II. REVIEW OF LITERATURE
REVIEW OF LITERATURE

The World Health Organization (WHO) estimated that about 80% of the world’s population still relies on plant-based medicines for their primary health care. This in fact is a clear indication for the role of medicinal plants in the maintenance of health and treatment of diseases as therapeutic alternatives throughout the world, still in the late 20th and early 21st century (WHO, 2002).

There is a scientific discipline known as ethnobotany (or ethno pharmacology), whose goal is to utilize the impressive array of knowledge assembled by indigenous peoples about the plant and animal products they have used to maintain health (Georges, 1949; Rojas, 1992; Silva, 1996 and Vanden Berghe, 1986). Plants have an almost limitless ability to synthesize aromatic substances, most of which are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g. the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds. The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade.

Numerous plant based drugs have entered the international market due to pharmaceutical companies made renewed strategies in favor of natural product based drug development and discovery. It can be noted that these drugs were isolated from natural source many of which have been used by various cultures throughout history. Some of them are morphine, strychnine, quinine and colchicine etc (Brent Jason, 2005).
Pharmaceutical research took a major leap when alongside natural products chemistry; pharmacologists, microbiologists and biochemists began to unravel the chemistry of natural processes in human, animals, plants and microorganisms. Advances in synthetic organic chemistry led to the identification of many key chemical molecules that offered more opportunities to develop novel compounds. Many new drugs emerged by this route, particularly those now being used to treat infections, infestations, cancers, ulcers, heart and blood pressure conditions. Many drugs were developed through random screening of thousands of chemicals synthesized as dye-stuffs and the like; many others resulted from serendipity arising from sharp-eyed observations of physicians and scientists. Examples of such drugs include sulphonamides, isoniazid, anti-psychotics, anti-histamines and penicillin (Clark, 1996).

The review of analysis of the drugs developed between 1981 and 2003 showed that natural products or natural product derived drugs comprised 68% of all new chemical entities launched onto the market. In addition, 44% of these were semisynthetic or natural mimic compounds, based on the study of pharmacophores related to natural products (Newman et al. 2003). This much bulky percentage suggests that natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development. The large proportion of natural products in drug discovery has stemmed from the diverse structures and the intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more "drug-likeness and biological friendliness than totally synthetic molecules" (Koehn and Carter, 2005), making them good candidates for further drug development (Balunas and Kinghorn, 2005 and Drahl et al. 2005).
1. Pharmacognosy

The increasing demand for herbal medicines both in the developing and developed countries inevitably led to maintaining the quality and purity of the herbal raw materials and finished products. Further, unlike the past ages, safety today is an issue due to, complexity of herbal extracts specially when the identity of an herb is questionable or extraction method is new, possibility of contamination due to heavy metals, microbiological load, pesticide residues or mycotoxins. So it is important that plant drugs and their products are prepared following standardized procedure to ensure product uniformity and quality. For standardization and quality assurance purpose, authenticity, purity and assay are desirable. Authenticity corresponds to the right identity, purity pertains to devoid of adulteration and assay part of standardization is chemical and biological profiling by which the chemical and biological effects could be assessed and curative values are established. It is practically impossible to avoid some naturally occurring inorganic (sand and inorganic constituents of soil) and organic (other parts of the same plants or parts of the neighbouring plants of another species) contaminants. The evaluation of these standardization parameters gives a clear idea about the specific characteristic of crude drug under examination (Mukherjee, 2002).

The standardization problem relating to the herbal drugs arises from the complex composition of drugs that are used in the form of whole plant, plant parts or extracts obtained there from. To ensure reproducible quality of an herbal remedy, proper control of the starting material is utmost essential. Usually plants cannot be identified to species using only rhizomes, roots or barks, which for many medicinal plants are the parts found in market. As a result, the development of evermore sophisticated or molecular methods to be employed in quality control has become necessary, especially in such morphologically problematic species. However, such methods are costly enough that they should not be employed when simpler methods would serve. Pharmacognostic study involving morphological and organoleptic
Review of Literature

identification is the oldest, simplest and cheapest of all methods, thus to be preferred when its use is feasible along with the other parameters like ash value, extractive value and qualitative chemical tests serve as source of information. Hence, the studies on pharmacognostic parameters are useful tools to determine the purity of certain plants and to avoid adulteration in the process of commercialization of raw material.

The Pharmacognostic studies have been extensively studied on different plants viz. in *Jatropha curcas* an important Ayurvedic drug known as ‘Dravanti’ (Gupta, 1985); *Ligaria cuneifolia* (Loranthaceae) used as substitute for *Viscum albam*, which is used in elevated blood pressure (Wganar et al. 1998); Bassols and Gurni, (2000) studied the anatomical features of four species of *Lippia* (Fam: Verbenaceae), known under the trivial name “poleo”, in order to distinguish them from each other, Kola et al. (2003) investigated the comparative pharmacognostic and antimicrobial studies on leaves of two varieties of *Heinsia crinite*. Recently pharmacognostic investigations were carried out considering various parameters like organoleptic characters, microscopy, stomatal number, stomatal index, vein islet number, vein termination number, palisade ratio, soluble extractives, loss on drying, ash values & total foreign organic matter for the characterization of plants viz. *Coleus forskohlii* (Shrivastava et al. 2002); *Actaea racemosa* L. (Applequist, 2003), *Uncaria tomentosa* and *Uncaria guianensis* (Gattuso et al. 2004), *Maytenus ilicifolia*, (Duarte and Debur, 2005), *Gisekia pharmacioides* (Musa et al. 2006), *Crateva nurvala* (Sikarwar 2009), *Annona squamosa* Linn. (Sharma et al. 2009), *Capparis sepiaria* (Kalidass et al. 2009) and *Holoptelea integrifolia*, (Padmaja, 2009).

2. Phytochemistry

Phytochemistry deals with chemicals obtained from the plant source. These chemicals are the secondary metabolites synthesized in the plants, which most of the time protect the plants from insect and microbial attack.
However, these secondary metabolites are of medicinal interest as they have got a vast range of biological activity.

In the ancient times, substances obtained from plants and animals, with or without purification have been employed for medicine. But the pure medicinal chemicals obtained from these plants have got their own advantages, since the physiological effects of such compounds are fixed and definite. The actual active constituents of many crude drugs are still unknown. The pharmacological action of the crude drugs is determined by the nature of its active constituents. The plant species may contain vast range of compounds such as alkaloids, terpenoids, flavonoids, glycosides etc. These secondary metabolites are responsible for the desired therapeutic properties. In recent years, a renewed interest in obtaining biologically active compounds from natural sources has been observed, notwithstanding the impressive progress of new competing methodologies, as for example, combinatorial chemistry and high throughput screening or genetic engineering. Contributing to this world-wide attention towards formulations based on natural products are their low or absent toxicity, their complete biodegradability, their availability from renewable sources, and, in most cases, their low-cost if compared with those of compounds obtained by total chemical synthesis. In developed countries this could be connected with the trend favorable to the so-called ‘sustainable development’, and to some extent with the observed decline of patent applications in Organic Chemistry, paralleled by the rise of Life Sciences applications (Corrado, 2001).

This may reflect the switch in interest of the chemical industry from synthetic to biologically derived molecules and processes. In developing countries, this is sustained by the search for biologically active compounds obtainable from locally available plants, particularly with a view to reducing public health costs which have significantly been raised due to acquisition of synthetic drugs from industrialized countries. The study of active principles
involved in traditional medicine treatments can also lead to an improvement of these remedies. A further drive to the study of compounds obtainable from natural sources is the increasing consciousness that destruction or severe degradation of rain forests and other wild habitats, including seas and oceans, will unavoidably result in the loss of unexamined species and consequently of potentially useful compounds. In fact, individual plant species may contain over one thousand chemical substances and only a minor fraction of the estimated total of 250,000 to 300,000 plant species has been studied for biomedical application; on the other hand, the marine ecosystem is still, to a large extent, unexplored. Thus, the urgent need for protection of biodiversity is, at molecular level, a need for protection of the chemical diversity, that is the variety of natural ‘libraries’ of compounds not yet identified and characterised. Owing to this renewed attention to pharmaceuticals, agrochemicals and nutraceuticals (functional foods) obtained from natural sources, the study of bioactive secondary metabolites, traditionally carried out mainly by chemists, has increasingly attracted the attention of pharmacologists, pharmacognosists, biologists, botanists, agronomists, etc. stimulating cooperative work. Chemo diversity in nature, e.g. in plants, microorganisms and marine organisms, still offers a valuable source for novel lead discovery, but rapid identification of the bioactive compounds of natural product mixtures remains a critical factor to ensure that this tool of drug discovery can compete with recent developed technologies such as chemical compound libraries and high-throughput screening of combinatorial synthetic efforts. Rapid screening of natural product mixtures requires the availability of a library of reference of natural compounds and methods for simple identification of putative lead structural classes avoiding, to a large extent, the potential for false-positive results. The coupling of chromatographic methods such as high pressure liquid chromatography (HPLC) with diode array detection, mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR) or, and with, on-line bioactivity assays, is an important tool for high throughput screening of natural product mixtures. The
introduction of a dereplication step after extraction by using a reproducible preseparation method would enable the rapid elimination of false positives (Verpoorte, 1998). The effective use of automated procedures and databases in the isolation, identification and biological profiling of bioactive compounds from natural sources will be the best guarantee to the continued discovery of novel chemotypes from nature (Hook et al. 1997). Structure-activity studies of these leads, preferentially combined with computer graphic model building, should result in molecules with optimal activity and bioavailability, fewer side effects and an acceptable therapeutic index and, consequently in good candidates, for the development to new drugs.

Although the first chemical substance to be isolated from plants is benzoic acid in 1560 the search for useful drugs of known structure did not begin until 1804 when morphine was separated from Papaver somniferum L. Since then many useful drugs from higher plants have been discovered but less than 100 with defined structure are in common uses. But less than half of them are accepted as drugs in industrialized countries (Framesworth, 1984). Among some of the earliest successes in developing drugs from natural products, one can mention the isolation of the antimalarial agents such as the Cinchona tree alkaloids, pain relievers such as the morphine alkaloids as well as the development of aspirin. Quinine (Croteau, 2000) originally isolated from the bark of Cinchona trees, Cinchona succirubra, was one of the principal antimalarial agents. Morphine (Newman, 2000) the major alkaloid of Papaver somniferum was first isolated between 1803-1806. It was widely used for pain relief beginning in the 1830's, but was also recognized as addictive. The “Ebers papyrus”, the Egyptian pharmaceutical record, indicates the use of willow leaves as an antipyretic agent. Following on this knowledge, chemists began to isolate the compounds responsible for the remedy, and salicin was isolated from the bark of the white willow, Salix alba, in 1825-26 (Viktorin, 1999). It was subsequently converted to salicylic acid (Wallach, 1887) via hydrolysis and oxidation, and proved potent as an antipyretic that
was manufactured and used worldwide (Viktorin, 1999). To overcome the severe gastrointestinal toxicity of salicylic acid, it was converted into acetylsalicylic acid (ASA) (Ruzicka, 1953) via acetylation and started to be marketed under the trade name aspirin in 1899 (Viktorin, 1999). Aspirin is still the most widely used analgesic and antipyretic drug in the world. More recently, the vinca alkaloids, vinblastine (Loomis, 1973) and vincristine (Whittaker, and Banthorpe, 1972) were isolated as antineoplastic agents from the Madagascan periwinkle, Catharanthus roseus, and subsequently derivatized to vinorelbine and vindesine, the drugs that are currently in use for cancer treatment (Newman,. 2000). Similarly, a potent antimalarial drug, a sesquiterpenoid endoperoxide, named artemisinin (Croteau, 1981) was isolated from Artemisia annua as a remedy against the multidrug resistant strains of Plasmodium, following on the long use of this plant material as an antimalarial drug in the traditional Chinese medicine. Using the basic structure of artemisinin, semisynthetic compounds were synthesized with the aim of optimizing the pharmacology of the principal molecule leading to the identification of artemether (Bryant, 1969) and dihydroartemisinin (Roberts, 1972) as potent antimalarial agents that are now in a widespread use around the world (Newman, 2000). Paclitaxel, marketed as Taxol was initially isolated from the bark of the Pacific yellow tree Taxus brevifolia, is the best selling drug obtained from natural products. This drug was developed by ‘Bristol-Myers Squibb’ and marketed for the treatment of ovarian and mammary cancers, and became available for use in the USA in 1993 (Michael et al. 2004).

Several phytochemical investigations in plant Azima tetracantha resulted in the isolation of dimeric piperidine alkaloids like azimine, azacarpine and carpine (Rall et al. 1968). Venkatarao and Prasadrao, (1978) have reported isolation of triterpenoids from leaf and roots. Further, Bennet et al. (2004) have reported the presence of glucosinalates, flavonoids, alkaloids and other secondary metabolites in the tissues of Azima tetracantha.
The alkaloids present specifically in the leaves of *Cocculus hirsutus* are D-trilobine and DL-Coclaurine (Jagannadha and Ramachandra, 1961). The whole plant of *C. hirsutus* has been reported to contain essential oil, β-Sitosterol, Ginnol (Merchant *et al.* 1962), glycosides, sterols and alkaloids (Das *et al.* 1964). Two resins and unknown alkaloids also have been reported (Nadkarni, 1976). The alkaloids reported to be present are Hirsutine, Shaheenine (Rasheed *et al.* 1991a and b), Jamtine-N-Oxide, Cohirsinine, jamtinine, (Viquaruddin *et al.* 1987a, 1991 and 1993a), Cohirsine, Corsitinine and Haiderine (Viquaruddin *et al.* 1987b, 1992 and 1993b). The leaves also contain Isotrilobine, (+)-Syringaresinol and Protoquericitol (Viquaruddin and Tahir, 1986).

3. Pharmacology

In the present investigation, leaves of *Azima tetracantha* and *Cocculus hirsutus* were selected for studying various pharmacological activities such as hepatoprotective, antioxidant, antipyretic anti-inflammatory, analgesic, cardiovascular anthelmintic and antimicrobial activities of alcoholic extracts and the literature survey pertaining to these are explained here under.

**Hepatoprotective activity**

The liver is a vital organ present in vertebrates and some other animals. It is a reddish brown and largest organ of the human body with four lobes of unequal size and shape. Normally an adult liver weighs between 1.4-1.6 kg (Cotran, 2005) occupying on the right side of the abdominal cavity just below the diaphragm and is connected to two large blood vessels, one called the hepatic artery and one called the portal vein. The hepatic artery carries blood from the aorta whereas the portal vein carries blood containing digested food from the small intestine. These blood vessels subdivided into capillaries which then lead to a lobule. Each lobule is made up of thousands of hepatic cells which are the basic metabolic cells.
The liver is necessary for survival; there is currently no way to compensate for the absence of liver function. This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It produces bile, an alkaline compound which aids in digestion, via the emulsification of lipids. It also performs and regulates a wide variety of high-volume biochemical reactions requiring highly specialized tissues, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton, 1993).

**Hepatitis**

Hepatitis is an inflammation and/or necrosis of liver cells resulting in liver dysfunction. Chemical and biological contamination of food and water or deterioration in environmental conditions, eating fine and with less fiber contents are some of the important factors attributed for the rising Liver infection (viral hepatitis) and dysfunction which ultimately leads to manifestation of jaundice (Sinha and Shweta Sinha, 2001). There are several types of hepatitis,

**Acute Hepatitis:** Acute (sudden in onset) infection of the liver e.g. infective hepatitis, serum hepatitis and toxic hepatitis. The common causes of acute hepatitis are hepatotropic viruses A, B, C, D and E, hepatotoxins and drugs.

**Chronic Hepatitis:** The injury to the liver is long standing and continuous. Hepatic inflammation and necrosis continues at least for 6 months. Milder forms are nonprogressive or only slowly progressive, while more severe forms may be associated with scarring and architectural organization, which when advanced leads ultimately to cirrhosis. Many people with chronic hepatitis may not have any typical signs and symptoms
and may therefore feel healthy e.g. chronic alcoholism, carriers of hepatitis viruses C & D. It may only be detected through laboratory tests of liver function. Illness due to chronic hepatitis may last for more than six months.

**Drug induced hepatitis:** Jaundice due to drugs and chemicals arise as a result of accidental, suicidal or therapeutic exposure to the agent. The exposure may be of an acute or a slow, prolonged and chronic type. Certain drugs and chemicals, which are poisonous to human system, produce jaundice by hepatocellular damage. In mild cases jaundice may be absent or slight whereas in severe cases death may occur before jaundice becomes apparent. In other cases jaundice is seen in association with other manifestations like albuminuria, diarrhoea, and vomiting, renal failure or blood dyscrasias. In some cases increased haemolysis may contribute to jaundice of hepatocellular damage.

Carbon tetrachloride (CCL₄) is widely used in animal models to induce acute liver injury. Prolonged administration of carbon tetrachloride can lead to cirrhosis (Cameron and Karunaratne, 1936) and hepatic carcinoma (Reuber and Glover, 1970). Most of the acute and chronic hepatic injury appears to result from the action of metabolite of the toxin (Recknagel and Glende, 1973). Chemically CCL₄ is a simple, strongly non-polar molecule (Von Oettingen, 1964), which undergoes metabolism in the smooth endoplasmic reticulum. It is generally believed that the toxicity of CCL₄ results from its reductive dehalogenation by the cytochrome P₄₅₀ enzyme system into the highly reactive free radical trichloromethyl radical (Recknagel, et al. 1989). Liver fibrosis induced by the CCL₄ leads to the impairment in hepatocellular functions this in turn causes obstruction in detoxification mechanism leads to the clinical conditions such as hyperbilirubinemia, hypoproteinaemia, etc.

The investigations of Karandikar et al. (1963), Rubin et al. (1963), Vaishwanar and Kowale, (1976) and Recknagel, (1983) proved that
administration of CCl₄ in rats tend to cause centrilobular hepatic necrosis or toxic hepatitis and the injury caused by this toxic substance is similar to that of human infective hepatitis. Similarly, the other hepatotoxic substances like alcohol (Gulati, et al. 1995); paracetamol (Chattopadhay et al. 1992; and Ahmed and Khater, 2001); aflatoxin B₁ (Dwivedi et al. 1990); D-galactosamine (Anandan and Devaki, 1999) etc. were also known to cause hepatic cirrhosis or necrosis in albino rats. Human beings are exposed to these compounds through polluted environment, consumption of contaminated food, synthetic drugs during diseases etc. which produces toxic manifestations. Biochemical damage is produced by the reactive oxygen species and free radicals are fundamental aspects of liver injury. Conventional drugs are often inadequate and hence it’s necessary to screen for alternative drugs that can replace currently used drugs of doubtful efficacy and safety.

Despite tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help the regeneration of the parenchyma cells. While corticosteroids are immunosuppressive agents, the side effects of which are alarming, are the only drugs of choice in modern medicine for the management of liver ailments, plants and natural products are proving to be good hepatoprotectants. This is evident from the voluminous work published on their hepatoprotective activity (Handa, 1986). The importance of plant products in modern medicine even in a highly advanced society as that of the USA can be seen from the data of natural surveys (Dewan, 1990), where in it was found that 25% of all the prescriptions dispensed contained crude plant materials or crude plant extracts. About 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess liver protective activity and about 600 commercial herbal formulations with claimed hepatoprotective activity are being sold world wide of these about forty patent poly herbal formulations, representing various herbs. For centuries, indigenous drugs, either alone or in combination have advocated in
the traditional systems of medicine especially Ayurveda for the treatment of liver disorders.

Many investigators reported the hepatoprotective activity in the various plant extracts such as *Embelica officinalis Gaertn.* (Pillay and Iyer, 1958; Hemadri, 1984 and Sarwat sultana et al. 2005); *Picroorhiza Kurroa* (Chaturvedi and Singh, 1965 and Atal et al. 1985); *Phyllanthus amarus* (Thyagarajan and Jayaram, 1992); *Elephantopus scaber, E. mollis* and *Pseudoelephantopus spicatus* (Chun-Ching Lin et al. 1995); *Berberis aristata* (Janbaz and Gilani, 2000); *Cassia angustifolia* Vahl (Ilavarasan et al. 2001); *Apium graveolens* Linn; *Croton oblongifolius* Roxb. (Ahmed, et al. 2002); *Thespesia populnea* (Havarasan, et al. 2003); *Vitex negundo* (Srinivas et al. 2004); *Boerhaavia erecta* (Krishna and Shanthamma, 2004); *Diospyros cordifolia* (Krishna et al. 2005); *Polygala arvensis* (Dhanabal et al. 2006); *Beta vulgaris* (Agarwal et al. 2006) and *Pergularia daemia* (Sureshkumar and Mishra, 2007); *Cichorium intybus* (Madani et al. 2008) *Calendula officinalis* (Koregnath, 2009) and *Sphaeranthus indicus* (Brijesh and Khosa, 2009).

Silymarin is a flavonoid isolated from *Silybum marianum* that heralded widespread world research on hepatoprotective agents (Wagner et al. 1968; Abraham et al. 1970 and Pelter and Hansel, 1975). Silymarin has been reported to protect liver cells from a wide variety of toxins, including acetaminophen, ethanol, carbon tetrachloride, and D-galactosamine. Silymarin has also been found to protect liver cells from ischemic injury, radiation, iron toxicity, and viral hepatitis. The mechanisms which provide silymarin's hepatoprotective effects are many and varied, and include antioxidation, anti-lipid peroxidation, enhanced detoxification, and protection against glutathione depletion. Silymarin has been found to inhibit the formation of leukotrienes from polyunsaturated fatty acids in the liver, via its inhibition of the enzyme lipoxygenase. These leukotrienes are known to be some of the most damaging chemicals found in man. *Picrorhiza kurroa,* on
the other hand, though less well researched than silymarin, appears to have similar applications and mechanisms of action. When compared with silymarin, the hepatoprotective effect of picrorhiza was found to be similar, or in many cases superior, to the effect of silymarin. Other important antihepatotoxic drug discoveries from plant sources include cynarin from *Cynara scolymus* (Panizzi and Scarpati, 1954) and schizandrin from *Schisandra sphenanthera* (Liu et al. 1978). The discovery of diverse chemical compounds from the natural products and synthetic compounds used in protective liver therapy such as phospholipids, sugar alcohols, pyrimidine, purine derivatives, vitamins, cysteine, glutathione, corticoids, androgens, penicillamine, ricin etc. does not confine the activity to any particular class of compounds (Shirwaikar et al. 1991), but emphasizes once again the complexity of liver disorders in addition to the different action, mechanisms of different pharmaceutical preparations.

Search for hepatoprotective drug has been investigated by several workers utilizing different plant species. Visen et al. (1990) isolated an active constituent andrographolide from *Andrographis paniculata* which was found to possess the hepatoprotective activity against paracetamol induced hepatic damage. Two iridoid glycosides isolated from the plant *Picrorhiza kurrooa* exhibited significant hepatoprotective and anti-cholestatic activity against CCl₄ induced hepatitis (Dwivedi et al. 1991 and Saraswat, 1993). Lin and Shieh, (1997) studied the hepatoprotective activity of the constituents baicalein, baicalin and wogonin, isolated from *Scutellaria rivularis*. Koul and Kapil, (1999) observed that, the piperine isolated from black and long peppers has got significant hepatoprotective activity. Lim et al. (2000) has evaluated the hepatoprotective effect of bergenin a major constituent of *Mallotus japonicas* on CCl₄ intoxicated rats. Bergenin has a potent hepatoprotective action against CCl₄ induced hepatic damage in rats which was indicated by controlling the serum enzyme levels. Bhattacharya et al. (2000) reported that glycowithanolides, isolated from *Withania somnifera* have protective
Effective against iron induced hepatotoxicity. Coumestans isolated from the leaves of *Wedelia calendulacea* was evaluated in paracetamol induced liver damage by Emmanuel *et al.* (2001). Coumestans afforded a significant protective action in the alleviation of paracetamol induced toxicity by restoring the increased serum enzyme levels.

Murakami *et al.* (2001) isolated the principle constituent quercetin 3-sephorotrioside from the young seeds of garden peas (*Pisum sativum*). The active constituent was found to have protective effects on liver injury induced by D-galactosamine and carbon tetrachloride in mice. Hepatoprotective activity of isolated bioactive compound indigtone from *Indigofera tinctoria* (Singh *et al.* 2001) showed significant hepatoprotective activity against CCl₄ induced liver damage in rats and mice. Tran *et al.* (2001) also observed that the triterpene and saponins from *Panax vietnamensis* has showed the hepatoprotective activity. Khalid *et al.* (2002) isolated rutin from *Artemisia scoparia* and investigated its hepatoprotective activity against paracetamol and CCl₄ induced hepatotoxicity. Aniya *et al.* (2002) observed the free radicals scavenging action of medicinal herb *Limonium wrightii*. Yoshikawa *et al.* (2003) noticed that the triterpene and saponins isolated from *Panax notoginseng* has hepatoprotective effects.

Further, Young *et al.* (1986) reported that the methanol extract of *Wedelia chinensis* possesses a strong anti-hepatotoxic property against CCl₄ induced hepatitis, using primary cultured rat hepatocytes. The ethanol extract fractionation of *Cnidium monnieri* furnished two hepatoprotective sesquiterpenes, torilin and toriololone (Oh *et al.* 2002). Both the compounds showed the hepatoprotective effects on tacrine induced cytotoxicity in human liver derived HepG2 cells. Bioassay-guided fractionation of water extract of the seeds of *Psoralea corylifolia* furnished bakuchiol, a hepatoprotective compound on tacrin induced cytotoxicity in human liver derived HepG2 cells (Choi *et al.* 2006).
Antioxidant activity

Free radicals are atomic or molecular species with unpaired electrons. They are highly reactive and unstable as compared to similar ions. Free radicals play an important role in many biological processes including metabolic pathways, cell signaling, immune response, and a variety of pathophysiological conditions. Detection and quantification of these species is critical to decipher cellular pathways and mechanisms to understand disease and function. Free radicals are generated in the biological environment as a result of reactions associated with common biochemical pathways involving oxygen metabolism. Thus, their universal presence and their role as critical mediators of normal and pathophysiology have resulted in considerable development of techniques that can detect these radicals (Rao et al. 2010). Some of the free radicals and other important oxidants found in living organisms are shown in Table 2.1.

Table 2.1 Some important reactive oxygen species in living organisms

<table>
<thead>
<tr>
<th>Free radicals</th>
<th>Non radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl radical</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>Superoxide radical</td>
<td>Singlet oxygen</td>
</tr>
<tr>
<td>Nitric oxide radical</td>
<td>Hypochlorous acid</td>
</tr>
<tr>
<td>Lipid peroxyl radical</td>
<td>Ozone</td>
</tr>
<tr>
<td></td>
<td>H₂O₂</td>
</tr>
<tr>
<td></td>
<td>¹O₂</td>
</tr>
<tr>
<td></td>
<td>HOCl</td>
</tr>
<tr>
<td></td>
<td>O₃</td>
</tr>
</tbody>
</table>

Reactive oxygen species are produced continuously in the human body as a consequence of normal metabolic processes. Some reactions that lead to free radical formation are shown below (Fig 2.1). If free radicals are not
inactivated, their chemical reactivity can damage all types of cellular macromolecules, including proteins, carbohydrates, lipids, and nucleic acids. Figure 2.2 illustrates some of the types of damage that can result from the actions of free radicals. Several of these effects have been implicated in the causation of degenerative diseases. For example, destructive effects on proteins may play a role in the causation of cataracts, effects on DNA are involved in cancer causation, and effects on lipids apparently contribute to the causation of atherosclerosis (Lillian Langseth, 1995).

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. The major characteristic of an antioxidant is its ability to trap free radicals viz. phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in grains, oil seeds, fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported by Miller and Rigelhof et al. (2000a).
Methods

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods require special equipment and technical skills for the analysis. The different types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR) and chemiluminescence methods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the superoxide anion radical (O₂⁻), the hydroxyl radical (OH), or the peroxyl radical (ROO).
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**Target Proteins**

**Consequences**

- Increased turnover
- Decreased enzyme activity
- Membrane damage
- LDL damage
- Cell injury

**Secondary products** (aldehyde)

**DNA** → Mutation

**Carbohydrate** → Receptor alterations

**Atherosclerosis**

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**Fig. 2.2 Types of damages due to the action of free radicals (Lillian Langseth, 1995)**

The various methods used to measure antioxidant activity of plant extracts can give varying results depending on the specific free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested. The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) (Miller and Rigelhof *et al.* 2000b) assays have been used extensively since the 1950’s to estimate the peroxidation of lipids in membrane and biological systems. These methods can be time consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix *etc.* and may not be practical when large numbers of samples are involved. Antioxidant activity methods using free radical traps are relatively straightforward to perform. The ABTS [2, 2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (Miller and Rigelhof *et al.* 2000a &b) has been used to screen the relative radical-
scavenging abilities of flavonoids and phenolics. Prior et al. (1998) has used the Oxygen Radical Absorbance Capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxyl radical generator, 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and inhibition of the free radical action is measured using the fluorescent compound, B-phycoerythrin or R-phycoerythrin (Cao et al. 1995). Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages and are usually quantified employing Folin’s reagent.

Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. An easier way to present antioxidant activity of natural products would be to reference to a common standard Trolox, Vitamin C etc. serves this purpose.

In enzymatic models the study is done on male or female rats/mice, by damaging the liver with hepatotoxic substances like CCl₄, Paracetamol (higher dose) etc. The activity of various enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutathione (GSH) superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) activity are estimated in liver. While in non enzymatic models based on chemical reactions of the constituents with standard reagents and plant extracts the activities are determined like determination of total phenolic content, Free radical scavenging assays-DPPH assay, Super oxide anion (O₂⁻) radical scavenging, Peroxide (H₂O₂) radical scavenging, Nitric oxide (NO) radical scavenging, Hydroxyl radical (OH⁻) scavenging, Protein oxidation and total flavonoid concentration Lipid peroxidation (LPO) MDA assays (Ajay et al. 2007).

There are several examples of isolation and extraction of antioxidants from plant materials viz. Herbal Cigarettes, tea, and capsules, (Siegel, 1976);
Paetta indica and Osbeckia octandra (Thabrew et al. 1987); Chrysanthemum morifolium (Duh, 1999); Mutisia friesiana (Asteraceae); and Sanicula graveolens, (Viturro et al. 1999); Salvia reflexa (Malencic et al. 2000); Rubus idaeus, Rubus occidentalis, and Fragaria ananassa (Shiow and Hsin-Shan, 2000); Cordyceps sinensis (Li et al. 2001); Olive extracts (McDonald et al. 2001); Cetraria islandica, (Gulcin et al. 2002); Pluchea indica, (Sen et al. 2002); Allium cepa, Illicium religiosum, Fagopyrum esculentum, Origanum officinalis, Rosmarinus officinalis, Pyrus pyrifolia, Acanthopanax senticosus, Eugenia caryophyllata and Erigeron annuus (Young and Kyong, 2003); Ardisia compressa (Sonia and de Mejia, 2004); Aframomum danielli, Allium cepa, Allium sativa, Capsicum frutescens, Citrus sinensis, Curcuma longa, Justicia flava, Ocimum gratissimum, Piper guineense (Odukoya et al. 2005); Fagopyrum esculentum (Ting and Chi-Tang, 2005); Cytisus scoparius (Raja Sundararajan et al. 2006); Rhodiola sacra, Polygonum multiflorum and P. multiflorum (Chi-Chun et al. 2006); Lycium barbarum (Li et al., 2007); Zanthoxylum piperitum (Yamazaki et al. 2007), Coleus Blumei, Orthosiphon Stamineus, Ocimum basilicum and Mentha arvensis (Zuraini Zakaria et al. 2008) and Carya cathayensis (Chenggang Zhu et al. 2008), Boerhaavia diffusa (Rachh et al. 2009), Thymus vulgaris and Lavendula multifida (Ramchoun, 2009).

**Antipyretic activity**

Fever is a medical symptom characterized by an increase in internal body temperature to levels that are above normal (the common oral measurement of normal human body temperature is 36.8 ± 0.7°C or 98.2 ± 1.3°F). Fever is most accurately characterized as a temporary elevation in the thermoregulatory set-point, causing typical body temperature to rise, and effector mechanisms are enacted as a result. A feverish individual has a general feeling of cold despite an increased body temperature, and increases in heart rate, muscle tone and shivering, all of which are caused by the body's attempts to counteract the newly perceived hypothermia and reach the new
thermoregulatory set-point. A fever is considered one of the body's immune mechanisms to attempt a neutralization of a perceived threat inside the body, be it bacterial or viral.

Fever is a common symptom of many medical conditions:

- Infectious disease, e.g. influenza, HIV, malaria, infectious mononucleosis, or gastroenteritis
- Various skin inflammations, e.g. boils, pimples, acne, or abscess
- Immunological diseases, e.g. lupus erythematosus, sarcoidosis, inflammatory bowel diseases
- Tissue destruction, which can occur in hemolysis, surgery, infarction, crush syndrome, rhabdomyolysis, cerebral hemorrhage, etc.
- Reaction to incompatible blood products
- Cancers, most commonly kidney cancer and leukemia and lymphomas
- Metabolic disorders, e.g. gout or porphyria

Fever differs from hyperthermia, which is an increase in body temperature over the body's thermoregulatory set point (due to excessive heat production or insufficient thermoregulation, or both). The agents causing such upward resetting of hypothalamic thermostat seem to be high molecular weight polysaccharides called pyrogens, which have been isolated from bacteria and leucocytes. It is now generally accepted that pyrogens are the circulating mediators of fever and they induce changes in the CNS presumably in the region of the anterior hypothalamus.

Antipyretics (literally "against the fire") are drugs that reduce body temperature in situations such as fever. However, they will not affect the normal body temperature if one does not have a fever. Antipyretics cause the hypothalamus to override an interleukin-induced increase in temperature. The body will then work to lower the temperature and the result is a reduction in fever. The most common antipyretics are aspirin and acetaminophen
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(paracetamol), which are used primarily as pain relievers. NSAIDs are antipyretic, anti-inflammatory, and pain relievers (Satoskar, 1986b).

The screening of natural products has led to the discovery of so many potent antipyretic drugs. They can be screened by following models.

i. Yeast induced pyrexia method in rats (Smith and Hambourger, 1935)

ii. Antipyretic activity in Rabbit (Deloprate, 1993)


**Antiinflammatory activity**

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbial agents and is the body's effort to inactivate or destroy invading organisms, remove irritants, and set the stage for tissue repair (Mary, 1997). Upon interaction of foreign pathogens with innate immune cells like macrophage or monocytes, inflammatory immune response is trigger off. Inflammatory mediators elicit a complex series of cellular events upon interaction with invading
microorganisms, including increased permeability of vessels, exudation of fluids and migration of leukocytes into the inflammatory focus, resulting in phagocytosis and killing of the microorganisms (Heumann and Roger, 2002). The inflammatory responses are vigorous reactions that results in some collateral damage to the surrounding tissues but such effect is normally local and transient (Bosca et al. 2005).

Essentially there are two types of inflammation: acute and chronic. The classical signs of acute inflammation are warmth, redness, pain, swelling and loss of function. Chronic inflammation is also characterized by long lasting pain, redness and swelling and is caused by the persistence of an irritant, which may be biological, physical or chemical in nature.

Inflammation research involves a number of experimental models to study the anti-inflammatory activity. According to Lewis, (1989) there are two models viz. Acute and Chronic anti-inflammatory models. Acute models are designed to test drugs that modulate erythema, changes in vascular permeability, leukocyte migration and chemotaxis, phagocytosis-polymorphonuclear leucocytes and other phagocytic cells, measurement of local pain, antipyretic activity and local analgesic action and rat paw edema (Barbosa-Filho et al. 2006) while, Chronic models are designed to find drugs that may modulate the disease process and these include sponge and pellet implants and granulama pouches which deposit granulation tissue, adjuvant induced arthritis and rabbit monoarticular arthritis which have an immune etiology (Lewis, 1989).

Natural products have long been recognized as an important source of therapeutically effective medicines for antiinflammation (Cragg et al. 2003). Different approaches used to analyze the anti-inflammatory potential of plant and plant derived compounds have been developed in the past years. Further, traditional herbal medicines like Commiphora mukul, Boswellia serrata,
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Harpagophytum procumbens, and Pluchea indica have been used for antiinflammatory effect with success (Vohara and Dandiya, 1992).

Plants as anti-inflammatory agents

Practitioners of traditional Indian medicine, use formulation for anti-inflammatory action with considerable success. Dashmoola (combination of roots of ten plants) is standard Ayurvedic remedy for anti-inflammatory diseases (Sharma et al. 1973). The anti-inflammatory activity of many medicinal plants have been scientifically evaluated viz. Curcuma amada (Mujumdar et al. 2000) Gochnatia polymorpha (Moreira, 2000); Goniothalamus andersonii (Shigeo et al. 2001); Cassia angustifolia, Rheum palmatum, Coptis chinensis, Phellodendron amurense and Scutellaria baicalensis (Cuellar et al. 2001); Leucas aspera (Goudgaon et al. 2003); Clitoria fairchildiana (Pereira da Silva and Paz Parente, 2002); Calendula officinalis, Hypericum perforatum, Plantago lanceolata and Glycyrrhiza glabra (Herold et al. 2003); Alchornea cordifolia (Mavar et al. 2004); Erigeron floribundus (Asongalem, 2004); Synurus deltoids (Park et al. 2004b); Securidaca longipedunculata (Okoli, 2005); Vitex negundo (Rasadah et al. 2005) Bacopa monniera (Shabana, 2006); Andrographis Paniculata (Sheeja et al. 2006); Ruta graveolens (Ratheesh and Helen, 2007); Aloe buettneri (Metowogo, 2008), Putranjiva roxburghii (Wantana, 2009) and Magnolia ovate (Candida, 2009).

Analgesic activity

Pain is an unpleasant sensation which informs structural and functional changes in body and acts as a warning signal against disturbaces in the body. Even though pain is an unpleasant sensation, is mainly a protective mechanism for the body (Kanodia, 2008). It is a consequence of complex neurochemical processes in the central and peripheral nervous systems (Mary, 1997). Typically, it is a direct response to an event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise
independently of any obvious predisposing cause or it can also occur as a consequence of brain or nerve injury. The pain mechanism and pathways is illustrated in the Figure-2.3 (Heinz, 2005).

Fig. 2.3 Mechanism and pathways of pain

An analgesic (also known as a painkiller) is any member of the diverse group of drugs used to relieve pain (achieve analgesia). The word analgesic derives from Greek an- ("without") and algos ("pain"). Analgesic drugs act in various ways on the peripheral and central nervous systems; they include paracetamol (para-acetylaminoephophenol), the non-steroidal anti-inflammatory
drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others. In choosing analgesics, the severity and response to other medication determines the choice of agent; the WHO pain ladder, originally developed in cancer-related pain, is widely applied to find suitable drugs in a stepwise manner Anonymous (1990). The analgesic choice is also determined by the type of pain: for neuropathic pain, traditional analgesics are less effective, and there is often benefit from classes of drugs that are not normally considered analgesics, such as tricyclic antidepressants and anticonvulsants. (Dworkin, 2003). Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively. These drugs have serious limitations due to their side effects (Mary, 1997 and Sathoskar, 1986). A natural agent with reduced or no toxicity is therefore, essential.

Evaluation of analgesic agents is done by several methods, some prominent ones are - Physical stimulus (Tail-Clip method), Thermal stimulus, Hot plate method, Tail flick method, Tail immersion method, Chemical Stimulus, Writhing test, Writhing induced by 4% NaCl Solution and Writhing induced by aconitine etc.

In view of the side effects of the synthetic analgesic drugs, investigators on the lookout for the safer ways in plants have been well documented viz. Caesalpinia ferrea (Carvalho et al. 1996); Psidium guajava (Kulkarni et al. 1999); Tragia involucrata (Dhara et al. 2000); Parkia biglobosa (Kouadio et al. 2000); Piperomia pellucida (Peter et al. 2001); Enhydra fluctuans (Rahaman, et al. 2002); Polygonum hydropiper (Rahaman et al. 2002); Cleome viscosa (Parimaladevi et al. 2003a); Datura fastuosa (Abena et al. 2003); Carthamus lanatus (Bocheva, et al. 2003); Clitoria ternatea (Parimaladevi et al. 2003b); Spilanthes acmella (Chakraborty et al. 2004); Capparis zeylanica (Chaudhary et al. 2004); Zataria multiflora
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(Jaffary et al. 2004); Neem (Patel et al. 2005); Euphorbia decipiens (Ahmad, et al. 2005); Sida acuta; Stylosanthes fruticosa, Toona ciliata, Bougainvilla spectabilis, Ficus glomerata and Polyalthia longifolia (Malairajan, 2006); Teucrium stocksianum (Radhakrishnan, 2001); Mahonia oiwakensis, (Jung Chao, 2009); Argyreia speciosa, (Bachhav, 2009); Citrus decumana (Shailja, 2009) and Trapa natans (Anuj et al. 2010).

Cardiovascular activity

The heart is a muscular organ found in all vertebrates that is responsible for pumping blood throughout the blood vessels by repeated, rhythmic contractions. The term cardiac (as in cardiology) means "related to the heart" and comes from the Greek kardia, for "heart."

Ever since William Harvey defined an animal's heart as "the supreme ruler of everything within them, the sum of their microcosm," in De Motu Cordis in 1628, the heart has been one of the most widely studied organs of the body and fortunately so, given that heart disease is the world's leading cause of death (Birmingham, 2002). Despite of incredible advances in the diagnosis and treatment of cardiovascular diseases, the incidence, prevalence, morbidity and mortality resulting from these diseases continue to escalate Cardiovascular diseases (CVD). Increased mechanisation, Westernisation of lifestyle and genetic factors, coupled with an increase in life expectancy owing to control of infectious diseases, have contributed to its rise in the developing world as well. The prevalence of heart failure nearly doubles with each decade of life after the age of 50. Heart failure represents a significant cause of mortality in the senior population. Consequently, heart failure has become one of the most severe health problems around the world (WHO, 2000).

Although scientific study of the heart began four centuries ago, the past few decades have seen a paradigm shift in research. The fruitful combination of herbal drugs with state-of-the art has advanced the treatment and
understanding of many cardiovascular disorders. Plant research is at the forefront of using these new approaches to find an effective and, perhaps even more important, safe pharmacological treatment for cardiovascular disorders. The impressive advances in natural drug discovery and the basic science of cardiac disease will most likely over the next decade translate into significant impact on the clinical therapeutic opportunities available for treatment of cardiovascular diseases.

**Screening techniques**

The animal or organ-based assays, *e.g.* the Langendorff isolated heart, have long been used and still retain significance as secondary assays for the determination of the cardiotonic effect of a compound. However, for comparison of the relative effects of different compounds, the requirement is that these be tested under identical conditions (Repke *et al.* 1995). Other techniques, right atrial preparations, aortic strip superfusion method, measurement of diuretic action and cultured myocardial cells have also been used to test for cardioactivity.

**Ischaemic heart disease**

Ischaemic heart disease (IHD) develops largely because of the reduction in blood supply to the heart muscles, mostly caused by narrowing of the coronary arteries by atherosclerosis. Atherosclerosis is a chronic condition associated with accumulation of lipids in blood vessels, leading to the occlusion of blood flow and much focus has been on the role of low-density lipoprotein (LDL), and of oxidatively modified LDL, in the initiation and progression of this disease. LDL is in fact a metabolic end-product of the triglyceride-rich lipoproteins (*i.e.* very-low-density lipoproteins) but triglycerides are also implicated as contributors to atherosclerosis (Le and Walter, 2007). Atherosclerosis starts very early in life, but the rapidity with which the disease advances may vary from individual to individual, depending on their genetic make up and other modifiable risk factors, such as
diet, physical activity and dyslipidaemia. Therefore, lifestyle modifications, alone or in combination with drugs, are crucial in positively modulating this crucial factor of IHD (Genest, 2000).

Plant products having coronary vasodilator activity can be useful as ‘add-on’ therapy in established cases of IHD. The Langendorff in vitro method is widely used for this purpose and is probably the oldest method for screening usefulness of a drug candidate in IHD.

Langendorff (1895) set up the first isolated mammalian heart and the goal of experiments using it is to provide the isolated heart with oxygen and metabolites via a single cannula inserted into the ascending aorta. Blood or an oxygenated perfusate is flushed down the aorta towards the heart using an external pump. As a consequence of the retrograde perfusion of the aorta, the aortic valve closes, forcing the fluid into the coronary arteries during the diastolic period as it does in the normal cardiac cycle. The perfusate flows through the coronary system finally exiting via the coronary sinus in the right atrium.

Natural products as cardiovascular agents

Natural products have featured prominently in the clinical treatment of cardiovascular diseases and in providing lead compounds for the development of more efficient drugs. Digoxin, a steroid glycoside from Digitalis lanata, is the oldest of the commonly used compounds in the treatment of heart disease; its clinical history dates back to 1785 (Repke et al. 1995). Apart from its use in congestive heart failure, it also finds use in the treatment of arrhythmia. Quinidine, a stereoisomer of the antimalarial quinine, found in cinchona bark, is an adequate antiarrhythmic agent, but has recently been replaced by pacemakers and newer drugs. Slow heart beat (bradycardia) is treated with atropine, a metabolite of the solanaceous plant Atropa belladonna, or isoproterenol.
Cardiovascular drugs also include e.g. digoxin (Digitalis purpurea), reserpine (Rauwolfia serpentina), aspirin (bark of willow Salix spp.) and taxol (Taxus brevifolia), the last one being used in drug-eluting stents to prevent restenosis following coronary angioplasty. All these examples demonstrate the vast potential of herbal products, their extracts and active principles in the management of CVD (Mukherjee, 2009).

Many new drugs have been developed over the last three decades in attempts to improve on those in clinical use. However none of the digitalis, diuretics and ACE drugs, when used alone, satisfy all the criteria for optimally managing heart failure. Moreover, digitalis intoxication can be a problem due to the narrow therapeutic ranges of its compounds and efforts to improve on this are still a matter of concern.

Many plants, such as Eremophila alternifolia Marcella et al. 1995, Allium sativum (Banerjee et al. 2002 and 2003; Saravanan and Prakash, 2004) Emblica officinalis (Bhattacharya et al. 2002; Rajak et al. 2004) and Terminala arjuna (Karthikeyan et al. 2003; Gauthaman et al. 2005) have been shown to have significant antioxidant properties and Chronic administration of these agents in animals (rats) offers protection against subsequent in vitro and in vivo ischaemic injury and ischaemic-reperfusion injury. These agents augment the level of endogenous antioxidants of the heart on chronic administration, which subsequently prevented oxidative stress induced by ischaemic-reperfusion injury (Banerjee et al. 2003a; Narang et al. 2005 and Sood et al. 2005). Other plant extracts and their isolated compounds used for their cardiovascular activity are Nerium oleander (Adome et al. 2003), Ocimum basilicum Linn. (Muralidharan and Dhananjayan 2004), Mangiferin from Mangifera indica (Prabhu et al. 2006), Emblica ribes (Uma et al 2008), Guazuma ulmifolia (Gil et al. 2008), Rhodiola sacra (Cheng et al. 2008) and Piperine from Piper nigrum L. (Syed et al 2008) Cordia rothii (Malathi and
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Sulakshna, 2009) *Desmodium gangeticum* (Gino and Jose, 2009), and *Vitex nigondo* Linn (Pai et al. 2010).

**Anthelmintic Activity**

Helminthiasis is prevalent globally, but is more common in developing countries with poorer personal and environmental hygiene. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm multiple infestations in the same individual are not infrequent. In the human body, gastro intestinal tract is the abode of many helminthes, but some also live in tissues. They harm the host by depriving food, causing blood loss, injury to organs intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of ill health (Thripathi, 2001). The main reasons responsible for the widespread nature of this disease in the developing countries are the lack of adequate sanitary facilities and supply of water, coupled with poverty and illiteracy. The helminth infection can be acquired by contact with infected animal, ingestion of infected meat, animal or human excreta via ground water, by means of certain mosquitos.

Diseases caused by helminth parasites in livestock continue to be a major problem, especially in small ruminants in the tropics and subtropics (Perry et al. 2002). Infections by gastrointestinal helminth parasites of livestock are among the most common and economically important diseases of grazing livestock (Monteiro, 1998). Adulteration of anthelmintics has been found to be a common practice (Dano and Bogh, 1999). Illiteracy and unfamiliarity with synthetic anthelmintics, resulting in incorrect usage, are also a problem leading to the same consequences. Moreover, these drugs are relatively expensive. As a consequence of these problems and difficulties, pastoralists and small holder farmer have continued to use indigenous plants as livestock dewormers (Waghorn and McNabb, 2003). Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects (De Bairacli and Levy, 1991). For example, plants
that contain condensed tannins, have these effects. Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e. nematodes. They are of huge importance for human tropical medicine and for veterinary medicine.

Many plants have been used from ancient times to cure parasitic diseases of man and animals. For example *Caesalpinia crista* (Leguminosae; karanjwa), *Melia azedarach* (Meliaceae; bakain), *Saussurea lappa* (Compositae; qust-eshireen), *Moringa oleifera* (Moringaceae; sohanjna), *Trachelospermum jasminoides, Butea frondosa* (Leguminosae; Dhak) etc. have been quite commonly used (Nadkarni, 1954). The fruit of *Mallotus phillippinensis* (Euphorbiaceae) has been used as an anthelmintic; it has also been used in external applications for the control of parasitic infections of the skin, as an antiseptic for ears and systemically for urinary disorders (Chopra et al. 1956; Ikram & Hussain, 1978; Satyavati et al. 1987). Seeds of *Butea superba* are extensively used as sedative and anthelmintic in the indigenous system of medicine (Chopra, et al. 1958). The powdered seeds and various extracts of plant *Peganum harmala* have been used as narcotic, analgesic, and antispasmodic in colic and as a remedy against tapeworm infection in man and animals (Chopra, 1956 and Said, 1969). *Vernonia anthelmintica, Embellia ribes, Psoralea corylifolia* and *Punica granatum* have been reported to possess anthelmintic, laxative, expectorant, diuretic and tonic properties (Nadkarni, 1954; Chopra et al. 1956; Said, 1969; Ikram & Hussain, 1978; Awan, 1981, Akhtar & Riffat, 1985). Various parts of *Lagenaria siceraria* have been used to treat tapeworm infections in children (Awan, 1981). *Fumaria parviflora* is traditionally used as an antidiabetic, diaphoretic, diuretic, anthelmintic (Nadkarni, 1954 and Chopra et al. 1956). *Nigella sativa* is used as an anthelmintic, stimulant and diuretic (Nadkarni, 1954 and Said, 1969). The roots of *Morus* are considered as an anthelmintic and vermifuge,
whereas root bark and stem bark are reported to be vermifuge and purgative (Nadkarni, 1954).

Eventhough indigenous system of medicine reports a number of plants for their anthelmintic efficacy and their scientific evaluation as compared to commercial anthelmintics is limited. Alkaloid hydrochlorides extracted from seeds of *Butea frondosa* proved 100% lethal to earthworms within 24 h indicating their anthelmintic activity (Kalesaraj and Kurup, 1962). Garg and Atal, (1963) reported remarkable vermicidal activity of Calotropain (proteolytic enzyme isolated from the latex of *Calotropis procera*) and Bromelain (an enzyme obtained as a by-product from pineapple industry) against *Oesophagostomum columbianum* and *Bunostomum trigonocephalum* of sheep origin compared to phenothiazine.

*In vitro* anthelmintic activity is matter of several investigations in various plants viz. *Ananas sativus, Embellia ribes, Macuna prurita* and *Melia azedarach* has significant activity against *Taenia canina* and *Paramphistomum cervi*; *Macuna prurita* was especially quite active against trematodes (Neogi *et al.* 1964); Anacardic acid form *Semecarpus anacardium* and its sodium salt have been found to be potent anthelmintic agent (Chattopadhyaya and Khare, 1969); *Zingiber zerumbet, Allium sativum, Alpinia calcarata, Citrus acida, Citrus aromatic, Citrus medica, Cucuruma aromatic, and Punica granatum* (Kalesaraj, 1962); *Lantana camara var. aculeate*; (Avadhoot *et al.* 1980 and Girme *et al.* (2006); Eugenol from *Ocimum sanctum* (Asha *et al.* 2001); *Balanites roxburghii* (Basavaraj Padmashali *et al.* 2001); *Cordia dichotoma* (Kuppasta and Nayak, 2003); *Artemisia brevifolia* (Iqbal *et al.* 2004); *Bacopa monnieri,* (Ghosh *et al.* 2005), *Mentha piperita* (Girme *et al.* 2006); *Enhydra fluctuans, Ananas comosus,* *Azadirachta indica, Caesalpinia crista, Vernonia anthelmintica, Fumaria parviflora and Embelia ribes* (Hordegen *et al.* 2006), *Flemingia vestita* (Tandon and Das, 2007) and *Euphorbia tirucalli* (Asha *et al.* 2009);
Antimicrobial activity

Infectious diseases account for approximately one-half of all deaths in tropical countries. In industrialized nations, despite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns.

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al. 2003). Therefore, researchers are increasingly diverting their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Iwu et al. 1999).

Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (Tanaka et al. 2002). The plants possess innumerable number of secondary metabolites which are usually produced under stress conditions and often in response to infections. These secondary metabolites possess profound antimicrobial potency. Many workers have isolated different types of active constituents and studied for their antimicrobial potency. Alkaloids (Burdick, 1971); phenolic compounds
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(Mason and Wasserman, 1987); tannins (Scalbert, 1991); flavanones and flavonoids (Panilio et al. 1992); sesquiterpenes (Topcu et al. 1993); anthroquinone (Kazmi, 1994); flavonoid glycosides (Hasan and Ahmad, 1996); triterpene acid glycosides (Kirmizigul et al. 1996); monoterpenes (Meng et al. 2000); diterpenes (Ulubelen et al. 2000); triterpenes (Akbar and Malik, 2002). These active constituents isolated from medicinal plants showed significant antimicrobial effect. Hence these phytomedicines are effective in treating most of the infectious diseases caused by bacteria, fungi and virus (Cowan, 1999).

The efficacy of plant extracts against microorganisms is of considerable interest among various investigators. Many plant species has shown antimicrobial activities like Mitracarpus scaber (Ekpendu et al. 1994); Landolphia owrrience (Ebi and Ofoefule, 1997); Eupatorium perforiatum (Habtemariam and Maepherson, 2000); Enantia polycarpa (Ajali, 2000); Ricinus communis (Parameswari and Tulasi Latha, 2001); Bixa orellina (Castello et al. 2002); Melissa officinalis (Mimica-Dukie, 2004); Solanum stramoenifolium Jacq., S. seafortianum Andr. and S. violaceum Ortg (Manjunatha et al. 2004); Eupatorium glandulosum (Sasikumar et al. 2005); Quercus infectoria (Basri and Fan, 2005) Bacopa monnieri, (Ghosh et al. 2006), Althaea officinalis, Mentha longifolia, Melissa officinalis and Rosa damascene (Bassam Abu-Shanab et al. 2006); weeds of Euphorbia family Euphorbia tirucalli (Asha et al. 2009) and Carthamus tinctorious (Paramesha et al. 2009).