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ANTIMICROBIAL AND BACTERIA CULTIVATIVE VALUE OF *MORINGA OLIFERA* LEAF EXTRACT IN SELECTED ANIMAL PATHOGENS

MSC THESIS

BY

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SEPTEMBER, 2014

BISHOFTU, ETHIOPIA

ANTIMICROBIAL AND BACTERIA CULTIVATIVE VALUE OF *MORINGA OLIFERA* LEAF EXTRACT IN SELECTED ANIMAL PATHOGENS

A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Microbiology

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SEPTEMBER, 2014

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared byAddisu Demeke entitled:antimicrobial and bacteria cultivative value of *Moringa olifera* leaf extract in selected animal pathogens and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Veterinary Microbiology.

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STATEMENT OF AUTHOR

First, I declare that this thesis/dissertation is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University and College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AChAcetylcholine

Cfu Colony forming unit

DBPs Disinfection by-products

DF Dialyzable

DMSO Dimethyl sulfoxide

EBV-EA Epstein-Barr virus-early antigen

EBV-EA Epstein-Barr virus-early antigen

EIAR Ethiopian Institute of Agricultural Research

H. pylori Helicobacter pylori

LD50 Lethal dose 50

MEMO Methanol Extract of *Moringa oleifera*

MICMinimum Inhibitory Concentration

mm millimeter

NDF Non dyalizable

TPA Tetradecanoylphorbol-13-acetate

WHO World health Organization

ABSTRACT

The research was conducted to evaluate the antibacterial effect of various forms of the leaf extracts (*Moringa oleifera*) on the growth of four selected gram-positive and negative bacteria and to evaluate the bacterial cultivated value of the extract.The antibacterial activity of leaf extracts of *Moringa oleifera* Lam., was determined in vitro, using disc diffusion and minimum inhibitory concentration (MIC) determined against selected animal pathogenic bacteria. *M. oleifera*leave extract has been found to possess antimicrobial properties. In this work, four extraction solvents were used, i.e. aqueous extraction, fresh leave juice, ethanol and methanol. The methanol extract method of fresh and dried leaves displayed potential broad spectrum activity against all the tested organisms whereas the fresh leave juice extract which didn't have any inhibitory effect were evaluated for its bacterial cultivated value and it was obtained that the power form of this extract was optimal environment for the growth of both gram positive and gram negative selected bacteria.

The antimicrobial assay showed that the zones of inhibition produced using disc diffusion method ranged 3mm to 22mm for the four extraction methods with the highest value of 22mm obtained with ethanol extraction. The zones of inhibition for fresh ethanol extract was 4 to 22 mm, methanol extract from fresh leaf was 8 to 20 mm, and hot and cold water extract of fresh and dried leaves was 8 to 9,8 to 10,3 to 4 and 3 to 5 mm respectively. The MIC values were conducted for the different form of extracts against the tested bacterial pathogens and the highest concentration was recorded 25mg/ml for *S.auerus* while it was 30 mg/ml for *S.fecalis*. The bacterial cultivated value of *M.oleifera* powder extract of fresh leaf juice was determined by cultivating the four tested pathogens on the culture medium which was prepared from the plant extract with the proper concentration of agar powder in sterile distilled water and it was revealed that all the pathogens were successfully grown on it. The consequences of this investigation suggest that the extracts and juice of *M.oleifera* Lam. can be used to discover antibacterial agent for developing new pharmaceuticals to control animal pathogenic bacteria responsible for severe illness.

Keywords*: Antimicrobial, leaf, Moringa oleifera, Pathogenic bacteria.*

1. INTRODUCTION

Antimicrobial resistance has become a global problem. Strategies to improve the current situation include research in finding new and innovative antimicrobials. Antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistant forms. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources. In developing countries, due to the cost of efficient antimicrobials, a large proportion of the population utilizes medicinal plants for the treatment of infectious diseases. According to the WHO estimation, traditional healing provides the primary health care needs for a large majority (80%) of the population in Africa (Bharali*et al*., 2003).

Medicinal herbs are moving from fringe to mainstream use with a greater number of animal seeking for remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to eco-friendly and bio-friendly plants, which can prevent and cure different human and animal diseases. According to the World Health Organization reports, the use of traditional medicine in the first world countries is on the rise due to failure of conventional medicine that can cure chronic diseases, emergence of multi-drug resistant pathogens and parasites, adverse effects of chemical drugs, increasing cost and information of herbal medicine (Dubey*et al.,* 2004).

Antimicrobial potentiality of different medicinal plants is extensively studied all over the world (Ahmed *et al*., 1998; Arora and Kaur, 1999; Arora*et al*., 2009; Rojas *et al*., 2006). However, only a few studies have been carried out in a systematic manner. *Moringaoleifera* is medicinal species, belonging to monogeneric family Moringaceae (order Brassicales). It has 33 species of trees and shrubs distributed in sub-Himalayan ranges of India, Sri Lanka, North Eastern and South Western Africa, Madagascar and Arabia. Today, it has become naturalized in many locations of the tropics and is widely

cultivated in Africa, Ceylon, Thailand, Burma, Singapore, West Indies, Srilanka, Malaysia and the Philippines (Fahey, 2005).

The "*Moringa*" tree is considered one of the world's most useful trees, as almost every part of the *Moringa* tree can be used for food or has some other beneficial properties. In the tropics, it is used as forage for livestock, and in many countries, it is used as a micronutrient powder to treat various ailments. The fruit of the tree is quite popular as a vegetable in Asia and African countries. In India and other parts of the country the fruit called as drumstick. The leaves contain more Vitamin A compared to carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas, and that the protein quality of *Moringa* leaves rivals that of milk and eggs. The *Moringa* trees have been used to combat malnutrition, especially among infants and nursing mothers. The nongovernmental organizations in particular-trees for life, Church World Service and Educational Concerns for Hunger Organization have advocated *Moringa* as natural nutrition for the tropics (Irene *et al*., 1998).Its leaves as food supplement, recommended for children with moderate malnutrition between the ages of 6 months to 5 years. It has been claimed to have an unusually high content of calcium, iron, and vitamin A and high quality protein, and a low content of antinutrients (Natural or synthetic compounds that interfere with the absorption of nutrients) such as tannins and oxalates. The leaves have therefore been promoted as a potential low cost high quality food (Nikkon*et al.*, 2003).

Almost all the parts of this plant: root, bark, gum, leaf, pods, flowers, seeds and seeds oil have been used for the various ailments in the indigenous medicine (Odebiyi and Sofowora, 1999). It has been known to be anti helminthic activity, antimicrobial activityd etoxifier, immune booster and anti parasitic activity (Thilza *et al*., 2010). Antimicrob activities of various *M. oleifera* morphological parts against some pathogenic microorgnis ms have been reported (Caceres *et al.,* 1999; Doughari *et al*., 2007; Kekuda *et al.,* 210; Ni kkon *et al*., 2003). According to ethano botanical studies its roots are bitter, acrid, thermogenic, digestive, carminative, ananthelmintic, constipation, antiinflammatorymme nagogue, diuretic, ophthalm, expectorant and stimulant. They are useful in dys-

pepsia, anorexia, verminosis, diarrhea, colic, flatulence, paralysis, inflammations, vesicle and renal calculi. Moreover it is used in cough, asthma, bronchitis, pectoral diseases, sple nomegaly, epilepsy and cardiopathy. Leaves are anti inflammatory, anodyne, anthelminto phthalmic and rich in Vitamin A and C. They are useful in wounds, tumors, inflammation and helminthasis. Seeds are acrid, bitter, anodyne, anti inflammatory, purgative, antipyre tic and ophthalmic. They are useful in neuralgia, inflammations, intermittent fevers and opthalmopathy. Bark is regarded as an antiscorbic, and it exudes a reddish gum sometimes used for diarrhea (Greenwood, 1989).

The specific components of *Moringa* preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include 4 (4'O acetyl Lrhamnopyranosy l) benzyl isothiocy anate, benzyl isothiocyanate, niazimicin, pterygospermin, benzylisothi ocyanate, and benzyl glucosinolate. It is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the caroten oids (including β carotene or provitamin A) (Odebiyi and Sofowora, 1999). The frequ- ency of life threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries (Al-Bari *et al*., 2006). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Rojas *etal*., 2006). For a longtime, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies world-wide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana *et al*., 2007). Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds etc.

The practice of complementary and alternative medicine is now on the increase in developing countries in response to world health organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the

efficacy of many plants used in folk medicine to treat infections(Ahmed*et al*., 1998). However, despite the existence of potent antibiotic and antifungal agents, resistant or multiresistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Badgett, 1964).

It is therefore very necessary that the search for fewer antibiotic sources be a continuous process. Dougharie*t al*. (2007) were reported that Plants are the cheapest and safer alternative sources of antimicrobials. In this regard among the medicinal plant available i n the world *M. oleifera* is the one that is leading this role to the frontline. *Moringa oleifera* Lam. is the most widely cultivated species of a monogeneric family, the moringace ae that is native to Ethiopia. Though the plant is available in our country especially of the Konso area, the phytochemical constituents, the antimicrobial activity as well as the nutritive value of the plant is not studied yet.

General objectives:

The main objectives of this study were to determine the antimicrobial activity of various extracts of *M.olifera* plant for selected animal pathogens and to assess the bacterial cultivated values of the extract.

Specific objectives:

- To assess the antibiograms activity of the leave of *M. olifera* plant extract.
- To determine the antimicrobial activity of methanol, ethanol, aqueous and Powder from freshleaf juice extract of the plant in the growth of the selected two Gram positive (*S. auerus, S.fecalis* and two Gram negative (*E.coli and P.aeruginosa)*bacteria.
- To assess the phytochemical constituent of the leaf of the plant and to investigate bacterial cultivated values of the plant extract.

2. LITERATURE REVIEW

2.1. Botanic description

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Family: Moringaceae

Genus: *Moringa*

Species: *Moringaoleifera*

Scientific name: *Moringaoleifera*

Common names: Sajina, the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree (Lee*et al*., 2005).

Moringa oleifera is a small, graceful, deciduous tree with sparse foliage, often resembling a leguminous species at a distance, especially when in flower, but immediately recognized when in fruit. The tree grows to 8 m high and 60 cm width. Bole crooked, often forked from near the base. Bark smooth, dark grey; slash thin, yellowish. Twigs and shoots shortly but densely hairy. Crown wide, open, typically umbrella shaped and usually a single stem; often deep rooted. The wood is soft. Leaves alternate, the old ones soon falling off; each leaf large (up to about 90 cm long), with opposite pinnae, spaced about 5 cm apart up the central stalk, usually with a 2nd lot of pinnae, also opposite, bearing leaflets in opposite pairs, with a slightly larger terminal leaflet. Leaflets dark green above and pale on the under surface; variable in size and shape, but often rounded elliptic, seldom as much as 2.5 cm long (fig 1). Flowers produce

d throughout the year, in loose auxiliary panicles up to 15 cm long; individual flower stalks up to 12 mm long and very slender; 5 pale green sepals 12 mm long, finely hairy, 5 white petals, unequal, a little longer than the sepals; 5 stamens with anthers, 5 without; style slender, flowers very sweet smelling. Fruit large and distinctive, up to 90 cm long and 12 mm broad, slightly constricted at intervals, gradually tapering to a point, 3-(4) angled, with 2 grooves on each face, light brown. It splits along each angle to expose the rows of rounded blackish oily seeds, each with 3 papery wings. The generic name comes from the Sinhalese name ‘morunga’ (Josephine *et al*., 2010).

Fig 1.*Moringa olifera* Plant

Source: (Eilert, 1978).

2.2. Natural Habitat

Readily colonizes stream banks and savannah areas where the soils are well drained and the water level remains fairly high all the year round. It is quite drought tolerant but yields much less foliage where it is continuously under water stress. It is not harmed by frost, but can be killed back to ground level by a freeze. It quickly sends out new growth from the trunk when cut, or from the ground when frozen. The Shifaraw tree, in Amharic (*Moringastenopetala*), which also has other names as Haleko, Ben-oil tree, Cabbage tree, Horse-radish tree, African *Moringa* tree, Malung-gay, Murunga-Kai, and Sohnja is originally from India and Arabia but the variety *Stenopetala* is native of Ethiopia, Kenya and Sudan. It prefers low altitudes and wet and sandy soils, but it can tolerate drought. The flowers are light yellow and attract many insects like bees. The tree itself helps to protect the soil from erosion; it's used as shade, wind breaker and as a living fence. Even when it's cultivated near homes and plantations, in its wild form is nearly extinct since its natural habitat is rapidly disappearing (Arun and Purnachandra, 2011).

2.3. Cultivation

It grows well in hot, semi-arid and humid regions and in welldrained sandy or loamy soils. Seed must be relatively fresh to give a good germination. Warm temperatures are important for germination. Planted seeds would be kept out of reach of mice and wood lizards, as the seed is nutty and considered a tasty morsel by these little scavengers. Stem cuttings, 10-60 cm long, can also be struck in spring and summer. Trees are grown extensively in tropical, sub-tropical and warm temperature areas(Akhtar and Ahmad, 1995).

2.4. Geographical distribution

Moringaoleifera is the most widely cultivated species in the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (fig 2). This rapidly-growing tree was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. It is already an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands. All parts of the *Moringa* tree are edible and have long been consumed by humans (Jed, 2005).

Fig2. The distribution of *Moringa* plant

Source: (Fozia*et al.,* 2012)

In Ethiopia the most striking characteristic of the Konso agricultural system is the cultivation of the cabbage tree (*Moringastenopetala)*. The tree is densely planted within the villages and generally more widely spaced in the fields and terraces between 1600 and 1800 m. Its light green leaves and the conspicuous grey bark are characteristic features of the cabbage tree. Konso can be considered as the area where the tree was first cultivated. From here the cultivation has spread into neighboring areas where it is being used intensively as well. In the whole region the cabbage tree does not occur in the wild. The tree is raised from seed; it requires relatively good soil conditions and prefers wind-protected places. After 5-6 years the first leaves can be harvested. They are boiled and eaten as a vegetable with any warm meal. The leaves are rich in vitamins and are mainly harvested in the dry season when other vegetables are scarce. During the rainy season there are only a few leaves left on the tree and they do not taste good. The leaves are an important trading product in the local markets. Outside the villages, especially on the terraces, the cabbage tree plays an important role in reducing soil erosion. There are trials under way to use the tree for this purpose in other areas

of the country. Furthermore, some very promising medicinal uses have been found. A tea of dried leaves is reported to be very efficient in treating light cases of diabetes and it is said that the extract of fresh leaves can cure indigestion and even the cure of an amoebic dysentery has been reported. Eye inflammations are treated and a root extract helps against unconsciousness. Recently it has been reported that ground seeds can be used to clarify muddy water. Experiments have shown that this powder has the same effectiveness as the best technical water clarifying agents. It can be concluded that *Moringastenopetala* is a greatly underutilized and relatively unknown tree which deserves further investigations (Mehta*et al.*, 2003).

Moringa is a tropical tree with multiple uses and is resistant to drought. Among the 13 species known, *Moringaoleifera* is particularly easy to reproduce and its growth is very fast. The numerous economic uses of *Moringa oleifera* combined with its easy propagatio n have raised growing international interest for this tree which originated from India and is found in most tropical countries (Africa, Asia and America). *Moringa stenopetal* and other species from Eastern Africa and Madagascar also have a great potential even though they have been less explored so far(Morton, 1991).

The species *M. stenopetala* differs from *M. oleifera* because of its composed leaves (3.3 to 6.5 cm) that have a sharper apex, its pods are bigger and twisted when the fruits are fresh, the seeds are ellipsoidal rather than spherical, and their color is cream rather than dark brown(Tsaknis*et al*., 1999).

Moringastenopetala is endemic in East Africa, specimen are found in Djibouti, Uganda, Sudan, and mainly in northern Kenya and southern Ethiopia, in Kaffa, Gamo-Gofa andSidamo, between 500 and 1600 m, in the Qolla agro-climatic area. The tree prefers well drained sandy soils, but as it is also drought resistant we can find the specimen both in wet and arid zones (Trease and Evans,1989).

Konso (SNNP Region - Ethiopia) is one of the main areas of domestication for *M. stenopetala*. The Ethiopian Konso people use different indigenous plants by planting them on their farmlands and in their living compounds for different advantages as discussed(Odebeyi and Sofowara, 1978).

Different parts of *moringa* trees are used in Konso as a food source (for humans and animals), as building material, as fuel source, as a shelter to other plants, to the people and to the farmland soil from the sun's heat and in some other purpose. But it is certain that the primary use of the plant in Konso is the consumption of fresh leaves. In Konso area *Moringastenopetala* is widely grown for its edible leaves throughout the year from generation to generation. You can recognize a Konso settlement by the presence of *moringa*plants covering the compound, and protecting other plants from the burning rays of sun. The extension of the *Moringastenopetala* outside Konso is related to the migration of the Konso people who immediately plant *moringa* everywhere they go to live (Francis and Liogier1991).

In Konso, the leaves are a constant ingredient in the daily dish damaa (in AfaaKonso, it is also known as kurkufaa in Amharic), constituting a major element in the Konso diet, and for peasants it is un-thinkable to cook food without *moringa* leaves. In the drought season, it is possible to cook *moringa*leaves even without any cereals; the Konso women will take out the leaves from the fire and cool them before rolling into balls after squeezing out the liquid (Silver,1993).

Konso people say, "If there is no *moringa* there is no life"; we may assume that the Konso culture as it presents itself to our eyes today is the result of the domestication of *Moringa stenopetala*. Because the wild plant is found in the lowlands extending between the Konso highlands and the Kenya border, where the nomadic pastoralist know it as good fodder for their animals, the origins of Konso culture are interlinked with the domestication of the *moringa* and its introduction in the highlands, once covered by forests(Dixon, 1991).

Nowadays the Konso territory is deforested with the exception of some remaining forests, preserved because under the protection of religious and traditional authorities. In all the terraced fields, *moringa* is present, visible due to its silver bark and shining light green foliage. Its presence in the fields absolves to a dual task: to consolidate the terracing and provide shade to the most precious cash crop of the highlands coffee. In fact the

association of the two plants, moringa and coffee exists only in Konso, it is a specific cultural mark related to the deep link between the Konso and their ecosystem. But the villages (Konso villages are old settlements on top of rocky hills, surrounded of stone walls and are thickly populated) are also recognizable by the wood canopy protecting them from the sun. In terms of numbers, the primary plant to be found in all villages is the moringa which assure shade and then followed by coffee, its best associated plant (Tsaknis*et al*., 1999).

Each family may own at least four good leaf yielding moringa trees within the house compounds and at least ten trees on the farmland on an average, for the day to day family consumptions. Any extra productions of leaves are sold to other people who are in need to buy them. The sale can be made in the market or on any other convenient place (Kekuda*et al*., 2010).

Fig3.*Moringastenopetala,* the indigenous cabbage tree ("halako"), Konso, Ethiopia

Source:(WHO, 1987)

2.5. **Use of *Moringa* plant**

According to Fuglie (1999), the many uses for *Moringa* include: alley cropping (bio-mass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). *Moringa* seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products.In the West, one of the best known uses for *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water (Berger *et al*., 1984), but the seeds are also eaten green, roasted, powdered and stee ped for tea or used in curries (Gassens-chmidt*et al*., 1995). This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern (Nikkon*et al*., 2003).

2.5.1. Nutrition

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular trees for Life, Church World Service and Educational Concerns for Hunger Organization have advo-cated*Moringa* as "natural nutrition for the tropics." Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. *Moringa* is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce (Mead and Curnow, 1982).

Fig4.Nutritive values of *moringa Olifera* lam.in comparison with other food staffs

Source: (WHO, 1987)

A large number of reports on the nutritional qualities of *Moringa* now exist in both the scientific and the popular literature. *Moringa* leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas," and that the protein quality of *Moringa* leaves rivals that of milk and eggs(fig 4). The oral histories recorded by Lowell Fuglie in Senegal and throughout West Africa, who reports countless instances of lifesaving nutritional rescue that are attributed to *Moringa* (Fuglie, 2000). In fact, the nutritional properties of *Moringa* are now so well known that there seems to be little doubt of the substantial health benefit to be realized by consumption of *Moringa* leaf powder in situations where starvation is imminent. Nonetheless, the outcomes of well controlled and well documented clinical studies are still clearly of great value (Sampson, 2005).

In many cultures throughout the tropics, differentiation between food and medicinal uses of plants (e.g. bark, fruit, leaves nuts, tubers, roots, flowers), is very difficult since

plant uses span both categories and this is deeply ingrained in the traditions and the fabric of the community (Lockett *et al*., 2000).

Table1.The vitamin analysis of leaf of *Moringaolifera*(Dahot, 1998).

(All values are per 100 grams of edible portion.)

	Fresh Leaf	**Dried Leaf**
Vitamin A (Arginine Carotene)	6.78 mg	18.9 mg
Vitamin B1 (Thiamin)	0.06 mg	2.64 mg
Vitamin B2 (Riboflavin)	0.05 mg	20.5 mg
Vitamin B3 (Niacin)	0.8 mg	8.2 mg
Vitamin C	220 mg	17.3 mg
Vitamin E	448 mg	0mg

Various parts of the *Moringa* tree are outstanding sources of minerals necessary to maintain good health. Along with vitamins and amino acids, the dried leaves can provide a large percentage of the daily requirements for calcium, iron, magnesium, potassium and zinc. As a result, the leaves are often used to supplement the diets of expecting and nursing mothers and young children. Lack of iron can lead to anemia and other serious medical problems for these vulnerable individuals, while calcium deficiencies can cause muscular and skeletal problems for mothers and children alike. *Moringa* leaves can help to provide the needed calcium, iron and other minerals to avoid these nutritional problems, especially in areas where prenatal health care is difficult to obtain. The mineral content present in Moringa leaves, seed pods, and roots can be used to supplement the regular diet of undernourished populations (Sharon *et al*., 2006). The tree allows everyone to enjoy better health, even in remote and undeveloped areas of the world (Clark, 1987).

Table 2. The mineral analysis of leaves of *Moringaolifera* (Bennett *et al*., 2003).

(All values are per 100 grams of edible portion.)

	Fresh Leaf	**Dried Leaf**
Calcium	440 mg	2,003 mg
Copper	0.07 mg	0.57 mg
Iron	0.85 mg	28.2 mg
Magnesium	42 mg	368 mg
Phosphorus	70 mg	204 mg
Potassium	259 mg	1,324 mg
Zinc	0.16 mg	3.29 mg

Amino acids are considered the building blocks of proteins and are necessary elements of a healthful diet. Scientists have identified 22 standard amino acids capable of naturally forming polypeptide compounds (Trease and Evans, 1989). The *Moringaoleifera* tree contains all eight essential amino acids, which cannot be synthesized by the body and must be obtained from dietary sources to provide a solid basis for physical health. The leaves and seeds of this useful plant are especially high in amino acids and provide a significant percentage of the recommended daily requirements for valine, lysine, methionine, cysteine, leucine, phenylalanine, threonine and isoleucine, the eight essential amino acids. These vital amino acids are necessary for proper brain, muscle and nervous function as well as providing the raw materials to allow the body to synthesize protein materials for further growth. *Moringa* seed pods and leaves provide all of the essential amino acids as well as 18 of the 22 standard amino acids and offer superior food value for undernourished populations in developing countries (Sanchez's ,2009).

Table 3. The amino acids and other nutritive analysis of leaf of *Moringaolifera* (Bennett *et al*., 2003).

(All values are per 100 grams of edible portion.)		
	Fresh Leaf	**Dried Leaf**
Arginine	406.6 mg	1,325 mg
Histidine	149.8 mg	613 mg
Isoleucine	299.6 mg	825 mg
Leucine	492.2 mg	1,950 mg
Lysine	342.4 mg	1,325 mg
Methionine	117.7 mg	350 mg
Phenylalanine	310.3 mg	1,388 mg
Threonine	117.7 mg	1,188 mg
Tryptophan	107 mg	425 mg
Valine	374.5 mg	1,063 mg

(All values are per 100 grams of edible portion.)		
	Fresh Leaf	**Dried Leaf**
Calories	92 cal	329 cal
Carbohydrates	12.5 g	41.2 g
Fat	1.70 g	5.2 g
Fibers	0.90 g	19.2 g
Protein	6.70 g	29.4 g

2.5.2. Cattle Fodder Supplement

In Nigeria showed that supplementing cattle feed with the leaves and green stems of *Moringa* can increase milk production by 43-65%, and increase daily weight gain in cattle by up to 32%. Some of them also found out that *Moringa* can be grown intensively as a field crop which is equivalent to 100 to 110 metric tons of dry mass, 17.5 metric tons of pure protein, 7000 kg of lipids, with 65% being omega-3 fatty acids, 10 metric tons of fermentable sugars, approximately 8 metric tons of starch, 45 metric tons of hemicelluloses and cellulose (Haristoy*et al*., 2005).

Sanchez's, 2009 studied the cultivation of this plant without irrigation and with much less fertilizer, and resulted in a total of 100 tons of green mass harvested from four crops in a year. However, milk production and cattle weight increased substantially in most of the studies. All these factors may make *Moringa* leaves and green stems very attractive and inexpensive as a cattle fodder supplement (Bennett *et al*., 2003).

2.5.3.Use of Moringaoleifera in Water Disinfection

The gravest of all dangers to which water supplies can be exposed is contamination by pathogenic organisms. Disinfection is a chemical process for eliminating pathogenic microbes from an environment. Chemical agents that have been used as disinfectants include halogens, phenols, alcohols, heavy metals, dyes, soap and detergents, ammonia compounds, hydrogen peroxide, and various alkalis and acids . The most common of these are the oxidizing chemicals, and chlorine is the most universally used. However, chlorine has problem of decay and reduced concentration as the water flows through the distribution network. It also has the potential for forming carcinogenic and mutagenic disinfection by-products (DBPs). Disinfectants and their by-products may also be associated with increased risks of cardiovascular diseases, cancers, and birth defects. Although such risks are low, Bennett *et al*. (2003) 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 Thilza, *et al.* (2010), identified the presence of an active antimicrobial agent in *Moringaoleifera* seeds.

2.5.4.Use of Moringaoleifera in water softening

Softening is the removal of ions which cause hardness in water. Hardness is caused mainly by calcium and magnesium ions, or at times, by iron, manganese, strontium, and aluminum ions. Hardness causes excessive soap consumption and scale formation in hot water pumps, boilers and pipes. Public water supplies should not exceed 300 to 500mg/l of hardness; although, aesthetically, hardness greater than 150mg/l is unacceptable. Because the cost of chemicals for softening is high, local materials are being considered

as substitutes. *M. oleifera* seed extract has been identified as a potential softening agent.Thilza, *et al*., (2010), reported that initial hardness of water varying from 80300mg/l CaCO was found to have been reduced to between 50-70% after coagulation and softening with *M. oleifera.*Sani (2009), using water samples from Watari and Challawa rivers, and from Yarimawa and KofarKabuga wells, reported total hardness reduction from 54mg/l to 25mg/l CaC033 for river water while using 40-200mg/l *M. oleifera* dosage.

2.5.5. As Sorbent

Moringa seeds could be used as a less expensive bio-sorbent for the removal of cadmium (Cd) from aqueous media (Sharma *et al*., 2006). The aqueous solution of *Moringa* seed is a heterogeneous complex mixture having various functional groups, mainly low molecular weight organic acids (amino acids). These amino acids have been found to constitute a physiologically active group of binding agents, working even at a low concentration, which because of the ability to interact with metal ions is likely to increase the sorption of metal ions. The proteinases amino acids have a variety of structurally related pH dependent properties, generating negatively charged atmosphere and play an important role in the binding of metals (Sharma *et al*., 2006).

2.5.6. Antidiarrheal activities

Among medicinal plants, *Moringaoleifera* Lam. has been recommended for several disorders in folk medicine (Caceres*et al.*, 1999). Indian *MateriaMedica* describes the use of roots of *Moringa oleifera* Lam in the treatment of a number of ailments, including asthma, gout, lumbago, rheumatism, enlarged spleen or liver, etc. (Kebreab *et al*., 2005). Nevertheless, no pharmacological studies of *Moringa oleifera* Lam root have thus far eva luated for its antidiarrheal activity(Vijaya and Ananthan, 1997).

2.5.7. Hypotensive and Spasmolytic Activities

Bioassay directed fractionation of an ethanol extract of *Moringaoleifera*(MO) leaves resulted in the isolation of four pure compounds, niazinin, niazinin, niazimicin and niazi minin. Intravenous administration of either one of the compounds (10 mg/kg) produced hypotensive and bradycardiac effects in anaesthetized rats. Pretreatment of the animals wi th atropine (1mg/kg) completely abolished the hypotensive and Brady cardiac effects of acetylcholine (ACh), whereas cardiovascular responses to the test compounds remain-ned unaltered, ruling out the possible involvement of muscarinic receptor activation. In isolated guinea-pig atria all the compounds (50–150 μg/ml) produced negative inotropic and chronotropic effects. Each compound inhibited K+ induced contractions in rabbit aorta as well as ileal contractions induced by Ach or histamine at similar concentration. Spontaneous contractions of rat uterus were also inhibited equally by all compounds. These data indicate that the direct depressant action of these compounds exhibited on all the isolated preparations tested is probably responsible for its hypotensive and Brady cardiac effects observed in vivo. Moreover, spasmolytic activity exhibited by the constituents of the plant provides a scientific basis for the traditional uses of the plant in gastrointestinal motility disorders (Sharma *et al*., 2006).

Anti-fungal activity Investigations were carried out to evaluate the therapeutic properties of the seeds and leaves of *Moringaoleifera* Lam. as herbal medicines. Ethanol extracts showed anti-fungal activities in vitro against dermatophytes such as *Trichophytonrubrum*, *Trichophytonmentagrophytes*, *Epidermophytonfloccosum*, and *Microsporumcanis.* GC-MS analysis of the chemical composition of the essential oil from leaves showed a total of 44 compounds. Isolated extracts could be of use for the future development of anti-skin disease agents(Sharma *et al*., 2006).

2.5.8. Ant nociceptive activities

The fresh leaf juice and ethanol extract of the leaves of *Moringaoleifera*were administered orally at doses of 25, 50,100 mg/kg in mice and were tested for ant nociceptive activities using three models: Acetic acid induced writhing, formalin induced paw licking and tail flick test using analgesicmeter. Amongst all doses, a dose of 100mg/kg of both the administered extracts showed a significant ant nociceptive activity

in mice. The effect was significantly reversed by the opioid receptor antagonist naloxone indicating the role of both the central and peripheral opioid receptors in alleviating pain(Muyibi and Evison, 1995b)

2.7.9. Hypolipidemic activities

The leaves of *Moringaoleifera* Lam., Moringaceae, are used by the Indians in their herbal medicine as a hypolipidemic agent in obese patients.Albino Wistar rats were fed with methanol extract of *M. oleifera* (150, 300 and 600 mg/kg, p.o.) and simvastatin (4 mg/kg, p.o.) along with hyperlipidemic diet for 30 days. *Moringa oleifera* and simvastati n were found to lower the serum cholesterol, triacylglyceride, and atherogenic index, but were found to increase the HDL as compared to the corresponding high fed cholesterol diet group (control). *Moringaoleifera* was found to increase the excretion of fecal cholesterol. Thus, the study demonstrates that *M.oleifera* possesses a hypolipidemie ffect(Bennett *et al*., 2003).

2.6.10. Anti-inflammatory activities

The seeds of *Moringaoleifera* were extracted with distilled ethanol and concentrated under reduced pressure at 40^{o}C. The resulting extract was partitioned between hexane, ethyl acetate, butanol and water. The solvent fractions were likewise concentrated under reduced pressure. The crude ethanol extract of dried seeds inhibited the carrageenan-induced inflammation in the hind paw of mice by 85% at a dosage of 3 mg/g body weight while the mature green seeds by 77%The hexane fraction of the crude ethanol extract of the dried seeds also inhibited inflammation by 77% at the same dosage while both butanol and water fractions inhibited inflammation by only 34%These results indicate the strong anti-inflammatory activities of the ethanol extract and the hexane fraction. On the other hand, the ethyl acetate fraction caused a 267% increase in inflammation and exhibited toxicity. The mice died after oral administration of the fraction. The crude ethanol extract also inhibited the formation of Epstein-Barr virus-early antigen (EBV-EA) induced by 12-0-tetradecanoylphorbol-13-acetate (TPA). At a dosage of 100 g/ml,

the extract inhibited EBV-EA formation by 100% suggesting its antitumor promoting activity (Bennett *et al*., 2003).

2.7.11. Larvicidalactivities

Biological effects of the water extract of *Moringa oleifera* seeds (WEMOS) were assessed on eggs and 3rd instar larvae of *Aedes aegypti* and on its toxicity upon laboratory animals (Daphnia magna, mice and rats)(Dixon*et al*.,1984). Crude extract showed a LC50 value of 1260µg/ml, causing 99.2 ± 2.9% larvae mortality within 24 h at 5200µg/ml, though this larvicidal activity has been lost completely at 80◦C/10 min. WEMOS did not demonstrate capacity to prevent egg hatching. After extensive dialyses of the crude WEMOS into water soluble dialyzable (DF) and non dialyzable (NDF) fractions, only DF maintained its efficacy to kill larvae. Acute toxicity evaluations on daphnids (EC50 of 188.7µg/ml) and mice (LD50 of 446.5 mg/kg body weight) pointed out to low toxicity (Haristoy*et al*., 2005).

2.7.12. Antibiotic Activity

This is clearly the area in which the preponderance of evidence both classical scientific and extensive anecdotal evidence is overwhelming. The scientific evidence has now been available for over 50 years, although much of it is completely unknown to western scientists. Benzyl isothiocyanate was already understood at that time to have antimicrobial properties. This group not only identified pterygospermin, but performed extensive and elegant characterization of its mode of antimicrobial action in the mid 1950's (Jed, 2005).

Fahey (2005) identified a number of glycosylated derivatives of benzyl isothiocyanate (e.g.compounds containing the 6-carbon simple sugar, rhamnose). The identity of these compounds was not available in the refereed scientific literature until "rediscovered" 15years later by Asres (1995). They re isolated and confirmed the identity of 4 (aLrhamnopyranosyloxy) benzyl glucosinolate (Asres, 1995) and its cognate isothiocyanate and verified the activity of the latter compound against a wide range of bacteria and fungi (Abuye*et al*., 1999).

Extensive field reports and ecological studies forming part of a rich traditional medicine history, claim efficacy of leaf, seed, root, bark, and flowers against a variety of dermal and internal infections. Unfortunately, many of the reports of antibiotic efficacy in humans are not supported by placebo controlled, randomized clinical trials. Again, in kee ping with Western medical prejudices, practitioners may not be expected to embrace *Moringa* for its antibiotic properties. In this case, however, the in-vitro (bacterial cultures) and observational studies provide a very plausible mechanistic underpinning for the plethora of efficacy claims that have accumulated over the years (Jed, 2005).

Aware of the reported antibiotic activity of 4-(-L-rhamnopyranosyloxy) benzyl isothiocyanate, benzyl isothiocyanate, and other isothiocyanates and plants containing them, it was also determined whether some of them were also active as antibiotics against Helicobacter pylori. *H. pylori*are an omnipresent pathogen of human beings in medically underserved areas ofthe world, and amongst the poorest of poor populations worldwide. It is a major cause of gastritis, and of gastric and duodenal ulcers, and it is a major risk factor for gastric cancer (having been classified as a carcinogen by the WHO i n1993). Cultures of *H. pylori*, it turned out, were extraordinarily susceptible to 4 (L - rhamnopyranosyloxy) benzyl isothiocyanate, and to a number of other isothiocyanates (F ahey *et al*., 2002; Haristoy *et al*., 2005). These compounds had antibiotic activity against *H. pylori* at concentrations up to 1000-fold lower than those which had been used in earlier studies against a wide range of bacteria and fungi. The extension of this finding to human *H. pylori* infection is now being pursued in the clinic, and the prototypical isothiocyanate has already demonstrated some efficacy in pilot studies(Fahey, 2005).

2.7.13. Cancer Prevention

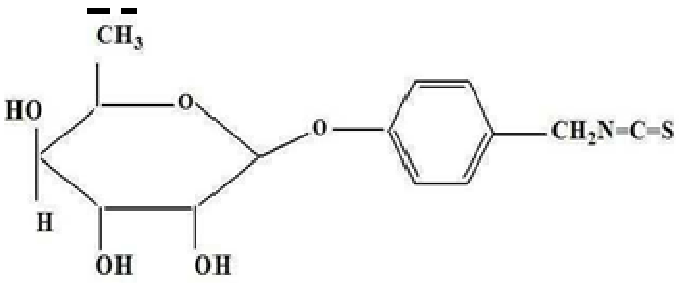
Since *Moringa* species have long been recognized by folk medicine practitioners as having value in tumor therapy (Hartwell, 1967), we examined compounds Oacetyl L rha mnopyranosyloxy) benzyl isothiocyanate and 4 (L rhamnopyranosyloxy) benzyl isothioc yanate for their cancer preventive potential. Recently, 4 (4' O acetyl L rhamnopyranosylo xy)benzyl isothiocyanate and the related compound niazimicin were shown to be potent inhibitors of phorbol ester (TPA) induced Epstein-Barr virus early antigen

activation in lymphoblastoid (Burkitt's lymphoma) cells (Murakami *et al*., 1998; Guevara *et al*., 1999). In one of these studies, niazimicin also inhibited tumor promotion in a mouse two-stage DMBA-TPA tumor model (Murakami *et al*., 1998). In an even more recent study, Bharali and colleagues have examined skin tumor prevention following ingestion of drumstick (Moringa seedpod) extracts. In this mouse model, which included appropriate positive and negative controls, a dramatic reduction in skin papilloma was demonstrated.

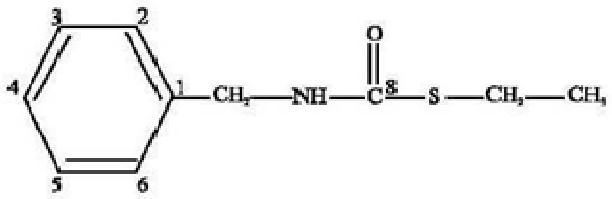
2.6. Phytochemistry

Phytochemicals are, in the strictest sense of the word, chemicals produced by plants. Commonly, though, the word refers to only those chemicals which may have an impact on health, or on flavor, texture, smell, or color of the plants, but are not required by humans as essential nutrients. An examination of the phytochemicals of *Moringa* species affords the opportunity to examine a range of fairly unique compounds. In particular, this plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolatesand isothiocyanates (Bennett *et al*., 2003; Fahey *et al*., 2001).

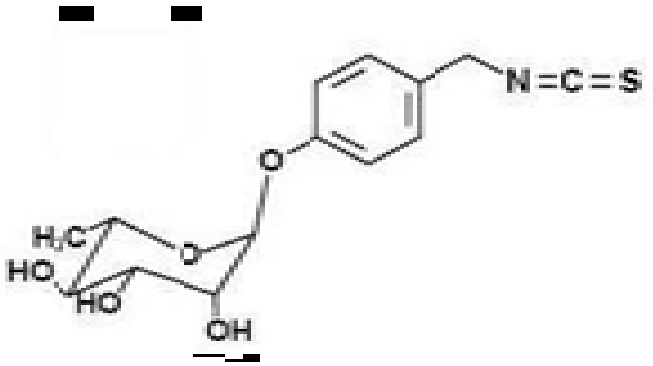
For example, specific components of *Moringa* preparations that have been reported to ha ve hypotensive, anticancer, and antibacterial activity include (fig 5) 4 (4'O acetyl a - rhamnopyranosyloxy) benzyl isothiocyanate (Abrams *et al*., 1993), 4 (aL rhamnopyranos yloxy) benzyl isothiocyanate (Abuye *et al*., 1999), niazimicin (Akhtar and Ahmad, 1995), pterygospermin (Anderson *et al*.,1986), benzyl isothiocyanate (Anwar and Bhanger, 200 3), and 4 (aL rhamnopyranosyloxy) benzyl glucosinolate (Asres, 1995).



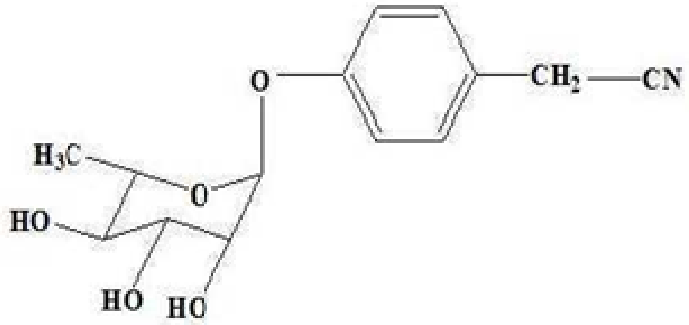
4-(a-L-rhamnosyloxy) benzyl isothiocyanate

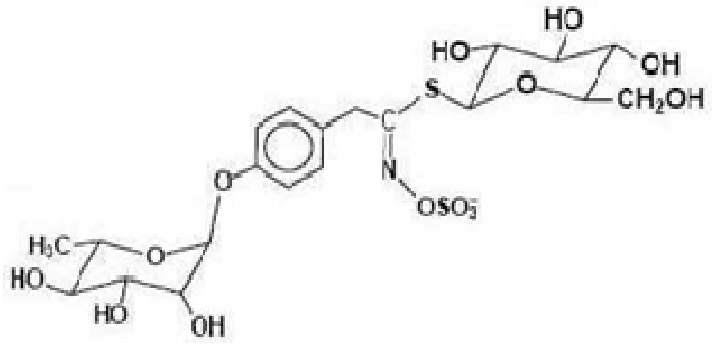


Aglycone of Deoxy-Niazimicine (N-benzyl, S-ethyl thioformate)

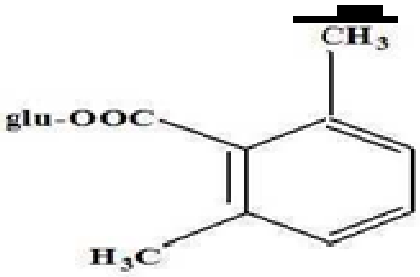


4-(4'-O-acetyl-a-L-rhamnopyranosyloxy) benzyl isothiocyanate





4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate



Moringyne

Figure 5. Structures of selected phytochemicals from *Moringa* spp: 4(4'O acetyl a Lrham nopyranosyloxy) benzyl isothiocyanate [1], 4(Lrhamnopyranosyloxy)benzyl isothiocyana te [2],niazimicin [3] pterygospermin [4], benzyl isothiocyanate [5], and 4 (aLrhamnopyra nosyloxy) benzyl glucosinolate [6].

Source:(USDA, 2003)

While these compounds are relatively unique to the *Moringa* family, it is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including b-carotene or pro-vitamin A). These attributes are all discussed extensively by Lockett and Calvert (2000)and others, and will be the subject of a future review in this series (Jed, 2005).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted at Debre Zeit city which is located 45kms south east of Addis Ababa. The area is located at 9°N latitude and 40°E longitudes at an altitude of 1850 meters above sea level. It has an annual rainfall of 866mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26°C and 14°C respectively with mean relatively humidity of 61.3% (EIAR, 2007).

Fig6. Map showing the study area

Source:(EIAR, 2007)

Fig7.*Moringa* farm – Debre Zeit, Ethiopian Institute of Agricultural Research (EIAR)

3.2. Collection, authentication and processing of plant materials

The fresh leaf of *M. oleifera* was collected from Debre Zeit, Ethiopian Institute of Agricultural Research (EIAR).The plant materials were identified and authenticated by a Botanist at the plant Science Department, Confirmation of taxonomic identity of the plants was achieved by comparison with voucher specimens kept at the Herbarium of the Department of plant Sciences, and use of documented literature from (Annex 5) (Cheesbrough, 2002).

3.3. Antimicrobial Disc preparation

Discs of about 6mm diameter were made from Whitman's No.1 filter paper using a paper puncher. Batches of 200 discs (minimum discs needed, 15x8=120) were transferred into Bijou bottles and sterilized in the oven at 121^{o}C for 15minutes.

3.4. Test microorganisms

Four bacterial strains were used in the study; among these were two Gramnegative, namely, *E. coli* (BUE1013), *Pseudomonas aeruginosa* (BUE1007), and twoGram positiv

e, namely, *Staphylococcus aureus* (BUE1002) and *S. fecalis* (BUE1003). All the tested strains are reference strains, and were collected from National Veterinary Institute (NVI). Bacteria were grown in nutrient agar medium and maintained on nutrient agar slants at 4°C.

3.5. Media for test organisms

27g of Muller Hinton Agar was added to 100ml of sterile distilled water and autoclaved at 121^0C for 15 minutes at 15lbs. 1.0g of dextrose was added to 10ml of sterile distilled water and steam sterilized for 15 minutes. After cooling both the content was mixed and poured into sterile petriplates approximately 4mm and allowed to set at ambient temperature and used.

3.6. Standardization of Inoculum

The inoculums were prepared from the stock cultures, which were maintained on nutrient agar slant at 4^OC and subculture onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determine d by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002).

3.7. Preparation of the extract

3.7.1. Preparation of aqueous extracts

i. Cold aqueous Extracts of Fresh and dried Leaves

Twenty five grams (25g) of fresh leaves of *M. olifera* Lam. were weighed out and crushed directly by grinder and was suspended into 100ml of cold distilled water in a conical flask and was covered with rubber cork and left for 7 days with occasional shaking. It was filtered off using sterile filter paper (Whitman no. 1) into another clean conical flask. The standard extracts obtained was then stored in the refrigerator at 4^oC for antibacterial activity test (Akueshi*et al*., 2002). In another case, the well air-dried fresh leaves was ground and 25g was weighed out and was dipped into 100ml cold distilled

water in a conical flask stoppered with rubber corks and was left for 7 days with occasional shaking. The other steps were the same as followed in case of cold water extracts of fresh leaves.

ii. Hot aqueous Extracts of Fresh and dried leaves

Here, the same step was used as in cold water treatment with 30-minutes boiling while plant material was dipped in distilled water.

3.7.2. Methanol extraction

The freshly collected leaves of *M.oleifera* were first washed with distilled water and air dried at 30±20^{0}C.Then dry in table drier under controlled condition and powdered. 50gm powdered plant leaf material was mixed with 200ml of methanol (50%).The mixtures were kept for 24hrs in tights sealed vessels at room temperature protected from the sun light and mixed several times with a sterile glass rod.This mixture wasfiltered through Whitman no.1 filter paper and the residue adjusted to the required concentration (50ml of methanol for the residue of 50g of powdered plant materials) with the extraction for fluid for the further extraction and it was repeated trice and clear colorless supernatant extraction liquid was finally obtained. The extracted liquid was subjected to rotary evaporation in order to remove the methanol. The semisolid extract produced was kept in a freezer at -80^{o}C overnight and then subjected to freezer drying for 24hr at -60^{o}C in 200ml vacuum. Then the extract was stored in air tight container at 4^{o}C in a refrigerator for further use.The concentrated extracts were labeled as MFLM(*Moringa*fresh leaf methanol extract) and MDLM(*Moringa*dried leave methanol extract). Similar procedure was followed for ethanol.

3.7.3. Powder from fresh leaf juice

One hundred grams of fresh leaves of *M. oleifera* Lam. were crushed directly by grinder without adding any solvent, and leaf juice was collected in a clean airtight bottle, and stored for antibacterial activity test. 8 ml of leaf juice were air-dried and 0.94g fine

powder was obtained, followed by dissolving in dimethyl sulfoxide (DMSO), and stored in airtight bottle for antibacterial activity test.

3.8. Antibacterial Assay

Antibacterial activity of the eight different samples; cold water extract of fresh leaves, hot water extract of fresh leaves, cold water extracts of dried leaves, hot water extracts of dried leaves, ethanol and methanol extracts of fresh leaves and dried leaves and powder from fresh leave juice were individually tested against the studied organisms. In vitro, antibacterial test was then carried out by disc diffusion method (Bauer, 1996; Barry, 1980) using 25µl of standardized suspension of tested bacteria (10^8cfu/ml) spread on Mueller-Hilton agar plates. The dried and sterile disc (6mm in diameter) impregnated with 0.1ml of standard plant extracts were placed on the seeded agar plates with the aid of sterile forceps. The plates were incubated at 37^0C for 24 hours. Antibacterial activity was evaluated by measuring in millimeter (mm) the zones of inhibition against the tested bacteria (Bauer, 1986).

DMSO was taken as control for all extracts. Sterile distilled water was taken a control for aqueous extracts. The dissolution of organic extracts (methanol) was aided by 1 % (V/V) DMSO and that of aqueous extracts with water which did not affect the growth of microorganisms in accordance with our control experiments(Bauer, 1986).

3.9. Minimum Inhibitory Concentration (MIC)

MIC of the different samples of the extracts of *M. oleifera* Lam was determined by two-fold serial dilution method. Five different concentrations of the samples were prepared, 30, 25, 20, 15 and 10 mg/ml. The above mentioned concentrations of the extracts were impregnated on 6mm size disc placed on sterile medium each containing test organisms and were incubated for 24 h at 37^oC. The lowest concentration of the crude extract that showed zone of inhibition is the Minimum Inhibitory Concentration.

3.10. Phytochemical tests

The analysis of phytochemicals from the solvent free extract of *Moringa olifera*was

individually performed using different quantitative tests for alkaloids, carbohydrates and glycosides, flavonoids, steroids, terpenoids, saponin, and tannin.

i. Test for alkaloids

The small portion of extract treated separately with a few drops of dilute hydrochloric acid and then filtered. The filtrate was treated with Mayers reagent and Dragendorffs reagent with the formation of creamy precipitate and orange brown precipitate respectively.

ii. Tests for carbohydrates and glycosides

Small amount of extract was dissolved separately in 5 ml of distilled water and then filtered. The filtrate may be subjected to Molischs test to detect the presence of carbohydrates. Another small portion of extract was hydrolyzed with dilute hydrochloric acid for few hours in water bath and was subjected to Liebermann-Burchards test to detect the presence of different glycosides. The pink to red colour indicated the presence of glycosides.

iii. Test for flavonoids

Five milliliter of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by the addition of concentrated sulfuric acid. A yellow coloration absorbed in extract indicated the presence of flavonoids.

iv. Test for steroids

Two ml acetic anhydride was added to 0.5 g different *M.oleifera* extract with 2 ml sulfuric acid. The change of colour from violet to blue indicated the presence of steroid.

v. Test for terpenoids

Five ml extract was mixed with 2 ml chloroform and 3 ml concentrated sulfuric acid was added carefully to form a layer. The formation of reddish brown colouration of the interface indicated the presence of terpenoids.

vi. Test for saponin

One ml extract and one ml alcohol diluted with 20 ml distilled water and shaken well for

15 minutes. The formation of 1 cm layer of foam indicated the presence of saponin.

vii. Test for tannin

Plant extract was treated with vanilline hydrochloric acid reagent. The formation of pinkish red colour indicated the presence of tannin.

viii. Test foranthraxquinones

To 1 mg of the extract, 2 ml of 25% ammonia solution was added and shaken. A cherish-red solution indicated the presence of emodols.

3.11. Cultivated values of *Moringa* powder extract of fresh leave juice

The bacterial cultivated values of *Moringa olifera* powder extract of fresh leave juice was determined by cultivating the four tested organisms on the culture medium which was prepared from the mixture of the above form of the plant extract with the proper concentrations of a solidifying agent synthetic agar powder in sterile distilled water.The culture medium were prepared from various concentrations of the plant extract(i.e. 10mg,20mg and 30mg) which were mixed with the average 25g weighing agar powder dissolved on 200ml distilled water. Before it was purred to the peteri plate each volume of the solutions were tested for their pH. Generally a total of 36 plates were prepared and each containing test organisms and were inocul-ated for 24 h at 37^{o}C.

3.12. Data Management and Analysis

Data were recorded and coded in Microsoft excel spread sheets before transferred to statistical software for analysis (Stata^{tm} 7.0 and SPSS 20).A one way analysis of variance was done to establish the presence or absence of variability between the antimicrobial activities of the four extracts namely: Aqueous, ethanol, methanol,fresh leaf juice and the control tetracycline. For individual chemical extracts of *Moringa oleifera*, were calculated as the mean value of the zone of inhibition obtained against all i ndividual bacteria. To measure the strength of the associationsbetween the growth of the organisms and the concentration of the plant extract used, univariable logistic regression

was applied to calculate Odds ratio. For all analysis, a P-value of less than 0.05 was taken as significant.

4. RESULTS

4.1. Antibacterial Assay

The antibacterial activity of the cold and hot water extract of fresh and dried leaves, ethanol and methanol extracts of fresh and dried leaves of *Moringaoleifera* are presented in table 5, 6 and 7. The antibacterial activity of the extract was greater againstgr am positive species (*S. aureus* and *S. faecalis*) than against gram-negative strains (*E. coli and P. aeruginosa*).

In the present study, Fresh leaf ethanol extract of *M. oleifera* was sensitive to three of the tested pathogens; *Streptococcus faecalis, Staphylococcus aureus and P. aeruginosa*, with varying degree of inhibition. Their individual diameter zones of inhibition were presented in table 5. Also, all the pathogens were sensitive to ethanol extracts of fresh and dried leaves with their respective diameter zones of inhibition as presented in the table 5, with the exception of *E. coli*and *P.aeruginosa*which showed no inhibition on the extract of the dried leaves.

The cold aqueous extracts of dried leaves exhibited antibacterial effect on *S. aureus* and*St reptococcus faecalis*, their diameter zones of inhibition were 9 and 8 mm respectively. This extracts was unable to inhibit the growth of *E. coli* and *P. aeruginosa*. Also, hot aqueous extracts of dried leaves displayed similar antibacterial effect on *S. aureus* and *Streptococcus faecalis,* and has no inhibition effect on *E. coli* and *P. aeruginosa*.

The methanol extract of *M.oleifera*was tested against two gram positive and gram negative bacterias and the extract was inhibitory to some of the tested pathogens with the respective diameter zone of inhibition as presented in table 6. The dried leaf of methanol extract was not inhibitory for *E.coli* and *P.aeruginosa*. It is also revealed that among the four tested pathogens,*S.auerus* was highly sensitive ($p<0.05$) for both form of the extract (fresh and dried) with the diameter zones of inhibition, 20±0.08 and 10±0.04mm

respectively. Generally in this form of extract DMSO(dimethylsulfoxide) and tetracycline were used as negative and positive control respectively.

The antibiogram activity of the fresh leave juice was also tested for the selected two gram negative and gram positive bacteria's and was revealed that both form of extract (Liquid and powder dissolved in DMSO) has no inhibitory effect on all of the tested pathogens).

In all cases, the activity of the extracts was compared with standard antibiotic tetracycline In this study, methanol and ethanol extract of fresh leaves exhibited higher antibacterial activity compared to tetracycline.

4.2. Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration of the different extracts against the tested bacterial pathogens isshown in table 9, 10, 11, 12 and 13 below. The fresh aqueous extract became inactive at concentration less than 20 mg/ml. The highest minimum inhibition co ncentration was 25mg/ml for *Staphylococcusaureus*, while it is 30 mg/ml for *S. faecalis*. The aqueous extract of both cold and hot water extract of fresh and dried leaves were also found to be susceptible to *S.auerus* and *S. faecalis* and their respective MIC values ranged from 20-25mg/ml.

The ethanol extracts of both the fresh and dried leaves have inhibitory activity against three of the tested pathogen; *S.auerus*, *S. faecalis* and *P.aeruginosa* and their MIC values ranged from 15- 25mg/ml.

The methanol extract of both the fresh and dried leaves have inhibitory activity against all of the tested organisms with their MIC value ranged from 15 30mg/ml. The highest mi nimum inhibitory concentration was 25mg/ml for *S.auerus*, while it is 15mg/ml for *S.fecalis*. The maximum inhibitory concentration was 30mg/ml for *S.aureus*.

Table 4. Antibacterial activity of various forms of extract of leaf of *M.oleifera* lams.against some animal pathogenic bacteria.

Name of Bacteria	Extraction Solvents			
	Aqueous	**Ethanol**	**Methanol**	**Fresh leaf Juice**
S.auerus	-/+	++	+++	-
S.faecalis	-/+	++	++	-
E.coli	-/+	-	-/+	-
P.aeruginosa	-/+	+	-/+	-

Key:

(-/+) variable under different forms of the extract

(-) not inhibitory (zero inhibition zone)

(+) mildly susceptible(inhibition zone <10mm)

(++) moderately sensitive (inhibition zone 10-20 mm)

(+++) highly Susceptibility (inhibition zone > 20 mm)

Table5. Antibacterial activity of ethanol extract of fresh and dried leaves of *M.oleifera*lam.against some animal pathogenic bacteria.

Name of Bacteria		Zones of inhibition (mm) Mean ±SE			LOS
		Ethanol extract of		Tetracycline (Standard)	
		Fresh leaf	Dried leaf		
Gram Positive	***S.auerus***	22 ±0.25	11 ±0.25	18 ±0.32	S
	S. fecalis	11 ±0.32	20 ±0.21	20 ±0.04	S
Gram Negative	***E.coli***	N	N	12 ±0.13	Ns
	P. aeruginosa	5 ±0.23	4 ±0.31	18.3 ±0.12	Ns

LOS level of significance, Ns =No significance (P>0.05)

S= Significant (P<0.05)

Table 6. Antibacterial activity of methanol extract of fresh and dried leaf of *M. oleifera* lams.against some animal pathogenic bacteria.

Name of Bacteria		Zones of inhibition (mm) Methanol extract means ±SE				LOS
		Fresh	Dried	DMSO	Tetracycline (Standard)	
Gram Positive	***S.auerus***	20 ±0.08	10 ±0.04	N	18 ±0.32	S
	S.fecalis	12 ±0.07	9 ±0.03	N	20 ±0.04	Ns
Gram Negative	***E.coli***	13 ±0.02	N	N	12 ±0.13	Ns
	P. aeruginosa	8 ±0.06	N	N	18.3 ±0.12	Ns

Data are presented as means ±SE, Value of triplicate experiment

Diameter of zone of inhibition including diameter of disc 6mm.

DMSO – Dimethylsulfoxide

LOS -level of significance, Ns =No significance (P>0.05)

S= Significant (P<0.05)

Table 7. Antibacterial activity of aqueous extract of fresh and dried leaves of *M.oleifera* lam against some animal pathogenic bacteria.

Name of Bacteria		Zone of inhibition (mm) aqueous extraction means ±SE						
		Fresh leave		Dried leave		Sterile distilled water	Tetracycline (Standard)	LOS
		Hot	Cold	Hot	Cold			
Gram Positive	***S.auerus***	8 ±0.02	10 ±0.28	4	3	N	18 ±0.32	Ns
	S.fecalis	9 ±0.04	8 ±0.29	3	5	N	20 ±0.04	Ns
Gram Negative	***E.coli***	N	N	N	N	N	12 ±0.13	Ns
	P.aeruginosa	N	N	N	N	N	18.3 ±0.12	Ns

Diameter of zone of inhibition including diameter of disc 6mm.

Data are presented as means ±SE,Value of triplicate experiment.

LOS =level of significance, Ns=No significance (P>0.05).

N = No inhibition

Table8.Antibacterial activity of fresh leave juice extract of *M.oleifera* lam against selected animal pathogenic bacteria.

Name of Bacteria		Zone of inhibition Mean(mm) ±SE			
		Fresh leaf juice			
		Liquid	Powder dissolved in DMSO	Tetracycline	DMSO
Gram Positive	***S.auerus***	N	N	18 ±0.32	N
	S.fecalis	N	N	20 ±0.04	N
Gram Negative	***E.coli***	N	N	12 ±0.13	N
	P.aeruginosa	N	N	18.3 ±0.12	N

N= no inhibition

DMSO = Dimethylsulfoxide

Table 9. Minimum inhibitory concentration of methanol extract of fresh and dried leaf of *M.oleifera* lams against some animal pathogenic bacteria.

Name of Bacteria		Minimum inhibitory concentration					
		Methanol extract of					
		Fresh leaf			Dried leaf		
		Min	Max	Mean	Min	Max	Mean
GramPositive	***S.auerus***	25	30	27.5	25	30	27.5
	S.fecalis	15	20	17.5	15	20	17.5
Gram Negative	***E.coli***	15	25	20	N	N	N
	P.aeruginosa	15	20	17.5	N	N	N

N =No inhibition recorded, Min=Minimum, Max =Maximum

Table10. Minimum inhibitory concentration of ethanol extract of fresh and dried leaf of *M.oleifera* lams against some animal pathogenic bacteria.

Name of Bacteria		Minimum inhibition concentration(mg/ml)					
		Ethanol extract of					
		Fresh leaf			Dried leaf		
		Min	Max	Mean	Min	Max	Mean
Gram Positive	***S.auerus***	25	30	27.5.	25	30	27.5
	S.fecalis	15	20	17.5	15	25	20
Gram Negative	***E.coli***	N	N	-	N	N	-
	P.aeruginosa	15	20	17.5	15	25	20

N= no inhibition,

"-" not determined

Table 11. Minimum inhibitory concentration of aqueous extract of fresh and dried leaf *M.olifera* lams against some animal pathogenic bacteria.

Name of Bacteria	Minimum inhibitory concentration (mg/ml)											
	Aqueous extract Fresh leafDried leaf											
	Hot			Cold			Hot			Cold		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
S.auerus	20	25	22.5	20	25	22.5	20	25	22.5	25	30	27.5
S.fecalis	25	30	27.5	25	30	27.5	25	30	27.5	25	30	27.5
E.coli	N	N	-	N	N	-	N	N	-	N	N	-
P.aeruginosa	N	N	_	N	N	_	N	N	_	N	N	_

N= no inhibition,

"-" not determined

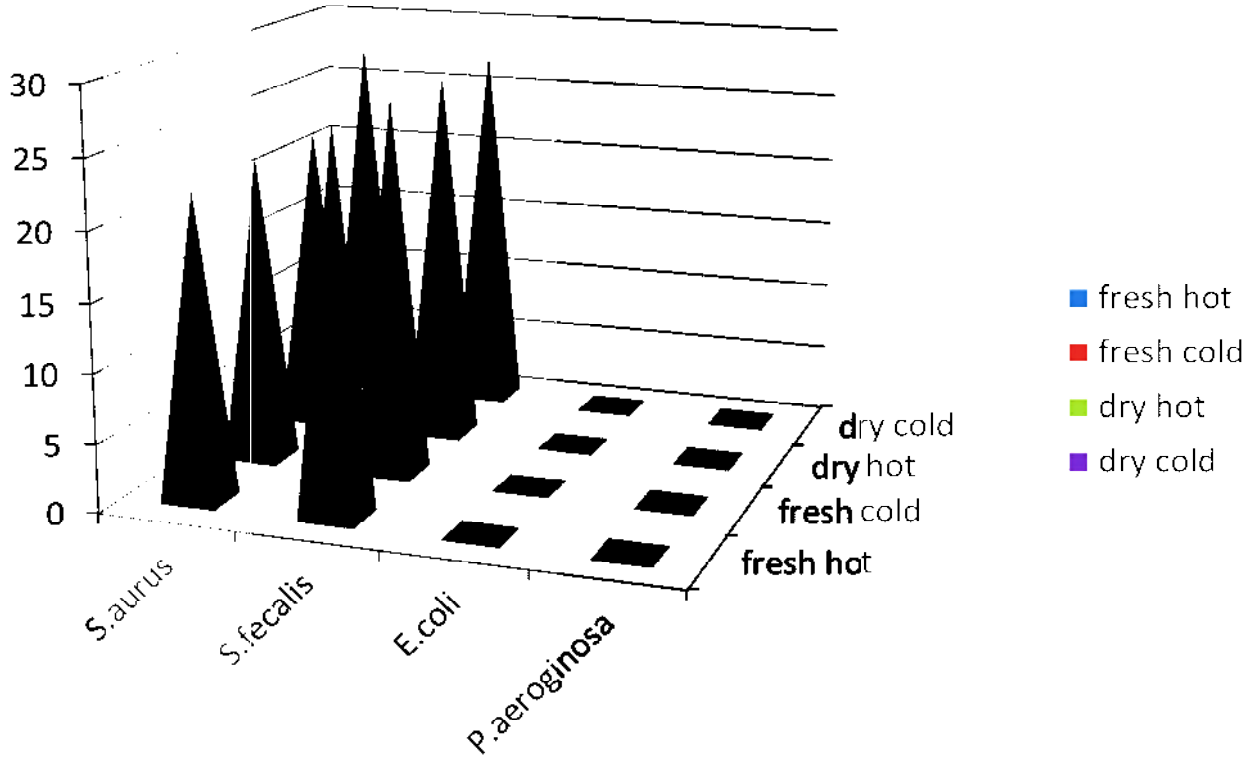


Fig8.Antimicrobial activity of *M. olifera* leaf (aqueous extract) against the tested species of bacteria (Values indicate the mean inhibition zone in mm).

Table12. Susubtability of the selected bacterial pathogens on ethanol and methanol extract of fresh and dried leaf of *M.oleifera* lam under different concentration.

Name of Bacteria		mg/ml	Inhibition zone in mm			
			Ethanol based extract		Methanol based extract	
			Fresh leaf	Dried leaf	Fresh leaf	Dried leaf
Gram Positive	*S.auerus*	10	-	-	-	-
		15	-	-	-	-
		20	-	-	-	-
		25	22	11	20	10
		30	24	13	21	11
	S.fecalis	10	-	-	-	-
		15	11	20	12	9
		20	20	21	13	11
		25	-	23	-	-
		30	-	-	-	-
Gram Negative	*E.coli*	10	-	-		
		15	-	-	13	-
		20	-	-	14	-
		25	-	-	15	-
		30	-	-	-	-
	P.aeruginosa	10	-	-		
		15	5	4	8	-
		20	9	6	9	-
		25	-	7	-	-
		30	-	-	-	-

Table 13. Susceptibility of the selected bacterial pathogens on aqueous extract of fresh and dried leaf of *M.olifera*lam. under different concentration.

Name of bacteria		mg/ml	Inhibition Zone in mm aqueous based dried leaf extract	
			Hot	Cold
Gram Positive	*S.auerus*	10	-	-
		15	-	-
		20	3	-
		25	3	3
		30	-	4
	S.fecalis	10	-	-
		15	-	-
		20	-	-
		25	3	5
		30	4	5
Gram Negative	*E.coli*	10	-	-
		15	-	-
		20	-	-
		25	-	-
		30	-	-
	P. aeruginosa	10	-	-
		15	-	-
		20	-	-
		25	-	-
		30	-	-

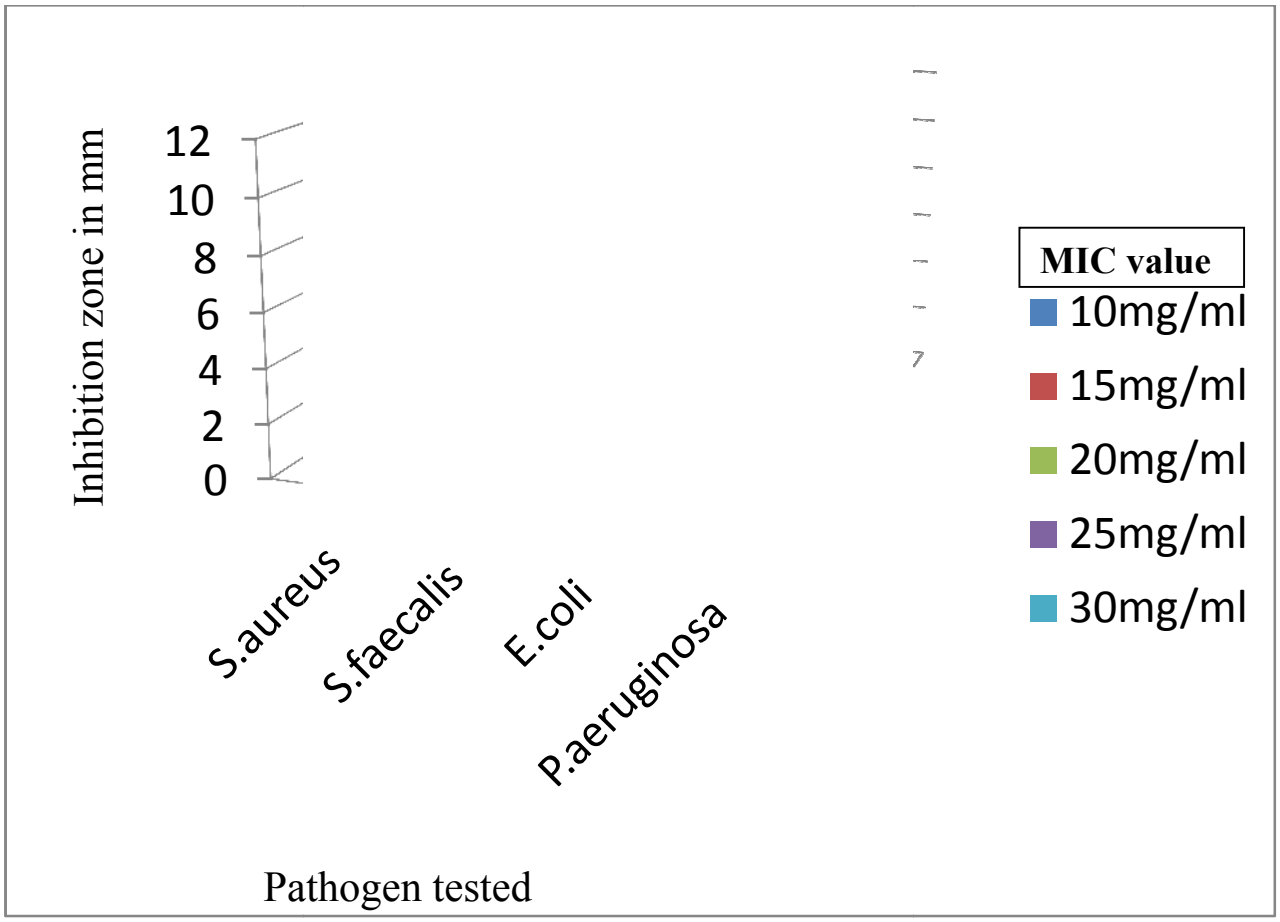


Fig9.Antimicrobial activity of *M.olifera*leaf (aqueous fresh hot leaf extract) against the tested species of bacteria (Values indicate the mean inhibition zone in mm, and the concentration tested in ml.

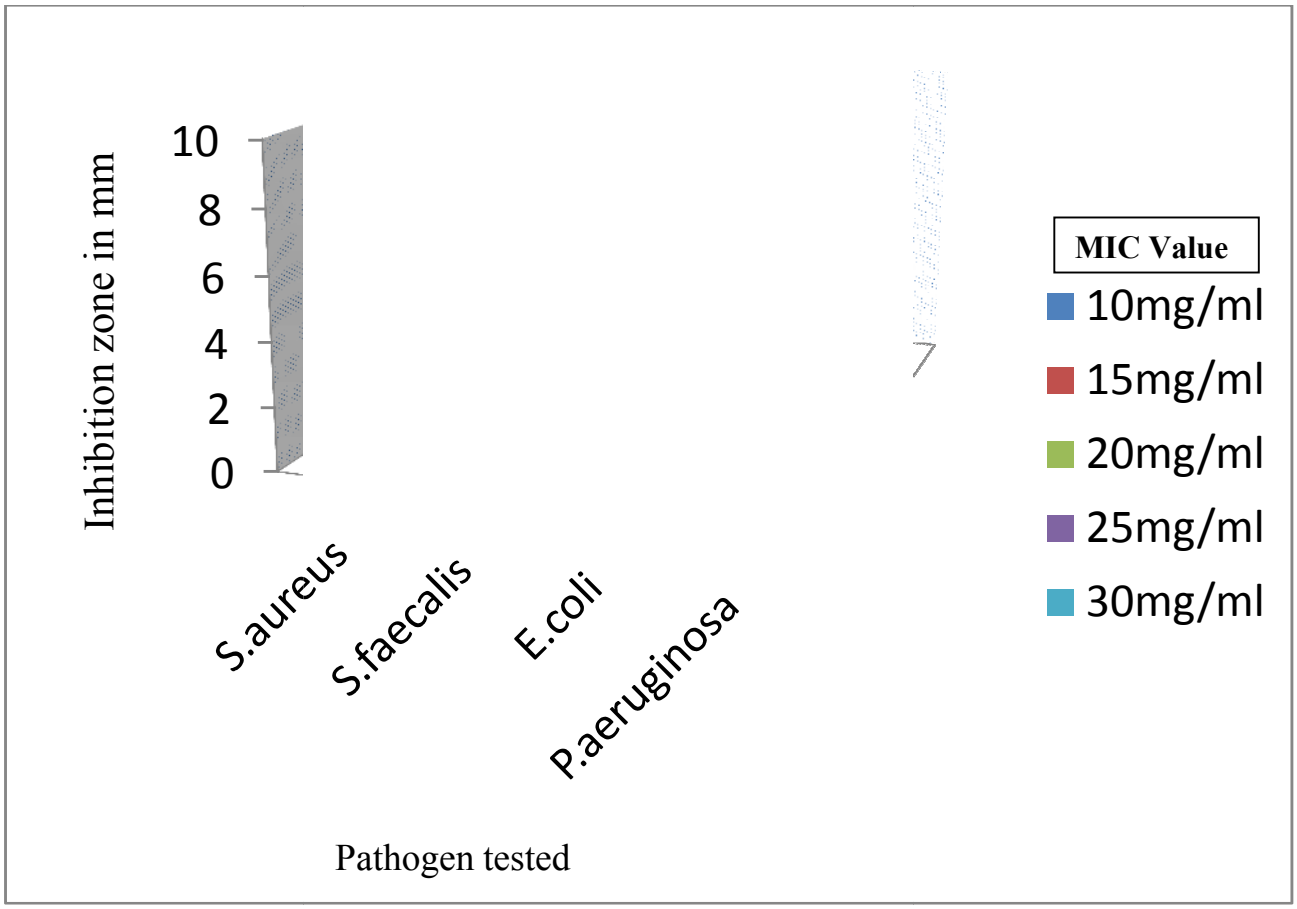


Fig10.Antimicrobial activity of *M.oleifera* leaf (aqueous fresh cold leaf extract) against the tested species of bacteria (Values indicate the mean inhibition zone in mm,and the concentration tested in ml).

Fig 11. Antimicrobial effect of 50% ethanol extracts of *M. oleifera* lam. fresh leaf extract

(The reference antibiotic disc used in this experiment was only Tetracycline which is represented as Kana).

4.4. Phytochemical analysis of *Moringa olifera* leaf extract

Phytochemical screening revealed that the phytochemical constituent of the plant varied on the method of extract used.Most of the phytochemical constituents were extracted on the organic solvents used as compared with water. Meanwhile, all of the bioactive constituents were absent in fresh leave juice extract (Table 14).

Table 14. Phytochemical analysis of *Moringa olifera* leaf extract

Name of Phytochemicals	***Moringa olifera* leaf extract**								
	Methanol		**Ethanol**		**Water**				**Fresh leaf Juice**
					Fresh leaf		**Dry leaf**		
	Fresh leaf	**Dry leaf**	**Fresh leaf**	**Dry leaf**	**Hot**	**cold**	**Hot**	**cold**	
Alkaloids	+	+	+	+	+	+	+	+	-
Glycosides	+	+	+	+	+	-	-	+	-
Flavonoids	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	-	+	-	-	-
Terpenoids	+	-	+	-	-	-	-	-	-
Saponin	+	-	+	-	+	+	-	-	-
Tannin	+	+	+	-	-	-	-	-	-
Foranthraxquinones	+	+	+	+	-	-	-	-	-

+ indicates presence or positive reaction, - indicates absence or negative reaction

4.5. Bacterial cultivated value of the fresh leaf juice powder extract of *M.oleifera*

Table14. Results obtained about the consistency of the medium

Amount of *M. olifera* powder used	Amount of agar mixed	Out put	P –value
10mg	5gm	-	0.25
	10gm	+	
	15gm	+	
	20gm	+	
15mg	5gm	-	0.21
	10gm	+	
	15gm	+	
	20gm	+	
20mg	5gm	-	0.31
	10gm	+	
	15gm	+	
	20gm	+	
25mg	5gm	-	0.32
	10gm	+	
	15gm	+	
	20gm	+	

Key: - not solidify

+ solidify

Data on table 14 provides information about the solidity and the texture of the culture medium which was prepared from the plant extract. In this experimentthe output indicated that the solidity was determined mainly with the agar powder and only the

culture mediums which were prepared at the concentration of 5gm agar powder mixed didn't solidify the medium among the four different concentrations were tested.

Table15. Ingredients, physico-chemical properties, cultivated value of the medium prepared from the fresh leaf juice powder form of extract.

Bacteria	Amount of *Moringa olifera* powder used	Amount of agar used	PH $[H^+]$	Cultivation temperature	Zone of inhibition	Tetracycline (Standard) (mm)	OR	95%CI of OR [Lower-Upper]
S.auerus	10mg	25gm[a]	6.6-7.00	33-37^{o}C	N	18 ±0.32	1	-
	15mg						2.1	0.22-2.44
	20mg						2.3	0.346-4.93
S.fecalis	10mg	25gm[a]	6.6-7.00	33-37^{o}C	N	20 ±0.04	1	-
	15mg						2.2	0.34-5.45
	20mg						2.4	0.12-6.56
E.coli	10mg	25gm[a]	6.6-7.00	33-37^{o}C	N	12 ±0.13	1	-
	15mg						2.3	0.34-4.56
	20mg						3.9	1.23-7.42
P.aeruginosa	10mg	25gm[a]	6.6-7.00	33-37^{o}C	N	18.3 ±0.12	1	-
	15mg						2.4	2.32-5.45
	20mg						4.5	0.11-7.34

N = no inhibition,mm = millimeter, gm. = gram,CI = Confidence Interval, mg = milligram, m[a] = mean

Data on table 15 provides information about the bacterial cultivated values of the culture medium which was prepared from *Moringa olifera*leaf juice powder

extract.From this experiment the output reveled that all of the tested pathogens were succ essfully and promptly grown with the temperature range of 35±2^{o}C at 6.8±0.2 optimum PHover the prepared medium. The appearances of the colonies where compared with their growth on nutrient agar medium and all do have similar characters (Fig 12).

Fig12. Media prepared from the *Moringa* leaf juice extract (showing the *E.coli*colonies)

5. DISCUSSION

5.1. Antibacterial Assay

The present study was conducted to obtain preliminary information on the anti-bacterial activity of the Fresh leaf juice, water, ethanol and methanol extract of *Moringa olifera* lam. leaf and the bacterial cultivation value of the extract depend on the anti-bio gram output of this experiment. The result obtained from this work indicated that all the extracts showed varying degrees of antimicrobial activity on the microorganisms tested.

The ethanol extracts of fresh and dried leaves have strong antibacterial activity in the growth of the organisms tested except on *E. coli* were it has non inhibitory effect as compared with the standardized control tetracycline ($p>0.05$). Ethanol extract of fresh leaf showed the extensive antibacterial effect against the tested Gram-negative bacteria except *E. coli* and Gram-positive bacteria (*S. auerus* and *S. fecalis*) and their respective diameter zones of inhibition were 5±0.23, 22±0.25, and 11±0.32mm respectively. The inhibitory effect of ethanol extracts of dried leaves was also noticed. This work is in agreement with Adesokan*et al*., 2007, who found the ethanol dried leave *Moringa* extract to be ineffective against *S. fecalis* isolated from clinical samples on the other hand, their extracts produced bacteriostatic and bactericidal effect on S. *aureus*. However, *Staphylococcus aureus*, and *Streptococcus*spp, has been reported to be less susceptible to plant extracts in earlier study conducted by other researchers (Kuhut*et al*., 1994; Afolayan and Meyer, 1995).

The hot and cold aqueous extract of the fresh and dried leaves showed appreciable antibacterial activity on *S. aureus* and *S. faecalis*, while it has no effect on *E.coli* and *P.aeruginosa*.The cold water extract of fresh leaves displayed a relatively better antibacterial effect against *S. fecalis* and *S. aureus*, with their individual diameter zones of inhibition recorded 8±0.25, 10.0±0.28 mm respectively. In earlier research done by other researchers, hot water extracts could not inhibit any organisms (Kuhut*et al.*, 1994 and Afolayan, 1995). But in this study, hot water extract of fresh and dried leaves were

able to inhibit two organisms: *Staphylococcus aureus*and *Streptococcus faecalis.*This result is interesting because in the traditional method of treating a bacterial infection, decoction of the plant part or boiling the plant in water is employed.

In this study, methanol and ethanol extract of fresh leaves exhibited higher antibacterial activity in the growth of Gram positive bacterias tested as compared to tetracycline. This is because methanol and ethanol can extract the active ingredient of the plant more than aqueous extracts. This corroborates the work of *Nair et al.* (2005) who had similar fi ndings. In the other direction, comparing the two form of extract, the methanol extract of fresh leave exhibited the broad antibacterial activity against all the tested bacteria (*S.auerus,S.fecalis,E.coli and P.aeruginosa*).

The activity of the plant against both Gram-positive and Gram negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds in the leaf of the plant (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007).

Though there was the remarkable inhibitory activity observed in some of the extracts, there was significant difference in the inhibition zones recorded in this and other previous researches. Farooq*et al.* (2007) reported that plants occur in varying habitats, a great magnitude of variation in the concentration and composition of phytochemical ingredients in the different parts of such plant is expected. Moreover, Yanaka*et al*. (2005) reported that phytochemicals are produced in response to perceived threats by the plants, therefore variation exist in the production of these phytochemicals depending on the type and amount of threat encountered by the plant.

Generally the antibacterial activity of the crude plant extracts on the test organisms justifies the active principle observed in herbal preparations and the antibacterial potential of the bioactive substance was highly comparable with that of antibiotic used in sensitivity test. Moreover, the activity of plants in the growth of the selected bacterias may be indicative of the presence of broad spectrum antibiotic compounds in the leaf of the plant. Thus, *M. oleifera* Lam. could become promising natural antimicrobial agents w ith potential applications in pharmaceutical industry for controlling the pathogenic

bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity should be taking into consideration.

Failure of some of the extracts to exert antibacterial effect on the test organism is not enough to conclude that the extract does not contain substances that can exert antibacterial activity against the test organism because the potency of the extract depends on the method used to obtain the extract (Unaeze, 1986). Research has also shown that the difference in antimicrobial properties of the plant extracts might be attributable to the age of the plant used, freshness of the plant material, physical factor (temperature, light or water), contamination by field microbes, and incorrect preparation of the plant etc. (Siddluraja and Becker, 2003; Vaghasiya and Chanda, 2007).

5.2. Minimum inhibitory concentration (MIC)

The aqueous extract of both the fresh and dried leaves were inactivated against *S.auerus* below 20mg/ml with the maximum inhibitory concentration of 25mg/ml except for dried leaves extracted which ranges 9-10mg/ml.In the other corner this form of extract becomes inactivated for *S. fecalis* below 25mg/ml with the maximum inhibitory concentr ation 30mg/ml. This work is in agreementwith the findings published by (Fahey, 2005) who reported the aqueous extracts of both the fresh and dried leaves to be effective against *S. fecalis* isolated from the animal sample with the maximum inhibitorco ncentrations of 35mg/ml.

The ethanol extracts of both the fresh and dried leaves have inhibitory activity against three of the tested pathogens:*S.auerus,S. fecalis*and *P.aeruginosa* and their MIC values were in agreement with the findings published by (Fahey, 2005) who reported the MIC values for *S. auerus* and *S.fecalis* which were isolated from clinical samples with the maximum inhibitory concentrations of 30mg/ml.

The methanol extract of the fresh leaf have inhibitory activity against all of the tested organisms with the lowest range of MIC value as compared with the other forms of extract. This corroborates the work of Akueshi*et al*. (2002)who had similar findings.

5.3.Phytochemical analysis of *Moringaoleifera* leaf extract

Phytochemically, the study revealed the presence of certain phytoconstituents. Alkaloids and glycosides were the dominant from of the extract obtained in this research.Meanwhile, several authors have characterized and reported several chemical compounds present in the leaf. Faizi*et al.* (1994) reported the isolation of two nitrile glycosides from the ethanolic extracts of *M. oleifera* leaf, niazirin and niazirinin and three mustard oil glycosides, 4-[(4'-Oacetylalpha-L- rhamnosyloxy) benzyl] isothiocyanate, niaziminin A, and niaziminin. Faizi*et al*. (1995) isolated six new glycosides and three synthetically known phenolics from the leaf of *M. olifera.* Most of the glycosides were, bearing thiocarbamate, carbamate or nitrile groups, are fully acetylated glycosides, which are very rare in nature.

Tannin, saponinand terpenoids were isolated from the organic solvent extract.This corroborates the work of Murakami *et al.* (1998) who had isolated tannin and a terpenoids from the leaves of *M. olifera.* Bennett *et al.* (2003) also isolated various glucosinolates,saponin and steroids compounds from various parts of *M. olifera*.

5.4. Bacterial cultivated value of the fresh leaf juice powder extract of *M.oleifera*

The strain *E.coli,S.auerus,S.fecalis* and *P.aeruginosa* all were resistant to the powder and liquid form of the *Moginga* fresh leave juice extract.The observed resistance matches findings from a study on the antibacterial properties of Indian*Moringa*plants showing this form of *Moringa* extract are ineffective against *E.coli* and *P.aeruginosa*. Fahey (2005) also reported that *S.auerus* to be resistant to this form of the extract .The author attributed the resistance to the absence of the saponin, tannic, phenolic and alkaloid phyto-

constitutions. However, (Bauer *et al*., 1986) observed inhibition halos up to 8 mm when cultivating *S.fecalis* with this form of extract which is in disagreement with this study.

The susceptibility profile of the tested pathogens over the fresh leave juice of the plant extract open the door for further investigation on this plant in assessing the bacterialcultivated value for the selected pathogens.

The media was nearly similar physical appearance and chemical state with nutrient agar media except some minor difference like the color and odor of the media. This difference may be due to the phytochemical constituents of the plant extract.

The result obtained from this experiment indicated that all the tested pathogen (*S.auerus*, *S.fecalis, E. coli and P. aeruginosa)* were promptly and successfully grown on the culture medium prepared. The primary and secondary cultured also revealed that the form of growth (the appearance of colonies) didn't vary across the different concentrations of agar and plant powder was tested, however denser colonies were observed at higher concentration (25mg) of the plant powder used. This is may be due to the difference in the amount of the nutrient available in the different concentration of the extract. The two pathogens *E. coli* and *P. aeruginosa* were grown on the medium whi ch was prepared at the concentration of 20mg (OR = 5.9 and 4.5) had a higher odd for growth as compared with reaming two gram positive bacteria (*S.auerus* and *S.fecalis*) with the OR of 2.3 and 2.4 respectively.

6. CONCLUSIONS AND RECOMMENDATIONS

In recent times the use of plants as a source of novel compounds to combat microbial infections has gained prominence. The necessity to search for plant based antimicrobial is increasing due to high cost, reduced efficacy and increased resistance to conventional medicines. Various findings suggest a new pathway in elucidating a potent antimicrobial agent from *Moringa olifera*. Generally though the active principle of the plant were not assessed under this research the result indicated that the active principle of the plant which were inhibitory in the growth of the tested pathogens could become the promising natural antimicrobial agents with the potential applications in pharmaceutical industri-es for controlling the pathogenic bacteria. In the contrary the powder form of the plant extract which was derived from fresh leave juice lacks those active principles which are of inhibitory for the growth of the tested pathogens, but the nutritive value of the extract was safe for the growth of the tested bacteria which brings to an idea of the medium may do have replace role of the synthetic general purpose medium.

Thus, based on the above concluding remarks, the following recommendable points are forwarded:

- ✓ Awareness should be created in the traditional use of the plant in the treatment of different infections in the area.
- ✓ People ought to be advised on possible way of cultivation of the plant.
- ✓ Since this plant naturally occurs in varying habitats, it is naïve to expect a great magnitude of variation in the concentration and composition of chemical ingredients in different parts of the tree. However, the extent to which the chemical composition varies in the plant adapted to varying habitats is not known. Thus, detailed studies are required to examine this aspect.
- ✓ Further studies should be conducted to assess the antibacterial activities of *M. olifera* extracts other than the selected pathogens using in vitro antibacterial screening techniques.

- ✓ *Moringa stenopetala*is the other species of the plant which is found in Ethiopia and Kenya so that the antimicrobial value of this plant should be evaluated just in comparison with the *M.olifera.*
- ✓ Investigation should be undertaken toidentify the possible factors affecting the antimicrobial effect of the plant extract.
- ✓ It should be assess the antimicrobial effect of other parts of the plant like: ponds, roots, seeds etc.

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8. ANNEXES

Annex1:Composition and preparation of media used for the study

Mueller Hinton Agar (Oxoid, ® Hampshire, England).

Typical Formula gm. /liter

Beef, dehydrated infusion 300

Casein hydro lysate 17.5

Starch 1.5

Agar 17

PH 7.3 ± 0.1 @ 25°C

Directions

Add 38g to 1 liter of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

Nutrient Broth Culture

1.Light your Bunsen burner.

2.In one hand hold both the stock culture and the broth culture to be inoculated. Loosen the tube caps

3.In your other hand hold the inoculating loop.

4.Flame the inoculating loop to redness by holding it pointed down into the flame, starting near the handle and then moving the loop into the flame. This technique sterilizes the loop and, if wet with a culture, heats up the loop without spattering bacteria into the air and onto the surrounding area.

5.Let the loop cool a minute. A hot loop will damage the bacteria cells.

6.Using the fingers of the "loop hand" remove the cap from the stock culture tube and flame the tube mouth.

7.Insert the cooled sterilized loop into the culture tube being careful to not touch the sides of the tube. Touch the loop to the culture. You need not scrape a visible amount from the culture. Hold the tube as horizontal as possible to preclude particles from the air settling into the tube BUT do watch out for any condensate in the bottom of slant cultures.

8.Remove the loop being careful again to not touch the tube sides.

9.Flame the tube mouth and replace the cap.

10. Remove the cap of the broth tube. Flame the top. Remember to hold the top in your fingers.

11. Insert the loop into the broth and shake to remove the bacteria.

12. Withdraw the loop, flame the tube mouth and replace the cap.

13. Resterilizes the inoculating loop and places it on the table.

14. Return the stock culture to the rack or holder.

15. Gently shake your broth culture. Label it with: today's date, microbe's name, and your last name & section letter. The label should be placed so that it will not be in the way of later observations.

Inoculate two broths, one each from the two different stock cultures provided. Incubate your broth cultures as directed.

Nutrient Agar Slant Culture

1.Hold the tubes, flame the tops, and transfer the culture as outlined for the broth culture. Use the inoculating needle instead of the loop. Remember to sterilize it before and after each use.

2.Inoculate your slant by moving the needle gently up the surface of the agar in a snake-like fashion. Be careful to not gouge the agar surface. If there is any liquid in the bottom of the slant tubes avoid sticking the needle into this condensate.

3.Label it with: date, microbe's name, your last name & section letter. The label should be placed so that it will not be in the way of later observations.

Annex2:*Moringa olifera* leave extracts

Harvested *moringa olifera* leave

Hot aqueousextract of *Moringa olifera*leave

A B

Cold aqueous extract of Fresh and dried leave (A &B) of *M.oleifera*


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Moringa olifera leave powder Extract

Fresh leave juice extract of *M. oleifera* lam.

Annex 3: Kirby-Bauer disk diffusion susceptibility test protocol

Kirby-Bauer disk diffusion susceptibility test protocol

Purpose

The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds in order to assist a physician in selecting treatment options for his or her patients. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.

Determination of bacterial resistance to antimicrobials is an important part of the management of infections in patients. The disk diffusion method of Kirby and Bauer has been standardized and is a viable alternative to broth dilution methods for laboratories without the resources to utilize the newer automated methods for broth micro dilution testing.

When a 6-mm filter paper disk impregnated with a known concentration of an

antimicrobial compound is placed on a Mueller-Hinton (MH) agar plate, immediately water is absorbed into the disk from the agar. The antimicrobial begins to diffuse into the surrounding agar. The rate of diffusion through the agar is not as rapid as the rate of extraction of the antimicrobial out of the disk, therefore the concentration of antimicrobial is highest closest to the disk and a logarithmic reduction in concentration occurs as the distance from the disk increases. The rate of diffusion of the antimicrobial through the agar is dependent on the diffusion and solubility properties of the drug in MH agar (2) and the molecular weight of the antimicrobial compound. Larger molecules will diffuse at a slower rate than lower molecular weight compounds. These factors, in co mbination, result in each antimicrobial having a unique breakpoint zone size indicating sus ceptibility to that antimicrobial compound.

If the agar plate has been inoculated with a suspension of the pathogen to be tested prior to the placing of disks on the agar surface, simultaneous growth of the bacteria and diffusion of the antimicrobial compounds occurs. Growth occurs in the presence of an antimicrobial compound when the bacteria reach a critical mass and can overpower the inhibitory effects of the antimicrobial compound. The estimated time of a bacterial suspension to reach critical mass is 4 to 10 hours for most commonly recovered pathogens, but is characteristic of each species, and influenced by the media and incubation temperature. The size of the zone of inhibition of growth is influenced by the depth of the agar, since the antimicrobial diffuses in three dimensions, thus a shallow layer of agar will produce a larger zone of inhibition than a deeper layer.

The point at which critical mass is reached is demonstrated by a sharply marginated circle of bacterial growth around the disk. The concentration of antimicrobial compound at this margin is called the critical concentration and is approximately equal to the minimum inhibitory concentration obtained in broth dilution susceptibility tests.

Zone size observed in a disk diffusion test has no meaning in and of itself. The interpretation of resistance and susceptibility to antimicrobials is determined through in

vivo testing of blood and urine to calculate the obtainable level of a given antimicrobial that results in resolution of an infection. This information is correlated with zone sizes resulting in the interpretive standards.

Use of the McFarland standard in the Kirby-Bauer procedure.

1. Prior to use, vigorously agitate the barium sulfate standard on a mechanical vortex mixer and inspect for a uniformly turbid appearance. Replace the standard if large particles appear. If using a standard composed of latex particles, mix by inverting gently, not on a vortex mixer.

2. As the student adds bacterial colonies to the saline in the "preparation of the inoculum" step of the procedure, he or she should compare the resulting suspension to the McFarland standard. This is done by holding both the standard and the inoculum tube side by side and no more than 1 inch from the face of the Wickersham card (with adequate light present) and comparing the appearance of the lines through both suspensions. Do not hold the tubes flush against the card. If the bacterial suspension appears lighter than the 0.5 McFarland standards, more organisms should be added to the tube from the culture plate. If the suspension appears denser than the 0.5 McFarland standards, additional saline should be added to the inoculum tube in order to dilute the suspension to the appropriate density. In some cases it may be easier to start over rather than to continue to dilute a bacterial suspension that is too dense for use.

Protocol on Preparation of Mueller-Hinton plate

1. Allow a MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.
2. If the surface of the agar has visible liquid present, set the plate inverted, ajar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 35°C incubator or
in a laminar flow hood at room temperature until dry (usually 10 to 30 minutes).

3. Appropriately label each MH agar plate for each organism to be tested.

Preparation of inoculum

1. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.
2. Suspend the organism in 2 ml of sterile saline and Vortex the saline tube to create a smooth suspension.

4. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.

5. Use this suspension within 15 minutes of preparation.

Inoculum preparation

Organisms to be tested must be in the log phase of growth in order for results to be valid. It is recommended that subcultures of the organisms to be tested be made the previous day. Never use extremes in inoculum density. Never use undiluted overnight broth cultures or other unstandardized inocula for inoculating plates. If the organism is difficult to suspend directly into a smooth suspension, the growth method of preparing the inoculums should be used. However, the recommended organisms listed in this procedure all produce smooth suspensions with little difficulty.

Inoculation of the MH plate

1. Dip a sterile swab into the inoculum tube.
2. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet
3. Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum
4. Rim the plate with the swab to pick up any excess liquid

5. Discard the swab into an appropriate container.

6. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

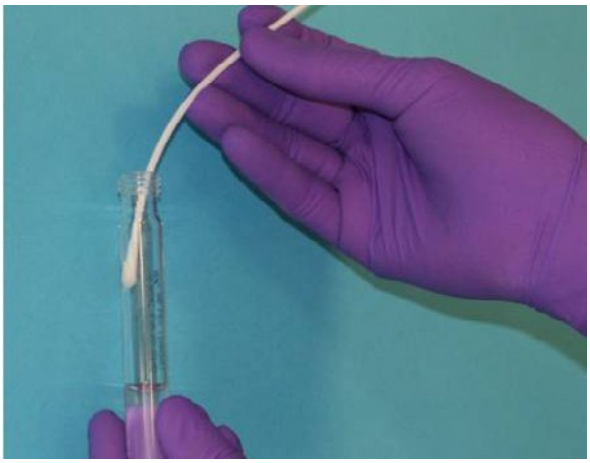
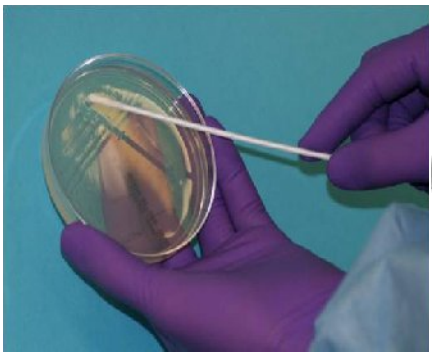
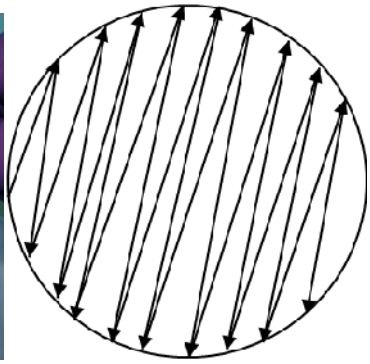


Fig 46. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the test plate. Step 2. Rotate the swab against the side of the tube while applying pressure to remove excess liquid from the swab prior to inoculating the plate.





A B

Fig 47. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the Mueller-Hinton agar plate. Step 3. (A) Inoculate the plate with the test organism by streaking the

swab in a back-and-forth motion very close together as you move across and down the plate. Rotate the plate 60° and repeat this action. Rotate the plate once more and repeat the streaking action. This ensures an even distribution of inoculum that will result in a confluent lawn of growth. (B) Diagram illustrating the pattern the swab should follow as i t is drawn across the plate.

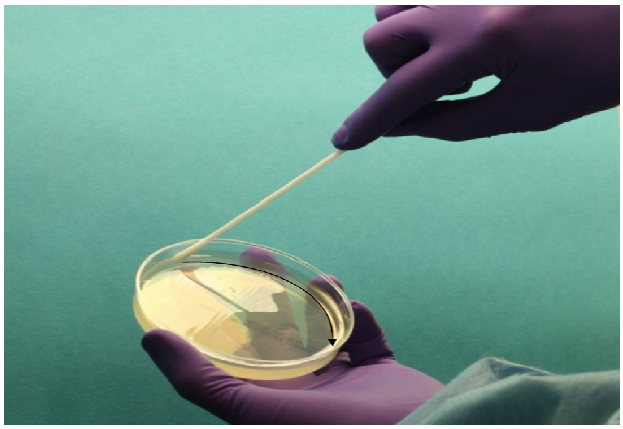


Fig 48. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the Mueller-Hinton agar plate. After streaking the Mueller-Hinton agar plate as described in Step 3, rim the plate with the swab by running the swab around the edge of the entire the plate to pick up any excessive inoculum that may have been splashed near the edge. The arrow indicates the path of the swab.

Placement of the antibiotic disks

1. Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multi disk dispenser to dispense multiple disks at one time. (See steps a. through d. for the use of the multi-disk dispenser or steps e. through g. for individual disk placement with forceps.

a. To use a multi disk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid.

b. Place the dispenser over the agar plate and firmly press the plunger once to dispense the

disks onto the surface of the plate.

c. Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petri dish lid as static electricity may

cause the disks to relocate themselves on the agar surface or adhere to the lid.

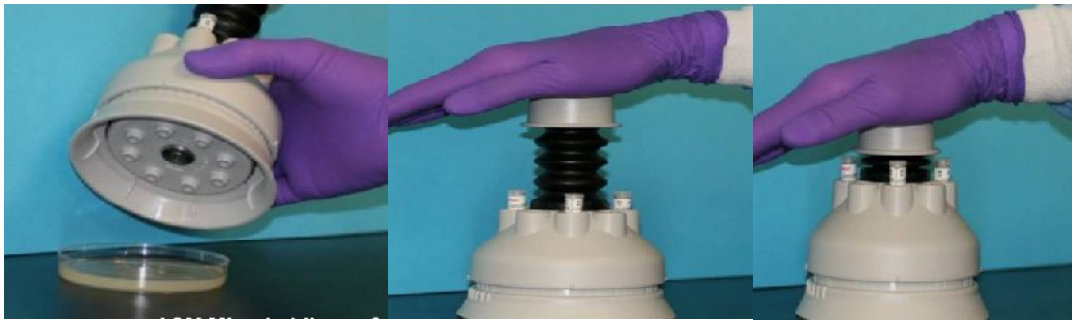
d. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact w ith the agar. To add disks one at a time to the agar plate using forceps, place the MH plate on the template provided in this procedure. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.

f. Using the forceps carefully removes one disk from the cartridge.

g. Partially remove the lid of the petri dish. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air.

h. Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in steps f. and g. above.

2. Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours.

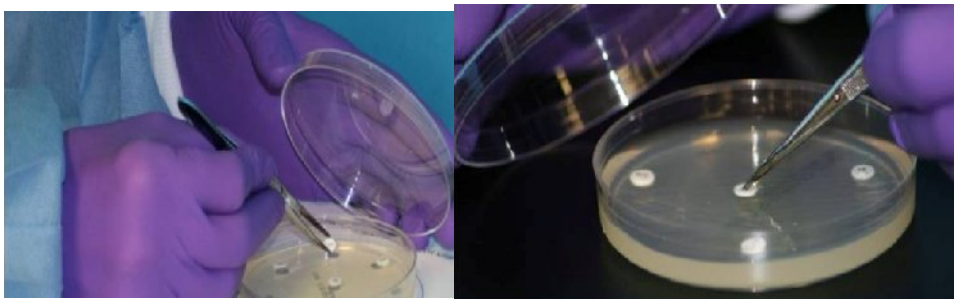


A B C

Fig 49. Kirby-Bauer disk diffusion susceptibility test protocol, placement of antibiotic disks

using an automated disk dispenser. Step1, a. through d. An automatic disk dispenser can be used to place multiple disks simultaneously on a MH agar plate. Set the dispenser over the plate. (B) Place the palm of your hand on the top of the handle. (C) Press down firmly and completely to dispense the disks. The spring loaded handle will return to the original position when pressure is removed.

A B



C D

Fig 50. Kirby-Bauer disk diffusion susceptibility test protocol, placement of antibiotic disks using forceps to manually place the disks. Step 1, e. through h. Antibiotic disks can be manually placed on the MH agar plate if desired. (A) Place the Mueller-Hinton agar plate over the disk template. (B) Remove one disk from the cartridge using forceps that have been sterilized. (C) Lift the lid of the plate and place the disk over one of the positioning marks. (D) Press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid of the plate between disks to minimize exposure to air-borne contaminants.

Table 1. Zone diameter interpretative standards for *Staphylococcus* species

***Staphylococcus* species**
(Zone Diameter, nearest whole mm)

	Resistant	Intermediate	Susceptible
Cefazolin (30 µg)	≤14	15-17	≥18
Clindamycin (2 µg)	≤14	15-20	≥21
Erythromycin (15 µg)	≤13	14-22	≥23
Gentamicin (10 µg)	≤12	13-14	≥15
Oxacillin (1 µg)	≤10	11-12	≥13
Penicillin G (10 µg)	≤28	--	≥29
Tobramycin (10 µg)	≤12	13-14	≥15
Vancomycin (30 µg)	--	--	≥15

Table2.Zone diameter interpretative standards for Pseudomonas aeruginosa and other non-fermenting gram-negative rods

***Pseudomonas aeruginosa* and other non-fermenting Gram Negative Rods (Zone Diameter, nearest whole mm)**

	Resistant	Intermediate	Susceptible
Amikacin (30 µg)	≤14	15-16	≥17
Cefoperazone (75 µg)	≤15	16-20	≥21
Cefotaxime (30 µg)	≤14	15-22	≥23
Gentamicin (10 µg)	≤12	13-14	≥15
Piperacillin (100 µg)	≤17	--	≥18
Tetracycline (30 µg)	≤14	15-18	≥19
Ticarcillin (75 µg)	≤14	--	≥15
Tobramycin (10 µg)	≤12	13-14	≥15

Table 3. Zone diameter interpretative standards for *E. coli* and other enteric gram-negative rods

***E. coli* and other enteric Gram Negative Rods**
(Zone Diameter, nearest whole mm)

	Resistant	Intermediate	Susceptible
Amikacin (30 µg)	≤14	15-16	≥17
Ampicillin(10 µg)	≤13	14-16	≥17
Cefazolin (30 µg)	≤14	15-17	≥18
Gentamicin (10 µg)	≤12	13-14	≥15
Tetracycline (30 µg)	≤14	15-18	≥19
Ticarcillin (75 µg)	≤14	15-19	≥20
Trimethoprim (5 µg)	≤10	11-15	≥16
Tobramycin (10 µg)	≤12	13-14	≥15

Annex4: Preparation of McFarland Standards

Purpose

McFarland Standards are turbidity standards that are used to gauge approximately how many bacteria are present in a liquid suspension. The standards are used to visually compare the turbidity of a suspension of bacteria with the turbidity of the appropriate standard. Standards are prepared by adding barium chloride to sulfuric acid to obtain a barium precipitate. The volumes of the two reagents are adjusted to prepare standards of different turbidity that represent different concentrations of bacteria.

Reagents

1. Sulfuric acid, 1%
2. Barium Chloride, 1.175%

Supplies

1. Acid washed glass screw-cap tubes comparable to that used for test
2. Sterile serological pipettes and pipette bulb
3.Para film or paraffin

Equipment

1.100ml volumetric flasks
2. Vortex
3. Spectrophotometer
4. Magnetic stirrer and stirring rod

Procedure for the Preparation of a 0.5 McFarland Standard

1. Add approximately 85 ml of 1% sulfuric acid (H_2SO_4) to a 100ml volumetric flask.
2. Using a volumetric pipette, add 0.5ml of 1.175% anhydrous barium chloride ($BaCl_2$) drop wise to the 1% sulfuric acid (H_2SO_4)while constantly swirling the flask.

3. Bring the volume to 100ml with 1% H_2SO_4.

4. Stir or mix for approximately 3 to 5 minutes while examining visually, until the solution appears homogeneous and free of clumps. A magnetic stirrer can be used for this step if available.

5. Check optical density.

6. If the optical density is acceptable, dispense 2 to 7 ml volumes (depending on volumes routinely used in test) into each glass screw- cap tube.

7. Label the tubes appropriately including the expiration date and the initials of the person preparing the standards. Make sure that the labeling is positioned so that it does no t interfere with spectrophotometer readings.

8. Cap the tubes tightly.

9. Draw a line to mark the meniscus on each tube. This mark can be used as a guide to check for evaporation at a later time.

10. Seal the tubes with paraffin or Para film.

11. Store the prepared standards in the dark at room temperature for 3 months or longer as per QC acceptability.

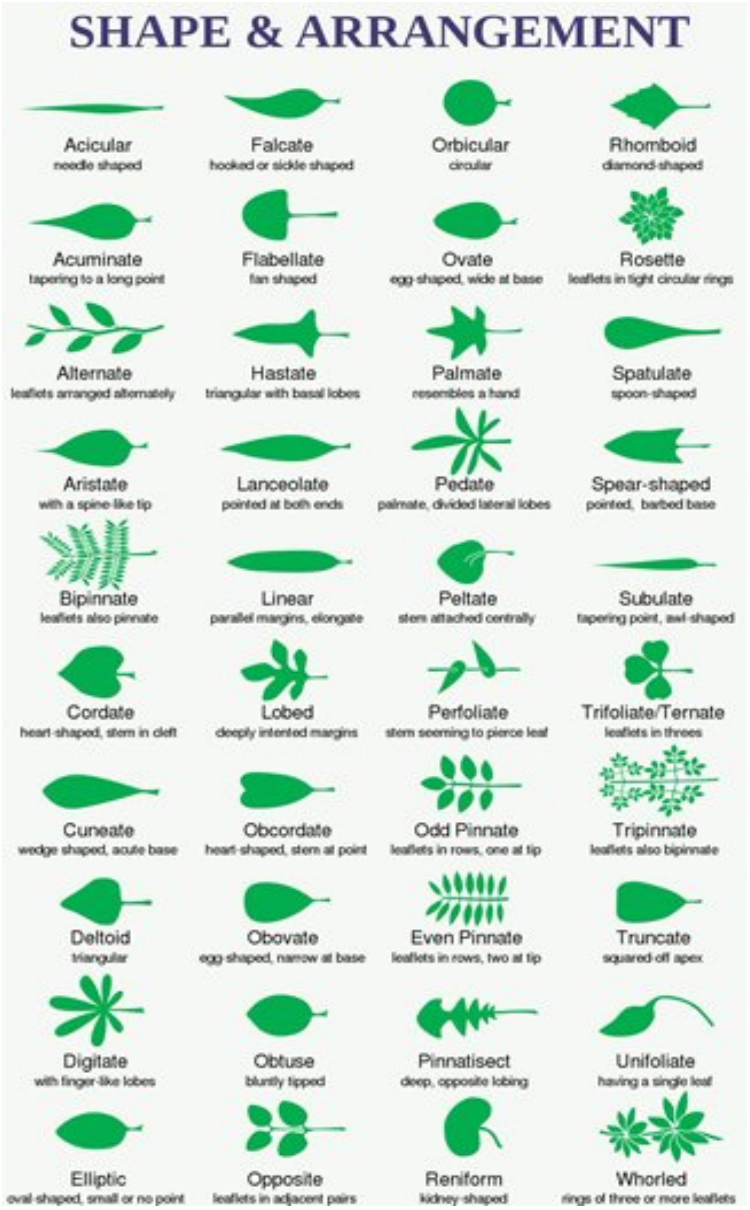
Guide for the Preparation of McFarland Standards

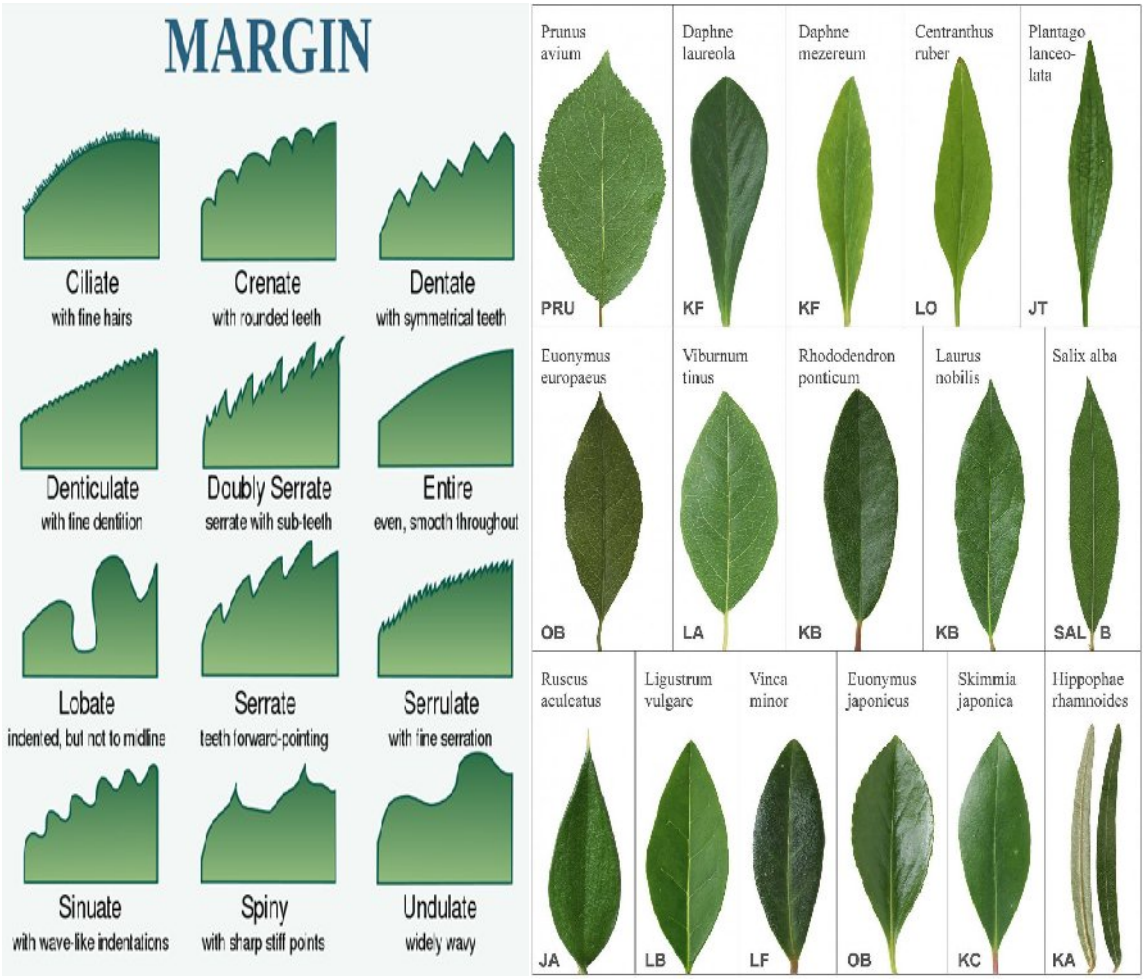
Volume in mL			
Standard	**1% $BaCL_2$**	**1% H_2SO_4**	**Number of Bacteria/ mL/(10^8) represented**
0.5	0.5	99.5	1.5
1	1.0	99.0	3
2	2.0	98.0	6
3	3.0	97.0	9
4	4.0	96.0	12
5	5.0	95.0	15
6	6.0	94.0	18
7	7.0	93.0	21

8	8.0	92.0	24
9	9.0	91.0	27
10	10.0	90.0	30

Annex 5.Identification and characterization *Moringa olifera* lam. Leaves

The leave of *M.oleifera* was determined by looking the Margin, shape, arrangement, venation etc.as described in (Cheesbrough, 2002).


SHAPE & ARRANGEMENT
Acicular
needle shaped
Falcate
hooked or sickle shaped
Orbicular
circular
Rhomboid
diamond-shaped
Acuminate
tapering to a long point
Flabellate
fan shaped
Ovate
egg-shaped, wide at base
Rosette
leaflets in tight circular rings
Alternate
leaflets arranged alternately
Hastate
triangular with basal lobes
Palmate
resembles a hand
Spatulate
spoon-shaped
Aristate
with a spine-like tip
Lanceolate
pointed at both ends
Pedate
palmate, divided lateral lobes
Spear-shaped
pointed, barbed base
Bipinnate
leaflets also pinnate
Linear
parallel margins, elongate
Peltate
stem attached centrally
Subulate
tapering point, awl-shaped
Cordate
heart-shaped, stem in cleft
Lobed
deeply intented margins
Perfoliate
stem seeming to pierce leaf
Trifoliate/Ternate
leaflets in threes
Cuneate
wedge shaped, acute base
Obcordate
heart-shaped, stem at point
Odd Pinnate
leaflets in rows, one at tip
Tripinnate
leaflets also bipinnate
Deltoid
triangular
Obovate
egg shaped, narrow at base
Even Pinnate
leaflets in rows, two at tip
Truncate
squared-off apex
Digitate
with finger-like lobes
Obtuse
bluntly tipped
Pinnatisect
deep, opposite lobing
Unifoliate
having a single leaf
Elliptic
oval-shaped, small or no point
Opposite
leaflets in adjacent pairs
Reniform
kidney-shaped
Whorled
rings of three or more leaflets


MARGIN
Ciliate
with fine hairs
Crenate
with rounded teeth
Dentate
with symmetrical teeth
Denticulate
with fine dentition
Doubly Serrate
serrate with sub-teeth
Entire
even, smooth throughout
Lobate
indented, but not to midline
Serrate
teeth forward-pointing
Serrulate
with fine serration
Sinuate
with wave-like indentations
Spiny
with sharp stiff points
Undulate
widely wavy
Prunus avium
PRU
Daphne laureola
KF
Daphne mezereum
KF
Centranthus ruber
LO
Plantago lanceo-lata
JT
Euonymus europaeus
OB
Viburnum tinus
LA
Rhododendron ponticum
KB
Laurus nobilis
KB
Salix alba
SAL B
Ruscus aculeatus
JA
Ligustrum vulgare
LB
Vinca minor
LF
Euonymus japonicus
OB
Skimmia japonica
KC
Hippophae rhamnoides
KA