2. REVIEW OF LITERATURE

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparation, as those described in ancient texts and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Diallo et al., 1999). The pharmaceutical industry, all over the world is engaged in discovery and development of drugs for disease such as malaria, filariasis, leishmaniasis, tuberculosis etc. and also fertility regulation, aging related disorders, inflammation, allergies, depression, fungal infections, cancers, viral infections, nosomial and community acquired pathogens, new infectious diseases and several other unmet medical needs. In order to meet the challenges, the pharmaceutical research requires constantly changing strategies with wide spectrum (Prabhakaran et al., 2002).

There is a serious lacuna in scientific validation of herbs for pharmaceutical use, as there are about 2,50,000 to 7,50,000 species of higher plant (flowering plants) on earth. Many of the plants have not yet been botanically described and due to industrialization, many of them face extinction. Only about 1% of them with scientific validation regarding the therapeutic value have been acknowledged in in vivo trial models. So it is vital to undertake a “need based” approach to research on medicinal plants with traditional knowledge and then screening of plants for biological activity.

2.1. Meliaceae

Meliaceae, the mahagoni family of flowering plants, of the order Sapindales comprising 51 genera and about 575 species of trees and (rarely) shrubs. They are native to tropical and subtropical regions. Most members of this family have large compound leaves, with the leaflets arranged in the form of a feather, and branched flower clusters. The fruit is fleshy and coloured or a leathery capsule. The China tree (Melia azedarach), also called chinaberry, bead tree, and Persian
lilac, is an ornamental Asian tree with round yellow fruits, often cultivated in many tropical and warm temperate areas. Trees of the genera Swietenia and Entandrophragma, commonly called as mahagoni and of the genus, Cedrela (especially the cigar-box cedar, C. odorata) are economically important timber trees. The neem tree, also called the margosa tree (genus Azadirachta), grown throughout the Old World tropics, notably in India and Southeast Asia, is a source of timber and medicinal oils and resins.

It is known that the family Meliaceae is among the richest and most diverse sources of secondary metabolites among the angiosperms, and the species of Meliaceae are known to have intense antimalarial characters due to highly oxygenated terpenoids. The Meliaceae plant family has been given much attention due to its chemical characters called “limonoid” (Connolly, 1983). Current research has pointed out that limonoids are highly oxygenated, modified terpenoids with wide range biological activities especially action against the insects. Not only insecticidal activity it has antibacterial, antifungal, antimalarial, anticancer, antiviral and other clinical activities on humans (Roy and Saraf, 2006). In the exploration of biological activities, the family Meliaceae has attracted extensive attention. Trichilia emetica particularly has been largely investigated. T. emetica, a plant native to Africa, is used in traditional medicine to treat various ailments such as abdominal pains, dermatitis, haemorrhoids, jaundice and chest pain. This species also known as Natal Mahagoni is used for its emetic, diuretic and purgative properties and for induction of labour. The extensive traditional use of this species has encouraged scientists to explore several biological activities including anti-infective, anti-inflammatory, anti-schistosomal, antiplasmodial, anticonvulsant, antitrypanosomal, antioxidant, antitussive, antimutagenic and hepatoprotective properties (McGaw et al., 1997; Diallo et al., 2003; Sanogo et al., 2006). The seeds of Swietenia mahagoni have been reported for its anti-inflammatory, antimutagenecity and antitumour activities. In Indonesia and in India, S. mahagoni seed used as folk medicine to cure diabetes. Azadirachta indica A. Juss. is of various medicinal uses, such as a contraceptive for intravaginal use, a mosquito repellent, and treatment of vaginal infections, treatment of gastric ulcers, cardiovascular disease, malaria, rheumatism and skin disorders, external applications for treatment of septic wounds,
ulcers and boils, treatment of allergic skin reactions, asthma, bruises, colic, conjunctivitis, dysmenorrhea, fever, gout, headache, itching due to varicella, kidney stones, leukorrhea, psoriasis, scabies, sprains and muscular pain, and wounds.

2.2. Swietenia mahagoni

Swietenia mahagoni locally known as mahagoni belongs to Meliaceae family that grows abundantly in the plain lands and hilly areas of Bangladesh. It is also distributed in India, Latin America and most of the tropical countries (Anonymous, 1989). It occurs natively in the Neotropics, from southern Florida, the Caribbean, Mexico and Central America south to Bolivia. The genus is named for Dutch-Austrian physician Gerard van Swieten (1700-1772) (Austin and Daniel, 2004). The genus was introduced into several Asian countries as a replacement source of S.mahagoni timber around the time it was restricted in its native locations in the late 1990s. Trade in Asian grown plantation mahagoni is not restricted. Fiji and India are the largest exporters of plantation S.mahagoni and wild S.mahagoni remains commercially unavailable to this day.

It is usually taken to consist of three species, geographically separated. They are medium-sized to large trees growing to 20–45 m tall, and up to 2 m trunk diameter. Usually, this plant is 30–40 meters in height and 3-4 meters in girth (Rastogi and Mehrotra, 1990). The leaves are 10–30 cm long, pinnate, with 3-6 pairs of leaflets, the terminal leaflet absent; each leaflet is 5–15 cm long. The leaves are deciduous to semi-evergreen, falling shortly before the new foliage grows. The flowers are produced in loose inflorescences, each flower small, with five white to greenish-yellowish petals. The fruit is a pear-shaped five-valved capsule 8–20 cm long, containing numerous winged seeds about 5–9 cm long.

The seeds and bark are used for the treatment of hypertension, diabetes, malaria, and epilepsy as a folk medicine in Indonesia and India (Kadota et al., 1990; Pullaiah, 2006). The bark is considered as astringent and is taken orally as a decoction for diarrhea, as a source of vitamins and iron, and as haemostyptic (Khare, 2007). The bark serves as antipyretic and tonic. Traditionally the bark decoction is used orally to increase appetite, to restore strength in cases of tuberculosis, to treat
anaemia, diarrhea, dysentery, fever and toothache (Anonymous, 1986). The leaf decoction is used against nerve disorders, the seed infusion against chest pain and a leaf or root poultice against bleeding (Miroslav, 2005). The local people of East Medinipur, (West Bengal), Balasore (Orissa) traditionally use the aqueous extract of its seed and bark for curing psoriasis, diabetes, diarrhea and also used as an antiseptic in cuts and wounds.

Previous chemical investigations (Figure 4) on S. mahagoni resulted in the isolation of some tetranortriterpenoids, limonoids and related compounds like Swietenine acetate; 3,6-di-0- acetylswietenolide; 2-alpha-hydroxymexioanolide; 3,-0-tigloylswietenolide; 6-acetylswietenine; 6-acetyl-3-tigloylswietenolide, etc. (Kadota et al., 1990).

Figure 4: Phytochemicals of Swietenia mahagoni

Limonoids Swietenine Tetranortriterpenoids

Earlier pharmacological reports on different parts of Swietenia mahagoni

The pharmacological properties of S. mahagoni which had been reported earlier after researches carried out in its different parts are presented in (Table 5). S. mahagoni is an important medicinal plant and has various types of medicinal values like antimalarial and antidiarrhoeal effects (Maiti et al., 2007; Munoz et al., 2000). The plant extracts possess antibacterial and antifungal activities (Majid et al., 2004). S. mahagoni extract showed agonistic activity to PPAR (gamma) and gave ameliorative effects on diabetic mice (Li et al., 2005). The seed extract of S. mahagoni has also been found to inhibit platelet-activating factor (PAF)-induced platelet aggregation (Ekimoto et al., 1991).
Table 5: Earlier pharmacological reports on different parts of Swietenia mahagoni

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pharmacological property</th>
<th>Plant part</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-inflammatory activity</td>
<td>Seeds (Methanol extract)</td>
<td>Ghosh et al., 2012</td>
</tr>
<tr>
<td>2</td>
<td>Analgesic activity</td>
<td>Seeds (Methanol extract)</td>
<td>Ghosh et al., 2012</td>
</tr>
<tr>
<td>3</td>
<td>Antipyretic activity</td>
<td>Seeds (Methanol extract)</td>
<td>Ghosh et al., 2012</td>
</tr>
<tr>
<td>4</td>
<td>Hepatoprotective activity</td>
<td>Leaves (Aqueous extract)</td>
<td>Udem et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stem bark (Methanol extract)</td>
<td>Haldar et al., 2011</td>
</tr>
<tr>
<td>5</td>
<td>Antiulcer activity</td>
<td>Leaves (Ethanol extract)</td>
<td>Radahe et al., 2012</td>
</tr>
<tr>
<td>6</td>
<td>Antibacterial activity</td>
<td>Leaves (Ethanol extract)</td>
<td>Udoumoh et al., 2011</td>
</tr>
<tr>
<td>7</td>
<td>Wound healing activity</td>
<td>Leaves (Ethanol extract)</td>
<td>Udoumoh et al., 2011</td>
</tr>
<tr>
<td>8</td>
<td>Anticonvulsant activity</td>
<td>Stem bark (Methanol extract)</td>
<td>Panda et al., 2010</td>
</tr>
<tr>
<td>9</td>
<td>Antidiabetic activity</td>
<td>Seeds (Ethanol extract)</td>
<td>Mahid- Al-Hasan et al., 2011; De et al., 2011; Hajra et al., 2011</td>
</tr>
<tr>
<td>S.No.</td>
<td>Pharmacological property</td>
<td>Plant part</td>
<td>Reference</td>
</tr>
<tr>
<td>------</td>
<td>------------------------------------------</td>
<td>------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>10</td>
<td>Antidiarrhoeal activity</td>
<td>Seeds (Ethanol extract)</td>
<td>Hajra et al., 2012</td>
</tr>
<tr>
<td>11</td>
<td>Cytotoxicity</td>
<td>Stem bark, seeds, leaves (Ethanol extract)</td>
<td>Ahsanul Akbar et al., 2009; Sahgal et al., 2010</td>
</tr>
<tr>
<td>12</td>
<td>Antioxidant</td>
<td>Seeds (Ethanolic extract)</td>
<td>Sahgal et al., 2009; Hajra et al., 2011</td>
</tr>
<tr>
<td>13</td>
<td>Xanthine oxidase inhibiting activity</td>
<td>Seeds (Ethanolic extract)</td>
<td>Sahgal et al., 2009</td>
</tr>
<tr>
<td>14</td>
<td>Anticandidal activity</td>
<td>Seeds (Methanolic extract)</td>
<td>Sahgal et al., 2011</td>
</tr>
<tr>
<td>15</td>
<td>Antibacterial activity</td>
<td>Limonoids</td>
<td>Rahman et al., 2009</td>
</tr>
<tr>
<td>16</td>
<td>Acaricidal activity</td>
<td>Leaves, stem bark (Ethanol extract)</td>
<td>Zalabani et al., 2012</td>
</tr>
</tbody>
</table>
2.3. Pharmacological investigation

Due to lack of thorough perpetuation of knowledge on medicinal plants, there is chance for people to go wrong in their right identification. Further, it is important to check the quality and purity of the drug to avoid adulteration. This can be achieved by analyzing their characters by pharmacological studies.

2.3.1. Acute toxicity

Toxicology is the science that deals with the study of the potential harmful effects of chemicals and drugs on living organisms. It is a branch of pharmacology and today it has developed into a full and independent discipline. The scope of toxicology encompasses the qualitative determination of poisons/chemicals, their deleterious (injurious) effects on the living organisms, their incidence, mechanisms, factors modifying them and reversibility (treatment) of such adverse effects. Theoretically, all substances are poisons and there is none that is not a poison. The right dose differentiates a poison and a remedy. All drugs are capable of producing harmful as well as beneficial effects (Manahan, 2002).

2.3.2. Antioxidant activity

Oxidative stress

Oxidative stress can be regarded as an imbalance between pro-oxidant/free radical production and opposing antioxidant defenses. Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological condition but become deleterious, when not being eliminated by the endogenous antioxidant systems (Fang et al., 2002). Free radicals are produced in normal and pathological cell metabolism considering that oxidation is essential to most living organisms, for the production of energy to fuel biological processes (Soares, 2009). However, the uncontrolled production of oxygen-derived free radicals are involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis, as well as in degenerative processes associated with aging (Mau et al., 2002).
Production of free radicals

A number of pathophysiological process in human body lead to the generation of a series of oxygen centred free radicals and other reactive oxygen species as by-products. The generation of reactive oxygen species is unavoidable in aerobic metabolism of the body. Mitochondria which consume more than 90% of oxygen in aerobic living organisms are the main source of reactive oxygen species and free radical. Oxygen in mitochondria is reduced to water by four sequential steps (Ames et al., 1993). Peroxyl radicals or its ionized form, the superoxide anion is the first reduced intermediate of oxygen. Hydrogen peroxide and hydroxyl radical are inevitable intermediates from oxygen to water reduction steps in body. Approximately 1% to 5% of the oxygen consumed by mitochondria is reduced and converted into these reactive oxygen species (Ames et al., 1993). Apart from normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells (Halliwell, 1994). Exogenous source of free radicals includes tobacco smoke, ionizing radiation, organic solvents and pesticides (Pepas, 1996).

Kinds of free radicals

Free radicals are defined as molecules having an unpaired electron in the outer orbit. Reactive oxygen species (ROS) can be classified into oxygen-centered radicals and oxygen centered non-radicals. Oxygen-centered radicals are superoxide radical anion (O₂ -•), hydroxyl radical (OH•), alkoxy radical (RO•), and peroxyl radical (ROO•). Oxygen-centered non-radicals are hydrogen peroxide (H₂O₂) and singlet oxygen (1⁡O2). Other reactive species are nitrogen species such as nitric oxide (NO•), nitric dioxide (NO₂•), and peroxynitrite (OONO•) (Fang et al., 2002). Reactive oxygen species or free radicals in biological systems can be formed by prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants, and glycoxidation (Halliwell, 1997; Stief, 2003; Lee et al., 2004). Some transitional metals, such as iron and copper, have many numbers of unpaired electrons and can also act as free radicals.

Superoxide ion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in
living systems (Stief, 2003). The superoxide anion can react with nitric oxide and form peroxynitrite (ONOO−), which can generate toxic compounds such as hydroxyl and nitric dioxide radical (Halliwell, 1997).

Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions such as copper or iron (Korycka-Dahl and Richardson, 1978). Unlike superoxide, the hydroxyl radical cannot be eliminated by an enzymatic reaction. It has a very short half-life and will react with all molecules in its vicinity. Because of its high reactivity it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins especially thiamine and guanosine (Ashok and Ali, 1999).

Peroxy radicals and alkoxyl radicals are formed by a direct reaction of oxygen with alkyl radicals (R•). Irradiation of UV light or the presence of transition metal ions can cause homolysis of peroxides to produce peroxy and alkoxyl radicals. Peroxyl and alkoxyl radicals are good oxidizing agents (Decker, 1998). Aromatic alkoxyl and peroxy radicals are less reactive than respective open chain radicals because of the delocalization of electrons in the ring (Lee et al., 2004). Some peroxy radicals break down to liberate superoxide anion or can react with each other to generate singlet oxygen (Halliwell and Gutteridge, 1985).

Nitric oxide is a free radical with a single unpaired electron. Nitric oxide is formed from L-arginine by NO synthase (Fang et al., 2002). It is believed to be essentially a beneficial metabolite and indeed it may react with lipid peroxides and function as an antioxidant (Hogg et al., 1993). The over production of NO is involved in ischemia reperfusion, neurodegenerative and chronic inflammatory diseases. Nitric oxide, exposed in human blood plasma, can deplete the concentration of ascorbic acid and uric acid, and initiate lipid peroxidation (Halliwell, 1996a). Nitric dioxide is formed from the reaction of peroxy radical and smoking (Noguchi and Nike, 1999). Nitric dioxide easily adds to double bonds and abstract labile hydrogen atoms, initiating lipid peroxidation and production of free radicals. It also oxidizes ascorbic acid (Papas, 1999a).

Hydrogen peroxide can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and
xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions. It can generate the hydroxyl radical in the presence of metal ions and superoxide anion (Halliwell, 1997). Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloramines in living systems (Stief, 2000; 2003).

Singlet oxygen is formed by our immune system. Compared with other reactive oxygen species, singlet oxygen is rather mild and nontoxic for mammalian tissue. In human, singlet oxygen is both a signal and a weapon, with therapeutic potency against various pathogens such as microbes, viruses and cancer cells (Stief, 2003). Lycopene, a biologically active carotenoid, exhibits the highest physical quenching rate constant with singlet oxygen (Di Mascio et al., 1989).

Over about 100 disorders like rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, metabolic disorders, and gastrointestinal ulcerogenesis have been reported as ROS mediated (Jha et al., 1995; Govindarajan et al., 2005). Further, ROS is also implicated in the pathogenesis of a vast variety of conditions including inflammatory diseases (Halliwell, 1994; Darley-Usmar et al., 1995), cancer (Eze et al., 1993; Halliwell, 1994), atherosclerosis (Cook and Samman, 1996), diabetes mellitus (Lee, 2006), malaria (Eze et al., 1993; Dey et al., 2009), neurodegenerative diseases (Halliwell, 2001), HIV/AIDS (Pocernich et al., 2005; Masia et al., 2007), and aging (Rattan, 2006). Damage caused by ROS may be due to their attack on membrane lipids, intracellular proteins/enzymes, carbohydrates, and nuclear DNA in cells and tissues. These include undesirable oxidation causing damage to membrane, protein modification, DNA damage, and cell death induced by DNA fragmentation and lipid peroxidation (Singh et al., 2004).

Biological role of free radicals

Free radicals may play an important role in the origin of life and biological evolution implicating their benefical effects on the organism (Mc Cord, 2000). Benign function of free radicals have been reported, including the activation of nuclear transcription factors, gene expression and a defense mechanism to target
tumor cells and microbial infections (Simon et al., 2000). Superoxide anion may serve as a cell growth regulator (Halliwell, 1997). Singlet oxygen can attack various pathogens and induce physiological inflammatory response (Stief, 2003). Nitric oxide is one of the most widespread signaling molecules and participant in every cellular and organ function in the body. It acts as a neurotransmitter and an important mediator of the immune response (Fang et al., 2002). However, the overproduction of ROS is also harmful to the body because the oxidation induced by ROS can result in cell membrane disintegration, membrane and protein damage and DNA mutation, which can further initiate or propagate the development of many diseases (Freidovich, 1999; Mc Cord, 2000).

Antioxidants

In biological systems, an antioxidant has been defined as any substance that when present at low concentrations compared to that of an oxidisable substrate, significantly delays or prevents oxidation of the substrate (Halliwell, 1990). However in food systems, antioxidants have been classified as substances, which are able to prevent or retard the oxidation of easily oxidisable materials, such as lipids in small quantities (Chipault, 1962). Almost all organisms have natural antioxidant properties and can repair oxidative damage in their systems, but these systems are unable to prevent the damage completely. Antioxidants are substances that can delay or prevent oxidation of cellular oxidisable substrates (Grice, 1988; Qi et al., 2005). Antioxidants play important roles in the scavenging of free radicals and/or chain breaking of the oxidation reactions both in vivo and in vitro (Ebrahimabadi, 2010).

Enzymatic antioxidants

The removal of free radicals is achieved through enzymatic and non-enzymatic reactions. The principal defense systems against oxygen free radicals are glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), and antioxidant nutrients (Fang et al., 2002). Antioxidant enzymes convert ROS into nonreactive oxygen molecules. SOD converts superoxide anion into hydrogen peroxide and oxygen. Glutathione peroxidase is the most important hydrogen peroxide removing enzyme existing in the membrane. Catalase is involved in cellular detoxification and can convert
hydrogen peroxide into water and oxygen. Glutathione disulfide reductase is a flavoprotein that permits the conversion of oxidized glutathione (GSSH) to reduced glutathione (GSH) by the oxidation of NADH to NAD+ (Papas, 1999b). The antioxidant defense systems in the body can only protect it when the amount of the free radicals is within the normal physiological level. But when this balance is shifted towards more of free radicals, it leads to oxidative stress, which may result in tissue injury and subsequent diseases (Finkel and Holbrook, 2000).

Synthetic antioxidants

The antioxidant molecules can be classified into synthetic and natural antioxidants. Many synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are very effective and are used for industrial processing, but they may possess some side effects and toxic properties to human health (Hou et al., 2003; Tepe et al., 2005; Anagnostopoulou et al., 2006). For this reason and because of the growing consumer preferences for natural products, there is an increasing interest in the investigation of naturally occurring antioxidants from plants (Vichi et al., 2001; Hou et al., 2003; Galvez et al., 2005). Natural antioxidants are healthier and less subject to hazards and demand for them is increasing compared to their synthetic counterparts (Farag et al., 1986). Medicinal plants are considered as important source of antioxidant compounds, and recently there has been considerable interest in finding natural antioxidants from plant materials to replace synthetic ones (Mehdipour et al., 2006).

Polyphenolic compounds as antioxidants

 Phenolic compounds are secondary metabolites in plants. They are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Rice-Evans et al., 1995). The antioxidant effect of plant products is mainly attributed to phenolic compounds, such as flavonoids, phenolic acids, tannins, and phenolic diterpenes (Pietta, 2000). Therefore, these substances have been proposed as health-promoting natural products (Formica and Regelson, 1995; Lee et al., 2003; Atoui et al., 2005; Capecka et al., 2005). Several studies have reported the antioxidant activity of plant extracts.
and their relationship with the phenolic compound content (Aaby et al., 2004; Silva et al., 2005; Sun and Ho, 2005; Yuan et al., 2005; Singh et al., 2007).

Over the past 10 years, there has been increasing interest in phenolics compounds and their role in human health and nutrition (Tapiero et al., 2002). Phenolic compounds are known to exhibit a range of biological activities, including anticancer, antibacterial, antioxidant and anti-inflammatory properties (Cuvelier, et al., 1994; Lu and Foo, 2000). Some of them present in natural products have higher antioxidant activities than those of synthetic antioxidants (Lu and Foo, 2000). An increasing interest in phenolics in recent years is because of their significant bioactivities such as scavenging free radicals, chelating metals, regulating enzyme activity, and modulating cell proliferation (Rice-Evans et al., 1996; Virgili et al., 1998). The protection afforded by plants against oxidative stress has been attributed to various phenolic antioxidants which are increasingly becoming of interest in the food industry because they retard oxidative degradation of lipids and thereby improve food quality (Kahkonen et al., 1999).

Several Indian medicinal plants have been reported for their antioxidant potential, for example cabbage and broccoli extracts (Fang et al., 1987), Phyllanthus amarus, P. debilis (Sane et al., 1995), Echinacea root (Hu and Kitts, 2000), Cassia tora (Yen and Chuang, 2000), Aegle marmelos (Sabu and Kuttan, 2001), Punica granatum (Singh et al., 2002), Lettuce varieties (Antonio and Marino, 2005), Ocimum sanctum (Thambi et al., 2005), green tea polyphenols (Metro et al., 2006), Capsicum annum (Deepe et al., 2006), Glycyrrhiza glabra (Visavadiyva et al., 2006), Bauhinia galpinii (Aderogba et al., 2007), Stevia rebaudiana (Tadhani et al., 2007), Asparagus officinalis (Sun et al., 2007), Pseudarthria viscida (Gincy and Sasikumar, 2008), Leucas aspera (Meghashri et al., 2010), Mangifera indica (Kim et al., 2010) and Solanum surattense (Joseph et al., 2011). There is a growing interest in natural antioxidants present in medicinal and dietary plants that might help attenuate oxidative damage (Silva et al., 2005). Many medicinal herbs exhibiting good antioxidant activities have been employed as sources of natural antioxidants. In recent years great efforts have been made to make safe and potent natural antioxidants from plant resources.
2.3.3. Anti-inflammatory activity

Non-steroidal anti-inflammatory drugs (NSAID)

Non-steroidal anti-inflammatory drugs (NSAID) are prescribed worldwide for the management of pain, inflammation and fever, as well as cardiovascular protection. Although NSAID are highly effective, their use can be associated with high occurrence of intestinal side effects and mucosal erosions that can progress into ulcers and lifethreatening complications as perforation and hemorrhage. Also kidney damage, increase in blood pressure and some other cardiovascular problems are seen with NSAID (Burke et al., 2006). However, the clinical treatment of inflammatory diseases is dependent on nonsteroidal or steroidal chemical therapeutics (Rainsford, 2007).

Mechanism of action of NSAID

Non-steroidal anti-inflammatory drugs (NSAID) reduce the pain and inflammation by blocking the metabolism of arachidonic acid by inhibiting cyclooxygenase enzyme (COX), and thereby the production of prostaglandin (Vane, 1971). Since prostaglandins are cytoprotective, long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their non-selective inhibition of both isoforms of the COX enzyme, the constitutive (COX-1) and the inducible (COX-2) isoforms (Robert, 1976; Peskar, 1977; Tapiero et al., 2002). On the other hand, fully selective and reversible COX-2 inhibitors with reduced gastro-intestinal toxicity have been associated with adverse cardiovascular effects (Dogné et al., 2005). Furthermore, the use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects (Schäcke et al., 2002; Reinke et al., 2002). Therefore, developing new agents with more powerful analgesic and anti-inflammatory activities and with lesser side effects is, at present, of great interest. During the past decades many researchers have focused on medicinal plants with analgesic and anti-inflammatory effects.

Indomethacin, one of the NSAIDs, is known to activate polymorphonuclear granulocytes and to induce gastrointestinal damage including bleeding, ulceration and perforation in both animal and human models (Naito et al., 1998a; Sagar and Ahamed, 1999). These pathologies have been attributed to damage of the mucosa
membranes. A well-known mechanism responsible for gastric damage induced by indomethacin is the inhibition of cyclooxygenase (COX), a rate limiting enzyme in the synthesis of prostaglandins. However, recent studies have suggested that NSAIDs such as indomethacin have pro-oxidant activity and initiate lipid peroxidation by generating reactive oxygen species (ROS), thereby interfering with endogenous antioxidant systems of the mucosa cells (Naito et al., 1998b; Takeuchi et al., 1991).

**Analgesic and anti-inflammatory activity**

Pain management for patients with chronic pain is a difficult task because of the risks associated with toxicity due to the drug intervention (Sam, 2008). Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed medications for the management of painful conditions, but they are frequently causing gastrointestinal damage (Fiorucci et al., 2001). Ethnobotanical practice to treat diseases is a therapeutic modality, which has stood the test of time for the treatment of various ailments (Gilani and Rahman, 2005). To explore new, effective and safe analgesic for the management of different painful conditions, the crude methanol extract was screened in various pain models. Intraperitoneal injection of acetic acid produced an abdominal writhing response by activating the chemosensitive nociceptors in animals (Stai et al., 1995) or by irritation of the visceral surface, leading to the production of algogenic substances (Schowb and Dubost, 1984). The analgesic effects of the extracts may be due either to their action on visceral nociceptors sensitive to acid, to the inhibition of the production of algogenic substances or the inhibition, at the central level, of the transmission of painful messages. It is also known that antihistaminic (Naik et al., 2000), myorelaxant and anti-inflammatory substances (Koyama et al., 1997) and opioids such as codeine (Schwob and Dubost, 1984; Aydin et al., 1996) are able to reduce pain induced by acetic acid. It becomes evident that this model of experimental pain is unable to indicate the mechanism of analgesic effects of the test substances. Collier et al., (1968) proposed that acetic acid acts indirectly by releasing endogenous mediators that stimulate the nociceptive effect but also stimulates neurons that are sensitive to other drugs such as narcotics and centrally acting agents. The writhing test has long been used as a screening tool for the assessment
of analgesic or anti-inflammatory properties of new substances (Collier et al., 1968). Though this method presents a good sensitivity, it shows poor specificity.

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmic fluid and blood cells. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs that pose a major problem during their clinical uses. Therefore, development of newer and more substantial anti-inflammatory drugs with lesser side effects is necessary (Shukla, et al., 2010). For an anti-inflammatory effect, it is important to estimate the activity of a treatment in both the acute and the chronic phases of inflammation. Accordingly, the carrageenan test was selected because of its sensitivity in detecting active anti-inflammatory agents, particularly in the acute phase of inflammation (DiRosa et al., 1971; DiRosa, 1972). It is evident that carrageenan induced edema is mediated by the release of histamine and 5HT in the early stage, followed by kinin protease release and then by prostaglandin in the later phase (Castro et al., 1968). The early phase, named non-inflammatory pain, is a result of direct stimulation of nociceptors and reflects centrally mediated pain; the late phase, named inflammatory pain, is caused by local inflammation with a release of inflammatory and hyperalgesic mediators (Hunskaar and Hole, 1987). On the other hand, a cotton pellet granuloma is a model of chronic inflammation (Ismail et al., 1997b) and the dry weight has been shown to correlate with the amount of granulomatous tissue formed (Swingle and Shideman, 1972).

Herbal extracts as analgesic and anti-inflammatory agents

The anti-inflammatory activity is closely related to analgesic activity (A´vila-Pena, 2007). Various flavonoids such as rutin, quercetin, luteolin, hesperidin, as well as biflavonoids produce significant antinociceptive and/or anti-inflammatory activities (Galati et al., 1994; Ramesh et al., 1998; Bittar et al., 2000; Calixto et al., 2000). There are few reports on the role of tannins in antinociceptive and anti-inflammatory activities (Starec et al., 1988). The anti-inflammatory effects of triterpenes (i.e., oleanolic and ursolic acids) have been attributed to various
mechanisms including: inhibition of lipoxygenase and cyclooxygenase activities, inhibition of elastase and inhibition of complement activity, possibly through the inhibition on C3-convertase of the classical complement pathway (Singh et al., 1992). The continued search for effective and safe anti-inflammatory agents cannot be over emphasised. The use of herbal extracts and nutritional supplements either as alternative or complimentary medicine to the conventional chemotherapy for treatment of inflammatory diseases is well documented in Ayurveda, which is an alternative medicinal system that has been practiced primarily in the Indian subcontinent for 5000 years (Dahanukar et al., 2000).

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history (Almeida et al., 2001). Many plant species have been reported for their analgesic and anti-inflammatory activities and to name a few are Teucrium stocksianum (Radhakrishnan et al., 2001), Moringa pterygosperma (Medhi et al., 2003), Zingiber officinale (Ojewole, 2006), Blechnum occidentale (Nonato et al., 2009), Baccharis dracunculifolia (Santos et al., 2010) and Cnestis ferruginea (Ishola et al., 2011). Over the years, natural products have contributed to the development of important therapeutic drugs used currently in modern medicine and should still be seen as a fruitful and logical research strategy, in the search for new analgesic and anti-inflammatory drugs.

2.3.4. Hepatoprotective activity

Hepatotoxicity

Liver is the organ for metabolism and detoxification of various components entering into the body. As it is involved in a wide range of functions, it is exposed to toxic substances and drugs absorbed from the intestine. Apart from the toxins and drugs (Marina, 2006), viral infections and microbial infections of Entamoeba histolytica (Sharma and Ahuja, 1997) also cause damage to the hepatocytes. Paracetamol, a common antipyretic and analgesic agent, can produce fatal hepatic necrosis in man, rats and mice at toxic doses (Mitchell et al., 1973; Kuma and Rex, 1991; Eriksson et al., 1992; Amar and Schiff, 2007). Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigators (Visen et al., 1993; Singh and Handa, 1995; Ahmed and
Khater, 2001). These toxicants mainly damage liver by producing reactive oxygen species. Therefore, numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional medicines in India

Paracetamol induced hepatotoxicity

Paracetamol is metabolically activated in the liver by cytochrome P450 to form a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) that covalently binds to protein (Vermeulen et al., 1992). NAPQI is detoxified in the liver by glutathione (GSH) to form a paracetamol–GSH conjugate. When NAPQI production exceeds GSH detoxification capacity, NAPQI covalently binds to various cell proteins to form inactive conjugates (Graham et al., 2005). These conjugates can lead to irreversible hepatic cell injury and liver necrosis by various mechanisms. Besides, NAPQI binds to sulphhydryl groups of enzymes and in that way may contribute to metabolic alterations in the liver (Yamamoto et al., 2007). Migration and activation of neutrophils in the liver (Xu Liu et al., 2006), production of cytokines (TNF-α i.e., tumor necrosis factor-α) interferon-γ (IFN-γ) and chemokines (Ishida et al., 2002), and platelet activating factor (PAF) (Grypioti et al., 2006) are additional mechanisms that have been reported to be important in the development of paracetamol hepatotoxicity. Oxidative stress is considered to play a prominent causative role in many diseases including liver damage (Kiso et al., 1984). In short, there is a dynamic relationship between reactive oxygen species and antioxidants in the human body. The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, catalase and GPX. These enzymes constitute a mutually supportive team of defense against ROS (Venukumar and Latha, 2002).

Plant based drugs for liver disorders

In the absence of reliable liver protective drugs, considerable interest has been evinced by researchers and medicinal professionals regarding the use of indigenous drugs in the treatment of diseases. Natural compounds that reduce enzymes related to bioactivation of chemicals could be considered as good candidates for protection against chemical induced toxicity. Plant derived natural
products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activities. Nearly 170 phytoconstituents from 110 plants and a number of polyherbal formulations have been claimed to possess liver protective activity.

Globally, plant based drugs like Silybum marianum (Scott Luper, 1998), Picrorhiza kurroa (Chander et al., 1990) and Phyllanthus emblica (Gulati et al., 1995) are widely and successfully used in the treatment of liver disorders. Silymarin is a standardized seed extract of Silybum marianum, which contains flavonolignans. Hepatoprotective activity of silymarin has been demonstrated by various researchers from all over the world against partial heptectomy models and toxic models in experimental animals by using acetaminophen, carbon tetrachloride, ethanol, D-galactosamine and Amanita phalloides toxin. Rats with partial heptectomy, where 70% of liver is removed, when subjected to silymarin pretreatment showed increased synthesis of DNA, RNA, protein and cholesterol, suggesting the regeneration of liver (Sonnenbitchler et al., 1986; Srivastava et al., 1996). The antioxidant and free radical scavenging activities of many substances have been assessed, and many substances that possess anti-hepatotoxic activity also show strong antioxidant activity (Hwang et al., 1996; Luper, 1998). Some hepatoprotective drugs are antioxidants and can produce such benefical effects by way of membrane stabilization, neutralization of free radicals and immunomodulation. Natural antioxidants, vitamins and flavonolignan materials have an effect in this way (Fehe’r et al., 1998).

Phenolics and flavonoids (rutin and quercetin), are well known hepatoprotective agents (Khalid et al., 2002). Ellagic acid is a polyphenol known to possess antiproliferative, hepatoprotective and antioxidant properties in a number of in vitro and small animal models (Singh et al., 1999; Seeram et al., 2005; Han et al., 2006). Ferulic acid also has a definite hepatoprotective role (Rajagopalan et al., 2004). Many plant species like Cassia occidentalis (Jafri et al., 1999), Cassia fistula (Bhakta et al., 1999; Bhakta et al., 2001), Emblica officinalis (Jose and Kuttan, 2000), Hedyotis corymbosa (Sadasivan et al., 2006), Indigofera tinctoria (Singh et al., 2006), I. aspalathoides (Rajkapoor et al., 2006), Butea monosperma

62
(Sehrawat et al., 2006), Bauhinia variegata (Bodakhe and Ram, 2007), Solanum fastigiatum (Sabir and Rocha, 2008), Clerodendron inerme (Gopal and Sengottuvelu, 2008) and Kyllinga nemoralis (Somassundaram et al., 2010) have been reported for their hepatoprotective activity.

Several anti-inflammatory, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity (Perry et al., 1999; Lin and Huang, 2002; Repetto and Liesuy, 2002). Some of the plants reported to possess both hepatoprotective and antioxidant properties are Phyllanthus maderaspatensis (Asha et al., 2004), Bauhinia racemosa (Gupta et al., 2004a), Eucalyptus maculata (Mohamed et al., 2005), Momordica dioica (Jain et al., 2008), Ginkgo biloba (Naik and Panda, 2007), Terminalia catappa (Kinoshita et al., 2007), Vernonia amygdalina (Iwalokun et al., 2006), Punica granatum (Kaur et al., 2006) and Hygrophila auriculata (Shanmugasundaram and Venkataraman, 2006).