CHAPTER-2

REVIEW OF LITERATURE

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continue to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (Owolabi et al., 2007). Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo et al., 2008). The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Cowman, 1999; Adesokan et al., 2008). The ancient texts like Rig Veda (4500-1600 BC) and Atharva Veda mention the use of several plants as medicine. The books on ayurvedic medicine such as Charaka Samhita and Susruta Samhita refer to the use of more than 700 herbs (Jain, 1968). According to the World Health Organization (WHO, 1977) “a medicinal plant” is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes (Okigbo, 2009). The term “herbal drug” determines the part-parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines. In India, the ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their
morphological and pharmacological or pharmacognostical characters can provide a better understanding of their active principles and mode of action.

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects (Brahmachari, 2001). Herbal drugs constitute a major share of all the officially recognized systems of health in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy. More than 70% of India's 1.1 billion populations still use these non-allopathic systems of medicine (Vaidya and Devasagayam, 2007). In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicines may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. Use of herbal medicines in developed countries has expanded sharply in the latter half of the twentieth century. In India, herbal drugs are an integral part of The Indian System of Medicine (Ayurveda) which is an ancient and mainstream system (Rai, 2005).

2.1 Moringa

*Moringa oleifera* is one of the best known medicinal plant. The *Moringa* plant has been consumed by humans (Iqbal *et al.*, 2006). It is one of the richest plant sources of Vitamins A, B, C, D, E and K (Anwar and Bhanger, 2003; Babu 2000; Caceres *et al.*, 1992; Dayrit *et al.*, 1990; Delisle *et al.*, 1997). The vital minerals present in *Moringa*
include Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc. It has more than 40 natural anti-oxidants. *Moringa* has been used since 150B.C. by ancient kings and queens in their diet for mental alertness and healthy skin. The leaves, pods, seeds, gums, bark and flowers of *Moringa* are used in more than 80 countries to relieve mineral and vitamin deficiencies, support a healthy cardiovascular system, promote normal blood-glucose levels, neutralize free radicals, provide excellent support of the body's anti-inflammatory mechanisms, enrich anemic blood and support immune system. It also improves eyesight, mental alertness and bone strength. It has potential benefit in malnutrition, general weakness, lactating mothers, menopause, depression and osteoporosis. It is also used to make an efficient fuel, fertilizer and livestock feed. *Moringa* leaf has been purported to be a good source of nutrition and a naturally organic health supplement that can be used in many therapeutic ways (McBurney *et al.*, 2004; Fahey, 2005; DanMalam *et al.*, 2001).

*Moringa* was highly valued in the ancient world. The Romans, Greeks and Egyptians extracted edible oil from the seeds and used it for perfume and skin lotion. In the 19th century, plantations of *Moringa* in the West Indies exported the oil to Europe for perfumes and lubricants for machinery. People in the Indian sub-continent have long used *Moringa* pods for food. The edible leaves are eaten throughout West Africa and parts of Asia. For centuries, people in many countries have used *Moringa* leaves as traditional medicine for common ailments. Clinical studies have begun to suggest that at least some of these claims are valid. With such great medicinal value being suggested by traditional medicine, further clinical testing is very much needed. A study was done in Pakistan to examine the physio-chemical characteristics of *M. oleifera* seeds and seed oil from a wild provenance of Pakistan. The *Moringa* seeds exhibited an oil yield of 34.80%. Protein, fiber, moisture and ash contents were 31.65, 7.54, 8.90 and 6.53%, respectively.
Nikolaus Foidl and Dr. Gabrielle Foidl, two Austrian scientists living in Nicaragua, have developed intensive methods of cultivating *Moringa*. They along with their associate Leonardo Mayorga, have been conducting their research in Nicaragua since the early 1990s. They have collaborated with the University of Hohenheim, Germany and with Dr. Michael Kreuzer, ETH (Swiss Federal Institute of Technology) Zurich, Switzerland. Their intensive cultivation methods were developed under experimental conditions on plots ranging in size from 0.5 to 4 hectares. Foidl and his associates have experimented with various uses of *Moringa* leaves and green stems, including their use in cattle fodder. Following the Foidl study, a study was conducted by Dr. Nadir Reyes Sanchez. Dr. Reyes is on the Faculty of the Veterinary Medicine and Animal Science Department of Animal Nutrition and Management at the Swedish University of Agricultural Sciences in Uppsala, Sweden (Foidl *et al.*, 2001). These two studies in Nicaragua showed that supplementing cattle feed with the leaves and green stems of *Moringa* can increase milk production by 43-65%, and increase daily weight gain in cattle by up to 32%. Recently a new benefit of *Moringa* was suggested: the leaves seem to contain a substance that stimulates plant growth and increases crop production. Several years ago, Mr. Nikolaus Foidl came across a reference to a study by a Mr. Singh of India. It said that an extract from *Moringa* leaves seemed to stimulate the growth of plants.

About two decades ago, in the southern states of India, and especially in Tamilnadu, *M. oleifera* was cultivated as single trees in homesteads, round cattle sheds, on farm boundaries, and as isolated plants in fences and as groups of trees on village waste lands. In the early 1990s in southern Tamilnadu people started growing perennial types - Moolanoor as an intercrop on field scale and their allies were cropped with vegetables and Sorghum. This system evolved as *Moringa* offered some protection to alley crops from drying winds during summer and *Moringa* provided some additional
income. With the migration of people from south to north India, the demand for *Moringa* products increased. In all the places concerned, with their differing conditions, cultivation of *M. oleifera* was not given the required attention and systematic production practices were not followed as people failed to notice that it was a commercially viable alternate crop in Arid Zone Horticulture. (Anbarassan *et al.*, 2001). In the Indian sub-continent *M. oleifera* has long been cultivated for its edible fruit: today these are exported, fresh and in tins, to consumers in Asia and Europe. The edible leaves of the tree are very nutritious and are consumed throughout West Africa as well as in some parts of Asia. Powder from seed kernels works as a natural coagulant which can clarify even very turbid water, removing up to 99% of the bacteria in the process. We need to explore therapeutic, nutritional and benefit of this gift of nature reported to be one of the world's most useful trees. *Moringa* has received attention in many countries in the tropics and sub-tropics and its leaves, pods and seeds form part of the traditional cuisine in these countries. Although *Moringa* is used in West, Central and East Africa and although it grows in some parts of South Africa, the plant itself, as well as its uses, are mostly unknown to South Africans in general (National Research Council, 2006).

Literature study and a few informal discussions held in Tshwane and Mokopane in the Gauteng and Limpopo provinces of South Africa respectively indicated that although some people use *Moringa* in their diets (mostly Indians) its usage is not documented in South Africa. However, the listing of *Moringa* as an herb in South Africa in a recent publication (Roberts, 2007) may be an indication that awareness of the plant in South Africa is on the increase. There is therefore an opportunity to introduce *Moringa* as a food source, which could lead to an increase in diversity of the dietary intake, especially among rural populations of South Africa. It has been shown in a recent survey that 33% of South African children under the age of six suffer from vitamin A deficiency (Coovadia, 2003). Ramachandran *et al.*, (1980) reported the vitamin A content of
Moringa as 11,300 IU per 100 g edible portion. The original source did quote the value as beta carotene, which should read 11,300 IU beta carotene per 100 g edible portion (McBurney et al., 2004). Babu (2000) reported vitamin A content as 3767 IU per 100 g edible portion. A publication of Kuhnlein (2003) quoted Moringa in Niger as containing 5880 μg beta-carotene per 100 g edible portion. This data of Kuhnlein (2000) is recommended by McBurney et al., (2004). An initiative was launched by FAO to analyse the nutrient composition of traditional leafy vegetables so as to standardise the nutrient content per 100 g edible portion (FAO, 2008).

2.2 Applications

According to (Verma et al., 1976) M. oleifera is a fast growing tree being planted in India on large scale as a potential source of wood for the paper industry. The wood provides a pulp that is considered suitable for paper, wrapping, textiles and cellophane. In Jamaica, exudate is used for blue dye.

All of the parts of the M. oleifera can be used in a variety of ways as food. It is full of nutrients and vitamins. The leaves, especially young shoots, are eaten as greens, in salads, in vegetable curries, and as pickles. In India, Moringa extracts are commonly used as a phytotherapeutic agent. The leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Dried or fresh leaves are also used in foods such as soups and porridges (Lockett et al., 2000), curry gravy and in noodles, rice or wheat (Abilgos et al., 1999). Farmers have added the leaves to animal feed to maintain a healthy livestock (Sarwatt et al., 2002; Fahey, 2005; Sanchez et al., 2006) while utilizing the manure and vegetable compost for crop growth (Fahey, 2005). Newer applications include the use of Moringa powder as a fish food in aquacultural systems (Dongmeza et al., 2006). In the West, one of the best
known uses for *Moringa* is the use of powdered seeds to flocculate contaminants and purify drinking water (Berger et al., 1984; Gassenschmidt et al., 1995; Olsen, 1987) but the seeds are also eaten green, roasted, powdered and steeped for tea or used in curries (Gassenschmidt et al., 1995).

*Moringa* leaves are used as feed for cattle, pigs and poultry. When *Moringa* leaves constituted 40 to 50% of feed, it was found in research studies that milk yields for dairy cows and daily weight gains for beef cattle increased by 30%. The birth weight of calves increased by 3 to 5 kg. Some animals, such as chickens will not voluntarily consume *Moringa* leaves or *Moringa* leaf powder (Price, 2000).

The seed of *M. oleifera* contains high quality edible oil (up to 40% by weight). In Haiti, the oil has been used as general culinary and salad oil. It resembles olive oil in its fatty acid composition (Abdulkarim et al., 2005). The oil is also used as a lubricant for fine machinery, such as timepieces, for its little tendency of deteriorating and becoming sticky (Foidl et al., 2001). Moreover, the oil has the capacity to absorb and retain volatile substance and is therefore valuable in the perfume industry.

After oil extraction of *Moringa oleifera* seeds, the left press cake contains water soluble proteins that act as effective coagulants for water purification. One to two seeds per litre are required for water purification. Seed powders are mixed with water, after hours, the water is filtered to get purified water. The charged protein molecules can serve as nontoxic natural polypeptide to settle mineral particles and organics in the purification of drinking water, vegetable oil, depositing juice (sugarcane) and beer (Foidl et al., 2001). Recently, there is an increasing trend to evaluate some indigenous cheaper material for wastewater treatment. Current studies report that *Moringa* seeds and pots are effective sorbets for removal of heavy mental and volatile organic compounds in the
aqueous system (Akhtar et al., 2006, Sharma et al., 2006). It can be added in oxidation lagoons of wastewater treatment units to coagulate algae as well. The algae are removed by sedimentation, dried and pulverized, and then are used as protein supplement for livestock (Foidl et al., 2001). The unique characteristic of Moringa seeds could be a possible solution for the developing countries which are suffering from lack of clean drinking water.

Moringa could be used as green compost. The juice from the fresh leaves can be used to produce an effective plant growth hormone (Price, 2000; Foidl et al., 2001). This hormone increases the yield by 25 – 30 % for nearly any crop including onion, bell pepper, soya, maize, coffee, tea and other plants. The active substance is zeatin; a plant hormone from the cytokinines group, which is available as a spray.

2.3 Antibacterial activity of Moringa

Bacteria are listed at first position among the microorganisms causing opportunistic diseases (Kone et al., 2004). Innumerable antibacterial agents are currently employed in treating bacterial infections. However, the widespread and indiscriminate use of antibacterial agents resulted in development of drug resistance among many virulently pathogenic bacterial species (Berkowitz, 1995). Many of the currently used antibacterials are associated with adverse effects such as toxicity, hypersensitivity, immunosuppression, and tissue residues posing public health hazard. Further, the newer broad spectrum antibiotics are cost prohibitive and are not within the reach of poor Indian farmer. These disadvantages undermine the therapeutic utility of the currently available antibacterials and thus necessitating the need for finding alternative remedies for treatment of bacterial diseases. As the global scenario is now changing towards the use of non-toxic and eco-friendly products, development of modern drugs from traditional
medicinal plants should be emphasized for the control of various human and animal diseases. *M. oleifera* is one such plant which is reported to possess several medicinal properties. The different parts of this plant viz. leaves, stem bark, root bark, flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments (Chopra *et al.*, 1956; Nadkarni, 1976). During recent years considerable work has been done to investigate the pharmacological actions of the leaves and seeds of *M. oleifera* on scientific lines but only limited work has been reported so far on antibacterial activity of *M. oleifera* root bark though it is reported to possess varied medicinal properties. Therefore, it was considered worthy to investigate the antibacterial activity of *M. oleifera* root bark. Bark used to cure Dental Caries/Toothache, Common cold, External Sores/Ulcer, Anti-Tumor, Snakebite, Scorpion bite, Digestive, Headache, Antinutrietional factors and Scurvy (Fahey, 2005).

In the late 1940’s and early 1950’s a team from the University of Bombay (BR Das), Travancore University (PA Kurup), and the Department of Biochemistry at the Indian Institute of Science in Bangalore (PLN Rao), identified a compound they called pterygospermin a compound which they reported readily dissociated into two molecules of benzyl isothiocyanate (Kurup and Rao, 1952, 1954; Kurup and Rao, 1954; Venkataraman *et al.*, 1954; Das *et al.*, 1954, 1957). Benzyl isothiocyanate was already understood at that time to have antimicrobial properties. This group not only identified pterygospermin, but performed extensive and elegant characterization of its mode of antimicrobial action in the mid 1950’s. Although others were to show that pterygospermin and extracts of the *Moringa* plants from which it was isolated were antibacterial against a variety of microbes, the identity of pterygospermin has since been challenged (Eilert *et al.*, 1981) as an artifact of isolation or structural determination. Subsequent elegant and very thorough work, published in 1964 as a PhD thesis by Bennie Badgett (a student of the well known chemist Martin Ettlinger), identified a number of
glycosylated derivatives of benzyl isothiocyanate (e.g. compounds containing the 6-carbon simple sugar, rhamnose) (Badgett, 1964). The identity of these compounds was not available in the refereed scientific literature until “re-discovered” 15 years later by Kjaer and co-workers (Kjaer et al., 1979). Seminal reports on the antibiotic activity of the primary rhamnosylated compound then followed, from U Eilert and colleagues in Braunschweig, Germany (Eilert, 1978; Eilert et al., 1981). They re-isolated and confirmed the identity of 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate and its cognate isothiocyanate and verified the activity of the latter compound against a wide range of bacteria and fungi. Extensive field reports and ecological studies forming part of a rich traditional medicine history, claim efficacy of leaf, seed, root, bark, and flowers against a variety of dermal and internal infections. Unfortunately, many of the reports of antibiotic efficacy in humans are not supported by placebo controlled, randomized clinical trials.

Aware of the reported antibiotic activity of and other isothiocyanates and plants containing them, we undertook to determine whether some of them were also active as antibiotics against Helicobacter pylori. This bacterium was not discovered until the mid-1980’s, a discovery for which the 2005 Nobel Prize in Medicine was just awarded. H. pylori is an omnipresent pathogen of human beings in medically underserved areas of the world, and amongst the poorest of poor populations worldwide. It is a major cause of gastritis, and of gastric and duodenal ulcers. Cultures of H. pylori, it turned out, were extraordinarily susceptible to and to a number of other isothiocyanates (Fahey et al., 2002; Haristoy et al., 2005). These compounds had antibiotic activity against H. pylori at concentrations up to 1000-fold lower than those which had been used in earlier studies against a wide range of bacteria and fungi. The extension of this finding to human H. pylori infection is now being pursued in the clinic, and the prototypical isothiocyanate has already demonstrated some efficacy in pilot studies (Galan et al., 2004; Yanaka et al.,
Faizi et al. (1994) reported the isolation of two nitrile glycosides from the ethanolic extracts of *M. oleifera* leaves, niazirin and niazirinin and three mustard oil glycosides, 4-[(4'-O acetylalpha- L- rhamnosyloxy) benzyl] isothiocyanate, niaziminin A, and niaziminin B. Niazirinin is a new compound. Niaziminins A and B have previously been obtained from the left extract as a mixture. This is the first report of the isolation of nitriles, an isothiocyanate, and thiocarbamates from the same plant species. Faizi et al. (1995) isolated six new and three synthetically known glycosides from the leaves of *M. oleifera*, employing a bioassay-directed isolation method on the ethanolic extract. Most of these compounds, bearing thiocarbamate, carbamate or nitrile groups, are fully acetylated glycosides, which are very rare in nature. Elucidation of the structures was made using chemical and spectroscopic methods, including 2D NMR techniques. Murakami et al. (1998) isolated niaziminin, a thiocarbamate from the leaves of *M. oleifera*. Bennett et al. (2003) isolated various glucosinolates and phenolic compounds from various parts of *M. oleifera*. Karuna Shanker et al. (2007) isolated nitrile glycosides (niaziridin & niazirin) from the leaves, pods and bark of *M. oleifera* by reverse phase HPLC. Singh et al. (2009) reported presence of gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaempferol, quercetin and vanillin from the aqueous extracts of leaves, fruits and seeds of *M. oleifera*. The leaves also contains quercetin-3-O-glucoside and quercetin-3-O-(6''-malonyl-glucoside), and lower amounts of kaempferol-3-O-glucoside and kaempferol-3-O-(6''-malonyl-glucoside), 3-caffeoylquinic acid and 5-caffeoylquinic acid.

The *Moringa* plant has been the object of much research due to its multiple uses and well-known bactericidal potential (Suarez et al., 2003; Ghebremichael et al., 2005). According to Bezerra et al., the *Moringa* tree is native to northeastern India. It is rich in nutrients and, apart from a range of industrial and medicinal applications, is used to
purify water for human consumption (2003). Not surprisingly, as explained by Makkar and Becker (1997), the *Moringa* is of economic importance in the production of several commodities, such as oils, foods, condiments and medicines. Traditional medicine has a long history of serving peoples all over the world (Cheng, 2000). Medicinal plants are an important element of indigenous medical systems that has persisted in developing countries. The plant kingdom was estimated to produce over 500,000 natural products and about 40 to 80 thousand per plant species (Bhatt, 1995). Recently, the use of traditional medicine based on plants has received considerable interest (Han *et al.*, 2002). There are national and indigenous rights over plant derived resources. Basic scientific investigations based on medicinal plants and indigenous medical systems have increased. It has been estimated that 1 to 10% of the large diversity of 250,000 to 500,000 plant species on the Earth have been studied chemically and pharmacologically for their medicinal properties (Farnsworth, 1991; Verpoorte, 2000). Recently a new benefit of *Moringa* was suggested: the leaves seem to contain a substance that stimulates plant growth and increases crop production. Several years ago, Mr. Nikolaus Foidl came across a reference to a study by a Mr. Singh of India. It said that an extract from *Moringa* leaves seemed to stimulate the growth of plants.