African Journal of Biotechnology Vol. 11(11), pp. 2797-2802, 7 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.686
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Antimicrobial activities of *Moringa oleifera* Lam leaf extracts

Moyo, Busani[1], Masika, Patrick Julius[2]* and Muchenje, Voster[1]

[1]Department of Livestock and Pasture Science, University of Fort Hare, Alice 5700, South Africa.
[2]Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare, Alice 5700, South Africa.

Accepted 7 September, 2011

Plants have been reported to contain important preventative and curative compounds. Studies were conducted to determine the antimicrobial activities of *Moringa oleifera* extracts using *in vitro* antimicrobial screening methods. The acetone extract of *M. oleifera* leaves at a concentration of 5 mg/ml showed antibacterial activities against *Escherichia coli*, *Enterobacter cloace*, *Proteus vulgaris*, *Staphylococcus aureus* and *Micrococcus kristinae*. *M. kristinae* was the most susceptible as its growth was inhibited at 0.5 mg/ml. On the other hand, *M. oleifera* acetone extract did not exhibit any inhibition on *Streptococcus faecalis*, *Bacillus pumilus*, *Klebsiela pneumonia*, *Bacillus cereus* and *Pseudomonas aeruginosa*. The acetone extract was bactericidal on *E. coli* and *M. kristinae*. It was also bacteriostatic on *S. aureus*, *E. cloace* and *P. vulgaris*. However, the water extract showed no activity at the highest concentration (5 mg/ml) tested. Furthermore, both the acetone and aqueous extracts did not exhibit any antifungal activity against the fungal species of *Candida albicans*, *Pennicillium notatum*, *Aspergillus flavus* and *Aspergillus niger* even at the highest concentration of 10 mg/ml. The ability of acetone extract to inhibit the growth of some strains of bacteria is an indication of its antibacterial potential which may be employed in the management of microbial infections.

Key words: Antibacterial, antifungal, bacteriocidal, bacteriostatic, Gram positive.

INTRODUCTION

Over the years, plants have been used as valuable sources of natural products for maintaining animal and human health. Plants have been reported to contain large varieties of chemical substances that possess important preventative and curative therapies (Nascimento et al., 2000). About 80% of individuals from developed countries use traditional medicines which have compounds derived from medicinal plants (Igbinosa et al., 2009). Despite the presence of various approaches to drug discovery, plants still remain the main reservoir of natural medicines (Mahomed and Ojewole, 2006).

Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance. This resistance could be attributed to indiscriminate use of commercial drugs or not taking an antibiotic prescription according to the instruction for example not taking all the prescription in the treatment of infectious diseases (Aliero and Afolayan, 2006). In addition, certain antibiotics present undesirable side effects such as nausea, depression of bone marrow, thrombocytopenic purpura and agranulocytosis leading to the emergence of previously uncommon diseases (Marchese and Shito, 2001; Poole, 2001). This has given scientists the impetus to search for newer and alternative microbial compounds from medicinal plants (Aliero and Afolayan, 2006). Besides, the high cost of conventional drugs, particularly in resource limited communities has led to the increased use of plants as an alternative for treatment of infectious diseases. Plant extracts and phytochemicals with antimicrobial properties are of great significance in therapeutic treatments. Their antimicrobial properties are due to compounds synthesized in the secondary metabolism of the plant. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotic protypes (Afolayan, 2003).

Bacteria and fungi are of human and veterinary

*Corresponding author. E-mail: Pmasika@ufh.ac.za. Tel: +27 40 602 2317. Fax: + 27 406022583.

importance as outlined later. *Bacillus cereus* has been implicated in food-borne intoxication (Granum and Lund, 1997). *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortions and upper respiratory complications (Fraser, 1986). *Streptococcus faecalis* is a pathogenic bacteria commonly found in the intestines of birds (Granum and Lund, 1997). *Aspergillus niger* has been reported to cause lung diseases, aspergillosis and otomycosis. Similarly, *Aspergillus flavus* is a human and livestock pathogen associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic and nasoorbital infections. They produce significant quantity of aflatoxin (Samson et al., 2001; Klich, 2007). *Penicillium notatum* induces hypersensitivity, pneumonitis in animals. *Candida albicans* is reported to cause vaginitis and yeast mastitis. These necessitate searching for antibiotics that could be used against microbes.

Moringa oleifera Lam is one of the best known, widely distributed and grown species of a monogeneric family moringaceae (Anwar et al., 2007). The plant is referred to as drumstick tree or the horse radish tree (Anwar et al., 2007). It is native to the western and sub-Himalayan parts of Northwest India, Pakistan and Afghanistan. The species is now widely cultivated across some African countries, South America and South-east Asia (Ben Salem and Makkar, 2009). It is a drought tolerant plant that thrives best under the tropical climate and tolerates different soil types (Fahey, 2005). In South Africa, the plant is found in few localities: Limpopo, Kwazulu Natal and Mpumalanga Provinces (van Jaarsveld, 2006). The plant is highly valued since almost every part of the tree (leaves, roots, bark, fruit flowers, immature pods and seeds) is used as food with high nutritional value (Anwar et al., 2007; Chuang et al., 2007). In addition, the plant has been reported to possess antimicrobial properties and this explains the reason for its wide use in the treatment of human diseases (Lockett et al., 2000; Anwar et al., 2007). To the best of our knowledge, there is little or no information on the antimicrobial activities of the South African ecotype of *M. oleifera.* The objective of the current study was, therefore, to determine the anti-bacterial and antifungal activities of *M. oleifera* extracts using *in vitro* antimicrobial screening methods.

MATERIALS AND METHODS

Plant material and extract preparation

The plant leaves were collected in April at Sedikong sa Lerato in Tooseng village, Ga-Mphahlele (24°26′57.10″S, 29°33′47.02″E), Limpopo Province of South Africa. The mean annual rainfall of the area is approximately 300 mm and the mean annual temperature is 15°C. The plant was authenticated at University of Fort Hare by Professor Grieson and voucher specimen (BM 01/2009) was prepared and deposited in the Giffen Herbarium of the University of Fort Hare. The leaves were harvested green, air-dried under shade and milled into powder. The two solvents: acetone and water were used and in all cases equal volumes of solvents were used.

One hundred grams of powdered leaves were soaked in 500 ml of each solvent which were acetone and distilled water. There were left shaking for 48 h at 30°C, on an orbital shaker (Stuart Scientific Orbital shaker, UK). Acetone used was of high analytical grade; which is less lethal to the test organisms (Eloff, 1998). The extracts were filtered separately through Whatman no.1 filter paper and the acetone extract was evaporated to dryness under reduced pressure at 40°C using a rotary evaporator (Laborator 4000-efficient, Heidolph, Germany). Water extract was freeze-dried using Savant refrigerated vapor Trap, (RVT4104, USA), and stored at 4°C. The yields of acetone and water extracts weighed 16 and 13%, respectively. They were stored in air-tight glass bottles before use and later re-dissolved in their respective solvents to the desired concentrations for the various experiments. Each test was replicated three times.

Origin of strains

The bacteria strains used were those recommended by the National Committee for Clinical Laboratory Standards. The selection of organisms depended on availability and were as follows: *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Micrococcus kristinae* (AI5), *Pseudomonas aeruginosa* ATCC (19582), *Escherichia coli* (ATCC25922), *Enterobacter cloace* (ATCC 13047), *Klebsiela pneumonia* (ATCC 10031) and *Proteus vulgaris* (ATCC 6830). The bacteria were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. Organisms were chosen based on reports of their human and livestock pathogenicity.

Minimum inhibitory concentration (MIC)

The bacteria species were maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth (Oxoid) and incubated at 37°C for 18 h. Before use, each bacteria culture was diluted 1:100 with fresh sterile nutrient broth (Grierson and Afolayan, 1999).

The bacteria were streaked in a radial pattern on the agar plates (Meyer and Afolayan, 1995), which were incubated at 37°C under aerobic conditions and examined after 24 and 48 h. Complete suppression of growth by specific concentration of an extract was declared active (Mathekga et al., 2000). Each extract was tested at a concentration of 5.0, 1.0, 0.5 and 0.1 mg/ml. Streptomycin and chloramphenicol were used as standard (positive) controls with pure solvents (acetone and water) and sample free solutions as blank controls. Each test was replicated three times. Acetone has been reported to be non-toxic to the organism at the concentration used (Meyer and Afolayan, 1995).

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the plant extracts was determined by the modified method of Spencer and Spencer (2004). The samples were sub-cultured from MIC plates that showed no growth after 24 h onto a fresh extract-free solid medium and incubated further for 18 to 24 h. The highest dilution (least concentration) that yielded no single bacterial colony on a solid medium was taken as MBC. The MBC was not determined for the water extract since it did not exhibit antibacterial activity. It should also be noted that the condition of evaluation for extract effectiveness was similar for all the bacterial and fungal species used.

Antifungal activity assay

The antifungal activity of *M. oleifera* was investigated using four

Table 1. Antibacterial activity of the leaf extracts of *M. oleifera*.

Bacteria species	Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (mg/ml)				
	Gram reaction	Water extract	Acetone extract	Streptomycin (µg/ml)	Chloramphenicol (µg/ml)
Bacillus cereus(ATCC 10702)	+	na	na	< 2	< 2
Bacillus pumilus (ATCC 14884)	+	na	na	< 2	< 2
Staphylococcus aureus (ATCC 6538)	+	na	5 (5)	< 2	< 2
Streptococcus faecalis (ATCC 29212)	+	na	na	< 2	< 2
Micrococcus kristinae §	+		0.5 (1)	< 0.5	< 2
Escherichia coli (ATCC 25922)	-	na	5(5)	< 2	< 2
Pseudomonas aeruginosa (ATCC 19582)	-	na	na	< 5	< 20
Enterobacter cloacae (ATCC 13047)	-	na	5(5)	< 2	< 2
Klebsiella pneumoniae (ATCC 10031)	-	na	na	< 2	< 2
Proteus vulgaris (ATCC 6830)	-	na	5(5)	< 2	< 2

na = Not active; MBC, values in bracket; § = environmental strain.

fungal species (*A. niger* (ATCC 16404), *A. flavus* (ATCC 9643), *P. notatum* (LIO) and *C. albicans* (ATCC10231), which were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. The selection of fungi used in the study was based on history of being pathogenic to humans and livestock. The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxid) at 25°C until they sporulated. Thereafter, the fungal spores were harvested by pouring a mixture of sterile glycerol and distilled water to the surface of the plate. Later, the spores were scraped with a sterile glass rod. The harvested fungal spores were standardized to an OD_{600nm} of 0.1 before use. The standardized fungal spore suspension (1000 µl) was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were bored into the agar media using a sterile 6 mm cork borer and the wells were filled with the solution of the extract (0.2 ml), taking care not to allow spillage of the solution onto the surface of the agar medium. Acetone and water extract concentrations used were 0.1, 0.5, 1.0, 5.0 and 10 mg/ml. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 h and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with amphothericin B and miconazole at a concentration of 1 mg/ml (Igbinosa et al., 2009).

Statistical analysis

Diameter of fungal growth was measured and expressed as means of percentage growth inhibition of three replicates. Significant differences within the means of treatments and controls were calculated using the LSD statistical test.

RESULTS

Antibacterial activity

The leaf acetone extract of *M. oleifera* at 5 mg/ml showed antibacterial activities against *E. coli* (ATCC 25922), *E.cloacae* (ATCC 13047), *P. vulgaris* (ATCC 6830), *S. aureus* (ATCC 6538) and *M. kristinae* § at 0.5 mg/ml, while reference antibiotics streptomycin and chloramphenicol had antibacterial activity at 2 µg/ml (Table 1). As indicated in Table 2, the *M. oleifera* acetone extract was bactericidal on *E. coli* (ATCC 25922) and *M. kristinae*, while it was bacteriostatic on *S. aureus* (ATCC 6538), *E cloacae* (ATCC 13047) and *P. vulgaries* (ATCC6830). Although, the MBC value for the *M. oleifera* acetone extract against *M. kristinae* was higher (1.0 mg/ml) than its 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. The water extract did not show any activity at the highest concentration (5 mg/ml) tested.

Antifungal activity

Both the *M. oleifera* acetone and aqueous extracts did not exhibit antifungal activity against the fungal species, *C. albicans* (ATCC10231), *P. notatum* (LIO), *A. flavus* (ATCC 9643) and *A. niger* (ATCC 16404).

DISCUSSION

The susceptibility of some bacteria strains to the extract *M. oleifera* may be a pointer to its

Table 2. Bacteriostatic and bactericidal activities of *M. oleifera* acetone extract.

Bacterial species	Gram +/-	Bacteriostatic (mg/ml)	Bactericidal (mg/ml)
Staphylococcus aureus (ATCC 6538)	+	5.0	na
Micrococcus kristinae §	+	na	1.0
Proteus vulgaris (ATCC 6830)	-	5	na
Escherichia coli (ATCC 25922)	-	na	5
Enterobacter cloacae (ATCC 13047)	-	5	na

na = Not active; § = environmental strain.

potential as a drug that can be used against these susceptible bacterial strains. Furthermore, antibacterial resistance, especially, among Gram-negative bacteria is an important issue that has created problems in the treatment of infectious diseases and necessitates the search for alternative drugs or natural antibacterial remedies (Khosravi and Behzadi, 2006). The difference in bacterial response was possible due to the nature of the bacterial species. It is noted that the acetone extract of *M. oleifera* leaves exhibited antimicrobial effect against both Gram-positive and negative bacteria (broad spectrum activities). The *M. oleifera* acetone extract, however, showed greater anti-bacterial activity against Gram-negative bacteria than Gram-positive bacterial strains. These contrasts with most researchers' findings who reported that most plant extracts have more activity against Gram–positive bacteria (Aiyegoro et al., 2008; Boussaada et al., 2008; Ashafa and Afolayan, 2009). Noteworthy, is the ability of the *M. oleifera* acetone extract to inhibit the growth of *M. kristinae* at 0.5 mg/ml which is the lowest MIC value in comparison to other bacterial strains. This suggests that *M. kristinae* was more sensitive to the *M. oleifera* acetone extract and could be used as an antibiotic against diseases that are caused by *M. kristinae*. This observation can best be explained by the fact that *M. kristinae*, which is an environmental strain, has a low incidence of antibiotic resistant genes as compared to most clinical bacterial strains; hence its susceptibility to the extract at a lower MIC value as compared to clinical strains (Aiyegoro et al., 2010).

The non activity of the water extract against microbes investigated in this study is in agreement with previous works which showed that aqueous extracts of plants generally exhibited little or no anti-microbial activities (Aiyegoro et al., 2008; Ashafa et al., 2008). Masika and Afolayan (2002) reported that Gram-negative bacteria are more resistant to water extracts. Furthermore, most researchers (Paz et al*.,* 1995; Vlientinck et al*.*, 1995; Martin and Eloff, 1998) have generally reported that water extracts of plants do not have much activity against bacteria. The reason might be that water extracts which is different from other solvents do have myriads of compounds that may interact antagonistically in their overall activities. It is also suggested that the active principles from plant materials are not readily extractable in water. In this experiment, acetone is a better solvent than water in extracting the active constituents from the leaves of *M. oleifera* (Eloff, 1998). Compounds like tannins and polyphenol which are found in *M. oleifera* are soluble in acetone (Makkar and Singh, 1992) and have been reported to possess antibacterial activity (Khosravi and Behzadi, 2006). Our findings, however, differ from the study by Dahot (1998) who reported that *M. oleifera* water extracts had antimicrobial activity against *E. coli*, *S. aureus* and *B. subtilis*. The difference could be attributed to variation in the environment where the plant was collected, the season and the physiological stage of the plant when leaves were harvested (Taylor and van Staden, 2001). This affects the chemical composition and the amount of compounds in the plant. In general, water extracts are the commonly used and are affordable to resource-limited farmers. The curative advantage is that consumers including animals tend to consume the plant material in large quantities and in high concentrations. This suggests its ability to meet the required physiological levels to inhibit the pathogen growth *in situ*. Yang et al*.* (2006) reported that the inclusion of *M. oleifera* leaf meal in Broiler feeds reduced the *E. coli* bacteria count in the ileum. In addition, *M. oleifera* leaf water extracts exhibited antimicrobial properties through the inhibition of the growth of *S. aureus* strains isolated from food and animal intestines (Yang et al., 2006). This point to the potential of *M. oleifera* as antimicrobial peptides to replace antibiotics in feeds.

In our study, the *M. oleifera* acetone extract had bactericidal properties against *E. coli,* which is mostly known to be multi-resistant Afolayan (2003). The ability of the acetone extract to kill *E. coli* is noteworthy even though it was at the highest concentrations (5.0 mg/ml) tested. Moreover, Gram-negative bacteria have been reported to be resistant to antibiotics (Boussaada et al., 2008). According to several authors, these bacteria are generally less sensitive to the activity of plant extracts (Pintore et al., 2002; Wilkinson et al., 2003; Boussaada et al., 2008). Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan and Abu-Hasan, 1998). The bactericidal and bacteriostatic activities of the *M. oleifera* acetone extract against *E. coli, M.*

kristinae, *S. aureus, E cloacae* and *P. vulgaris* was established. Compounds like pterygospermin, benzyl glucosinolate and benzyl isothiocynate have, however been isolated from *M. oleifera* leaves and these compounds have been reported to have antimicrobial properties against a wide range of bacteria which could partly explain the observed bacteriostatic and bactericidal activity (Fahey, 2005). It should be noted that for plant materials, there is actually no standard concentration as a model measure for determining the antibacterial activity but Aliero and Afolayan (2006), Ashafa et al. (2008) and Aiyegoro et al. (2009), considered 5 mg/ml as their highest concentration level.

The leaves of *M. oleifera* have also been known to contain a number of phytochemicals such as flavonods, saponins, tannins and other phenolic compounds that have antimicrobial activities (Sato et al., 2004; Cushine and Lamb, 2005; Mboto et al., 2009). This would suggest that the antimicrobial activities observed in this study could be attributed to such compounds. The mechanisms of actions of these compounds have been proven to be via cell membranes perturbations (Esimone et al., 2006). This coupled with the action of β-lactams on the trans-peptidation of the cell wall could lead to an enhanced antimicrobial effect of the combinations (Esimone et al., 2006).

According to Dahot (1998), *M. oleifera* leaf extracts contain small peptides which could play an important role in the plant's antimicrobial defense system. The proteins/ peptides are believed to be involved in a defense mechanism against phytopathogenic fungi by inhibiting the growth of micro-organisms through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Chuang et al., 2007). Antimicrobial peptides probably interact with the membranes in two stages. Firstly, cationic amino acids are attracted by negative charges such as phospholipid head groups on the surface. Secondly, hydrophobic and positively charged patches of the peptide interact with the aliphatic fatty acids and the anionic components, respectively (Zasloff, 2002; Koczulla and Bals, 2003). 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 the peptide and blocking of anionic cell components or the triggering of autolytic enzymes (Zasloff, 2002). Another strategy used by plants to thwart invaders is based on the localized production of antimicrobial; low molecular weight secondary metabolites known as phytoalexins (Maher et al., 1994; Dahot, 1998). The antibacterial activity of *M. oleifera* acetone extract validates some medicinal uses of *M. oleifera* (Fahey, 2005; Fugile, 2005).

In our study, none of the extracts showed any antifungal activity. Similar to our results is the report of Dahot (1998), whereby the *M. oleifera* water extracts were found to be inactive against the growth of *Aspergillus fumigates*, *A. flavus* and *Penicillium expansum* and moderately active against *A. niger* (Dahot, 1998). Variation in the antimicrobial activity of *M. oleifera* water extract could be attributed to the plant's ability to produce a wide range of selective antimicrobial compounds. This could be either in a constitutive or an inducible manner to protect themselves against pathogens (Cammue et al., 1992; D´Haeze and Holsters, 2004). Moreover, the synthesis of many presumed defense related compounds are induced when plants are exposed to pathogens (Linthorst, 1991). Antibacterial activity showed by the acetone extracts might justify the reports that *M. oleifera* have medicinal properties. Interestingly, the plant's nutritional compound assists the consumer to enhance their immune system against wide range of pathogens (Oiyegoro et al., 2009). Moreover, its ability to inhibit the bacterial growth enables the antibodies generated to destroy the invading pathogens.

Findings of the current study suggested that acetone extracts of *M. oleifera* leaves have potential as antimicrobial compounds against pathogens and their ability to either block or circumvent resistance mechanisms could improve treatment and eradication of microbial strains. Thus, plant extracts could be used in the treatment of infectious diseases caused by resistant microbes. The obtained results could therefore, form a basis for investigating which compound in the *M. oleifera* have antimicrobial activity.

ACKNOWLEDGEMENTS

The authors are grateful to the Govan Mbeki Research and Development Centre (GMRDC) of the University of Fort Hare for financial support, Department of Agricultural and Rural Development and Research Institute (ARDRI), Department of Biochemistry and Microbiology, Department of Livestock and Pasture Science and Department of Botany, all in the University of Fort Hare, for providing the necessary facilities. We are also indebted to Sedikong sa Lerato drop-in centre for supplying us with the material (leaves) for the study.

REFERENCES

Adwan K, Abu-Hasan N (1998). Gentamicin resistance in clinical strains of *Enterobacteriaceae* associated with reduced gentamicin uptake. Folia Microbiol. 43: 438-440.

Afolayan AJ (2003). Extracts from the shoots of *Arcotis arctotoides* inhibit the growth of bacteria and fungi. Pharm. Biol. 41: 22-25.

Aiyegoro OA, Akinpelu DA, Afolayan AJ, Okoh AI (2008). Antibacterial activities of crude stem bark extracts of *Distemonathus benthamianus* Baill. J. Bio. Sci. 8(2): 356-361.

Aiyegoro OA, Afolayan AJ, Okoh A I (2009). *In-vitro* antibacterial time kill studies of leaf extracts of helichrysum longifolium. J. Med. Plants Res. 3(6): 462-467.

Aiyegoro OA, Afolayan AJ, Okoh AI (2010). Interactions of antibiotics and extracts of *Helichrysum pedunculatum* against bacteria implicated in wound infections. Folia Microbiol. 55(2): 176-180.

Aliero AA, Afolayan AJ (2006). Antimicrobial activity of *solanum tomentosum*. Afr. J. Biotechnol. 5 (4): 369-372
Anwar F, Latif S, Ashraf M, Gilan AH (2007). *Moringa oleifera*: A Food plant with Multiple Medicianl uses. Phytother. Res. 21:17-25.
Ashafa AOT, Afolayan AJ (2009). Screening the root extracts from *Biden pilosa* L. var. *radiata* (Asteraceae) for antimicrobial potentials. J. Med. Plant Res. 3(8):568-572.
Ashafa AOT, Grieson DS, Afolayan AJ (2008). Antimicrobial activity of extract from *Felicia muricata* Thunb. J. Bio. Sci. 8(6): 1062-1066.
Ben Salem H, Makkar HPS (2009). Defatted *Moringa oleifera* seed meal as a feed additive for sheep. Anim. Feed Sci. Technol. 150: 27-33.
Boussaada O, Ammar S, Saidana D, Chriaa J, Chraif I, Daami M, Helal AN, Mighri Z (2008). Chemical composistion and antimicrobial activity of volatile components from capitula and aerial parts of *Rhaponticum acaule* DC growing wild in Tunisia. Microbiol. Res. 163: 87-95.
Cammue BPA, De Bolle MFC, Terras FRG, Proost P, Damme JV, Rees SB, Vanderleyn J, Broekaert WF (1992). Isolation and characterization of a novel class of plant antimicrobial peptide from *Mirabilis jalapa* L. seeds. J. Biol. Chem. 267: 2228-2233.
Chuang P, Lee C, Chou J, Murugan M, Shieh B, Chen H (2007). Antifungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. Bioresour. Technol. 98: 232-236.
Cushine TPT, Lamb AJ (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrobial. Agents, 26(5): 343-356.
D´Haeze W, Holsters M (2004). Surface polysaccharides enable bacteria to evade plant immunity. Trends Microbiol. 12(12): 555-561.
Dahot MU (1998). Antimicrobial activity of Small Protein of *Moringa oleifera* leaves. J. Islam. Acad. Sci. 11(1): 27-32.
Eloff JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 60: 1-8.
Esimone CO, Iroha IR, Ibezim EC, Okeh CO, Okpana EM (2006). *In vitro* evaluation of the interaction between tea extracts and penicillin G against *Staphylococcus aureus*. Afr. J. Biotechnol. 5 (11): 1082-1086.
Fahey JW (2005). *Moringa oleifera*: A review of the Medical evidence for its nutritional, Therapeutic and prophylactic properties. Part 1. http://www.TFLjournal.org/article.php/20051201124931586. accessed 15/03/2009.
Fraser CM (1986). The Merck Veterinary Manual (sixth ed.), Merck, New Jersey.
Fugile LJ (2005). The Moringa Tree: A local solution to malnutrition? Church World Service in Senegal.
Granum PE, Lund T (1997). *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett. 157: 223-228.
Grierson DS, Afolayan AJ (1999). Antibacterial activity of some indigenous plants used for the treatment of wound in the Eastern Cape, South Africa. S. Afr. J. Ethnopharmacol. 66: 103-106.
Igbinosa OO, Igbinosa EO, Aiyegoro OA (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). Afr. J. Pharm. Pharmacol. 3(2): 058-062.
Khosravi AD, Behzadi A (2006). Evaluation of the antibacterial activity of the seed hull of *Quercus brantii* on some gram negative bacteria. Pak J. Med. Sci. 22(4): 429-432.
Klich MA (2007). Aspergillus flavus: The major producer of aflatoxin. Mol. Plant Pathol. 8(6): 713-722.
Koczulla AR, Bals R (2003). Antimicrobial peptides: current status and therapeutic potential. Drugs, 63(4): 389-406.
Linthorst HJM (1991). Pathogenesis related proteins of plants. Crit. Rev. Plant Sci. 10: 123-150.
Lockett CT, Calvet CC, Grivetti LE (2000). Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria. Int. J. Food Sci. Nutr. 51(3): 195-208.
Maher EA, Bate NJ, Elkin WNIY, Dixon RA, Lamb CJ (1994). Increased disease susceptibility of transgenic tobacco plants with suppressed level of preformed phenyl propanoid products. Proc. Natl. Acad. Sci. USA. 91: 7802-7806.
Mahomed IM, Ojewole JAO (2006). Anticonvulsant activity of *Harpagophytum procumbens* DC (Pedaliaceae) secondary root aqueous extract in mice. Brain Res. Bull. 69: 57-62.
Makkar HPS, Singh B (1992). Detannification of oak leaves: treatment and the optimization. Anim. Feed Sci. Technol. 36: 13-27.
Marchese A, Shito GC (2001). Resistance patterns of lower respiratory tract pathogens in Europe. Int. J. Antimicrobial Agents 16: 25-29.
Martin N, Eloff JN (1998). The preliminary isolation of several antibacterial compounds from *Combretum erythrophyllum*-(Combretaceae). J. Ethnopharmacol. 62: 255-263.
Masika PJ, Afolayan AJ (2002). Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. J. Ethnopharmacol. 83(1-2): 129-134.
Mathekga ADM, Meyer JJM, Horn MM, Drews SE (2000). An acylated phloroglucinol with Antimicrobial properties from *Helichrysum caespititum*. Phytochemistry, 53: 93-96.
Mboto CI, Eja ME, Adegoke AA, Iwatt GD, Asikong BE, Takon I, Udo SM, Akeh M (2009). Phytochemical properties and antimicrobial activities of combined effect of extracts of the leaves of *Garcinia Kola*, *Vernonia amygdalina* and honey on some medically important microorganisms. Afr. J. Microbiol. Res. 3(9): 557-559.
Meyer JJM, Afolayan AJ (1995). Antibacterial activity of *Helichrysum aureonites* (Asteraceae). J. Ethnopharmacol. 47: 109-111.
Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemical on antibacterial-resistant bacteria. Braz. J. Microbiol. 31(4): 247-256.
Paz EA, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, Vazquez A, Vero S, Zunio L (1995). Screening of Uruguan medicinal plants for antimicrobial activity. J. Ethnopharmacol. 45: 67-70.
Pintore G, Usai M, Juliano C, Boatto G, Tomi F, Mario C, Riccardo C, Joseph C (2002). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardina and Corsica. Flavour Fragrance J. 17: 15-19.
Poole K (2001). Overcoming antibiotic resistance by targeting resistance mechanisms. J. Pharm. Pharmacol. 53: 283-294.
Samson RA, Houbraken J, Summerbell RC, Flannigan B, Miller JD (2001). Common and important species of fungi and actinomycetes in door environment In: Micro-organisms on home and indoor work environments. New York: Taylor and Francis, pp. 287-292.
Sato Y, Shibata H, Arai T, Yamamoto A, Okimura Y, Arakaki N, Higuti T (2004). Variation in synergistic activity by flavones and its related compounds on the increased susceptibility of various strains of methicillin-resistant *Staphylococcus aureus* to β-lactam antibiotics. Int. J. Antimicrob. Agents, 24(3): 226-233.
Spencer ALR, Spencer JFT (2004). Public health micro-biology: Methods and Protocols. Human Press Inc. New Jersey. pp. 325-327.
Taylor JLS, van Staden J (2001). The effect of age,season and growth conditions on anti-inflammatory activity in *Eucomis autumnalis* (Mill.) Chitt. Plant extracts. Plant Growth Regul. 34(1): 39-47.
van Jaarsveld E (2006). Plants of Southern Africa. http://www.plantafrica.com/plantklm /moringoval.html.
Vlientinck AJ, van Hoof L, Lasure A, Vanden D, Rwangabo PC, Mvukiyumwami J (1995). Screening of a hundred Rwandese medicinal plants for ant-microbial and antiviral properties properties. J. Ethnopharmacol. 46: 31-47.
Wilkinson JM, Hipwell M, Ryan T, Cavangh HMA (2003). Bioactivity of *Backhousiacitrodora*: antibacterial and antifungal activity. J. Agric. Food Chem. 51: 76-81.
Yang R, Chang L, Hsu J, Weng BBC, Palada C, Chadha ML, Levasseur V (2006). Nutritional and Functional properties of Moringa leaves-from germplasm, to Plant, to food, to health. Moringa and other highly nutritious plant resources: Strategies, standards and markets for a better impact on nutrition in Africa, Accra, Ghana, November 16-18, 2006.
Zasloff M (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870): 389-395.