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Bioprospecting of *Moringa* (Moringaceae): Microbiological Perspective

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Plants produce primary and secondary metabolites which encompass a wide array of functions. Some of these have been subsequently exploited by humans for their beneficial role in a diverse array of applications. However, out of 750,000 species available on earth, only 1 to 10 % is being potentially used. *Moring*a is one such genus belonging to the family of Moringaceae, a monotypic family of single genera with around 33 species. Most of these species have not been explored fully despite the enormous bioactivity reports concerning various potentials such as: cardiac and circulatory stimulants; anti-tumor; antipyretic; antiepileptic; anti-inflammatory; antiulcer; antispasmodic; diuretic antihypertensive; cholesterol lowering; antioxidant; antidiabetic; hepato protective; antibacterial and antifungal activities. They are claimed to treat different ailments in the indigenous system of medicine. Surprisingly, some of the species have been reported to be extinct from the face of earth before their exploration and exploitation for economic benefits. This review focuses on the bio-prospects of *Moringa* particularly on relatively little explored area of their microbiological applications

Keyword: Applied microbiology, Antimicrobials, *Moringa* species.

1. Introduction

Plants have been and will remain vital to mankind, animals as well as environment. They produce primary and secondary metabolites which encompass a wide array of functions[1] many of which have been subsequently exploited by humans for their beneficial role in a diverse array of applications[2]. The most important of these bioactive constituents of plants are the secondary metabolites which include alkaloids, phenolic compounds, tannins, phytosterols, and terpenoids. Infectious diseases are the leading cause of death worldwide in the current scenario where clinical efficacy of many conventional antibiotics is being threatened by the emergence of multidrug resistant pathogens[3] and for this, phytomedicine is becoming popular in developing and developed countries owing to its natural origin and lesser[4] side effects. Plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs[5,6]. The list of benefits of plants' bioactive compounds to human health such as anti- cancer, anti-hypertension, anti-hypoglycaemia, anti-oxidants and antimicrobial activities have been reported[7-14].

The search for plants as a source of potential candidate for drug development is still unsound. Out of 250,000 to 500,000 species available on earth only 1-10% percent are being potentially used[15]. *Moringa* is one such genus whose various species have not been explored fully despite the enormous reports concerning the various parts of a few species' potentials such as: cardiac and circulatory stimulants; anti-tumor; antipyretic; antiepileptic; anti-inflammatory; antiulcer; antispasmodic; diuretic antihypertensive; cholesterol lowering; antioxidant; antidiabetic; hepato- protective; antibacterial and antifungal activities. These are also being used for treatment of different ailments in the indigenous system of medicine[16-21]. The indigenous knowledge and use of *Moringa* is referenced in more than 80 countries and it's known in over 200 local languages. *Moringa* has been used by various societies including the Roman, Greek, Egypt, India and many others for thousands of years with writings dating as far back as 150 AD. The history of *Moringa* dates back to 150 B.C. where ancient kings and queens used *Moringa* leaves and fruit in their diet to maintain mental alertness and healthy skin. Ancient Maurian warriors of India were fed with *Moringa* leaf extract in the warfront. The Elixir drink was believed to add them extra energy and relieve them of the stress and pain incurred during war. The *Moringa* species are currently of wide interest because of their outstanding economic potential. Amongst these species, *M. oleifera* is the most prevalent for its nutritious and numerous medicinal uses that have been appreciated for centuries in many parts of its native and introduced ranges[22-24].Recently, a few others like *M. stenopetala, M. peregrina* and *M. concanensis* have been discovered to be having equal potential such as nutritious vegetables, high-quality seed oil, antibiotics and water clarification agents just like the *M. oleifera*. In this review we focus on the bio-prospects of *Moringa* species particularly on relatively little explored area of their microbiological applications and ascertain the prevailing gaps.

2. Moringaceae Family

The family Moringaceae is a monotypic family of single genera with around 33 species (Table 1) of which 4 are accepted, 4 are synonym and 25 are unassessed[25]. Out of these, 13 species, native of old world tropics[26] are documented. (Table 2, Figure1 a, b, c)

2.1. Taxonomic classification: [27]

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Eudicots
Subclass	:	Rosids
Order	:	Brassicales
Family	:	Moringaceae
Genus	:	*Moringa*

Table 1: Different species of *Moringa*

Species	Authorship	Taxonomic status
Moringa amara	Durin	Unresolved
M. aptera	Gaertn.	Unresolved
M. arabica	Pers.	Unresolved
M. arborea	Verdc.	Unresolved
M. borziana	Mattei	Unresolved
M. concanensis	Nimmo ex Dalzell & A.Gibson	Unresolved
M. concanensis	Nimmo	Unresolved
M. domestica	Buch.-Ham.	Unresolved
M. drouhardii	Jum.	Unresolved
M. edulis	Medik.	Unresolved
M. erecta	Salisb.	Unresolved
M. hildebrandtii	Engl.	Unresolved
M. longituba	Engl.	Unresolved
M. Moringa	(L.) Millsp.	Synonym
M. myrepsica	Thell.	Unresolved
M. nux-eben	Desf.	Unresolved
M. octogona	Stokes	Unresolved
M. oleifera	**Lam.**	**Accepted**
M. ovalifolia	**Dinter & A.Berger**	**Accepted**
M. ovalifolia	Dinter & Berger	Unresolved
M. ovalifoliolata	Dinter & A. Berger	Synonym
M. parvifolia	Noronha	Unresolved
M. peregrine	**(Forssk.) Fiori**	**Accepted**
M. polygona	DC.	Unresolved
M. pterygosperma	Gaertn.	Synonym
M. pygmaea	Verdc.	Unresolved
M. rivae	Chiov.	Unresolved
M. robusta	Bojer	Unresolved
M. ruspoliana	Engl.	Unresolved
M. stenopetala	**(Baker f.) Cufod.**	**Accepted**
M. streptocarpa	Chiov.	Unresolved
M. sylvestris	Buch.-Ham.	Unresolved
M. zeylanica	Burmann	Synonym

Source data[25]

Table 2: Geographic distribution of documented 13 *Moringa* species and their morphotypes

Species	Geographical location
Bottle trees	
M. drouhardii Jum	Madagascar
M. hildebrandtii Engl.	-do-
M. ovalifolia Dinter & A. Berger	Namibia and S.W. Angola
M. stenopetala (Baker f.) Cufod	Kenya and Ethiopia
Slender trees	
M. concanensis Nimmo.	India
M. oleifera Lam.	-do-
M. peregrina (Forssk) Fiori	Red Sea, Arabia, Horn of Africa
Tuberous shrubs and herbs of North Eastern Africa	
M. arborea Verdc.	North Eastern Kenya
M. borziana Mattei	Kenya and Somalia
M. longituba Engl.	Kenya, Ethiopia, Somalia
M. pygmaea Verdc.	North Somalia
M. rivae Chiov.	Kenya and Ethiopia
M. ruspoliana Engl	Kenya, Ethiopia, Somalia

Source data[28]

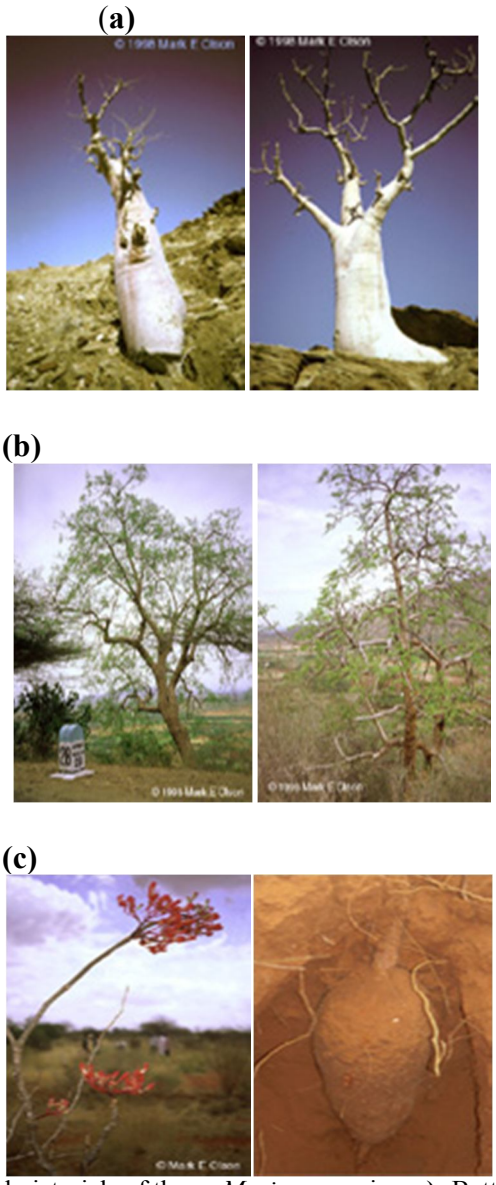

Figure 1: Morphological pictorials of three *Moringa* species; **a):** Bottle Trees; **b):** Slender Trees; **c):** Tuberous shrubs. Reprinted with permission from[28]

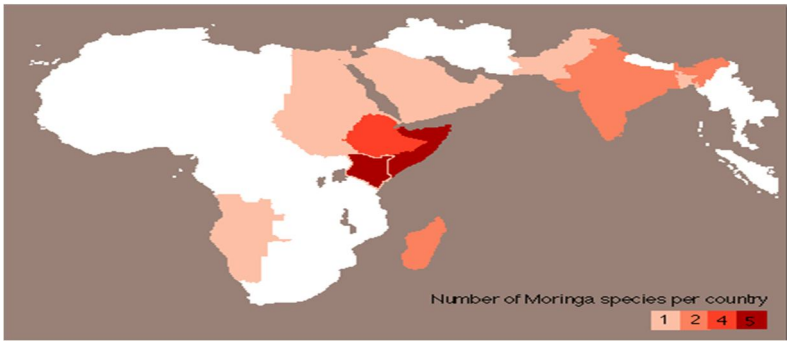

Figure 3. Distribution of *Moringa* in the old world tropics. Reprinted with permission from[28]

3. Bioactivity feasibility of *Moringa*

3.1. Indigenous claims of unexplored Moringa species

Out of the 33 species listed in Table 1, only documentation of 13 species is available. Though the information is not of scientific background, the claims by various indigenous people on their medicinal uses are an indication of the untapped potential (Table 3).

Table 3: Unexplored species of *Moringa*

Species	Morphological characteristics	Indigenous claims	Geographic location
M. drouhardii Jum.	Bloated bright white trunk	Scented bark used for Colds and coughs	Madagascar
M. ovalifolia Dinter & A. Berger	Bloated white trunk	Not documented	Namibia
*M. Pygmaea**	Tuberous shrub with tiny leaflets and yellow flowers	Not documented	Somalia
M. rivae Chiovenda*	Large spray of pale pink & wine Red flowers	Not documented	Kenya, Ethiopia
M. ruspoliana Engler	A small tree with thick, tough & large Leaves, large flowers, thick taproot which becomes more globose as the plant ages	Not documented	Somalia, Ethiopia, Kenya
M. longituba Engler	Bright red flowers, large tuber	Root extracts used for treating intestinal infections of domestic animals	Kenya, Ethiopia, Somalia
M. arborea Verdcour	Large sprays of pale pink & wine red flowers	Unspecified medicinal uses	Kenya, Ethiopia
M. borziana Mattei	Bears one or two stems which die back to the tuber every few years, sometimes due to variation of environmental parameters the plant grows into a small tree, Greenish cream to yellow flowers with brown Used for treatment of smudges on the petal tips	Used for treatment of different ailments	Kenya and Somalia
M. hilderbrandtii Engler	A massive water storing bloated trunk, deep red stem tip of young plants, large spray of small whitish flowers[29,30]	Unspecified medicinal uses	Madagascar
M. Peregrine (Forssk) Fiori	Erect trunk and white bark, small long and remote leaflets, pendulous pods with angled nut like white seeds	Used as an analgesic in the ancient world[31]	Egypt

*: Extinct species; Source data[28]

3.2. Documented Moringa species and their known bioactivities

Moringa oleifera Lamarck (Lam.) also known by different common names as per the different countries' vernacular names and can be found in the following link: http:/www.treesforlife.org/our-work/our-initiatives/*Moringa*. It is a small, fast growing evergreen or deciduous tree that usually grows up to 10 or 12m in height. It has a spreading, open crown of drooping, fragile branches, feathery foliage of tripinnate leaves and thick corky, whitish bark [21,22,32]. The uses of its roots, root bark, stem bark, exudates, leaves, flowers and seeds in the treatment of a wide variety of ailments have been discussed in the Sanskrit texts on medicinal plants[24] and the tree continues to have an important role particularly as counter-irritant in the

indigenous medicine in Asia and West Africa[33,21,34]. Based on the indigenous claims, *Moringa oleifera* (the drumstick tree) has been prevalently the subject of much research and development. *M. stenopetala* (Baker f.) Cufodontis is another species in focus due to its equal potential as *M. oleifera*. It is an important food plant in southwestern Ethiopia, where it is cultivated as a crop plant. *Moringa concanensis,* Nimmo. Tree commonly known as Horseradish tree, Drumstick tree, Never die tree, West Indian ben tree, and Radish tree which is native through the sub- Himalayan tracts of India[35,36]. It has a very strong central trunk that is covered with an extremely distinctive layer of very furrowed bark. The flowers also have distinctive green patches at the tips of the petals and sepals. It is commonly known as Kattumurungai by tribal peoples of Nilgiris hill region in Tamil Nadu, India, and widely used since the Ayurveda and Unani medicinal systems for the treatment of several ailments[37,24]. In Microbiological perspective, the various parts of these plants have been explored and reported for their potential in medical, food industry, agriculture and the environment as explained further;

3.2.1. Moringa in Water Treatment

Water is vital to life; however, due to indiscriminate human activities its quality has deteriorated causing about 80% of diseases which plague the human race especially in many developing countries. Promising water treatment techniques are far much costly for the 'have nots' and many disinfectants currently used can be harmful. For instance, it has been indicated that the chemicals used for water purification can cause serious health hazards if mishandled in the course of treatment process[38,39]. These reports suggested that a high level of aluminium in the brain may be a risk factor for Alzheimer's disease. Several researchers have raised doubts about the advisability of introducing aluminium into the environment by the continuous use of aluminium sulphate as a coagulant in water treatment[39,40,41]. This has aggravated the search for safer organic alternatives. *Moringa* seeds have been commonly known and used in many rural areas for clarification of drinking water to reduce the health risks associated with excessive turbidity. Turbidity has also been found to have a significant effect on the microbiological quality of drinking water and can interfere with the detection of bacteria and viruses[42].Though not a direct indicator of health risk, a strong relationship between turbidity removal and protozoa removal has been established[43].

A simplified, low cost, point of use, low risk drinking water treatment protocol using *M. oleifera* seeds has been invented for use by the rural and pre – urban people living in extreme poverty who are presently using highly turbid and microbiologically contaminated water. Systematic research has shown that *M. oleifera* seeds acts as an effective water clarifying agent across a wide range of various colloidal suspensions[44]. They yield water soluble organic polymer also known as natural cationic (net positive charge) poly-electrolyte[45]. It was confirmed later to be a low molecular weight water soluble protein with a positive charge acting as a coagulant responsible for binding with predominantly negative charged particulate matter that make raw water turbid[46]. In an investigation on *Moringa oleifera* (Drumstick) seed as natural absorbent and antimicrobial agent for river water treatment, a reduction of microbial colonies on plate with increase in concentration of sample was observed[47]. This endorsed the previous study[34,48] as 4 alpha rhamnosyl oxybenzyl isothiocyanate and presently known as glucosidal mustard oil which coagulates the solid matter in water and removes a good portion of suspended bacteria. Similar studies

show that *Moringa* flocculants are basic polypeptides with molecular weight between 6 and 16kDa with an isoelectric pH of 10 to11[49].The natural poly-elecrolytes released from the crushed seed kernels function as natural flocculating agents, binding suspended particles in a colloidal suspension, forming larger sedimenting particles (flocs) in which microorganisms are generally attached. Hence, treatment employing *M. oleifera* seed (press cake) can remove 90% to 99% of fecal coliforms bacterial load[50]. However, it has to be noted that after several hours of storage, temperature-induced bacteria might regrow within the storage container and there's no guarantee for 100% virus and /or bacteria-free water immediately after treatment or storage hence additional disinfection process may be required. Similarly, a group of researchers in their study on traditional water purification using *Moringa oleifera* seeds discovered a steroidal glycoside – strophantidin as a bioactive agent which was more efficient in the clarification and sedimentation of inorganic and organic matter in raw water and reduced 55% and 65% microbial and coliform load respectively after 24 hours whereas alum achieved 65% and 83% reduction under similar conditions[51]. The difference in efficacy as shown in both cases above may be attributed to the effect of oil on the bioactive agent. It forms an emulsion of film coating which may inhibit its contact with the surface of reaction and thus reduce floc formation[46]. Similarly, the difference in location of cultivation as reported[52,53] may cause the variation.

The seeds of *Moringa stenopetala* have been found to have flocculating and anti-microbial properties. The active substances are found only in the cotyledons of the seeds[54]. In a recent study on the antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seeds extracts exhibited highest inhibition of *E. coli, S. typhi* and *V. cholera* and the samples were active in low doses[55]. This could be due to less oil in low concentrations and it goes well with observations made[46] regarding the effect of oil on the bioactive agent which forms an emulsion of film coating that inhibits the reaction. *Moringa* seeds contain an antibiotic principle known as pterygospermin which is responsible for destruction of micro-organisms in water[56]. In Sudan though not proven scientifically, the seeds of *M. peregrina* have been used as coagulant to purify water[57].

3.2.2. Moringa a Source of Pharmacological Products

3.2.2.1. Alternative Medicine for Human Pathogens

An escalating antibiotic resistance by the pathogenic bacteria has been observed since last decade and the adverse effects of conventional antibiotics calls for a friendly alternative. Out of the 250,000 to 500,000 species of plants on earth[15], *Moringa* is one of the 10% which have a profound potential in pharmaceutical industry as a source of bioactive constituents for drug development. Chen[58] in her studies on the synergistic effect of *M. oleifera* seeds and chitosan (an essential and abundant component of exoskeletons – the mucoadhesive polymer which is derived from chitin) on antibacterial activity against *Bacillus subtilis* and *Pseudomonas putida* found the individual samples to be more effective than the two combined. However, in another study to determine antimicrobial potential of different plant seed extracts against Multidrug Resistant Methicillin Resistant *Staphylococcus aureus* (MDR – MRSA), it was established that *Moringa oleifera* seeds had a synergistic potential to restore the effectiveness of B – lactam antibiotics against MRSA[59]. The synergistic properties could be attributed to β – lactamase inhibition by the Flo peptide (a specific polypeptide

found in *Moringa oleifera* that is both a flocculent and a biocide). The cationic Flo peptide supposedly serves as a highly efficacious immunity response, interacting with the anionic cell membranes of bacterium[60]. This interaction destabilizes the bacterial membrane, causing leakage of cytoplasmic content and killing the bacterial cell. Antimicrobial peptides, such as the Flo peptide, have been reported to act directly and non-specifically upon bacterial membranes, thus hindering their ability to develop resistance. However, antimicrobial peptides rarely affect the membranes of cells in multicellular species[61] an indication that they are ineffective against eukaryotes especially fungal pathogens. Recently, an *in vitro* antimicrobial activity of *Moringa oleifera* L. seed extracts prepared in aqueous and organic solvents against *Staphylococcus aureus*, *Bacillus subtilis*, *Escheriachia coli, Pseudomonas aeruginosa, Aspergillus niger and Candida albicans* exhibited antimicrobial properties[62]. However, this is partly contradicted by the findings in our laboratory using up to 20% aqueous extracts of the same plant (unpublished data) where one of the seeds sample collected from a different locality exhibited less active and to a few test organisms among Gram positive, Gram negative and yeast pathogens used which may be due to differences in source of the samples as depicted earlier[52,53]. The variation may also be brought about by environmental changes such as effect of pathogens[63], allelopathy and herbivory[64] which may trigger production of high levels of secondary metabolites. On the other hand, water availability, exposure to soil pathogens and variations of soil pH and nutrients affect the accumulation of secondary metabolites[65]. Similarly, environmental factors such as temperature, rainfall, day length and edaphic factors affect the efficacy of the medicinal properties of different plants[66].

Urinary tract infections are the second most common type of infection in the world. It is mainly a bacterial infection that affects peoples throughout their lifespan[67]. These are more common in women than men, leading to approximately 8.3 million doctor visits per year. *Proteus mirabilis* is a small Gram negative bacillus, facultative anaerobe belonging to the family of Enterobacteriaceae that commonly cause urinary tract infections and formation of stones[68].

In a study on the antibacterial effect of *Moringa oeifera* leaves extracts prepared in different solvents, petroleum ether extracts demonstrated the highest activity against clinical samples and environmental samples of *Proteus mirabilis*[69]. However, in a separate study chloroform extracts[70] showed broad spectrum potential than that of petroleum ether an indication of the presence of different active principles. *In vitro* studies on different extracts of the root bark of *Moringa oleifera* against *Staphylococcus aureus, Echerichia coli, Salmonella gallinarum, Pseudomonas aeruginosa* among others showed that ethyl acetate and acetone extracts exhibited maximum activity as compared to other solvents[71,72] which shows that active compounds are polar in nature. Antimicrobial activity studies of stem bark of *Moringa oleifera* against some human pathogens demonstrated methanolic extracts to be the most effective among other solvents used[73,74]. In most recent study on *in vitro* antibacterial and antifungal potential of *Moringa oleifera* stem bark against ten bacterial strains and six fungal strains, petroleum ether extract was reported inactive[75]. To this point, it emanates that the different plant parts of *Moringa* contain a vast array of bioactive constituents of varying polarity which can be potential candidates as drug leads/ development.

Still within this genus, a project on development of pharmaceutical products from medicinal plants was carried out in Ethiopia by the institute of Pathobiology, Addis Ababa University in 1996, *Moringa Stenopetala* was one of the plants assessed. It was reported that the biological active compounds isolated from both leaves and seeds of the plant by a bioassay guided fractionation exhibited antimicrobial activity against *Staphylococcus aureus, Salmonella typhi, Shigella* and *Candida albicans*. A number of Laehiums prepared by herbal venders in South India was tested for antimicrobial activity. It was reported that ethanol, petroleum ether, hexane (prepared in 1000 ppm) and aqueous extracts (20%) resins of *Moringa concanensis* (which were traditionally used for treatment of fire burns) exhibited antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*[76].

3.2.2.2. Synthesis of Nanoparticles

In addition to above searches, the possibility of using *Moringa* in nanotechnology is being explored for useful products. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. Metal nanoparticles which have a high specific surface area and a high fraction of surface atoms have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties[77]. Nanoparticles have a long list of applicability in improving human life as well the environment and among this drug delivery technology. The technology has come into spotlight due to its benefits such as shorter development periods and lower costs compared to the development of a new drug[78-80]. The ideal nanoparticle materials are those which do not undergo chemical changes, satisfy the conditions of biodegradability, bio-compatibility and delivery speed of the drug[81,82]. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained[83,84]. Generally metal nanoparticles are synthesized and stabilized by using chemical methods such as chemical reduction[85,86] which are too costly and hazardous. For this reason, cheaper and environmental friendly biological methods are sought for. Synthesis of nanoparticles within the biological means using bacteria and fungi[87,88], has been proved efficient and safer. However, it has been established that use of plant leaves extract is cheaper[89] as it reduces the costs and does not require any special culture preparation and isolation techniques. Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provides advancement over chemical and physical method as it is cost effective and eco-friendly. It can be easily scaled up for large scale synthesis and there is no need to use high pressure, energy, temperature and toxic chemicals. In this regard, investigations in the biofabrication of Ag nanoparticles using *M. oleifera* leaves extract revealed the leaves to have the potential of producing Ag nanoparticles extracellularly by rapid reduction of silver ions (Ag^{+} to Ag^{0}) which were quite stable in solution[90]. In subsequent testing for antimicrobial activity against a number of pathogens, this Ag nanoparticles suspended hydrosol showed considerable antimicrobial activity in comparison to chloramphenicol and ketoconazole antibiotics.

3.2.2.3. Bio- Enhancing Properties

Some parts of this multipurpose genus have also been associated with bio-enhancing properties. Bio-enhancers are molecules which do not possess drug activity of their

own but promote and augment the biological activity or bioavailability or the uptake of drugs in combination therapy, resulting in reduced drug associated toxicity, reduced cost and duration of chemotherapy. Isolated plant biomolecules or their semisynthetic derivatives have provided useful clues in the production of medicines[91]. Recently, in a pre-clinical study on the influence of *Moringa oleifera* pods on Pharmacokinetic disposition of rifampicin using HPLC- PDA method established that the active fraction isolated from air dried pods of the plant when mixed with rifampicin and administered to the experimental animals enhanced systemic availability of the drug and suppression of the drug metabolizing cytochrome P-450[92]. In another study, a bioenhancing property of *M. oleifera* pods extract was reported. It was found that niaziridin rich fraction of *M. oleifera* pods enhances the bioactivity of commonly used antibiotics such as rifampicin, tetracycline and ampicillin against Gram positive and Gram negative bacteria. It also facilitated the absorption of drugs, vitamins and nutrients through the gastro-intestinal membrane thus increasing their bio-availability[91]. This lowering of the dosage level and shortened treatment course of rifampicin as an anti-tuberculosis drug minimize its associated side effects to the advantage of the patients.

3.2.2.4. Other Pharmacological Potentials

Though the review focuses on the economic potentials of *Moringa* from the Microbiological perspective, it will be worth listing the prospects of this genus in other pharmacological fields (Table4)

3.2.3. Moringa in Food Preservation

Protection of food from microbial or chemical deterioration has traditionally been an important concern in the food industry. Chemically synthesized preservatives have been classically used to decrease both microbial spoilage and oxidative deterioration of food[106]. However, in recent years; consumers are demanding partial or complete substitution of chemically synthesised preservatives due to their possible adverse health effects. This fact has led to an increasing interest in developing more "natural" alternatives in order to enhance shelf-life and safety of the food[107]. Though not so extensive work in this field in regard to the plant under review, a recent study indicated that seeds exhibited the potential as sanitizers/preservatives by inhibiting the growth of organisms such as *E. coli, S. aureus, P. aeruginosa, S. typhi, S. typhimurium* and *E. aerogenes* which range from pathogenic to toxigenic organisms liable to cause food – borne illnesses to spoilage-causing organisms liable to spoil food products[108].

3.2.4. Moringa in Agriculture

The practice of using plant derivatives or botanical insecticides in agriculture dates back to at least two millennia in ancient China, Egypt, Greece, and India [109,110]. What is clear from recent history is that synthetic insecticides effectively relegated botanicals from an important role in agriculture to an essentially trivial position in the marketplace among crop protectants. However, history also shows that overzealous use of synthetic insecticides led to numerous problems unforeseen at the time of their introduction: acute and chronic poisoning of applicators, farm workers, and even consumers; destruction of fish, birds, and other wildlife; disruption of natural biological control and pollination; extensive groundwater contamination, potentially threatening human and environmental health; and the evolution of resistance to pesticides in pest populations[111-114]. Trials on the potential of *Moringa oleifera* for agricultural and

Table 4: Scientific / Traditional claims of pharmacological potentials of Moringa species

Species	Therapeutic Effects	Plant Part	Reference
a	b	cL,F,B,R,P,S,O,G,	35, 93- 97
d	Leishmaniasis trypanosomiasis	LR	98,99
	Anti-fertility, oxytocic, activity, hypoglycaemic activity, cholesteral reduction	L	99-101
e	Cosmoceutical	O	
	Fire burn wounds	G	102
	Ophthalmic, goitre, venereal infections, glycosuria, lipid disorder	S	76
	Anti-inflammatory, analgesic, Antipyretic,	F	103
	Cholesterol reduction * and body weight, ophthalmic, menstrual pains, women fertility, aphrodisiac, jaundice, fatigue, diabetes blood pressure*,constipation* ,skin tumor	L	
	Thyroid disorders, leucorrhea, abortion	F	
	Spleenomegaly*	L_T	
	Bloat	B_s	
	Intestinal worms	S	
	Head ache*	G	
	Spinal cord pain	R	104
f	Analgesic Abdominal Burns Constipation Febrifuge Laxative Headache	NS**	31
	Cosmoceutical , abdominal pain	O	
	GNS***	S	57
g	GNS***	W	
h	GNS***	W	30
****	Cosmoceutical	O	
	Colds and coughs	BW	105
	-	-	-

a: *M. oleifera;* **b**: Comprehensively covered; **c**: L- leaves, L_T- tender leaves, F-flowers, B-bark, Bs- stem bark, R-roots, P-pods, S-seeds, O-oil (from seeds), G-gum, W-wood; **d**: *M. stenopetala*; **e**:M. *concanensis*; **f**:*M. peregrina*; **g**: *M. hildebrandtii*; **h**: *M. drouhardii*;*: Combination therapy; **NS: Not specified; ***GNS: General not specific; ****: Other species not documented

industrial use have shown that the leaves of this plant contains a bioactive substance which when sprayed on crops indicates accelerated growth of young plants, which become more resistant to pests and diseases[115]. Though no further literature is available in this regard, the potential of *Moringa* in agriculture is obvious in this study and needs extensive exploration.

4. Toxicological reports on some *Moringa* species

Toxicological evaluation of medicinal plants has often been neglected by many traditional healers with the notion that plants are harmless and therefore historical usage of such products cannot always be a reliable guarantee of safety. It is difficult for these practitioners to detect or monitor delayed effects (e.g. mutagenicity), rare adverse effects arising from long-term use[116] as in food supplements and nutraceuticals e.g. *Glycyrrhiza glabra*, which is used for conditions like bronchitis and peptic ulcers causes not only hypertension, weight gain and hypokalaemia but also low levels of aldosterone and anti-diuretic hormone on excessive or prolonged usage[117]. Many widely used medicinal plants have been implicated as possible causes of long-term disease manifestations such as liver and kidney diseases for instance widespread use of *Scenecio, Crotalaria* and *Cynoglossum* has been implicated in the occurrence of liver lesions and tumours, lung and kidney diseases in certain areas of Ethiopia [118]. Similarly, reports are available on accidents due to mistakes of botanical identification, plants that interfere with pharmacological therapy (such as those containing coumarinic derivatives, high tyramine content, those containing oestrogenic compounds, those that cause irritation and allergy, those containing photosensitive compounds)[119-123].

In light of the above, there is scanty literature on *Moringa* genus in regard to toxicological studies. However, the few reports on *M. oleifera* and *M. stenopetala* available are not exhaustive. Adedapo *et al*.[124] established in their study that aqueous extract of *Moringa oleifera* leaves was nontoxic in rats both oral and sub-acute on haematological biochemical and histological parameters. In a similar study, Ashong and Brown[125] observed no impact and short term toxicity of aqueous leaf extracts of various concentration on feed intake of poultry. The ethanolic and aqueous extracts of *M. oleifera* bark were found to have no adverse effect on growth related and biochemical parameters in rats, an indication that neither steroids, triterpenoids, saponins, alkaloids nor carbohydrates phytoconstituents identified were toxic [126]. Kasolo *et al.*[127] in their *in vivo* study established that acute toxicity tests with aqueous and ethanol extracts of M. *oleifera* roots exhibited a safe range where the LD_{50} for aqueous extracts was 15.9mg/kg and for ethanolic extract was 17.8mg/kg. However, in the most recent studies on the effect of methanolic extracts of *M. oleifera* roots on histology of kidney and liver on Guinea Pigs was found to distort the histo-architecture of both the organs and the effects were time as well as dose dependent[128]. Several *in vivo* studies indicate aqueous extracts of M. *oleifera* seeds to be safe[129,130]. However, Oluduro and Aderiye[131] contradicted these findings in their study on the effect of *M. oleifera* aqueous seed extracts on vital organs and tissue enzyme activities of male albino rats where their findings suggested that prolonged consumption of water treated with $\geq$ 2mg/ml of *M. oleifera* seeds may lead liver infarction. Similar observations though with methanolic extracts were made in a different study which confirmed that administration of these seeds extracts appears relatively nontoxic to animals at low

doses. However, at high dosages, the alterations observed in various parameters tested suggested a dose sensitive toxicity when repeatedly consumed on a daily basis for a prolonged time[132].

The cytotoxicity of extracts from a widely used species of plant, *Moringa stenopetala*, was assessed in HEPG2 cells, by measuring the leakage of lactate dehydrogenase (LDH) and cell viability. The functional integrity of extract-exposed cells was determined by measuring intracellular levels of ATP and glutathione (GSH). The ethanol extracts of leaves and seeds significantly increased ($p < 0.01$) LDH leakage in a dose- and time-dependent manner. However, aqueous extract of leaves and ethanol extract of the root did not increase LDH leakage. A highly significant ($p < 0.001$) decrease in HEPG2 viability was found after incubating the cells with the highest concentration (500µg/ml) of the ethanol leaf and seed extracts. The water extract of the leaves did not alter GSH or LDH levels or affect cell viability, suggesting that it may be non-toxic. This was an indication that not all compounds of these morphological parts tested were toxic but only those extractable by ethanol[98]. In another study to establish the effects of *M. stenopetala* on Blood parameters and histopathology of liver and kidney in mice, it was reported that the extract did not show any morphological changes in the liver cells as well as no histopathological changes in the kidneys of the treated mice with all doses used (600,750,900mg/kg) [101]. From these studies we cannot make a haste conclusion of safety or toxicity of the sample under study since different solvents are responsible for extracting different compounds. Therefore, a compound of interest should be tested on its own or a systematic extraction (using all possible solvents) and subsequent toxicity testing of the compound of interest is of paramount importance. However, it calls for intervention of more rapid and less expensive approaches to work at the *in vitro* as well as *in vivo* toxicity.

5. Phytoconstituents of *Moringa*

Out of the 13 species of *Moringa, Moringa oleifera* has been given much publicity including its phytoconstituents. A few others such as *M. stenopetala, M. peregrina, M. concanensis* have been reported. Nevertheless, the various studies reported (Table 5) are not exhaustive and much work is needed to establish the comprehensive phytoconstituents of these and other *Moringa* species, and further explore and exploit their antimicrobial properties not forgetting to ascertain the safety of the active principles.

In light of the limitations in the various studies reported herein, looking at different parameters in a systematic way in this context and not just a single assay for determining the biological efficacy of plants is of essence based on the following facts:

- Some compounds which show good activity *in vitro* may be metabolized *in vivo* into inactive metabolites. Alternatively, extracts may only show *in vivo* activity due to the metabolism of inactive compounds into active forms [140].
- Similarly, the pharmacological investigation of drug interactions in multi-compound preparations is difficult due to the presence of several constituents where some may show less specific activity and some may camouflage the toxicity and activity of the more therapeutically effective compounds.
- There is no one solvent which can extract all the phyto-constituents and thus several of them should be used for better comparison.
- Some of the most common side effects are difficult to recognize in animal models e.g. nausea, nervousness, lethargy, heartburn, headache, depression, stiffness, etc.
- *In vitro* findings may not extrapolate into *in vivo* models such as animals and humans. Therefore a thorough investigation and trials

to authenticate such findings is the need of tomorrow.

- Toxicological studies are mandatory at the initial stages of pharmacological studies to avoid waste of resources on unsuitable compounds.
- Efforts are required for wholesome research including elucidation of structure of responsible compound/s and establishing the mechanism of action.

Table 5: Phytoconstituents of various *Moringa* species

Plant Species	Phytoconstituents and their bioactivity		Reference
[a]	**Stem bark**		
	Methanol	NR***	
	Moringly linoleneate,	-do-	
	β- sitosterol, epilupeol,	-do-	
	oleiferyl capriate,	-do-	
	glyceropalmityl phosphate,	-do-	
	glycero-oleiostearyl phosp.	-do-	133
	Standard methods		
	Alkaloids	-	
	Saponins	-	
	Triterpenoids	Antimicrobial	
	Diterpenoids	-do-	
	Flavonoids	-do-	
	Cardiac glycoside	-do-	
	Tannins	-	
	Phytosterols	-	Arora and Onsare (unpublished)
	Ethanol		
	Steroids	NR***	
	Triterpenoids	-do-	
	Saponins	-do-	
	Alkaloids	-do-	
	Carbohydrates	-do-	
	Distilled Water		
	Saponins	-do-	
	Carbohydrates	-do-	
	Alkaloids	-do-	126
	Seeds		
	Ethanol		
	Sterols	NS*	
	Glycosides	-do-	
	Carbohydrates	-do-	
	Alkaloids	-do-	
	Flavonoids	-do-	
	Petroleum ether		
	Sterols	-do-	
	Carbohydrates	-do-	
	Flavonoids	-do-	
	Chloroform		
	Carbohydrates	-do-	
	Distilled water		
	Carbohydrates	-do-	
	Alkaloids	-do-	
	Flavonoids	-do-	134
	Roots		
	Ether		
	Gallic tannins	NR***	
	Steroids	-do-	
	Triterpenoids	-do-	
	Anthraquinones	-do-	

Ethanol		
Catechol Tannins	-do-	
Steroids	-do-	
Triterpenoids	-do-	
Anthraquinones	-do-	
Alkaloids	-do-	
Carbohydrates	-do-	
Distilled Water		
Saponins	-do-	
Alkaloids	-do-	
Carbohydrates	-do-	127
Leaves		
Petroleum ether		
Alkaloids	NS**	
Flavonoids	-do-	
Tannin	-do-	
Phenolic compounds	-do-	
Carbohydrates	-do-	
Acetone		
Tannin	-do-	
Phenolic compounds	-do-	
Carbohydrates	-do-	
Isopropyl alcohol		
Alkaloids	-do-	
Flavonoids	-do-	
Tannins	-do-	
Phenolic compounds	-do-	69
Ethanol		
Flavonoids	NS**	
Tannin	-do-	
Glycoside	-do-	
Terpenoids	-do-	
Chloroform		
Alkaloids	-do-	
Saponins	-do-	
Tannin	-do-	
Distilled Water		
Flavonoids	-do-	
Saponins	-do-	
Tannin	-do-	
Glycoside	-do-	
Terpenoids	-do-	135
Seeds' coat		
Standard methods		
Alkaloids	-	
Triterpenoids	Antimicrobial	
Flavonoids	-do-	
Diterpenoids	-do-	
Cardiac glycoside	-do-	
Phytosterols	-	
Tannins	-	
Pods' husks		
Standard methods		
Alkaloids	-	
Triterpenoids	Antimicrobial	
Flavonoids	-do-	
Diterpenoids	-do-	
Cardiac glycoside	-	
Phytosterols	-	
Tannins	-	Arora and Onsare (unpublished)
Leaves		
Standard methods		

b	Chromophers	Hypotensive	
	Polyphenols	-do-	
	Saponins	-do-	
	Physteroides and withanoids	-do-	
	Flavonoids	-do-	
	Tannins	-do-	
	Alkaloids and anthraquinone	-do-	
	Glycosides	-do-	136
c	**Seeds**		
	4-(α-L-Rhamnosyloxy) benzyl-isothiocyanate	NR***	48
	4-(4'-*O*-Acetyl- α-L-rhamnosyloxy)-benzyl isothiocyanate	-do-	
	2-Propyl isothiocyanate	-do-	
	2-Butyl isothiocyanate	-do-	
	2-Methylpropyl-isothiocyanate	-do-	
	5,5-Dimethyl-oxazolidine -2-thione	-do-	137
	Roots		
	4-(α –L- Rhamnosyloxy) Benzyl-isothiocyanate	-do-	138
	Stem bark		
d	**Hydroalcoholic**	-do-	
	Carbohydrates	-do-	
	Alkaloids	-do-	
	Glycosides	-do-	
	Tannins	-do-	
	Saponins	-do-	
	Terpenoids	-do-	
	Flavonoids	-do-	
	Proteins	-do-	
	Chloroform		
	Alkaloids	-do-	
	Tannins	-do-	
	Terpenoids	-do-	
	Ethyl acetate		
	Carbohydrates	-do-	
	Glycosides	-do-	
	Tannins	-do-	
	Flavonoids	-do-	
	Petroleum ether		
	Steroids	-do-	
	Terpenoids	-do-	
	Ethanol		
	Carbohydrates	-do-	
	Glycosides	-do-	
	Tannins	-do-	
	Saponins	-do-	
	Flavonoids	-do-	
	Proteins	-do-	139
e*****			

a: *M. oleifera*; **b:** *M. stenopetala*; **c:** *M. peregrine*; **d:** *M. Concanesnsis*; **e:** Other species; NS*: Anti-inflammatory reported but not specified; NS**: Antibacterial reported but not specified; NR***:Not reported; NT**** :Not yet tested; *****: Literature not available.

6.0. Strength, Opportunities and Threats

Out of the 33 species of *Moringa,* 13 have been documented where *M. oleifera, M. concanensis, M. peregrina* and *M. stenopetala* and the unexplored ones have been proven scientifically as well as untapped indigenous claims to have prospects as potential candidates for development of useful products. Hence, the establishment of the location as well as continual documentation of information of the various species of this family is a breakthrough towards exploration and exploitation of their potentials. However, amid the much work on the Indian *Moringa oleifera,* gaps in comprehensive microbiological studies in this as well as the other unexplored species of the genus still exist. A systematic research of this prospective Moringaceae family will lead to potential bio-products of vast array applications in fields such as; water, pharmaceutical, food and agricultural industries thus,

1. A great hope to the two million people who die from diseases caught from contaminated water every year, with the majority of these deaths occurring among children under five years of age.
2. Safer and eco-friendly products.
3. Empowerment to the 'have nots' and hope to unemployed.
4. It should be noted with concern that some of the species are reported to be extinct from the face of the earth. Human activities and the aggravating effects of climate change may lead to loss of the remaining species unless appropriate measures are taken into consideration. Similarly from the technical point of view, due to lack of systematic research in addition to limitations already heighted, the lives of many may be at stake especially those who rely on herbal products whose toxicity has not been established.

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8. Conflict of Interest

The authors declare no conflict of interest in the contents of this paper.

9. References

1. Croteau R, Kutchan TM, Lewis NG. Natural products (secondary metabolites). In: B. Buchanan, W. Gruissem, R. Jones, (Eds.), Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant Physiology, 2000, 1250-1318.
2. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. Science 1985; 228, 1154-1160.
3. Bandow JE, Botz H, Leitchert LIO, Labischinski H, Hecker M. Proteomic approach to understanding antibiotic action. Antimicrob. Agents Chemother. 2003; 47, 948-955.
4. Brahmachari UN. The role of science in recent progress of medicine. Curr. Sci. 2001; 81: 15- 16.
5. Robbers J, Speedie M Tyler V. Pharmacognosy and pharmacobiotechnology. Williams and Wilkins, Baltimore, 1996, 1–14.
6. Hammer KA, Carson CF Riley TV. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 1999; 86, 985-990.
7. Arora DS, Kaur J Antimicrobial activity of spices. Int. J. Antimicrob. Ag. 1999; 12, 257-262.
8. Bandyopadhyay U, Biswas K, Sengupta A, Moitra P, Dutta, P, Sarkar D, Debnath P, Ganguly CK, Banerjee RK. Clinical studies on the effect of Neem (*Azadirachta indica*) bark extract on gastric secretion cancer. Life Sci. 2004; 75, 2867-2878.

9. De-Boer HJ, Kool A, Broberg A, Mziray WR, Hedberg I, Levenfors JJ. Antifungal and anti-bacterial activities of some herbal medicines from Tanzania. J. Ethnopharmacol. 2005; 96:461-469.
10. Surveswaran S, Cai YZ, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem. 2007; 102: 938-953.
11. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potent application in the prevention and treatment of oral diseases. Evidence- based Complem. Alternat. Med. 2009; 6, 1-15.
12. Darwish RM, Aburjai TA. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli*. BMC Comp. Alt. Med. (2010)**10**: 1
13. Chandra P, Arora DS. Optimization of antioxidant potential of *Penicillium granulatum* Bainier by statistical approaches, ISRN Microbiol. 2012; 1-10.
14. Arora DS, Chandra P, Kaur GJ.Optimization and assay of antioxidant potential of two *Penicillium* spp by different procedures. Curr. Biotechnol. 2012; 1: 2-10.
15. Cowan MM. Plant products as antimicrobial agents, Clin. Microbiol. Rev. 1999; 12: 564 – 582.
16. Morton JF. The horseradish tree, *Moringa pterigosperma* [*Moringa*ceae], a boon to arid lands. Econ. Bot. 1991; 45: 318-333.
17. Caceres A, Saravia A, Rizzo S, Zabala L, Leon ED, Nave F. Pharmacologic properties of *Moringa oleifera* 2: Screening for antispasmodic, anti-inflammatory and diuretic activity, J. Ethnopharmacol. 1992; 36: 233–237.
18. Pal SK, Mukherjee PK, Saha BP. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. Phytother. Res. 1995; 9: 463–465.
19. Mughal MH, Ali G, Srivastava PS, Iqbal M. Improvement of drumstick [*Moringa pterygosperma* Gaertn.] – a unique source of food and medicine through tissue culture. Harmdad Med. 1999; 42: 37 – 42.
20. Guevara AP, Vargas C, Sakurai H. An antitumor promoter from *Moringa oleifera Lam*. Mutat. Res. 1999; 440: 181–188.
21. Ramachandran C, Peter KV, Gopalakrishnan PK. Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. Economic Botany, 1980; 34: 276- 283.
22. Nikkon F, Saud ZA, Rehman MH, Haque ME. *In vitro* antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. *Pak.* J. Biol. Sci. 2003; 22: 1888–1890.
23. Mossa JS. A study on the crude antidiabetic drugs used in Arabian folk medicine. Int. J. Crude Drug Res. 1985; 23:137-145.
24. Booth FEM Wickens GE. Non- timber uses of selected arid zone trees and shrubs in Africa.FAO Conservation Guide 19, Rome, Food and Agriculture Organization, 1988, 176.
The Plant List **(**2010). Published on the Internet; Version **1;**http://www.theplantlist.org/ (accessed 1st January).
25. Mabberley DI. The Plant Book. Columbia University Press, Cambridge, New York, 1987
26. Olson ME. Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). Syst. Bot. 2002; 27: 55–73.
27. Olson ME (1999). The home page of the plant family Moringaceae, Available at: www.mobot.org/gradstudents/oslon/*Mo ringa*home.html.
28. Mark EO, Sylvain GR. *Moringa hildebrandtii* (Moringaceae): a tree extinct in the wild but preserved by indigenous horticultural practices in Madagascar, Adansonia 2000; 22, 217- 221.
29. Olson ME, Razafimandimbison SA.. *Moringa hildebramdtii* (Moringaceae): a tree extinct in the wild but preserved by indigenous horticultural practices in Madagascar. Adansonia, 2000; 22: 217-221.
30. Batanouny KH. "Wild medicinal plants in Egypt". (With contribution of: E.Aboutabl, M. Shabana & F. Soliman). With support of the Swiss Development Co-operation (SDC). Academy of Scientific Research and

Technology, Egypt. The World Conservation Union (IUCN), Switzerland, 1999, 60-64.
31. Jahn S, Azharia AI, Musnad HA, Burgstaller H. The tree that purifies water. Cultivating multipurpose *Moringa*ceae in the Sudan, Unasylva 1986; 38:23-28.
32. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants Council of Scientific and Industrial Research Press, New Delhi India, 1956, 330.
33. 3Szolnoki TW. Food and fruit trees of the Gambia.
Hamburg, Bundesforschungsanstalt fur Forst – und Holzwirtschaft, 1985, 132.
34. Fahey JW. *Moringa oleifera*: A review of the medicinal evidence for its nutritional, therapeutic and prophylactic properties. Part 1. J. Phytochem. 2005; 47: 123-157.
35. Jose TA, Oliveira SB, Silveira A. *Moringa*; compositional and nutritional attributes of seeds from the multiple purpose tree *Moringa oleifera* Lamarck. J. Sci. Food Agric. 1999; 79: 815-20.
36. Anwar F. Rashid U. Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. Pakistan J. Biol. Sci. 2007; 39:1443-1453.
37. Crapper DR, Krishnan SS, Dalton AJ. Brain aluminium distribution in alzheimer's disease and experimental neurofibrillary degeneration, Science 1973; 180: 511-513.
38. Miller RG, Kopfer FC, Ketty KC, Stober JA Ulmer NS. The occurrence of aluminium in drinking waters, J. Am. Water Works Assoc. 1984; 76: 84-91.
39. Mallevialle J, Brichet A, Fiessinger F How safe are organic polymers in water treatment. J. Am. Water Works Assoc. 1984; 76, 87-93.
40. Letterman RD Driscoll CT. Survey of residual aluminium in filtered water. J. Am. Water Works Assoc. 1988; 82,154-158.
41. Lechevallier MW, Babcock RM, Lee RG. Examination and characterization of distribution system biofilms, Appl. Environ. Microbiol. 1987; 54, 2714 -2724.
42. E.P.A. Guidance manual for compliance with the interim enhanced surface water treatment rule: Turbidity provisions. Prepared by SAIC for the USEPA, Office of ground water and drinking water, Washington, D.C., 1999.
43. Lea M. Bioremediation of turbid surface water using seed extract from *Moringa oleifera* Lam. (Drumstick) Tree, Curr. Protoc. Microbiol. 2010; 16: 1G.2.1 – 1G.2.14.
44. Marobhe NJM. Water supply in Tanzania and performance of local plant material in purification of turbid water. Ph.D.,Thesis. University of Stockholm, Sweden, 2008.
45. Malusare CN, Milind RG. Study of *Moringa oleifera* extracts in water treatment. A national seminar vision 2025, technological developments in biological sciences. Patkar – verde college, Mumbai – India, 2011.
46. Mangale SM, Chonde SG, Jadhav AS, Raut PD. Study of *Moringa oleifera* Drumstick) seed as natural absorbent and antimicrobial agent for river water treatment. J. Nat. Prod. Plant Resou. 2012; 2: 89-100.
47. Eilert U, Wolters B, Nahrstedt A. The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. Planta Med. 1981; 42: 55-61.
48. Jahn SAA. Using *Moringa* seeds as coagulants in developing countries. J.Am. Water Works Assoc. 1988; 80: 43-50.
49. Madsen M, Schlundt J, Omer EF. Effect of water coagulation by seeds of *Moringa oleifera* on bacterial concentrations. J. Trop. Med. Hyg. 1987; 90: 101-109.
50. Olayemi AB, Alabi RO. Studies on traditional water purification using *Moringa oleifera* seeds. *Afr. Study Monogr*. 1994; 15: 135- 142.
51. Narasiah KS, Vogel A, Kramadhari NN. Coagulation of turbid waters using *Moringa oleifera* seeds from two distinct sources. Water Sci. Technol. Water Supply 2002; 2: 83 – 88.
52. Nwaiwu NE, Mshelia F, Raufu IA. Antiseptic and coagulative properties of crude extracts of *Moringa oleifera* seeds from North East of Nigeria. J. Appl. Phytotechnol. Environ. Sanit. 2012; 1: 51-59.
53. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestree Database:a tree reference and selection guide version 4.0., 2009.(http://www.worldagroforestry.org/af/treedb).

54. Atieno W, Wagai S, Arama P, Ogur J. Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n- hexane seed extracts on bacteria implicated in water borne diseases. Afr. J. Microbiol. Res. 2011; 5: 153-157.
55. Aney J, Rashmi T, Maushumi K, Kiran B. Pharmacological and pharmaceutical potential of *Moringa oleifera*, Rev. J. Pharm. Res. 2009; 2: 1424-1426.
56. Munyanziza E, Yongabi KA. *Moringa peregrina* (Forssk.) Fiori In: van der Vossen HAM and Mkamilo GS, (Editors). PROTA 14: Vegetable oils/Oléagineux. [CD- Rom]. PROTA, Wageningen, Netherlands, 2007.
57. Chen M. Elucidation of bactericidal effects incurred by *Moringa oleifera* and Chitosan. J U.S. SJWP. 2009; 4: 65-79.
58. Karthy ES, Ranjitha P, Mohankumar A. Antimicrobial potential of plant seed extracts against Multidrug Resistant Methicillin Resistant *Staphylococcus aureus* (MDR – MRSA). Int. J. Bio. 2009; 1:34-40.
59. Suarez M, Haenni M, Canarelli S, Fisch F, Chodanowski P, Servis C, et al. Structure-function characterization and optimization of a plant- derived antibacterial peptide. Antimicrob. Agents Chemother. 2005; 49, 3847-3857.
60. Fisch F. Flo antibacterial peptide from the tropical tree *Moringa oleifera*: A template for novel antibacterial agents. PhD Thesis. Lausanne, Universite de Lausanne. 2004.
61. Abdulmoneim MS, Abu IE. An *in vitro* antimicrobial activity of *Moringa oleifera ISRN Microbiol* L. seed extracts against different groups of microorgamisms, Aust. J. Basic Appl. Sci. 2011; 5:129 - 134.
62. Fluck H. The influence of climate on the active principles in medicinal plants. J. Pharm. Pharmacol. 1955; 7: 361 -383.
63. Gershenzon J. Changes in the levels of plant secondary metabolites under water and nutrient stress. Recent Adva. Phytochem. 1984; 18, 273 -320.
64. Economakis C, Skaltsa H, Demetzos C, Sokovic M, Thanos CA. Effect of phosphorus concentration of the nutrient solution on the volatile constituents of leaves and bracts of *Origanum dictammus*. J. Agric. Food Chem. 2002; 50: 6276 – 6280.
65. Dubey NK, Kumar R, Tipathi P. Global promotion of herbal medicine: India's opportunity. Curr. Sci. (India) 2004; 86:37-41.
66. Sowmiya S, Soundarapandian P, Rajan S. Bioactive studies of *Mangifera indica* against bacteria isolated from urine samples. Curr. Res. J. Biol. Sci. 2009;1:139-143.
67. Marte HP, Georgina SA, Roberto MS. Microbial resistance to antibiotics used to treat urinary tract infections in mexican children. Proc. Western Pharmacol. Society 2004; 47: 120-121.
68. Arun T, Rao PCH. Phytochemical screening and antibacterial activity of *M. oleifera* Lam. against *Proteus mirabilis* from urinary tract infected patients. Int. J.PharmTech. Res. 2011; 3: 2118 – 2123.
69. 7Devendra BN, Srinivas N, Prasad.Talluri VSSL, Latha PS. Antimicrobial activity of *Moringa oleifera* Lam. Leaf extract against selected bacterial and fungal strains.Int. J. Pharma. Bio. Sci. 2011; 2: B13 –B18.
70. Dewangan G, Koley KM, Vadlamudi VP, Mishra A, Poddar A, Hirpurkar SD. Antibacterial activity of *Moringa oleifera* (drumstick) root bark. J. Chem. Pharm. Res. 2010; 2: 424-428.
71. Anitha JR, Velliyur KG, Sangilimuthu AY, Sudarsanam D. Antimicrobial activity of *Moringa oleifera* Lam. root extract. J. Pharm. Res. 2011; 4: 1426-1427.
72. Renu S, Manvi M, Sapna B. Evaluation of antibacterial potential of stem bark of *Moringa oleifera* Lam. The Biosc. 2010;1: 89-94.
73. Bolin C, Satyabrat G, Antibacterial activities of the methanolic extract of stem bark of *Spondias pinnata, Moringa oleifera* and *Alstonia scholaris*. Asian J. Trad. Med. 2011; 6: 163-167.
74. Das J, Biswas SK, Chowdhury A, Sharif SR, Hannan MA,. *In vitro* antibacterial and antifungal potentials of petroleum ether extract of *Moringa oleifera*. J. Pharmacol Toxicol. 2012; 7; 110- 113.
75. Chitravadivu C, Bhoopathi M, Balakrishnan V, Elavazhagan T, Jayakumar S.Antimicrobial activity of Laehiums prepared by herbal venders, South India. Am. Euras. J. Sci. Res. 2009; 4: 142-147.

76. Catauro M, Raucci MG, De Gaaetano FD, Marotta A. Antibacterial and bioactive silver-containing $Na_2O.CaO.2SiO_2$ glass prepared by sol– gel method. J. Mater. Sci. Mater. Med. 2004; 15:831-837.
77. Howey DC, Bowsher RR, Brumelle RL, Woodworth JR. [Lys (B28), Pro (B29)] – Human insulin: a rapidly absorbed analogue of human insulin, Diabetes, 1994; 43:396-402.
78. Howey DC, Bowsher RR, Brumelle RL, Woodworth JR. [Lys (B28), Pro(B29)] – Human insulin: effect of injection time on postrandial glycemia, Clin. Pharmacol. Ther. 1995; 58: 459.
79. Terbraak EW, Woodworth JR, Bianchi R, Cerimele B, Erkelens DW, Thijssen JH,et al. Injection site effects on the pharmacokinetics and glucodynamics of insulin lispro and regular insulin. Diabetes Car. 1996; 19: 1437- 1440.
80. Kang S, Brange J, Burch A, Volund A, Owens DR.. Comparison of subcutaneous soluble human insulin and insulin analogues (AspB9, GluB27; AspB10; AspB28) on meal- related plasma glucose excursions in type I diabetic subjects, Diabetes Car. 1991; 14: 571 – 577.
81. Campbell RK, Campell LK White JR. Insulin lispo: It's role in the treatment of diabetes mellitus. Ann. Pharmacother. 1996; 30:1263 – 1271.
82. Langer R. Biomaterials in drug delivery and tissue engineering: One laboratory's experience, Accounts of Chem. Res. 2000; 33: 94-101.
83. Bhadra D, Bhadra S, Jain P Jain NK. Pegnology: A review of PEG-ylated systems. Pharmazie 2002; 57: 5-29.
84. Balantrapu K, Goia D. Silver nanoparticles for printable electronics and biological applications, J. Mater. Res. 2009; 24, 2828-2836.
85. Tripathi RM, Antariksh S, Nidhi G, Harsh K, Singh RP. High antibacterial activity of silver nanoballs against *E. coli* MTCC 1302, *S. typhimurium* MTCC 1254, *B. subtilis* MTCC 1133 and *P. aeruginosa* MTCC 2295. Digest J. Nanomater. Biostruct. 2010; 5: 320- 330.
86. Kuber C, Souza SF Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*, Colloids Surf. B 2006; 47:160-164.
87. Lengke M, Southam G. Bioaccumulation of gold by sulfate-reducing bacteria cultured in the presence of gold (I) - thiosulfate complex. Acta 2006; 70: 3646-3661.
88. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J. Colloid Interface Sci. 2004; 275: 496-502.
89. Prasad TNVKV, Elumalai EK. Biofabrication of Ag nanoparticles using *M. oleifera* extracts and their antimicrobial activity. Asian Pacif. J. Trop. Biomed. 2011; 439 – 442.
90. Khanuja SPS, Arya JS, Tiruppadiripuliyur RSK, Saikia D, Kaur HSingh M. Nitrile glycoside useful as a bioenhancer of drugs and nutrients, process of its isolation from *Moringa oleifera*. United States Patent 6,858,588; 2005. .
91. Pal A, Bawankule DU, Darokar MP, Gupta SC, Arya JS, Shanker K, Gupta MM, Yadav NP, Singh Khanuja SP. Influence of *Moringa oleifera* on pharmacokinetic disposition of rifampicin using HPLC-PDA method: a pre-clinical study. Biomed. Chromatogr. 2010; 25:641-645.
92. Mahmood KT, Tahira M, Ikram UH. *Moringa oleifera*: a natural gift-A review. J. Pharm. Sci. Res. 2010; 2: 775-781.
93. Sachan D, Jain SK Nandlal S. I*n-vitro* and *in-vivo* efficacy of *Moringa oleifera* plant constituents in urolithiasis as antilithiatic drug. Int. J. Pharmac. Sci. Res. 2011; 2:1638- 1644.
94. Patel S, Thakur AS, Chandy A, Manigauha A. *Moringa oleifera*: A Review of their medicinal and economical importance to the health and nation. Drug Inv. Today 2010; 2: 339-342
95. Mishra G, Singh P, Verma R, Kumar S, Srivastav S, Jha KK et al. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. Der Phamacia Lettre, 2011; 3: 141 – 164.
96. Goyal RB, Mehta AA, Agrawal BB Goyal KR. Phyto-pharmacology of *Moringa oleifera* Lam.ó an overview. Nat. Prod. Rad. 2007; 6: 347-353.

97. Mekonnen N, Houghton P, Timbrell J. The toxicity of extracts of plant parts of *Moringa stenopetala* in HEPG2 cells *in vitro*. Phytother. Res. 2005; 19: 870–875.
98. Mekonnen Y. Effects of ethanol extract of *Moringa stenopetala* leaves on guinea pig and mouse smooth muscle. Phytother. Res. 1999; 13: 442-444.
99. Makonnen E, Hunde A, Damecha G. Hypoglycaemic effect of *Moringa stenopetala* aqueous extract in rabbits, Phytother. Res. 1997; 11: 147-148.
100. 101. Ghebreselassie D, Mekonnen Y, Gebru G, Ergete W, Huruy K. The effects of *Moringa stenopetala* on blood parameters and histopathology of liver and kidney in mice. Ethiop. J. Health Dev. 2011; 25: 51-57.
101. Kale S, Gajbhiye G, Chaudhari N. Formulation and *in- vitro* Evaluation of *Moringa concanensis*, Nimmo. Seed Oils Sunscreen Cream. Int. J. PharmTech. Res. 2010; 2: 2060-2062.
102. Jayabharathi M, Chitra M. Evaluation of anti-inflammatory, analgesic and antipyretic activity of *Moringa concanensis* Nimmo, J. Chem. Pharmac. Res. 2011; 3: 802-806.
103. Anbazhakan S, Dhadapani R, Anadhakumar P, Balu S. Traditional medicinal knowledge on *Moringa concanensis* Nimmo of Perambalur District, Tamilnadu. Anc. Sci. life 2007; 24: 42-45.
104. Munyanziza E. *Moringa* drouhardii Jum. In: van der Vossen HAM and Mkamilo GS (Editors). PROTA 14: Vegetable oils/Oléagineux. [CD-Rom]. PROTA, Wageningen, Netherlands, 2007.
105. Gould GW. Biodeterioration of foods and an overview of preservation in the food and dairy industries. Int. J. Biodeter. Biodegr. 1995; 36:267- 277.
106. Roller S. The quest for natural antimicrobials as novel means of food preservation: status report on a European research project. Int. J. Biodeter. Biodegrad. 1995; 36:333-345.
107. Buker A, Uba A, Oyeyi TI. Antimicrobial profile of *Moringa oleifera* Lam. Extracts against some food – borne microorganisms. Bayer J. Pure Appl. Sci. 2010; 3:43-48.
108. Ware GW. Pesticides theory and application. *San Francisco*: Freeman 1883; 308.
109. Thacker JMR. An introduction to arthropod pest control.Cambridge, UK: 101 Cambridge Univ. Press, 2002; 343.
110. Marco GJ, Hollingworth RM, Durham W. Eds. Silent Spring Revisited, American Chemical Society, Washington, DC, 1987, 214.
111. Forget G, Goodman T, de Villiers A (ed). Impact of pesticide use on health in developing countries, Ottawa:International Development Research Centre, 1993,335.
112. Perry AS, Yamamoto I, Ishaaya I, Perry RY. Insecticides in agriculture and environment: Retrospects and prospects. Berlin: Springer-Verlag, 1998, 261.
113. National Research Council. The future role of pesticides in US agriculture. Washington, DC: National Academic Press, 2000, 30.
114. Foidl N, Makkar HPS, Becker K. The potential of *Moringa oleifera* for agricultural and industrial uses. In: "The miracle tree/ the multiple attributes of *Moringa*" (Ed. Lowell J Fuglie). CTA. USA, 2001, 45-76.
115. Ernst E. Harmless herbs? A review of the recent literature. Am. J. Med. 1998; 104:170- 178.
116. Newall CA, Anderson LA, Phillipsen DJ. Herbal Medicines: A Guide for Health Care Professionals, The Pharmaceutical Press, London, 1996, 296.
117. Addae-Mensah I. Towards a rational scientific basis for herbal medicine – A phytochemist's two-decade. Accra, Ghana University Press, 1992.
118. *Pereira* SMN. *Occurrence of accidents with toxic plants*. *Infarma 1992; 16 – 19*..
119. Gilbert B, Ferreira JLP, Almeida MBS, Carvalho ES, Cascon V, Rocha LM. The official use of medicinal plants in public health. Cieˆncia e Cultura, J. Braz. Assoc. Adv. Sci. 1997; 49: 339–344.
120. Simoes JA, Giraldo PC, Faundes A. Prevalence of cervicovaginal infections during gestation and accuracy of clinical diagnosis. Infections Obstet. Gynaecol. 1998; 6:122- 133.

121. Catalun˜a P, Rates SMK. The traditional use of the latex from *Euphorbia tirucalli* Linnaeus (Euphorbiaceae) in the treatment of cancer in south Brazil, Acta Hortic.1999; 501: 289–295.
122. Schenkel EP, Zannin M, Mentz LA, Sal B Irgang B. Poisonous plants. In: C.M.O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz and P.R. Petrovick. Pharmacognosy: the medicinal plant, Porto Alergre, University/ UFRGS and Florianopolis, Editor of UFSC, 2000, 755 -788.
123. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of *Moringa oleifera* in rats. J. Med. Plants Res. 2009; 3: 586-591.
124. Ashong JO, Brown DL. Acute toxicity of aqueous extracts of *Moringa oleifera* leaf in growing poultry. J. Anim. Sci. 2011; 89: 577-580.
125. Lambole V, Kumar U. Phytochemicals and acute toxicity of *M. oleifera* barks in rats. Int. J. Biochem. Res. 2011; 2: 548-553.
126. Kasolo JN, Bimenya GS, Ojok L, Ogwal-okeng JW. Phytochemicals and acute toxicity of *M. oleifera* roots in mice. J. Pharmacog. Phytother. 2011; 3: 38- 42.
127. Paul CW, Didia BC. The Effect of Methanolic Extract of *Moringa oleifera* Lam. Roots on the histology of kidney and Liver of guinea pigs. Asian J. Med. Sci. 2012; 4: 55-60.
128. Ferreira PMP, Carvalho AFU, Farias DF, Cariolano NG, Melo VMM, Queiroz MGR, et al. Larvicidal Activity of the Water Extract of *Moringa oleifera* Seeds Against *Aedes Aegypti* and its Toxicity upon Laboratory Animals. Ann. Braz. Acad. Sci. 2009; 81:207- 216.
129. Ayotunde EO, Fagbenro OA, Adebayo OT. Toxicity of aqueous extract of *Moringa oleifera* seed powder to Nile tilapia *Oreochromis niloticus* (LINNE I779), fingerlings. Int. Res. J. Agric. Sci. Soil Sci. 2011; 1:142-150.
130. Oluduro AO, Aderiye BI. Effect of *Moringa oleifera* seed extract on vital organs and tissue enzymes activities of male albino rats. Afr. J. Microbiol. Res. 2009; 3:537-540.
131. Ajibade TO, Olayemi FO, Arowolo ROA. The haematological and biochemical effects of methanol extract of the seeds of *Moringa oleifera* in rats. J. Med. Plants Res. 2012; 6: 615-621.
132. Maria K, Mohammed A. Phytochemical investigation of the stem bark of *Moringa oleifera* Lam. Int. J. Res. Ayurv. Pharm. 2011; 2: 1577-1579.
133. Usman MRM, Barhate DS. Barhate. Phytochemical investigation and study of anti- inflammatory activity of *M. oleifera* Lam. Int. J. Pharmac. Res. Dev. 2012; 3: 114- 119.
134. Manivasagaperumal R, Vonoth B, Balamurugan S. Phytochemical analysis and antibacterial activity of *Moringa oleifera* Lam. Int. J. Res. Biol. Sci. 2012; 2: 98 – 102.
135. Mekoya M. Hypotensive effects of aqueous extract of *Moringa stenopetala* in both *in vivo* and *in vitro* animal models. M. Sc. Thesis. Addis Ababa University, School of Graduate Studies, 2007.
136. Kjaer A, Malver O, El-Menshawi B, Reisch J. Isothiocyanates in myrosinase-treated seed extracts of *Moringa peregrine*. Phytochem. 1979; 18: 1485-1487.
137. Dayrit FM, Alcantar AD Villasenor IM . Studies on *Moringa oleifera* seeds, Part I: The antibiotic compound and its deactivation in aqueous solution. Philippine J. Sci. 1992; 119: 23.
138. Ravichandran V, Arunachalam G, Subramanian N, Suresh B. Pharmacognostical and phytochemical investigations of *Moringa concanensis* (Moringaceae) an ethno medicine of Nilgiris. J. Pharmacog. Phytother. 2009; 1: 76-81.
139. Farnsworth NR. Biological approaches to the screening and evaluation of natural products. In: Rasoanaivo P, Ratsimamanga-Urverg S, (eds.) Biological Evaluation of Plants with Reference to the Malagasy Flora,Monograph from the IFS- NAPRECA Workshop on Bioassays. Madagascar, 1993, 35– 43.