CHAPTER 1

INTRODUCTION

1.1 MOTIVATION AND BACKGROUND

Natural products are chemical substances present in nature inherent with distinct pharmacological effects. They derive their origin from plants, animals and microorganisms. These naturally occurring pharmacological products have been known to humankind since centuries and are used in the treatment of several diseases. Biologically active principles obtained from plants, in particular, are considered to be of greater reliability due to their renewable nature. A number of documents are available which establish how plant originated biochemical constituents or their derivatives are absolutely essential in the development of modern drugs (Kinghorn et al. 2011; Newman and Cragg 2012).

Herbal medicine has been acknowledged by the WHO as a vital part of primary healthcare and 11% of the basic 252 drugs consumed by mankind are obtained from plants (Taylor 2000). These medicines include digoxin from *Digitalis* sp., quinine and quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna*, and morphine and codeine from *Papaver somniferum*. Various plant-derived drugs such as paclitaxel, camptothecin-derived analogues, arteether, galanthamine, tiotropium are in clinical use today while some others are undergoing Phase II and Phase III clinical trials (Jachak and Saklani 2007).
Bioactive compounds are commonly considered as plant-derived secondary metabolites which exert a pharmacological effect on the human body system (Abdelkarim Guaadaoui et al. 2014). Within the category of secondary metabolites, polyphenolic compounds exhibit pharmacological properties that are related to their total antioxidant potential (Elfalleh et al. 2011). Polyphenols serve as antioxidants enhancing the body’s resistance against oxidative damages and bringing about a substantial improvement in health conditions (Pandey and Rizvi, 2009). The antioxidant capacity of phenolic compounds occurs in response to their redox properties which allow them to perform as singlet oxygen quenchers or metal chelators besides being reducing agents and hydrogen donors. In addition to their antioxidant potential, polyphenolic compounds are considered to be very safe for consumption because of their plant origin (Elfalleh et al. 2011).

Over the last couple of decades, there has been a growing use of naturally occurring plant-based polyphenolic compounds in the treatment of manifold degenerative diseases due to their positive and active therapeutic properties like antioxidant, antimicrobial, anticancer and anti-inflammatory activities. However, a vacuum exists owing to insufficient data documentation in relation to the antioxidant properties of plants that are not entirely exploited in medicine and nutrition (Sadia et al. 2014). In light of this, there is a need to conduct a detailed study of unexplored polyphenolic rich plants for evaluation of their bioactive principles and biological effects which in turn will open a new avenue in discovering new sources for natural antioxidants, functional foods and nutraceuticals.

Mulberry is a phytonutrient rich plant which belongs to the genus Morus and family Moraceae. It has been widely used as one of the conventional medicinal plants for centuries due to its chemical composition and pharmacological utility (Ramesh et al. 2014). The mulberry has been an integral part of traditional Chinese
medicine due to its low toxic levels and excellent therapeutic functioning (Li 1998), and its curative benefits are abundantly described in various traditional Chinese medicinal records. Numerous studies have brought to light the existence of multiple bioactive constituents, namely alkaloids (Asano et al. 2001), carotenoids and flavonoids (Hassimotto et al. 2007), vitamins, fats (mainly linoleic acid), palmitic acid, oleic acid, sugars (glucose and fructose) and minerals (Ercisli and Orhan 2007). Plants belonging to the genus Morus comprise diverse flavonoids including rutin, quercetin and isoquercitin (Ercisli and Orhan 2007; Ozgen et al. 2009), apigenin, luteolin, morin, caffeic acid, gallic acid, umbelliferone, chlorogenic acid and kaempferol (Chu et al. 2006).

The entire plant of Morus alba L., including roots, bark, branch, leaves and fruits, possesses multiple medicinal values. The fruits have been traditionally used as a remedy for dysentery and as a laxative, odontalgic, anthelmintic, expectorant, hypoglycaemic and emetic. They have also been used as a popular antidote in conventional Chinese herbal medicine to alleviate dental and oral diseases, diabetes, hypertension, arthritis and anaemia (Liang et al. 2012). In recent times, there has been a marked escalation in the production and consumption of mulberry fruits due to their aromatic taste, nutrition benefits, bioactive compound content and biological functions (Liang et al. 2012). The health benefits of mulberry plant are, nevertheless, obtained not only from their fruits but also their leaves which have revealed extensive biological properties. Infact, a comparison with mulberry fruits clearly shows leaves to display a higher content in phenolic compounds and antioxidant activity (Salcedo et al. 2015).

Mulberry leaves are utilized as animal and insect feed as they are highly nutritious, palatable and non-toxic and are stated to improve milk feed when fed to dairy animals (Sastri 1962). They have been an integral part of traditional medicine to treat diabetes and hyperlipidemia, and lower blood pressure, high cholesterol and
neutral fat (Assy et al. 2000; Steinberg et al. 1989). Literature analysis reveals that mulberry leaf contains anticancer properties and deters hyperglycaemia (Zhou et al. 2001). Numerous studies have submitted findings showing it to contain potential antioxidant activity (Kim et al. 1999; Arabshahi-Delouee and Urooj 2007; Katsube et al. 2006). The leaves help to alleviate the risk and aid in the treatment of type 2 diabetes, diseases of the cardiovascular system, urinary system, nervous system (Alzheimer’s disease), as well as facilitate weight loss.

The beneficial effects of mulberry leaves exerted on the human body are the result of a huge presence of flavonoids, steroids, triterpenes, aminoacids, vitamins and other trace elements (Deshmukh et al. 1993). Latest reports suggest *Morus alba* L. leaves to be a rich source of polyphenolic substances, including phenolic acids and flavonoids such as caffeic acid, caffeoylquinic acids, kaempferol-3-O-(6-malonyl)-glucoside, quercetin-3-O-(6-malonyl)-glucoside, and quercetin-3-O-glucoside (Memon et al. 2010; Thabti et al. 2012). A few flavonol glycosides such as antioxidant components, including rutin, isoquercitrin, astragalin and quercetin-3-(6-malonyl) glucoside are seen to be present in these leaves (Katsube et al. 2006).

Beyond their medicinal usage, mulberry leaves are also consumed as part of the daily diet in some Asian countries. For instance, mulberry leaf powder is added to wheat flour in the preparation of paratha, an item regularly eaten during breakfast and dinner in India (Srivastava et al. 2003). The intake of mulberry leaves as infusion and powdered juice is common in Korea and Japan with their demand steadily increasing (Desmukh et al. 1993; Katsube et al. 2009; Thabti et al. 2012). Infact, in Japan, the consumption of mulberry leaf tea is a growing phenomenon and mulberry green tea is served as one of the health drinks (Katsube et al. 2006).
Mulberry leaves can be regarded as potential sources of phytochemical compounds with verified biological properties. With a steady rise in food products made from mulberry leaves and a rapid increase in reports on their biological properties, further exploration of their phytochemical composition would facilitate a renewed interest in this vegetative material in order to produce mulberry leaf-derived products rich in phenolics.

In India, the mulberry has largely been used as a valuable resource for sericulture purposes. These include white mulberry (M. alba) and M. indica as their leaves are tender, highly nutritious and succulent resulting in better cocoon production. Sericulture Institutes under the aegis of Central Silk Board (CSB) have focused on those aspects of mulberry leaves primarily related to the silkworm industry, engaging in the collection of mulberry germplasm, their morphological identification and the development of new cultivars through breeding programmes with improved commercial traits for sericultural practices. However, a gap of research exists as there is no comprehensive/scientific database on the morphology, physiology and biochemistry of the leaves of mulberry species/varieties available in the country.

Mulberry plant remains an unexplored phytonutrient rich plant in India. Literature survey has revealed a dearth of study on phytochemical evaluation that identifies the bioactive principles inherent in the leaves of different varieties/species of mulberry and their pharmacological effects. Among the total available species/varieties of mulberry unused for sericulture purposes, some could be explored for the production of secondary metabolites due to the presence of flavonoids and polyphenols in them (e.g. M. latifolia, M. multicaulis, M. serrata, M. laevigata, M. australis, etc.).
Mulberry is widely cultivated in Tamil Nadu, one of the main centres for cultivation of different species/varieties of mulberry in India. Hence, its easy availability rendered it expedient to select this plant for study. Furthermore, India being home to several species and natural variants of mulberry plants, there was an urgent need to choose this plant and explore its therapeutic potential which would consequently enhance the significance of mulberry beyond its traditional eminence as exclusive forage of silkworms.

1.2 AIM

Metabolic profiling of different indigenous species/varieties of mulberry in order to identify biomolecules of economic and therapeutic interest and study of their selective therapeutic properties.

1.3 OBJECTIVES OF THE RESEARCH

The objectives of the study are:

I) Morphological characterization and documentation of various *Morus* species/varieties

II) Bioprospecting of biomolecules from mulberry leaves and their antioxidant properties

III) Animal model studies using polyphenolic extract of mulberry leaves with special reference to lifestyle diseases (diabetes and obesity)

IV) Molecular docking of bioactive polyphenolic molecules on marker compounds for diabetes and obesity