Chapter 1
Introduction
1. Introduction

The humans have relied on nature for their basic needs such as foods, shelter, clothing, fertilizers, flavours, fragrances, and medicines from prehistoric time. This practice, the great civilizations of the ancient Indians, Chinese, Arabians and North Africans provided written evidence of man’s ingenuity in utilizing plants for the treatment of a wide variety of diseases (Phillipson, 2001). Thus, plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide humankind with new remedies (Gurib–Fakim, 2006). Through periods of trial, error and success, the ancient man and their followers have accumulated a huge knowledge about medicinal plants (Iwu et al., 1999). The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BC. Among the substances that used were oils of Cedrus species (Cedar) and Cupressus sempervirens (Cypress), Glycyrrhiza glabra (Liquorices), Commiphora species (Myrrh) and Papaver somniferum (poppy juice), all of which are still in used for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation (Gurib–Fakim, 2006).

1.1 Traditional medicines

The knowledge of the medicinal plants and the methods of application for particular ailments were passed down through oral tradition. Eventually information regarding medicinal plants was recorded traditional medicine. Many traditional systems do not have the scientific insight to explain and predict the curative action of plants. It is based on the assumption that the appearance of plants may give clues to their medicinal properties—it is interpreted as God’s signature on the plant. For example, Red juice and sap, considered to be associated with blood and menstrual ailments; yellow flowers with bile and jaundice; the human shape of certain roots with the female form of fertility and so on. However, sometimes this concept worked: Chelidonium majus, contains yellow flowers and a yellow alkaloid containing latex, and has been used successfully to treat jaundice (Gurib-Fakim, 2006).

Historically, all medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc (Krishnaraju et al., 2005). The importance of medicinal plants and traditional health
systems in solving the health care problems of the world is gaining increased attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. The Indian traditional medicines have had practiced successfully in modern age, have hardly been studied for scientific validation.

1.1.1 Indian Systems of Medicine

India has a rich cultural heritage of traditional medicines which chiefly comprised two widely flourishing system of treatments i.e. Ayurveda and Siddha system of medicine (Surana et al., 2008). These systems are considered not just as an ethnomedicine but also as a complete medicinal system that takes in to consideration physical, psychological, philosophical, ethical and spiritual well being of mankind. It lays great importance on living in harmony with universe and harmony of nature and science, this universal and holistic approach makes it a unique and distinct medicinal system. The principle behind diagnosis and treatment are unique and are still valid in today’s modern era. It based on foundational principles of panchamahabhutha (five basic elements of nature), tridosha (three humours) and prakrithi (individual constitution) (Venkatasubramanian, 2007). The tridosha in balanced condition ensure body healthy condition, but when they are unbalanced, leading to diseased state.

1.2 Liver

Liver is an indispensable organ composed of hepatocytes, plays a pivotal role in regulating various physiological processes such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful principles (Shanmugasundaram and Venkataraman, 2006). It also involved in biotransformation, excretion of drugs and other xenobiotics from the body (Saleem et al., 2010).

During detoxification and excretion of xenobiotic (Phase I and Phase II reactions) highly reactive oxygen species (ROS) like peroxides, epoxides and other reactive radicals are formed. Physiologically, liver synthesizes endogenous antioxidant enzymes and factors (GSH, ascorbic acid, and vitamin E and antioxidant enzymes such as SOD, catalase, and GPx) to balance the condition. The excessive ROS generation causes oxidative stress (Kohen and Nyska, 2002), which is responsible for hepatic disease and damage.
1.3 Liver diseases

In present scenario, liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. The common liver diseases are…..

1.3.1 Hepatitis

It is nothing but inflammation of the liver, caused mainly by viral infection (viral hepatitis) but also by some liver toxins (e.g. alcoholic hepatitis), autoimmunity (autoimmune hepatitis) or hereditary conditions. According to WHO, globally 170 million people are chronically infected with hepatitis-C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis-B virus (HBV) and over 5 million are getting infected with acute HBV annually (Negi et al., 2008).

1.3.2 Alcoholic liver disease

It is any hepatic manifestation of alcohol over consumption, including fatty liver disease, alcoholic hepatitis, and cirrhosis. Analogous terms such as "drug-induced" or "toxic" liver disease are also used to refer to the range of disorders caused by various drugs and environmental chemicals.

1.3.3 Fatty liver disease (Hepatic steatosis)

It is a reversible condition in which large vacuoles of triglyceride fat accumulate in liver cells. Non-alcoholic fatty liver disease is a spectrum of disease associated with obesity and metabolic syndrome, among other causes. Fatty liver may lead to inflammatory disease (i.e. steatohepatitis) and, eventually cirrhosis.

1.3.4 Cirrhosis

It involves the development of fibrous tissue (fibrosis) at the place of liver cells resulting the death of liver cells. Furthermore, it is associated with viral hepatitis, alcohol over consumption, and other forms of liver toxicity. Cirrhosis cause chronic liver failure.

1.3.5 Liver cancer

Primary liver cancer most commonly manifests as hepatocellular carcinoma and/or cholangiocarcinoma; rarer forms include angiosarcoma and hemangiosarcoma of the liver. Many liver malignancies are secondary lesions that have metastasized from
primary cancers in the gastrointestinal tract and other organs, such as the kidneys, lungs, breast, or prostate. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 250000 new cases each year.

1.3.6 Drug/chemical-mediated hepatic injury

Hepatic injury is the common sign of drug toxicity (Lee, 2003) and accounts for greater than 50% of acute liver failure cases. Hepatic damage is the largest obstacle to the development of drugs and is the major reason for withdrawal of drugs from the market (Cullen and Miller, 2006). Drug-induced liver disease can be predictable (high incidence and dose-related) or unpredictable (low incidence and may or may not be dose-related) and referred as idiosyncratic.

1.3.7 Other types:

- **Primary biliary cirrhosis** is a serious autoimmune disease of the bile capillaries.
- **Budd-Chiari syndrome** is the clinical picture caused by occlusion of the hepatic vein, which in some cases may lead to cirrhosis.
- **Hereditary diseases** that cause damage to the liver include hemochromatosis, involving accumulation of iron in the body, and Wilson's disease, which causes the body to retain copper. Liver damage is also a clinical feature of alpha 1-antitrypsin deficiency and glycogen storage disease type II.
- **Gilbert's syndrome**, a genetic disorder of bilirubin metabolism found in about 5% of the population, can cause mild jaundice.

Oxidative stress has been implicated in the pathogenesis of various liver disease including alcoholic liver disease, non-alcoholic fatty liver disease, and chronic hepatitis-C etc (Seki et al., 2005; Kitase et al., 2005).

1.4 Liver function test

Liver involved in multiple functions and it also has great power of regeneration; these makes estimation of the presence or absence of hepatic dysfunction is complicated. The series of biochemical test were used to assess the liver function:
The biochemical tests includes the estimation of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), total bilirubin, protein, triglyceride, total cholesterol, HDL and LDL. Antioxidant assay includes estimation of superoxide dismutase (SOD), catalase, glutathione peroxidase, lipid peroxidation and glutathione transferase in liver tissues.

The pathophysiology of almost all the liver diseases is same. The main cause is oxidative stress and other includes ROS induced inflammatory responses. In the treatment of liver diseases, anti-inflammatory plays vital role.

1.5 Free Radicals and oxidative stress

Free radicals are molecules or molecular fragments containing one or more unpaired electrons. The presence of unpaired electrons confers a considerable degree of reactivity to free radicals (Valko et al., 2004). ROS is a collective term including both oxygen radical-centered free radicals and non-radical oxidants. ROS include superoxide (\(\cdot O_2\)), hydroxyl (\(\cdot OH\)), hydroperoxyl (HOO\(\cdot\)), alkoxy radicals (RO\(\cdot\)) and peroxyl radicals (ROO\(\cdot\)) (Hu et al., 2005). The free radicals are produced within living cells and are part of the cell’s normal metabolic processes, including detoxification processes and immune defenses system. The excessive generation of the free radicals and ROS damages cell, affect the cell membrane and the nuclear DNA damage. In aerobic organisms, the defense system against these free radicals and ROS is provided by free radical scavengers which act as anti-oxidants. Free radical scavengers function by donating an electron to the free radical, the latter of which pairs with the unpaired electron and thereby stabilizing it. Anti-oxidant defense involves both enzymatic and non-enzymatic mechanisms. Enzymatic mechanisms utilizes specific enzymes such as superoxide dismutase, catalase and glutathione peroxidase, while nutrients and minerals are utilizes non-enzymatic mechanisms (Aggarwal et al., 2005). An increased level of oxidants (free radicals and ROS) in the cell can result in oxidative stress. This is responsible for the pathogenesis liver disease including alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis-C and other several diseases including atherosclerosis, cardiovascular disease, cancer (Seki et al., 2005; Kitase et al., 2005).

1.6 Antioxidants
Antioxidants are substance having potential to quench free radicals and significantly delay or inhibit oxidation of the substrate, thus protect biological systems against potential harmful effects of free radicals; in low concentrations. Synthetic antioxidants are phenolic compounds with varying degrees of alkyl substitution but their usage is being restricted and suspected to have negative health effects. It has been observed that natural antioxidants are safer than synthetic antioxidants (Su et al., 2009). Therefore, there is an increasing interest natural antioxidants. Enzymatic antioxidants are important for intracellular defenses, while non-enzymatic antioxidants are the major defense mechanism against extracellular oxidants. Natural antioxidants can be phenolic compounds (tocopherols, flavonoids, anthocyanins and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), or carotenoids as well as vitamin C and E, and phospholipids. Plants produce a large number of antioxidants to control the oxidative stress caused by sunbeams and oxygen, thus plants contains potent compounds with antioxidant activity (Scartezzini and Speroni, 2000). These compounds are able to scavenge reactive oxygen species due to their electron donating properties (Re et al., 1999). In search of novel anti-oxidants with low toxicity over past few years, medicinal plants have been studied extensively for their radical scavenging activity. The several categories of compounds showed predominant antioxidant potential. The few of them discussed below.

1.6.1 Phenolic Acids

Hydroxybenzoic and hydroxycinnamic acids are predominant phenolic acids found in plants. Differences between their derivatives consist in the different patterns of hydroxylations and methoxylations of their aromatic rings.

Gallic acid is one of the common hydroxybenzoic acids. Its dimeric condensation product and related dilactone, ellagic acid, are commonly found in plants.
Hydroxycinnamic acids are also commonly found in foods of plant origin. p-Coumaric, caffeic, ferulic and sinapic acids are major hydroxycinnamic acids found in fruits.

1.6.2 Flavonoids

Flavonoids represent the most common and widely distributed group of plant phenolics. Their common structure (C6-C3-C6) consists of two aromatic rings (A ring and B ring) linked through a three carbon bridge that is usually an oxygenated heterocycle (C ring).

In the nature, flavonoids occurs as anthocyanidins, chalcones, flavanols, flavanones, flavones, flavonol, and isoflavones. The variability of the flavonoids is based on the hydroxylation of the pyrone ring, absence or presence of double bond, the number of hydroxyls in the A ring and B ring, and/or a double bonded oxygen atom attached to position C-4 of the C ring.

1.6.2.1 Flavanols and Flavonols

Flavanols are known as flavan-3-ols, and they are the subunits of proanthocyanidins, which have a hydroxyl group attached to the C-3 position of the C ring, no positive charge on the oxygen atom and no double bond in the C ring.
The flavonols are very similar to those of flavanols, except that there is a double-bonded oxygen atom attached to position C-4 of the C ring and a double bond in the C ring.

1.6.2.2 Flavanones and Flavones

Flavanones and flavones have structures similar to those of flavanols and flavonols, respectively. However, in each case there is no longer a hydroxyl group attached to the 3 position of the C ring.

It is well documented that flavonoids possess antioxidant properties \textit{in-vitro} and \textit{in-vivo}. The flavonoids contain a number of phenolic hydroxyl groups attached to ring structures, which confer the antioxidant activity. Catechin and their epimers serve as powerful antioxidants for directly eliminating superoxide anion radicals. Kaempferol, quercetin derivatives from this plant showed strong antiradical activity (Braca et al., 2002). Epicatechin, epigallocatechin, epicatechin gallate and procyanidin B1 and B2 reported to posses strong DPPH radical scavenging activity. The antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule and the activity can be strengthened by steric hindrance. The electron withdrawing properties of the carboxylate group in benzoic acids has a negative influence on the H- donating abilities of the hydroxy benzoates. Hydroxylated cinnamates are more effective than benzoate counterparts (Rice-Evans et al., 1996).

1.6.3 Steroids and triterpenoids
These are another group of phytoconstituents widely distributed in higher plants and organisms. Several thousand steroids and terpenoids occur with wide chemical diversity, accumulated as glycosides (saponins) or non-glycoside form in extensive amounts in plants (James and Dubery, 2009).

The phytosterols in the plant are found as campesterol, β-sitosterol, stigmasterol, campestanol, brassicasterol, ergosterol and sterol esters. The most abundance are campesterol, β-sitosterol, stigmasterol, campestanol.

β-sitosterol

The common pentacyclic triterpenoids compounds are found as oleananes (also known as β-amyrin derivatives), ursanes (also known as α-amyrin derivatives), lupanes, hopane and fridelane, Special groups comprises cucurbitacines and limonoids, triterpenoids of Asteraceae (taraxasterol, arnidiol, faradiol).

The recent studies have shown the in-vivo antioxidant potential of commonly found β-sitosterol by neutralizing free radicals and ROS, which could be effective in preventing protein damage caused by oxidative stress (Gupta et al., 2011). This antioxidant potential provides the cytoprotective ability (Baskar et al., 2010).
The oleananes, ursanes and lupanes are commonly distributed in the angiopermic plant. These types of triterpenoids were recently well explored for their therapeutic potential. Surprisingly they are coming out as panacea. Some earlier studies were carried out on natural and semi synthetic derivatives of oleananes and lupane, showed the potent *in-vivo* and *in-vitro* antioxidant activity with anti-inflammatory and cardioprotective ability (Pawar and Bhutani, 2003; Vannini et al., 2007; Silva et al. 2012).

The *in-vitro* and *in-vivo* studies on ursolic acid established it as potent antioxidant triterpenoid (Ramachandran and Prasad, 2008).

### 1.7 Treatment in liver disease

In spite of phenomenal growth of modern medicine, there is scarcity of safe and effective synthetic drugs for the treatment of hepatic disorders (Ramachandra Setty et al., 2007). The treatments available for the cure from liver diseases are modern medications and traditional medications. The modern medications includes anti-inflammatory, immunomodulatory (Kamath and Kim, 2007; Worman, 1997), corticosteroid, antiviral drugs (Toniutto et al., 2008), sadenosyl- L-methionine (Wang et al., 2006), α-Lipoic acid (Packer et al., 1995), dipeptide caspase inhibitor (Ueno et al., 2009) and ursodeoxycholic acid (UDCA). A hydrophilic bile acid, with putative immunomodulatory capacities (Miyaguchi and Mori, 2005; Rolandi et al., 1991), and prednisone in combination with azathioprine is also preferred for treating liver diseases (Ishibashi et al., 2007).

Contrary, in Indian systems of medicine, integer of herbs and herbal formulations are recommended. These formulations are time tasted, with good efficacy and efficiency. Amongst the herbs only few herbs has special intention for search for hepatoprotective medicine, while other remains with the text of reference books.
Some of drugs are *Boerhavia diffusa*, *Picrorhiza kurroa*, *Phyllanthus niruri*, *Phyllanthus emblica*, *Aloe vera* etc (Saleem et al., 2010).

The most successful liver protective natural product is silymarin, mixture of flavonolignan from milk thistle *Silybum marianum*. Silymarin useful in treatment of all types of liver diseases, as it protects liver cells from a wide variety of toxins, from ischemic injury, radiation and viral hepatitis. Silymarin mechanism includes antioxidant, anti-lipid peroxidant, anti-inflammatory and antifibrotic effects (Li and Friedman, 1999; Kiso et al., 1987). Silymarin has drawn increasing attention because of its antifibrogenic properties; it reduces collagen accumulation by 30% in secondary biliary fibrosis in rats. Due to its antioxidant activity it decreases hepatic injury by both cytoprotection and inhibition of kupffer cells activation. A clinical trial involving patients with alcoholic liver cirrhosis indicated a slight survival advantage of treated compared with untreated controls.

### 1.8 Selection of traditional medicinal plant

Conventional modern medicine treatment does not provide efficient remedies for liver diseases. The current modern therapeutic strategies are not efficient enough for the complete removal of liver hazard, without provoking adverse drug reactions and a curative agent has not yet been found in modern medicine. The current usage of corticosteroids and immunosuppressive agents only brings about symptomatic relief. However, their usage is associated with risk of relapses and danger of side effects. In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, and offer protection to the liver from damage or help regeneration of hepatic cell. Hence, the ultimate treatment of severe liver damage is surgical liver transplantation.

Recently, the use of herbal product has gained interest globally. The indigenous systems of medicine in India have a long tradition of treating liver disorders with plant drugs (De et al., 1993). Indian Systems of Medicine (ISM) has recommended more than eighty-seven hepatoprotective plants and some of them are used clinically. There are several herbs/herbal formulations claimed to possess beneficial activity in treating hepatic disorders, but yet to validate scientifically.

The traditional therapies claims include the complete cure of the liver disease with no side effects and cost effectiveness. These claims are however, not backed by well-
documented scientific data. The modern medicines used in the treatment of liver
diseases are often inadequate and can have serious adverse effects. Henceforth, there
is an unmet need for alternative drugs for the treatment of liver disease, to replace the
currently used drugs of doubtful efficacy and safety (Ahmed and Khater, 2001).

In the present study, the selection of traditional medicinal plants was carried out based
on recommendation for treatment of liver diseases. The two traditional medicinal
plants, that are bark of *Ficus microcarpa* L. fil. (Syn: *Ficus retusa* L.) (Moraceae) and
pericarp of *Luffa acutangula* (L) Roxb. var. *amara* (Cucurbitaceae) were selected
through extensive review from the standard book. These plants were strongly
recommended for treatment of liver disease. The bark *F. microcarpa* (FMB)
recommended as juice of fresh bark or dried powder in lukewarm milk of *F.
microcarpa*; While, *nasya* of fresh fruits or decoction of dried fruit without seed
(pericarp) of *L. acutangula* (L) Roxb. var. *amara* recommended for three to four days
for treatment of liver diseases (Kirtikar and basu, 1987a, b; Despande, 2002,
Nadkarni, 1992). Despite of strong traditional medicinal claims, these plants were not
evaluated scientifically for their hepatoprotective potential. Therefore, the present
study were undertaken to evaluate the hepatoprotective effect of *F. microcarpa* and *L.
acutangula* (L) Roxb. var. *amara* and to validate their traditional use.
1.9 References


CHAPTER 1

Introduction


