Chapter 2

Review of Literature
2. REVIEW OF LITERATURE

Age old traditional medicine to current modern drugs

Since down the historical times plants are used as resource of drugs by mankind, they are major principal sources of drugs. Rig Veda has mention about remedial properties of some herbs which appears to be the prehistoric document in India. Medicinal plants have been used to a great extent by health management in two different sectors; conventional medicinal system and contemporary medicinal system. Functioning of conventional medicinal system is by two modes, which are (1) Folk medicine and, (2) Ayurveda, Siddha and Unani which comes under well organized Indian medicine. The emergence of the agro dependent cities of Indus Valley civilization around 4500 BC existed together with medicinal systems in India at least 5000 BC ago. (Mazid et al., 2012).

Documented drawings and scripts about the use of medicinal plants have discovered by people from the areas of Tigris and Eufrat which suggest that people of those regions were well aware of using medicinal plants in their culture. Similarly, engraved old rocks have many plants on them, which support the assumption of the use medicinal plants in their life time.

About 3000BC ago, German Egyptologist, Georg Ebres who studied a large number of documents of luxurious culture of Arabic people. He mentioned the usage of plants for therapeutic purpose by Arabs in his book ‘Papyrus Ebers’. His documents consists the knowledge about Babylonians who use to prepare 800 medicinal recipes and details of 700 plants like they are of foreign origin or native plants. Few of plants amongst Ebres documents are aloe, absinth, Arabic gum, colocynth, cumin, garlic, Indian hemp (cannabis) juniper, peppermint, opium poppy, ricinus seeds.
The record Pen tsao left by Chinese emperor, Shen Nung, who lived around 3000 B.C., reveals that some of the same plants were used in different cultures who stayed far apart during the same period, and several of these plants are still in use today. Descriptions of plants such as opium poppy, liquorice, ergot, rhubarb, gentian and valerian are mentioned by Shen Nung.

Great number of scripts which explains the production methods, use of medicinal plants and recipes used for treating diseases were recorded in the ancient documents left by the supposed antique Greek Roman period.

Greece based Hippocrates who lived 460-377 BC is called as the father of Mediical art. In his 60 documents, he described about the medicinal plants used by him for the treatment of various diseases. Theophrastus (370-287 BC) a medical person and philosopher was one of the famous successors of Hippocrates. Theophrastus wrote the important documents “De causis plantarum” and “Historia plantarum” for describing plants, their growth and uses, including their use in medicinal preparation. Out of 8 volumes “De causis plantarum” 6 are available even today.

“Historia naturalis” is a medical document written by Plinius the elder, (23 A.D. – August 25, 79) a Roman Empire. This book was referred by many scholars of different countries, who are related to the study of plants, plant products and medicines.

Aulus Cornelius Celsus (25 BC—50 AD) wrote a reference work, De medicina, describing the use of 250 medicinal plants. This book was used as a source for knowledge on diet, pharmacy, surgery and other related topics for a long time.

Pedanius Dioscorides who lived at 40-80 AD was a celebrated Greek physician, botanist, pharmacologist and surgeon in the period of Roman Emperor
Nero. He was considered as a well known medical person of the Roman period. To make the people unconscious by anaesthesia during surgery by the extracts of mandragora. When he treated extracts of mandragora to people they did not lose consciousness, but gives them feeling of absentia. His document “De Materia Medica” which explains the properties of 600 medicinal plants considered to be the precursor of the Pharmacopoeias is a recent document on medicinal plants and medicines.

Claudius Aelius Galenus (129 – 200 A.D.), was a famous Roman physician and philosopher, also known as Galen of Pergamum was considered as one of the best medical scientists of the Roman period. Over a millennium, the theories of Galen dominated Western medical science. In those days Galen explained the methods of making herbal medicines, techniques of coating pills. He has brought the name to one of the most important pharmaceutical science of today. His Galenical pharmacy, deals with the production and the processes behind the preparation of medicinal pills. Galen left documentations on 130 medicinal plants and recipes.

Avicenna has written a document called Canon Avicenna, which is also known as Canon of medicine. He was a well known philosopher in the Arabic and Monastery period. Avicenna systematically illustrated the knowledge of medicine and pharmacology during 980 – 1037 A.D. It is the Avicenna who explained first about coating of pills, distillation of alcohol, preparation of juices, production of juices, tinctures and extract preparation from herbs. The systematic experimentation and quantification in physiology was introduced by Avicenna as evidence-based medicine which recognises the importance of a proper diet and also the influence of climate and the environment on health (Hoeg 1984, Nordal 1960, Paulsen 2001, Askel, 2010).
The texts of prehistoric cultures of Egypt, Mesopotamia and India illustrate and explain the utilization of plant products which are having therapeutic values. The rich history of entire Middle East explains about herbal therapeutics. The prescription of 876 herbal drugs are prepared from more than 500 various herbal substances was mentioned in the Ebers Papyrus which is written around 1500 BC ago and it also contains very earlier knowledge of herbal medicines. Ebers Papyrus is one of the most valuable manuscripts, and it is preserved safely by Egyptian manuscripts.

The text of Jami of Ibn Baiar (1248 A.D.) is the chief treasure of the Muslim materia medica. It enlists more than 2,000 substances; including many plant products. The principal Ayurvedic book on internal medicine, the Chark Samhita, describes 582 herbs. The main book on surgery, the Sushruta Samhita, has the record of some 600 herbal remedies. Most experts agree that these books are at least 2,000 years old. (Chattopadhaya, 2010).

Paracelsus Phillipus Aurelius was a great medicinal reformer in his time. He was a clinician, an alchemist and an astrologer. Paracelsus said that he did not want to recommend a remedy for treatment of illnesses that he had no proof for working. He was the first to carry out clinical trials and used several herbal products as a doctor to confirm their effects and initiated the research in the area of clinical studies. His contributions to the field of development of surgical methods, medicinal chemistry, and theory for development of medicines and their properties are remembered till today. The theories of Paracelsus about diseases and their remedial methods are very interesting. He defines the poison as –“Everything is poison; it is just the concentration that will decide if something is nontoxic.” this definition is acceptable even today. Paracelsus says: “The body is a conglomerate of chemical compounds
that have to be in equilibrium with each other. If they are not so, the body is ill, and other chemical components must be added to get the body in balance again."

The modern “scientific” era began after Paracelsus which has laid the foundation for today’s contemporary medicine and is assumed to be started 1600 year ago, and now also we practice the same science. Few scientists are remembered forever for their remarkable contributions to human health and they are great examples for modern scientists.

The role of unique identification and systemic classification was realised by a Swedish botanist Carl von Linne (23 May 1707 – 10 January 1778) from university of Uppsala; he was well known for plant systematic. His system of classification of plants made easier to identify large number of plants. Linne explained various rules to identify different characters of plant and categorize the plants into different classes based on these rules. He is best known icon for the introduction of plant systematic that included rules for how to examine different features of the plants based on the features to segregate the plants and place into a definite system of classification that made it easier to identify them.

Even today also his system is valid for classifying and studying plants, and name of Linne was still mentioned with plants after “the system of Linné”. Though the present day classification of plants is advanced to the molecular levels such as advanced gene technologies and cladistic approaches, nature of proteins expressed by plant machinery, but Linné system of classification remains to be correct method in most of the times.

The foundation for chemistry of recent times was laid by Swedish scientist, pharmacist Carl Wilhelm Scheele (9 December 1742 – 21 May 1786) by discovering elements like oxygen, nitrogen, and many more. Knowledge of synthesizing urea,
isolation of several chemical elements was first reported by a German chemist Friedrich Wöhler (31 July 1800 – 23 September 1882).

Salvarsan the first synthetic drug used to treat Syphilis was prepared by Paul Ehrlich (14 March 1854 – 20 August 1915). The German based scientist Ehrlic also worked on various fields like haematology, immunology, and chemotherapy. His research work on autoimmunity has recognised and awarded a Nobel Prize to him in medicine.

Another biologist and pharmacologist from Scotland, Sir Alexander Fleming (1881 –1955) made revolution in the field of medicine by the rediscovery of antibiotics. In the year 1928 Fleming earned the Nobel Prize for identification of antibiotic substance penicillin from the fungus Penicillium notatum. Prior to this he discovered lysozyme in the year 1923. Large numbers of people were died during world war. The discovery of penicillin strengthened the treatment of several microbial diseases (Hoeg 1984, Nordal 1960, Paulsen, 2010, Askel 2010).

The knowledge of herbal medicine was widely spread all over Europe in seventeenth century. In 1820 the first United States Pharmacopoeia was published, further it was regularly revised and in 1906, it became the officially authorized standard reference for medical compounds. The first volume of pharmacopeia explains about catalogue, properties, uses, dosages and purity of herbal medicines.

Countries like India, Pakistan, China and other countries of the world realized the value of ethno medicinal plants which are the store house of number of potential medicines. To search the novel drugs and their biological properties of medicinal plants several nations across the world started the assessment of traditional medicinal plants scientifically and experimentally to confirm their effects.
The ancient traditional systems of medicine has been identified and accepted as complementary system of medicine in primary health care system to treat few chronic diseases. Benzoic acid was the first chemical substance, which was isolated from plants in 1560. Morphine was isolated from *Papaver somniferum* L. (Opium) in the year 1804 then the whole scientific community affirm to engage themselves in search for valuable drugs and their structure.

From the discovery of morphine the current scientific field has made an advanced innovation in drug discovery from higher plants. Generally there are 100 drugs with known structure and functions are useful in common. In western medicine practice there are around 55 drugs used to treat all ailments. The drugs such as aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine morphine, physostigmine, pilocarpine, quinine, quinidine, resperine, taxol, tubocurarine, vincristine and vinblastine are the examples of modern drugs which we unearthed from medicinal plants since the past (Chattopadhaya, 2010).

**Medicinal plants and Antimicrobials**

In the ilieu of development of resistance by micro-organisms against pharmaceutical agents, treatment of diseases to these drugs is slowly losing its position and thus people are focusing on the use of natural products for their healthcare. In order to determine the specific plants and its parts used as drugs for the treatment of various ailments, more exploration is ongoing at this direction. A variety of biological functions of phytomedicines have been found consequently in medical research, mainly the functions include estrogenic activity, anti-inflammatory, antimicrobial, enzyme inhibition, antiallergic activity, antitumour activity, vascular activity, antioxidant activity, diabetes mellitus, skin infection and antimalaria activity (Bojase et al., 2002). Interest in phytomedicine usage is increased by consumers and
food manufacturers due to their medicinal properties which include their possible function in the treatment of several diseases including cardiovascular and cancer. The beneficial effects of vegetables, fruits, red wine and tea is attributed to the bioactive compounds present in them other than nutrients and vitamins. The phytochemicals especially flavonoids have an antioxidant, antiplasmodial, anti-inflammatory and antimicrobial activities that most often exist in nature (Edewor, 2013).

**Antibacterial activity**

Bacteria are prokaryotic microorganisms found enormously at large number in nature. They were first discovered in 1676 by Anton Van Leeuwenhoek (Beak et al., 1999). Not all the bacteria are pathogens but some have shown to possess beneficial properties and are rendered harmless by the body’s immune system when they enter into the body system. However, a few species of bacteria are pathogenic which cause various human diseases, infections and may result in human death (Volk et al., 1991).

There is decrease in the rate of mortality from bacterial infections from the 20th century by the advancement of drug discovery, development and clinical usage of antibiotics. There is also a regression in the introduction of new antibiotics mostly due to the huge investment involved in developing and testing of new drugs and there is crisis of increased resistance of bacteria to currently available antibiotics (Afolayan and Meyer, 1997). An improved effort is continued to determine and produce new antibacterial drugs that are efficient against pathogenic bacteria which have increased resistant to current antibiotics. Antibacterial activity has been screened for crude extracts from plants that are used in traditional folk medicine (Lin et al., 2000; Lindahl and Tagesson, 1993; Malterund et al., 1985; Serafini et al., 2009; Middleton and Chithan, 1993).
The acetone, hexane, ethanol, chloroform, methanol and aqueous extracts of roots and leaves from the bushy lippia (Lippia alba) was evaluated for the antimicrobial activities at the concentration of 2 mg/disc against Serratia marcescens, B. subtilis, E. faecalis, S. aureus, Micrococcus luteus, P. aeruginosa, E. coli, Monilia sitophila Mycobacterium smegmatis, and C. albicans has been observed by Aguiar et al., (2008). The results indicated that the extracts from roots such as chloroform, acetone and ethanol extracts prevented the growth of M. luteus, S. aureus, M. smegmatis, B. subtilis, C. albicans and M. sitophila, whereas the ethanol, hexane and methanol extracts from leaves inhibited the growth of M. smegmatis, M. luteus, B. subtilis, S. aureus and M. sitophila.

Silva Jr. et al. (2009) have demonstrated the antimicrobial activity of various extracts including ethyl acetate, hexane, dichloromethane and ethanol from phloem of Calophyllum brasiliense (guanandi), Bowdichia virgilioides (“sucupira”), Lafoensia pacari (“dedaleira”), Cariniana rubra (“jequitibá”), Stryphnodendron obovatum; rhizomes of Simaba ferruginea and C. urucurana latex were tested against bacteria and fungi. The results indicated that ethyl acetate and hexane extracts of C. brasiliense phloem has shown significant antibacterial activity against gram-positive bacteria such as S. epidermidis, S. aureus and S. agalactiae. Ethyl acetate and ethanol extracts of L. pacari phloem and B. virgilioides extracts has shown little effect on fungi. It was the first report on antifungal activities of extracts from C. rubra and S. ferruginea.

Ushimaru et al., (2007) have performed antibacterial activity against Enterococcus sp., E. coli, S. aureus and Salmonella utilizing extracts from A. sativum (bulbs), Z. officinale (rhizomes), Caryophyllus. aromaticus (flower buds), C. citratus (leaves), P. guajava (leaves) and M. glomerata (leaves). The observation reveals that
the extracts from garlic (A. sativum) and ginger (Z. officinale) have exhibited the highest activity against gram-negative bacteria at the concentrations for garlic ranged from 1.38 to 1.61 mg/mL while for ginger it has shown 6.97 mg/mL. Maximum susceptibility was for Gram-positive strains in guava extracts at concentrations of 0.77 and 1.74 mg/mL and clove extracts at concentrations of 0.46 to 1.24 mg/mL.

According to Mohamed et al., (2011), the antimicrobial activities of the roots of Arbutus unedo L. water and methanol extract and three phenolic fractions indicated that water and methanol extract are shown poor antibacterial activity against both Staphylococcus aureus and Pseudomonas aeruginosa bacteria. However water extract and phenolic fractions have shown moderate antibacterial activity against Escherichia coli and S. aureus respectively. They have concluded that the antibacterial activity of the extracts is due to the presence of anthocyanins, anthraquinones reducteurs compounds, quinones, tannins and flavonoids was found while screening the A. unedo. Even the quantitative phytochemical screening has shown that the roots contain high anthocyanins compounds (3.65 mg g\(^{-1}\)) followed by total flavonoids (0.56 mg g\(^{-1}\)), flavones & flavonols (0.17 mg g\(^{-1}\)).

Parekh et al., (2005) have screened some medicinal plants for potential antibacterial activity against five medically important bacterial strains namely Pseudomonas pseudoalcaligenes, Staphylococcus epidermidis, Proteus vulgaris, Bacillus subtilis and Salmonella typhimurium, they have selected twelve medicinal plants namely Ficus benghalensis, Caesalpinia pulcherrima, Abrus precatorius, Casuarina equisetifolia, Cardiospermum halicacabum, Delonix regia, Cynodon dactylon, Euphorbia tirucalli, Euphorbia hirta, Santalum album, Gmelina asiatica and Tecomella undulata. The antibacterial activity was determined by both agar disk diffusion and agar well diffusion method for aqueous and methanol extracts. The
results exhibited that methanol extract was more active than the aqueous extract among all the plants extracts tested and it was more active against gram-positive than against gram-negative bacteria. Majority of susceptibility was observed for *B. subtilis* followed by *S. epidermidis* and the most resistant bacteria was *P. vulgaris* followed by *S. typhimurium*. *Caesalpinia pulcherrima* Swartz. Which have shown the highest antibacterial activity and hence this plant is considered as important therapeutic antimicrobial agent.

The extracts of *Malva parviflora* L. and *Malvastrum coromandelianum* L. was assessed for their antibacterial, antifungal and irritant activities using hexane, chloroform and ethanol extracts. The *Amaranthus viridis* L. was tested for antibacterial and irritant activities using hexane, chloroform, ethanol and aqueous extracts. The results has shown that the extracts of *Malva parviflora* L. and *Malvastrum coromandelianum* L. have a good antibacterial activity against *Escherichia coli* but slight lower in the antibacterial response against *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus*. Chloroform extracts of both the plants have displayed a prominent antibacterial activity when compared to that of other extracts. Antifungal activities against *Aspergillus niger* and *Aspergillus oryzae* of all the extracts have shown almost same result. The antibacterial activity of ethanol extract of *Amaranthus viridis* L. was more prominent than aqueous extract and polar mass. In contrast the hexane extract has exhibited a higher antibacterial activity against Gram positive and Gram negative microorganisms compared to chloroform extracts (Islam et al., 2000).

The Mexican marigold (*Tagetes erecta*) leaf extract was screened for antibacterial effect by well diffusion method at room temperature against ten gram-positive multidrug resistant bacterial isolates such as *Erysipelothrix rhusiopathiae*,...
Streptococcus agalactiae, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecium, Streptococcus pneumoniae, Bacillus cereus, Propionibacterium acne, Staphylococcus saprophyticus and six gram negative multidrug resistant bacterial isolates such as Pseudomonas auregenosa, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Salmonella enteriditis and Alcaligen faecalis. The maximum antibacterial effect of Mexican Marigold leaf extract was obtained for Acinetobacter baumannii with Activity Index of 0.913333333, Propionibacterium acne with Activity Index of 0.906666667 and minimum for Streptococcus pneumoniae with Activity Index of 0.026666667. Based on the results they have concluded that the Tagetes erecta has antibacterial effect against airborne disease causing gram positive and gram negative bacteria mainly on skin infection causing bacteria. Hence this plant is considered as useful in developing drugs for diseases like dermatitis, acne, skin races and as antiseptic.

Antimicrobial activity of Baba and Onanuga (2011) have studied antibacterial activity using modified Kirby- Bauer disc diffusion and agar dilution techniques by using methanol extract of three Nigerian medicinal plants to determine the diameters of zone of inhibition and minimum inhibitory concentrations (MIC) of the extracts against five clinical bacterial isolates comprising two Gram-positive bacteria such as Bacillus subtilis and Staphylococcus aureus and three Gram-negative bacteria such as Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia. All the extracts have displayed moderate to high level of antimicrobial activities against all test microorganisms and phytochemical screening revealed the presence of some secondary metabolites such as tannins, alkaloids, anthraquinones, saponins and flavonoids. Thus these Nigerian medicinal plants are useful for cheap, safe and
culturally acceptable standardized herbal products and serve as a source of new molecules for broad-spectrum antimicrobial drugs.

The *in vitro* antimicrobial activities of different solvent fractions of ethanolic extract of *Cryptolepis sanguinolenta* was evaluated against eight standard bacteria and clinical isolates such as *Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi* and *Salmonella typhimurium* by using Kirby Bauer disk diffusion method to ascertain the antibiogram of the test organisms while the agar diffusion method was followed to investigate the antimicrobial properties of the crude plant extracts. The chloroform fraction has shown the highest activity followed by water, ethanol, petroleum ether and ethyl acetate respectively (Felix et al., 2012).

Tawfik et al., (2011) have demonstrated that the alcohol extract of *Atriplex semibacata* exhibits a significant antimicrobial activity against Gram negative bacteria, moderate activity against Gram positive bacteria. On the other hand the pet. Ether extract has showed marked activity against Gram positive bacteria and fungi, however, the Gram negative bacterium was greatly inhibited by the chloroform extract.

Antimicrobial activity of 18 ethnomedicinal plant extracts were evaluated against nine bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Ervinia sp, Proteus vulgaris* and one fungal strain *Candida albicans*. The results indicated that among 18 plants, antimicrobial activity was exhibited by 10 plants against tested microorganisms at three different concentrations of 1.25, 2.5 and 5 mg/disc. Among the plants tested, *Acalypha fruticosa, Peltophorum pterocarpum, Toddalia asiatica, Cassia auriculata, Punica*
*Phytochemical and Pharmacological Profiling of Ficus glomerata Roxb*

*grutanum* and *Syzygium lineare* were most active. The highest antifungal activity was exhibited by methanol extract of *Peltophorum pterocarpum* and *Punica granatum* against *Candida albicans* which are potential sources of new antimicrobial agents (Veeramuthu et al., 2006).

**Antioxidation**

The protection of cells against the damaging effects of reactive oxygen species, such as peroxyl radicals, singlet oxygen, hydroxyl radicals, superoxide and peroxynitrite is due to the presence of antioxidants compounds. If imbalance between antioxidants and reactive oxygen species occurs, it results in oxidative stress and leading to cellular damage. These species of ROS are produced by organism during oxygen metabolic reactions or by exogenous damage which play a role in the development and maintenance of cellular life (Chin et al., 2006; Cos et al., 1986). These free radicals due to reaction with endogenous molecules such as DNA, proteins and lipids continuously cause damage to the body cells and tissues.

Saeed et al., (2012) have screened various solvent extracts of whole plant of *Torilis leptophylla* to display the potent in vitro (DPPH, ABTS, superoxide, hydroxyl radicle) and in vivo (GSH, TBARS) antioxidant activity, total phenolic and flavonoid contents in order to evaluate the possible sources for future novel antioxidants in food and pharmaceutical formulations. Data from the present results revealed that *Torilis leptophylla* act as an antioxidant agent for possessing free radical scavenging and cytoprotective activity.

The study of Konrath et al., (2012) on the biological properties of the folk medicinal plants *Lycopodium clavatum* and *Lycopodium thyoides* has confirmed the antioxidant effects. The results indicated moderate in vitro antioxidant activity, estimated through the thiobarbituric acid reactive substances test (TBARS), catalase
(CAT) and superoxide dismutase (SOD). Chemically, the main alkaloids responsible for the antioxidant effects are found to be present in two species were identified as lycopodine and acetylidihidrolycopodine.

The objective of the study conducted by Rizwan and Sreemoy, (2013) was to investigate the phytochemical constituents, along with antioxidant evaluation of *Nelumbo nucifera* by the DPPH scavenging activity, total phenolic content and β catotene assay. The crude plant extract was subjected to evaluate the potential antioxidant and it was found that methanolic extract which has shown IC₅₀ (78 ± 4.32) and ethanolic extract IC₅₀ (177.66 ± 2.05) in DPPH free radical Scavenging assay.

The polyphenolics and oils obtained from ajwain, mustard, fenugreek and poppy seeds were analyzed for their DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging potential, ferric reducing ability and chelating power. The study has shown that the oil fractions were acted as strong antioxidants even in high concentration of polyphenols, though the oil seeds are used in very small quantity in food, but they are potential source of natural antioxidants and replace synthetic ones (Shagufta et al., 2013).

Matured green leaves of *Osbeckia stellata* belonging to family Melastomataceae was tested for antimicrobial activity. The results suggest that the petroleum ether extract has shown better antioxidant activity by scavenging DPPH free radical and concluded that this wild plant can be used as a clinically efficient antioxidant compound (Das et al., 2013).

In the study of Abul et al., (2013), in both *in vitro* and *in vivo* antioxidant activities of *Ganoderma lucidum* grown on germinated brown rice (GLBR) was revealed. The findings demonstrate the remarkable potential of GLBR extract as
valuable source of antioxidants which was proved by strong metal chelating activity, DPPH, ABTS, hydroxyl and superoxide radical scavenging activity.

Feng et al., (2010) have screened various extracts and fractions from the herbs of *Artemisia selegensis* Turcz (AST) by *in vitro* and *in vivo* assays. FRAP, DPPH and ABTS assays were made to evaluate the *in vitro* antioxidant activities. The results obtained out of three *in vitro* antioxidant assays, water extract was found to have the highest antioxidant activity by reducing oxidative stress in male mice. The *in vivo* antioxidant assay results showed the that high doses of water extract has significantly decreased the MDA level compared to normal group and the SOD activity of mice was the highest in water extract at high dose. Both *in vitro* and *in vivo* studies have demonstrated that the extracts, particularly the water extract from *Artemisia* had shown a significant antioxidant and free radical scavenging activities.

The *in-vivo* antioxidant potential of ethanolic extract of *Abutilon indicum* against CCl₄ induced toxicity in rats after pre-treatment with 500 mg/kg of ethanolic extract of *Abutilon indicum* improved the SOD, glutathione, catalase, and peroxidase levels significantly when compared to the control group indicating *Abutilon indicum* is having a significant *in-vivo* antioxidant activity and can be used to protect tissue from oxidative stress (Kaushik et al., 2011).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, nitric oxide radical and *invivo* antioxidant enzyme assay were carried out to evaluate the antioxidant potential of the ethanol extract from water spinach (*Ipomoea aquatical*) leaf and stem. The antioxidant activity varies with the concentration of the extract. In DPPH radical scavenging assays the IC₅₀ value of the extracts was 33.188 and 672.376 (μg/ml) for the stem and leaf respectively. The plant inhibited the nitric oxide radicals generated from sodium nitroprusside with IC₅₀ of 142.52 and 156.99 (μg/ml) for the stem and
leaf respectively compared to 0.0161 (μg/ml) for vitamin C. It is presumed that the total polyphenolic constituent present in the plant extract could be responsible for the antioxidant activity (James et al., 2009).

Sachin et al., (2012) have assessed the in-vivo antioxidant potential of ethanolic extract of Mentha Pulegium against CCl₄ induced toxicity in rats. The result indicates that the activities of glutathione, SOD, catalase, and peroxidase in group treated with CCl₄ reduced significantly than that of the control group. Based on these observations they have concluded that the ethanolic extract of Mentha Pulegium possesses antioxidant activity and can be employed in protecting tissue from oxidative stress.

Both in vitro and in vivo antioxidant activity of ethanolic leaves extract of Moringa oleifera Lam (Moringaceae) and its effect on lipid indices in male albino rats was investigated. The extract is having appreciable phenol content and exhibited in vitro antioxidant effect. The extract also exhibited modulating effect on enzymatic antioxidants, lipid-lowering ability and changes in lipoproteins of serum was observed (Ogbunugafor et al., 2012).

The study carried out by Dinanath et al., (2011) to evaluate the in-vitro antioxidant activities of ethanol, methanol, and hexane extracts of Ocimum basilicum Labiatae (sweet basil) at varying concentrations (10-50μg/ml) using DPPH radical scavenging activity, reducing power assay, hydroxyl radical scavenging activity and nitric oxide radical scavenging activity. The results obtained were analyzed and indicated that the ethanol extract of Ocimum basilicum is having high antioxidant activity than the standard antioxidant drug.

The investigation conducted with different solvent extracts such as methanol, chloroform and petroleum ether extracts of C. ternatea leaf extract for their in vitro
antioxidant potential by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. All the extracts have exhibited potent in vitro free radical scavenging activity that was increased with increasing extract concentrations. Similarly it is observed that the methanol extract was found to be the most potent followed by the chloroform and petroleum ether extracts (Rishov et al., 2012).

**Antimitotic activities of phytochemicals**

Now-a-days the people who are actively engaged in science and ethical activities are raising the issues for careful use of animals and conducting toxicology tests on animals. Alternatives to the use of animals in research, toxicology testing and education are being searched by the scientific community (Mukhopadhyay et al., 2004). Employing plants in genotoxicity assays reduces the experimental expenses which provide reliable and rapid results. Further it is found that the chemical induced chromosomal aberration in plant cells and animal cells are similar (Grant, 1978; Ma et al., 1994). The onion root tip assay is suitable method for studying genotoxicity effects of environmental pollutants and pesticides which induce chromosomal aberrations in meristematic cells of *Allium cepa* (Ma et al., 1994; Fernandes et al., 2007, Asita and Matebesi, 2010).

Asita and Matebesi (2010) have studied the cytotoxicity and gene toxicity effect of chemicals on root tips of *Allium cepa*. Based on mitotic index, the cytotoxic effect of test chemicals was calculated, similarly the genotoxicity was measured by anaphase-telophase cells per dose of chemical for assessing chromosome fragments, bridges, vagrant chromosome, c-anaphase, multipolarity and stick chromosomes and also by comparing the percentage of aberrant cells.

Maria et al., (2002) have reported the effects of aqueous extracts of *Typha domingensis* on *Allium cepa* L. seed germination and roots. The study was carried out
by conducting germination bioassay method and microscopic examination. Extracts of *Typha domingensis* has delayed and inhibited the growth of onion roots and significantly reduced the percentage of germinating seedlings after extract treatment. The aqueous extracts of *Typha domingensis* roots were having inhibitory effect compared to the aqueous extracts of stem. Extracts of leaves and leaves extracts which have lesser inhibitory effect. Other changes such as impaired cell proliferation, distraction of cell wall integrity, loss of root caps, severe dehydration, decrease in mitotic activity, was also observed.

The mutagenic effect of chloroquine on onion roots treated with various concentrations such as 20%, 50% and 100% at 6hr, 12hr and 18hr of interval respectively showed that chloroquine has induced cell mitotic abnormalities like anaphase, C-metaphase and clumping of chromosomes with above concentrations and different time of exposure authenticated chloroquine as a mitotic depressor and it is mutagenic to plant cell at higher concentrations (Nwangburuka and Oyelana, 2011)

Several investigators have worked on the observations of mitosis in root tip cells of *Triticum turgid* treating with ethidium bromide, the RNA synthesis inhibitor which induce abbreations of cells by forming incomplete chromatin condensation and nuclear envelop breakdown; causes delay in maturation of prophase microtubule band and assembly of microtubule in cells, displaying an interphase appearance of nucleus prevention of prophase spindle formation, disorganization of a typical pre nuclear metaphase spindle in cells; inhibition of the anaphase spindle formation and also chromosome movements; disorganization of the a typical mitotic spindle during transition from mitosis to cytokinesis. It is also reported that ethidium bromide treatment arrests dividing root tip cells of *Allium cepa* at prophase stage (Risuefio and Moreno 1978; Gonzfilez-Fernfindez et al., 1970; Galati and Apostolakos, 2000).
Research on the effect of benomyl a fungicide of *Allium cepa* studied on the mitotic cell division in onion root tip cells during germination at different concentrations caused several abnormalities in mitotic cell divisions and the mitotic frequency in the onion root tip cells decreased with the increase in the concentrations of benomyl solution (Dane and Dalgic, 2003).

The onion root tip model to study the regulation of mitotic index, treating with N1, N3 Bis (6-chlorobenzo (d) thiazol-2-yl) 2–substituted methyl malonamides. The experimental observations of both the compounds have shown considerable antimitotic activity in concentration dependent manner. The antimitotic activity was found to be increased by more than 20% when the test concentration was increased by two fold. However monomeric compounds did not show any such activity. These results clearly indicate that all the compounds possess significant antimitotic activity at both the test concentrations. In the series compounds IVj (Nitro) was found to be more potent with 54 and 74% inhibition of mitosis at 10 and 20µg/ml respectively. It was followed by the compounds IVc (Aniline) & IVe (Chloro) which have showed 70% inhibition at 20µg/ml concentration (Veni et al., 2013).

Mahamuni et al., (2012) have demonstrated done the cell growth inhibitory activity in onion root model to find out the lead extract. Seven different extracts of *Lagenaria siceraria* were used in search of lead extract. n-butanol, chloroform and ethyl acetate extract are shown higher antimitotic activity among seven extracts. The n-butanol extracts mitotic index was found to be good compare to others and selected for further studies.

The antimitotic activities of alcoholic extract of four different plants such as *K. galanga, C. viscosum, J. curcas and L. culnaris* at different concentrations in onion root tip model was studied by Bagya et al., (2011). The results of their study indicated
that standard drug vincristine showed a maximum activity at all concentrations, while alcoholic extracts of all four plants have inhibited mitosis in dose dependent manner.

Suggestions of Rai and Murthy (1986) on the antimitotic activities of some synthetic derivatives of podophyllotoxin in onion root tip for determining the antimitotic activities is quite valid and reliable for screening the chemical compounds.

The *Allium cepa* root tip meristem is used as a standard model to study the cytotoxic and antimitotic activites of *Calotropis procera* and podophyllotoxin comparing with standard cytotoxic drug cyclophosphamide and non-cytotoxic drugs Cyproheptadine and aspirin indicated that cyclophosphamide, *Calotropis procera* and podophyllotoxin have significantly inhibited the growth of roots and mitotic activity in a dose dependent manner, while podophyllotoxin acts as a more potent drug which induced root decay, where as Cyproheptadine and Aspirine have showed a marginal effect on the root growth and mitotic activity at very higher concentration (Sehagal et al., 2006). The in vitro antimitotic and genotoxic effect of *Trichozanthus dioica* root extract on *Allium cepa* root meristem treating with different concentration of the extracts under specific experimental conditions to determine the root growth inhibition and mitotic index. The observations revealed that inhibition of root length and number and reduction in mitotic index was concentration dependent indicating genotoxicity of the plant extract (Bhattacharya and Haldar, 2012).

Petroleum ether, chloroform, ethanol and water extracts of *Revia hypocrateriformis* used to test the antimitotic effect of successive chloroform and ethanol extracts which have inhibited the meristematic cell growth in different phases of cell cycle, on the other hand petroleum ether and water extracts have displayed a marginal activity. The mitotic index of chloroform and ethanol extracts were found to
be 14.24 and 12.14 respectively which was close to methotrexate a standard antimitotic drug (Shweta et al., 2012).

Investigation of Aslan turk., (2006) throws light on oxidative stress induced anti-mitotic and anti-genotoxic effect of *P. lanceolata* L. leaf aqueous extracts (15 g/L and 30 g/L) on *Allium cepa* L. root tip meristem cells. In their experiment they have used H₂O₂ as stress inducer in plant cells. They have conducted experiment by inducing oxidative stress with 0.7% hydrogen peroxide, before treating with plant extracts and after treating with plant extract. Bulbs of onions were treated with two different concentrations of 15 g/L and 30 g/L of *P. lanceolata* extracts for a time period of 24h. 0.7% H₂O₂ used as positive control and tap water used as negative control. From the experimental findings it was observed that aqueous extracts has reduced mitotic index and chromosome aberrations in treated groups when compare with control groups.

The result from the study of Tenimozhi and Rao (2011) revealed that the aqueous extract of *Solanum torvum* has shown excellent anti-mitotic activity that was comparable to the activity of methotrexate, in which maximum numbers of non-dividing cells were observed. The number of cells which are entering into prophase stage was found to be less. Hence, prophase cell population was less, it indicates reduced cell division. Methotrexate anticancer drug inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the tetrahydrofolate synthesis.

**Clot lysis activity of plant extracts**

A blood clot (thrombus) develops in the circulatory system due to failure of homeostasis which causes vascular blockage and while recovering leads to serious consequences in thrombotic diseases such as myocardial or cerebral problems, at times leading to death (Yamamoto et al., 2005). Thrombolytic agents that include
tissue plasminogen activator (t-PA), urokinase (UK), and streptokinase (SK) are used across the world for the treatment of these diseases. In India, though streptokinase and urokinase are widely used due to lower cost, (Dwivedi, 2007; Collen, 1990) as compared to other thrombolytic drugs. But due to the weak substrate specificity of these first generation drugs (streptokinase and urokinase), they lead to systemic fibrinolysis, anaphylactic reaction, and bleeding complications (hemorrhage) (Ahmed et al., 2014). Again, multiple treatments with streptokinase are restricted in a given patient as a result of immunogenicity (Furie and Furie 2008). Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs (Mucklow, 1995; Rajkumar et al., 2009; Londonkar and Kirankumar, 2014).

Sikder and co workers (2013) have studied on invitro thrombolytic activity of four Bangladeshi medicinal plants Sansevieria trifasciata, Justica gendarussa, Hydnocarpus kurzii and Mesua nagassarium by the modified kupchan partitioning method to prepare soluble fractions of Mesua nagassarium, CCl₄ soluble fractions of Hydnocarpus kurzii, aqueous soluble fractions of metahnolic extract of Sansevieria trifasciata have shown significant thrombolysis activity with a clot lysis value of 50.86%, 47.50%, and 47.10% respectively. On the other hand the petroleum ether and CCl₄ soluble fractions of metahnolic extracts of Justica gendarussa have shown moderate thrombolytic activity compare with standard streptokinase exhibited 61.50% of lysis and negative control vehicle treated have shown 2.56% clot lysis.

Reports of Hossain et al., (2012) demonstrated the thrombolytic activity of ethanol extract of Swertia chirata along with its anti-inflammatory activity and phytochemical screening. The crude ethanol extract and chloroform fractions have showed average clot lysis of 46.38% while the standard streptokinase has showed
69.35%. The percentage of clot lysis was found to be significant when compared with the vehicle treated control.

Many researchers worked on the crude methanolic extract of *Bougainvillea glabra* to evaluate its possible thrombolysis activity. Plant material was extracted by employing hot extraction method. The *Bougainvillea glabra* crude methanol extract has shown appreciable clot lysis potency with a maximum activity of 94.5% at a concentration of 800µg/ml with incubation period of 76hrs, on the other hand standard drug streptokinase has shown 98.5% with same incubation period. Negative control water dissolved the clot up to 52.4%. (Elumalai et al., 2012).

Islam et al., (2013) have displayed the in vitro thrombolytic potential of crude methanolic extract of *T. crispa* stem using human blood. The study relies on cheap and simple technique which suits to the underdeveloped countries like Bangladesh. *T. crispa* stem extracts was prepared in a saline at different concentrations (2.5mg/ml - 20mg/ml) and mixed with blood clots and kept for incubation about 90 minutes at room temperature. Clot lysis activity of extracts was observed average to good (14.81%-25.73%) and standard thrombolytic agent streptokinase dissolved the clot to 50.1%.

The methanolic extract of leaf of *Pothos scandens* was screened for cytotoxic assay using brine shrimps and in-vitro thrombolytic activity. In thrombolytic assay the *P. scandens* methanolic extract has diluted the clot to a value of 19.45%. Compared to positive control streptokinase (SK) which has shown 69.48% and negative control water has demonstrated 3.06% thrombolysis (Yusuf et al., 2013).

The organic soluble extractives of three *Bridelia species*, *B. verrucosa*, *B. stipularis* and *B. tomentosa* growing in Bangladesh were subjected to screening for free radical scavenging activity, total antioxidant capacity, total phenolic content and
thrombolytic activity. All the extractives of three plants were also studied for their thrombolytic potential. Among the three plants the carbon tetrachloride soluble fraction and methanol extract of leaf and aqueous soluble fraction of bark of *B. tomentosa*, methanol extract of bark of *B. stipularis* and carbon tetrachloride soluble fraction of leaf of *B. verrucosa* exhibited highest thrombolytic activity with clot lysis value of 41.46%, 34.85%, 37.04%, 36.45% and 33.72%, respectively. Standard streptokinase was used as positive control which exhibited 61.50% lysis of clot while the negative control water revealed 2.56% lysis of clot. (Rashid et al., 2013).

Thrombolytic, cytotoxic and antidiabetic Effects of *Paederia foetida* L. Leaf extract in in-vitro thrombolytic assay, *P. foetida* extract lysed the clot 21.40 ± 1.39%. This percentage of clot lysis was statistically different from the maximum clot lysis 81.42 ± 0.88% for the positive control streptokinase (SK) and 4.63 ± 0.31% for negative control. However, the combined effect (32.25 ± 0.64%) of SK and *P. foetida* was close to that of *P. foetida* alone and the effect was comparable to SK. The mean difference in clot lysis percentage between positive and negative control was also very significant (Ahmed, 2014).