CHAPTER II

Review of Literature
Medicinal plants are known to be an initial line of defense against various diseases (Deepa et al., 2014). They have been found to possess significant medicinal properties that regulate various vital cell signaling pathways. A huge number of compounds, responsible for therapeutic properties of the plant have been isolated, processed and developed as efficient pharmacological products with potent anticarcinogenic, antioxidative, anti-infective, antiangiogenic, anti-inflammatory properties etc. Few of such plant products have also found their application as lead molecules to obtain highly biologically similar semisynthetic pharmacological derivatives (Ramana et al., 2014).

2.1 Usage of medicinal plants in treating different diseases in India

Several studies have been carried out by different researchers to understand the use of traditional wisdom and skills followed by people for treating various diseases (Gupta et al., 2014). As such the ethno-botanical and phytoconstituent evaluation of 70 plants of Ladakh, North India, was carried out by Gupta et al., 1981. Srivastava & Kapahi, (1991) explored Sikkim Himalaya and reported nearly 400 plants of aromatic and medicinal values. Certain plant species of Kashmir Himalaya belonging to different families was reported for their ethnomedicinal value by Khan et al., 2004. Medicinal plants used in Arunachal Pradesh to treat various diseases like malaria, cancer, jaundice, tuberculosis etc., have been studied and have been documented by Das & Tag, 2006. Gautam et al., (2011) have explored the traditional, medicinal and edible uses of medicinal plants of Himachal Pradesh. Numerous reports have been documented by various researchers regarding the ethnobotanical knowledge of Western Ghats (Upadhya et al., 2012). Upadhya et al., (2012) has analyzed and documented the traditional knowledge on use of plants involved in the treatment of bone fracture in north-central Western Ghats of India. Bagul, (2013) has reported the medicinal plants used in Maharashtra against various diseases. The study by Sivasankari et al., (2013) revealed some of the important medicinal plants, their significance and utilization in Tamilnadu, South India. Santhosh et al., (2014) has documented medicinal plants incorporated by local herbal practitioners of Chikmagalure district of Karnataka, South India. Policepatel & Manikrao, (2013) has documented the medicinal plants and their mode of use in treating skin diseases in Hyderabad Karnataka region. Similarly a large number of research literatures are
available emphasizing the medicinal properties on natural botanicals studied throughout India.

Samy et al., (2008) has reviewed some of the indigenous medicinal plants used in India to treat different diseases. Some among them are *Acorus calamus* (Baje) was used to treat memory loss, mental fatigue, anxiety and bronchitis; *Eclipta alba* (Bhringaraj) used against memory disorder, liver disorder, viral hepatitis, hair and skin care and to strengthen spleen; *Mucuna pruriens* (Kapikachchha) for treating parkinson’s disease, nervous disorder and general weakness; *Ocimum sanctum* (Tulasi) was used against ringworm, common cold, bronchitis, nausea and tuberculosis, and *Phyllanthus amarus* (Bhumi amalaki) used against chronic liver disorders, chronic colitis, irritable bowel syndrome, jaundice, viral hepatitis etc. *Oroxylum indicum* is known to be widely used by the Indians in treating cancer (oral cancer) etc., (Kainsa et al., 2012).

India along with its rich natural wealth also consists of enormous number of tribal groups and they have been known to incorporate a huge number of medicinal plants to treat various ailments like jaundice, skin diseases, rheumatism, wounds, snakebite, ulcers, tumour, dysentery, fever, cough, indigestion etc., (Harsha et al., 2002; Sharma & Kumar, 2012). Certainly these people have proper knowledge on the healing properties of particular plants (Sharma and Kumar, 2012) and their usage continues from long time till to date (Harsha et al., 2002). Iyer, (1992) has reported some of the plants commonly used against skin infection by different tribal communities in India and few plants among these have been proven for their antiallergic and antimicrobial properties. Sharma & Kumar, (2012) has reported the uses of Bauhinia Species, an important medicinal plant used by tribes of Rajasthan. This species of plants have also found their place in the traditional system of medicine. Choudhary et al., (2011) has studied and documented the traditional herbal remedies incorporated by tribals like Bhil, Bhilala, Gond and Korku, inhabiting central Narmada valley of Madhya Pradesh, to cure skin diseases.

Karnataka also consists of enormous number of tribal communities. The tribals like Siddis, Gowlis, Halakkis and Kunabis inhabit the semi-evergreen forest of Uttar Kannada district, which is one among the rich biodiversity center of Western Ghats (Harsha et al., 2002). Harsha et al., (2002) has surveyed and documented the
medicinal plants used to treat various human diseases by Kunabi tribe of Uttar Kannada district of Karnataka. Guruprasad et al., (2013) has reported the medicinal plants utilized by Iruliga tribe of Western Ghats against various diseases. Medicinal plants used in the treatment of various ailments by local herbal healers and tribes of Biligiri Rangana hills of Chamarajanagar district of Karnataka, have been documented by Gireesha & Raju, 2013. The Soliga tribe harbour the hill and are known to use more than 300 herbs for their therapeutic purpose (Gireesha & Raju, 2013). Some among them are Ruta chalepensis, Solanum indicum, Vitex negundo etc., (Gireesha & Raju, 2013). As such, many works are reported by several researchers on ethnomedicinal/traditional uses of natural botanicals of various geographical areas. These data highlights the enormous exploitation of medicinal plants traditionally for treating various diseases.

2.2 Plants and their traditional usage all over the world

The medicinal value of the roots of Rauwolfia serpentine, was known by ancient people and had been administered it in curing mental disorder for at least 2500 years, before its recognition by western countries for treating mental problems. Vinca rosea, also called ever bloom possessing pink or white flowers had been used in treating leukemia. It had also found its use in treating diabetes mellitus traditionally (Gupta et al., 2014). Melia azadirachta is known for its potent health alleviating property and also act as astringent. Its detoxifying ability has resulted in its usage from thousands of years. The organ systems like digestive system, circulatory system and respiratory system have been greatly benefited with the usage of this plant (Pandey et al., 2013). As such, numerous medicinal plants have found their application in indigenous system of medicine all over the world and some are presented in table 2.1.

2.3 Secondary metabolites of Plants

The therapeutic potential of natural botanicals lies in the presence of various phytochemicals called plant secondary metabolites (bioactive compounds). These metabolites have found to possess their absence in the fundamental role in maintaining the life process of plant, but are very much essential for the plants to interact with their environment (Ramakrishna & Ravishankar, 2011).
Table 2.1 Medicinal plants and their therapeutic properties

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Medicinal Plants</th>
<th>Therapeutic properties</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Azadirachta indica</em></td>
<td>used to treat diabetes, wounds, leprosy, tumor and skin diseases</td>
<td>Biswas et al., 2002</td>
</tr>
<tr>
<td>2.</td>
<td><em>Salvia miltiorrhiza</em></td>
<td>used for the treatment of angina pectoris, hyperlipidemia and acute ischemic stroke</td>
<td>Walden &amp; Tomlinson, 2011</td>
</tr>
<tr>
<td>3.</td>
<td><em>Nardostachys jatamansi</em></td>
<td>used for its effectiveness in mental health</td>
<td>Pandey et al., 2013</td>
</tr>
<tr>
<td>4.</td>
<td><em>Sida cordifolia</em></td>
<td>used to treat dysentery, leucorrhoea and wounds</td>
<td>Jain, 1991</td>
</tr>
<tr>
<td>5.</td>
<td><em>Gmelina arborea</em></td>
<td>used as antidote, blood purifier and to treat bronchitis, cholera and malaria</td>
<td>Jain, 1991</td>
</tr>
<tr>
<td>6.</td>
<td><em>Withania somnifera</em></td>
<td>used to treat asthma, skin disorders, epilepsy and chest complaints</td>
<td>Jain, 1991</td>
</tr>
<tr>
<td>7.</td>
<td><em>Piper longum</em></td>
<td>used against spleen complaints and paralysis</td>
<td>Jain, 1991</td>
</tr>
<tr>
<td>8.</td>
<td><em>Terminalia chebula</em></td>
<td>used to treat measles, diabetes, stomach complaints, menstrual complaints and constipation</td>
<td>Jain, 1991</td>
</tr>
</tbody>
</table>

Various environmental factors like humidity, temperature, light intensity, water supply, mineral supply etc., have been reported to influence the growth of a plant and its secondary metabolite production (Ramakrishna & Ravishankar, 2011). Specific pathways are followed by these metabolites for their production and their site of synthesis varies between plant species and type of compounds (Yazdani et al., 2011). These include the following classes of compounds.

**Phenolic compounds** are secondary metabolites widely produced by plants and are generally called as phenols or phenolics or polyphenols. The defense against
ultraviolet radiation and aggression by pathogens in plants are encountered by these compounds. These are grouped into different classes based on the number of phenol rings present and on the basis of structural elements that bind these rings to one another. Phenolic acids, flavonoids, stilbenes and lignans are the main classes of polyphenols. All these classes of polyphenols arise from a common intermediate phenylalanine or a close precursor shikimic acid (Pandey & Rizvi, 2009). This class of plant compounds are known to possess antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiinflammatory properties etc., (Sermakkani & Thangapandian, 2010).

**Flavonoids** are the group of plant polyphenols and the basic structure consists of two benzene rings (A and B) linked through a heterocyclic pyran ring (C) (three carbon atoms) (Kumar et al., 2013). These are classified into six subclasses, based upon their variation in heterocycle ring and include flavonols, flavanones, flavanols, flavones, anthocyanins and isoflavones. The variation in the hydroxyl groups arrangement, number and the extent of alkylation and/or glycosylation has resulted in the further difference in each class of flavonoids (Pandey & Rizvi, 2009).

Their essential role in plant-bacterial and plant-insect interactions have made them an attractive class of phytoconstituents (Tapas et al., 2008). They possess several properties which improve the human health and help to reduce the risk of diseases (Kumar et al., 2013). They are potent antioxidants with stronger metal chelating ability (Tapas et al., 2008). They also act as a good anti-inflammatory, anticarcinogenic, antiallergic, antithrombotic, hepatoprotective and antiviral agents (Tapas et al., 2008). Due to their antioxidant property, flavonoids act as excellent source for food preservatives, with antiviral and antibacterial ability they acts as a source for antibiotics and with allelopathic property may found to occupy the place of chemical pesticides and insecticides (Materska, 2008). These are also considered as important nutraceutical ingredients of plants which is a toxic less supplement of food extract, with scientifically proven health benefits for both the treatment and prevention of diseases (Tapas et al., 2008). Ubiquitous presence, natural origin and huge medicinal properties of flavonoids have made them to be an excellent compound for therapeutic purpose. Quercetin, kaempferol, myricetin, rutin etc., are some of the well known flavonoids of plant kingdom and exert a wide array of therapeutic properties (Tapas et al., 2008).
**Alkaloids** are the secondary metabolites of plants with lower molecular weight, containing nitrogen-atom and are derived from amino acids. They are known to protect the plant against herbivores and pathogens attack. They have been found to exert therapeutic properties in humans and have found their application as an antiamoebic e.g., emetine, as gout suppressant e.g., colchicines, as antimicrobial e.g., sanguinarine, as narcotic analgesic e.g., morphine, as narcotic analgesic and antitussive e.g., codeine and as anticancer e.g., vinblastin. They are also used as muscle relaxant, as antineoplastic, antinociceptive and anticholinergic agents (Ramawat et al., 2009; Seifu et al., 2012).

**Steroids** also called as plant sterols are plant secondary metabolite containing C28 and C29 carbon steroid alcohols and are the integral components of cell membrane of the plant (Bruce & Grattan, 2013). They usually occur in many forms and steroidal glycosides also referred to as ‘cardiac glycosides’ are one of the most naturally occurring plant steroid. Steroids are known to exhibit cardiotonic, insecticidal and antimicrobial properties. They have found their application in the field of herbal medicine, nutrition and cosmetics (Sermakkani & Thangapandian, 2010). Some of the examples of plant steroids include cevadine, diosgenin etc., (Doughari, 2012). Steroids like campesterol, β-sitosterol and stigmasterol are found in the diet obtained from plants (Bruce & Grattan, 2013).

**Terpenoid** also called as terpenes or isoprenoids are one of the secondary metabolites of plant and are derived from five-carbon isoprene units assembled and modified in thousands of ways. They are responsible for numerous vital function of plants and as such act as a electron carriers (plastoquinone, ubiquinone), as hormones (abscisic acid, gibberellins), as pigments of photosynthesis (carotenoids) and as membranes of structural components (phytosterols). They have been reported for their biological properties like anticancerous, antioxidant, antimicrobial, antiinflammatory, cholesterol synthesis inhibition, antihyperglycemic and immunomodulatory activities (Seifu et al., 2012). Examples of terpenoids includes menthol, thujone, eugenol, taxol etc., (Doughari, 2012).

**Glycosides** are water soluble constituent of plants containing colourless, crystalline carbon, hydrogen and oxygen atoms. These include such compounds which act on heart called cardiac glycosides/steroidal glycoside, for treatment of skin diseases.
called anthracene glycosides, having anticancer property called chalcone glycoside etc. Some of the examples are strophanthidin isolated from *Strophanthus*, digitoxin from *Digitalis*, barbaloin from *Aloes* etc. Glycosides have also found their application to promote appetite and to help digestion. These are also used as astringents and as antiprotozoans (Doughari, 2012).

**Tannins** are plant secondary metabolite and possess the ability of inhibiting insect growth in plants (Cowan, 1999). They are well known to exhibit antimicrobial and antioxidant properties (Peteros & Uy, 2010). They are used in the treatment of inflammation, leucorrhoea, gonorrhea, burn, piles, diarrhea and are also used as antidote (Hussain et al., 2011). Tannins have been recently reported to posses cytotoxic and antineoplastic properties (Sermakkani & Thangapandian, 2010). Daidezein, glycitein and genistein are some of the well known examples of tannins (Doughari, 2012)

**Saponins** are secondary metabolites found in plants and are of high molecular weight compounds in which, sugar molecule is combined with triterpene or steroidal aglycone. Saponins have found to be therapeutically important due to their hypolipidemic and anticancerous properties. They are known for their antimicrobial, hepatoprotective, antiinflammatory and antiulcer activities (Hussain et al., 2011; Sermakkani & Thangapandian, 2010). They have also found their application against hypercholestrolaemia, hyperglycemia, weight loss etc., (Sermakkani & Thangapandian, 2010). Diosgenin and hecogenin are the examples of plant saponins (Doughari, 2012).

**2.4 Bioactive compounds isolated from medicinal plants**

The traditional use of medicinal plants formed the basis for the isolation of most of the pure plant compounds. An alkaloid, morphine isolated in the pure form from the plant *Papaver somniferum* in 1805, became the first pharmacologically active molecule to be isolated. Then after, the later century marked the isolation of active compounds from plants (Salim et al., 2008). This resulted in the starting of the modern phase of pharmacology, where pure isolated components became the standard treatment for diseases, instead of the extracts (Lahlou, 2013).
Thereafter the discoveries of plant based drugs (secondary metabolites) speeded with isolation of digoxin, a heart stimulant from flowers of *Digitalis lanata* (Lahlou, 2013), serpentine from roots of *Rauwolfia serpentine*, an Indian plant, for hypertension and lowering of blood pressure, vinblastine from *Catharanthus roseus* against hodgkins, choriocarcinoma, non-hodgkins lymphomas and leukemia in children (Pandey et al., 2011), taxol from *Taxus brevifolius* for ovarian and lung cancer treatment (Verma & Singh, 2008), teniposide from Podophyllum species for testicular and lung cancer treatment, lupeol acetate from the roots of Indian sarsaparilla *Hemidesmus indicus* for neutralizing the pathophysiological changes induced by *Daboia russellii* venom etc., (Hasan et al., 2009). As such many drugs are isolated from plant kingdom.

The two decades 1950-1970 became an important segment in the field of natural medicine by introducing approximately 100 different plants based new drugs into the USA drug market. Drugs like ectoposide, eguggulsterone, plaunotol, lectinan, artemisinin etc., were introduced during 1971-1990. From 1991 to 1995 drugs such as, paciltaxel, toptecan, irinotecan etc., found their way into drug market (Hasan et al., 2009). As such a total of 122 drug compounds have been isolated and identified from plants and 80% of them are used for the same (or related) ethnomedical purposes (Fabricant & Farnsworth, 2001).

Nearly 60 % of the commercially available drugs or drugs under clinical trial used to treat tumour and infections are of natural origin and most of these yet cannot be economically synthesized and rely on natural plants (Rates, 2001). Also, few of plant compounds, like atropine (anticholinergic), codeine (cough suppressant), colchicine (antigout), ephedrine (bronchodilator), morphine (analgesic), pilocarpine (parasympathomimetic), and physostigmine (cholinesterase inhibitor) are presently being widely used solely or in combination as a drug (Salim et al., 2008). Here lies the importance of medicinal plants.

The active compound isolation, characterization and identification along with elucidation of the action mechanism is of paramount importance and various techniques like thin layer chromatography, column chromatography, High Performance Liquid Chromatography (HPLC), spectroscopical analysis like UV-visible spectroscopy, Infrared spectroscopy (IR), Nuclear Magnetic Resonance spectroscopy (NMR) and Mass Spectroscopy (GC-MS and LC-MS) analysis aids in
the isolation and characterization of pure compounds (Sharma & Singh, 2013).

Along these lines high through put screening, combinatorial chemistry, bioinformatics, genomics and proteomics have been evolved in recent decades and have contributed their wider support in drug discovery research. The advancement in the field of NMR, designing of compound library, physicochemical concepts, other technologies like 3D QSAR in modern drug design, drug toxicity detection using computers are also increasing the research on drugs from natural origin. Thus, the plant products have been and will be an important source of new pharmaceutical compounds without any quires (Lahlou, 2013).

Arteether – a potent antimalarial drug isolated from *Artemisia annua*, Nitisinone – drug against an inherited disease, tryrsinaemia, obtained from the modification of leptospermone isolated from *Callistemon citrus* and Tiotropium – drug for treatment of chronic obstructive pulmonary disease (COPD) obtained based on ipratropium, a derivative of atropine isolated from *Atropa belladonna* are some of the drugs of plant origin introduced to the market during last decade (Balunas & Kinghorn, 2005).

### 2.5 Antibacterial agents of plant origin

Increasing incidence of the morbidity and mortality with the general population, especially in developing countries, is mainly due to the infectious diseases caused by microorganisms (Silva & Junior, 2010). This makes the necessity of their inhibition and is usually rendered with antibiotics. Unfortunately, the microorganisms have developed resistance towards various antibiotics of present use. The horizontal resistant genes transfer among bacterial species and mutations have diminished the clinical usefulness of antibiotics (Stefanovic et al., 2012) and moreover, most of the antibiotics have considerable drawbacks in terms of serious side effects, limited antimicrobial spectrum and systemic toxicity. All these make the necessity to redesign drug discovery.

Treatment of human diseases caused by pathogens, with plant based product has various advantages. They are easily available, are of lesser cost and are mainly biodegradable without producing any adverse effect both on human and environment (Mwitari et al., 2013). Thus plants have become the best choice for the isolation of effective antibacterial drug. Enormous number of medicinal plants have been
explored for their antibacterial ability and have been proven by various researches all over the world, for instance of such literature include the studies of Samy et al., 1998 on Indian medicinal plants; Sokmen et al., 1999 on Turkish medicinal plants; Chah et al., 2006 on Nigerian medicinal plants etc.

Ray et al., (2004) has reported various plants like *Azadirachta indica*, *Camelia sinensis*, *Hypericum perforatum*, *Allium sativum* to exhibit considerable antibacterial activity. Dabur et al., (2007) has documented that aqueous extract of *Acacia nilotica*, *Justicia zelanica*, *Lantana camara* and *Saraca asoca* to exhibit good antibacterial activity against human pathogenic bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris* with the MIC in range of 9.375-37.5 µg/ml concentration. Sharma et al., (2009) has recorded the antibacterial activity of numerous plant species like *Andrographis paniculata*, *Abutilon indicum*, *Ocimum sanctum*, *Terminalia chebula*, *Zingiber officinale* etc., against 33 UTI isolates like *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Klebsiella pneumoniae* etc.

The work of Panghal et al., (2011) showed that out of ten medicinal plants eight of them namely *Asphodelus tenuifolius*, *Asparagus racemosus*, *Balanites aegyptiaca*, *Eclipta alba*, *Murraya koenigii*, *Pedalium murex*, *Ricinus communis* and *Trigonella foenum* to possess potent bacterial growth inhibiting ability with most of the tested cultures like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Proteus mirabilis*, *Klebsiella pneumoniae* etc. The MIC was obtained in the range of 31-500 µg/ml.

Mwitari et al., (2013) has reported that plants such as, *Withania somnifera*, *Warbugia ugandensis*, *Plectranthus barbatus* and *Prunus africana* to be used in Kenya traditionally to treat microbial infection and cancer. These plants when tested proved that extracts of *Withania somnifera* and *Warbugia ugandensis*, to have bactericidal activity. Thus these data justifies the traditional use of these plants to treat infections.

The work of Farjana et al., (2014) revealed the growth inhibitory property of aqueous and methanol extracts of *Psidium guajava*, *Camelia sinensis*, *Azadirachta indica* and *Calendula officinalis* against *Pseudomonas* sp., *Klebsiella* sp., *Salmonella* sp., and *Vibrio* sp. In-vitro antibacterial activity of sixteen different medicinal plants used in Nepal traditionally were determined against 13 bacterial isolates including meticillin
resistant *Staphylococcus aureus*, imipenem-resistant *Pseudomonas aeruginosa*, multidrug resistant *Salmonella typhi* and *Salmonella typhimurium* and have been reported to possess antibacterial activity by Marasini et al., 2015. The essential oils of various plants have also been proven for their antibacterial activity. For instance, the essentials oils of *Piper nigrum*, *Szygyium aromaticum* and *Pelargonium graveolens* have show inhibition against both Gram-positive and Gram-negative bacteria (Dorman & Deans, 2000).

As such, a lot of research has been done and is going on in this field. The antibacterial ability of plants in the form of both the extracts and isolated active compounds, has led researchers to investigate the mechanism of action of active plant compounds.

The plant extracts containing various secondary metabolites (active compounds) determined to exhibit antibacterial action by disintegrating cytoplasmic membrane of bacteria, by destabilizing the proton motive force, active transport, electron flow and by coagulating the cell contents. It is not necessary that all above action works on specific targets, and some sites may be affected due to other mechanism (Silva & Junior, 2010). The sites of the bacterial cells where natural products exert their action is presented in figure 2.1

![Figure 2.1 Site of action of natural compounds on cellular components of bacteria](Silva & Junior, 2010)

This ability of plants has made possible their extensive exploitation in the treatment of infection caused by pathogens and in the search of newer antibacterial drugs (Ncube et al., 2008) by various researchers. In this context the present study made an attempt to screen a collection of medicinal plants for their antibacterial activity.
2.6 Antioxidants of plant origin

Oxygen consumed by human becomes a part of potentially damaging molecule commonly called “free radicals”. These radicals are highly reactive, unstable having unpaired electron and are electrically charged molecules. Due to their instability they always try to become stable by abstracting electron from other molecule. Even though the first attack causes the free radical to become stabilized, the chain reaction of free radicals continues with another free radical production in the process. Until these subsequent free radicals are stabilized or neutralized, large number of free radical reactions occurs within seconds of the initial reaction and attacks the healthy cells of the body, causing them to lose their structure and function (Percival, 1998). Free radicals/reactive species include both “Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).

Though, ROS/RNS are toxic to cell leading to pathological situation, small amount of endogenously produced ROS and RNS are essential for life, being involved in many different physiological cell functions (Prousek, 2007). The beneficial physiological cellular use and pathological situation of ROS/RNS has been briefly presented in figure 2.2.

![Diagram of Beneficial and Damaging Role of ROS/RNS](image)

**Figure 2.2 Beneficial and damaging role of ROS/RNS** (Nirmala et al., 2011; Prousek, 2007)
ROS/RNS are produced continuously on normal cellular processes like respiration, enzyme action, phagocytosis etc., of aerobic organism and also by various external sources like exposure to UV-rays, radiations, pollutants etc., (Prousek, 2007; Kunwar & Priyadarsini, 2011) and induce multiple chemical changes in the cellular organelles like membrane lipids, DNA, proteins and eventually leads to cell damage. Under the pathological conditions, iron the transition metal ion work as a catalyst in the synthesis of ROS \textit{in-vivo} (Prousek, 2007). Iron inside human body is required to be bound to either ferritin or transferring or other proteins. in its unbound state it can leads to the formation of free radicals (http://projecthealthychildren.org/wp-content/uploads/2012/03/2012-06-18-PHC-Iron-FINAL-FINAL-REVISED.pdf).

Along with iron other transition metal ion like copper is also known to play a role in free radical formation (Lu et al., 2010). Superoxide radical is an initial radical produced and upon its interaction with other molecules (metal ions) of human body it produces secondary ROS, such as hydrogen peroxide, hydroxyl radical etc., (Prousek, 2007). Thereby many radicals and non radicals are produced and exert their action. Figure 2.3 shows the causes and consequences of ROS/RNS.

\textbf{Figure 2.3 Causes and consequences of ROS} (Cooke & Evana, 2003)
When these free radicals are over produced, they result in the condition called oxidative stress leading to oxidation of various biomolecules. Interaction of these reactive species with nucleic acids may lead to a wide variety of nucleobase products, deoxyribose products like 8-oxo-guanine, 5-hydromethyluracil etc., thereby resulting in strand breaks and DNA cross links. Many of these modifications are substrates for DNA repair. However, a consequence of unrepaired damage is, potentially, mutations which can lead to cancer (Cooke & Evana, 2003).

Another consequence of ROS/RNS is the reaction with lipids leading to their peroxidation (lipid peroxidation), which is the oxidative deterioration of lipids containing carbon-carbon double bonds. These include unsaturated fatty acids, glycolipids, phospholipids, cholesterol esters etc. ROS attacks the multiple double bond and the methylene – CH₂ group with reactive hydrogen atom, in the unsaturated fatty acid and initiates the lipid peroxidation chain reaction (Lu et al., 2010) resulting in the formation of various secondary products like malondialdehyde (MDA) and 4-hydroxynonenal, which are mutagenic and cytotoxic, in nature (Siddique et al., 2012). Lipid peroxidation by free radicals is most important and frequent, due to the occurrence of lipids in the cell membrane and consequently affects the genetic material (Repetto et al., 2012). Figure 2.4 tries to briefly explain the phenomenon of lipid peroxidation by ROS resulting in production of various intermediate radicals and toxic metabolites eventually leading to biomolecule and cell damage.

ROS, can also effect proteins by modifying amino acids by the process of nitration or chlorination and result in various products having ability to damage biomolecules. Superoxide radical an initial radical reacts with nitric oxide in human body and produces peroxynitrite. The RNS (peroxynitrite) produced is stronger than the superoxide radical and damages the cell proteins. This damage results in protein function and structure alteration, including the enzyme activity inhibition leading to several medical issues (Lu et al., 2010). Finally the oxidation of various biomolecules by free radicals, results in the implication of several diseases like cancer, atherosclerosis, neurological disorders, diabetes, liver disorder, nephrotoxicity, inflammation, rheumatoid arthritis, ageing etc., (Verma et al., 2008; Zargar et al., 2011).
Unfortunately, human body is evolved with a protective system which is complex and sophisticated in such a way to protect its cells and organs against the attack of free radicals and is known as endogenous antioxidants. A balance is maintained between the free radicals formed and endogenous antioxidants, under normal healthy condition. But, under pathological conditions or during stress, radiation injury etc., the balance is lost and the excess of free radical formed will overwhelm the endogenous antioxidant system. This leads to the oxidative stress causing damage to various biomolecules resulting in triggering of number of human diseases. There arouses the need for external source of antioxidants to fight oxidative stress (Lobo et al., 2010).

The increasing risk factor of human to various diseases caused by free radicals and the adverse effects of synthetic antioxidants has led to the use of natural source to obtain therapeutic antioxidants (which possess a wide array of medicinal property) (Papas, 1999; Lobo et al., 2010). There exists many evidences reporting the correlation between lower incidence of morbidity and mortality with increased intake of dietary antioxidants (Devasagayam et al., 2004). In this view, huge works has been reported and have been going on in the search for natural antioxidants. Medicinal plants
belonging to different families, particularly Apiaceae (fennel, cumin), Lamiaceae (sage, basil, mints, rosemary etc.,) and Zingiberaceae (turmeric, ginger) have been reported for their antioxidant activity worldwide (Skrovankova et al., 2012). The work of various researchers like Dapkevicius et al., (1998) on some aromatic herbs grown in Lithuania; Jadhav & Bhutani, (2002) on some Indian medicinal plants; Govindarajan et al., (2003) on Desmodium gangeticum; Chen et al., (2008) on Zingiberaceae plants in Taiwan etc., emphasis the well established antioxidant property of medicinal plants.

70 different medicinal plants have been worked upon and reported for their antioxidant capacity by Katalinic et al., 2006. Among 70 plants evaluated few such as Melissae folium, Spiraea herba and Rubi fructose proved to be potent with determined in-vitro antioxidant assay (Ferric reducing antioxidant power assay). Inflammation results in the release of ROS from activated neutrophils and macrophages (Conforti et al., 2008). When these ROS are released/produced in larger number they results in tissue injury by damaging lipid macromolecule and thus have to be deactivated. In this view Conforti et al., (2008) has studied and reported the antioxidant property using DPPH assay, bovine brain peroxidation assay and beta carotene bleaching test of five hydroalcoholic extracts of edible plants from Calabria region (Italy), which are used in local traditional medicine for the treatment of inflammatory diseases. Among the tested plants, Mentha aquatic appeared to be most active (Conforti et al., 2008).

Some of the plant species used in Ayurvedic medicine against various ailments were determined for their ability to neutralize ROS/RNS, using different methods like ferric thiocyanate assay and thiobarbituric assay by Zahin et al., 2009. Plumbago zeylanica, Acorus calamus, Hemidesmus indicus and Holarrhena antidysenterica were the plant screened and Plumbago zeylanica proved to be potent (Zahin et al., 2009). Desmodium gangeticum (Linn.), Eclipta alba (Linn.), Piper longum (Linn.), Amaranthus caudatus (Linn.), Ocimum sanctum (Linn.) and Solanum nigrum (Linn.) were determined for their antioxidant ability by Veeru et al., (2009) using DPPH radical scavenging method and the methanol extract of Desmodium gangeticum (Linn.) proved to be potent and was followed by other test plants.
Previous research works have suggested the involvement of oxidative stress in age-related neurodegenerative disorders and various studies have documented the positive benefits of antioxidants against the disorder. Adewusi et al., (2011) has studied some of the plants like *Salvia tilifolia*, *Chamaecrista mimosoides*, *Buddleja salvifolia* and *Schotia brachypetala* used in the traditional treatment of neurodegenerative diseases for their antioxidant and acetylcholinesterase inhibition ability. The results showed that, the tested plants exhibited potent antioxidant and enzyme inhibition property, concluding to play a role in the treatment of certain neurodegenerative diseases (Adewusi & Steenkamp, 2011).

The well known medicinal plants like *Withanis somnifera*, *Ocimum sanctum*, *Piper nigrum*, *Daucus carrota*, *Allium sativum*, *Ginkgo biloba*, *Vitis vinifera* etc., have been reported to possess antioxidant property (Sharma et al., 2013). Neuroprotective, hepatoprotective, antinecrotic, antiinflamatory drugs have been discovered to possess antioxidant property and this emphasis the importance of antioxidants (Lavanya et al., 2010). As such wide works have been done and continues, to determine the ROS deactivating ability of plants. Thus the present study tried to screen some of the medicinal plants for their antioxidant property and to isolate the active compound.

Antioxidant compound deactivates free radicals by donating or accepting electrons to stabilize the unpaired condition of the radicals. Numerous mechanisms have been possessed by antioxidant molecules to stop the free radical chain reaction and thereby protect biomolecules. Such mechanism includes reducing capacity, ability as antioxidative enzymes, oxidative enzyme inhibition capacity, metal chelating property and ability to scavenging/neutralize ROS directly (Boligon, 2014; Lu et al., 2010). During this process antioxidant molecules may become radical itself. But these newly formed free radicals will be usually less reactive and less dangerous than those radicals they have neutralized. Further they may be scavenged by other antioxidant molecules or their radical state may be eliminated by other mechanisms (Lu et al., 2010).

### 2.7 Immunomodulators of plant origin

The protection of human body against the deleterious effect of microorganisms/foreign particle is offered by its immune system, which comprises innate and acquired mechanism of immunity. Various components like macrophages, neutrophils, natural
killer cells, antigen presenting cells, complement system constitutes the innate immune system and are involved in immediate non-specific response to foreign particles. When the antigen bypasses this innate immune system, acquired immune system, comprising humoral and cell mediated components will acts against the invaders and protects human against foreign substances (Tan & Vanitha, 2004). But, when this defense system of the body fails to work properly against infection, it results in primary immunodeficiency diseases, which is a class of disorders involving an intrinsic defect in the human immune system (Hovermale et al., 2011). This necessitates the need for immune system modulation and such substances which induce modulation are called as immunomodulators.

The concept of immune system modulation becomes important and involves the activation of the function and efficiency of macrophages, granulocytes, complement system, natural killer cells and also work by either enhancing cytokine secretion, or by directly stimulating B- or T-lymphocytes (Uttara & Mishra, 2009; Tan & Vanitha, 2004). Natural adjuvants and synthetic agents have found their wide application as immunomodulators. But, the general uses of synthetic immunomodulators have major limitations. For instance, Cyclophosphamide (synthetic immunosuppressor) leads to myelosuppression, levamisole and thalidomide (synthetic immunostimulators), have resulted in various health issues like hepatotoxicity, nephrotoxicity, gastrointestinal disturbance etc., (Nagarathna et al., 2013; Rawat et al., 2012).

With this background, as an alternative to conventional chemotherapy, immunomodulation using natural botanicals can be preferred. Medicinal plants are known to be incorporated for healing, preventing, curing and for immunomodulating properties by people since long back (Ranjith et al., 2008). Thus, immunomodulation using medicinal plants to alleviate the diseases has been of interest for many years and these with their enormous substance are determined to induce paraimmunity and nonspecific immunomodulation (Shariffiar et al., 2009).

In this aspect several plant species have been exploited by various researchers like Thabrew et al., 1991; Makare et al., 2001; Bishayi et al., 2002; Jiang & Xu, 2003; Kovacevic et al., 2006; Gabhe et al., 2006 etc., for their immunomodulation ability. Plants like Asparagus racemosus, Azadiracta indica, Boerhaavia diffusa, Curcuma
long, Ocimum sanctum, Tinospora cordifolia, Withinia somnifera etc., are reported for their immunomodulatory activity (Kumar et al., 2011).

Oladunmoye, (2006) has reviewed the stimulatory effect of Tridax procumbens on humoral immunity. It showed to stimulate phagocytosis and provided protection against Pseudomonas aeruginosa determined using Swiss albino rat with haematological test and urine analysis. Ghule et al., (2006) has reported the immunomodulation effect of ethanolic and aqueous extract of Capparis zeylanica. The result showed that aqueous extract was more potent in causing neutrophil adhesion (immunomodulation ability) than that of ethanol extract. Immunomodulatory activity of Gymnema sylvestre, leaves have been determined and reported by Gupta et al., (2010) using in-vitro methods like neutrophil locomotion and chamotaxis assay, qualitative nitroblue tetrazolium test etc., and the result concluded that aqueous extract of Gymnema sylvestre to be stronger in exhibiting potent immunomodulating ability.

Immunostimulatory activity of Couropita guianensis on both specific and non-specific immune mechanism was determined and reported using hypersensitivity reaction, hemagglutination reaction and phagocytosis of Candida albicans by Pradhan et al., 2009. The result reveled Couropita guianensis to be potent with immunostimulating ability. Kuumar et al., (2011) has reported the effect of Tinospora cordifolia, extracts on the activation of lymphocytes and natural killer cells. Sahu et al., (2013) has reported Habenaria intermedia to possess immunomodulatory ability, with its ethanol extract. The ethanol extract at higher concentration showed to increase delayed type hypersensitivity response and also showed to improve phagocytic index. Djafoua et al., (2015) has determined and revealed the immunomodulatory ability of medicinal plants like Adenocarpus mannii, Caucalis melanantha, Ocimum gratissimum, Asystasia intrusa and Clematis chinensis using in-vitro and in-vivo models. The work resulted in plants such as Adenocarpus mannii, Asystasia intrusa and Clematis chinensis to produce stimulation of blood lymphocytes, whereas extracts of Caucalis melanantha and Ocimum gratissimum exhibited inhibition effect.

Alternanthera tenella extracts determined for its immunomodulation property by various in-vitro and in-vivo methods like antibody assay, plaque forming assay etc.,
resulted in possessing both immunostimulatory and immunosuppressive activity (Carla et al., 2008). As such the research is rising in the search of both immunostimulators and immunosuppressors of natural origin. In this view the present work endeavors to screen some of the medicinal plants for their immunomodulation ability.

A great number of medicinal plants have been studied and are reported by various researchers all over the world for their bioactivities like antibacterial, antioxidant and immunomodulatory properties and some among them are presented in table 2.2 with their active compounds.

Irrespective of specific bioaction, the research on the therapeutic properties of medicinal plants is increasing. This shows the renewed interest of research on various aspects of medicinal plants and has been also supported by the major research project received by University of Mysore, Mysore, Karnataka, India, working on bioprospecting of medicinal plants.

However, the knowledge on therapeutic and non therapeutic essentials of medicinal plants due to their secondary metabolites, estimation of medicinal plants and their bioactive compounds studied so far and their advantage over synthetic ones has encouraged us to isolate and characterize the bioactive compound from the medicinal plant, which could be a drug for future.

In this concern the present study aimed to screen, isolate and characterize the bioactive compound from any of the selected medicinal plant extracts with potent activity, such that the isolated compound may act as a drug or as a precursor to reduce microbial infection or act against oxidative stress or possess immunomodulation property, which could represent significant segment of pharmaceutical market with safe and healthy life. As such the medicinal plants namely *Canthium parviflorum*, *Digera muricata*, *Cleome gynandra* and *Amaranthus spinosus* were selected based upon their ethno directed approach and their local availability for isolating bioactive compound.
Table 2.2 Medicinal plants with their active compounds and their bioactivities (Cowan, 1999 - SL. No.1-5; Panchawat et al., 2010 - SL. No. 6-10; Roshan & Savitri, 2013 - SL. No. 11-15)

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Medicinal plants</th>
<th>Secondary metabolite</th>
<th>Bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Papaver somniferum</td>
<td>Opium</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td>2</td>
<td>Camellia sinensis</td>
<td>Catechin</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td>3</td>
<td>Rauvolfia serpentine</td>
<td>Reserpine</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td>4</td>
<td>Glycyrrhiza glabra</td>
<td>Glabrol</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td>5</td>
<td>Curcuma longa</td>
<td>Curcumin</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td>6</td>
<td>Acacia arabica</td>
<td>Catechin, epicatechin, quercetin and gallic acid.</td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td>7</td>
<td>Echium amoenum</td>
<td>Rosmarinic acid</td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td>8</td>
<td>Citrus lemon</td>
<td>Citral</td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td>9</td>
<td>Rosmarinus officinalis</td>
<td>Borneol</td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td>10</td>
<td>Mentha arvensis</td>
<td>L-menthol</td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td>11</td>
<td>Clerodendrum phlomidis</td>
<td>Hispidulin, Apigenin, luteolin</td>
<td>Immunomodulatory activity</td>
</tr>
<tr>
<td>12</td>
<td>Terminalia belerica</td>
<td>Gallic acid, ellagic acid, ethyl gallate, chebulic acid</td>
<td>Immunomodulatory activity</td>
</tr>
<tr>
<td>13</td>
<td>Alstonia boonei</td>
<td>a &amp; b ammyrin and lupeol</td>
<td>Immunomodulatory activity</td>
</tr>
<tr>
<td>14</td>
<td>Acacia catechu</td>
<td>Catechin and epicatechin</td>
<td>Immunomodulatory activity</td>
</tr>
<tr>
<td>15</td>
<td>Premna integrifolia</td>
<td>Premnine, Ganikarine,</td>
<td>Immunomodulatory activity</td>
</tr>
</tbody>
</table>
2.8 Medicinal plants selected

*Canthium parviflorum*

Kingdom  Plantae  
Family  Rubiaceae  
Genus  *Canthium*  
Species  *parviflorum*

**Synonym:** *Canthium coromandelicum, Plectronia parviflora* (Patro et al., 2014).

**Vernacular names:** *Canthium parviflorum* is known as Nagabala in Sanskrit, Carrycheddie in English and Karemullu in Kannada (Patro et al., 2014).

**Occurrence:** Is found in peninsular India, in scrub forests, coramandel coast and in dry plains (Kala et al., 2012).

**Morphology:** Is a thorny subscandent shrub with spreading branches, edible leaves and fruits. Leaves are small, obviate, opposite with interpetiolar stipules and axillary spines (Kala et al., 2012). Fruits are obovate and are red or brown in colour (Patro et al., 2014).

**Ethnomedicinal uses:** In Ayurvedic medicine the plant is used to treat cough and tumor and in Siddha medicine it is used against gout (Prabhu et al., 2013). The roots and leaves are known to possess diuretic, astringent, febrifuge, thermogenic and anthelmintic properties (Kala et al., 2012). This plant is also used against obesity, diabetes and respiratory disorder (Prabhu et al., 2013). It has also found its application in the treatment of snake bite, wounds, intestinal worms, scabies, ring worm infection, diarrhea, fever, leukorrhoea, indigestion, nausea etc., (Patro et al., 2014).

**Determined pharmacological properties:** Several studies have reported *in-vivo* and *in-vitro* anticancer activity (Prabhu et al., 2011), wound healing activity (Dinesh et al., 1997), antioxidant activity (Kala et al., 2014), antibacterial and antifungal activity (Haroled et al., 2011) and anti–HIV activity of the plant (Patro et al., 2014).
**Digera muricata**

Kingdom  
Plantae

Family  
Amaranthaceae

Genus  
Digera

Species  
muricata

**Synonyms:** *Digera arvensis, Achyranthes muricata* (Sharma & Vijayvergia, 2013).

**Vernacular names:** *Digera muricata* is known as Aranyaastuka in Sanskrit, False amaranth in English and Chenchali soppu in Kannada (Sharma & Vijayvergia, 2013).

**Occurrence:** Is found to be present in eastern tropical Africa and subtropical Asia (Sharma & Vijayvergia, 2013).

**Morphology:** Is an annual herb and grows upto 20-70 m tall. Leaves are linear to ovate and are alternately arranged, flowers are long and hairless and fruits are subglobose in nature (Sharma & Vijayvergia, 2013).

**Ethnomedicinal uses:** The entire plant has been used as crude drug against renal and urinary tract disorders (Sharma & Vijayvergia, 2013). Locally this plant is used to cure inflammation and prevent pus formation. It is also well known for its hepatotoxicity treatment (Anar et al., 2013). It has also found its application as an alternative for secondary infertility and boiled root infusion is used to enhance lactation in humans (Khan et al., 2011; Mety et al., 2011).

**Determined pharmacological properties:** The plant has been previously reported for its antioxidant (Mety et al., 2011) antimicrobial, anthelmintic, antidiabetic and hepatoprotective activities (Anar et al., 2013).


**Cleome gynandra**

Kingdom  
Plantae

Family  
Cleomaceae

Genus  
Cleome

Species  
gynandra

**Synonyms:** Gynandropsis gynandra, Gynandropsis pentaphylla, African spider flower, cat whisker (Mishra et al., 2011).

**Vernacular names:** Cleome gynandra is known as Ajagandha in Sanskrit, Dog mustard in English and Narambele soppu in Kannada (Mishra et al., 2011).

**Occurrence:** Is found to occur throughout world in tropical and subtropical regions (Mishra et al., 2011).

**Morphology:** Is an annual herb, erect, growing upto 25-60 cm tall. Leaves are palmately compound, flowers are bisexual white or with little purple colour and the plant bear capsule form of fruit (Mishra et al., 2011).

**Ethnomedicinal uses:** In Ayurveda and other medicine system, the plant has found its use to treat cough, snake bite. It is considered as anthelmimitic and as rubefacient (Chopra et al., 1956). This plant has also found its application in the treatment of malaria, gonorrhea, tumour, arthritis, wounds and sepsis (Mishra et al., 2011).

**Determined pharmacological properties:** The plant has also been previously reported for its immunomodulatory, antioxidant, antiinflammatory, antidiabetic and anticancerous activities (Mishra et al., 2011).
Amaranthus spinosus

Kingdom: Plantae
Family: Amaranthaceae
Genus: Amaranthus
Species: spinosus

Synonyms: Galliaria spinosa, Galliaria spitosa
(http://www.theplantlist.org/tpl/record/kew-2633107)

Vernacular names: Amaranthus spinosus is known as Tanduliuyah in Sanskrit, Prickly amaranth in English and Mulluharive soppu in Kannada (Kumar et al., 2014).

Occurrence: It occurs widely throughout the tropics and warm temperate regions of Asia from Japan to Indonesia to India (Kumar et al., 2014).

Morphology: It is an annual or perennial herb, containing spines, growing upto 1 m tall and is with green or purple colour. The leaves are simple and are alternate; flowers are 1-1.5 mm long and the plant posses ovoid shaped fruits (Kumar et al., 2014).

Ethnomedicinal uses: In Ayurveda, the plant is known for its application as antipyretic, febrifuge, laxative and diuretic. The roots and leaves are used to treat abscesses, burns, wound, inflammation, gonorrhea, gall bladder inflammation and are also used against snake bite, to cure dysentery, jaundice, leprosy, malaria, blood diseases, hallucination and rheumatism (Kumar et al., 2014).

Determined pharmacological properties: The plant has been previously reported for its antidiabetic, antipyretic, antitumor, antioxidant, antiinflammatory, antimicrobial, hepatoprotective and immune stimulating activities (Kumar et al., 2014).

These works of different researchers imparts the knowledge on therapeutic properties of medicinal plants (Jadhav & Bhutani, 2002).
Objectives of the present work

With this background of medicinal plants the following objectives were planned and executed.

1. Phytochemical screening of medicinal plants from family Amaranthaceae, Rubiaceae and Cleomaceae

2. Evaluation of antibacterial activities of test plant extracts

3. a. Evaluation of *in-vitro* antioxidant activities of test plant extracts

   b. Evaluation of *in-vitro* immunomodulatory activities of test plant extracts

4. Isolation, Characterization and Identification of bioactive compound