ANTIBACTERIAL AND WATER PURIFICATION ACTIVITIES OF *MORINGA OLEIFERA*

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DECLARATION

I declare that the work in this thesis entitled ANTIBACTERIAL AND WATER PURIFICATION ACTIVITIES OF *MORINGA OLEIFERA*, has been performed by me in the Department of Microbiology under the supervision of Dr. S.E. Yakubu and Dr. J.B. Ameh.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis entitled ANTIBACTERIAL AND WATER PURIFICATION ACTIVITIES OF *MORINGA OLEIFERA* by Sani, Fatima Gwarzo meets the regulations governing the award of the Degree of Masters in Microbiology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to my beloved mother, Hajiya Ummulkhair, My father Alhaji Sani Gwarzo, my husband Muntari Salisu Aliyu and my little daughter Maimuna (Amirah). May God forgive my mother and make her soul rest in perfect peace Ameen.

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ABSTRACT

The *Moringa oleifera* seeds and leaves extract using hexane and ethanol as solvents were screened and found to contain important bioactive components, including alkaloids, steroids, tannins, reducing sugar, glycosides, flavonoids, saponnins, resins, and higher fatty acids. They were also screened for their antibacterial activity on *Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhi*, *Staphylococcus aureus, Bacillus cereus*, and *Enterococcus faecalis*, using disc diffusion method. The ethanolic and hexane leaves extract at 10% (100µg/ml) and 20% (200µg/ml) were inhibitory to *Pseudomonas aeruginosa*, while hexane leaves extract at 20% (200µg/ml) was found to be inhibitory to *Enterococcus faecalis*. Ethanolic Leaves extract at 20% (200µg/ml) was found to be inhibitory to *Staphylococcus aureus*. On further dilutions, MIC values were 12.00µg/ml for hexane extract and 25.00µg/ml for ethanol extract against *Pseudomonas aeruginosa* for hexane and ethanolic extracts respectively and the activity was found to be bacteriostatic at the above concentrations. There was no inhibitory activity against all the six bacteria when ethanol and hexane seeds extract are used. The study also revealed the purification activity of seeds of *Moringa oleifera*, when A.B.U. Dam water was used as raw water. Physico-chemical analysis on water samples after treatment with *Moringa* seeds

(suspension) showed that, there was reduction in turbidity from 5NTU (raw water) to 0.81 NTU (water treated with 15mg/l concentration of the seeds suspension). There was also a reduction in hardness from 34mg/l $CaCO_3$ (raw water) to 10-21mg/l $CaCO_3$ of water samples treated with seeds suspension. The pH was found to be independent for both hardness and seed concentrations, it ranges from 6.9-7.4. There was decrease of colour intensity, i.e. $20H^o$ (raw water) to $5H^o$-$15H^o$ for water treated with seed suspension. Water treated with seed suspension at 10mg/l was the clearest, then followed by that with 15mg/l and 20mg/l and they were all found with lesser turbidity. There was also a reduction in bacterial load from water samples treated with seed and with alum. Paired t test Analysis showed that, there was significant difference between the means of colonies formed between raw water and water treated with seeds at 10^{-2} i.e. (37.18) ($P>0.05$, 95% confidence interval). Also there was significance between raw water and water treated with alum at 10^{-2} i.e. (16.48). However the alum activity was significantly low compared to that of seeds suspension.

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Chapter 1 :

INTRODUCTION

Moringa oleifera is a member of *Moringaceae* family which grows throughout the tropics. It is a native to sub-Himalayan tribes of North West India, Pakistan, Bangladesh and Afghanistan, but now distributed worldwide in the tropics and found in many countries of Africa and South America. It was well known to the ancient world, but recently has been “rediscovered” as a multipurpose tree with tremendous variety of potential uses (Lowell, 2001).

The leaves, flowers, fruits and roots of *Moringa* are used as vegetables; the seeds for water purification, and the oil extracted is used for cooking, soap manufacture, as a cosmetic base and to provide illumination. Other uses include cattle fodder, biogas production, and all plant organs are used as constituent in traditional medicine (John and Maria, 1962). In Africa the cooked leaves of *Moringa* are eaten as principal ingredients of sauce, but in Northern Nigeria the leaves are used as vegetable in soup preparation, while the root poultice is used to treat inflammation especially swelling tissue or *oedema* (Lowell, 2001).

Bacteria are among the major microbial groups that are able to cause different types of illness such as waterborne diseases, airborne diseases and food borne diseases. Some of the disease causing bacteria include;*Pseudomonas, Staphylococcus aureus, Shigella species and legionella.*

Antibiotics are substances produced by living organisms, which are active against other living organisms (Chessbrough, 2000). The various antibacterial agents differ considerably in their range of action, that is, in number and variety of species against which they are effective. An antibiotic should have a lethal or inhibitory effect on the microorganisms attacking but its therapeutic concentration should have little or no toxic action on the host tissue (It's only to kill or only destroy the bacteria at which it was aimed) (Brock and Madigan 1988).

Antimicrobial resistance has now become a serious public health concern. Most of the disease causing bacteria are resistant to modern chemotherapeutic agents. The solution is made more difficult in developing countries such as Nigeria where antimicrobial drugs are misused and the good ones are very expensive. Therefore poor people end up taking fake drugs, and these

constrains lead to higher prevalence of drug resistance among different types of species and the use of herbal medicines such as *Moringa oleifera* plant.

JUSTIFICATION OF THE STUDY

Problem of resistance to modern antimicrobial drugs has lead to the wide use of traditional medicine, and many plant extracts with antimicrobial activities have provided a scientific basis for their use in treatment of many diseases and infections with promising results (Sofowora, 1993).

Waterborne diseases still remain one of the major causes of human morbidity and mortality in developing countries, especially in the rural areas, where there is inadequate or no pipeborne water supply. The possibility of use of plants that are inexpensive and easily available such as *Moringa oleifera* "Zogale" to remove turbidity, colour and reduce the microbial load in raw water, a function played by chemicals such as alum, will provide a cheap source of portable drinking water.

AIMS AND OBJECTIVES

- Determine the antibacterial activity of "Zogale" (*Moringa oleifera*) extract.
- Determine the minimum inhibitory concentration and MBC among the fractions of the extract.

- ❖ Determine whether the coagulant activity of “Zogale” seeds has any effect on the microbial content of water to be purified.

Chapter 2:

REVIEW OF LITERATURE

LITERATURE ON MORINGA OLEIFERA

2.1 **Botanical Character**

Moringa oleifera (Syn. M. pterygosperma Gaertn) is the botanical name for Horse radish tree which is also known as Drumstick or Ben oil tree. It belongs to *Moringaceae* family Genus *Moringa.* The tree is native to Arabia and India where it is frequently planted in compounds. It is now widely distributed in the tropics and in Africa. In Nigeria it is locally known as "Zogalegandi" in Hausa, Ewe Igbale in Yoruba, 'Okire ó Yibo" in Igbo, "Gaware" in Fulfulde, "Chigban wawa" in Nufe, Agamago in Tiv and "Alim" in Shuwa Arab (Dalziel, 1955 and Keay, 1988).

> *Moringa* plant is a small graceful tree, 7-12meter in height and sometimes even 15m. 60cm in girth with thick, smooth, gray back, fragrant white flowers, sparse foliage and long green pods, which gives the tree its name as drumstick tree [describing the shape of the pods] or horseradish tree [describing the taste of the roots]. It resembles a leguminous species at a distance especially when in flowers but immensely recognized when in fruits. The tree is deciduous, leaves are alternate, up to the branches and leaflets are dark green above and pale underneath. Flowers grow throughout the year, and are cream in colour (Keay, 1988).

2.2 History

The *Moringaceae* family consists of 13 known species that belong to *Moringa genus. Moringa stenopetals* is native to Ethiopia and Northern Kenya. *M. peregrine* is found in Sudan, Egypt, the Arabian peninsula as far North as the Dead Sea. *M. ovalipolis* grows in Angola and Namibia. However, the best known member of the genus is the *Moringa oleifera*, a fast growing, drought resistant tree, native to sub-Himalayan trade of North India but now distributed worldwide in the tropics and the sub-tropics (Lowell, 2001). It can also grow rapidly in poor soil (Sengupta and Gupta, 1970 and Morton, 1991).

2.3 Uses

Booth and Wickens in 1988 categorized uses of *Moringa oleifera* as follows;

Alley Cropping: With their rapid growth, long taproots, few lateral roots, minimal shade and large production of high protein biomass, *Moringa* trees are well suited for use in alley cropping systems.

Animal Forage: Leaves are readily eaten by cattle, sheep, goats, pigs and rabbits. leaves can also be used as food for carp and other

fish. The seed cake could be used as animal feed if it is pre-tested to remove its alkaline and saponin content.

Biogas: *Moringa* leaves provide an excellent material for production of biogas. (Nouala, at. al. 2006).

Domestic Cleaning Agent: Crushed leaves are used in some parts of Nigeria to scrub cooking utensils or clean walls.

Dye: The wood yields a blue die which was used in Jamaica and Senegal.

Fencing: A common use of *Moringa* trees is a living support for fencing around gardens and compounds.

Fertilizer: The seed cake can be used as protein rich plant fertilizer.

Foliar Nutrient: Juice extracted from the leaves can be used to make foliar nutrient capable of increasing yields by up to 30%.

Green Manure: Cultivated intensively and then ploughed into soil, *Moringa* can act as a natural fertilizer for other crops.

Gum: The gum produced from a cut tree trunk has been used in calico printing, in making medicines and as a bland-tasting condiment.

Honey Clarifier: Powdered seeds can be used to clarify honey without boiling. Seed powder can also be used to clarify sugar cane juice.

Medicine: Every part of the plant is widely used to make a wide variety of traditional medicines.

Ornamental: Incorporating *Moringa* leaves into the soil before planting can prevent damping off disease (*Pythium debariyanum*) among seedlings.

Pulp: The soft spongy wood makes poor firewood, but the wood pulp is highly suitable for making newsprint and writing paper.

Rope-Making: The bark of the tree can be beaten into fiber for production of ropes or mats.

Tanning: The bark and gum can be used for tanning hides.

Water Purification: Powdered seed kernels act as natural flocculant, able to clarify even the most turbid water.

2.4 Nutritional Importance of *Moringa oleifera*

Malnutrition is the major cause of high infant mortality in the tropics and sub-tropic countries. In the poorest countries, as many as one child in five will die during infancy. Church world services (CWS) 1999 in the research estimated that worldwide, seven million people die each year due to chronic malnutrition. Several researches conducted on nutritional value of *Moringa*

oleifera leaves and pods by Zija (2005) revealed that leaves and pods of *Moringa oleifera* can be an extremely valuable source of nutrition for people of all ages, for a child aged between 1 –3 years a 100gram serving of fresh leaves would provide all his/her daily requirements of calcium, about 75% of iron and half of protein needs, as well as important supplements of potassium, B-complex vitamins, copper and all the essential amino acids. As little as 20 grams of fresh leaves would provide a child with the vitamins A and C. he/she needs NANA (2000).

For pregnant and breast feeding women, *Moringa oleifera* leaves and pods can do much to preserve the mother's health and pass on strength to the fetus or nursing child. One portion of leaves could provide a woman with over a third of her daily need of calcium and give her important quantities of iron, protein, copper, sulfur and B vitamins. Just 20 grams of fresh leaves will satisfy all her daily requirements of vitamins C, for both infants and mothers, pods can be an important source of fiber, potassium, copper, iron, chlorine, vitamin C and all the essential amino acids. Malnourished children can benefit from addition of *Moringa oleifera* leaves to their diet. The high concentrations of iron, protein, copper, various vitamins and essential amino acids present in *Moringa oleifera* leaves make them a virtual ideal nutritional supplement.

2.5 Medicinal Plant Phytochemistry.

The plant usually contain physiologically active substances which are technically referred to as drugs and over the years these drugs have been exploited in traditional medicine for treatment of various ailments disturbing man. In 1986 Sofowora noted that plant produce many organic substances such as alkaloids, tannins, saponins, resin and many others and concluded that these chemicals differ from one specie to another and are the ones utilized for the treatment of diseases. According to a survey conducted by the United Nations Commission for Trade and Development (UNCTAD) (1974) more than 33% of modern drugs and medicinal products are derived from plants (Ghani., 1990). The usual term used for chemical substances present in plants is “constituents”, therefore Phytochemistry is concerned with the chemical study of plant constituents.

2.5.1 Alkaloids

Alkaloids are basic nitrogenous compounds (usually derived from amino acids) which posses a marked physiological action on man and other animals

and are usually derived from plant sources. Most alkaloids are colorless, well defined chystaline substances which unite with acids to form salt. They are bitter tasting and form a water soluble salt. They contain carbon, nitrogen and oxygen and occur in plants both as free bases and salt. Others may occur in nature as terpenoids and some may be steroidal like aromatic types. They give a precipitate with heavy metals (George and Pandalai 2002). Most of the natural drugs used in pharmacy and medicine are obtained from alkaloid containing plants. The steroidal alkaloids may elicit pharmacological effect on kidney, bladder, skin, heart and lungs.

2.5.2 Steroids

Steroid are a group of natural components that are widely distributed throughout plant and animal kingdom. They contribute to a wide range of therapeutic applications such as cardiotonic vitamin D precursors, oral contraceptive agents, anti inflammatory agents and anabolic agents. Also they are used in replacement therapy, reduction of lymphatic tissue, suppression of *lymphoporosis, lymphomos rheumatoid arthritis, cerebral oedema* and raised intracranial pressure (Trease and Evans, 1992). Steroids can be obtained from animal or plant sources. Those obtained from plant source are called phytosterol while those obtained from animal source are called zoosterol

2.5.3 Tannins

Tannins are complex chemical substances, which consists of mixture of polyphenols. Most of the time they occur in glycopsidic combinations with sugars. They readily combine with proteins rendering their resistance to proteolytic enzymes. They also precipitate protein, gelatin and alkaloid from solutions and form dark blue or greenish black compounds with ferric chloride.

Tannins are widely distributed in plant kingdom and are mainly present in unripe fruits and also in leaves. They are mostly used in the tanning industry for conversion of animal hides to leather also they posses antiseptic properties which protect the leather from decay (Health Note, 2005).

2.5.4 Reducing sugar

Reducing sugars are polyhydroxyl aldehyde or ketone alcohol containing carbon, hydrogen and oxygen. (Caceres et al., 1991).

2.5.5 Glycosides

Glycosides are acetals formed by the combination of one or more sugar units with a non-sugar component through oxide rings. The non-sugar component of glycoside is known as the aglycone and the sugar component is called the glycone. The aglycone involved in the formation of glycoside may belong to any group of natural chemical substances and any type of sugar may constitute the glycone part of this group of plant constituents. Large variety of glycosides occur in plants and most of them serve as important medicinal substances (Ghani, 1990).

2.5.6 Flavonoids

Flavonoids are a class of water soluble plant pigments. They are classified into isoflavones, anthoflavans, flavonols, flavones, and flavanones (Peterson, 1998). They are not considered as essential nutrients but some flavonoids help in health by strengthening capillaries and other connecting tissues, and function as anti-inflammatory, anti-histaminic and antiviral agent. It has been reported to block the “sorbitol pathway” that is linked to maintenance problems associated with diabetes. Flavanoids can be found in a wide range of food or plant products (*Health Notes*, 2005).

2.5.7 Saponins

Saponins are steroidal compounds which are widely distributed in higher plants. They form colloidal solutions with water that foam upon shaking. They posses a bitter acid taste (Caceres et al., 1991).

2.5.8 Resins

Resins are chemically non-nitrogenous compounds containing six carbon rings. They are oils but differ from essentials oils in being usually in solid, only slightly volatile and more likely to be pungent or burning in flavour than aromatic. Most of them are found in plants but associated with other plant constituents due to its adhesive quality. It is used in wound dressing and filling of cavities in teeth (Thomas, 1949).

2.5.9 High fatty acid

Are chemical specie of carboxylic acid. They are main constituent of acid that contain both highly hydrophobic (water repelling) and highly hydrophilic (water soluble) region. They are non-polar solvents (Health Notes, 2005).

2.6.1 Medicinal uses of *Moringa oleifera*

In Africa many plants are used in traditional medicine as anti microbial agents but only small proportion of such are documented. Also there has been little trial of these drugs thereby listed as “native drugs of probable value”. However, resistance to modern antimicrobial drugs such as *gentamycin, augumentin* and others which has been reported in many centers, lead to the wider use of traditional medicines (Murray, 1992).

Moringa oleifera plant is one among the plants that are highly esteemed by people in the tropics and sub-tropics for its numerous medicinal properties. Some of these traditional uses reflect the nutritional content of the various tree parts (Verma et al., 1976). The following are about some of the ways the plant is used in different parts of the world. (Lowell, 2001).

- In India juice made from leaves taken orally is believed to have stabilizing effect on blood pressure and is used to treat anxiety.

- Poultice made from fresh leaves is applied externally to reduce glandular swelling.

- Root juice taken orally is used as carminative (promotes gas expulsion from alimentary canal, against intestinal pains spasms.

- Paste made from ground bark is considered effective in treating boils by applying on the affected area.

- Stem and root bark are taken orally as appetizer and digestive.

- In Senegal, an infusion of leave juice taken orally is commonly used to control glucose levels in cases of diabetes, and sometimes is used as a purgative.

- In Nicaragua, leaves and young buds are rubbed on temple for headache, while in Philippines eating of leaves is believed to increase a woman's milk production and sometimes prescribed for anaemia.

- In Malaysia, the leaf poultice is applied on the abdomen to expel intestinal worms, while in Arabia, a treatment used against warts is to cover them with a paste made from crushed seeds.

2.6.2 Common uses of *Moringa* are as follows:-

- Paste made from roots and salt or gum or seeds oil are applied externally on the affected area to relieve articular pain and also swelling in cases of gout or rheumatism and at the same time taking four ripe *Moringa* seeds kernels daily (two in the *Morning* and two at night) serve the same function.

- Infusion made from flowers and that made from stem bark powder or leaves juice when applied into the eye are believed to cure eye diseases (i.e. conjunctivitis) and also serve as an eye wash.

- Gum mixed with sesame oil or juice made from leaves or root bark are applied into the ear to relieve ear aches.

- Paste made from leaves, juice made from roots or gum are applied externally as rubefacient (skin toning) and vesicant (agent to reduce blistering) while bark paste or the seeds oil are believed to cure or treat many skin diseases such as scurvy.

- Leaves mixed with honey and followed by a cup of coconut milk 2 or 3 times a day is used treat diarrhea, dysentery and colitis (inflammation of the colon).

- Juice made from leaves or infusion made from flowers are taken to alleviate sore throat, catarrh and bronchitis.

- A snuff made from roots is inhaled to relieve tooth ache, while juice from root bark is placed in a tooth ache cavity as a pain killer, also gum is used for dental cavities.

- Leaf juice, sometimes with carrot added or eating of fresh leaves used as diuretic (to increase urine flow)

- Decoction from flowers, root bark or roots, taken orally has been used to treat hysteria. Also application of paste made from leaves, bark or seeds oil externally is believed to cure hysteria, epilepsy and nervures.

- Leaves, gum or roots are believed to be useful against intermittent fever, while roots juice mixed with milk is considered useful against hiccup and asthma.

Some of the above traditional remedies have been supported by recent laboratory studies (Ezeamuzie et al., 1996). Among these include:

(1) *Moringa* leaves extract has been shown to be effective in lowering blood sugar levels within a space of 3 hours albeit less effectively than the standard hypoglycaemic drug glibenclamide. Effects increased with larger doses (Makonnen et al., 1997). Pal et al., (1995) and (Dahiru, et. al. 2006) reported that extracts taken from dried leaves showed impressive ability to heal ulcer in laboratory animals. The crushed whole seeds or presscake remaining after oil extraction from *Moringa* seed is found to be a natural coagulant in raw and

wastewater treatment practices (Sutherland et al., 1994) and (Ndabigengesere and Narasiah, 1998).

(2) An extract made from powdered leaves was shown to have a very potent depressive effect on the central nervous system, resulting in significant muscle relaxation, decreased body temperatures and increased sleep time among laboratory mice. Subjects receiving the highest dosages spent twice as much time asleep as the control group (Pal et *al*, 1996). Also extracts from dried roots, applied orally to laboratory mice demonstrated clearly that the roots posses anti-inflammatory properties (Ezeamuzie et al., 1996).

(3) An infusion made from seeds demonstrated an ability to inhibit intestinal spasms, as well as some diuretic activity. However, other plant parts (leaves, roots, stalk and flowers) showed no significant anti-spasmodic activity (ibid). An in-vitro study showed that an aqueous extract made from seeds is effective against *Pseudomonas aeruginosa*, Staphylococcus aureus and *Escherichia coli* (Caceres, 1991). Umar (1998). in his study noted that the aqueous extract of *Moringa oleifera* leaves posses significant antimicrobial activities against some gram-positive and gram-negative bacteria and some fungal species (Kautharaja and Dodd, 1991).

2.6.3 Other uses of *Moringa Oleifera*

The leaves, flowers and roots of the tree are used as vegetables. In Africa the cooked leaves of *Moringa oleifera* are eaten as principal ingredients of sauce, but in northern part of Nigeria, the leaves are used as vegetables, in soup preparation. (Sofowora, 1993). The oil extracted from the seeds is used for cooking, soap manufacture, as a cosmetic base and to provide illumination (John and Maria, 1962). The trunk is used in the paper industry (Verma et al., 1976). The characteristics of the oil from seeds of *Moringa oleifera* variety of Mbobololo showed that this oil could be utilized successfully as source of edible oil for human consumption. It contains a high ratio of mono unsaturated to saturated fatty acids and might be acceptable substitute for highly mono saturated oils such as olive oil in diets (Tsaknis et al., 1999). But in (1974) Ibrahim et al., reported that the oil content and its properties show a wide variation, depending mainly on the specie and the environmental conditions.

In Saudi Arabia the hot water extracts of dried fruits is taken orally for treatment of ulcer, abdominal tumor, paralysis and diabetes (Mossa, 1985). In Europe the seed oil extracted from *Moringa oleifera* which is known as Ben oil is used as lubricant for fine machinery (Anon, 1904). It is also use as antipyretic and for wound healing (Hukkeri and Nagathan,2006).

6.4 Coagulation activity of *Moringa oleifera*

The microbial contamination of drinking water is a serious problem that can result to a large outbreak of waterborne diseases. United Nations International Children Emergency Fund (UNICEF) (1996) reported that waterborne disease cause an estimated 3 million deaths annually, mostly children and 1 billion episode of illness every year. The major concern in water treatment is the need to remove disease causing organisms (e.g. bacteria, viruses and protozoans) from water before its consumption and main purpose of water supply distribution is to deliver to consumers safe, adequate and aesthetically acceptable quality water. Drinking water should be aesthetically acceptable; it should be free from apparent turbidity, color and odour from any objectionable taste, it should be of reasonable temperature, water meeting these conditions is called “potable” meaning that it may be consumed in any desired amount without concern for adverse effect on health (Fredrick, 1990).

Reduction in the occurrence of waterborne diseases in some developing countries cannot be fully achieved due to the fact that up to now many areas do not have access to pipe borne water supply. World Environmental Development Conference (WEDC) (1994) reported that for many communities in developing countries, the use of conventional method

(coagulation, flocculation and sedimentation) of water treatment is inappropriate because of high cost and low availability of chemical constituents such as aluminum sulphate (alum) and ferric salt. UNICEF in (1993) stated that approximately three out of every five persons in developing countries do not have access to drinking water. Research conducted by (Dada et al., 1990) found out that the quality of water of water delivered to consumers in Zaria did not meet the World Health Standard Organization's standard in terms of faecal Coliforms, content or residual chlorine concentration.

Moringa oleifera crushed seeds suspension or presscake remaining after oil extraction from the seed has been found to be a natural coagulant in raw water and waste water treatment and also a coagulant aid (Madsen et al., 1987; Karser et *al*., 1990). In Sudan, dry *Moringa oleifera* seeds are used in place of alum by rural women to treat highly turbid Nile water (Jahn, 1986). Sutherland et al., (1994) in their pilot and full scale trials in Malawi have demonstrated the effectiveness of *Moringa oleifera* seeds coagulant activities for the clarification of highly turbid river water as inlet turbidities of 270 – 380 NTU were consistently reduced to below 4 NTU in the finished water. Also Sulayman and Charles (1994) in their findings on coagulation of low

turbidity surface water with *Moringa oleifera* seeds noted that *Moringa oleifera* seed has potential for use as a coagulant and with alum as primary coagulant for treatment of low turbidity surface water. Their result indicated that up to 83% turbidity removal was achieved when *Moringa oleifera* seeds was used as a primary coagulant.Mataka et.al. 2006 in their study of streams in Malawi noted that *Moringa oleifera* apart from its clarifying activities it was also found to be effective in heavy metal detoxification of water.

2.7 Microbes and Antimicrobial Resistance

Man has been able to score some notable success in his fight against infection; infective agents continue to have great impact on mankind's mortality and morbidity. The development of Salvarsa by Paul Ehrlich in 1910 was the first documented example of a chemical used successfully as an antimicrobial medication (Nester et al, 2001). In many developing countries microbial infections are still directly responsible for nearly half of all deaths and the financial management and the control of infections is very substantial. The infections encountered in medical practice are caused by a broad spectrum of pathogens which range from smallest viruses to mould and yeast (Denyer and Baird, 1990).

An antibiotic or antimicrobial agent is a chemical substance produced by microorganisms or plants that kills or inhibits the growth of microorganisms, such substances which kill organisms are often called “cidal” agents with a prefix indicating the kind of organism it kills. As a result of that the agent may be bactericidal, fungicidal, or algicidal while an antimicrobial agent that stop or inhibits the action of an organism is called “static” (Brock and Madigan, 1988).

The performance of antibiotics over the years has unfortunately not been sufficient justification for the burst of enthusiasm which greeted their introduction into medical practice. Many of the pathogens which were in the beginning very susceptible to some antibiotics have now become resistant and the effectiveness of these antibiotics has consequently diminished very substantially. The situation with regards to the development of antibiotic resistance is in some cases so serious that it is now difficult to treat some infectious diseases. Bacteriostatic drugs after inhibiting the growth of a pathogen may lead to the death of the targeted organism. Bactericidal drugs are more effective chemotherapeutic agents than bacteriostatic drugs since they are able to sterilize the tissue by themselves. (Tom, et al., 1997).

Nowadays most of the antimicrobial resistance are due to the extensive use and misuse of antimicrobial drugs which have favoured the emergence and survival of resistant strains of microorganisms. Drug resistant strains are common among *Staphylococcus,* Gonococci, Meningococci, Pneumocci and Gram negative bacteria such as *Salmonella, Shigella spp., Klebsiella, Pseudomonas* and *Mycobacteria* tuberculosis (Chessbrough, 2000).

Bacteria become resistant to antimicrobial agent by a number of mechanisms but this resistance can be an inherent property of microorganisms or it can be acquired. (Geo, et al.1995).

The component mechanisms of inherent resistance are:

1. The organism may lack structure which an antibiotic inhibits for example some bacteria like mycoplasma lack a typical bacterial cell wall and are resistant to penicillin.
2. The organism may be impermeable to the antibiotic.
3. The organism may be able to change the antibiotic to an inactive form by production of certain enzymes which inactivate the organism. For instance in penicillin resistance, group A enzymes called or β-

lactomases destroy the β-lactam ring of penicillin thus rendering it inactive (Brock and Madigan, 1988).

Acquired resistance of microorganisms to an antibiotic is multifactorial namely:-((Chigbu and Ezeoenye, 2003).

(a) Overuse or misuse of an antimicrobial agent.

(b) Poor compliance.

(c) Self medication.

(d) Counterfeit drugs.

(e) Poor control of antimicrobials in hospital.

(f) Poor standard of hygiene, personal or environmental in hospitals.

(g) Use of antibiotic in animal husbandry, horticulture and aquaculture.

(h) International trade and travel (Chigbu and Ezeonye, 2003).

Chessbrough (2000) grouped antibacterial agents by their mode of action as:-

1. Inhibitors of bacterial cell wall synthesis e.g. penicillin and glycopeptide.
2. Inhibitors of bacterial nucleic acid synthesis e.g. Metronidazole and co-trimazole.
3. Inhibitors of protein synthesis e.g. Chloranphenicol and tetracycline.

2.8 Organisms and their Characteristics

Microorganisms are organisms that are too small to be perceived clearly by an unaided eye (i.e. about 0.1 – 1μm in diameter) ; therefore the existence of these microbial world was unknown until the invention of the microscope. The discoverer of this microbial world was by a Dutch merchant called Antony van Leeuweenhock (Roger, 1998). Microorganisms have a taxonomic distribution; metazoans, many algae, fungi, bacteria and viruses. They are unicellular and occur everywhere in nature for example in air, water, surface of our body and that of objects, as a result of their diverse distribution and ability to survive in almost every environment, therefore they are closely associated with the health and welfare of human beings. Some are beneficial to man for example those that are involved in the making of yoghurt, cheese and wine, in production of antibiotics (e.g. penicillin) while some play important role as agents of infectious diseases, some spoil food and deteriorate materials like iron pipes, glass lenses and wood pilling (Michael et al., 2002)

Bacteria are one of the microorganisms that are very small, (approximately 0.2 – 1.0μm in diameter). Bacterial shape is governed by their rigid cell wall (some are cocci, bacillus and spiral). Based on the type of cell wall and grams reaction, bacteria are further divided into Gram-positive and Gram-negative and the difference between the two is that the cell wall of Gram-negative bacteria is more complex than that of the Gram-positive bacteria.(Tom et al., 1997).

Pseudomonas aeruginosa, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Shigella flexneri*, and *Enterococcus faecalis* are among the organisms that are usually found to be resistant to many antibiotics. For example more than 70 percent of the bacteria that causes hospital aquired infections i.e *Pseudomonas* (Joanna, 2000) are resistant to atleast one of the antibiotics most commonly used to treat them. Therefore the resistance effects of such Microorganisms may cause problems in the hospitals such as that of long stay, more toxic effect due to drug side effects and more expenditure.

Secondly some of the above mentioned organisms also contributed in the spread of water borne diseases eg.*Salmonella typhi* (Park, 2002)while some causes foodborne infections such as *Bacillus cereus*(Granum et. at.1996 and Kramer et. al.1998)therefore the use of a plant that is available in our Enviroment and inexpensive i.e *Moringa oleifera* will help in reducing the occurance of such diseases and illnesses in our society. Selection of organisms with different response to gram staining, due to their nature of cellwall ensures that the plant will work both on gram positive as well as on gram negative microorganisms.

Chapter : 3

MATERIALS AND METHODS

3.1 COLLECTION OF SAMPLES

The seeds and leaves of *Moringa oleifera* plant were collected from botanical garden of Department of Biological Science, Ahmadu Bello University Zaria, where a voucher specimen of the plant is retained

3.2 Preparation of material

The seeds and leaves were air dried ground into a powdered form using mortar and pestle and stored at room temperature in a sterile bottle.

3.3 Extraction

Maceration method was used to get the extracts using n-hexane and ethanol as solvents as follows: 500g of powered seeds and Leaves were soaked in 100ml of 99% n-hexane for 32 hours with agitation at 6 hours interval.The solutions were filtered and evaporated to dryness so as to eliminate the excess solvent then concentrated. The residue was collected in a clean sterile bottle and stored at 4^0c in a refrigerator until required (Trease and Evans, 1989).

The above procedure was repeated with ethanol as a solvent and the two extract obtained were screened for bioactive agents according to the method described by Silvers *et al*., (1998).

3.4 **Preliminary phytochemical screening**

3.4.1 **Test for Alkaloids**

An aliquot of 0.5g of each extract was dissolved in 1% aqueous HCl on a 10ml on a water bath and then filtered. A few drop of Dragendoff's reagent was added to 1ml of filtrate. Formation of an orange precipitate indicated that Alkaloids are present.

3.4.2. **Test for Tannins.**

A total of 0.5g of each extract was dissolved in 10ml of distilled water and filtered. Few drops of Ferric Chloride solution were added to the filtrate. Formation of blue precipitate indicated the presence of Tannins.

3.4.3 **Test for Saponins**.

A total of 0.5g of each extracts was dissolved in 5ml of distilled water; the mixture was shaken vigorously for 2 minutes. Frothing was observed and this shows that saponin was present.

3.4.4 **Test for flavonoid.**

Little amount of metallic magnesium power and few drops of concentrated HCl were added to 10ml of each extract. Observation of red colour in the solution indicated the present of flavonoid.

3.4.5 **Test for Resins**

To 10ml of solution of extract sufficient acetic anhydrides solution was added and few drops of concentrate H_2S0_4 was added into the solution by the side of the tube,until A reddish-purple colour was obtained. The reddish-purple colour indicated the present of Resins.

3.4.6 **Test for Steroids**

A total of 0.5g of each extract was dissolved in 5ml of chloroform and 3ml of concentrated H_2S0_4 was carefully added down the tube. Presence of reddish-brown colour at interface was taken as evidence for the presence of Steroids.

3.4.7 **Fehling's test for Reducing Sugar.**

A total of 0.5g of each extract was dissolved in 2ml of distilled water and then 1ml of Fehling's (1+11) solution were added and heated. A brick-red precipitate indicated the presence of reducing sugar.

3.6. Antibacterial screening test by agar diffusion method

3.6.1. A disc diffusion method of Jorgensen *et al., (*1999) was used to determine the antibacterial activities of the extracts. Whatman No 1 filter paper was cut into small sizes,(6mm in diameter using a puncture) sterilized in an autoclaved at 121^{o}C for 15 minutes introduced into a beaker containing 10ml of extracts at two different concentrations(10% w/v) = (100μg/ml and (20% w/v) = 200μg/ml of seeds and leaves extract). The impregnated Whatman filter papers were stored at 4^{o}C until needed for analysis.

Mueller-Hinton agar was used as growth medium for the microbes. It was prepared according to the manufacturer's instruction.(Oxoid company). Standard strains of 6 selected bacteria species namely *Pseudomona, aeruginosa, Shigella flexneri, Salmonella typhi, Staphylococcus aureus , Bacillus cereus* and *Enterococcus faecalis* were collected from Mr. Adewumi, Department of Microbiology , Ahmadu Bello University,Zaria.

The isolates were formed into different concentrations according to McFarland turbidity standard. Culture of this isolates made from such concentration were inoculated over each Mueller Hinton agar – plates by spread plates method. After 30 minutes of diffusion, prepared disc were inoculated and incubated at 37^{o}c for 24 hours.

The antibacterial effects were assessed based on zone of inhibition which appeared as a clear area around the disc, size was measured with a ruler, the diameter (mm) regarded as proportional to the activity of a the amount of antimicrobial agent added to the disc.

3.6.0. **Determination of minimum inhibitory concentration (MIC) of the extracts**

Different concentrations (i.e. 0.097, 0.195, 0.39, 0.78, 1.56, 3.13, 6.25, 12.50, 25.00, and 50.00mg/ml) of the plant extract were introduced into test tubes containing a nutrient broth which was prepared according to manufacturer's introductions. Each tube was inoculated with an overnight culture of the test organisms. The least concentration of plant extract from the above concentrations, that did not permit visible growth of the inoculated test organism was regarded as the MIC in each case (Bauer et al 1996).

3.7 Determination of minimum bacteriostatic concentration (MBC)

Cultures which showed no turbidity were plated out on a fresh nutrient agar plate, the lowest concentration which showed no colonies after 24 hours at 37^{o}C incubation period was regarded as the minimum bactericidal concentration. Appearance of growth on such plants indicated that the extract is only bacteriostatic (Bauer et al 1996).

3.8 Water purification activity

3.8.1 Collection of water sample.

Raw water samples were collected from A. B. U. Dam in a sterile, screw cap bottles and transported to the Department immediately. The pH and temperature were taken at collection site, and then after the experiment.

3.8.2 Physico-chemical analysis of water.

- Turbidity was measured using HACH 2100 N turbidity meter machine:- Water sample was poured into one tube and distilled water into another, distilled water was placed into a hole on the machine to zero it (balance it). Distilled water was removed and sample was placed on the hole.

Reading was taken. The same procedure was repeated for the remaining water samples.

- Colour was measured using Lovibond comparator apparatus (machine):-

  A disc (HAZEN NSA with different matching colours) was inserted into the machine. Water sample was poured into a tube and placed into a hole on the machine. The disc was rotated till the colour on the disc corresponds with colour of the water sample in a tube, and that colour number of the matching disc was taken as the colour of the water sample.

- Temperature was taken using thermometer by deepening it, into the water sample.

- pH was measured by the Henna pH Meter. The base end of the pH meter was immersed first into a buffer solution to sensitize and then into the water sample inside a beaker, and reading was taken from the meter.

- Hardness was measured by titration method as follows:

- Water sample was shaken thoroughly, and 25ml of the water sample was diluted to 50ml with distilled water; 2 drops of Eriochrome black indicator was added to the solution and titrated with (Ethylene diamine

tetra acetic acid) EDTA within 5 minutes. The blue colouration was regarded as the end points, and the titre was read as final reading minus initial reading.

Calculation

Formula used for the calculation of hardness in mg/l$CaCO_3$

$$\frac{\textbf{A x B x 1000}}{\textbf{ml sample}}$$

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

B = mg $CaCO_3$ equivalent. to 1.00ml EDTA titrant.

3.8.4 **Bacteriological analysis of water**

Powder of "zogale" seeds at different concentration in mg/l (5, 10, 15, 20, 25, 30, 35, 40 and 50) was each made into a suspension and introduced into 1 liter each of raw water (A.B.U. Dam water). A liter of raw water was kept aside as control. Another 1 liter of distilled water was also kept as control. The water samples were stirred and allowed to settle and observed after 22 hours. The same procedure was repeated using alum. Total bacterial count of the raw water was recorded before and after application of seeds and alum.

Total bacterial count was carried out by pour plate method, and oxoid agar was used as follows:

Water sample was diluted into three dilutions 10^{-1}, 10^{-2} and 10^{-3}, 1.0ml from 10^{-2} and 10^{-3} dilutions were transferred into sterile Petri-dish. Water and the agar were mixed thoroughly by gentle rotation (clockwise and counter clockwise, and rocking back and forth). The agar and the contents was allowed to solidify and incubated at 37^{o}C for 24 hours. Average bacterial count from the triplicate plates was taken, and the bacterial content of the water was recorded from the known dilutions and multiplied by the dilution factor as shown by the formula below.

Equation = (c/vxm) where as:

C = mean colony count

V = volume of plate

M = dilution e.g. (10^{-2} and 10^{-3})

3.8.5 **Statistical analysis**

Collected data were reduced using descriptive statistics by means of graphs and tables. Comparative analysis between means performance of alum and seed purification activities were also carried out using paired t test to determined the significance of the result obtained

Chapter : 4
RESULTS

4.1 PRELIMINARY PHYTOCHEMICAL SCREENING

The screening carried out on leaves and seeds extract of *Moringa oleifera* using two different solvents (hexane and ethanol) indicated the presence of reducing sugars, glycosides, tannins, flavonoids steroids, resins, saponins, alkaloids and high fatty acid. Steroids and resins were found to be present in both hexane and ethanolic extracts of all the plant parts, while reducing sugars, saponins, and alkaloids were found in the ethanolic extracts of both leaves and seeds only. Flavonoids, tannin, and glycosides were found in the ethanolic extract of the leaves only while high fatty acids were found in hexane extracts of both leafves and seeds only, as shown (Table 1.)

Table 2 shows the inhibitory activities of the hexane leaves extract on six different species of the selected bacteria. The table shows that the hexane leaves extract had a mean inhibitory activity of 36mm against *Pseudomonas aeruginosa* at 100 µg/ml and a mean of 51mm inhibitory activity at 200 µg/ml, while the hexane leaves extract had no activity against *Shigella flexineri*, *Salmonella typhi*, *Staphylococus aureus* and *Bacillus cereus* at any tested concentration (100 µg/ml and 200 µg/ml).However, there was an inhibition at 200 µg/ml concentration on *Enterococus faecalis*. Zone of

inhibition with hexane leaves extract at 200 µg/ml was found to be slightly higher than that at 100 µg/ml.

TABLE 1: Preliminary phytochemical screening of leaf and seed extracts of *Moringa oleifera* using hexane and ethanol as solvents.

Chemical Constituent	Hexane Extract of		Ethanolic Extract of	
	Leaves	Seeds	Leaves	Seeds
Alkaloids	-	-	+	+
Steroids	+	+	+	+
Tannins	-	-	+	-
Reducing sugar	-	-	+	+
Glycosides	-	-	+	-
Flavoniods	-	-	+	-
Saponins	-	-	+	+
Resins	+	+	+	+
High Fatty Acid	+	+	-	-

Key:

(-) = Negative (absence of constituent)

(+) = Positive (presence of constituent)

TABLE 2: Antibacterial activity of leaves extracts using hexane as a solvent.

	Zone of inhibition in mm					
	10% (100µg/ml)			20% (200 ug/ml)		
Test Organisms	**A**	**B**	**M**	**A**	**B**	**M**
Pseudomonas aeruginosa	35	38	37	53	48	51
Shigella flexneri	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-
Stephylococcus aureus	-	-	-	-	-	-
Bacillus cereus	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	15	12	14
Control	-	-	-	-	-	-

Key:

(-) = No zone of inhibition

Control = plate with no organism on it

A - = First plate

B- = Second plate

M – = Mean of duplicate plates

Table 3 shows that, hexane seeds extract had no activity on all the six tested organisms at the two selected concentrations (100 µg/ml and 200 µg/ml).

Table 4 shows that, ethanolic leaves extract had a mean inhibitory activity of 31mm at 100 µg/ml and 42mm at 200 µg/ml against *Pseudomonas aeruginosa*. Also the extract had inhibitory activity of 15mm against *Staphylococcus aureus* at 200 µg/ml but with none activity against *Salmonella typhi, Shigella flexneri, Bacillus cereus and Enterococus faecalis.*

Table 5 shows that the ethanolic seeds extract had no activity against all the six selected bacteria species at two different concentration (100 µg/ml and 200 µg/ml).

TABLE 3: Antibacterial activity of seeds extracts using hexane as a solvent.

Test organism	Zone of inhibitions in mm					
	10%(100µg ./ml)			20% (200µg/ml)		
	A	B	M	A	B	M
Pseudomonas aeruginosa	-	-	-	-	-	-
Shigella flexneri	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-
Staphylococcus aureus	-	-		-	-	-
Bacillus cereus	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-
Control	-	-	-	-	-	-

Key: :-

(-) = No zone inhibition

Control = Plate with no organism on it

A = First plate

B = Second plate

M = Mean of duplicate plates

TABLE 4: Antibacterial activity of leaf extract using ethanol as a solvent

Test Organism	Zone of inhibitions in mm					
	10%(100µg/ml)			20% (200µg/ml)		
	A	B	M	A	B	M
Pseudomona aeruginosa	30	32	31	43	42	43
Shigella flexneri	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	14	16	15
Bacillus cereus	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-
Control	-	-	-	-	-	-

Key:

(-) = No zone of inhibition

Control = Plate without an innoculum

A = First plate

B = Second plate

M = Mean of duplicate plates

TABLE 5: Antibacterial activity of seeds extract using ethanol as a solvent

Test Organisms	Zone of inhibition in mm					
	10% (100µg/ml)			20% (200µg/ml)		
	A	B	M	A	B	M
Pseudomona aeruginosa	-	-	-	-	-	-
Shigella flexneri	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-
Staphylococeus aureus	-	-	-	-	-	-
Bacillus cereus	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	-	-	-
Control	-	-	-	-	-	-

Key:

(-) = No zone of inhibition

C = Control (plate without an innoculum)

A = First plate

B = Second plate

M = Mean of duplicate plates

4.1.1 Results of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the two best extracts (hexane leaves and ethanolic leaves extract) on *Pseudomonas aeruginosa* was determined to be at 25.00 µg/ml for ethanolic extract and 12.50 µg/ml with hexane leaves extract. The above two concentrations are regarded as the least concentration of the extract of which the inhibitory activity completely kills or inactivates the growth of the test organism. Control 1 (broth only) served as organism sterility, control 2 (broth + organism) served as organism viability and control 3 (extract only) serve as sterility of the extract (Table 6).

4.1.2 Results of minimum bactericidal concentration (MBC)

Table 7 shows that the growth appearance on the re-inoculated plates (2 to 9) with concentration at 25.00 µg/ml ethanolic extract and 12.50 µg/ml of hexane extract were only bacteriostatic because they only inactivated the growth of *Pseudomonas aeruginosa* at the 2 MIC, therefore the inhibitory activity is not bactericidal. Plate 1 served as organism control, because appearance of growth ensured that the organism was viable. Plate 10 served as media and extract sterility control because absence of growth on the plate ensured that the media and the extract were sterile.

TABLE 6: Minimum inhibitory concentration (MIC) of leaves extract on

Pseudomonas aeruginosa

Concentration of *Pseudomonas aeruginosa* µg/ml	**Concentration MIC of ethalonic leaves extract**	**Mean of (MIC) of hexane leaves extract.**
50.00	-	-
25.00	- (MIC)	-
12.50	+	-MIC
6.25	+	+
3.13	+	+
1. 56	+	+
0.78	+	+
0.39	+	+
0.195	+	+
0.097	+	+
$Control_1$	-	-
$Control_2$	+	+
$Control_3$	-	-

Key:

(-) = No zone of inhibition
(+) = Activity
$Control_1$ = Broth only
$Control_2$ = Broth + organism
$Control_3$ = Extract only

TABLE 7: Minimum bacterostatic concentration (MBC) of leaves extract of Moringa oleifera on *Pseudomonas aeruginosa*.

Plate Number	1	2	3	4	5	6	7	8	9	10
Test organism	Ps	Ps	Ps	Ps	Ps	Ps	Ps	Ps	Ps	-
Concentration of ethalonic leaf extract in the Nutrient medium (ML)	00.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Concentration of Hexane leaf extract in nutrient medium (ML)	00.00	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
Observation	+	+	+	+	+	+	+	+	+	-

Key:

Ps = *Pseudomonas aeruginosa*
(+) = Bacterial growth
(-) = No growth
Plate 1 = serve as organism control
Plates 2 – 9 = re-inoculated Plate with hexane extracts *Pseudomonas aeroginosa* at 2 MIC (25.00 µg/ml and 12.50 µg/ml of ethanol and hexane extracts respectively.

Plate 10 = serve as media sterility control

4.2 Results of purification activities of *Moringe oleifera* seeds extract

The result of physico-chemical analysis (colour, turbidity. odour, pH, and hardness) of water sample from the A. B. U. dam was noted and is presented in Table 8.

4.2.1 **Turbidity**

Table 8 shown that the raw water was found to have the highest turbidity of 5.00 NTU while water containing 10mg/L of seed extract had the lowest turbidity of 0.81 NTU and that treated with alum had 1.99 NTU. The relationship between the turbidity and color, extract concentration, pH and hardness is demonstrated in Figures 1 to 4.

4.2.2 **Colour**

The result generally showed that the colour of the water ranged from 5 – 20 H^o using Lovibond comparator with a disc – Hazen NSA. The raw water has the highest colour intensity ($20H^o$) followed by water containing 40 – 50 µg/l with $15H^0$. the water containing 5 and 25mg/L together with water treated with alum at 50mg/L have $10H^o$. the least colour intensity was observed from water containing 10, 15 and 20mg/L of the seed extract.

4.2.3 **Odour**

Water samples with concentration of 5 mg/l, 25mg/l and 30mg/l of the seeds extract were found to be slightly smelling after 22 hours while water samples with 35mg/l to 50mg/l were found to be strongly smelling. Water samples treated with 10 – 20mg/l of seeds extract, and that of water treated with alum and raw water were found odourless after 22 hours.(Table 8).

4.2.4 **Temperature**

Temperature of the water samples before and after treatment remains almost the same. Range from 34.0^0C to 35.05^0C

4.2.5 **pH**

Table 8 shows that, the pH of raw water and that of those treated with seeds extract at different concentrations (5mg/l – 50mg/l) ranges from 6.9 – 7.6. However pH of the water treated with alum at 50mg/l was found to be 5.0.

4.2.6 **Hardness**

Table 8 revealed that. Raw water had the highest hardness of 34mg/l as $CaCO_3$ while water treated with seeds extract at 50mg/l had the lowest hardness of 10mg/l as $CaCO_3$. Water treated seeds extract at concentration ranges from 5 – 45mg/l and that of water treated with alum at 50mg/l had hardness which ranges from 13 – 22mg/l as $CaCO_3$.

TABLE 8: Physico-chemical analysis of water samples treated with *Moringa oleifera* seeds powder at different concentrations.

Water samples with different concentration of *Moringa seeds* mg/ml	Colour Matching Disc H^o	pH	Hardness mg/L as $CaCO_3$	Turbidity NTU	Odour assessed using nostril
5	10	6.9	21	1.88	Slightly smelling
10	5	7.4	20	0.81	Odourless
15	5	7.2	18	1.25	Odourless
20	5	7.5	17	1.38	Odourless
25	10	7.3	16	1.70	Slightly smelling
30	10	7.3	16	1.85	Slighty smelling
35	10	7.4	15	2.05	Strongly smelling
40	15	7.1	14	2.90	Strongly smelling
45	15	7.3	13	2.98	Strongly smelling
50	15	7.4	10	3.05	Strongly smelling
$Control_1$ 50	10	5.0	22	1.99	Odourless
$Control_2$ 0	20	7.3	34	5.00	Odourless

Key:

H^o = **Colour intensity**

$Control_1$ = Water sample with Alum at 50mg/ml as a control

$Control_2$ = Raw water sample only as a control.

Table 9 showed the mean bacterial count in colony forming unit (CFU) of raw water before treatment, water samples treated with *Moringa* seeds extract and water treated with Alum at concentration ranges from 5-50mg/liter at10^{-2} dilution. The result indicated that the raw water had more colonies ranges from 170-210 colonies (1.7×10^{-4} to2.1×10^{-4} CFU) while water treated with seeds extract had 16-31 colonies (1.6×10^{-3} to 3.1×10^{-3} CFU) and water treated with Alum had100-130 colonies (1.0×10^{-4} to 1.3×10^{-4} CFU) therefore water treated with seeds extract had the lowest colonies.

TABLE 9: Total bacterial count of raw water before treatment, water treated with seeds powder and alum by pour plate count method.

Bottle number	Seeds powder/alum concentration in mg/l	**Total bacterial count of raw water before treatment, water treated with seeds powder and alum by pour plate count method.**		
		Raw Water Powder	Seeds	Alum
1	**5**	**2.1×10^4**	**3.1×10^3**	**1.3×10^4**
2	**10**	**1.9×10^4**	**3.0×10^3**	**1.2×10^4**
3	**15**	1.9×10^4	**3.0×10^3**	**1.2×10^4**
4	**20**	1.9×10^4	**2.6×10^3**	**1.2×10^4**
5	**25**	**1.7×10^4**	2.4×10^3	**1.1×10^4**
6	**30**	**1.8×10^4**	2.2×10^3	**1.1×10^4**
7	**35**	**1.7×10^4**	2.0×10^3	**1.1×10^4**
8	**40**	**1.8×10^4**	2.0×10^3	**1.1×10^4**
9	**45**	**1.9×10^4**	1.8×10^3	**1.0×10^4**
10	**50**	**1.8×10^4**	1.6×10^3	**1.0×10^4**
Control	**-**	**-**	**-**	**-**

Key:

Control = distilled water

Note: Values are means of duplicate readings.

Figure 1: shows the relationship between concentration of the seeds extract applied and the turbidity of the treated water samples. The raw water had turbidity of 5NTU, turbidity reduced to 1.88NTU when 5mg/l of the seeds extract was applied to the water sample. It was also reduced to 0.81NTU in the water sample containing 10mg/l of the seeds extract, but the turbidity continues to increase i.e 1.65NTU to 3.05NTU from water samples containing 15 – 50mg/l concentration of the seeds extract.

Figure 2: Illustrates the relationship between concentration and hardness. The higher the concentration of the seeds extract applied the lower the hardness. The raw water sample had hardness of 34mg/l as $CaCO_3$, but lowers to 10mg/l as $CaCO_3$ by the addition of 50mg/l of the seeds extract.

Chapter : 5

DISCUSSION

5.1.1 **Preliminary Phytochemical Screening.**

The result obtained from this study (Table 1) indicated that seeds and leaves of *Moringa oleifera* possess all the nine tested chemical constituents using two different solvents (hexane and ethanol). These results agree with Sofowora findings in 1982, where he noted that plants produce many organic substances such as resins, alkaloids, flavonoids, saponins, and steroids. Again the presence or absence of a particular constituent in a plant or plant part also was justified as plants vary in their chemical constituents depending on age, species and agroforestry(*Sofowora*, 1993).

Some of the chemical constituents found in this study are usually associated with medicinal purposes, It has been documented that alkaloid, tannins, glycoside, are known to display physiological activities against many microorganisms (Rajas et al., 1992).For example alkaloid is one of the natural drugs used in pharmacy, because of its effect on kidney, bladder, skin, heart, and lungs (*Tyler et al.*, 1981). Therefore the presence of alkaloid may be

responsible for its use in treating diabetes, blood pressure problems and skin and infections.

Tannins were confirmed to posses an antiseptic properties (astringent properties) (Ezeamuzie,1996).It may be that is why the juice prepared from *Moringa* leaves as is use skin antiseptic.Resins are found to be important laxatives and cathartic drug (Ghani 1990). This may be the reason, the plant is traditionally employed in treatment of anxiety. Thomas (1947) noted that one of the properties of resins is that, they are usually associated with adhesive quality, which make them valuable for wound dressing and filling cavities in curing tooth ache. Flavonoids are known with the ability to block "sorbitol" pathway and may be this property is the one which makes *Moringa* plant a drug for treating diabetes. Also flavonoid has the capacity of strengthening capillaries and connecting tissues. Therefore, the use of the plant in treating inflammation, tumor and allergy will be possible due to the presence of flavonoid. (Heathnote, 2005). Steroids are known to contribute to a wide range of therapeutic application such as cardiotonic vitamin D precursors, oral contraceptive agents anti inflammatory, agents ananabolic agent, therefore traditionally the use of the plant in treating some of the above mentioned problems may be due to the presence of steroids (Trease and Evans, 1992).

5.1.2 **Discussion on Antimicrobial Screening**

Moringa oleifera plant has been found to be a plant with various medicinal properties, that is why it is referred to as a "miracle tree" (Lowell, 2001). This study noticed that hexane and ethalonic seed and leaves extract possess inhibitory activities against some of the bacteria species tested in this work. Inability to have inhibitory activity against, *Shigella flexneri, Bacillus cereus* and *Salmonella typhi* does not mean that, it will not have inhibitory activities at higher concentrations or when different solvents are used for extraction. The results also indicated that the higher the concentration of the plant extract the higher the inhibitory activity (Tables 2 and 4) This agrees with research finding of Kurosaki and Nishi (1983). Makonnen et al., (1997) noted that effect of an antimicrobial agent is usually increased with larger doses. Tables 2 and 4 also show that the leaves of *Moringa oleifera* possess an antimicrobial activity, This agrees with Sutherland (1994) findings, that aqueous extract of *Moringa oleifera* leaves possess significant antimicrobial activities against some Gram positive and Gram negative bacteria and some fungal species. The effect of inhibitory activity of the extract correlated with the finding's of (El-

faraly et al.1983). where they found out that an antimicrobial agent with low activity against an organism have high MIC while a highly active antimicrobial agents gives low MIC.

5.1.3 Discussion on water purification activity

The function of any coagulant is to remove the substances that are responsible for turbidity and colour in water. The result obtained from this work revealed that, this function has been found to be performed by *Moringa oleifera* seeds as shown in Table 8. This agrees with the findings of Sulayman and Charles (1994). In addition to that, it has ability to reduce the microbial load (Tables9).

The turbidity between the raw water and that of water treated with seeds and with alum is not much because turbidity removal was found to be more effective in very turbid water than in water with low turbidity. This was in agreement with studies carried out by Jahn, (1988), Sutherland et al. (1990) and Kaser et al. (1991). They found out that, with the seeds extract turbidity removal activity was found to be more effective in raw water with high turbidity. And Weber (1972) noted that *Moringa oleifera* seeds is a poly electrolyte therefore it may not be effective as a primary coagulant for low turbidity waters because such waters contain low concentrations of colloidal particles. The turbidity was found generally, low (NTU).

The pH (6.9-7.5) of water treated with seeds extract was found to be slightly alkaline and in general, this pH is within the recommended standards (WHO 1994) unlike the pH of water treated with alum which was found to be slightly acidic (pH 5.0). Well (1994) reported that, there was no evidence suggesting any acute or chronic side effects on human, when seeds of *Moringa oleifera* was used in water treatment at low doses.

Chapter 6 :

CONCLUSION

In conclusion this study reveals that seeds and leaves of *Moringa oleifera* possess chemical constituents that are usually associated with physiological activity. The antibacterial activity shown by the leaves gives promises of their potential application in treatment of various types of illness and are already an alternative to the orthodox medicine. *Moringa* plant was also found to possess water purification properties comparable to the alum in conventional use. Therefore it provides an alternative agent for water purification especially in our rural areas where majority of people depend on well and stream water. Hexane and ethanol were found to have an extractive properties and therefore, they can be used as solvents for extraction of chemical constituents from *Moringa* plant and others.

RECOMMENDATIONS

Based on this work the following recommendations are made:-

- The use of plant in preparation of modern drugs in our developing countries will reduce the risk of resistance and drug abuse among the population. High cost and side effects of modern drug will also be

reduced. Therefore, it is recommended here that the government and non governmental organization should work seriously on our herbal drugs.

- Enlightenment campaign and health talk on the importance of *Moringa oleifera* to rural people will probably help to reduce the rate of death as a result of waterborne diseases and also in solving some problems which are usually encountered on health issues.
- Government should be using *Moringa* seeds in water treatment plants instead of alum or other chemicals which are not easily available and they are also expensive.
- Lastly, there is a need for any one wishing to conduct projects or researches similar to this one to work on the shelf life of water treated with *Moringa* seeds.

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Appendix

Statistical Analysis

A

H_0: μ1 = μ2 (i.e. there is no significant difference in means of colonies formed between Raw water and water treated with seeds suspension at 10^{-2} dilutions)

Ha: μ ≠ μ1 (i.e. there is significant difference in means of colonies formed between Raw water and water treated with seed suspension at 10^{-2} dilutions).

Rejection Rule = Reject Ho if $t_{cal} \geq t_{\alpha}\, n_1 - n_2 - 2$

$t_{\alpha}\, n_1 + n_2 - 2 = t_{\alpha}.18 = 2.10$

P<0.05, DF = 20 – 2 = 18

Formular:

$$t = \frac{X_1 - X_2}{\sqrt{SP\left(\frac{n_1 + n_2}{n_1 n_2}\right)}}$$

X_1 = 185.0000 X_2 = 23.7000

S_1 = 11.7851 S_1 = 5.3759

S_1^2 = 138.8886 S_2^2 = 28.9003

n_1 = 10 n_2 = 10

$$Sp = \frac{(10-1)138.8886 + (10-1)\,28.9003}{10+10-2}$$

$$= \frac{1249.9974 + 260.1027}{18}$$

$$= \frac{1510.1001}{18}$$

$$= 83.8945$$

$$|t| = \frac{185.0000 - 23.7000}{\sqrt{83.8945}\ \frac{(20)}{100}}$$

$$= \frac{161.3000}{4.0962}$$

$$|t| = 39.3779$$

Decision Accept H_a.

Conclusion Reject H_o

B

H_0: μ1 = μ2 (i.e. there is no significanst difference in means of colonies formed between Raw water and water treated with Alum at 10^{-2} dilutions)

Ha: $\mu 1 \neq \mu 2$ (i.e. there is significant difference in means of colonies formed between Raw water and water treated with Alum at 10^{-2} dilutions).

Formular:

$$t = \frac{X_1 - X_2}{\sqrt{SP\left(\frac{n_1 + n_2}{n_1 n_2}\right)}}$$

$X_1 = 185.0000$ $X_2 = 114.0000$

$S_1 = 11.7851$ $S_2 = 8.4327$

$S_2^2 = 138.8886$ $S_2^2 = 71.1104$

$$Sp = \frac{(10-1)138.3886 + (10-1)\,71.1104}{18}$$

$$= \frac{1313.9883}{18}$$

$$= 72.9934$$

$$|t| = \frac{185.0000 - 114.0000}{\sqrt{72.9934\ \frac{(20)}{100}}}$$

$$= \frac{71.0000}{3.8209}$$

$$|t| = 18.5820$$

Decision Accept H_a.

Conclusion Reject H_o