Antibacterial properties of *Moringa oleifera*

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Received 22 August 2017; Received in revised form 12 November 2018; Accepted 7 December 2018

**ABSTRACT**

*Moringa oleifera*, considered a medicinal tree by many civilizations, owes its repute to its medicinal attributes, and in particular, to its antimicrobial properties. Different parts of this plant, including pods, stem bark, leaves, roots, and seeds contain bioactive agents such as quercetin, phenolic acids, alkaloids, flavonoids, etc. The modes of action of these bioactive agents are varied, ranging from the inhibition of enzymes to the disruption of bacterial cell membranes. The various antimicrobial properties of the plant are manifested in extracts of methanol, ethanol, hexane, chloroform, and water. Such extracts, which also display antibacterial activity against biofilms, can serve as therapeutic agents against multidrug resistant pathogens, including both Gram-negative and Gram-positive bacteria.

*Keywords:* *Moringa oleifera*, antimicrobial properties, Gram-positive bacteria, Gram-negative bacteria, bioactive agent

**INTRODUCTION**

Plants have been the source of many natural antimicrobial compounds since the history of mankind (Arora and O'shone, 2014). An example of such plants is *Moringa oleifera* Lam. (synonymous to *Moringa pterygosperma* Gaertn), a highly valued medicinal plant that is often considered a "miracle tree". It belongs to the monogenic plant family Moringaceae and grows to a height of 5-10 meters (Morton, 1991).

It consists of 33 species, among which *M. oleifera* and *Moringa peregrine* (forsk) flor, are well known species. The synonym of *M. oleifera* is *M. pterygosperma* Gaertn. Other names used for *M. peregrine* include *Moringa aptera* Gaertn., *Moringa arabica* (Lam.) Pers., *Moringa zeylanica* Sieb. and *Balanusmyrepiscia* Blackm. (Lalas et al., 2012; Arora and O’shone, 2014).

The tree is widely distributed (Nadkarni, 1976; Ramachandran et al., 1980). It is commonly found near sandy beds of rivers and water bodies (Qaiser, 1973), but it adapts to a wide range of environments, ranging from the humid tropics to hot dry lands. Accordingly, it is little affected by drought (Morton, 1991) and thrives in rainfall between 250 mm to 3000 mm. It tolerates both acidic and alkaline soils, adapting to soil pH of 5.0-9.0 (Palada and Changi, 2003; Anwar et al., 2007). While the tree is found in the wild, *Moringa* can be cultivated easily in household gardens.

According to Arora et al. (2013), the history of *Moringa* dates back to 150 B. C. It is mentioned in ancient scriptures of the Egyptians, Greek, Romans, and Indians. The tree was used by ancient kings and queens in their royal diet to maintain good physical and mental health. Its leaves were given to ancient Maurians of India to remain fit and healthy in times of war. Having been used as a medicinal tree in more than 80 countries, *Moringa* has been reported in more than 200 local languages in the literature (Mossa, 1985; Booth and Wickens, 1988; Nikkonet al., 2003; Arora et al., 2013).

The taxonomic classification of *M. oleifera* is given below.

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

Taxonomic classification of *M. oleifera* (Arora et al., 2013).
The occurrence of Moringa is widespread. It has been reported in Pakistan, India, Asia minor, Africa and Saudi Arabia (Mughal et al., 1999), the Philippines, Cambodia, Central, North and South America, and the Caribbean (Morton, 1991; Anwar et al., 2007).

As a “miracle tree” (Jerri et al., 2012), different common names are used for Moringa in different countries. In Pakistan, it is called as “Sohanjana” (Qaiser, 1973; Anwar et al., 2005), whereas in the Nile valley, people called it “Shagara al Rauwaq” which means “tree for purifying” (von Maydell, 1986). Other names include the drumstick tree, horse radish tree, kelor tree, and medicinal tree (Anwar and Bhanger, 2003; Anwar et al., 2007).

**MORINGA OLEIFERA AS A MEDICINAL TREE**

Plants produce primary and secondary metabolites (Croteau et al., 2000; Arora et al., 2013) that have applications for different purposes, e.g. curing diseases, healing injuries, etc. Such secondary metabolites are bioactive compounds that include alkaloids, phenolics, tannins, phytosterols and terpenoids (Balandrin et al., 1985; Arora et al., 2013). The importance of different parts of the Moringa plant as a source of these useful compounds cannot be ignored (Walter et al., 2011). In many cultures, they would be considered a part of “natural nutrition”. The flowers, leaves, seeds, pods, stem, and bark of the tree have high nutritional value (D’souza and Kulkarni, 1993; Anwar and Bhanger, 2003; Anwar et al., 2005; Anwar et al., 2007).

**Medicinal properties of M. oleifera**

All parts of M. oleifera plants possess medicinal properties. These are discussed briefly below. Figure 1 shows the M. oleifera tree and its parts.

**Leaves**

The leaves of M. oleifera are a rich source of calcium, potassium, protein, β-carotene, antioxidants (ascorbic acid, flavonoids, phenolics, carotenoids) (Dillard and German, 2000; Siddhuraju and Becker, 2003). Their inclusion in the diet increases milk production in women, and hence, the tree is often referred to as “mother’s best friend” in the Philippines. Some doctors also recommend Moringa leaves to anemic patients (Estrella et al., 2000; Siddhuraju and Becker, 2003). Another medical application of Moringa leaves is as a treatment for fevers, piles, bronchitis, sore throat, ear and eyes infections, catarrh. They are also used to control blood sugar levels, reduce swelling of glands, and as a purgative. The leaves are rubbed on the temples for headaches and applied as a poultice to sores (The Wealth of India, 1962; Dahot, 1988; Morton, 1991; Makonnen et al., 1997; Fuglie, 1999; Anwar et al., 2007).

**Figure 1**: Figure showing M. oleifera tree and its different parts; (a) leaves (b) flowers (c) stem bark (d) gum (e) seeds (f) roots (g) tree.

**Roots**

Known for their medicinal properties (The Wealth of India, 1962; Padmarao et al., 1996; Dahot, 1988; Ruckmani et al., 1998; Anwar et al., 2007), Moringa roots are useful for the treatment of numerous ailments, e.g. paralytic afflictions, rheumatism, inflammations, articular pains, lower back or kidney pain and constipation. Besides being useful as a laxative, they are also act as an antilithic agent, helping the body to prevent the formation of stones in the gall bladder and kidney, as well as aiding the body in their removal. Moringa roots are also used as a carminative agent, they help to prevent or relieve flatulence. Moringa roots are useful also as antifertility, anti-inflammatory, and abortifacient agents (Anwar et al., 2007).

**Bark**

The bark of the Moringa stem is used to cure eye diseases. It is used also for the treatment of delirious patients, as a medication to prevent enlargement of the spleen, formation of tuberculous glands of the neck, and
as an agent that destroys tumors and heals ulcers. The juice from the root bark is put into the ears to relieve earaches and also placed in a tooth cavity as a pain killer. It is reputed to have anti-tubercular activity (Bhatnagar et al., 1961; Siddharaju and Becker, 2003; Anwar et al., 2007).

Gums

The gums extracted from Moringa are used for treatment of dental caries. It is an astringent. If the gum is mixed with sesame oil, it is effective for the relief of headaches, fevers, intestinal complaints, dysentery and asthma. It is sometimes used as an abortifacient, and to treat syphilis and rheumatism (Fuglie, 1999; Anwar et al., 2007).

Flowers

The flowers of Moringa are reported to possess high medicinal value as a stimulant, an aphrodisiac, an abortifacient and a chologogue. It is used to treat inflammation, muscle diseases, hysteria, tumors, enlargement of the spleen, and to lower serum cholesterol, phospholipids, triglycerides, VLDL, LDL cholesterol to achieve a desirable phospholipid profile. It improves the atherogenic index, decreases lipid profile in hypercholesterolaemic rabbits and increases the excretion of fecal cholesterol (Bhattacharya et al., 1982; Dahot, 1998; Mehta et al., 2003; Siddharaju and Becker, 2003; Anwar et al., 2007).

Seeds

Moringa seed extracts exerts their protective effect by decreasing liver lipid peroxides. They contain antihypertensive compounds Thio carbamate and isothiocyanate glycosides have been isolated from the acetate phase of the ethanolic extract of Moringa pods (Faizi et al., 1998; Lalas and Tsaknis, 2002; Anwar et al., 2007).

ANTIMICROBIAL SIGNIFICANCE OF M. OLEIFERA

The medicinal properties of this plant have been attributed to a wide range of antimicrobial compounds present in its tissues (Kostova and Dinchev, 2005; Vieira et al., 2010). The cell walls of Gram-negative bacteria do not allow passage of many antibiotics, and thus act as a barrier to their action (Tortora et al., 2001). Accordingly, Moringa extracts tend to be more effective against Gram-positive than Gram-negative bacteria (Kudi et al., 1999; Ali et al., 2001; Palombo and Semple, 2001; Vieira et al., 2010). The widely reported antimicrobial action of this plant (Siddharaju and Becker, 2003; Vaghasiya and Chanda, 2007; Mashiar et al., 2009; Walter et al., 2011) is mediated through the inhibition of enzyme activity or disruption of cell membranes (Siljestro et al., 2000; Suarez et al., 2003; Anwar et al., 2007). According to Cáceres et al. (1991), fresh leaf juice and aqueous seed extracts inhibit Pseudomonas aeruginosa and Staphylococcus aureus growth. Folkard and Sutherland (2005) find that Moringa seeds destroy Salmonella typhi living in the human intestine. Oil in the seeds contains the antibiotic pterygospermin (Aney et al., 2009) that form a thin film over the walls of the intestines, thus protecting them from penetration by microorganisms (Caceres and Lopez, 1991; Cáceres et al., 1991; Nwosu and Okafor, 1995; Walter et al., 2011). The Moringa seeds also contain a gum like compound which is antityphoid in nature (Fuglie, 1999; Harristoy et al., 2005).

Antimicrobial properties of different plant parts

As mentioned earlier, all parts of M. oleifera plant exhibit antimicrobial properties (Arora and Onsare, 2014) (Table 1). The following paragraphs outline the antibacterial properties in the different parts of the tree.

Pods

The pod husks are found to have antibacterial properties effective against S. aureus, Staphylococcus epidermidis, Salmonella typhimurium, E. coli and Klebsiella pneumoniae. The major phytochemicals found in the pods are flavonoids, alkaloids, tannins, triterpenes, diterpenes and cardiac glycosides. Among these, the antimicrobial compounds are flavonoids and diterpenes (Arora and Onsare, 2014). According to Nantanchit (2006), purified extracts of capsules have been found to be effective against both Gram-positive and Gram-negative bacteria. The vegetable peels of M. oleifera have been observed to be active against Enterobacter aerogenes, K. pneumoniae, S. aureus, Staphylococcus subflava, Proteus mirabilis, Cryptococcus luteolus, Clostridium rubrum, S. typhimurium (Chanda et al., 2010).

Roots

In different civilizations including India, Vietnam and Pakistan, Moringa roots are held in high esteem owing to their antibacterial properties (Rao et al., 2001). In 1957, Das et al. reported that the antibiotic compound pterygospermin from Moringa roots were found to exhibit antifungal as well as antibacterial properties. After the 1950s, different researchers all over the world reported various antimicrobial components from the Moringa root. For example, Eilert et al. (1981) reported 4-α-L-rhamnosyl oxybenzyl isothiocyanate as a major antimicrobial constituent. In 2003, Nikkon et al. stated that the aglycone of deoxy-niazimicine (N-benzyl, S-ethyl thioformate) isolated from the chloroform fraction of an ethanol extract of the root bark was responsible for antibacterial and antifungal activities observed. The root bark was also reported by Dewangan et al. (2010) to show antibacterial activity against S. aureus, E. coli, Staphylococcus gallinarum and P. aeruginosa. Raj et al. (2011) observed antimicrobial activity against P. aeruginosa, S. aureus, E. coli.
| Chemical structural classes | Major subclasses | Normal function | Antibacterial mode of action | References |
|-----------------------------|-----------------|----------------|-----------------------------|------------|
| Phenolics                   | Simple phenols  | Protection against oxidative stress | Inhibition of ATP production. Inhibition of enzymes (urease, proline dehydrogenase). Inhibition of nucleic acid synthesis i.e. DNA or RNA. | Tholl, 2006; Simões et al., 2009 |
|                             | Phenolic acids  | -              | -                           | Bumke-Vogt et al., 2014 |
|                             | Flavonoids      | Antioxidants, anti-inflammatory, and anticancer activities | -                           | Mirzoeva et al., 1997; Plaper et al., 2003; Tholl, 2006 |
|                             | Flavonols       | Attraction for pollinating insects to flowers, protection against UV damage (flavonoids, anthocyanins) | Quercetin-increase in permeability of inner bacterial membrane, dissipation of membrane potential, binding of GyrB subunit of E. coli DNA gyrase and inhibition of the enzyme ATPase activity. | Cowan, 1999; Brehm-Stecher and Johnson, 2003; Simões et al., 2008 |
| Terpenoids                  | Terpenoids      | Plant-environment interaction; also plant fragrance, an essential oil | Alteration of membrane permeability, disruption of cytoplasmic membrane. | Simões et al., 2008 |
| Essential oils              | Essential oils  | Aroma and fragrance | Alteration of ion transport processes. Inhibition of respiration. Disturbance of membrane embedded protein. Interference with membrane expansion. Increase in membrane fluidity and permeability. | Simões et al., 2008 |
| Alkaloids                   | Alkaloids       | Inhibition of multidrug resistance | Inhibition of efflux pumps, interaction with bacterial cytoplasmic membrane. | Gibbons and Udo, 2000; Simões et al., 2008 |
| Lectins                     | Lectins         | Pathogen invader. Involvement in plant physiology and functionality | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
| Polypeptides                | Peptides        | Signalling agents, working as intracellular chemical messengers | -                           | Bergey et al., 1996; Pearce et al., 2001 |
| Quinones                    | Quinones        | Pathogen invader. Involvement in plant physiology and functionality | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
| Tannins                     | Tannins         | Structural constitutive function, found in almost every plant part (bark, wood, leaves, fruits, roots). Responsible for astringent taste of wine or unripe fruit. Components of flower color and autumn leaves | -                           | Bergey et al., 1996; Pearce et al., 2001 |
| Polyacetylenes              | Coumarins       | Pathogen invader. Involvement in plant physiology and functionality | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
| Miscellaneous               | Polyketides     | -              | -                           | Bergey et al., 1996; Pearce et al., 2001 |
|                             | Polyamines      | -              | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
|                             | Isothiocyanates | -              | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
|                             | Sulfides        | -              | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
|                             | Thiosulfimates  | -              | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
|                             | Glycosides      | -              | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
|                             | Phenanthrenes   | -              | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
|                             | Stilbenes       | -              | -                           | Cowan, 1999; Dorman and Deans, 2000; Newman et al., 2000; Stavri et al., 2007; Simões et al., 2009 |
Flowers

Pterygosperminis also present in the flowers of M. oleifera. Thus, the flowers have antimicrobial properties which are effective against many microorganisms, including bacteria and fungi (Rao et al., 2001).

Bark

The stem of M. oleifera has been a drug of choice for treating urinary tract infection (UTI) as it is cheap and easily available (Chetia and Gogoi, 2011). It is reported to contain 4-hydroxyxymellin and octacosanoic acid (Faizi et al., 1994), two alkaloids (namely moringine and moriningine), vanillin, β-sitosterol, β-sitosterone, and phenolics (Kumbhar et al., 2012), procyanandin (Atawodi et al., 2010), and 4-[(1-hexamethylenoxy)-benzylic glucosinolate (Bones and Rossiter, 1996; Maurya and Singh, 2014). It showed prominent effect against E. coli (MIC 64 µg/mL), which is a major causative agent of UTI. Maurya and Singh, (2014) also concurred regarding its activity against E. coli (Chetia and Gogoi, 2011). Zaffer et al. (2014) found that the bark of M. oleifera has broad spectrum antibacterial properties effective against both Gram-positive and Gram-negative bacteria (Maurya and Singh, 2014). E. coli involved in UTI is killed by bark extract at MIC 64 µg/mL (Chetia and Gogoi, 2011; Maurya and Singh, 2014).

Leaves

The Moringa leaves contain protein that is reported to contain antimicrobial activity against E. coli, K. aerogenes, S. aureus and Bacillus subtilis (Dahot, 1998).

Seeds

Eilert et al. (1981) identified 4-alpha-rhamnosol-oxy-benzyl isothiocyanate being an active antimicrobial agent in M. oleifera seed powder which helped in reducing Most Probable Number (MPN) and Standard Plate Count (SPC) at a dosage of 100-150 mg/L (Mangale et al., 2012). According to Padia et al. (2012), M. oleifera contains the antibacterial compound 4-(α-L-rhamnosol-oxy) benzyl isothiocyanate. It was effective against Mycobacterium phlei and B. subtilis at concentrations of 12.5 µg/mL and 17.5 µg/mL respectively (Eilert et al., 1981). The antimicrobial peptides in seeds of Moringa are discussed further below.

Oil

A study on the chemical composition of oil from leaves via GC and GCMS analysis found that hexacosane (13.9%), pentacosane (13.3%) and heptacosane (11.4%) were the main constituents (Marruo et al., 2013). UPLC-DAD analysis also detected the presence of the flavonoids quercetin (126 µg/g) and luteolin (6.2 µg/g). The bacteria B. cereus and P. aeruginosa were found to be sensitive to Moringa oil, with luteolin showing biological and pharmacological properties like antimicrobial, antiallergic, anticancer, antiplatelet and antioxidant characteristics.

Antimicrobial peptide from seeds

The crushed kernels of Moringa seeds are reported to possess antimicrobial water-soluble peptides (Suarez et al., 2005). These proteins have residues at their terminals, which can bind to any suspended particulate matter, including microorganisms, present in the medium. One such well-known antimicrobial peptide (AMP) is Flo. The positive charge of this cation enables it to interact with the negative charges present on the surface of microorganisms. This allows upon the peptide the role of the first line of defense against many pathogens. The amphiphilic structures of the AMP allow their incorporation into the cellular membrane, leading to transmembrane channel formation and the consequent leakage of cellular components out of the cell. Depolarization of bacterial membrane follows, resulting in uneven distribution of lipids between cell membrane layers, leading eventually to intracellular damage (Zhang et al., 2001; Suarez et al., 2005). Cell death has been hypothesized in the following ways (Suarez et al., 2005).

Barrel Stave Model: By formation of pores by amphipatic helices of the peptide in the lipid bilayer, either in the lipid core region or aqueous environment within the pore (Boheim, 1974; Ehrenstein and Lecar, 1977; He et al., 1995).

Aggregate Channel Model: By formation of pores constituted by peptide and phospholipid heads from bent membrane (Matsuzaki et al., 1996; Oren and Shai, 1998).

Carpet Model: By a more general disorganization of the membrane hydrophobic and hydrophilic layers, leading to their disruption (Pouny et al., 1992; He et al., 1996; Bechinger, 1999; Heller et al., 2000).

The strong antimicrobial activity of the Moringa AMP is under investigation in many laboratories. Improvement to the AMP has been achieved either by mutating the primary sequence, for instance, to augment its amphipathic character (Dathe et al., 1996; Friedrich et al., 2000; Dathe et al., 2001; Tachi et al., 2002; Farnaud et al., 2004), or by multimerizing the active sequence (Azuma et al., 1999; Lee et al., 2002; Tam et al., 2002).

Recommended dosage

The recommended doses of different parts of M. oleifera plant are: intact form of seed: 5-10 grams, root bark: 2-5 grams, leaf (aqueous juice): 10-20 ml, powder of seed, root, bark, leaf, fruit and flower: 1-3 grams. (API; Mishra et al., 2011).
In order to understand the exact mechanism of a bioactive compound from *M. oleifera* against microorganisms, it is necessary to have some insights into the phytochemistry of the plant. The phytochemical profile for *M. oleifera* plants includes alkaloids, saponin, glycoside, flavanoids, tannins and terpenoids. Various chemical compounds, including tannins, saponins, flavonoids and steroids, are found in its leaves, seeds and flowers. Among the compounds present in extracts of *Moringa* that are responsible for the plant’s antimicrobial activity are tannins (Scalbert, 1991), glycosides (Abaoba and Efuwape, 2001), saponins (Hostettman and Nakaniishi, 1979), terpenoids and flavonoids (Leven et al., 1979), and alkaloids (Karou et al., 2005; Gami and Parabia, 2011). The phytochemicals found in seeds have been found to be effective against *K. pneumoniae*, *S. aureus* and *Streptococcus*. The agent present in the flower is effective against *E. coli*, *K. pneumoniae* and *P. mirabilis*. The leaf compounds are effective against *E. coli*, *K. pneumoniae*, *Enterobacter*, *P. aeruginosa* and *S. aureus* (Nepolean et al., 2009). The details of phytochemical constituents in *M. oleifera* are given in Table 2.

### Antibacterial mode of action of different phytoconstituents

The antimicrobial modes of action of different constituents from *M. oleifera* are given below (Table 2) while Figure 2 shows a graphical summary of mode of action of different parts of the *M. oleifera* plant.

### Essential Oils

In the plant kingdom, many species produce essential oils, for example thymol, terpenoids, carvacol etc. that are responsible for the characteristic flavor and aroma of the plant. Although their exact mechanisms of action are not known, they are considered to be involved in the disruption of bacterial membranes through formation of lipophilic products that could increase membrane permeability and membrane fluidity, alter the ion transport processes, inhibit the electron transport chain, interfere with membrane-embedded proteins, alter membrane size, etc. (Mendoza et al., 1997; Griffin et al., 1999; Simões et al., 2009). Any of the said mechanisms or a combination of them could induce the deaths of Gram-positive and Gram-negative bacteria (Simões et al., 2009).

### Alkaloids

Alkaloids are known to inhibit efflux mechanism by intercalating with DNA. Essentially, alkaloids can disrupt bacterial cytoplasmic membrane, resulting in antibacterial action (Jennings and Ridler, 1983; Khan et al., 2006; Simões et al., 2009). Khan et al. (2006) reported alkaloids as antimicrobial agent against *S. aureus*.

### Phenols and Phenolic Acids

Phenol and phenolic compounds inhibit enzymes like polyphenol oxidase by binding covalently to functional residues of proteins including sulfhydral and primary amino groups, resulting in disruption of energy production (Mason and Wasserman, 1987; Simões et al., 2009). The inhibition of enzymes like urease or proline dehydrogenase by phenolics has been reported (Simões et al., 2009). The inhibition of proline dehydrogenase results in the disruption of energy production at the plasma membrane of Gram-negative bacteria such as *Helicobacter pylori* (Li et al., 2005; Simões et al., 2009).

Plants are hosts to many antioxidants that include phenolics which act as free radical terminators or metal chelators (Shahidi et al., 1992). In plant seeds, they are commonly present in bound form (Arranz et al., 2009), cross linking with the cell wall through ester or ether linkages involving their hydroxyl and carboxyl groups (Yu et al., 2001). These bound phenolics are released when seeds are subjected to treatments with enzymes, acids or alkalis (Durkee and Thivierge, 1975; Krygier et al., 1982; Dvořáková et al., 2008). According to Negi, (2012), phenolic compounds that display antibacterial potential include quercetin, cinnamic acid, protocatechuc acid, caffeic acid, vanillin, ferulic acid, p-coumaric acid, catechin, epicatechin, and gallic acid (Singh et al., 2013).

### Flavonoids

Flavonoids that include various polyphenols are known to inhibit the synthesis of the nucleic acids of Gram-positive and Gram-negative bacteria. Flavonoids contain a B-ring which plays the role of an intercalating agent by creating hydrogen bond in stacks of nucleic acid. In this manner, flavonoids play a significant role in inhibiting the synthesis of DNA or RNA (Mori et al., 1987; Mirzoeva et al., 1997; Cushnie and Lamb, 2005; Simões et al., 2009).

Crude extracts of flavonoids from selected plant species and tissue calli have been tested for their antimicrobial activity against the Gram-positive bacteria *B. subtilis*, *S. aureus*, the Gram-negative bacteria *E. coli*, *K. pneumoniae* and the fungal pathogen *C. albicans*. Flavonoids extracted especially from tissue calli show high antimicrobial activity against microorganisms because of the higher concentrations of flavonoids in such material (Talreja, 2010; Talreja et al., 2012; Onsare and Arora, 2014).

### Antibacterial activity in different extracts

The presence of secondary metabolites in plants confers the following advantages to their hosts: they protect against biotic or abiotic stresses, they serve as defense mechanisms by virtue of their repellent properties, and they maintain tissue structural integrity. Secondary metabolites of plants, including phenolic compounds, alkaloids, flavonoids, terpenoids, can be extracted in organic or aqueous solvents (Paiva et al., 2010). The extracts are categorized on the basis of their polarity:
1. Polar solvents: e.g. organic acids.
2. Solvents of intermediate polarity e.g. methanol, ethanol, acetone, dichloromethane.
3. Low polarity solvents e.g. hexane, chloroform.

Many studies have been undertaken to investigate medicinal plants in order to validate their ethnopharmacological claims. The extract of the same plant part in solvents of different polarities may exhibit different biological properties (Tepe et al., 2005; Paiva et al., 2010). Almost all the plant parts of Moringa can be used for organic solvent extraction. Organic extracts may have antibacterial property against only Gram-negative bacteria or Gram-positive bacteria, or both. As mentioned earlier, Gram-negative bacteria have an outer membrane containing lipopolysaccharides which makes it impermeable to lipophilic solutes, while the presence of porins act as a selective barrier to hydrophilic solutes with a cut off value of 600 Da. Solutes greater in size than 600 Da cannot pass through porins (Paiva et al., 2010). On the other hand, the presence of an outer peptidoglycan layer on Gram-positive bacteria makes it more susceptible to solutes as it is not considered an effective barrier (Arias et al., 2004; Paiva et al., 2010). The solvents used for extraction can be hexane, acetone, methanol, ethanol, etc. According to previous literature (Jabeen et al., 2008; Khesorn, 2009), ethanolic, methanolic, and dichloromethane extracts show the highest antimicrobial activities against various Gram-negative and Gram-positive bacteria.

**Acetone extract**

The acetone extract of Moringa contains various phytochemicals, including phenolics, flavonoids, tannins, glycosides (Korithu et al., 2011), alkaloids, carbohydrates, proteins, amino acids, saponins and triterpenoids (Gami and Parabia, 2011). These agents have been shown to be effective against S. typhii, S. aureus, E. coli and B. subtilis (Korithu et al., 2011). Masika et al. (2012) reported the antimicrobial effect of leaf acetone extract at 5 mg/ml against both Gram-negative and Gram-positive bacteria, including E. coli, Enterobacter cloacae, P. vulgaris, S. aureus and Micrococcus kristinae. M. oleifera acetone extract was bactericidal on E. coli and M. kristinae, while it was bacteriostatic on S. aureus, E. cloacae and Proteus vulgaris. Although, the minimal bactericidal concentration (MBC) value for the M. oleifera acetone extract against M. kristinae was higher (1.0 mg/mL) than its minimal inhibitory concentration (MIC) value of 0.5 mg/mL, it is interesting to note that the MIC and MBC values (5 mg/mL) against the inhibited bacteria were the same (Masika et al., 2012).

**Figure 2:** Overall summary of mode of action of different parts of M. oleifera plant.
### Table 2: Antibacterial property of phytoconstituents present in *M. oleifera* plant parts.

| Plant parts        | Constituents                                                                 | Antimicrobial property | Microorganisms against which it is active | References                          |
|--------------------|-------------------------------------------------------------------------------|------------------------|-------------------------------------------|-------------------------------------|
| Whole plant        |                                                                               |                        |                                            |                                     |
|                    | Alkaloid                                                                      | Yes                    | Both Gram + & -                           | Fahey et al., 2001; Bennet et al., 2003 |
|                    | Moringine                                                                     | -                      | -                                         | Kerharo, 1969; Faizi et al., 1994a   |
| Stem bark          | Vanillin                                                                       | -                      | -                                         |                                     |
|                    | β-sitosterol                                                                  | -                      | -                                         |                                     |
|                    | β-sitosterene                                                                 | -                      | -                                         |                                     |
|                    | 4-hydroxymellin                                                               | -                      | -                                         |                                     |
|                    | Ocatcosanoic acid                                                             | -                      | -                                         |                                     |
| Whole gum exudate  | L-arabinose                                                                    | -                      | -                                         | Bhattacharya et al., 1982           |
|                    | L-galactose                                                                   | -                      | -                                         |                                     |
|                    | L-glucuronic acid                                                             | -                      | -                                         |                                     |
|                    | L-rhamnose                                                                    | -                      | -                                         |                                     |
|                    | L-mannose                                                                     | -                      | -                                         |                                     |
|                    | L-xylose                                                                       | -                      | -                                         |                                     |
| Degraded gum exudate | L-galactose                                                                    | -                      | -                                         | Bhattacharya et al., 1982           |
|                    | L-glucuronic acid                                                             | -                      | -                                         |                                     |
|                    | L-mannose                                                                     | -                      | -                                         |                                     |
| Flowers            | Nine amino acids                                                              | -                      | -                                         | Faizi et al., 1994a                 |
|                    | Sucrose                                                                        | -                      | -                                         |                                     |
|                    | D-glucose                                                                      | -                      | -                                         | Siddhuraju and Becker, 2003         |
|                    | Traces of alkaloids                                                           | -                      | -                                         |                                     |
|                    | Wax                                                                            | Yes                    | Both Gram + & -                           | Becker, 2003                       |
|                    | Quercetin                                                                      | -                      | -                                         |                                     |
|                    | Kaempferate                                                                    | Yes                    | Both Gram + & -                           |                                     |
|                    | Flavonoid pigments                                                            | -                      | -                                         |                                     |
|                    | Alkaloids                                                                      | Yes                    | Both Gram + & -                           |                                     |
|                    | Kaempferol                                                                     | -                      | -                                         |                                     |
|                    | Rhamnetin                                                                     | -                      | -                                         |                                     |
|                    | Isoquercitrin                                                                  | -                      | -                                         |                                     |
|                    | Kaempferitrin                                                                 | -                      | -                                         |                                     |
| Pods               | Thiocarbamate                                                                  | -                      | -                                         | Faizi et al., 1998                  |
|                    | Isoctitanate glycoside                                                         | -                      | -                                         |                                     |
| Fruit              | Cytokinin                                                                     | -                      | -                                         | Nagar et al., 1982                  |
| Leaves             | Ascorbic acid                                                                  | -                      | -                                         | Anwar et al., 2005; Makkar and       |
|                    | Flavonoid                                                                      | -                      | -                                         |                                    |
|                    | Phenolics                                                                      | Yes                    | Both Gram + & -                           | Becker, 1996                       |
| Roots              | Ptterygospermin                                                                | Yes                    | Both Gram + & -                           | Das et al., 1957; Ellet et al., 1981;|
|                    | 4-α-L-rhamnosylxybenzyl isothiocyanan                                        | Yes                    | Both Gram + & -                           | Rao et al., 2001; Dewangan et al., 2010|
|                    | 4-(α-L-rhamnosylxy)-benzyl isothiocyanate                                     | Yes                    | Both Gram + & -                           |                                     |
|                    | Niazimicin                                                                     | Yes                    | Both Gram + & -                           |                                     |
|                    | 3-α(6'-O-oleyl-β-D-glucopyranosyl)-β-sitosterol                                | -                      | -                                         |                                     |
|                    | β-sitosterol-3-α-β-D-glucopyranoside                                          | -                      | -                                         |                                     |
|                    | Niazin                                                                         | -                      | -                                         |                                     |
|                    | β-sitosterol                                                                   | -                      | -                                         |                                     |
|                    | Glycerol-1-(9-octadecanoate)                                                   | -                      | -                                         |                                     |
| Seed               | Campestereol                                                                   | -                      | -                                         | Tsaknis et al., 1999; Lala and       |
|                    | Stigmasterol                                                                   | -                      | -                                         | Faiz et al., Anwar et al., 2005      |
|                    | β-sitosterol                                                                   | -                      | -                                         |                                     |
|                    | Δ^4-avenasterol                                                                | -                      | -                                         | Anwar and Bhangar, 2003              |
| Oil (seed)          | Clerosterol                                                                    | -                      | -                                         |                                     |
|                    | 24-methylene cholesterol                                                       | -                      | -                                         | Anwar et al., 2005                  |
|                    | Δ^1-campesterol                                                                | -                      | -                                         |                                     |
|                    | Stigmastanol                                                                   | -                      | -                                         |                                     |
|                    | 28-isoavenasterol                                                              | -                      | -                                         |                                     |
Ethanolic Extract

Bukar et al. (2010) reported that M. oleifera leaf ethanolic extract had the broadest spectrum of activity on the test bacteria, a finding that was in agreement with Doughari et al. (2007). The ethanolic extract of M. oleifera was reported to be effective against B. subtilis and S. aureus, E. coli and K. pneumoniae (Talreja, 2010). Peixoto et al. (2011) found it effective against S. aureus, V. parahaemolyticus, E. leacalis and A. caviae. Doughari et al. (2007) reported an ethanolic extract of the plant at 100 mg/ml demonstrated the highest activity, while the aqueous extract showed the least activity. Renitta et al. (2009) reported highest antimicrobial activity from the ethanolic extract of leaves, seeds and flowers of M. oleifera against microorganisms like E. coli, K. pneumoniae, Enterobacter spp., P. mirabilis, P. aeruginosa, S. typhi A, S. aureus, Streptococcus spp. and C. albicans (Kalpana et al., 2013). One study showed that whereas the seed aqueous extract was not effective against Gram-negative bacteria (E. coli, Shigella flexneri and S. typhi), the seed ethanolic extract showed zones of inhibition against the same (Cáceres et al., 1991; Jamil et al., 2007; Lar et al., 2011; Mishra et al., 2011; Saadabi and Abu, 2011; Farooq et al., 2012). Ethanol based extracts were also effective against S. aureus and Vibrio cholera (Vieira et al., 2010) and against E. coli, S. flexneri, and S. typhi (Lar et al., 2011).

Ethyl acetate extract

In one study, an ethyl acetate extract of Moringa leaf recorded the highest antimicrobial activity against four microorganisms namely, S. epidermidis, S. aureus, P. aeruginosa and Bacillus cereus, respectively (Abdallah, 2016).

Butanol extract

In the same study, a butanol Moringa leaf extract was active only against S. epidermidis and S. aureus (Abdallah, 2016).

Aqueous extract

Abalaka et al. (2012) found that the MIC of an aqueous leaf extract on E. coli and S. typhi were 1.667 mg/ml and 0.417 mg/ml respectively, but no MIC was reported for the extract on P. aeruginosa. Mehta and Agrawal, (2008) also reported antimicrobial activity of an aqueous extract of seeds against E. coli, S. aureus and P. aeruginosa. The water-based extract was similarly found to be effective against S. aureus and V. cholera (Vieira et al., 2010). According to Abdallah, (2016) an aqueous leaf extract was active only against S. epidermidis. Unlike leaves, the aqueous extracts of seeds did not show any activity (Cáceres et al., 1991; Jamil et al., 2007; Jabeen et al., 2008; Lar et al., 2011; Mishra et al., 2011; Saadabi and Abu, 2011; Farooq et al., 2012). Another Moringa species, M. peregrine, showed activity in its aqueous seed extracts and seed oil against E. coli, C. albicans, S. aureus, S. epidermidis, P. aeruginosa (Spiliotis et al., 1997; Lalas et al., 2012).

Hexane extract

Antibacterial activity of M. oleifera seeds in a hexane extract was reported against S. typhi, V. cholera and E. coli (Walter et al., 2011).

Chloroform extract

Nikkon et al. (2003) isolated the aglycon of deoxy-niazimicine (N-benzyl,S-ethyl thiophionate) from M. oleifera crude chloroform extract. It exhibited antimicrobial activity against S. aureus, Shigella dysenteriae, Shigella boydii, S. typhi and P. aeruginosa. The antibacterial effect of the chloroform extract was also reported against S. aureus (Abdallah, 2016). The MICs of the chloroform leaf extract on E. coli, S. typhi and P. aeruginosa were 1.667 mg/mL, 0.417 mg/mL and 0.417 mg/mL, respectively (Abalaka et al., 2012).

Methanol extract

A methanol extract of Moringa leaves was found effective against a broad range of microorganisms, including K. pneumoniae, P. aeruginosa, Enterobacter aerogenes, Salmonella typhi, Salmonella paratyphi A, S. aureus, Micrococcus luteus and B. Subtilis (Gami and Parabia, 2011). The methanolic extract of dried leaf of M. oleifera was found to possess potent phytochemicals with high inhibitory activities on bacteria of UTI origin (Okiki et al., 2015).

Phytochemicals and biofilms

When bacterial cells start to grow on a solid surface, the colony may take the form of a film that is known as a biofilm (Simões et al., 2009). The plant-microbe interaction can be exploited for the production of phytochemicals that can interfere with biofilm formation (Costerton et al., 1987; Ramey et al., 2004; Jain et al., 2007; Simões et al., 2009). Biofilm formation involves the attachment of bacterial cells by extracellular appendages and hydrophobicity of cell surface. If this step is disturbed, then biofilm formation cannot take place. This initial attachment can be disturbed by flavonoids obtained from M. oleifera seed coat (Cushnie and Lamb, 2005). This step is concentration and exposure time dependent (Simões et al., 2008).

Antibacterial mode of action

The presence of the lipopolysaccharide membrane in Gram-negative bacteria makes it impermeable to exogenous molecules (Nikaido and Vaara, 1985; Gami and Parabia, 2011). The significance of the cell membrane is already known. It is involved in various processes like uptake and transport of solute molecules, cell signaling, metabolism regulation, energy transducing...
processes, and maintenance of osmotic pressure (Sikkema et al., 1995). In Gram-positive bacteria, the cell membrane has low lipid content and is high in lipoteichoic acids. When an antibacterial agent enters the cell, imbalance of ions (K+ and H+) occurs, resulting in disturbance of metabolism which leads to protein and enzyme denaturation and, eventually, cell death. According to Singh et al. (2013), phenolic compounds disturb the enzymatic machinery of the cell, thereby inducing an antimicrobial effect. In Gram-negative bacteria, the presence of the outer lipopolysaccharide membrane makes entry of exogenous compounds difficult. If antibiotic compounds gain entry, cell membrane disruption takes place due to interference in ATP generation from dextrose (Gill and Holley, 2004). Cushnie and Lamb, (2005) reported that phenolic compounds like quercetin and luteolin which are found in Moringa extracts interfere with the bacterial DNA gyrase activity.

CONCLUSIONS

A serious threat of multi-drug resistance has arisen this century. In such a scenario, natural remedies are increasingly under investigation to validate their reputed pharmaceutical properties in the quest for new drugs. M. oleifera, despite being regarded by many as a miracle tree, has not been well researched or validated for its many potential medicinal applications. Reports on its antimicrobial properties, for example, are few and dispersed in different publications. This review is, therefore, an effort to collect all the antimicrobial information related to the plant’s parts, extracts and phytochemistry of M. oleifera. In summary, it can be said that all parts of the Moringa plant that are extracted in any solvent exhibit antimicrobial properties against Gram-positive and Gram-negative bacteria. The Moringa plant offers natural and safe protection against a broad spectrum of pathogens.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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Malays. J. Microbiol. Vol 15(3) 2019, pp. 244-259
DOI: http://dx.doi.org/10.21161/mjm.113117

ISSN (print): 1823-8286, ISSN (online): 2231-7538
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