African Study Monographs, 15(3): 135 – 142, November 1994

STUDIES ON TRADITIONAL WATER PURIFICATION USING *Moringa oleifera* SEEDS

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ABSTRACT The efficacy of *Moringa oleifera* Lam seeds paste for water purification was investigated. Chemical analysis found the seed to contain 34.1% protein, 15% carbohydrates and 15.5% lipids. Phytochemical tests and spectral studies led to the elucidation of a steroidal glycoside-strophantidin as a bioactive agent in the seed. Comparative studies with alum showed that the seed paste was effective in the clarification and sedimentation of inorganic and organic matter in raw water. It reduced the total microbial and coliform counts by 55% and 65%, respectively, after 24 hours whereas alum achieved 65% and 83% reduction under similar conditions.

Key Words: *Moringa oleifera*; Purification; Bacteria; Sedimentation; Coliforms.

INTRODUCTION

Moringa oleifera Lam, is a deciduous plant native of India, but introduced into the tropics generally (Hutchinson & Dalziel, 1966). The tree is fast-growing and has been planted in many tropical countries. Its common names are horse-radish tree, drumstick (English), Samarin danga (Hausa), Ewe ile (Yoruba), and Okwe oyibo (Ibo), Yevutsi (Ghana), Bagaelean (Togo), Kangaluni (Malawi), Mborongi (Kenya) and Mrongo (Somalia).

The capsule of the fruit, about 20–45 cm long, are pendulous, linear, acuminate and ribbed. It contains numerous globular seeds, about 1 cm wide with three membranous wings at the base and apex.

Scattered African nomads have, in the past, used the paste of the seeds as a natural coagulant for water purification in the Sudan and other parts of Africa (Eleirt et al., 1980; Anonymous, 1987). The plant is also known to have a wide application in therapy ranging from its use as an antiscorbutic and anti-irritant in Nigeria, for the treatment of diarrhea, rheumatism and goitre in Mauritius to its use for treating nervous debility and leprosy in India (Sofowora, 1984). The seeds can as well be consumed after drying and used as a condiment and garnish. Furthermore, they are not toxic. As a multi-purpose tree, it is also of great environmental interest for plantations, in compounds, around fields, and on nursery plots.

The practice of using the seed for water purification has long died out in Africa. However, the flocculating value of the plant has recently attracted the attention of non-governmental organisations (NGO) and research centres in developing coun-

tries. Further research into its efficacy in water purification is desirable so that it may be employed in providing easy and safe means of potable water for rural population, since modern technology for potable water is limited, very expensive, and unavailable in those areas. This will also allow for easy transfer of laboratory experience to village needs.

In this study, preliminary studies were carried out to assess its coagulating potential by optical density and total solid measurements after treating the water with the seed paste. The effect of *Moringa* on total bacterial and coliform loads in water was determined by the standard plate count. To elucidate the active agent, a study of the spectral data was carried out.

MATERIALS AND METHODS

(1) Plant material

Moringa oleifera seeds were collected from the residential quarters of the University of Ilorin, Nigeria. A voucher sample was deposited at the Biological Sciences Herbarium of the University.

(2) Preparation of seed paste, crude filtrate and seed extract

Twenty grams of the dried seeds of *Moringa oleifera* were ground into powder and a stock was kept from which appropriate amounts were taken for experiments.

Five grams of the seed powder were dissolved into 100 ml of sterile distilled water and filtered using Whatsman Paper No. 1. This served as the crude extract. Five grams of the seed powder was extract using 80% ethanol and the yield was evaporated in a rotary evaporator.

(3) Chemical analysis of seeds

The seed powder was analysed for moisture, ash, minerals, total carbohydrate, lipids and protein content using the standard methods (A.O.A.C., 1980). The content of K^+ and Na^+ was also determined by the flame photometric method.

(4) Phytochemical and spectral studies

In order to elucidate the functional groups and structure of the bioactive compound of the seed, a study of spectral data was carried out. The ultra violet (U.V.) spectrum was obtained on a Pye Unicam Model 648657 recording spectrophotometer in MeOH, and the infrared (ir) spectrum was determined on a Pye Unicam Model 641749 recording spectro-photometer in KBr pellets. The nuclear magnetic resonance spectrum (^{1}H-nmr) was recorded on Brucker-WH-90 spectrophotometer (60 MHz) in $CDCl_3$, with deuterated MeOH with tetramethylsilane (TMS) as the internal standard and chemical shifts reported in lambda (ppm) units.

A Liebermann-Buchard reaction was performed on the seed paste to test for the presence of steroids (Herbuorne, 1973). To 50 milligrams of seed powder, five drops of acetic anhydride and a drop of concentrated H_2SO_4 were added. This was then steamed for one hour and neutralized with NaOH followed by the addition of chloroform. The appearance of a blue-green colour indicated the presence of steroid.

(5) Effect of *Moringa* on clarification

One hundred milligrams of the seed powder were crushed and made into a paste with a small quantity of the raw river water. This was then made up to one litre by stirring it in a one-litre measuring cyclinder of raw river water. An equal amount of alum was used for a similar preparation. Raw Asa River water served as a control.

The degree of clarification was measured by taking the optical absorbances of the treated and the raw water samples using the Pye-Unicam Model SP6-500 U.V. spectro-photometer. Ten ml were taken from the upper layer of the water column which represented the treated water portion, and measured at 0, 24, 48, and 96 hours respectively.

(6) Effect of *Moringa* on sedimentation of total solids

A clean platinum evaporating dish was dried in the oven at 103°C for 1 hour until a constant weight was obtained after cooling in a dessicator. A 250 ml volume of Asa River sample was thoroughly mixed, from which 100 ml was measured into the dish and evaporated in the oven for 1 hours at 103°C. It was then cooled in the dessicator and weighed. The total solids in ppm were calculated as:

$$\frac{\text{increase in weight (gm} \times 1{,}000{,}000)}{\text{ml of sample}}$$

To test the comparative effects of alum and *Moringa* powder, 100 mg of each substrate were weighed into 1 litre of the river water sample and thoroughly mixed. The total solids content at 0, 24, 48 and 96 hours were determined as described above by using 100 ml from the treated water sample.

(7) Effect of *Moringa* on total bacterial and coliform load

Total bacterial and coliform counts of the raw water was estimated by serial dilution using the plate method. One millilitre of a 10^{-4} dilution was plated on duplicate plates of Yeast Extract Agar (Oxoid) and incubated at 37°C for 48 hours after which the total coliforms were enumerated on Eosin Mythylene Blue (Oxoid) agar.

At 0, 24, 48, and 96 hours, 1 ml was carefully measured from the treated portion of the water layer, serially diluted and enumerated on the same media.

The same procedure was followed for alum treated water.

RESULTS

The percentage chemical composition of *Moringa oleifera* seed powder (Table 1) consisted of protein (34), carbohydrate (15), lipids (15.5), with more sodium (0.7) than potassium (0.04).

The U.V. spectrum exhibited maximum absorption at 440 nm in MeOH showing the presence of unsaturated conjugated bonds. Their v max (KBr) of the seed extract showed absorptions at 3,300, 3,300–2,700, 1,730 and 1,650 cm^{-1}, revealing multiple hydroxyl, lactone and unsaturated ketone groups (Table 2). The H-nmr spectrum exhibited signals at lambda 0.5–1, 4 and 5.3 (Table 3).

Table 1. Chemical composition of crude powder of *Moringa oleifera*.

Component of Seed	%
Dry matter	95.0
Moisture	5.0
Ash	11.8
Protein	34.1
Carbohydrate	15.0
Lipids	15.5
Sodium	0.71
Potassium	0.04

Table 2. Infra-red (IR) spectrum characteristics *Moringa oleifera* seed extract.

Bond	Wave length (cm^{-1})
$(O\text{-}H)_n$; nl	3,300
C-H stretch C=0	3,300–2,700
C=0	1,730
C=0	1,650

Table 3. Nuclear Magnetic Resonance (MNR) shift-characteristics of *Moringa oleifera* seed extract.

Type of Proton/group	Range of Shift
Multiple CH_3 and CH_2 groups	0.5–1.0
-OH base	4.0
C=C-H	5.3

The characteristics of spectral studies and the positive result from Liebermann-Burchard test led to the elucidation of strophantidin, a steroidal glycoside as the bioactive compound.

Significant degrees of clarification and sedimentation of total dissolved solids were effected by the *Moringa* seed paste, comparative to alum. After a holding time of 24 hours, 37% reduction was achieved by the seed paste as against 48% recorded by alum. These corresponded with 58% and 65% reduction in optical densities by the seed and alum respectively (Fig. 1, 2).

Similarly, the bacterial load of raw water treated with the seed paste and alum were reduced after 24 hours by 55% and 65% respectively. The total coliform populations of raw water sample were also reduced by 63% and 83% by the seed paste and alum (Fig. 3, 4).

CONCLUSION

A number of bioactive agents have been isolated from different parts of the *M. oleifera* plant. Pterygospermin, an antifungal substance has been extracted from

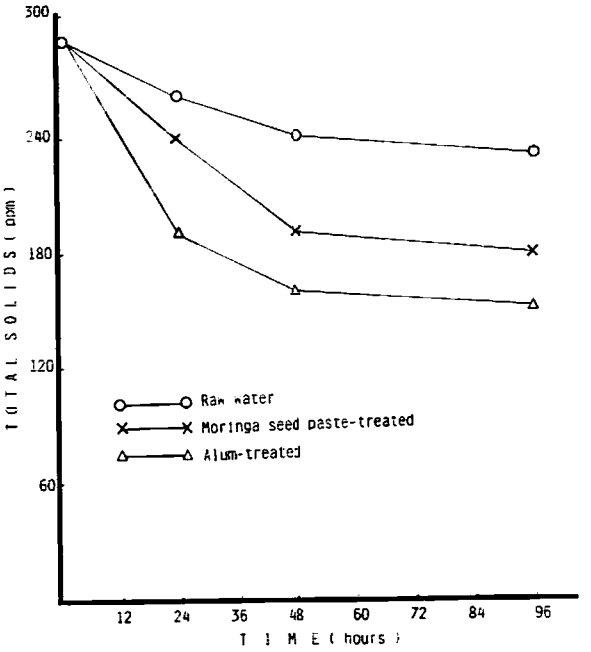

Fig. 1. Sedimentation rates of total solids in water treated with *Moringa* seed paste and alum.

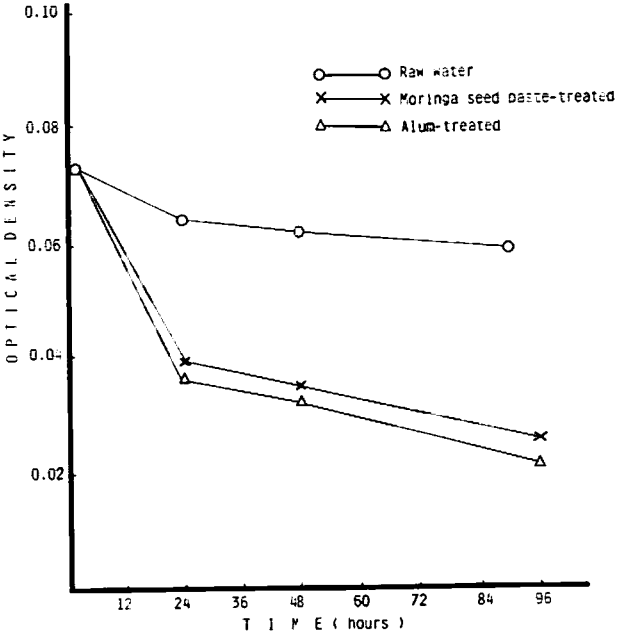

Fig. 2. Clarification of water by *Moringa* seed paste and alum.

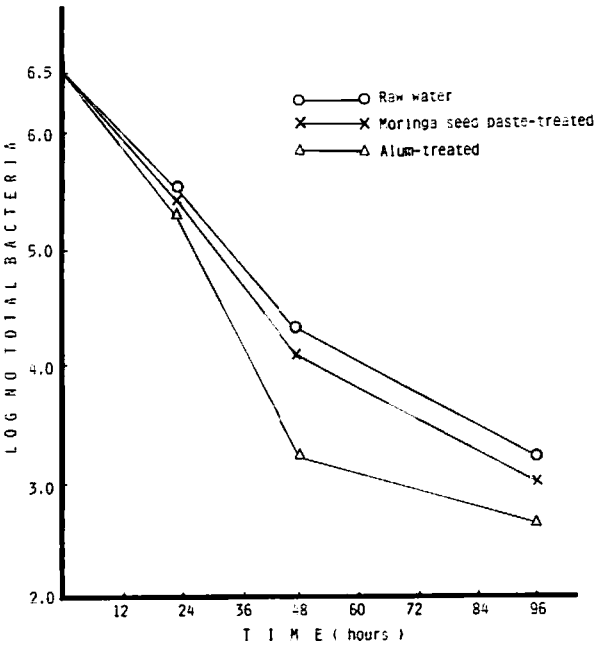

Fig. 3. Effects of *Moringa* seed paste and alum on total bacteria in Asa River water.

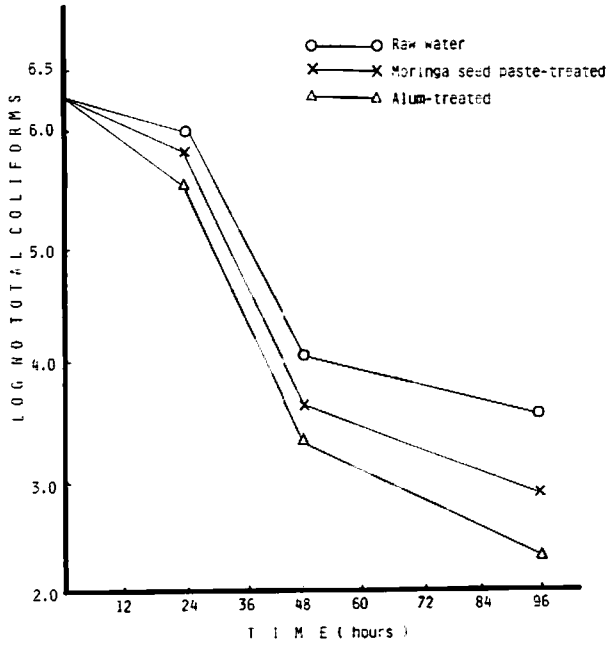

Fig. 4. Effect of *Moringa* seed paste and alum on total coliforms in Asa River water.

the flower. 4-hydroxy-mellein, vanillin, β-sitostenone and β-sitosterol have been characterised from the stem (Sofowora, 1984). From the root bark, stimulants and diuretics identified as moringine and moringinine have been isolated (Eleirt et al., 1980). Also from the seeds, 4(β-L-rhansoxyloxy) benzyl isothiocyanate and moringyne have been elucidated (Memon et al., 1985).

In this study, strophantidin was identified. Strophantidin, a cardenolide is a C_{23} steroidal glycone with α, β unsaturated five membered lactone ring and a C_{14} hydroxyl group. This bioactive ingredient, like other cardiotonic glycosides such as digoxigenin and strophantidol, has been extracted from *Digitatis* species and other plants. These complex glycosides are known to inhibit bacteria and fungi (Myant, 1981).

The reduction observed in bacterial populations of raw water treated with the seed paste can be attributed to the antibacterial properties of the bioactive ingredient. Narashima-Rao (1984) recorded similar observations with a component of pterygospermin present in flower. The observation here agrees with the preliminary findings of the 'Water Aid for Africa' workers who reported a 95% reduction of total bacteria by the seed paste. These encouraging findings support the recommendation that of the seed paste is a cheap and easy alternative for purifying water, especially in remote areas.

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———Accepted *September 14, 1994*

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