Effect of Extraction Method on the Antimicrobial Activity of *Moringa Oleifera* Seeds Extract

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Abstract: Moringa Oleifera seeds extract has been found to posses antimicrobial properties. The mechanism of its action in this regard is still largely un-determined. In this work, six methods of extracting the active ingredients from the Moringa seed powder were considered. They were: Normal aqueous extraction (M1), Salt extraction (M2), Oil removal followed by aqueous extraction (M3), oil removal followed by salt extraction (M4), oil removal followed by aqueous extraction and microfiltration (M5), and oil removal followed by salt extraction and microfiltration. The antimicrobial action of each extract on *Escherichia Coli* was determined using Disc Diffusion and Agar Well methods. An average of 639.77±16.72gm of seed kernel was obtained per Kg of raw Moringa seed processed. The results also showed that 72.28±4.84% cake was produced for each 10gm of the <210 μm seed powder de-fatted and the seed oil produced was 28.75±1.68%. The zones of inhibition produced using disc diffusion method ranged 6.5mm – 9.0mm for the six extraction methods with the highest value of 9.0mm obtained with method M3. The Agar well method yielded 6.75mm – 12.38mm zone of inhibition diameter with the highest value of 12.38mm obtained with method M3 of seed extraction. The investigation thus concluded that the M3 was the best extraction method. The Minimum Inhibitory Concentration (MIC) for the Moringa disinfection Solution M3 was determined to be 200μL/mL while the Minimum Bacterial Concentration (MBC) was 210μL/mL. Optimization of the operating conditions was suggested for the subsequent application of this method of seed processing in water disinfection.
[Bichi, M. H., Agunwamba, J. C., Muyibi, S. A., and Abdulkarim, M.I. **Effect of Extraction Method on the Antimicrobial Activity of *Moringa Oleifera* Seeds Extract.** *J Am Sci* 2012;8(9):450-458]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 63

Keywords: Moringa Oleifera, Seed Extract, Extraction Method, Disinfection, Antimicrobial Activity.

1. Introduction

The provision of portable water satisfying modern quality requirements is an enamours task of most water supply agencies the world over. Whereas the demand for water is increasing, the recurrent costs of water supply agencies is increasing (Muyibi, et al, 1995) and the quality of the raw water from the various sources is decreasing due to increased industrialization (Bichi, 2000; Dan'azumi & Bichi, 2010a,b). Moreover, the cost of treatment chemicals, especially in developing countries, is increasingly becoming beyond the reach of most water supply agencies (Muyibi & Evison, 1995a,b), which often leads to under-dosing with the concomitant production of low-quality waters. In addition, the use of chemical additives for water treatment raises a lot of concerns over safety issues (Arbuckle et al., 2002; Goveas, et al, 2010).

Chlorine, for instance, is being widely used as a disinfectant. However, chlorine has problem of decay and reduced concentration as the water flows through the distribution network (Devarakonda, et al, 2010). It also has the potential for forming carcinogenic and mutagenic disinfection by-products (DBPs) (Goveas, et al, 2010). Disinfectants and their by-products may also be associated with increased risks of cardiovascular diseases, cancers, and birth defects. Although such risks are low, Arbuckle et al., (2002); Bove et al., (2002); and Woo, et al., (2002) noted that associations with such diseases could not be ruled out.

These, and the high cost of chlorine, especially in developing countries where it needs to be imported, makes it imperative to look for cheaper alternatives that are also environmentally friendly. Studies by Eilert at al (1981); Suarez, M., et al (2003), Suarez, M., et al (2005), Fisch, et al (2004), Thilza, et al (2010), and Bukar, et al (2010) identified the presence of an active antimicrobial agent in *Moringa Oleifera* seeds.

Moringa Oleifera (*Zogale*) is cultivated across the whole of tropical belt and used for a

variety of purposes (Jahn, 1986). Many researchers (Bina, 1991; Muyibi and Evison, 1995a; Buthelezi, et al 2009) have reported its great potential for water treatment. Many researchers have reported its use as a coagulant (Jahn and Dirar, 1979; Muyibi and Evison, 1995a; Jahn, 1988; and Folkard et al, 1992; Okuda, et al 2001a,b); a softening agent (Muyibi and Evison 1995 a,b); and a bactericidal agent (Madsen, *et al,* 1987; Eilert, *et al* 1981; Kalogo, et al, 2000; Bukar, et al , 2010; Thilza, et al, 2010).

Eilert *et al* (1981) identified 4α-*4-rhamnousyloxy-benzyf-isothiocynate* as an active antimicrobial agent in *M. Oleifera.* This is readily soluble to water at 1.3umol/l and is non-volatile. In a study using pure *4α-4-rhamnotyloxy-benzyl-sothiocynate* isolated from defatted *M. Oleifera* seeds, the antimicrobial action of *M. Oleifera* was investigated on three bacteria species - *Bacillus Subtilis* (gram -ve) , *Serratia Marcescens* (gram -ve) and *Mycobacterium Phe4.* The result showed that B. *Subtlis* was completely inhibited by 56μmol/l and *M. Pheli* by *40μ*mol/L. Only partial inhibition was observed for S. *Macesscens* in the range of concentration considered.

Madsen *et al* (1987), in a study carried out on turbid Nile water in the Sudan, observed turbidity reduction of 80-99.5% paralleled by a bacterial reduction of 1-4 log units (90-99.99%) within the first one to two hours of the treatment with *M. Oleifera* seeds, with the bacteria being concentrated in the coagulated sediments.

The effect of residual turbidity on the antimicrobial action of *M. Oleifera* was also reported. Folkard (1989), using extract of *M. Stenopetala,* was able to achieve 90% reduction of *Herpes simplex* virus and *Orf* virus. Whereas re-growth of *Serratia Marinatubra* occurred at high dosage (800rng/l), no re-growth w*as* observed at lower seed dosage. In each case, the initial sample turbidity was between 20 to 25 NTU with residual turbidities in the range of 3-8NTU. However, Jahn (1982) reported that residual turbidities greater than 100NTU was companied by bacterial removal of only 0-36%.

Thilza, et al (2010) reported that Moringa leaf stalk extract had mild activities against E.Coli and *Entrobacter aerogenes*. Bukar, et al (2010) also studied the antimicrobial activities of Moringa Seed Chloroform extract and Moringa Seed Ethanol extract. They found both to have inhibitory effects on the growth of *E.Coli* and determined the Minimum Inhibitory Concentration (MIC) to be >4mg/ml.

This suggests that Moring seed extracts may be promising alternatives to the currently used chemical water treatment agents. In this paper, six methods of extracting the active ingredients in the Moringa Oleifera seeds are presented, and their performance with respect to antimicrobial action investigated. The method producing the highest antimicrobial action is evaluated.

2. Materials and Methods

Processing of *Moringa Oleifera* Seeds Powder: The dry *M. Oleifera* seeds used for the studies were obtained locally from the villages surrounding the Bayero University (New Campus) Kano, Nigeria. The seeds were air freighted to the Biotechnology Engineering Research Unit (BERU) of the Department of Biotechnology Engineering, Kulliyya of Engineering, International Islamic University (IIU), Kuala Lumpur, Malaysia where the laboratory investigation was carried out. Good quality dry seeds of *M. Oleifera* were selected and the seed coat and wings were removed manually. The kernel was ground to fine powder using the coffee mill attachment of the Moulinex domestic food blender. The ground powder was then sieved through 210 μm sieve. Appropriate quantity of the powder was weighed using an analytical balance for each of the tests to be carried out. The flow chart for the production of the Moringa seeds powder is shown in Figure 1.

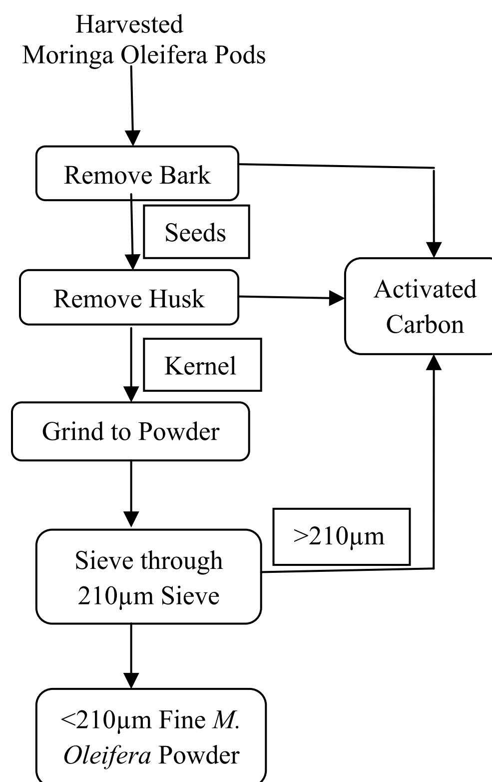

Figure 1: Flow Chart for Production of Moringa Oleifera Seeds Powder

Extraction of Bio-active Constituents of Moringa Oleifera Seeds Powder: Six different methods of extracting the bio-active constituents from the Moringa seeds powder were used (Figure 2). The <210µm Moringa powder was divided in to three portions 1(a), 1(b), and 1(c). Portion 1(a) was used for normal aqueous extraction to produce Moringa disinfection powder/solution (M1). Portion 1(b) was used for salt extraction to produce Moringa disinfection powder/solution (M2).

The third portion (1c) was used for oil extraction methods. After extracting the oil, the filtrate can be used as animal feed and fertilizer and the cake residue was used in the production of Moringa disinfection solution/powder. The de-fatted cake residue was then divided in to two equal portions (2a and 2b).

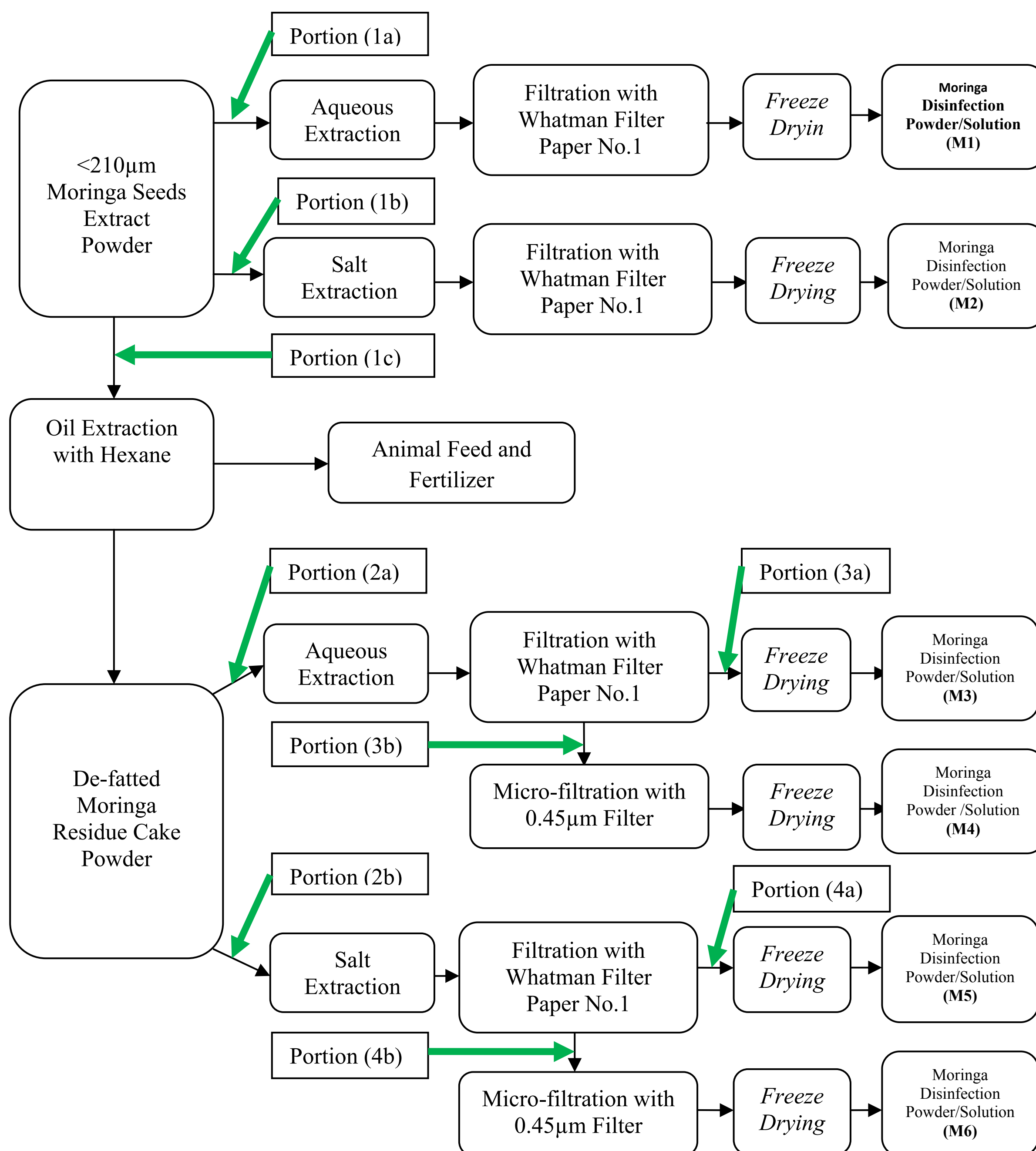

Figure 2: Flow Chart for *Moringa Oleifera* Seeds Extraction Methods

Aqueous extraction was applied to portion 2(a) after which the mixed liquid was filtered with Whatman No.1 filter paper. The resulting filtrate was then further divided in to two portions 3(a) and 3(b). Portion 3(a) was the Moringa disinfection solution (M3). Portion 3(b) was applied to micro-filtration with 0.45µm filter cartridge and the filtrate yielded Moringa disinfection solution (M5), which can also be freeze-dried to give Moringa disinfection powder (M5).

Salt extraction method was applied to portion 2(b). After the salt extraction, the solution was filtered through No.1 Whatman filter paper and the resulting liquid further splitted in to two portions 4(a) and 4(b). Portion 4(a) was the Moringa disinfection solution (M4) which could be freeze-dried to produce Moringa disinfection powder (M4) while portion 4(b) was passed through 0.45µm micro-filter and the filtrate became Moringa disinfection solution (M6) which could be freeze-dried to give Moringa disinfection powder (M6).

Normal Aqueous Extraction of Bio-active Constituents (Method M1): To extract the active ingredients using aqueous extraction method (M1), 10gms of the <210µm fine *Moringa Oleifera* seed powder was measured in to 500mls of sterile distilled water in a beaker. 200mls distilled water was added and put in a high speed mixer (ATO MIX, MSE) and blended for 30minutes at 150rpm to extract the active ingredients. This was then put through No.1 Whatman filter paper and the filtrate made-up to 500mls with distilled water. This stock solution was used directly for the disinfection studies and some could be freeze dried to produce Moringa disinfection powder (M1).

Normal Salt Extraction of Bio-active Constituents (Method M2): This was carried out by adding 1M NaCl to 5gm of the sample and mixed for 30 minutes at room temperature (23 ± 2°C) using the magnetic stirrer (ERLA, ERLA Technologies (M) Sdn. Bhd. Malaysia) as described by Ali (2010). The extraction solution was centrifuged at 6000 rpm for a period of 10 minutes using the centrifuge (Eppendorf, 5804R, Germany), and the supernatant filtered through Whatman filter paper No.1. The retentate can be used as animal feed and/or fertilizer. The extracted liquid was used for the study and could also be freeze-dried in to a powder to give Moringa disinfection powder (M2).

Oil Extraction of Bio-active Constituents Followed by Aqueous Extraction (Method M3): ***Oil Extraction***: Oil extraction was done by adding hexane to the seed powder. To separate the oil from the seeds, the electro thermal Soxhlet was used and the procedure was as follows: Weighing of 10 gm of *Moringa Oleifera* seed powder and setting it in the thimbles of the electro thermal soxhlet extraction chamber; Adding 170 ml of Hexane in the heating chamber; Evaporating of hexane within three cycles each to ensure the extraction of oil from the seeds (until the hexane became colourless); Drying of *Moringa Oleifera* cake residue from the soxhlet thimbles and weighting the dry sample (Muyibi et al. 2003). The *Moringa Oleifera* cake residue stock after oil extraction was used in this and subsequent methods of seeds processing. The Moringa cake residue stock after oil extraction was divided in to portions (2a) and (2b) for use in aqueous and salt extractions respectively.

Aqueous Extraction: The active ingredient in the cake powder (2a) was then extracted using the normal aqueous extraction procedure earlier described in Method (M1). The supernatant after filtration was then divided in to two portions 3(a) and 3(b). Portion 3(a) was the Moringa disinfection solution (M3) and portion 3(b) was passed through micro-filtration procedure to produce Moringa disinfection solution (M5). The other portion (2b) of the cake residue was used in the salt extraction.

Oil Extraction Followed by Salt Extraction of Bio-active Constituents (Method M4): The salt extraction was carried out as described in Method (M2). The extracted liquid after filtration with Whatman paper was divided in to two portions (4a) and (4b). Portion (4a) was the Moringa disinfection solution (M4) and could be freeze-dried in to a powder to produce Moringa disinfection powder (M4). Portion 4(b) was used to produce Moringa disinfection powder (M6).

Oil Extraction Followed by Aqueous Extraction and Micro-filtration (Method M5): The oil and aqueous extractions were carried out using the procedure earlier described in Method (M3). The other portion (3b) of the supernatant was used for the Mico-filtration. To perform the miro-filtration technique, the molecular weight cut off was needed. Ali (2010) found the molecular weight to be 670, 158, 44, 17, and 1.35 kDa, respectively. Filter paper (Waters GHP 0.45 µm) was used to filter the sample before injecting into HPLC. Thus 0.45 µm filter cartridge was used for the cross-flow filtration.

Cross Flow Filtration: The bio-active constituent was separated using micro-filtration techniques. The equipment used for this process was QuixStand Bench top System with peristaltic pump (Watson-Marlow Bredel Pumps, Falmonth Cornwall TR 11 4RU, England. The microfiltration cartridge (CFP-4-E-3MA) was used for sample filtration with pore size of 0.45 µm. This has a fibre ID 1mm, membrane area 110 cm^2, and nominal flow path

length 30 cm, and is a polysulfone membrane which operates in a vertical orientation complete process fluid drainage. The supernatant produced after microfiltration produced Moringa disinfection solution (M5).

Oil Extraction Followed by Salt Extraction and Micro-filtration (Method M6): The portion (4b) of the supernatant was used for the Mico-filtration. This was done using the procedure earlier described in Method (M5). The supernatant produced after this micro-filtration was the Moringa disinfection solution (M6).

Preparation of Synthetic Water for Disinfection Studies: The microorganism used was *Escherichia coli* ER2566 which was obtained from the Bioenvironmental Engineering Laboratory of the Department of Biotechnology Engineering, Kulliyya of Engineering, International Islamic University, Kuala Lumpur, Malaysia. This was maintained on nutrient agar slants and stored at 4°C. The *E.Coli* strain was cultured on nutrient agar slants for 24 hours at 37°C. A loop-full of the culture was diluted in sterile saline solution (0.85g $100mL^{-1}$) to give a final concentration of approximately 10^8cfu mL^{-1} (Chamsai, et al 2009).

Preparation of Nutrient Agar: McConkey agar and bacteriological peptone agar were prepared as stipulated (HI media lab PVT Ltd.). Weigh 28 grams of nutrient agar and dilute in 1.0 litre of distilled water in a clean beaker. Heat slightly, to dissolve, and sterilize in autoclave at 15 PST for 15 minutes.

Disinfection Studies: The disinfection studies was aimed at applying the *Moringa Oleifera* seeds extract disinfection solutions produced using the six different methods (M1, M2, M3, M4, M5 and M6) of seeds preparation in the disinfection of synthetic water using *E. Coli* as an indicator organism. The disinfection studies for antimicrobial susceptibility of the Moringa seeds extract were carried out using the Disc Diffusion Method (DDM) and the Agar Well Method.

Disc Diffusion Method (DDM): The agar plates were prepared by pouring 10 to 20 ml of melted nutrient agar solution maintained at 44°C to 46°C on to petri dishes and allowing the medium to solidify within 10 minutes on a level surface in a laminar flow hood. The determination of the antimicrobial activity was carried using the procedures described by Walter, et al (2011) and Chamsai, et al (2009).

Sterile wire loop was dipped in the *E.Coli* bacterial suspension and evenly streaked in three directions over the entire surface of the agar plate to obtain uniform inoculums. 6 mm diameter sterilized paper discs made from Whattman filter paper were impregnated with the test extracts and placed into the surface of the inoculated media. Plates set with sterile distilled water serve as negative control while that with chlorine solution serve as positive control. The experiment was replicated three times. The set up was allowed to stand for 24 hours in an incubator at 37°C. The plates were then inspected for the presence of zones of inhibition around the discs (if any) which was interpreted as indication of antibacterial activity. The diameter of the zone of inhibition was measured in each case. The procedure was repeated for each of the extract solutions produced using the six different methods, as well as the control solutions.

Agar Well Method: This was carried out using the procedure described in Ugbgu & Akukwe (2009) and Oluseyi & Francisca (2009). Determination of antibacterial activity of the extracts was carried out as follows: Nutrient agar was poured into Petri dishes, allowed to set and bored with a sterilized Durham tube. Bacterial culture was used to inoculate each of the agar plates after which about 0.01 ml of the extract was added. Incubation was done at 37°C for 24 h after which the plates were inspected for zones of inhibition. The procedure was repeated for each of the extract solutions produced using the six different methods. Sterile distilled water was used as negative control while 5.5% chlorine solution was used as positive control.

Determination of MIC and MBC: The minimal inhibitory concentration (MIC) and a minimal bactericidal concentration (MBC) of the Moringa seeds extract produced using the best method was determined using the broth dilution method in test tubes as described by Chamsai, et al (2009). Fifty microlitres of each substance at a concentration of 200-1000 µL/mL was added to 5 ml of nutrient broth (NB) tubes containing inoculums of 10^8 cfu mL−1. Different dilutions of the Moringa extract were made with sterilized distilled water. The tubes were then incubated at 35°C for 24 hr on an incubator shaker (Gallenkamp, Loughborough, England) to evenly disperse the extract throughout the broth. The lowest concentration that showed no visible growth was regarded as the MIC. Cells from the tubes showing no growth were sub-cultured on NA agar plates to determine if the inhibition was reversible or permanent. The MBC was determined as the lowest concentration at which no growth occurred on the plates.

3. Results

Raw Seed Processing: The raw seed was processed by manually removing the wings and the seed coat to produce Moringa Oleifera seed kernels.

One kilogram of the seed was used in each case and the weight of seed kernels produced in each case is given in Table 1.

Table 1: Weight of Moringa Kernel Produced per Kg of Raw Moringa Seed

S/NO	Raw Seed (Kg)	De-husked Seed Kernel (gm)
1	1	653.40
2	1	616.23
3	1	649.70
Average		639.77±16.72*

*Standard Deviation

On average, a Kilogram of raw Moringa seed produced 639.77±16.72gm of seed kernel. The kernel, after grinding using the domestic blender, was sieved through 210µm sieve with a sieve shaker to produce Moringa seed powder. For each 10gm of the <210 µm seed powder, the de-fatted seed cake produced averaged 72.28±4.84% after oil extraction. The extracted oil was separated from the Hexane using the Rotary Evaporator (BUCHI Rotavapour R-210/215). The yield of the extracted oil from the seed powder averaged 28.75±1.68%.

Antimicrobial Susceptibility Tests Results: The six Moringa disinfection solutions were subjected to antimicrobial susceptibility tests. Two methods were used for the test: The disc diffusion method (DDM) and the Agar Well method. The results obtained using the two methods are presented in Tables 2 and 3. In each of the two methods, the highest zone of inhibition was obtained with Moringa disinfection solution (M3).

Table 2: Results for Disc Diffusion Susceptibility Test (Av, Average; EC1, E.Coli plate for Test Run No. 1; EC2, E.Coli plate for Test Run No. 2)

Test Runs		Inhibition Zone Diameter(mm) For Moringa Disinfection Solutions							
		M1	M2	M3	M4	M5	M6	C+	C-
E.C 1	*1*	7	7	9	7	8	7	22	6
	2	7	7	7	8	7	7	22	6
	3	7	7	8	7	7	7	23	6
	Av	7	7	8	7.3	7.3	7	22.3	6
E.C 2	*1*	6	8	10	7	6	6	34	6
	2	6	8	10	8	6	6	30	6
	3	6	9	10	7	6	6	28	6
	4	6	7	10	7	6	6	28	6
	Av	6	8	10	7.3	6	6	30	6
Av. Result (mm)		6.5	7.5	9.0	7.3	6.7	6.5	26.15	6.0

Table 3: Results for Agar Well Susceptibility Test (Av, Average; EC1, E.Coli plate for Test Run No. 1; EC2, E.Coli plate for Test Run No. 2)

Test Runs		Inhibition Zone Diameter (mm) For Moringa Disinfection Solutions							
		M 1	M2	M3	M4	M5	M6	C+	C-
E.C 1	*1*	6	9	13	7	7	7	35	6
	2	6	7	13	7	7	7	36	6
	3	6	12	13	9	7	10	36	6
	Av.	6	9.3	13	7.6	7	8	35.6	6
E.C 2	*1*	6	7	12	9	10	10	35	6
	2	6	8	11	8	10	8	35	6
	3	12	7	12	9	11	8	35	6
	4	6	9	12	8	10	8	35	6
	Av.	7.5	7.8	11.8	8.5	10.3	8.5	35	6
Av. Result (mm)		6.8	8.5	12.4	8.1	8.6	8.3	35.3	6.

Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC): The Moringa disinfection solution M3, determined as the best method of seed processing, was used for the determination of MIC and MBC. Concentrations of 1000, 800, 600, 400, 200, and 0µL/mL were sued for the determination. After incubation at 37°C for 24hrs, the tube containing 200µL/mL of the extract was the lowest concentration that showed no visible growth. This was thus taken as the Minimum Inhibitory Concentration (MIC). The cells from this and subsequent tubes were sub-cultured on agar plates to determine if the growths were reversible. No growths were observed and as suggested by Chamsai, et al (2009), the MBC was taken as 10µL/mL higher than the MIC value. Thus The MBC was taken as 210µL/mL.

4. Discussions

Moringa Seeds powder is traditionally prepared by manually removing the wings and grinding the kernel in to powder. In this study, one kilogram of the seed yields 635±16gm of the seed kernel or 63.5%. The quantity of fine seed powder produced, however, depends on the amount of oil contained in the seed as this tends to interfere with the sieving process by blocking the sieve.

10gm of the <210µm fine seed powder produced 2.7±0.3gm of oil and 6.8±3.2gm of the cake powder. This result agrees with the findings of Okuda (1999) and Ali (2010). Many seed oils have been shown to possess antimicrobial activity (Ugbogu, et al, 2006; Ugbogu & Akukwe, 2009). Although Moringa seed oil has been found to possess some antimicrobial properties (Bukar, et al, 2010; Thilza, et

al, 2010), it may not be suitable for application in water disinfection because of its insolubility.

The result of the disc diffusion antimicrobial susceptibility tests indicated zone of inhibition of 6.5mm (including 6mm disc diameter), 7.5mm, 9.0mm, 7.28mm, 6.65mm and 6.5mm using Moringa extract solutions, M1, M2, M3, M4, M5, and M6 respectively. Thus Moringa disinfection solution M3 indicated the largest zone of inhibition.

The agar well susceptibility test method also indicated zone of inhibition around the wells of 6.75mm, 8.53mm, 12.38mm, 8.05mm, 8.63mm, and 8.25mm respectively for Moringa disinfection solutions M1, M2, M3, M4, M5, and M6. The salt crystallizes around the well holes and there was no bacterial growth within this area. For disinfection solutions M4 and M6, the area immediately after the salt crystal also indicated some susceptibility within about 2mm (10mm diameter). Again using this method, the M3 disinfection solution indicated the largest zone of inhibition.

The negative control used was sterilized distilled water and no zone of inhibition was indicated from both methods. The positive control used was 5.5% hypochlorite solution and this indicated average zones of inhibition of 26.15mm and 35.3mm diameter using the disc diffusion and agar well methods respectively.

As noted by Duncan (2005), the disc diffusion test is used to approximate the Use Dilution Test (Testing the chemical under actual conditions of use). Even though the zones of inhibition have not been standardized, a comparison of zone sizes for the same chemical among organisms will provide and approximate effectiveness of the chemical. The disc method showed lower zones in all cases may be because of low concentration of the extract in the discs. In comparison, the agar well method might have indicated larger zones because the amount of extract in the wells is relatively more than that soaked in the discs.

Suarez, M., et al (2003) found that Moringa Oleifera seeds contain polypeptides which act as a water clarification agent that coagulates particles and bacteria in suspension. The study showed that it also possess an antibiotic activity that leads to growth inhibition and killing of bacteria, including antibiotic-resistant human pathogens, and it might represent environment-friendly substitute to commonly used disinfecting agents. This was corroborated by later findings of Bukar, at al (2010) and Thilza, et al (2010).

Zika (1988) had earlier noted that extracting solvents could bring about variation in specie extractive components, which may influence their antimicrobial activities. Oluseyi & Francisca (2009) for instance, reported that Hexane extraction of antimicrobial agent of *Buchholzia Corcea* (Wonderful Kola) showed inhibitory zone of 21mm for E.Coli and methanolic extract showed inhibitory zone of 30mm for *E.Coli*. Thilza, et al (2010) using extract from Moringa leaf stalk, found that at dilutions of 1000mg/ml, 700mg/ml, 400mg/ml, and 200mg/ml, only mild activity against *E.Coli* and *Entrobacter Aerogenes* was noticed. They also found that the highest activity was produced by *E.Coli* at 1000mg/ml which comparatively was less than that of the standard drug tetracycline (250mg/ml).

For compounds requiring large contact times for bacterial inactivation, Minimum Inhibitory Concentration (MIC) is commonly used to characterize their effect on controlling microbial growth (Cunningham, et al, 2008). The MIC is known as the smallest amount of antimicrobial agent needed to inhibit growth. The MIC for the Moringa disinfection Solution M3 was determined to be 200µL/mL which is equivalent to 4mg/mL. Bukar, et al (2010) also found that the MIC for Moringa Seed Chlorofom (MSC) extract and Moringa Seed Ethanol extract were >4mg/mL. Earlier, Suarez, M., et al (2003) reported that 1 – 6mg/mL of flo were found to decrease viable cell counts by several orders of magnitude and concluded that E.Coli incubation with 2mg/mL of either Moringa seed extract or flo resulted in strong inhibition of bacterial growth. The finding in this study thus agreed with the earlier results. The Minimum Bacterial Concentration (MBC) was determined to be 210µL/mL which is equivalent to 4.2mg/mL.

The results indicated that the largest zone of inhibitions of 9mm and 12.38mm were produced with the Moringa Disinfection Solution M3. In comparison, Thilza, et al (2010), using extract from Moringa leaf stalk, found zone of inhibition of 10mm with 1000mg/ml for *E.coli*. The Minimum Inhibitory Concentration (MIC) was determined to be 200µL/mL while the Minimum Bacterial Concentration (MBC) was 210µL/mL. This is therefore the best method of seed preparation for the application of Moringa seeds extract in water disinfection. It is suggested that this should be used to determine the optimal operating conditions that will give the best result for practical application.

Acknowledgements:

The funding for this research work was provided by the Bayero University, Kano-Nigeria through the MacArthur Foundation Grant. The work was carried out in the laboratories of the Biotechnology Engineering Research Unit (BERU),

Department of Biotechnology Engineering, International Islamic University, Malaysia. The contributions of Assoc. Prof. Dr. Parveen Jamal and Aliyu Salihu in the microbiological investigations and other aspects of the work are hereby acknowledged.

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8/8/2012