Capsule Formulation of *Moringa Oleifera* seed for River Water Clarification

Okubanjo Omotunde.O#, Olokede T.

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy. University of Lagos, P.M.B 12003 Lagos, Nigeria.

ABSTRACT

Moringa oleifera (MO), is a tropical tree, whose seeds have been reported to contain high water-soluble proteins that act as effective flocculant and coagulant for water treatment. This study seeks to design an encapsulated formulation of the seed powder of MO which is standardized and can be easily applied for water clarification in rural setting thus enabling a greater chance of treating water with doses within standard limits especially in rural areas of developing and underdeveloped countries in line with World Bank report recommendations. Optimal extraction of oil using n-Hexane was carried out and the residual seed powder collected was encapsulated in a gelatin capsule. Treatment of sample water was carried out by directly using the encapsulated defatted seed cake powder of *Moringa oleifera*. The effective dose of encapsulated MO seed powder of 50mg/L to150 mg/L for reducing the Total Dissolved Solid, hardness, chloride, turbidity was evaluated. The powder filled into gelatin capsules was brown and spherical in shape with excellent flowing properties evidenced by an angle of repose of 27.02° and a Carr's index of 5.02%. The weight uniformity of the capsules were within official limits over a 30day period and the capsules remained stable over the same period at room temperature. At a concentration of 150mg/L the encapsulated MO exhibited excellent antimicrobial, flocculant as well as coagulant properties. The formulation of capsulated *Moringa oleifera* seed-cake powder has been shown to be effective in water clarification

Keywords: Clarification, Water Treatment, Capsules, *Moringa oleifera* seed cake powder, Dosage form

INTRODUCTION

Humans use water for several purposes but the level of purity of the water being consumed is very crucial since it has a direct effect on health. Due to limited alternatives, in rural settings in developing nations, surface water either from rivers or rain fed ponds has become one of the main sources of water supply. This water is vulnerable to various forms of pollution generated from different sources mainly households, agriculture and industries (Oria-Usifo et al, 2014). This has a range of serious health implications (WHO Guidelines, 2011) and since one of the Millennium Development Goals (MDGs) call for reducing by half the proportion of people without sustainable access to safe drinking water and basic sanitation by 2015 (UN MDG Fact Sheet, 2013), it has become imperative for researchers to focus on providing sustainable and cost effective solutions water flocculation and clarification. Although, aluminium is the most commonly used coagulant in the developing countries, studies have linked it to the development of neurological diseases (e.g. pre-senile dementia or Alzheimer's disease) due to the presence of aluminium ions in the drinking water (Patrick et al., 2004).

Consequently, there is a need to develop alternative, cost effective and also environmentally friendly coagulants. A number of effective coagulants from plant origin have been identified such as Okra (Raji et al, 2015); red bean, sugar and red maize (Gunaratna et al., 2007) and *Moringa oleifera* ((Kumar et al. 2010)). The seeds of *Moringa oleifera* are one of the most effective sources of coagulant for water treatment (Kumar et al., 2010). The powder from crushed MO seed kernels work as natural flocculants and coagulant that aid in binding the solids in water and causing them to sink to the bottom (Beltrán-Heredia & Sánchez-Martín, 2009); Ali 2004). Comparative coagulation studies between alum and MO have also been studied (Sarpong & Richardson 2010). Compared to the commonly used coagulant chemicals, MO is relatively cheap. It produces lower biodegradable sludge volume without affecting the pH of the water thus making it an environmentally friendly alternative with significant potential both in developing and developed countries.

Corresponding author: mokubanjo@unilag.edu.ng ; omotundeokubanjo@yahoo.co.uk

Phone: +234- 803-714-7599, +234 -807-059-0910

Though aluminium sulfate stands out as, the most effective chemical coagulant used in many countries due to its effectiveness and relative low cost, their use has been associated with acceleration of the degenerative process of Alzheimer's disease (Cardoso et al, 2008).

Due to lack of access to effective water treatment methods, agents, formulation and/or facilities in developing and underdeveloped countries, formulation of a safe biodegradable and standardized water clarification dosage form has become imperative (Yarahmadi, 2009).

This study seeks to design an encapsulated formulation of the seed powder of MO which is standardized and can be easily applied for water clarification in rural setting thus enabling a greater chance of treating water with doses within standard limits.

MATERIALS AND METHOD

Materials

Moringa oleifera seed pods, n–Hexane, Ethylene diamine tetra acetic acid (EDTA), Eriochrome black indicator, were obtained from Sigma Aldrich, GmbH, Silver Nitrate solution and potassium chromate solution were purchased from R&M Chemicals, Essex, U.K., all other reagents and chemicals used were of analytical grade.

Collection of Sample

Mature (brown) fruit pods from *Moringa oleifera* tree, along with its leaves and bark were harvested from a forest field in Ibadan, Oyo State. These were identified and authenticated by Mr. Oyeleke of the Department of Pharmacognosy Department of the Faculty of Pharmacy, University of Lagos, and stored as herbarium specimens PCG/MO/100.

Extraction of Oil

A 210g sample of seeds obtained from *Moringa oleifera* seed pods were weighed transferred into a mortar and crushed using a pestle until a cake-like mixture was achieved. Optimal extraction of oil using n-Hexane was carried out as reported by Nzikou et al., 2009 and the residual seed powder collected in a clean amber bottle and stored in a desiccator until required.

Capsulation of Moringa Oleifera Seed Powder

Moringa oleifera seed powder was evaluated for moisture content, particle size distribution, bulk and tapped density and angle of repose prior to encapsulation. Total plate count of *Moringa oleifera* seed powder was also carried out prior to capsulation.

Empty gelatin capsules shells of size 1 and volume 0.5ml obtained from Walmat® Nigeria Limited, Lagos were manually filled using the following method. The empty capsule shells were weighed W1, then, 150mg of MO seed cake powder with a spatula was gathered together on a weighing paper to form a powder bed. The cap of the shell was removed and the empty capsule body held between thumb and forefinger was repeatedly dipped into the powder until all the powder got into the shell body. The cap was replaced and the filled capsule was weighed, W2. Thereafter, W1 was subtracted from W2 and for every capsule a final weight difference of 150mg was ascertained. Thereafter, the capsules were cleaned with a napkin and stored in an air-tight glass amber bottle. The encapsulated seed powder was visually inspected and evaluated weight uniformity. The capsules were placed in airtight amber glass bottle with a screw cap as the secondary packaging material to prevent the possible effect of light on the stored capsules. Since the capsules are not intended for oral use, a red label was put on the container bearing the capsules and had printed on it the strength of each capsule (150mg), the direction for use (empty one capsule into one litre of water to be treated, stir or shake very well, leave for 1 hour and pour off the clear water into a clean container) and storage condition (to be stored in cool and dry place).

Water clarification activity

Water (5L) was collected from Obawole river located within Ifako- Ijaiye Local Government Area of Lagos State, Nigeria in a plastic container and immediately transported to the Department of Pharmaceutics and Pharmaceutical Technology, University of Lagos, Idi–Araba where it was stored at 4°C until it was ready for use.

Clarification Test Procedure

Treatment of sample water was carried out by directly using the encapsulated defatted seed cake powder of *Moringa oleifera*. According to Mangale et al. 2012, the effective dose of MO seed powder is 50mg/l and 100 mg/L for reducing the Total Dissolved Solid, hardness, chloride, turbidity; while when parameters considered included Standard Plate Count and Most Probable Number, the effective dose of *Moringa oleifera* seedcake powder was 150 mg/L. Thus, the concentration of seed powder utilized for the study was150mg/L.

One capsule containing 150mg of powdered seed extract of MO was dispersed into one litre of river water to be treated, upon stirring for a minute, the water was allowed to stand undisturbed for about an hour, after which the resulting clean water was carefully decanted off leaving the coagulated particles.

Physico-chemical and microbiological analysis of water were carried out to include turbidity, pH, hardness, chloride content, and total dissolved solids and the results were compared with that obtained using distilled water.

Turbidity

Turbidity of untreated and treated water sample was measured using HACH 2100 N turbidimeter, Hach U.S.A. The turbidimeter was first sensitized and balanced by pouring a sample of water to be treated into one sample cell and distilled water into another. Observations were made and readings were taken from the screen. The same procedure was repeated twice for both untreated and treated water sample.

pH determination

The pH of the treated and untreated water samples, was measured using a S20 SevenEasy pH Meter, Mettler Toledo,U.S.A. The base end of the pH meter was immersed into the water sample inside a beaker and reading was taken from the meter. Measurement was done before and after treatment in triplicates.

Hardness

Hardness of treated and untreated samples was measured using the titration technique.

Treated water sample (25mls) was shaken thoroughly and diluted to 50ml with distilled water; 2 drops of Eriochrome black indicator was added to the solution and titrated with EDTA (Ethylene diamine tetra acetic acid). Within 5mins, the blue colouration observed was regarded as the end point and the titre value was read off. The same method was repeated for untreated water sample in triplicates. Formula used for the calculation of hardness in mg/lCaCO3 = $\frac{A \times B \times 1000}{\text{Sample (ml)}}$

Where, A = final reading minus initial reading = ml of titrant.

B = mg CaCO3 equivalent to 1.00ml EDTA titrant.

Chloride content

Chloride content was measured using Mohr's method. Dry sodium chloride of weight 20mg was measured with an analytical balance and dissolved in 100ml of distilled water. This was used to standardize the silver nitrate solution. The pH of this solution was measured and it was adjusted to an alkalinity value of between 8 and 10 by adding sodium hydroxide after which 1.0ml of 5% potassium chromate was added as indicator. This solution was titrated with silver nitrate solution (0.01M) until a permanent orange pink colour was obtained. Untreated water (100ml) was measured and then filtered. Thereafter, 1.0ml of potassium chromate was added as indicator and titrated against the standardized silver nitrate solution. This procedure was done for treated samples. All titrations were done in triplicates.

Formula used for the calculation of the chloride content is given below;

Chloride concentration (mg/L) = (A – B) ml × M(mmol/L) × 35.5 (mgCl/mmol) 100ml × 1L

Where, A = ml of the silver nitrate used for sample

B = ml of silver nitrate used for blank; and

M = Molarity of silver nitrate.

Total dissolved solids

Total dissolved solids was measured by weighing the amount of dissolved solids present in a known volume of the water sample. This was done by weighing an empty beaker with an analytical balance, filling it with 50ml of water sample treated that has previously been filtered with a filter paper, evaporating the water in an oven at 105°C and completely drying the residue. The beaker containing the residue was then weighed. The total dissolved solids concentration is the difference between the weight of the beaker with the residue, and the weight of the beaker without. This procedure was done for both the untreated and treated water sample.

Microbiological analysis involving total plate count of untreated and treated water was carried out after clarification was done. The same procedure was repeated for water samples that were not treated with the seed powder as negative control and distilled water as positive control.

RESULTS

Phytochemical Tests

The screening carried out on the seeds extract of MO indicated the presence of reducing sugars, glycosides, tannins, flavonoids steroids, resins, saponins, alkaloids and high fatty acid.

Table 1: Preliminary phytochemical screening of seed powder of Moringa Oleifera using hexane as solvent.

Chemical constituent	**Hexane extract of seeds**
Alkaloids	+
Steroids	_
Tannins	+
Resin	+
Glycosides	+
Flavonoids	+
Saponins	_
Fatty acids	+
Peptides	+

Key:

(-) = Negative (absence of constituent)
(+) =Positive (presence of constituent)

Physical Parameters of river water treated with MO seed cake powder.

The table below shows the results obtained from the physical parameters studied before and after treating the river water (1L) with 150mg capsules of MO seed cake powder. Fig 1 also shows the clarification potential of the capsulated MO seed cake powder.

Table 2:Parameters studied before and after treatment of 1Litre river water with 150mg capsule of MO seed cake powder.

Parameters	**Before Treatment**	**After Treatment with MO (150 mg/l)**	**WHO Standard**
pH	8.2 ±1.00	7.1 ± 0.06	6.5 - 8.5
Turbidity (NTU)	10.7 ± 0.09	3.0 ± 0.03	*HDL-200 #MPL -500
TDS (mg/l)	602 ± 0.33	210 ± 0.25	*HDL- 200 #MPL- 500
Colour	Cloudy	Colourless	Colourless
Hardness as $CaCO_3$ (mg/l)	101 ± 1.12	54 ± 1.02	*HDL - 200 #MPL -600
Chloride Content (mg/l)	12 ± 0.57	5 ± 0.57	*HDL -200 #MPL- 600
Bioburden or TPC/100ml	3.5×10^5± 0.56	0.9×10^2 ± 0.05	* HDL - 200 #MPL - 1×10^6

NTU- Nephthelometric Turbidity Units;
TDS- Total Dissolved Solids;
TPC- Total Plate Count;
WHO - World Health Organisation
*HDL -Highest Desirable Limit;
#MPL- Maximum Permissible Limit

Untreated water **Treated water**

Figure 1 Water clarification before and after treatment with capsule containing MO seed cake powder; 150mg

The histogram plot (Figure 2) shows the size distribution of the particles of the MO seedcake powder.

The angle of repose, bulk density and tapped density determines the flow rate property of the powder. The results are as shown below in Table 3.

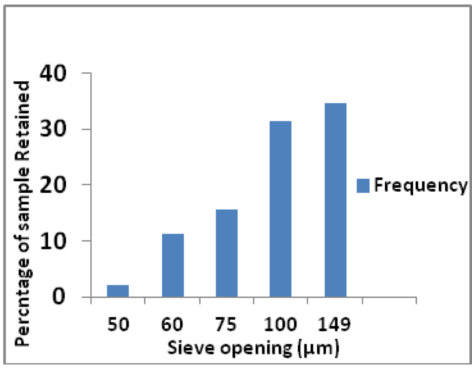

Figure 2 Histogram plot of particle size distribution of sample powder.

Table 3: Other Formulation parameters investigated

Angle of Repose	27.02° ± 0.45
Bulk Density	0.91g/ml ± 0.01
Tapped Density	0.98g/ml ± 0.02
Carr's Index	7.4% ± 0.01

DISCUSSION

The result obtained from the phytochemical testing indicates that the MO seed cake powder possessed flavonoids, alkaloids, glycosides, tannin, resin, fatty acid and peptides using hexane as extraction solvent. The results are in agreement with results obtained from investigations carried out by previous researchers like Abalaka et al., (2012). Hexane was employed as the extracting solvent because it has been shown to be the most effective extraction solvent with respect to oil-content removal which when present, impedes the coagulant-flocculant efficacy of MO seed powder, Nzikou et al., 2009. The presence of resins, flavonoids and peptides has been associated with the clarifying and antibacterial activity of MO seedcake powder (Suarez et al., 2005); Ghebremicheal et al., 2005). It has also been documented that the presence of alkaloids, tannins and glycosides are responsible for MO seed cake powder's activities against many microorganisms (Oluduro, 2012). Other reported functions of MO seedcake powder includes anti-asthmatic, antitumor (Farooq et al, 2012), antipyretic, antiulcer, immune stimulant (Mehta et al., 2011) and nutritional supplement (Ijarotimi et al., 2013). Similarly, resins (as part of the constituents present) has been reported by Mataka et al., 2010 to be associated with adsorbing properties, which explains their probable adsorption of particles suspended in water to promote clarification and also make them valuable for wound dressing and filling cavities in curing tooth ache.

Physico-chemical and microbiological investigation of MO seedcake powder showed that the powder effectively reduced the turbidity of the river water.

Total dissolved substances were effectively reduced by the utilized concentration to a value that is within the acceptable range of W.H.O. It is believed that the seed powder due to its positivity causes a disturbance in the existing but loose interaction between the dissolved substances and water molecules, hence causing insolubility and thereafter coagulation. This effect may also assist in removing the unpleasant color of the water.

Total dissolved substances were effectively reduced by the MO seedcake powder concentration utilized. The particles in the river water are clumped together by the powder and settle at the bottom of the container, thereby bringing about clarity evidenced by the turbidity result as seen in Table 2. The turbidity of water sample reduced significantly to a value that agrees with the W.H.O standard hence, declaring the treated water aesthetically safe. There was no significant change in pH of the water sample. This result correlates that reported by Abalinawo et al., 2008.

Total or standard plate count / bio-burden refer to quantitative counting of total number of bacteria. Moringa seed powder was seen to significantly reduce the microbial load as reported earlier by different scholars (Abalinawo et al., 2008, Oria-Usifo et al., 2014). According to the USP29-NF24 (2006), the dry sieving process for estimating the particle size distribution of a pharmaceutical powder is generally intended for use where at least 80% of the particles are larger than 75µm.The MO powder mass falls under this category of powders as 84% of the particles were larger than 75µm. The MO powder mass can be said to be essentially coarse as seen by the larger percentage of bigger granules in the powder. However, in spite of this predominant granular morphology, the sieving method employed enabled the choice of a finer powder bed. It is in view of this and also to minimize segregation that the particle mass retained of sieve size 75µm was employed in the formulation process. The histogram chart reveals the powder distribution as a non-symmetrical one showing skewness towards lower size ranges. Table 3 shows that the powder bulk has an angle of repose of 27.02º with the fixed height funnel method of angle determination. This clearly indicates the powder possesses a good flow property. The flow property recorded with the powder can be attributed to the spherical nature of the individual particles, which facilitates their gliding over one another. The Carr's index value also corroborates the result that MO seed cake powder possesses a good flow rate property. According to Aulton 2001, a powder with its Carr's index falling in the range of 5 – 15% has a good flowing property. Hence, the powder, at industrial scale, will not have flow rate challenges, which will further reduce the necessity for glidants. This is a major factor in production since it is to be dispersed in portable water. The less excipients used, the better the clarifier as the cumulative presence of some of these excipients in the human body may lead to health complications (Stein, 2010)

The powder has a bulk density of 0.91mg/ml and a tapped density of 0.98mg/ml, depicting the presence of air-filled volume in the bulk, which was reduced with tapping to give an increased density.

Each filled capsule contained 150mg of MO seedcake powder and the idea of presenting the final product in this pharmaceutical dosage form was born out of the rationale to standardize the concentration of the MO powder that is utilized for water treatment as well as discourage the usual indiscriminate use of the powder or pulverized seeds. Dispersible tablets would also have been acceptable but for the need to incorporate different excipients, also, capsules are aesthetically appealing and user-friendly.

There was no significant difference between keeping the capsules in the container in a refrigerator and keeping it at room temperature Katayon et al., (2006). The ambient temperature option is preferred over refrigeration, as most communities that are faced with the challenge of poor quality of drinking water do not have access to refrigerator due to absence or poor electricity supply.

CONCLUSION

A powder product packaged as capsules of Moringa seed-cake has been investigated for the clarification of water. These capsules have the potential of serving as an alternate and convenient method of river water clarification in rural areas since quantification of the amount of Moringa seed-cake powder in each capsule against the quantity of water to be purified has been elucidated.

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