General Introduction
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G.1 Plants: Natural therapeutic resources

Plants with a wide variety of chemical constituents offer a promising and rich source of agents with innumerous biological properties of great value. Besides constituting the basis of traditional medicine systems, plants continue to be untiring natural resources of a variety of potent biochemical products that provide mankind with nutritious food supplements and novel therapeutics (Farnsworth, 1984). The high costs and risks associated with the current therapies have compelled the need for alternative cost-effective and non-toxic measures to minimize the complications. Medicinal plants and their products being more convenient and greatly accepted by the users for their minimum side effects, low cost and easy availability, seem to offer a promising solution to this problem.

Medicinal plants have always been an important therapeutic armor of mankind (Hostettmann and Marston, 2002). Plants constitute the prime source of about 25% of the drugs prescribed worldwide from which 121 active compounds are being used currently. About 11% of the 252 drugs considered as basic and essential by the World Health Organization (WHO), are exclusively of plant origin and a significant number of synthetic drugs are obtained from natural precursors. Phytotherapy is an important part of the WHO’s health programs which encourage and suggest basic procedures for the validation and development of drugs from plant origin in developing countries.

While herbal medicine industry is being well-established in Eastern countries, such as China and India, investments are being made in Latin American and European countries in medicinal plant research programs, standardization and regulation of phytomedicinal products. Recently, specific departments dedicated to the study of new drugs from natural sources have been included even in large pharmaceutical companies, such as Merck, CIBA, Glaxo, Boehringer and Syntex. However, higher plants have not been explored
completely for their potential use as a source of new drugs. It is found that out of 250,000–500,000 plant species estimated, only a small percentage has been phytochemically studied and even a smaller percentage has been properly investigated (Rates, 2001). In recent years, considerable attention is directed towards natural ingredients from plant extracts which are believed to be safer and healthier than their synthetic counterparts. Thus, it is essential and worthwhile to investigate and scientifically validate such medicinally important plants.

G.2 Prostate cancer: The dreadful disease of men

Prostate cancer is an abnormal, uncontrolled growth of cells that results in the formation of a tumor in the prostate gland, a part of male reproductive system that helps in the synthesis and storage of seminal fluid. The prostate in adult men is located in pelvis and surrounds a part of urethra involved in urination and ejaculation (Aumuller, 1979; Moore and Dalley, 1999). Prostate cancer is the most prevalent cancer afflicting men over the age of fifty (Siegel et al., 2011). Earlier, half of the people with prostate cancer in India died within ten years (Jaubert de Beaujeu and Chavrier, 1976).

The presence of the disease may be indicated by physical examination, tumor markers or biopsy. While a few patients diagnosed with prostate cancer may have symptoms that include frequent and painful urination, two thirds may not have symptoms (Miller et al., 2003). Although a complete understanding of the causes of prostate cancer remains elusive (Hsing and Chokkalingam, 2006), the primary risk factors may be obesity, age, genetic background and family history. It is also found that men with high blood pressure are more likely to develop prostate cancer (Martin et al., 2010).

Surgery, radiation therapy, hormone therapy and chemotherapy are the usually adopted therapeutic measures of aggressive prostate cancers (Hong et al., 2010; Peyromaure et al., 2009). The disease advances with the metastasis of the cancer cells from the prostate to other parts of the body, particularly the
bones and lymph nodes (Ummanni et al., 2008). Aggressiveness of the cancer is rated based on Gleason Score. The development of cancer is grouped into four grades or stages (Staging and Grading – Prostate Cancer Foundation of Australia, 2013).

Stage 1 - the cancer is small and within the prostate.
Stage 2 - the cancer is larger and may be in both lobes of the prostate, but is still confined to the organ.
Stage 3 - the cancer has spread beyond the prostate and may have invaded the adjacent lymph glands or seminal vesicles.
Stage 4 - the cancer has spread to other organs, or to bone.

The cancer may turn out to androgen-independent stage which is also termed as the castration-resistant or hormone refractory prostate cancer when the disease becomes no longer responsive to castration or hormone treatment. The risk increases as the cancer progresses from androgen-dependent to androgen-independent stage (Bruckheimer and Kyprianou, 2000). Clinical studies have indicated that progression to androgen independence remains the main obstacle to improving survival for patients with advanced prostate cancer (Farhat et al., 2000).

G.3 PC3: The cell lines of choice

The human prostate cancer cell lines, PC3 seem to be useful in investigating the biochemical changes in advanced staging of the disease and in assessing their response to chemotherapeutic agents. They are the classical in vitro androgen-independent models of prostate cancer with high metastatic potential (Nachshon-Kedmi et al., 2004; Younghun Jung et al., 2012). They have been established in 1979 from bone metastasis of grade IV of prostate cancer in a 62 year old Caucasian male. Karyotypic analysis by quinacrine banding has revealed the cells to be completely aneuploid with a modal chromosome number in the hypo triploid range. At least 10 distinctive marker chromosomes have been identified. The overall karyotype and the marker chromosomes are found to be distinct from those of the Henrietta Lacks
(HeLa) cells derived from cervical cancer cells taken on February 8, 1951 from the patient Henrietta Lacks (Scherer et al., 1953).

Electron microscopic studies of PC3 cells have revealed many features common to neoplastic cells of epithelial origin including numerous microvilli, junctional complexes, abnormal nuclei and nucleoli, abnormal mitochondria, annulate lamellae, and lipoidal bodies. The functional and morphologic characteristics of PC3 cells are those of a poorly-differentiated adenocarcinoma. The cells have a greatly reduced dependence upon serum for growth when compared to normal prostatic epithelial cells and do not respond to androgens, glucocorticoids, or epidermal or fibroblast growth factors (Kaighn et al., 1979).

The metastatic potential of PC3 cell lines is higher than that of other prostate cancer cell lines namely the androgen-independent DU145 cell lines derived from brain metastasis and the androgen-sensitive Lymph Node Carcinoma of the Prostate (LNCaP) cell lines from the left supraclavicular lymph node metastasis in a 50-year-old Caucasian male in 1977. DU145 cells have a moderate metastatic potential and LNCaP cells have low metastatic potential (Pulukuri Sai MuraliKrishna et al., 2005). PC3 cells have low acidic phosphatase and testosterone-5-alpha reductase activity. They do not express Prostate Specific Antigen (PSA) and are Prostate Specific Membrane Antigen (PSMA)-negative (Arundhati Ghosh et al., 2005; Alimirah et al., 2006).

The growth of PC3 cells become androgen unresponsive and refractory to hormonal therapy due to the poor expressed levels of functional androgen receptors (AR) and PSA. Thus, the PC3 cell lines provide an appropriate model to study the effect and mechanism of action of plant products as therapeutic aids for androgen-independent prostate cancer.

G.4 Moringa oleifera and its flowers: The plant source of study

In a pharmacological study, selecting a suitable plant is a very important and decisive step. This is usually done based on the careful understanding of the
ethnobotany and ethnopharmacology of the plant. Also, availability, ease of access and ability of the plant to meet the experimental needs are taken into account for selection. *Moringa oleifera* Lam. (synonym: *Moringa pterygosperma* Gaertn.) is a highly valued plant, distributed in many countries of the tropics and subtropics. It is a predominant Indian nutritional plant with a wide range of medicinal properties. It is the most widely cultivated species of the family Moringaceae with the only single genus, *Moringa* (Anwar et al., 2007).

**TAXONOMIC CLASSIFICATION** (Garima Mishra et al., 2011)

**Kingdom** – Plantae  
**Sub kingdom** – Tracheobionta  
**Super Division** – Spermatophyta  
**Division** – Magnoliophyta  
**Class** – Magnoliopsida  
**Subclass** – Dilleniidae  
**Order** – Capparales  
**Family** – Moringaceae  
**Genus** – Moringa  
**Species** – Oleifera

*M. oleifera* is an evergreen or deciduous tree that usually grows upto a height of 9 m with fragrant, bisexual, yellowish white flowers (Figure G.1) borne on slender, hairy stalks in spreading or drooping axillary panicles 10–25 cm long. The flower buds are ovoid in shape. Each flower has 5 petal-like sepals and 5 petals. The 2 dorsal sepals and 1 dorsal petal that usually remain un-reflexed form a projecting keel, while the rest of the perianth reflexes downwards to form a ‘banner’ at right angles to the keel. Sepals are elongated in shape, 7–15 mm long by 5–6 mm wide, and have obtuse apices. They are white or cream, sometimes with yellow streaks in the centre, and are usually puberulent. The petals are slightly spoon-shaped, 1–2 cm long by 5–6 mm wide, with prominent veins. The 5 fully formed stamens, about 10 mm long have
Figure G.1: *Moringa oleifera* flowers

Yellowish white flowers of *Moringa oleifera* (along with buds) borne on slender stalks in drooping axillary panicles.
waxy yellow or orange anthers and alternate with a row of 5 staminodes, about 7 mm long. These stamens and staminodes have filaments that are hairy at the base. The hairy ovary is oblong, about 5 mm long with a single locule containing numerous ovules. It is topped with a single slightly hairy style and a minute stigma. Flowering phenology varies widely among varieties and with location. In locales with more constant seasonal temperatures and rainfall regimes, flowering may occur more or less continuously throughout the year. The flowering season usually begins in January and continues through to March and often within 6 months after planting (Parrotta, 2009; Sheldon Navie and Steve Csurhes, 2010).

*M. oleifera* is rich in a number of vitamins, minerals and specific phytochemicals, reported to have hypotensive, anticancer and antibacterial activities (Fuglie, 2001). It has been also reported to have anti-ulcerogenic activity (Akhtar and Ahmad, 1995). Being both a medicinal and functional food, it finds use in the form of “Miracle vegetable” (Verma *et al*., 1976). The plant is rich in compounds containing rhamnose, glucosinolates and isothiocyanates (Bennett *et al*., 2003; Fahey *et al*., 2001). Phytochemicals such as pterygospermin (Kurup and Rao, 1954), moringine and moringinine (Chopra, 1958), fatty acids and amino acids (Ramiah and Nair, 1977; Farooq and Bhanger, 2003; Abdulkarim *et al*., 2005), steroids (Saluja *et al*., 1978; Giovanna *et al*., 2002), carbamates and thiocarbamates (Faizi *et al*., 1995; Faizi *et al*., 1997; Murakami *et al*., 1998; Francis *et al*., 2004), flavonoids (Stavros and John, 2002; Bennett *et al*., 2003) have been isolated from the root, root bark, leaves and stem.

The tree's fruits, flowers, leaves, pods, young shoots and roots are all consumed as food and have an impressive range of medicinal uses (Umberto Quattrocchi, 2000). The fruit cures leucoderma and tumor. It also improves sperm count and motility. The seeds are capable of disinfecting heavily contaminated water. The seed oil is used as an external application for rheumatism. The root is diuretic, anti-inflammatory agent, laxative and used to
treat stomatitis and obstinate asthma (Kirtikar and Basu, 1975). The root bark is used in heart complications and eye disorders. Both root and bark act as abortifacients (Satyavati and Gupta, 1987). The leaves are hypolipidemic, antiatherosclerotic with immune-boosting and tumor-suppressive properties (Udupa and Kulkarni, 1998; Prakash et al., 1988; Faizi et al., 1995). The flowers are used to make tea for colds and improve the quality and flow of breast milk. They possess good amounts of both calcium and potassium (Fahey, 2005). They are chlolagogue, stimulant, tonic and diuretic (Chopra et al., 1956). They are also used to cure inflammations and muscle diseases. Folk medicine supports the use of *M. oleifera* flowers for treating cancerous tumors (Harwell, 1971).

Besides meeting the global needs for safe and efficient drugs, the exploration of this natural resource preserves the natural diversity and environment. The plant is commonly available and grows rapidly. Though flowers produce seeds which assist in propagation, the plant is typical such that it is easily and preferably propagated by stem cuttings rather than being raised from seeds as the latter reduces the flowering pace and produces fruits of inferior quality (Ramachandran et al., 1980). On the other hand, these flowers are very light that a number of healthy flowers often shed from the plant. These dropped flowers instead of going as a waste, can be processed for their therapeutic values. Several decades of research have been more on the other parts of this medicinal plant. But only limited research is done on the flowers. Therefore, in the current study, focus is laid on the *M. oleifera* flowers to explore their therapeutic potential.

**G.5 Research problem**

To identify suitable candidates from *M. oleifera* flowers to treat androgen-independent prostate cancer.
G.6 Objectives

- To investigate the antioxidant activity of *M. oleifera* flower extracts and the effect of geographical properties on the phytochemical composition and antioxidant activity.
- To examine the potential of the selected extract to inhibit the growth of cancer cells in PC3 cell lines.
- To predict the bioactive components in the extract.
- To analyze the structure of drug targets.
- To interpret the mutation in the target protein and the effect of ligands on the target proteins.
- To determine the molecular properties and pharmacokinetics of the ligands.
- To study the ligand interactions with the drug targets and their pharmacodynamics.

G.7 Conceptual and Methodological details

The study is an attempt to explore the molecular aspects of androgen-independent prostate cancer as well as the drug candidates derived from *M. oleifera* flowers. The flower sample has been analyzed phytobiocemically for its biological activities and bioconstituents that can be of therapeutic value against the drug targets in PC3 cells. The biocomputational methods are mainly harnessed to reduce the time, cost and risk associated with Drug Discovery (Shoba *et al.*, 2010). Prediction of three dimensional (3D) models of drug targets and their docked conformations with ligands play a vital role in determining their biological significance. The successful drug-like ligand is determined by its effective interaction with the drug targets at their active sites and by its molecular properties. The whole of the molecular studies that have been followed revolve around the basic molecular structures of the drug targets and ligands. Relevant methodologies have been adopted to match the research design. While the first two chapters deal with the *in vitro* phytobiocemical studies, the next two chapters are concerned with the *in silico* molecular studies.
G.8 Bibliography


