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REVIEW ARTICLE

A Review on the Phytochemical Profile and the Antibacterial Susceptibility Pattern of Some Clinical Isolates to the Ethanolic Leaves Extract of *Moringa oleifera* LAM (*Moringaceae*).

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Abstract

Several reports on the phytochemical screening and antibacterial assays on *M. oleifera* leaves extract have been documented but none of the reports showed the fractionation and separation of the phytoconstituents by solvent extraction and thin layer chromatographic procedures, respectively. The ethanolic leaves extract of *M. oleifera* of the family *moringaceae* was reviewed for the antibacterial susceptibility pattern, preliminary phytochemical analysis, solvent extraction and TLC analysis, which were carried out using standard procedures. The results of the preliminary phytochemical screening revealed the presence of saponins, condensed tannins, flavonoids, terpenoids, steroids, phenolics, alkaloids, phlobatannins, cardiac glycosides and reducing sugars. The TLC separation of the phytoconstituents using chloroform-methanol solvent system resolved the fractionated extract into compounds with R_f values; 0.32, 0.53, 0.54, 0.55, 0.69, 0.89, 0.95 and 0.97. The antibacterial susceptibility pattern portrayed broad activity spectrum against the test microbes with comparable inhibitory zones by standard antibiotics. The MIC ranged between 0.1mg/ml and 90mg/ml for all the organisms. The results from this review have shown the antibacterial susceptibility pattern of *M. oleifera* implying that the extract could help as a chemotherapeutic agent or might be a lead compound for the development of new efficacious antibacterial agents.

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Introduction

Medicinal plants are plants whose extracts could be used directly or indirectly for the treatment of different diseases and the use of tradomedicine and medicinal plants in most developing countries as a basis for the maintenance of good health has been observed. According to the World Health Organization(WHO, 1977), medicinal plants are described as plants containing one or more structural organs with active principles that could be used for therapeutic purposes, as lead compounds or precursors for drug synthesis. Many pharmaceutical preparations are based on plants. An analogy could be drawn from the Chinese herb, *Artemisia annua* L.,

which had been a useful chemotherapeutic agent for plasmodiasis. From the plant was isolated a phytochemical constituent called artemisinin, a sesquiterpene lactone with an intramolecular peroxidic linkage.

Nature has blessed us with numerous great things. Aside the sun, moon, stars, mountains, oceans, etc., that are great and also look great, there are things that are deceptively simple and unassuming but are capable of doing enormous good to us that even a detailed study may not tell fully their greatness. One such thing is *M. oleifera* Lam. plant, the miracle tree of life. Rev. 22:1-3 says "and He showed me a pure river of water of life in the midst of the street of it and on either side of the river was there a tree of life

which bare twelve manner of fruits and yielded her fruit every month; and the leaves of the tree were for the healing of the nations, and there shall be no more curse”, amen.

There are global problems of multiple antibiotics resistance (Albuquerque *et al.*, 2007) as well as the emergence of new diseases and the resurrection of previously eradicated diseases. Most of the current antimicrobial agents simply induce bacteriostasis and some of them are very toxic to the hepatic, renal, haematopoietic and the central nervous systems (Tatli and Akdemir, 2005). Antimicrobial resistance among enteric pathogens is becoming a matter of serious concern (El Mahmood *et al.*, 2008) and poses a great threat to global human health. Moreso, new microbial strains are being continuously discovered, which are refractory to the current arsenal of drugs (Erturk *et al.*, 2006). This is because antimicrobial resistance leads to therapeutic failures of empiric therapy (Parekh and Chanda, 2007). As a result, it has become imperative to combat the emerging and re-emerging infectious diseases with a view to discovering and inventing new agents of greater therapeutic profile to mitigate frequent outbreaks of diseases which had posed a new threat to the global health security (Mohanta *et al.*, 2007).

An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several chemotherapeutic agents from these plants. The use of medicines from plants in the form of local medicine had been an age-long practice and the medicinal values of these plants may be due to the presence of minute doses of bioactive principles called phytochemicals.

The continuous evolution of bacterial resistance to the currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds to which these microbes are yet to develop resistance (El-Mahmood *et al.*, 2008). With the rising problems of side effects, cost and limited efficacy of antibiotic drugs, there is an urgent need for the development of alternative antimicrobial substances and researchers are nowadays turning to natural products from plants (Nitta *et al.*, 2002), as their main source of bioactive compounds with antimicrobial properties to complement the existing synthetic antimicrobials that are gradually becoming less potent against pathogenic microorganisms possibly due to impaired antimicrobial influx and efflux pump mechanisms, altered binding sites or the acquisition of mutant genes (Omojate C.G., 2012). Hence, there is the need for a review on the phytochemical profile and the antibacterial

susceptibility pattern of some clinical isolates to the ethanolic leaves extract of *M. oleifera*.

M. oleifera Lam. is the most cultivated species of a monogeneric family, the *moringaceae* that is native to the sub-Himalayans regions of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree, also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, Baijhan, Mulangay, Sajna or Ben oil tree, was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated in the tropical and subtropical regions (<http://www.tfljournal.org/article.php/2005/201124931586>).

In the 1940s, a team from the University of Bombay, Travancore University and the Department of Biochemistry at the Indian Institute of Science in Bangalore, identified a compound which dissociates into two molecules of benzylisothiocyanate and have antimicrobial activities. However, an *in vitro* bacterial cultures and observational studies provided a plausible mechanistic underpinning for the plethora of efficacy claims on *M. oleifera*.

In a common sight in rural parts of India, the plant is justifiably described as the miracle tree. It is known as murungai in Tamil, Nadu and Kerala. In East Africa, it is described as mother's best friend (<http://www.dynamicyouth.org/index.php?>). The plant has an impressive range of medicinal uses with high nutritional value. Different parts of this plant such as the leaves, roots, seeds, barks, flowers, and immature pods contain a quantum of crucial phytoconstituents such as tannins, saponins, alkaloids, steroidal aglycones, reducing sugars, terpenoids, and so on, that act as a cardiac and circulatory stimulants, possess antitumour, antipyretic, anticonvulsant, anti-inflammatory (Kumar, *et al.* 2009), antiulcer, antispasmodic, antidiabetic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, antifungal, abortifacient, antibacterial, (Anwar and Rashid, 2007; Ghebremichael, *et al.*, 2005; Lockette *et al.*, 2000; Walter *et al.*, 2011), antiretroviral, antispeticemic, antidiarrhoeal, hepatorenal disorders, cardiovascular, gastrointestinal and haematological disorders (Paliwal *et al.*, 2011), anxiety, asthma, bronchitis, cough, diarrhoea, conjunctivitis, cephalgia, arthralgia, psoriasis, semen deficiency, helminthiasis, lactation, pregnancy and diabetes (Nikkon *et al.*, 2003), and they are being employed for the treatment of different ailments in the indigenous system of medicine (Posmontier, 2011; Fahey, 2005; Fakurazi *et al.*, 2008). The plant contains more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than banana, lowers blood sugar in diabetes and

lowers blood pressure in hypertension (Fuglier, 1999). This is why *M. oleifera* is praised as

nutritional and medicinal cornucopia (<http://www.itdnow.co/benefits>).

Plant Classification (Taxonomy)

Kingdom	Plantae — plants
Subkingdom	Tracheobionta — vascular plants
Superdivision	Spermatophyta — seed plants
Division	Magnoliophyta — flowering plants
Class	Magnoliopsida — Dicotyledons
Subclass	Dilleniidae
Order	Cappareles or Violes
Family	Moringaceae — Horseradish tree
Genus	Moringa Adans — Moringa
Species	<i>M. oleifera</i> Lam — Horseradish tree

(<http://www.plants.usda.gov/javalprofile?symbol=moo1>)

A pharmacognostic profile of *M. oleifera* is described below:

Botanical Name:	<i>M. oleifera</i> Lam.
Family Name:	Moringaceae
Common Names	Moringa, Horseradish tree, Drumstick tree, Sahijan.
Nigerian Names:	
Yoruba:	Ewe ile, ewe Ighale, Adagba malero, Idagbo monoye (the tree that grows crazily).
Hausa:	Bagai-uwar maka, Bagaruwar maser, Barambo, Koraukin zaila, Shipka hali, Shuka halinka, Rimin nacara, Rimin turawa, Zogall, Zogalla-gandi.
Ibo:	Odudu oyibo, Okwe oyibo.
Fulani:	Gawara, Habiwal hausa, Konamarade, Rini maka, Konamarade (Imnakoya, 2005).

Moringa Species

M. oleifera, *M. arborea*, *M. drouhardii*, *M. peregrine*, *M. longituba*, *M. rivaie*, *M. borziana*, *M. concanensis*, *M. hildebrandtii*, *M. ruspoliana*, *M. stenopetala*, *M. ovalifolia*, *M. pygmaea* (Mahmood *et al.*, 2012).

Botanical Description

M. oleifera Lam. is a slender softwood tree that branches freely and can be extremely fast-growing. Although it can reach 3 heights in excess of 10 metres and a diameter of 0.2-0.4m, it is considered a small-to-medium-size tree (Raovich T., 2009). The stem is normally straight but occasionally is poorly formed. The tree grows with a short, straight stem

that reaches a height of 1.5-2m before it starts branching but can reach up to 3m (Foidl *et al.*, 2001).

The extended branches grow in a disorganised manner and the canopy is umbrella-shaped. The tripinnate compound leaves are feathery with green to dark green elliptical leaflets 1 long. The alternate, twice or thrice pinnate leaves grow mostly at the branch tips. They are 20-70cm long, greyish downy when young, long petiole with 8- 10 pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, 1-2cm long (Morton, 1991).

The flowers are conspicuous, lightly fragrant borne on inflorescences 10-25cm long, and are generally

white-to-cream coloured, 2.5cm in diameter, borne in sprays with five at the top of the flower, although they can be tinged with pink in some varieties. The flowers which are pleasantly fragrant and 2.5cm wide are produced profusely in axillary, drooping panicles 10-25cm long (Sachan *et al.*, 2010). They are white or cream coloured and yellow-dotted at the base. The five-reflexed sepals are linear-lanceolate. The five petals are slender-spatulate. They surround the five stamens and five staminodes and are reflexed except for the lowest (Proyecto B., 1996).

The fruits are trilobed capsules and are frequently referred to as pods. Immature pods are green and in some varieties have some reddish colour. Pods are pendulous, brown, triangular, splitting lengthwise into three parts when dry, 30-120cm long, 1.8cm wide containing about 20 seeds embedded in the pith, pod tapering at both ends, 9-ribbed. The seeds are round with a brownish semi-permeable seed hull, with 3 papery wings. Seed hulls are generally brown to black, but can be white if kernels are of low viability. Viable seeds germinate within two weeks (Paliwal *et al.*, 2011).

Ethnobotanical Distribution

M. oleifera of the *moringaceae* is native to the sub-Himalayan parts of India, Bangladesh, Afghanistan, and Pakistan (Sharma *et al.*, 2011). It is a fast-growing tree which was used extensively by the antiquated Greeks, Romans and Egyptians.

Presently, it is extensively cultivated in the tropics. The plant is a perennial softwood tree with timber of low quality, but which for years has gained traditional, medicinal and industrial applications in India, Ethiopia, the Philippines and the Sudan and it is now cultivated on a large scale in East, West and South Africa, Latin America, Tropical Asia, Florida, Caribbean and the Pacific Islands (Fahey J.W., 2005; Sachan *et al.*, 2010).

Phytochemical Constituents of *M. oleifera*

Investigations into the phytochemical constituents of *M. oleifera* revealed the presence of an array of unique compounds with several medicinal, nutraceutical and pharmaceutical properties. The compounds include rhamnose, glucosinolates and isothiocyanates, 4-(4'-O-acetyl-alpha-L-rhamnopyranosyloxy) benzylisothiocyanate, niazimicin, pterygospermin, benzylisothiocyanate, and 4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolate, minerals, vitamins such as carotenoids (including pro-vitamin A). (Fahey W.J., 2005). However, the presence of alkaloids such as moringine, moringonine and spirachin had been documented (Gianina M.A., 2003).

In the table below is contained some phytoconstituents in *M. oleifera* found in various parts of the plant.

Table 1: Some Phytoconstituents of *M. oleifera* (Kamal M., 2008)

Plant Part	Phytoconstituents
Root	Moringine, moringinine, spirachin, 4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolate and benzylglucosinolate 10.
Bark	4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolate 10.
Gum exudates	D-galactose, L-rhamnose, L-arabinose, D-glucuronic acid, D xylose, D-mannose and Leucoanthocyanin 12-13.
Stem	B-sitosterol 11, 4-hydroxymellein, Vamchin octacosanic acid and beta-sitosterone.
Leaves	Niaziminin A and B 14-15, Niazirin, niazirin, and 4-(4'-O-acetyl- alpha -L-rhamnosyloxy).
Pods	Isothiocyanate, Nitrites, thiocarbamates, 0- (1 heptenyloxy) propyl undecanoate, 0-ethyl-4-(alpha-L-rhamnosyloxy) benzyl carbamate, methyl-P-hydroxybenzoate and Beta – sitosterol 14-15.
Flowers	Protein, D-mannose, D-glucose, polysaccharide 16, quercetin, isoquercitin, kaempferol, kaempferitrin and ascorbic acid.
Seeds	Crude fat, crude protein, methionine, cysteine, 4-(alpha-L- rhamnopyranosyloxy) benzylglucosinolate, Moringine, benzylglucosinolate, di-oleic triglyceride 10 and mono-palmitic acid.
Seed oil	β -carotene, Vitamin A.

General Applications of *M. oleifera*

M. oleifera is a plant with many uses. According to Fuglie L.J. (1999) and Fahey J.W. (2005), the uses of the plant include but not limited to; as biogas (leaves), alley cropping (biomass production), animal forage (leaves and treated seed-cake), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed cake), foliar nutrient (leaves juice), green manure (leaves), gum (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 seedlings' damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). The seed oil (30-40% w/w) also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. The Ben oil is used in salads, as smooth motor lubricants and in perfumery and hair finishing products. In the West African countries such as Nigeria, pulverized seeds are used to remove contaminants present in water and its use in water purification is an age-long practice. However, the seeds could be eaten green, roasted, pulverised and steeped for tea.

Several phytochemical and antibacterial surveys on different plants have been carried out and the major chemical constituents of interest in these surveys were the alkaloids and steroidal saponins. However, other diverse groups of naturally occurring phytochemical constituents such as phenolics, saponins, flavonoids, terpenoids, tannins and reducing sugars, are present in varied amounts in the plant extracts.

In a research, the ethanolic leaves extract of *M. oleifera* of the family *moringaceae* was investigated for preliminary phytochemical analysis, antibacterial activity as well as the Stas-Otto solvent extraction and TLC analysis. These were carried out using standard procedures. Qualitative phytochemical screening of the crude extract was performed according to Parekh and Chanda (2007) to identify the various active chemical constituents. Alkaloids were detected by Mayer's reagent, Dragendorff's reagent and Wagner's reagent, while for cardiac glycosides, the Keller-Killiana test was carried out. Steroids were detected using the Salkowski's test and Liebermann- Burchard test. The frothing and emulsion tests were used to detect the presence of saponins. Terpenoids were detected using the Salkowski's reaction, reducing sugars using Fehling's reaction and tannins' detection was done using Ferric chloride solution. The results of the preliminary phytochemical screening revealed the presence of condensed tannins, flavonoids, alkaloids,

phenolics, terpenoids, phlobatannins, reducing sugars, saponins, steroids, and cardiac glycosides. The fractionation of the phytochemical constituents in the crude plant extract using solvent extraction procedures gave five fractions; A, B, C, D and E. On phytochemical screening of Fraction A, tannins were identified. Screening of Fraction B gave positive results with flavonoids, reducing sugars and phenolics, and Fraction C indicated the presence of steroidal aglycones. Fraction D gave positive results with alkaloids and phlobatannins while Fraction E showed the presence of saponins, phenolics and reducing sugars. The TLC analysis using chloroform-methanol solvent system resolved Fraction A into one component with R_f value 0.55, Fraction B into two components with R_f values 0.54 and 0.95, Fraction C into one component with R_f value 0.53, Fraction D into two components with R_f values 0.89 and 0.97, and Fraction E into three components with R_f values 0.32, 0.69 and 0.95. The antibacterial susceptibility pattern of *S. saprophyticus*, *E. coli*, *P. aeruginosa* and *K. oxytoca*, and the MIC as well as the MBC were also determined. The sensitivity-resistance pattern was found to be *K. oxytoca* (19mm), *E. coli* (17mm), *S. saprophyticus* (15mm) and *P. aeruginosa* (12mm) at 60mg/ml ethanolic extract concentration. The MIC ranged from 10mg/ml to 90mg/ml for all the organisms except *K. oxytoca* that was resistant at the various concentrations used. The result of the MBC of the MBC screening revealed that the MBC of the ethanolic leaves extract on *S. saprophyticus*, *E. coli*, and *P. aeruginosa* were 50mg/ml, 60mg/ml, and 90mg/ml respectively but there was no MBC on *K. oxytoca* at various concentrations utilized.

In a similar research, the antimicrobial profile of *M. oleifera* Lam. Leaves extracts against some food-borne microbes was studied. In that study, the chloroform and ethanol extracts of seeds and leaves of the plant were investigated for antimicrobial activity against some selected food-borne microbes as a test in the screening of the extracts for preliminary preservative properties on foods. The preliminary phytochemical screening and antibacterial assay were carried out using standard procedures. The results of the phytochemical screening revealed the presence of an array of phytoconstituents such as saponins, alkaloids, flavonoids, terpenoids, and reducing sugars, in the extracts. The antibacterial assay results showed that the ethanolic leaves extract exhibited broad spectrum of activity against the test organisms with *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes* susceptible. The study suggested that *M. oleifera* leaves extract have preservative potentials by inhibiting the growth of the test organisms which

range from food pathogens to spoilage-causing organisms in foods (Oyeyi *et al.*, 2010).

In another research, the fresh leaf juice and aqueous extract from the plant was found to inhibit the growth of *P. aeruginosa*, *S. aureus* and *Bacillus subtilis*. A compound 4-(alpha-L-rhamnosyloxy) benzylisothiocyanate isolated from the plant was reported to act on several bacteria (Caceres *et al.*, 1991). Rao and Kurup performed a series of experiments to establish pterygospermin isolated from the plant as a potent antimicrobial agent (<http://www.omicsgroup.org/journals/MAP/MAP-1-101.php>). Various compounds isolated from the leaves showed antibacterial properties (Nikken *et al.*, 2003). Recent findings have unveiled the cyanobactericidal potential in the seeds extract (Lurling M. and Beckman W., 2009).

In the 1940s, a team from the University of Bombay, Travancore University and the Department of Biochemistry at the Indian Institute of Science in Bangalore, identified a compound pterygospermin which dissociates into two molecules of benzylisothiocyanate, and have potent antimicrobial activities. However, *in vitro* bacterial cultures and observational studies provided a plausible mechanistic underpinning for the plethora of efficacy claims on *M. oleifera* (<http://www.tfljournal.org/article.php/20051201124931586>).

The antibacterial effects of *M. oleifera* extract against Gram negative and Gram positive bacteria have been studied. In the study, the antibacterial effects of the aqueous and ethanolic extracts from *M. oleifera* were examined against *S. aureus*, *Vibrio cholerae*, *E. coli* and *Salmonella enteritidis*. It was observed that *S. aureus*, *E. coli* and *Salmonella enteritidis*, were sensitive to the extract from the plant (Fernandes *et al.*, 2009).

The phytochemicals and uses of *M. oleifera* leaves in Ugandan rural communities have been documented. *M. oleifera* grown and used in many countries around the world is a multipurpose tree with medicinal, nutritional and socio-economic values. In Senegal land, moringa leaves are dispersed as powders at health facilities to treat moderate malnutrition in children. It established the medicinal uses of the plant in Uganda and identified phytochemicals present. It used quantitative and experimental methods to identify phytochemicals. The phytochemicals present include the alkaloids, steroids, tannins, anthraquinones, alkaloids, reducing sugars, saponins and triterpenoids. The presence of these phytochemicals in the extracts indicate possible prevention and curative properties of the leaves

(Kasolo *et al.*, 2010). In a similar research in Malaysia, studies on the phytochemical constituents of the plant revealed the presence of tannins, alkaloids, flavonoids, saponins, phlobatannins, and terpenoids. The significance of these phytoconstituents with respect to the role of this plant in traditional medicine have been documented (Devi, T. 2009).

Moreover, the antibiotic activity of *M. oleifera* against *Helicobacter pylori* has been documented. In the study, cultures of the pylori were extraordinarily susceptible to the plant extract. The extract had antibiotic activity against *H. pylori* at concentration up to 1000-fold lower than those which had been used in earlier studies against a wide range of bacteria. The extension of this finding to human *H. pylori* infection is now being pursued in clinics and the extract containing the prototypical isothiocyanate had already demonstrated some efficacy in pilot studies (Galan *et al.*, 2004).

In another research, the antimicrobial activity of crude seed extract of *M. oleifera* was investigated by Thin Layer Chromatography (TLC) bioassay against *E. coli*, *P. aeruginosa*, *S. aureus*, *Cladosporium cladosporoides* and *Penicillium sclerotigenum*, most of which were prominently inhibited by an isolate with R_f values 0.92- 0.96. The characterisation and identification of the extract revealed the occurrence of three bioactive compounds; 4-(alpha-L-rhamnopyranosyloxy) benzylisothiocarbamate, methyl-N-4-(alpha-L-rhamnopyranosyloxy) benzylcarbamate and 4-(beta-D-glucopyranosyl) benzylthiocarbamide. All these compounds at 5mg/ml had very high bactericidal activity against some test organisms even at 2-hour contact period. It was observed that 4-(beta-D-glucopyranosyl)-1 → 4-alpha-L-rhamnopyranosyloxy benzylthiocarbamate was the most potent with about 100% toward *Bacillus cereus*, *E. coli* and *Salmonella typhi* and 99.2% inhibition towards *Shigella dysenteriae* within 4 hours of contact (Oluduro *et al.*, 2009).

Moreso, the antibacterial potential of *M. oleifera* seeds extract has been studied. In the study, the ethanolic extracts were used for the evaluation of the antimicrobial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli* and *S. typhi* and the minimum inhibitory concentration (MIC) was also determined. The sensitivity-resistance pattern was found to be *S. aureus* (9.0mm), *B. subtilis* (7.3mm), *P. aeruginosa* (7.2mm), *E. coli* (6.8mm) and *S. typhi* (4.5mm) at 50mg/ml ethanolic extract concentration (http://www.biotech_asia.org/abstractphp?vabid=856).

In another study, the antimicrobial activity of small protein of *M. oleifera* leaves was investigated and three fractions obtained from the leaves of *Moringa oleifera* using Sephadex G-25 column chromatography was found to exhibit strong antibacterial activity against some clinical isolates. The antibacterial action of minute peptides was tested against *E. coli*, *Kl. aerogenes*, *Kl. pneumoniae*, *S. aureus*, and *B. subtilis*. Fraction P1, P2 and P3 showed strong inhibitory activity against *E. coli*, *S. aureus* and *B subtilis* but clear zone of inhibition was also noted against *Kl. aerogenes* with peptide 1. Fraction P2 portrayed significant zone of inhibition against *Aspergillus niger*. Based on this research, it could be concluded that *M. oleifera* contains an array of phytochemicals with potent antibacterial activities (Dahot U.M., 1998).

The antimicrobial activity of *M. oleifera chloroform leaves* extract against some clinical isolates had been documented (Devendra, *et al.*, 2011). According to the researchers, *M. oleifera* Lam (Moringaceae) is a very useful tree in tropical countries. In folklore, and ayurvedic all parts of the tree used in different healing procedures for different diseases. This plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. The antimicrobial assay was carried out using the modified agar well diffusion. Chloroform extract of the plant leaves showed good antimicrobial property promising inhibition zone diameter (IZD) against wide range of pathogens like *E. coli* (IZD = 8.8 mm), *P. aeruginosa* (IZD =9.5 mm), *S. aureus* (IZD =6.2 mm), *S. pyogenes* (IZD =7.0 mm). *Aspergillus niger* (IZD =7.3 mm), *C. albicans* (IZD =6.2 mm) along with positive controls. It was concluded that this plant extract has good healing properties without side effects which when compared with synthetic antibiotics would be useful chemotherapeutic agent.

Katariya *et al.*, (2005) studied on the phytochemical and antimicrobial screening of *M. oleifera* Lam methanolic leaves extract. The preliminary phytochemical investigation was done for the identification of various phytochemical constituents present in the extracts and also subjected to antimicrobial activity for the assessment of inhibitory effects of the methanolic extracts of these plants against eight medically important pathogenic microbes by in vitro agar diffusion method. The results of the preliminary phytochemical studies revealed the presence of alkaloids, phenols, flavonoids, tannins, steroidal aglycones, and saponins in the plant extract under assay. In addition, the extract exhibited significant zone of inhibition and good antimicrobial activity against the majority of the selected strains of microbes such as *S. aureus*, *P.*

aeruginosa, *E. coli* and *Klebsiella pneumoniae*. These presented results that led to the conclusion that *M. oleifera* Lam, would be a novel source of antimicrobial agents. These secondary plant metabolites were most likely responsible for the observed activity of the plant parts. Results obtained in this work justified the medicinal uses to which the plant had been employed traditionally in recipes for infections.

Discussion

Due to several intricacies of modern or the orthodox antibiotics, there has been a significant shift towards alternative therapy and herbal remedies. Antibiotic screening on natural products obtained from *M. oleifera* used in the Complementary and Alternative Medicine (CAM) is a major thrust of research and development. Therefore, in a bid to discover new antimicrobials that would be effective against multi-drug resistant microbial strains, phytochemical screening and investigations into the antibacterial profile of the ethanolic leaves extract of *M. oleifera* were carried out. It is suggested that this plant drug would have enormous health benefits with little untoward effects that is common with the synthetic drugs. During the course of this research, investigations have afforded many phytochemicals with promising antibacterial. The extraction of the pulverized *M. oleifera* leaves with 70% v/v ethanol gave a yield of 11.83%. The preliminary phytochemical screening of the 5% w/v extract revealed the presence of terpenoids, cardiac glycosides, alkaloids, phenols, phlobatannins, flavonoids, saponins, condensed tannins and reducing sugars but cardiac glycosides and terpenoids were absent in the ethanolic extracts heated under reflux at 100°C with 3M hydrochloric acid for 3 hours. The absence of these phytochemicals in the hydrolysed extract might be attributable to the acid hydrolysis of the functional groups in the phytoconstituents. Phytochemical screening is based on colour formation, precipitate formation, interfacial film formation, frothing or emulsion production. Alkaloids contain one or more nitrogen on their heterocycles which may be primary (mescaline), secondary (Nicotine) or tertiary (Quinine). Alkaloidal reagents are presumed to react with the nitrogen on the heterocycles resulting in coloured precipitate formation. Tannins contain phenolic hydroxyl groups which are reduced by reducing agents such as ferric chloride solution resulting in a blue-black, brown or red precipitate formation. Reducing sugars such as rhamnose produce a deep blue or green coloration on addition of Fehling's solution due to the reduction of

copper sulphate present in Fehling's reagent to copper (II) oxide. Non-reducing sugars do not convert copper sulphate to the corresponding copper (II) oxide. This forms the basis for the colour change. Saponins form colloidal solutions on hydrolysis in water, hence, they foam upon shaking. Saponins may be neutral or acidic on the basis of the structural moiety. Foam formation is based on saponification, a reaction involving soap formation which serves as the emulgent. This might be the basis for the emulsion test. Naturally occurring glycosides in the presence of mineral acids undergo hydrolysis into sugars and aglycones. The glycone moiety is reduced by the ferric chloride solution in the presence of an acid giving a characteristic colour formation. However, steroids contain keto functional groups which are reduced by sulphuric acid in the Salkowski reaction. This reduction forms the basis for the colour change. Many reviews and articles reporting the antibacterial activities of flavonoids (Pretorius J.C., 2003; Khan *et al.*, 2003), anthraquinones (Cowan *et al.*, 2000), polyphenols and phenols (Hatano, *et al.*, 2000; Funatoyawa, *et al.*, 2004), and tannins (Tominaga *et al.*, 2002), have been published in recent years. Several phenolic compounds have been identified and isolated from plants and they have shown promising bacterial inhibiting properties against specific and broad spectrum of cultured as well as clinical bacterial strains including Methicillin-Resistant *Staphylococcus aureus* (MRSA), and multi-drug resistant bacteria. The presence of alkaloids such as moringine, moringinine and spirachin, as well as pterygospermin have been shown to demonstrate antibacterial activity (Gianina *et al.*, 2003). Alkaloids, phenols, flavonoids and glycosides have a number of biological activities and strong antibacterial potentials (Robbers *et al.*, 1996). Alkaloids have exhibited promising activity against *H. pylori* (Hadi and Bremner, 2001) and a number of other bacterial strains (Sinha *et al.*, 2001; Saeed and Sabir, 2001; Khan *et al.*, 2001; Kren and Martinkova, 2001;

<http://www.iupac.org/symposia/proceedings/phuket97/sener.html>). Similarly, a few glycosides have presented with antibacterial activities (http://www.medical_ias.org/11-1/Dahot.html). The antibacterial potential of terpenoids have been documented. Terpenoids are bioactive molecules which are a part of plants' defence mechanisms as phytoprotectants (Morrissey and Osbourn, 1999). The fractionation of the phytochemical compounds in the plant extract under study using solvent extraction procedures was also carried. This afforded five fractions — A, B, C, D and E. On phytochemical screening of fraction A (strong acid), tannins were identified. Screening of fraction B gave positive

results with flavonoids, reducing sugars and phenolics and fraction C (neutral portion) indicated the presence of steroidal aglycones. Fraction D (basic fraction) gave positive result with alkaloids, and phlobatannins, while fraction E (aqueous portion) showed the presence of saponin, phenolics and reducing sugars. Thin layer chromatographic analysis using chloroform-methanol solvent system resolved fraction A into one component with R_f value 0.55, fraction B into two components with R_f values 0.54 and 0.95, fraction C into one component with R_f value 0.53, fraction D into two components with R_f values 0.89 and 0.97, and fraction E into three components with R_f values 0.32, 0.69 and 0.95. The plant extract under study had varied extents of antibacterial activities which were concentration-dependent. The extract had the largest diameter zone of inhibition on *E. coli* and *K. oxytoca* at 60 mg/ml. Also, it was observed that an increase in concentration of extract resulted in diminished activity on *S. saprophyticus* while reverse was the case with *P. aeruginosa*. The antibacterial activities of the extract suggest that the plant could be of relevance in the treatment of infections caused by these organisms. Ofloxacin, the control antibiotic had greater diameter zone of inhibition on the test microbes than that of plant extract. However, there was no significant difference ($P > 0.05$) between the diameter zones of inhibition of the extract and the control antibiotic. The statistical analytical method utilized in comparing the diameter zones of inhibition between the plant extract and the control antibiotic is the analysis of variance (ANOVA). Studies have shown that the antimicrobial potential of *M. oleifera* leaves extract may be attributable to the presence of an array of phytochemicals. Following structural elucidation using mass spectroscopy and nuclear magnetic resonance spectroscopic procedures, the presence of a short polypeptide named 4-(alpha-L-rhamnosyloxy) benzylisothiocyanate was investigated. It was argued that the peptide might act directly on microbes and result in growth inhibition by disrupting cell membrane synthesis, a mechanism of action similar to the Beta Lactam and cephalosporin antibiotics, or the inhibition of the synthesis of essential enzymes (Bukar *et al.*, 2010; Suarez *et al.*, 2003). The MIC obtained shows that different concentrations were effective against some of the test organisms. The most susceptible organisms to the antibacterial activity of the extract was *E. coli* while the least susceptible was *P. aeruginosa*. *K. oxytoca* was resistant at the various concentrations utilised. The MBC determined revealed that at a concentration of 50-90mg/ml bactericidal effect was observed. The mechanism of action by which the phytochemical constituents of *M.*

oleifera exert their antibacterial activity might be attributable to bacterial enzyme inhibition such as the sortase inhibitory effect, DNA replication, bacterial toxin action (Fakai *et al.*, 2002) and causing the lysis of bacterial cells. It had been suggested that pterygospermin acts by the inhibition of the transaminase enzyme (<http://www.atgchecker.com/pubmed/20341204>) and through cell membrane perturbations. This, when coupled with the action of Beta lactams on the transpeptidation of the bacterial cell wall could lead to an enhanced antimicrobial effect of the combinations. Antimicrobial peptides probably interact with cellular membranes in two stages. Firstly, cationic amino acids are attracted by negative charges such as phospholipoidal groups on the surface. Secondly, hydrophobic acid and positively charged patches of the peptides interact with the aliphatic fatty acids and anionic components respectively. This induces membrane destabilization and bacteria are thought to be killed by the leakage of cytoplasmic contents, loss of membrane potential, change of membrane permeability, lipid distribution, the entry of peptides and the occlusion of anionic cell components or the actuation of autolytic enzymes. Tannins are polyphenols with pronounced ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes (Kamba and Hassan, 2010). Saponins might act by altering the permeability of cell walls and hence exert toxicity on all organised tissues. They exert some antibacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Moyo *et al.*, 2012). It was suggested that polyphenols such as gallic acids act possibly by binding to bacterial dihydrofolate reductase (DHFR) enzymes, inhibition of supercoiling activity of *E. coli* bacterial gyrase by binding to the ATP binding site of gyrase B and binds to bacterial DNA thereby inducing topoisomerase IV enzyme-mediated DNA cleavage and growth inhibition (Sakharkar *et al.*, 2010).

Conclusion

The phytochemical screening and investigation into the antibacterial potential of the ethanolic leaves extract of *M. oleifera* Lam leaves showed or highlighted the antibiotic spectra of the plant extract under assay, suggesting a promising lead as an alternative antibiotic and it yielded scientific support to their use in traditional Ayurvedic medicine. The plant extract was found to exhibit comparable antibacterial activity with the standard antibiotic - Ofloxacin against some clinical isolates. This

antibacterial potential was stated to be attributable to the presence of an array of bioactive principles such as alkaloids, tannins, flavonoids, terpenoids, cardiac glycosides, phenols, steroids, saponins and reducing sugars. The separation of the phytoconstituents in the extract was carried out using thin layer chromatography following fractionation by the Stas-Otto solvent extraction procedures. The results obtained from the preliminary phytochemical screening and investigations into the antibacterial potentials of the ethanolic extract from *M. oleifera* Lam leaves revealed the presence of an array of bioactive principles called phytochemicals whose antibacterial potentials were comparable with those of the standard antibiotics - Ofloxacin, a DNA gyrase inhibitor against the Gram positive and Gram negative bacterial strains tested.

In concluding, *M. oleifera* Lam leaves extract could be a promising naturally occurring antibacterial agent with potential applications in the pharmaceutical industry for controlling the pathogenic bacterial infections such as urinary tract infections (UTI), respiratory tract infections and Septicaemia caused by the test microbial strains used in this research.

Based on the current research, promising chemotherapeutic moieties such as *M. oleifera* extract should be subjected to further advanced studies such as in vitro assays, clinical trials, and descriptive toxicological assays such as those for teratogenicity, carcinogenicity and mutagenicity, the later being simulated using the AME'S TEST. Candidate compounds identified from the extract under study should be analysed retro-synthetically in a bid to identifying and elucidating the readily available and/or accessible lead or starting materials for the pharmaceutical industry. For the future of chemotherapy, the pharmacokinetics, mechanisms of action and resistance should be taken into account in the appraisal of novel antimicrobials such as *M. oleifera* Lam leaves extract.

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