

Full Length Research Article

SYNERGY BETWEEN *Moringa oleifera* SEED POWDER AND ALUM IN THE PURIFICATION OF DOMESTIC WATER

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ABSTRACT

Moringa oleifera seeds were analyzed for chemical composition. Phytochemical screening indicates the presence of saponins (+(+)), flavonoids (+(-)) and alkaloids(+(-)). Instrumental analysis showed also the presence of sodium (15.21±0.10ppm), aluminum (12.21±0.012) potassium (14.21±0.013ppm) and sulphate (1.72±0.011 ppm). Similarly, commercial alum was also analyzed and the results showed the presence of sodium (10.47±0.1500), aluminum (18.17±0.024ppm), potassium (8.01±0.012ppm) and sulphate (3.73±0.010ppm). Jar test trials on raw water samples displayed favourably characteristics at 60% alum to 40% *M.oleifera* mg/l blend with total coliform count of 30ml⁻¹ and turbidity of 3.2NTU below the WHO maximum permissible limit of 5NTU. Other parameters determined also showed conformity with WHO standards for drinking water. The results indicate that *Moringa oleifera* has a double advantage compared to commercial alum because of the presence of phytochemicals which have been reported to possess antimicrobial properties with potentials for conjunctive use with alum for water purification in rural communities.

Keywords: Phytochemicals, coagulant, antimicrobial, total coliform count, turbidity, *Moringa oleifera*

INTRODUCTION

Several chemical coagulants have been used in conventional water treatment processes for portable water production that includes inorganic, synthetic organic polymer and naturally occurring coagulants (Okuda *et al.*, 2000). Generally, alum (Aluminum sulphate), an inorganic coagulant and its synthetic polymeric derivatives are widely used in water treatment (Najm *et al.*, 1998). However, there is a fear that aluminum may induce Alzheimer's disease and strong carcinogenic properties (Crapper *et al.*, 1973, Malleavialle *et al.*, 1984). On the other hand, there is evidence that the use of extracts from plant species possessing both coagulating and antimicrobial properties are safe for human health (Muyibi & Okuofu, 1995; Okuda *et al.*, 2000; Ali *et al.*, 2004; Akinnibosun *et al.*, 2008 & 2009).

Historically, the use of natural materials of plant origin to purify turbid surface waters has been practiced for long. Egyptians inscription afforded the earliest recorded knowledge of plant materials used for water treatment, dating back perhaps to 2000BC in addition to boiling and filtration (Fahey, 2005). Of the large number of plant materials that have been used over the years, the seeds from *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment especially in rural communities (Folkard *et al.*, 1993; Doer, 2005; Onwuliri & Dawang, 2006). Folkard *et al.*, (1993) reported that while aerating well water in rural areas of Sudan for the reduction of carbon dioxide prior to softening, numerous complaints of red water in hot water systems were received even when aeration was continued and the carbon dioxide neutralized with lime in the regular plant treatment process. These complaints ceased and did not reoccur as *Moringa* seeds was used. Palada & Chang (2003) described the morphology of the plant. It is native to India and widely grown in the tropics. It is also called horse radish or drumstick tree and known by many native names in Nigeria such as zogalle (in Hausa), okweoyibo (in Igbo), eweigbake (in Yoruba) and dogalla (in Taroh).

The present research investigates the proximate profiles of *Moringa oleifera* seeds and commercial alum and their synergistic blend purifying properties for domestic water treatment.

MATERIALS AND METHODS

Sample Collection: Riped fruits (pods) of *M.oleifera* were collected from Magama and Mabudi villages of Lantang South Local Government Area of Plateau State, Nigeria during the early rainy season and cracked to obtain the seeds. Commercial alum was purchased from Kwararafa market in Jos, Nigeria.

Sample Treatment: The seeds were peeled to obtain the nuts and dried in an oven for 1hr. Thereafter, the dried seeds were ground and sieved to mesh size of 150 µm. Commercial alum was also ground to mesh size of 150 µm.

Moisture and Crude Fat Contents: These were determined by conventional methods.

Determination of Crude Protein: 0.5 g of powdered *M.oleifera* seeds was weighed into Kjeldahl flask. 0.2 g catalyst system made up of anhydrous sodium sulphate, copper (II) sulphate and selenium dioxide in the ratio 98:1:1 was added to the substrate in

the flask.. 10 ml of pure analyte sulphuric acid was added and the mixture heated until floating was reduced. The solution was transferred into 100ml volumetric flask and diluted to 100ml. 10ml of the solution was placed in the markehan still and 20ml of 40% NaOH was then added. The mixture was steam distilled and 20ml of 2% boric acid containing screened methyl orange indicator was added and titrated with acid to end point. A blank determination was concurrently carried out. Percentage nitrogen was calculated as:

$$\begin{aligned} \text{\% nitrogen} &= \frac{14(T-B) \times M \times 100 \times 100}{1000 \times 10 \times \text{weight of sample}} \\ B &= \text{Blank} \\ T &= \text{volume of acid used} \\ 6.25 &= \text{Conversion factor} \\ M &= \text{Molarity of sulphuric acid} \\ \text{\% crude protein} &= \text{conversion factor} \times \text{percentage nitrogen} \end{aligned}$$

Determination of total carbohydrate: This was determined by L-cysteine sulphuric acid method (Christian,1986).

Phytochemical screening: The procedures of Sofowora (1993) were used for phytochemical screening.

Test for saponins: To small quantity of the powdered seeds, 90% of ethanol was added and boiled. The mixture was filtered hot and cooled and 2.5ml of the ethanol extract dissolved in 10ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 mins and then allowed to stand for another 30 mins. Honey comb froth was observed indicating the presence of saponins.

Test for flavonoids: 2g of the milled seed (powder) was soaked in enough quantity of acetone and allowed to stand for 15-20 mins. It was filtered and warmed over water bath to evaporate the acetone. Warm water was then added to the mark with swirling. This was filtered while hot and the filtrate allowed to cool for subsequence use for sub-lead acetate test. 5mls of the 10% lead acetate solution was added to the filtrate. A yellow coloured precipitate was observed which indicates the presence of flavanoids.

Test for alkaloids: 10g of the powdered seeds was moistened and mixed thoroughly to paste with sufficient quantity of concentrated ammonia and allowed to stand for 10mins. Sufficient quantity of chloroform was added to the paste, boiled and filtered through a plough of cotton wool. The filtrate was transferred into a separating funnel and 30ml of 10% sulphuric acid added and shaken. Two layers were observed. The lower chloroform layer was drained off and discarded while the acid layer was retained. To 2ml each of the acid layer in 5 different test tubes, drops of Meyers, Dragendorf, Waymers and Hawymers reagents were added. Precipitation occurs in most of these reagents indicating the presence of alkaloids.

Determination of Sodium, Potassium and Aluminum in the seed powder and commercial alum: This was determined using Atomic Absorption Spectrophotometer(AAS) analysis.

Determination of sulphate in the seed powder and commercial alum by colometric method: Samples of Moringa seed powder and commercial alum were acidified with 3ml concentrated HCl and

evaporated to dryness. The residues were dissolved in 3ml concentrated HCl, and 50ml of warm distilled water was added. The insoluble silica was filtered and the evaporating dishes with the residues were washed with several portions of hot distilled water. The filtrates obtained were diluted to convenient volumes with concentration HCl to the pink colour of methyl orange indicator and heated to boiling. Warm barium chloride solution was then added drop-wisely and a white precipitate was observed. When the solutions were brought near the standard solution, the colour changes confirmed the presence of sulphate in the solutions.

Jar Test Trials of Alum and Moringa seed powder blends: The jar test protocol was designed to simulate coagulation-flocculation and settling at the Plateau State water treatment plant in Jos. 1g each of powdered moringa seeds and alum was dissolved in separate 100ml of distilled water as stock solutions. 200ml of raw water were measured and introduced into 7 beakers labeled 1-7 with designated dose blends of alum to moringa as:

Jar 1=(2.0:0.0)ml, Jar 2=(1.6:0.4)ml, Jar 3=(1.2:0.8)ml, Jar 4=(0.8:1.2)ml, Jar 5=(0.4:1.6)ml, Jar 6=(0.0:2.0)ml, Jar 7(control)=(0:0).

With a calibrated pipette, each stock solution dosages of alum and moringa solutions were added onto the water samples in the beakers as rapidly as possible..Note: 1ml stock solution contains 0.01g solute \equiv 10mg. The sequence of addition was Moringa solution followed by Alum solution, with stirring paddles lowered into the beakers, and the jar tests mixer turned on. Flash fast mixing was done for 2 mins at a speed of 100rpm, followed by slow mixing for 8mins at 25 rpm. The beakers were observed and evaluated for specific dosages and floc quality. The jar test mixer was turned off and the flocs allowed to settle in the beakers for 30mins and flocs settling characteristics were observed.

Turbidity Test: This was determined by Nephelometric method using a turbidimeter.on water samples on the jar tests.

Calculation of Coagulation activity: This was calculated based on Lee *et al.*, (1995).

Determination of free Alkalinity: To 50ml watersample was added two drops of methyl orange indicator and titrated with 0.02M HCl to the pinkish end point. The titre value was then used to calculate the free alkalinity as described by Alpha-Awwa-Wpcf (1975).

Microbial Test: The microbial test was carried out using the total plate count (TCP) media, which was prepared by dissolving 20.5g of the TCP powder in 1litre of distilled water, autoclaved and allowed to cool. The TCP media was poured into Petri dishes labeled 1 to 7 and innoculated for 24hrs in an electro-thermal incubator. The growth of the microorganisms was then observed and counted per ml (number of microorganisms per ml of water samples).

RESULTS

Table 1 shows the percentage composition of both primary and secondary (phytochemicals) organic metabolites of Moringa seeds powder. Table 2 shows the results of comparative proximate inorganic composition of Moringa seeds powder and commercial

alum in parts per million (ppm) determined by AAS and colorimetric methods. Table 3 shows the results of Jar test trials on raw water samples (Jar numbers 1-7). The table also shows parameters such as floc marks, appearance and settling times of flocs, turbidity

levels, pH values, colour, free alkalinity, clarity, coagulant activities, number of micro-organisms/ml and odour of water samples from the various dose blends compared to parameters of the control (Jar 7).

TABLE 1. RESULTS OF PRIMARY ORGANIC METABOLITES AND PHYTOCHEMICAL SCREENING OF *Moringa oleifera* SEED POWDER

Parameter	A	B	C	D	E	Unknown	Saponnins	Flavonoids	Alkaloids
Compositon (%)	4.73 ±0.25	1.05 ± 0.18	38.0 ± 0.07	2.66± 0.28	5.13 ± 0.42	48.33	+(+) very much present	+(-) slightly present	+(-) slightly present
A-Moisture content B-Ash content	C-Fat content D-Protein		E-Carbohydrate						

TABLE 2. RESULTS OF INSTRUMENTAL ANALYSIS OF *M. oleifera* SEED POWDER AND COMMERCIAL ALUM.

Parameter	Concentration in (ppm)	
	Alum	Moringa seeds
Sodium (Na)	10.47 ± 0.015	15.21 ± 0.010
Potassium (K)	8.01 ± 0.024	14.21 ± 0.013
Aluminium (Al)	18.17 ± 0.024	12.21 ± 0.012
Sulphate (SO ₄ ²⁻)	3.73 ± 0.010	1.7.2 ± 0.011

TABLE 3. RESULTS OF JAR TEST TRIALS AND ANTIMICROBIAL ACTIVITIES OF TEST WATER SAMPLES

Parameters	Jar Numbers						
	1	2	3	4	5	6	7(Control)
Coagulant Dosage (mg/l) Alum: Moringa	100:0	80:20	60:40	40:60	20:80	0:100	0:0
Floc mark	5	6	5	4	3	2	0
Appearance time (mins)	<1	<1	<1	<1	>1	>1	
Settling time (mins)	<15	<15	<15	>15	>15	>15	
Temperature (°c)	27.0	27.0	27.0	27.0	27.0	27.0	26.0
Turbidity (NTU)	2.61	2.81	3.20	8.70	20.90	68.00	129.00
Clarity	Very clear	Very clear	Very clear	Clear	Cloudy	Cloudy	Very cloudy
Odour	Odourless	Odourless	Odourless	Slight	Slight	Slight	Offensive
Colour	Colourless	Colourless	Colourless	Light brown	Light brown	light brown	Dirty brown
pH	7.0	7.2	7.3	7.3	7.2	7.3	7.2
Alkalinity (mg/l)	8.0	8.8	12.0	16.0	22.0	30.0	8.9
Coagulant activity	0.9798	0.9782	0.9736	0.9326	0.89380	0.4729	0.0000
TPC/ml	280	100	30	41	43	50	>300

DISCUSSION

In nutritive terms, the percentage composition of carbohydrates, proteins and fats are reasonably obtained from the seeds of this tree in addition to its highly nutritive leaves (Jefrey, 2006). Yang *et al.*, (2006) reported the nutritional and functional qualities of Moringa, indicating a value of 5.7% for protein and 3% for carbohydrate in the leaves. Comparing these values with values of 2.66% for protein and 5.13% for carbohydrate obtained from the seeds in the present work suggests a reasonable comparative nutrient content of the seeds to the leaves as source of energy (Lockett *et al.*, 2000). Furthermore, Jiru *et al* (2006) reported that moringa oleifera is a good source of micronutrient. Earlier reports (Fuglie, 1999; Anwar & Bhangar, 2003) showed values of 30-40% crude fat content from Moringa seeds which is in agreement with our result of 38% crude fat. The same authors suggested that the fat content of the seeds could have additional uses as lubricating, cooking and soap making oil.

The results of proximate inorganic analysis of *M.oleifera* seeds along side that of Alum, indicate appreciable concentrations of sodium, aluminum, potassium and sulphate in the seeds compared to that of alum. It is known that the sulphate of these elements do confer on this seed a purifying property as evident in the process of softening of hard water by ion exchange whereby potassium, aluminium and sodium present in the ion exchange column are responsible for precipitating calcium and magnesium in the hard water, resulting in water softening (Muyibi & Evison, 1994; Olabode *et al.*, 2005). Therefore the presence of these metals in the seeds of *M. oleifera* makes it a good material for water softening comparable to alum or any synthetic polyelectrolytes (Van-Benchoston & Edzward, 1990; Okuda *et al.*, 2000).

Jar numbers 1, 2, & 3 showed better flocs as compared to numbers 4, 5 & 6. The higher floc number is an indication that a higher

alum:Moringa dose ratio forms better or denser flocs than a higher Moringa : alum dose ratio which forms sluggish or lazy flocs. Furthermore, jar numbers 1, 2, 3 & 4 formed flocs faster with appearance time of less than 1min while flocs from jars 5 & 6 appeared in more than 1min. Similarly, it was observed that the dosages of 100% alum, 80:20, & 60:40mg/l alum to Moringa dose ratios (jars 1,2 & 3 respectively) settled in less than 15 mins while those of 40:60, 20:80mg alum to Moringa dose ratios & 100% Moringa (jars 4, 5 & 6 in that order) settled in more than 15mins. These observations are in consonant with the definition of flocculation as a process of gentle and continuous stirring of coagulated water that enables the formation of flocs through the aggregation of minute particles present in water which can easily be removed by settling or filtration (Folkard *et al.*, 1993). This also agrees with Lee *et al.*, (1998), Najm *et al.*, (1998) & Luu (2000) who compared the turbidity removal methods for various polyelectrolytes including *M. oleifera* seeds and confirmed that the time required for the flocs to settle down the bottom of the container is an important factor in ensuring a regular supply of treated water. This means that alum in its pure form is a more powerful electrolyte because it aggregates and settles faster than Moringa seeds which in this study were used in the crude form (Muyibi & Evison, 1994).

The pH of all the dosages ranged from 7.0 to 7.3 which are within the WHO (1971 & 1976) permissible limits of 6.5-8.5 for drinking water. However, the 100% alum dose solution moved towards more acidity attributable to the fact that alum produces sulphuric acid in solution which lowers the pH values. This tendency towards increase in acidity could also be due to trivalent cation aluminum which serves as a Lewis acid that can accept a lone pair of electrons (Miller *et al.*, 1984). The reverse was observed when Moringa concentration of the dosing solution was increased. The pH increases slightly with increasing concentrations of the Moringa coagulant even though Muyibi & Evison (1994) observed very high doses (400-750mg/l) of Moringa in the complete removal of total hardness in water at no significant increases in pH values. However, as earlier mentioned, high dosage of alum in water treatment even though a better coagulant may lead to high acidity raising health concerns about alum related diseases reported by several investigators (Miller *et al.*, 1984, Martyns *et al.*, 1998 & Najm *et al.*, 1998). Doer (2005) reported that the action of *M. oleifera* as a coagulant lies in the presence of water soluble cationic proteins in the seeds (Table 2). This suggests that in water, the basic amino acids present in proteins of Moringa would accept a proton from water resulting in the release of a hydroxyl group making the solution basic. This accounted for the slight tendency towards basic pH values observed when the concentration of Moringa was increased compared to alum.

The turbidity tests show that the doses 100mg/l alum, 60:40mg/l & 80:20mg/l alum to Moringa dose blends reduce the water turbidity from 129 NTU to below 5NTU which is the maximum permissible limit World Health Organisation (WHO) standard for drinking water while the other dose blends 40:60mg/l, 20:80mg/l alum to Moringa & 100mg/l Moringa have turbidity values respectively higher than 5 NTU. Similarly, the number of microorganisms (Total Plate Count) reduces from 280 at 100% alum to a minimum of 30, below the 50 maximum permissible WHO limit at 60:40mg/l alum to Moringa dose is attributable to presence of phytochemicals mentioned

earlier. Thereafter very slow increases were observed in the coliform count up to 50 as the dose of Moringa increases from 60 to 100mg/l (jars numbers 4,5 & 6) as compared with that of the coagulant free sample (jar number 7) containing over 300 microbial count ml⁻¹. This slow increase in number of microorganisms as the dose of Moringa increases may be attributed to the enmeshment of the microorganisms by the high concentration of particulate matters still present in the Moringa treated water as observed by Amagloh & Benang (2007). Furthermore, Okuda *et al.*, (2000) have earlier observed the shortcoming of Moringa treated with distilled water (as stock solution in the present study) results in residual dissolved organic carbon (DOC) of the treated water. DOC is usually regarded as source of odour, colour and taste and precursor to disinfection by-products in drinking water treatment as observed in jars 4, 5 & 6 at 60, 80 & 100mg/l Moringa doses respectively.

From the results of the jar tests it was observed that the optimum dose for the coagulants blend from this study is 60:40mg/l alum to Moringa dose ratio (jar number 3) because it displays the best characteristic properties in terms of colour, odour, taste, floc mark, turbidity, settling time, pH clarity and total plate count in conformity with WHO standards. This optimum dose observation is in disagreement with the findings of Muyibi & Okuofu (1995) who reported optimum dose ratio of 20mg/l alum to 10mg/l Moringa with water having initial turbidity between 30 to 90NTU and also disagrees with that of Folkard *et al.*, (1993) who had earlier reported Moringa dosages between 10-200mg/l for turbidities ranging between <50 and >150NTU, moreover that these investigators did not report any microbial count and other parameters. WHO (1971 & 1976) standards for drinking water specify that, water intended for human consumption must be free from organisms and from concentrations of chemical substances that may be a hazard to health. In addition, supplies of drinking water should be as pleasant to drink as circumstance permit.

Blending alum and *M. oleifera* seed powder showed characteristic synergies in water treatment than using alum alone or Moringa alone. The conventional method of water treatment using expensive chemicals such as alum and calcium hypochlorate (chlorine) is expensive thereby making portable drinking water beyond the reach of rural dwellers that depend mainly on contaminated water sources such as wells, dams, streams, rivers e.t.c. exposing them to water borne diseases. The optimum dose observed in the present study of 60mg/l alum to 40mg/l Moringa seed powder reduces alum requirement by 40-60% and by implications the risk of alum related diseases by about 40-60% as well as the cost of water treatment (Muyibi & Okuofu, 1995). Blending alum and *M. oleifera* seed powder in water treatment can effectively improve water sanitation in third world countries at low cost because the plant is cultivated locally. However, isolating and purifying the active agent(s) from the plant and toxicological assessment of the seeds on various species of bacteria are necessary to evaluate its antimicrobial property.

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