Chapter 2

LITERATURE SURVEY
India is the largest producer of medicinal plants and is rightly called "Botanical Garden of the World". The medicinal plants have very important role in the health and vitality of human beings as well as animals. As per the WHO estimates, about three quarters of the world's population currently use herbs and other traditional medicines to cure various diseases, including liver disorders. Hence several phytomedicines (medicinal plants or herbal drugs) are now used for the prevention and treatment of various disorders. Although experimental studies have been conducted on a number of these plants and their formulations, however, only some plants have clearly shown the hepatogenic/hepatoprotective effects against liver diseases or hepatotoxicity caused by variety of hepatotoxic agents such as chemicals, drugs, pollutants, and infections from parasites, bacteria or viruses, etc. The medicinal plants contain several phytochemicals which possess strong antioxidant property, leading to antihepatotoxic activity (Pandey Govind, 2011).

From the beginning of time, plants have played a role in human affairs, influencing the evolution of civilization and cultures, human migration, medicine and healthcare, wars, art, mythology, religion and so on. Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki and Shanidar, 1975). Plants have been the almost exclusive therapy available to humans and form the primary source of substances to be developed as drugs in traditional medicinal system for thousands of years in countries such as China (Chang and But, 1986) and India (Kapoor, 1990).

Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grow wild in different parts of our country. In India, the use
of different parts of medicinal plants to cure specific ailments was in practice from ancient times (Bhattacharjee 1998). India is rich in medicinal plant diversity. All known types of agro-climatic and ecologic conditions exist within India, and is rich in all three levels of biodiversity, as species diversity, genetic diversity and habitat diversity (Zafar et al., 1999).

Tuberculosis is one of the fatal communicative diseases and is spread easily amongst people. Over one-third of the world's population is estimated to be infected with Mycobacterium tuberculosis and over 2 million people a year will die of the disease (Shishoo et al., 2001). Multi-drug resistant (MDR) strains of M. tuberculosis have emerged and a co-infection with AIDS was found out. This turned out that the WHO declared tuberculosis as ‘Global health emergency’ (Anon, 1997). The administration of isoniazid and rifampicin, the most common medication prescribed against tuberculosis, produces many metabolic and morphological aberrations in liver due to the fact that liver is the main detoxifying site for these antitubercular drugs such as INH and rifampicin (INH-RIF).

These antitubercular drugs induce hepatitis by a multiple step mechanism. It is characterized by a fall in serum albumin concentration and a rise in serum globulin concentration, which is related to the severity & duration of the disease. INH-RIF mediated oxidative damage is generally attributed to the formation of highly Reactive Oxygen Species (ROS), which acts as stimulator of lipid peroxidation and source for the destruction and damage to the cell membrane (Georgieva et al., 2004).

2.1. PREVIOUS EXPLORATION ON TRADITIONAL MEDICINAL PLANTS

The traditional systems of medicine are being practised among the people of our country for treating various ailments and for promotion of health. Deleterious side
effects and expenses in drugs of modern medicine necessitate the use of traditional
drugs for several diseases. Therefore, there has been increasing interest towards the
scientific study of formulations in traditional systems of medicine among
pharmacologists and botanists. Many herbal and traditional formulations have been
screened for their biological activity (Fulzele et al., 2002)

Plants are well known for their medicinal properties. Indian system of
medicine have a well documented account of several indigenous medicinal plants and
their therapeutic value. In recent years, systematic analyses are being undertaken to
validate the above practices and as a result active secondary metabolites of medicinal
plants are being purified, isolated and used in pharmaceutical preparations (Perry,
1980). Higher plants produce hundreds to thousands of diverse chemical compounds
with different biological activities (Hamburger and Hostettmann, 1991) and these
antimicrobial compounds produced by plants are active against pathogenic micro
organism (Mitscher et al., 1987)

Herbal medicine sometimes referred to as herbalism or botanical medicine, is
the use of herbs for their therapeutic or medicinal value. A herb is a plant or plant
part valued for its medicinal, aromatic or savory qualities. Herb plants produce the
variety of chemical substances that act upon the body. Herbal medicine is the oldest
form of health care known to mankind. It is the local heritage with global importance.
World is endowed with a rich wealth of medicinal plants. Herbs have always been the
principle form of medicine in India and presently they are becoming popular
throughout the developed world, as people strive to stay healthy in the face of chronic
stress and pollution, and to treat illness with medicines that work in concern with the
body's own defenses. People in Europe, North America and Australia are consulting
trained herbal professionals and are using the plant medicines. Medicinal plants also play an important role in the lives of rural peoples, particularly in remote areas of developing countries with few health facilities. According to World Health Organisation (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicines for some aspect of primary health care. This means that 3,300 million people use medicinal plants on regular basis (Farnsworth, 1994).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in same chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites such as alkaloids, steroids, tannins, flavonoids etc., which are synthesized and deposited in the specific parts of plants (Parikh et al., 2005).

The different extracts of *Apium graveolens* Linn. (Apiaceae) and *Croton oblongifolius* Roxb. (Euphorbiaceae) were tested for their hepatoprotective activity against CCl₄ induced hepatotoxicity in albino rats. The degree of protection was measured by using biochemical parameters like serum transaminases (SGOT and SGPT), alkaline phosphatase, total protein and albumin. The methanolic extracts showed the most significant hepatoprotective activity comparable with standard drug silymarin. Other extracts namely petroleum ether and acetone also exhibited a potent activity (Bahar Ahmed et al., 2002).

Ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine were studied for their antimicrobial activity against certain drug-resistant bacteria and
a yeast *Candida albicans* of clinical origin. Of these, 40 plant extracts showed varied levels of antimicrobial activity against one or more tested bacteria. Anticandidal activity was detected in 24 plant extracts. Overall, broad-spectrum antimicrobial activity was observed in 12 plants (*L. inermis*, *Eucalyptus* sp., *H. antidysentrica*, *H. indicus*, *C. equistifolia*, *T. beferica*, *T. chebula*, *E. officinalis*, *C. sinensis*, *S. aromaticum* and *P. granatum*). No correlation was observed between susceptibility of test strains with plant extracts and antibiotic resistance behaviour of the microbial strains (*Staphylococcus aureus*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*). Qualitative phytochemical tests, thin layer chromatography and TLC-bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents (Iqbal Ahmad *et al.*, 2001).

The ethanolic extracts from fresh apical stems of *Phyllanthus niruri* L. (Euphorbiaceae) cultured on Murashige and Skoog (MS) medium supplemented with IBA/BAP/Coconut milk for 1, 2, 4 and 6 months were phytochemically and biologically investigated and compared with intact plant part and whole plant extracts. Results from the *in vitro* antiplasmodial testing indicated that the ethanolic extract of a 1-month-old callus culture (*IC*<sub>50</sub> = 16.3±2.5 <i>g/ml</i>) exhibited a higher activity than the ethanolic extracts of the fresh apical stem (*IC*<sub>50</sub> = 18.2±2.4 <i>g/ml</i>) and callus cultures of 2-, 4- and 6-months-old (25 <i>g/ml</i> < *IC*<sub>50</sub> < 40 <i>g/ml</i>). These activities were however lower than that displayed by the ethanolic extract of the whole plant. The ethanolic extract of 1-month-old callus culture (the most active) was fractionated with solvents of different polarities. Its CH<sub>2</sub>C<sub>12</sub> fraction rich in terpenic constituents exhibited a higher antiplasmodial activity than its isoamylic alcohol fraction obtained at pH 2–3 rich in flavonoids. The activity of these two fractions was lower than that
displayed by the same fractions from the whole plant (2 g/ml < IC50 < 3 g/ml). Alkaloidic fractions from the whole plant and 1-month-old callus culture of fresh apical stem were considered as inactive (IC50 > 100 g/ml) (Cimanga, et al., 2004).

*Euphorbia fusiformis* Buch.-Ham. ex. D.Don (Euphorbiaceae) is a rare medicinal herb. Aqueous and organic solvent extracts of the leaves and rootstocks were investigated for anti-bacterial properties by using disc diffusion and well-in agar methods, against pathogenic strains of gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhii* A and *Salmonella typhii* B). The different extracts differed significantly in their antibacterial properties with the methanolic extract being very effective followed by acetone and chloroform extracts. Aqueous and ethanolic extract showed very least activity. The result highlights that rootstock extracts had good anti-bacterial properties than leaf extracts. The results of this study support the use of this plant in traditional medicine to treat fever, wound infections and intestinal disorders (Natarajan et al. 2005).

A study was designed to evaluate the antimycobacterial, antibacterial and antifungal activities of the methanol extract from the stem bark of *Thecacoris annobonae* Pax & K. Hoffm, that of aristolochic acid I (1) and other isolated compounds. The microplate alamar blue assay (MABA) and the broth microdilution method were used to determine the minimal inhibitory concentration (MIC) and minimal microbicidal concentration (MMC) of the above samples. The H+-ATPase-mediated proton pumping assay was used to evaluate a possible mechanism of action for both the methanol extract and aristolochic acid I. The results of the MIC determinations showed that the methanol extract and aristolochic acid I prevent the
growth of all studied organisms. The results obtained in this study also showed that the methanol extract as well as aristolochic acid I inhibited the H+-ATPase activity. The overall results provided evidence that the methanol extract of T. annobonae might be a potential source of new antimicrobial drug against tuberculosis, and some bacterial and fungal diseases, but should be consumed with caution, bearing in mind that the main active component, aristolochic acid I is a potentially toxic compound (Kuete et al., 2010).

The aqueous stem bark and leaf extracts of plant *Euphorbia hirta* (family-*Euphorbiaceae*) have potent molluscicidal activity. Sub-lethal doses (40% and 80% of LC$_{50}$) of aqueous stem bark and leaf extracts of this plant also significantly (P < 0.05) alter the levels of total protein, total free amino acid, nucleic acids (DNA and RNA) and the activity of enzyme protease and acid and alkaline phosphatase in various tissues of the vector snail *Lymnaea acuminata* in time and dose dependent manner. *Euphorbia hirta* (family-*Euphorbiaceae*) commonly known as Dudhi, is a common medicinal plant of India, which is used in variety of diseases i.e. cough, asthma, colic, dysentery, genito urinary diseases (Sunil Kumar Singh, et al., 2005).

Water and ethanol extracts of *Briaklia ferruginea* were examined for phytochemical and antimicrobial properties. The extracts, which were tested at a final concentration of 5 mg/ml, produced in vitro antimicrobial activities in assays against hospital strains of *Staphylococcus aureus*, *Candida albicans*, *Staphylococcus epidermidis*, *Escherichia coli*, *Streptococcus lactis*, *Proteus vulgaris*, *Proteus mirabilis*, *Streptococcus pyogenes* and *Klebsiella* sp. The zones of inhibition produced by the extracts in agar diffusion assays against the test micro-organisms ranged from 4 to 20 mm, while the chloramphenicol antibiotic control produced zones that
measured 15-36 mm. Preliminary phytochemical analysis of the plant extracts showed
the presence of phenols and tannins. Sesquiterpenes, anthroquinones, and saponins
were not detected in the water and ethanolic extracts. The gram-negative bacteria
appeared to be more susceptible (4-20 nun) to the antimicrobial effect of the extracts
than the gram-positive organisms (4-18 nun) (Irobia et al., 1994).

The in vivo hepatoprotective effects of Rhoicissus tridentata subsp. cuneifolia,
a traditional Zulu medicinal plant, against carbon tetrachlorideinduced acute liver
injury in rats were investigated. A group of male Sprague–Dawley rats were divided
into three subgroups. Two subgroups were injected with carbon tetrachloride (CCl₄)
and the other group with an equivalent amount of olive oil. Two hours after CCl₄
intoxication one of the two subgroups was administered with R. tridentata extract by
stomach tube. The subgroup that received olive oil was sacrificed after 2 h. Groups of
rats from the other two subgroups were sacrificed at 24, 48 and 72 h after the
respective treatments. The variables investigated were the enzymes alanine
aminotransferase (ALT), aspartate aminotransferase (ASP) and glucose-6-
phosphatase (G-6-Pase). In addition lipid peroxide (LPO) levels of liver homogenates
as well as liver microsomal fractions were determined as malondialdehyde (MDA)
levels. CCl₄ intoxication resulted in significant increases (P<0.05) in all the variables
investigated except G-6-Pase which was significantly decreased (P<0.05). The
administration of R. tridentata extracts after CCl₄ intoxication resulted in significantly
reduced (P<0.05) concentrations of ALT and ASP as well as the levels of LPO
whereas the concentrations of G-6-Pase were significantly increased (P<0.05). From
the results obtained during the present study it could be concluded that R. tridentata
has components that have hepatoprotective effects (Opoku et al. 2007).
Lipid peroxidation in biological systems has been considered as one of the major mechanisms of cell injury in aerobic organisms subjected to oxidation stress. Plants, among other functions, are considered to act as free radical scavengers and as antioxidants. Iron II (Fe2+), sodium nitroprusside (SNP) and nitropropionic acid stimulate the production of free radicals and lipid peroxidation. In this study, four commonly used tropical medicinal plants (Kigelia africana, Calotropis procera, Hibiscus sabdariffa and Alchornea cordifolia) were studied (in vitro) for their effects on the formation of thiobarbituric acid reactive substances (TBARS) induced by different pro-oxidants (10 mM FeSO4, 5 mM - sodium SNP and 2mM 3-nitropropionic acid) in rat liver homogenate. All the pro-oxidants significantly increased (P <0.05) the formation of TBARS, which indicates increased lipid peroxidation in the rat liver (in vitro). However, all the plant extracts statistically (P<0.05) reduced the production of TBARS in a concentration-dependent manner in all the tested pro-oxidant-induced oxidative stresses. *Alchornea cordifolia* appeared to offer the highest protection. The results of the present study suggested that the use of these plants in the treatment of various diseases, especially liver disease, is probably due to their ability to act as antioxidants (Mary Tolulope Olaye et al., 2007).

Aqueous extract of flowers of *Butea monosperma* (*Fabaceae*) was evaluated at different dose levels (200, 400, 800 mg/kg, p.o.) for its protective efficacy against *CCl4* (1.5 ml/kg i.p.) induced acute liver injury to validate its use in traditional medicines. The *CCl4* administration altered various biochemical parameters, including serum transaminases, protein, albumin, hepatic lipid peroxidation, reduced glutathione and total protein levels, which were restored towards control by therapy of *B. monosperma* Adenosine triphosphatase and glucose-6-phosphatase activity in the liver were decreased significantly in *CCl4* treated animals. Therapy of *B. monosperma*
showed its protective effect on biochemical and histopathological alterations at all the three doses in dose dependent manner. *B. monosperma* extract possess modulatory effect on drug metabolizing enzymes as it significantly decreased the hexobarbitone induced sleep time and increased excretory capacity of liver which was measured by BSP retention. Histological studies also supported the biochemical finding and maximum improvement in the histoarchitecture was seen at higher dose of BM extract (Neetu Sharma, et al., 2011).

The antioxidant activity of chloroform and methanol extract of roots and stems of *Rhubarb (Rheum ribes L.)*, which are used for medicinal purposes and also its fresh stems and petioles are consumed as vegetable, was studied. The antioxidant potential of both extracts of roots and stems were evaluated using different antioxidant tests, namely total antioxidant (lipid peroxidation inhibition activity), DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, superoxide anion radical scavenging, ferric reducing power, and cupric reducing power (CUPRAC), and metal chelating activities. Total antioxidant activity was also measured according to the β-carotene bleaching method, and all four extracts exhibited stronger activity than known standards, namely butylated hydroxytoluene (BHT) and a-tocopherol. Particularly, higher activity was exhibited by roots with 93.1% and 84.1% inhibitions of chloroform and methanol extracts, while 82.2% and 82.0% inhibitions by stem extracts, respectively. However, both roots and stem of methanol extracts exhibited higher DPPH radical scavenging activity than the corresponding chloroform extract, moreover, methanol extract of the stems showed better activity than BHT. In addition, both root extracts showed more potent superoxide anion radical scavenging activity than BHT, and comparable with well known radical scavenger L-ascorbic acid. Except chloroform extract of the roots, the other three extracts exhibited better metal
chelating activity than quercetin. Also, total phenolic and flavonoid contents in both extracts of the roots and stems of *R. ribes* were determined as pyrocatechol and quercetin equivalents, respectively (Mehmet Ozturk *et al.*, 2007).

Extracts of *Boerhaavia diffusa* leaves were evaluated for antioxidant and hepatoprotective properties in the acetaminophen-induced liver damage model. Antioxidative evaluation of ethanolic extract gave total phenolic content, total flavonoid content, vitamin C content and vitamin E content and the levels of selenium and zinc as 6.6 ± 0.2 mg/g tannic acid equivalent, 0.092 ± 0.003 mg/g quercetin equivalent, 0.21 ± 0.03 mg/g, 0.054 ± 0.002 mg/g, 0.52 ± 0.05 ppm and 9.28 ± 0.16 ppm, respectively. The DPPH scavenging capacity and the reductive potential were 78.32 ± 2.41% and 0.65 ± 0.02 mg/g ascorbic acid, respectively. Pretreatment with aqueous and ethanolic extracts decreased the activities of alkaline phosphatase, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and the level of bilirubin in the serum that were elevated by acetaminophen. The two extracts also ameliorated the elevation in the activities of the enzymes in the liver. Acetaminophen intoxication led to reduction in serum and liver albumin levels which were not significantly increased by pretreatment with the extracts. The extracts also protected against acetaminophen induced lipid peroxidation. These results indicated that leaf extracts from *B. diffusa* possess hepatoprotective property against acetaminophen-induced liver damage which may be mediated through augmentation of antioxidant defenses (Tolulope Olaleye *et al.*, 2010).

A review provides the status report on the scientific approaches made to herbal preparations used in Indian systems of medicine for the treatment of liver diseases. In spite of the availability of more than 300 preparations for the treatment of jaundice
and chronic liver diseases in Indian systems of medicine using more than 87 Indian medicinal plants, only four terrestrial plants have been scientifically elucidated while adhering to the internationally acceptable scientific protocols. In-depth studies have proved *Silybum marianum* to be anti-oxidative, antilipidperoxidative, antifibrotic, anti-inflammatory, immunomodulating and liver regenerative. *Glycyrrhiza glabra* has been shown to be hepatoprotective and capable of inducing an indigenous interferon. *Picrorhiza kurroa* is proved to be anti-inflammatory, hepatoprotective and immunomodulatory. Extensive studies on *Phyllanthus amarus* have confirmed this plant preparation as being antiviral against hepatitis B and C viruses, hepatoprotective and immunomodulating, as well as possessing anti-inflammatory properties. For the first time in the Indian systems of medicine, a chemo-biological fingerprinting methodology for standardization of *P. amarus* preparation has been patented (Thyagarajan et al., 2002).

2.2. PREVIOUS EXPLORATIONS ON *Aporosa lindleyana*

*Aporosa lindleyana* is found to be active as hypoglycemic, diuretic and antiviral and antipyretic (Bhakuni et al., 1988). A decoction of the root is used traditionally in jaundice, fever, headache, insanity, seminal loss and excessive thirst (Anonymous, 1985; Kirtikar and Basu, 1987; Chopra et al., 1992).

The 50% ethanol extract of *Aporosa lindleyana* exhibited hypoglycemic (Nadkani, KM. 1954) and the extract showed an LD$_{50}$ of more than 1.0 g/kg body weight (Dhawan et al., 1980). The cold and ethanolic and aqueous extracts of the root of *Aporosa lindleyana* showed positive results in invitro HBsAg binding studies. The minimum inhibitory concentration (MIC) determined for the ethanolic extract was
1.25mg/ml (Venkataraman et al., 2010). β-sitosterol and β-sitosterolglucoside were reported in *Aporosa lindleyana* (Venkataraman, 2000).

Antihyperglycemic and hypoglycemic effect of *Aporosa lindleyana* was investigated in normal and alloxan induced diabetic rats. The aqueous and alcoholic extracts of *Aporosa lindleyana* reduced the blood glucose level, compared with tolbutamide, an oral hypoglycemic agent (Jayakar and Suresh, 2003).

Four successive and two crude extracts of *Aporosa lindleyana* were tested for antioxidant activity in CCl₄ induced experimental rats, revealed strong antioxidant nature of this plant and the invitro cytotoxic studies against normal vero cell lines indicate the non-toxic nature of the root extracts (Shrishailappa Badami et al., 2005).

Antimicrobial and analgesic activities of bark extract of Aporosa lindleyana revealed the crude extract of this plant was encouraging and methanolic extract showed very good analgesic activity and pet ether showed considerable analgesic activity (Lingadahalli Srikrishna et al., 2008).

Hepatoprotective effect of ethanolic root extract of *Aporosa lindleyana* was tested on INH-RIF induced in rats and analysing various hepatic markers and the result showed this plant has hepatoprotective effect (Ramakrishnan and Venkataraman 2010).

Hepatoprotective effect of *Aporosa lindleyana* roots was tested on CCl₄ induced in experimental rats and analysing various hepatic markers and the result showed this plant has hepatoprotective effect (Akshatha, 2011).
SCOPE & OBJECTIVE
2.3. SCOPE AND OBJECTIVE

At present, the active principles from natural products have gain attention for the treatment of various ailments due to safe and lesser side effects than synthetic one. Among the natural products are attracting the interest because of their beneficial effects in human health. Very limited studies showed the beneficial effects of *Aporosa lindleyana* in various disease conditions. Although the study of various pharmacological effects has been described well, few reports evidenced on the phytochemical screening, antimicrobial activity on bark extract and hepatoprotective effect of *Aporosa lindleyana* in CCl₄-induced rats. Hence the present study was planned to unravel the effects of Ethanolic Root Extract of *Aporosa lindleyana* (EREAL) on INH-RIF induced in experimental rats.

Objective of the Present Investigation:

 ✓ Preliminary phytochemical screening of *Aporosa lindleyana*

 ✓ Separation and Identification of the root of *Aporosa lindleyana* through paper and thin layer chromatography patterns and identification of compounds by gas chromatography–mass spectrometry (GC–MS)

 ✓ To examine the antibacterial action of *Aporosa lindleyana* against various pathogenic microorganisms.

 ✓ Quantitative screening of biomolecules

 ✓ To determine *in vitro* free radical scavenging potential of the EREAL (Hydroxy, Hydrogen peroxide, Superoxide anion radical scavenging activity etc.)

 ✓ To study the hepatoprotective effect of EREAL in male albino wistar rats.