Chapter-1

INTRODUCTION
1. INTRODUCTION

Plant based natural products have been the components of phytomedicines throughout human history. In last few decades, plants have become an important source for the discovery of novel pharmaceuticals. According to current estimate, 80% of total world population is still dependent on plant based drugs (Pandey et al., 2011). Medicinal plants are one of the main source of new drugs and as such many new drugs have been isolated and characterized from medicinal plants. It was found that out of all new chemical entities launched in the market between the period of 1981-2002, 54% are either directly derived natural products or their derivatives (Newman et al., 2000). Plant based compounds are still evidencing to be a valuable source of medicines for human. Some of this plant derived compounds, such as Atropine, Colchicine, Digoxin, Vinblastine, Taxol, Morphine, Reserpine are directly used as drugs (Fabricant and Fransworth, 2001). The association of medicinal plants shows their protective effects for curing various diseases. The World Health Organization (WHO) estimates 35 million individuals in the world every year die from chronic diseases (Bengmark, 2006), out of which a significant portion is due to liver disease.

Liver, the largest organ of the body, plays a key role in metabolism. Its highly specialised tissues control high-volume biochemical reactions and continue the vital functions of the body. Because of its diverse functional roles, liver is a sensitive organ. Acute and chronic liver diseases interfere with the liver functions and substantially cause damage of it and
thereby cause symptoms. The most common liver diseases include various types of acute hepatitis, chronic or long duration hepatitis, fatty liver, scarring or cirrhosis and liver cancer. Liver cell (hepatic cell) injury also occurred due to oxidative damage by reactive oxygen species (ROS) (Cesaratto et al., 2004).

Nowadays, plant-based medicines gained priority compared to synthetic drugs. Plant-based remedies are used as hepatoprotective as well as antioxidant agents against different chemical induced liver diseases. Traditional medication systems in different parts of the world use different plants having hepatoprotective properties. Among them, Gundelia tournefortii L. has been used in Iran (Jamshidzadeh et al., 2005), Viburnum tinus L. has been used in Egypt (Mohamed et al., 2005), Morus bombycis Koidz., Capparis decidua Edgew., Newbouldia laevis Seem. have been used in Taiwan, Republic of Korea, Sudan and Nigeria, respectively (Yen et al., 2006; Jin et al., 2005; Ali et al., 2009; Hassan et al., 2010). The Indian traditional medicine system also reports numerous plants as excellent hepatoprotective agents. Some of these are Aegle marmelos (L.) Correa ex Roxb. (Vinodini, 2007; Saleem et al., 2010), Azadirachta indica A. Juss., Careya arborea Roxb. and Roxb., Wedelia calendulaecea Less., Cassia fistula L. (Bhakta et al., 2001), Taraxacum officinale [Weber] (Kumar et al., 2012). There are numerous plants that have been reported to possess antioxidant activity. Among them some are Hygrophila auriculata (Schumach.) Heine, Phaseolus trilobus Wall., Oroxyllum indicum Vent., Rubia cordifolia L., Careya arborea Roxb., Phyllanthus amarus Schumach., Phyllanthus polyphyllus Willd., Silybum marianum (L.) Gaertn., Cichorium intybus L., Solanum nigrum L., Aloe barbadensis Mill. (Shanmugasundaram and Venkataraman, 2006; Srinivasan et al., 2007; Fursule and Patil, 2010; Tenpe et al., 2009; Kalaivani and Mathew, 2009; Joharapurkar et al., 2003; Sharma and Sharma, 2010, Chandan et al., 2007).
A number of phytoconstituents which have hepatoprotective and antioxidant potentialities were also reported by several workers, among these, α-lapachone and β-sitosterol were isolated from *Dolichandrone falcatia* (Wall. Ex DC.) Seem (Aparna *et al.*, 2009), Protocatechuic acid, acteoside and jionoside-D were isolated from *Jacaranda caucana* Pittier (Martin *et al.*, 2009). But a very few isolated compound from documented plants have got scientific validation, Verbascoside and anthraquinone were isolated from *Newbouldia laevis* Seem.(Gormann *et al.*, 2006), C-glucosylxanthones isolated from *Arrabidaea patellifera* (Schltrl.) Sandwith (Martin *et al.*, 2008), iridoids isolated from *Crescentia alata* Kunth (Valladares and Rios, 2007), acteoside isolated from *Tecoma stans* Juss. (Kameshwaran *et al.*, 2013), betulinic acid isolated from *Tecomella undulata* Seem. (Kapadia *et al.*, 2012). But a very few isolated compound from documented plants have got scientific validation.

Plant’s secondary metabolites are responsible for curing any particular disease. The secondary metabolites are biosynthetically derived from primary metabolites. According to their chemical characteristics, the secondary metabolites can be classified into groups, such as — alkaloids, terpenoids, phenols, pigments, flavonoids, glycosides, steroids, etc.

**Alkaloids:** Alkaloids are distinguished from plant metabolites containing nitrogenous base in a heterocyclic ring. Alkaloids are present in the form of salts of organic acid, oxalic acid, melic acid, lactic acid, tartaric acid, aconitic acid in different parts of plants. Berberine, piperine, palmatine, Tetrahydropalmatine are the examples of Alkaloids. Alkaloids have anti-diarrhoeal, antimicrobial and anthelmintic effect (Wang *et al.*, 2010).

**Flavones, flavonoids and flavonols:** Flavones are phenolic structures containing one carbonyl group and a 3-hydroxyl group addition yields a flavonol. Flavonoids, the hydroxylated phenolic substances, occur as a C<sub>6</sub>–C<sub>3</sub> unit linked to an aromatic ring (Sutar
et al., 2010; Mute, 2009; Sharma and Sharma, 2010). Flavonoids and flavonols are generally responsible for anti-diarrhoeal and antimicrobial property. Flavonoids have been reported to possess antioxidant, hepatoprotective and anti-ulcer properties (Zaveri et al., 2008). **Tannins:** Tannin is responsible for plant pigment and is a group of polymeric phenolic substances. Tannins in plants inhibit insect growth and can be toxic to filamentous fungi, yeasts and bacteria. Ellagitannins, an example of tannin, has anti-diarrhoeal, antimicrobial and anthelmintic property (Patel, 2010). **Coumarins:** Coumarins are phenolic substances bearing pyrrole rings. Warfarin is a particularly well-known coumarin which is used both as an oral anticoagulant and it has anti-diarrhoeal property (Roy, 2010). Chromones are isomer of coumarin. Chromone 3-[2-(3, 5- dimethoxy) ethenyl] -2- methyl from *Eucaria microcarpa* Boiss. was proved as a scavenger for DPPH free radical (Haschem, 2000-2001). **Phenols:** Plant phytochemicals comprises of major groups of phenolics and polyphenols. There are several subclasses of polyphenols. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring, cinnamic acid and caffeic acid which are common in a wide group of phenylpropanederived compounds. Phenols have two or more hydroxyl groups (OH) bonded directly to an aromatic hydrocarbon group in the same molecule. Polyphenols have anti-diarrhoeal and antioxidant properties (Cowan, 1999; Mahmoud et al., 2006). **Steroids:** Steroids are responsible for anti-diarrheal activity (Hanwa et al., 2009). The mechanism of action of some antifungal drugs is that it binds to the cell membrane of pathogenic fungi in the presence of certain sterols, which subsequently disturb permeability and transport characteristics of the membrane, resulting in loss of intracellular cations (Maniyar et al., 2010). **Glycosides:** Glycosides are phytocompounds which have antioxidant and antidiarrhoal property. Glycosides are present in plant parts in an
inactive state, and the inactive glycosides are activated by enzyme hydrolysis. Glycosides can be classified by glycone, glycosidic bond and aglycone. If a glycone group of glycoside is glucose, then the molecule is a glycoside. Glycosides can be classified depending on the chemical nature of aglycone (Tiwari et al., 2011). The phytoconstituent Glycoside contain sugar and non carbohydrate moiety in their structure. Amygdalin and stereospermoside are the examples of Glycosides (Kanchanapoom et al., 2006).

**Terpenoids and Essential Oils:** Essential oil fraction is responsible for the fragrance of the plants. These oils are secondary metabolites, enriched in isoprene unit. When the compounds contain additional oxygen then they are termed as terpenoids. Terpenoids are synthesized from acetate units and as such they share their origins with fatty acids. Examples of terpenoids are campherol (monoterpenes), farnesol and artemisin (resquiterpenoids). Terpenoids have anti-diarrhoeal and antioxidant properties. (Tiwari et al., 2011; Ali, 2009).

**Saponin:** The phytocompound saponins are amphipathic glycosides and have antioxidant activities. In case of structural compositions, saponins contain one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Kalaivani and Matew, 2009).

In an experimental animal model for *in vivo* study, carbon tetrachloride (CCl₄) induced hepatic injury is commonly used to estimate the hepatoprotective and antioxidant activities of medicinal plants. The changes associated with CCl₄ induced liver damage are similar to that of acute viral hepatitis (Orhan et al., 2007, Suja et al., 2002; Rubinstein, 1962). Therefore, CCl₄ mediated hepatotoxicity is considered as an experimental model for liver injury. It has been established that CCl₄ accumulates in hepatic parenchymal cells and
metabolically activated by cytochrome p450 dependent monooxygenase to form a trichloromethyl free radical (CCl₃) which alkylates cellular proteins (including cytochrome p450) and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen (O₂) to produce lipid peroxides leading to liver damage (Recknagel, 1983; Recknagel and Ghosal, 1966).

On the other hand, ROS are inevitably generated due to the incomplete reduction of O₂ in electron transfer reactions as byproducts of biological reactions. ROS plays a significant role in regulation of cell growth, energy production and intercellular signaling (Lee and Lim, 2008; Halliwell, 1992; Ogunlana and Ogunlana, 2008) and over production of ROS results in loss of detoxification capacity of the body cell which causes extensive damage to DNA, protein, carbohydrate and lipids (Kowaltowski and Vercesi, 1999; Cesaratto et al., 2004).

Antioxidants, the substances which have the capacity to eliminate the pro-oxidants and scavenge free radicals (Velioglu et al., 1998) play a protective role against cell damage caused by oxidative stress. Natural antioxidants isolated from plant material have an excellent protective efficacy against such damages. Numerous medicinal plants and their crude extracts have been reported for their hepatoprotective and antioxidant activity (Yadav and Dixit, 2003), very insignificant number of drugs have come out from all those works till date. Silymarin, a plant based hepatoprotective drug isolated from *Silybum marianum* (L.) Gaertn. (Pradhan and Girish, 2006) got major attention for its curative efficacy and become both scientifically and commercially acclaimed. But this drug is not adequate in all respect to give liver protection. On the other hand synthetic drugs cause severe side effects. Therefore, it necessitates the search for alternative potent herbal drugs to combat these
deficiencies. Hence, it is always necessary to find suitable curative agents for the treatment of liver diseases from natural plants products (Chatterjee, 2000; Chattopadhyay, 2003; Bhandarkar and Khan, 2004).

_Pajanelia longifolia_ (Willd.) K. Schuman, belongs to the family Bignoniaceae, is traditionally known to be a very effective hepatoprotective agent and used in ailments for curing jaundice in Southern Assam. It is a terrestrial plant, medium in size, found both as wild and less cultivated. The bark of the plant is well known in folk medicine for exhibiting hepatoprotective activity. However, from literature survey it was found that there are less scientific studies have been carried out regarding the hepatoprotective activity and antioxidant activity of the plant. Therefore, it was our interest to investigate the hepatoprotective and antioxidant efficacy of the stem bark of mentioned plant.
Fig: 1.1 Closer view of the plant *Pajanelia longifolia* (Willd.) K. Schuman

Fig: 1.2. View of the plant *Pajanelia longifolia* (Willd.) K. Schuman from distance.