent Extraction

The residual debris from the hexane oil extraction process stated above from all the samples of the *Moringa oleifera* seed powder were used for solvent extraction. The method described by Nwaiwu *et al*. (2012 a) was adopted.

In this method, each of the debris from the oil extraction process was put into a thimble. 700ml of water was added to each sample. The mixture was then filtered using a muslin cloth. The resulting filtrates were termed crude extracts.

2.6 Determination of amount of extract in yield

This was carried out to know the effect of the particle sizes on the yield of coagulants after extraction. The amount of extract recovered after each extraction was calculated using the following formula;

$$\text{Extract yield (\%)} = \frac{\text{Weight of extract after extraction}}{\text{Weight of plant material before extraction}} \text{ x } 100 \quad (1)$$

2.7 Collection of Water samples

The water sample employed in this study was collected from River Ezu which is located between the villages of Ebenebe and Amansea both in Awka North Local Government Area of Anambra State, Nigeria. The water samples were conveyed in a plastic cooler with ice blocks to the laboratory prior to analysis.

2.8 Coagulation process

The optimum dosage of the *Moringa oleifera* seed crude extracts was determined using a conventional jar test apparatus. The jar test was performed using all the extracts. The jar test procedure was performed using these following steps:

Six (6) beakers were arranged on the jar test apparatus (1L capacity each).
Each beaker contained raw water with initial turbidity of 190 NTU.

0.4g/l, 0.6g/l, 0.8g/l, 1.0g/l and 1.2g/l of the *Moringa oleifera* seed crude extracts was added to the raw water samples in the 1L beaker before stirring began.

The solution was then mixed and agitated at varying mixing time and speed, the first being the rapid or fast mixing for 3 minutes at 350 revolutions per minute, and the second being the slow mixing for 20 minutes at 30 revolutions per minute following immediately.

After this period of agitation, the suspension was allowed to settle by gravity and the supernatants were then collected at different settling times (10, 20, 30, 40, 50 and 60 minutes).At the end, these withdrawn supernatants were tested for turbidity which represents the final concentration.

2.9 Turbidity Removal Efficiency Calculation

The efficiency of the coagulant in reducing the turbidity level of the water was calculated using the Equation;

$$\% \text{ Reduction } = \frac{C_0 - C_1}{C_0} \text{x } 100 \quad (2)$$

Where C_0 is the initial turbidity in NTU

C_1 is the residual turbidity in NTU

2.10 Statistical Analysis

A two – way analysis of variance (ANOVA) without replication was carried out to determine the presence or absence of statistically significant differences between the turbidity removal efficiencies of the six sample sizes namely: 300µm, 425µm, 600µm, 850µm, 1.18mm and 2.0mm. The analysis was done with Microsoft Excel Version 2007. The confidence limit was set at 95%.

III. RESULTS AND DISCUSSION

3.1 Sieve analysis of *Moringa oleifera* seed

The result of the sieve analysis of the blended *Moringa oleifera* seed sample is presented in Figure 1. The total dry weight of the sample was 4615.72g. The uniformity coefficient (C_U) value of 2.64 indicated that the ground *Moringa oleifera* seed sample was uniformly graded in the sense that the ground *Moringa oleifera* seed had identical size of the particles. The effective particle size (d_{10}) was found to be 0.36mm.

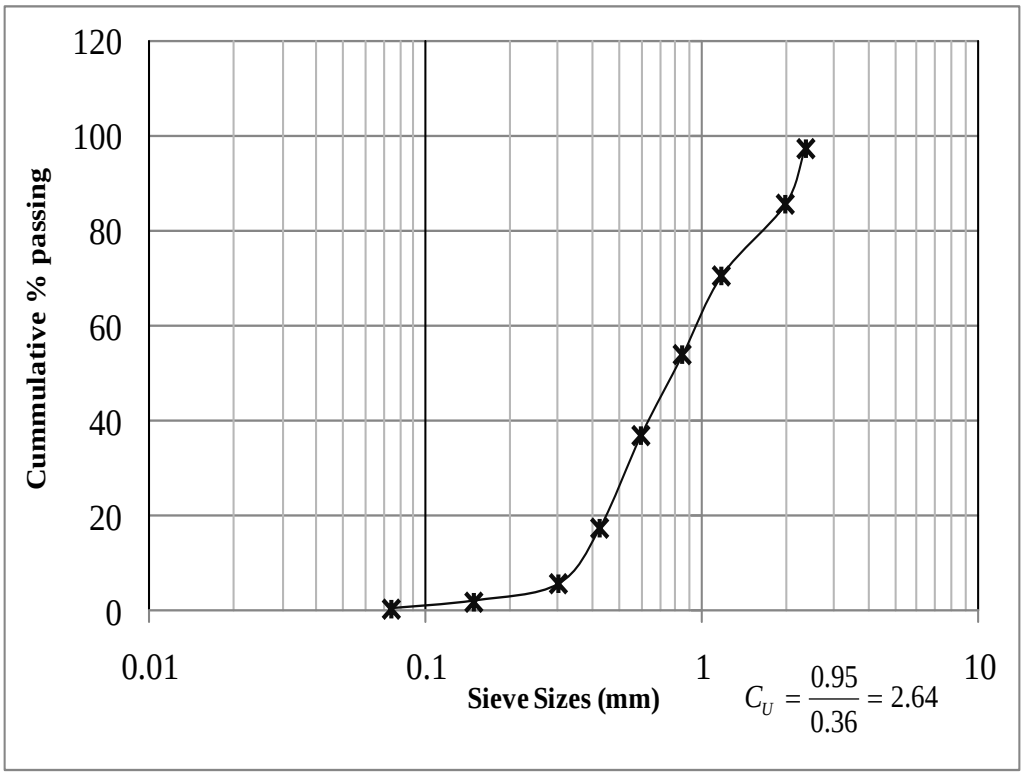

Figure 1. Particle size distribution of ground *Moringa oleifera* seed sample

It can be seen from Figure 1 that the cumulative percentage passing of the total weight of the sample decreased with a corresponding decrease in sieve size. Consequently, it was observed that 93.78% of the total weight passed through the 2.36mm sieve. 85.73% passed through the 2.00mm sieve, while 70.77% of the total weight passed through sieve 1.18mm. The least percentage passing was recorded against 75µm sieve (0.32%), with only 1.95% passing through the 150µm sieve, while greater values of percentage passing (54.0%) and (36.88%) passed through the 850µm and 600µm sieves respectively.

3.2Effect of particle size on phytochemical composition of *Moringa oleifera* seed

The quantitative determination of phytochemical constituents of the six sizes of *Moringa oleifera* seed is presented in Table 1. The results show that all the sizes of the *Moringa oleifera* seed contain substantial amount of saponin, tannin, alkaloid, flavonoid and phenol. The concentration of tannin found in the six sizes of the *Moringa oleifera* seed was higher compared with the level observed for the other constituents.

All five phytochemical constituents were observed to be higher in 600µm *Moringa oleifera* seed sample. The 600µm sample was high (238.17mg/100g) in tannin, comparably higher than the value obtained in 2.0mm sample (198.97mg/100g). The value of tannin was greater (235.05mg/100g) in 850µm sample than (228.65mg/100g) in 1.18mm sample, (217.6mg/100g) in the 4250µm sample, and (204.22mg/100g) in 300µm respectively. Similarly, the value of phenol was more (38.55mg/100g) in 600µm sample with the 425µm sample following closely with a value of 37.0mg/100g. The 2.0mm sample had the least amount of phenol (34.3mg/100g) compared with 35.34mg/100g in 1.18mm sample, 36.50mg/100g in 850µm sample, and 36.21mg/100g in 300µm sample.

Table 1: Quantitative Estimates of Phytochemical Properties of *Moringa oleifera* seed

Phytochemical constituents	Composition (mg/100g)/Particle size					
	2mm	1.18mm	850µm	600µm	425µm	300µm
Saponins	7.98	8.11	8.65	9.5	8.67	8.21
Tannins	198.97	228.65	235.05	238.17	217.6	204.22
Alkaloids	17.33	17.94	18.03	18.36	18.11	16.86
Flavonoids	4.2	4.88	5.04	5.62	4.97	3.92
Phenols	34.3	35.34	36.5	38.55	37	36.21

It was also deduced from Table 1 that 1.18mm sample contained 17.94mg/100g of alkaloid. This was higher than 16.86mg/100g in 300µm sample. Alkaloid was however more (18.36mg/100g) in the 600µm sample

than in 425µm sample (18.11mg/100g), 850µm sample (18.03mg/100g). The lowest concentration of alkaloid was observed in 300µm sample (16.86mg/100g). Saponin was highest (9.50mg/100g) in 600µm sample and lowest (7.65mg/100g) in the 850µm sample. Also, saponin was more in the 600µm sample (9.5mg/100g) than in 425µm sample (8.67mg/100g), and lower in 2.0mm sample (7.98mg/100g) than in 1.18mm sample (8.11mg/100g). The least concentration of flavonoid (3.92mg/100g) was found in the 2.0mm sample while the 600µm had the highest (5.62mg/100g). Flavonoid was more (5.04mg/100g) in the 850µm sample compared with 4.97mg/100g in 425µm sample, 4.88mg/100g in 1.18mm sample, and 4.2mg/100g in the 300µm sample.

The result in Table 1 reveals that the concentration of the phytoconstituents conformed to the findings of Ijarotimi *et al*. (2013) who reported the phytochemical properties of raw *Moringa oleifera* seed and disagrees with Fowoyo and Oladoja (2015) who concluded that alkaloid, flovanoid, phenol and tannin were absent from the seed of *Moringa oleifera* collected from Minna, Niger state, Nigeria. Phytochemicals such as alkaloid, flavonoid, tannin and saponin have also been found to be present in the dried seeds of *Moringa oleifera* obtained from Porthacourt, Nigeria (Aprioku and Onyeneturuchi, 2018). The result also showed that as the particle size decreased from 2.0mm to 600µm, the quantities of the phytochemicals increased, however, further decrease in the particle size from 600µm to 300µm resulted in a corresponding decrease in the quantities of the phytochemicals. Zhang *et al*. (2016) also reported that particle size influenced the total phenolic content of coarse and fine black tea powder. Green tea with particle sizes of 0.25-1mm has been reported to yield higher phytochemicals than the one with particle sizes greater than 1mm (Vuong *et al*. 2011). Lower sizes of plant materials leads to increase in surface area which makes it possible for mass transfer of active components from the plant material (Handa, 2008).According to Makanjuola(2017), the smallest particle size may not always result in high quantities of phytochemicals and the optimum particle size may also depend on the type of phytochemical being desired. However, a critical particle size is achieved whereby further reduction in the particle size would lead to a decrease in the quantities of phytochemicals obtained (Makanjuola, 2017). Similar report has been made by Brewer *et al*. (2014). This means that during the extraction of the phytochemicals, these small particles have the tendency to settle at the bottom of the extraction container which reduces the interaction between the material and the extraction solvent, therefore lesser quantities are given off (Vuong *et al*. 2011). Also, finer particles may become slimy or mucky which may be difficult to filter out during extraction (Makanjuola, 2017).

3.3 Effect of particle size on extract yield of *Moringa oleifera* seed

The percentage yield of the aqueous extracts of the six sample sizes of the *Moringa oleifera* seed were different and is shown in Figure 2. The percentage yield of the extracts ranged from 13.33% - 48.16%. The 300µm size sample had the highest yield (48.16%) while the lowest yield (13.33%) was obtained at 2.0mm size sample. With 425µm sample size, the percentage yield was 44.42%. This percentage yield however dropped to 38.5% as the sample size increased to 600µm. With sample sizes of 850µm and 1.18mm, the percentage yield obtained was 23% and 15.97% respectively. Similarly, Nwaiwu *et al*. (2012 b) found out that the percentage yield of the crude extracts from the seed of *Moringa oleifera* obtained from Borno, Yobe and Adamawa states in Nigeria were 21.56, 51 and 21.85(%) respectively. Also the crude extracts of *Moringa oleifera* seed obtained from Dala, Kano state, Nigeria was reported to yield 21.75% of extracts (Nwaiwu *et al*., 2012 a). From the forgoing result, it was clearly seen that the percentage yield of the extracts followed a rank order of 300µm > 425µm > 600µm > 850µm > 1.18mm 2.0mm. This implies that as the particle size increases, the percentage yield of extracts decreased (See Figure 2).This shows that smaller particle sizes reduces the obstacles to diffusion of solvent into the particles, and that of solute and solvent out of the particle pores (Martinez, 2013), which could result in extraction of active compounds that are high in coagulation and antimicrobial activity (Veggi, 2013).

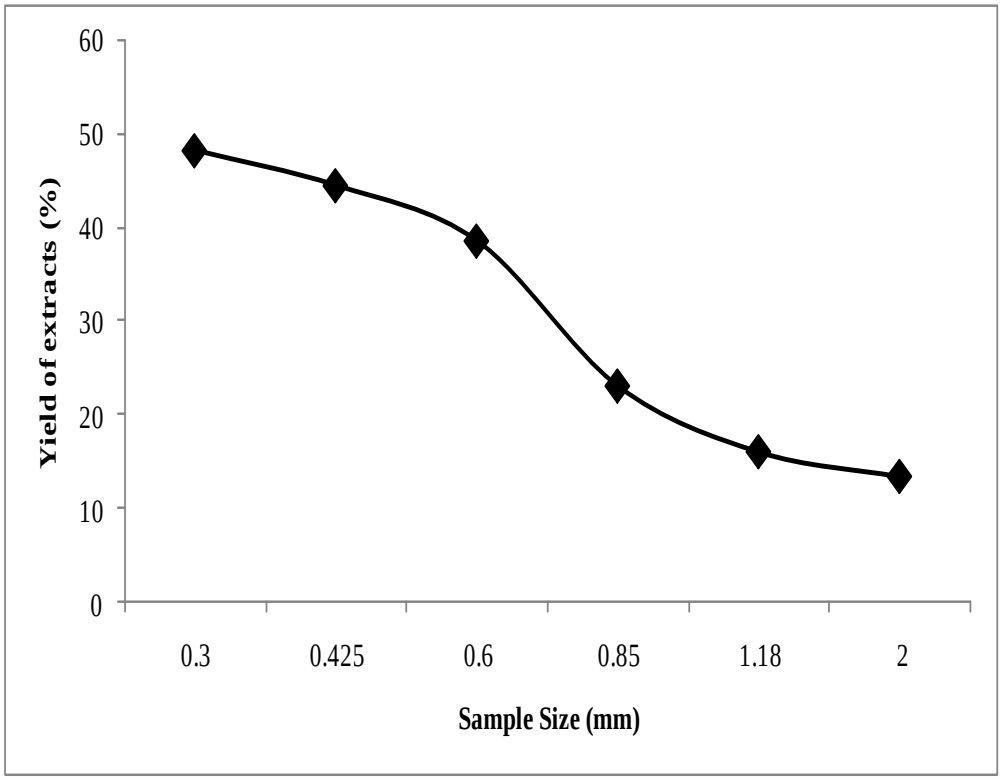

Figure 2. Yield of aqueous extracts of *Moringa oleifera* seed

3.4 Coagulation properties

3.4.1 Effect of particle size and initial coagulant dosage on turbidity removal efficiency of aqueous extracts of *Moringa oleifera* seed

Turbidity removal is a key factor in water purification. Figure 3 gives the effect of particle size on turbidity removal efficiency of *Moringa oleifera* seed extracts at varying coagulant dosage.

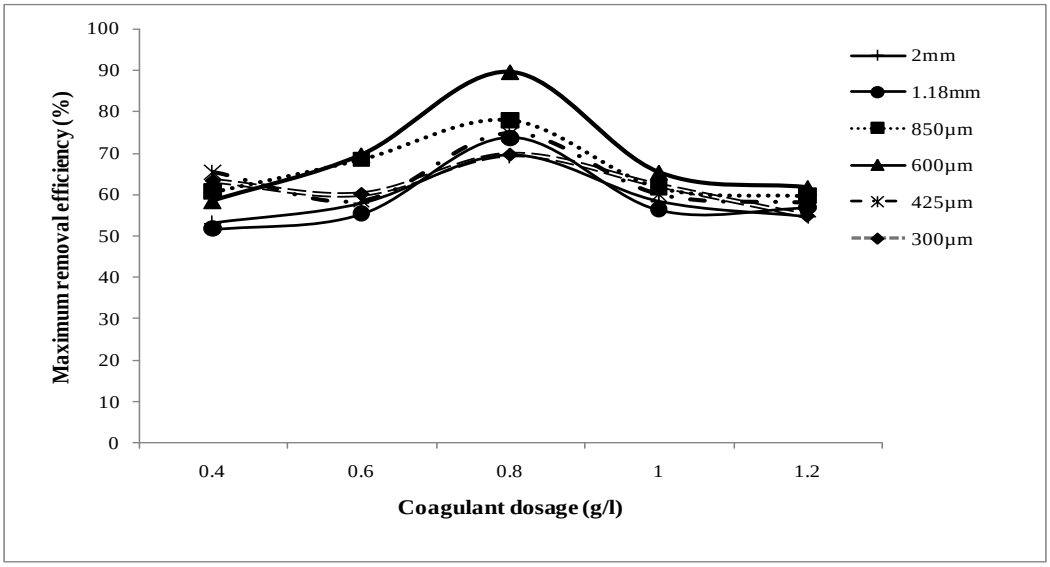

Figure 3. Turbidity removal efficiency with sample size and initial coagulant dosage

Different degrees of turbidity removal efficiencies have been exhibited by the six different sample sizes of the *Moringa oleifera* seed employed in the study. The result showed that on varying the coagulant dosage from 0.4g/l to 0.8g/l, the percentage removal of turbidity for the 2.0mm size sample increased from 53.2% to 69.5%. As the dosage increased to 1.2g/l, the removal efficiency reduced to 54.7%. With the 1.18mm sample

extracts, turbidity removal efficiency ranged between 51.6% and 73.7%. With 0.4g/l the 850µm reduced the turbidity concentration by 60.5%. However from 0.6g/l to 0.8g/l the removal efficiency increased from 68.4% to 77.9%. From 1.0g/l to 1.2g/l there was a reduction in removal efficiency from 61.6% to 59.5% for the 850µm size sample extract. The maximum turbidity removal efficiency of 89.5% was achieved with 600µm sample extracts with coagulant dosage of 0.8g/l. This removal efficiency however dropped to 61.6%at 1.2g/l. The 425µm sample extract had maximum removal efficiency of 74.7% at 0.8g/l and the lowest removal efficiency (57.9%) at 1.2g/l. Within the coagulant dosage range of 0.4g/l – 0.8g/l, the 300µm sample extract yielded turbidity removal efficiency of 69.5%. With increment in the coagulant dosage to 1.2g/l the removal efficiency reduced to 54.7% for the 300µm sample extract. At the end of the experiments, it was observed that the optimum coagulant dosage was 0.8g/l while the optimum particle size was 600µm.The result however agreed with the findings of Nwaiwu *et al*. (2012 a) who reported that the aqueous seed extracts of *Moringa oleifera* removed turbidity from surface water by 84.6%. In another study, Ugwu *et al*. (2017) noted that *Moringa oleifera* seed extracts reduced turbidity in sampled sullage (wastewater) by 91%, which is consistent with Rodríguez-Núñez *et al*. (2012) whose report showed turbidity was reduced by 88.9% in raw water sample with an initial turbidity of 118NTU. Similar reports have been made by Omodamiro *et al*. (2014) and Oria-Usifo *et al*. (2014).

The calculated F-value (5.33974) between the turbidity removal efficiencies obtained from the extracts of the six different sample sizes of the *Moringa oleifera* seed was higher than the tabulated value of F (2.71089) with p=0.00281 at 5% level of significance for degrees of freedom 4 and 5. This shows that particle size had significant effect on the removal efficiencies.

IV. CONCLUSION

This study has revealed the effect of particle size on turbidity removal potentials of aqueous extracts of *Moringa oleifera* seed in raw surface water. All employed sample sizes extract of *Moringa oleifera* seed showed varying turbidity removal efficiencies. This could be attributed to the presence of phytochemicals such as phenols, flavonoids, tannins, saponins and alkaloids. The 600µm sample extract exhibited the highest turbidity removal potential over the other five sample sizes at optimum coagulant dosage of 0.8g/l. Particle size had significant effect on the turbidity removal efficiencies ($p<0.05$). Findings of this study provide a new direction on the use of aqueous extracts of *Moringa oleifera* seed in turbidity removal to avoid wastages and make available more natural material that will serve the growing populace especially in the developing nations.

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