Traditional systems of medicine continue to be widely practiced for many reasons. Population rise, inadequate supply of medicines, prohibitive cost of treatments, side effects of several allopathic medicines and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for many human diseases. Global estimates indicate that 80% of about 4 billion population cannot afford the modern medicines and have to rely upon the use of traditional medicines which are mainly obtained from the plants.

Pharmaceutical research took a major leap when alongside natural products chemistry; pharmacologists, microbiologists and biochemists began to unravel the chemistry of natural processes in human, animals, plants and microorganisms. Advances in synthetic organic chemistry led to the identification of many key chemical molecules that offered more opportunities to develop novel compounds. Many new drugs emerged by this route, particularly those now being used to treat infections, infestations, cancers, ulcers, heart and blood pressure conditions. Many drugs were developed through random screening of thousands of chemicals synthesized as dye-stuffs and the like; many others resulted from serendipity arising from sharp-eyed observations of physicians and scientists. Examples of such drugs include sulphonamides, isoniazid, anti-psychotics, anti-histamines and penicillin (Clark, 1996).

Plant based drugs provide outstanding contribution to modern therapeutics; for example: serpentine isolated from the root of Indian plant *Rauwolfia serpentina* in 1953, was a revolutionary event in the treatment of hypertension and lowering of blood pressure. Vinblastine isolated from *Catharanthus roseus* (Farnsworth *et al*. 1967) is used for the treatment of hodgkins choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer. Vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymphosarcoma, small
cell lung, cervical and breast cancer (Farnsworth and Bingel, 1977). Taxol isolated from *Taxus brevifolius* is used for the treatment of metastatic ovarian cancer and lung cancer. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer.

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

**PHYTOCHEMISTRY**

Phytochemistry is the branch of natural product chemistry that deals with qualitative and quantitative analysis of herbal drugs. Phytochemistry in the strict sense of the word is the study of phytochemicals. These are the secondary metabolites synthesized by the plants as part of their normal metabolic activities. Many of these are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers.

Isolation of phytoconstituents is of great importance for the development of bioactive substances from medicinal plants. In the development of drugs and therapeutics, the use of plants as medicines has involved the
isolation of active constituents, beginning with the isolation of morphine from opium in 1803 (Samuelsson, 2004; Newman et al. 2004). Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, digitoxin, emetine, strychnine, and quinine in addition to morphine, of which some are still in use (Graul, 2001; Butler, 2004).

Drugs isolated from medicinal plants can serve not only as new drugs themselves but also as drug leads. In the recent past, many bioactive phytoconstituents were isolated or derived from natural products. Some of them are galanthamine (Trade name: Reminyl), a natural product discovered through an ethnobotanical lead and first isolated from Galanthus woronowii in Russia in the early 1950s. It is approved for the treatment of Alzheimer’s disease (Pirttila, 2004). Arteether (Trade Name: Artemotil) is a potent antimalarial drug and is derived from artemisinin, a sesquiterpene lactone isolated from Artemisia annua, a plant used in Traditional Chinese Medicine (Heinrich and Teoh, 2004). Tiotropium (Trade name: Spiriva) has been introduced to the US market for treatment of chronic obstructive pulmonary disease. Tiotroprium is based on ipratropium, a derivative of atropine isolated from Atropa belladonna (Mundy and Kirkpatrick, 2004). Vinfluinine is a modification of vinblastine from Catharanthus roseus for use as an anticancer agent with improved efficacy (Cragg and Newman, 2004) and camptothecin and its analogs initially discovered in the Chinese tree Camptotheca acuminata Decne as anti-cancer agents. (Kinghorn et al. 2011; Cragg and Newman, 2013).

Therefore, it is important to use the phytochemical methods to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. The plant species may contain a vast range of compounds such as alkaloids, terpenoids, flavonoids, glycosides etc. The study paves the way to the discovery of new therapeutic compounds as needed by the modern scientific approach of producing medicines. The phytochemical investigation of a plant involves:
• Authentication and extraction of the plant material.
• Separation and isolation of the phytoconstituents of interest.
• Characterization of the isolated compounds
• Investigation of the biosynthetic pathways to particular compounds and quantitative evaluations.

Mulberry is known for medicinal values and has been used by indigenous tribes to cure ailment of several kind. The genus *Morus* which comprises about a dozen of species has been the subject of intense experimentation to isolate compounds of therapeutic values. However most of these studies are mainly concentrated around white mulberry, *Morus alba*.

The plant is a very good source of ascorbic acid, of which over 90% is present in a reduced form and also contains carotene, vitamin B1, folic acid, folinic acid, isoquercetin, quercetin, tannins, flavonoids and saponins, which act as a good source of natural antioxidants (Anonymous, 1952). White mulberry leaf contains triterpenes (lupeol), Sterols (β-Sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids and organic acids. *Morus alba* leaves contain rutin, quercetin and apigenin as bioactive constituents (Doi et al. 2001). *Morus alba* leaf extract has been found to produce nitric acid, prostaglandin E2 and cytokines in macrophages. Many flavones were isolated from the root bark as active principles (Chu et al. 2007). Many biochemical compounds such as moranoline, albafluran, albanol, morusin, kuwanol, calystegin and hydroxymoricin are isolated from mulberry plants which play an important role in pharmaceutical industry (Bose, 1984). The plant is reported to contain the phytoconstituents tannins, phytosterols, sitosterols, saponins, triterpenes, flavonoids, benzofuran derivatives, morusimic acid, anthocyanins, anthroquinones, glycosides and oleanolic acid as the main active principles (Anonymous, 1952; Nomura et al. 1994; Kusano et al. 2002; Chen et al. 2004). Table 2.1 summarizes the details of phytochemicals present in different species of mulberry (Kumar and Chauhan, 2008).
Table 2.1. Phytochemicals in different *Morus* species

<table>
<thead>
<tr>
<th>Name of Species</th>
<th>Active Constituent</th>
<th>Part used</th>
<th>Medicinal Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Morus alba</em></td>
<td>Kwanon I, Kwanon I hexamethyl ether, Kwanon I octamethyl ether, 2’-hydroxy-2,4,4’-trimethoxychalcone and 2’-Hydroxy 3’ prenyl-2,4,4’-trimethoxychalcone III, Mulberrofuron T and Kwanon E, Morusin, Mulberrofuran D, g, k, Kwanon G, H, Mulberries A, Cis mulberries A, Oxyresveratrol, Isoquercetin, Kwanon G, Moracin E, F, G and H, Kwanon D, E, F, Deoxynojirimycin-1 etc.</td>
<td>Roots, Stem, leaves, fruit</td>
<td>Astringent, Anthelmintic, HIV, cough, anti-inflammatory, exudative, high blood pressure, diaphoretic, purgative, emollient, diarhoea, diabetes, atherosclerosis, anti-tumor, hypoglycemic etc.</td>
</tr>
<tr>
<td><em>Morus australis</em></td>
<td>Australone A, triterpenoid 3B-[(m-methyl benzol) oxy] urs-12-en-28-oic acid, morusin, Kwanon C, betunilic acid, B-amyrin, quercetin, ursolic acid, Mulberrofuran D, Sanggenols N and O etc.</td>
<td>Root, leaves, fruits</td>
<td>Astringent, anthelmintic, purgative, anti-platelet etc.</td>
</tr>
<tr>
<td>Morus laevigata</td>
<td>Citrulline, Hydroxyprolines, free amino acids</td>
<td>Fruits</td>
<td>Plaster for sores, cools the blood</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------------</td>
<td>--------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Morus nigra</td>
<td>Deoxynojirimycin</td>
<td>Roots, leaves, Fruits</td>
<td>Diabetic, AIDS, purgative, arterial pressure, vermifuge, cancer etc.</td>
</tr>
<tr>
<td>Morus serrata</td>
<td>B-Amyrin acetate, betunilic acid, cerylalcohol, quercetin and morin</td>
<td>Roots</td>
<td>-</td>
</tr>
<tr>
<td>Morus rubra</td>
<td>Rubraflavones A,B,C,D</td>
<td>Roots</td>
<td>Anti-dysentric, laxative, purgative vermifuge, urinary problems weakness etc.</td>
</tr>
</tbody>
</table>
PHARMACOLOGY STUDIES

In the present investigation, leaves of three different species of mulberry were selected for studying various pharmacological activities such as antioxidant, anticancer, analgesic, anti-inflammatory, wound healing, CNS depressant, anthelmintic and antimicrobial activities along with the in silico toxicology and in silico pharmacology studies and the literature survey pertaining to these are explained here under.

ANTIOXIDANT ACTIVITY

Molecular oxygen is an essential component for all living organisms particularly aerobic organisms. Oxygen sustains them, but it also poisons them through reactive intermediates called free radicals produced during respiration. Free radicals have been implicated in the etiology of several human diseases as well as aging. But they are continuously produced in the human body, as they are essential for:

- Generation of ATP from ADP in the mitochondria: oxidative phosphorylation.
- Detoxification of xenobiotics
- Apoptosis of defective cells.
- Killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes.
- Oxygenases for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

Sources of Free Radicals

Free radicals, the partially reduced metabolites of oxygen, are highly toxic, mutagenic and reactive. A free radical is a molecule with one or more unpaired electrons in its outer orbital. Many of these molecular species are oxygen (and sometimes nitrogen) centered. These highly unstable molecules tend to react rapidly with adjacent molecules donating, abstracting or even sharing their outer orbital electron(s). This reaction not only changes the adjacent target molecule, but often passes the unpaired electron along to the
target, generating a second free radical or other ROS, which can then go on to react with a new target and generates molecular chain of reactions (Gutteridge and Halliwell, 2000). They are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air-pollution, pesticides etc. (Li and Trush, 1994) (Table 2.2).

Generation of oxygen free radicals begins within mitochondrial inner membrane when cytochrome oxidase catalyzes the four electron reduction of oxygen ($O_2$) to water ($H_2O$). Intermediate between reactions of $O_2$ to $H_2O$, the following three partially reduced species of oxygen are generated depending upon the number of electrons transfer:

- Superoxide oxygen ($O_2^-$): one electron
- Hydrogen peroxide ($H_2O_2$): two electrons
- Hydroxyl radical ($OH^-$): three electrons.

**Table 2.2: Types of Free Radicals (Robert A. Jacob, 1999)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>$HO^-$</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>$HO_2^-$</td>
<td>Hydroperoxyl radical</td>
</tr>
<tr>
<td>$O_2^-$</td>
<td>Superoxide anion radical</td>
</tr>
<tr>
<td>$O^-$</td>
<td>Singlet oxygen</td>
</tr>
<tr>
<td>$RO^-$</td>
<td>Alkoxyl radical</td>
</tr>
<tr>
<td>$ROO^-$</td>
<td>Peroxy radical</td>
</tr>
<tr>
<td>$NO^-$</td>
<td>Nitric oxide radical</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>$HOC_1$</td>
<td>Hypochlorous acid</td>
</tr>
</tbody>
</table>
**Free radicals and diseases**

Free radicals are electrically charged unguided missiles that attack our cells, cause damage to biomolecules namely proteins, lipids, nucleic acids, enzymes *etc.* present in the body. This attack by free radicals is collectively known as oxidative stress. They react with serum lipoprotein (LDL), cell membrane lipid causing lipid peroxidation and resulting in the generation of further free radicals. Normally there is a balance between the amount of free radicals generated in the body and the antioxidant defense systems that scavenge/quench these free radicals preventing them from causing deleterious effects in the body (Nose, 2000).

A majority of the present day diseases are reported to be due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of the present day life, or due to the poor scavenging / quenching in the body caused by depletion of the dietary antioxidants (Dringen, 2000; Schulz *et al.* 2000). The antioxidant defense systems in the body can only protect the body when the amount of the free radicals is within the normal physiological level. But when this balance is shifted towards more of free radicals, it leads to oxidative stress, which results in tissue injury and subsequent diseases.

The role played by the oxidative stress has been implicated in the etiology of large number of human disorders and diseases like rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastro-intestinal diseases and AIDS as well as the process of aging. Some specific examples of ROS mediated diseases include Alzheimer’s disease, Parkinson’s diseases, atherosclerosis, cancer, Down’s syndrome and ischemic reperfusion injury in different tissues including heart, liver, brain, kidney and gastro-intestinal tract.
Antioxidants
Free radicals are not completely bad. The macrophages and neutrophils use them to destroy pathogens and other foreign invaders. However, excessive production or production in the wrong place can be harmful both acutely and chronically. Therefore, the body needs antioxidant compounds. An antioxidant is a substance that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate (Dormandy, 1978; Halliwell, 2000). These are also known as free radical scavengers. They function by offering easy electron targets for free radicals. In taming free radical, antioxidants “trap” (de-energize or stabilize) the lone free-radical electron and makes it stable enough to be transported to an enzyme which combines two stabilized free radicals together in order to neutralize them. They also can act at different levels of protection such as prevention, interception and repair. Antioxidants are required in the different compartments of the body, such as the cardiovascular system, the cell membranes, inside the cells and across the blood-brain barrier into the central nervous system to combat the damaging effects of free radicals in the body.

Classification of Antioxidants
Antioxidants may be classified into two major groups namely, endogenous and exogenous antioxidants (Venkat Ratnam et al. 2006).

Endogenous antioxidants
There are a large number of enzymatic and non-enzymatic physiological substances known to have “antioxidant-like” functions, but the important ones are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH).

SOD is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. It is the antioxidant enzyme that catalyzes the dismutation of $O_2\cdot-$ to $O_2$ and to the less-reactive species $H_2O_2$. 

CAT is an antioxidant heme enzyme, located in a cell organelle called the peroxisome. The enzyme very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen.

\[
\text{Catalase} \quad 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

GPx is a selenium containing enzyme which catalyses the reduction of \(\text{H}_2\text{O}_2\), lipid hydroperoxide, generated during lipid peroxidation, to water using GSH as substrate.

The other antioxidant enzymes are thioredoxin, heme oxygenase and biliverdin reductase. The hormone melatonin is also an important antioxidant.

**Exogenous antioxidants**

Plants (fruits, vegetables, medicinal herbs *etc.*) are the important sources of exogenous antioxidants which contain a wide variety of free radical scavenging molecules/antioxidant principles, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, carotenoids *etc.* (Larson 1988; Shahidi and Naczk 1995; Cotelle *et al.* 1996; Velioglu *et al.* 1998; Zheng and Wang, 2001; Cai *et al.* 2003). Among these, vitamin E (\(\alpha\)-tocopherol), vitamin C (ascorbic acid), lycopene and \(\beta\)-carotene are important and deficiency of these may lead to a number pathophysiological consequences. They possess lipid protection properties and are highly efficient in mopping up free radicals. In addition to these antioxidants, other antioxidants used by the body are trace elements, zinc, selenium, lipoic acid, albumin, uric acid, co-enzyme-Q10, bilirubin *etc.* Selenium is a trace mineral, which enhances the properties of vitamin E and is also required by the body for the synthesis of the enzyme glutathione peroxidase. Selenium and vitamin E both appear to be necessary for efficient scavenging of peroxides from cytosol and cell membrane. The endogenous antioxidant supply to the body is limited and is used up continuously in the process of neutralizing free radicals. Therefore, there is a constant need to replenish antioxidant resources either endogenously or through supplementation.
Methods for antioxidant activity

Several methods are used to measure the antioxidant activity of a biological material. The most commonly used ones are those involving chromogen compounds of radical nature that stimulate the reductive oxygen species. These methods are popular due to their ease, speed and sensitivity. The presence of antioxidants leads to the disappearance of these radical chromogens. The most widely used methods are free radical scavenging assays—DPPH (1,1-diphenyl-2-picryl hydrazyl) assay, Super oxide anion (O2•−) radical scavenging, peroxide (H2O2) radical scavenging, nitric oxide (NO) radical scavenging, hydroxyl radical (OH−) scavenging, Protein oxidation and total flavonoid concentration, malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assays for estimation of lipid peroxidation in membrane and biological systems, total antioxidant capacity, total phenolic contents etc. These are non-enzymatic models based on chemical reactions of the plant extracts/constituents with standard reagents (Indu and Geetha, 2006).

In enzymatic models the study is carried out in rats/mice, by damaging the liver with hepatotoxic substances like CCl4, Paracetamol etc. The activity of various antioxidant enzymes SOD, CAT, GPx and non-enzyme GSH are estimated in liver (Ajay et al. 2007).

Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate, IC50 or EC50 values (concentration which can achieve 50% scavenging) etc. The results are compared with the standard antioxidants viz. ascorbic acid (vitamin C), Butyl hydroxytoluene (BHT) etc.

Plants as a source of antioxidants and radical scavengers

In recent years, the use of natural antioxidants present in plants, vegetables, fruits and herbal drugs has attracted considerable interest due to their safety, nutritional and therapeutic value (Ajila et al. 2007). Nutraceuticals are going to hold the key to a healthy society in future. The quest for natural
antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades.

India has a rich history of using various herbs and herbal products for treating various diseases. Indian medicinal plants provide a rich source of antioxidants. There are also a number of ayurvedic formulations showing antioxidant activities namely Abana, Amrita bindu, C-phycocyanin, Centalaplus, Chapparal, Geriforte, Jigrine, Liv-52, Maharishi formulations, Muthu marunthu, Ophtacare, P55A, Sandhika, Student rasayana and Tamra bhasma.

However, there are still a large number of plants and ayurvedic formulations whose antioxidant activity need to be studied in relation to their potential therapeutic values. This will help to a great extent in identifying more potent compounds with potential applications in prevention and/or in the treatment of human ailments especially among people who do not have access to the use of expensive modern medicines.

There are several examples of isolation and extraction of antioxidants from plant materials viz. herbal cigarettes, tea and capsules, (Siegel, 1976); Paetta indica and Osbeckia octandra (Thabrew et al.1987); Chrysanthemum morifolium (Duh, 1999); Salvia reflexa (Malencic et al. 2000); Cetraria islandica, (Gulcin et al.2002); Ardisia compressa (Sonia and de Mejia, 2004); Cytisus scoparius (Raja Sundararajan et al. 2006); Zanthoxylum piperitum (Yamazaki et al. 2007); Carya cathayensis (Chenggang Zhu et al. 2008); Boerhaavia diffusa (Rachh et al. 2009); White grape (Anamarija et al. 2009); H. officinalis L. Var. angustifolius, V. odorata, B. hyrcana and C. speciosum (Ebrahimzadeh et al. 2010); Pandanus conoideus Lam (Rohman et al. 2010); Cassia occidentalis (Mehta et al. 2010); Tecomastans (Govindappa et al. 2011); Miconia albicans (Pieroni et al. 2011); Hypochaeris radicata L (Jamuna et al. 2012); Pongamia pinnata (Saiprasanna et al. 2012); Ganoder malucidum (Huihua et al. 2013); Coscinium fenestratum (Goveas et al. 2013); Arisarum vulgare (Kadri et al. 2013); Coriandrum sativum (Tang et al. 2013); Morinda
REVIEW OF LITERATURE

citrifolia (Kumar et al. 2014); Bixa orellana (Abayomi et al. 2014); Sonchus oleraceus (Jain and Singh, 2014); Alocasia indica (Swagata et al. 2014); Vaccinium corymbosum L (Contreras et al. 2015); Agave americana Linn (Mannasaheb et al. 2015); Hypericum perforatum (Ghosian et al. 2016); Acacia catechu (Saha et al. 2016) etc.

Screening for antioxidant potentials in mulberry particularly in fruits has been the matter of intense research since long time. Aysel Syvaci and Munevver Sokmen, (2004) investigated the seasonal changes of antioxidant activity in Morus nigra L and Morus alba L using stems. Takuya Katsube et al. (2006) isolated the antioxidant flavonol glycosides in mulberry (Morus alba L.) leaves based on antioxidant activity and reported that quercetin 3-(6-malonylglucoside) and rutin were the predominant antioxidants in the mulberry leaf. Antioxidant effect of Morus bombycis Koidzumi roots and the presence of purified compound 2,5-dihydroxy-4,3-di-trans-stilbene displayed dose dependent superoxide radical scavenging activity (Jin et al. 2006). Eight phenolic compounds purified from Morus yunanensis stem bark showed potential antioxidation activities (Cui et al. 2008). Nikkhah et al. (2008) evaluated in vitro screening for antioxidant activity of blackberry (Morus nigra) and demonstrated that anthocyanin pigment in blackberry is an excellent natural antioxidant and a free radical scavenger. Antioxidant activity of Morus leaf was tested using silica gel column chromatography and isolated constituents were analyzed in vitro for antioxidant activity against DPPH and ABTS radicals (Jiang et al. 2008; Wang et al. 2011). Four flavonoids were isolated and all these compounds showed DPPH and ABTS radical scavenging activity (Zhang et al. 2009). In an another study radical scavenging activity of different parts of mulberry (Morus alba L.) were determined methanol extracts and their fractions dose dependently increased radical scavenging activity of mulberry branches, roots and leaves (more than 70%). Study shows that mulberry fruits exhibited the highest radical scavenging activity (Chon et al. 2009). Antioxidant potential of fruits of four mulberry species namely Morus
alba, Morus nigra, Morus indica and Morus laevigata were studied and it was concluded that mulberry fruit is a potential source of radical scavenging activity (Imran et al. 2011). The antioxidant activity of leaf extracts was evaluated by measuring 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging activity, 2,2’-azino-bis-(3- ethylbenzthiazoline-6-sulphonic acid (ABTS•+) radical cation scavenging capacity and ferric ion reducing power. The investigated features reveal good antioxidant attributes significantly (Shahid et al. 2012). Methanolic extract of fruit showed a correlation with total phenolic constituents of the respective fruits. Ethanol and hexane extracts were investigated in an in vitro air dried leaves and fruit juice of some Ficus and Morus species (Awad et al. 2012). White mulberry stem bark, root bark, leaves and fruit content of methanolic extract was evaluated by in vitro method and results revealed that, among the extracts, stem bark showed highest antioxidant activity and hence, plant could serve as effective free radical inhibitor (Khan et al. 2013).

ANTICANCER ACTIVITY

Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources (Kinghorn et al. 2000). Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth (Krishnamurthi, 2007). Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism) (Divisi et al. 2006).

Natural products have been regarded as important sources of potential chemotherapeutic agents and many anticancer drugs have originated from natural sources. According to Cragg and Newman over 50% of the drugs in clinical trials for anticancer properties were isolated from natural sources or are related to them (Cragg et al. 2000). Several natural products of plant origin
have potential value as chemotherapeutic agents. The plant based drug discovery resulted mainly in the development of anticancer agents including plants (vincristine, vinblastine, etoposide, paclitaxel, camptothecin, topotecan and irinotecan), marine organisms (citabrine, aplidine and dolastatin) and micro-organisms (dactinomycin, bleomycin and doxorubicin). Beside this there is numerous agents identified from fruits and vegetables can used in anticancer therapy. The agents include curcumin (turmeric), resveratrol (red grapes, peanuts and berries), genistein (soybean), diallyl sulfide (allium), S-allyl cysteine (allium), allicin (garlic), lycopene (tomato), capsaicin (red chilli), diosgenin (fenugreek), 6-gingerol (ginger), ellagic acid (pomegranate), ursolic acid (apple, pears, prunes), silymarin (milk thistle), anethol (anise, camphor, and fennel), catechins (green tea), eugenol (cloves), indole-3-carbinol (cruciferous vegetables), limonene (citrus fruits), beta carotene (carrots) and dietary fiber (Pezzuto, 1993; Tan et al. 2006; Abhishek et al. 2011).

The preventive mechanisms of natural phytochemicals on tumor promotion ranges from the inhibition of genotoxic effects, increased antioxidants and anti-inflammatory activity, inhibition of proteases and cell proliferation, protection of intracellular communications to modulate apoptosis and signal transduction pathways (Soobrattee et al. 2006).

There are around 460 species of plants that can be used as herb for remedy, including plant healer for various types of cancer. Some of such plant are zedoary (Curcuma zedoaria), rodent tuber (Typhonium flagelliforme), god’s crown (Phaleria macrocarpa), madagaskar periwinkle (Catharanthus roseus), artocarpus integer (Selaginella corymbosa), bamboo grass (Loathatreum gràcies), handsome (Taraxacum mongolicum), fruit makasar (Brucca javanica), garlic (Allium sativum), echo China (Smilax china), sunflower (Helianthus annus), leunca (Solanum nigrum), Job’s Tears (Coix lachryma-Jobi), bamboo rope (Asparagus cochinchinensis) and others (Umadevi et al. 2013). Apart from these plants several other plants have also been reported to have anticancer activity like Ocimum basilicum (Hanan et al. 2010); Moringa
oleifera (Asima et al. 2012; Charoensin, 2014); Ageratum conyzoides L (Adebayo et al. 2010); Excoecaria agallocha (Patil et al. 2011); Argemone mexicana Linn (Kiranmayi et al. 2011); Michelia champaca (Lee et al. 2011); Withania somnifera, Oroxyllum indicum and Calotropis gigentia (Das et al. 2012); Calea pinnatifida (Marchetti et al. 2012); Polygala rosmarinifolia (Alagammal et al. 2012); Cynodon dactylon (Kanimozhi et al. 2013); Tillandsia recurvata L (Henry et al. 2013); Croton cajucara (Rumkinath et al. 2013); Barleria grandiflora (Manglani et al. 2014); Ficus carica L, Olea europaea L, Salvia officinalis L, Teucrium polium L, Vitis vinifera L (Alzeer et al. 2014); Dioscorea bulbifera (Ghosh et al. 2015); Aristolo chiatagala and Curcuma caesia (Hadem et al. 2015) and Impatiens glandulifera (Cimmino et al. 2016).

Mulberry also contains several anticancer compounds. The literature survey revealed the anticancer activities in different plant extracts of the genus Morus (Nikkhah et al. 2008; Jiang Hao et al. 2011; Preeti Karade et al. 2012; Bandna Devi et al. 2013).

Tumourigenesis in Swiss albino mice was studied and the efficacy of Morus indica (Methanolic extract) was found to be useful as a therapeutic agent for cancer control as it blocks or suppresses events associated with carcinogenesis (Prasad et al. 2004). Prenylated flavanone, separated from ethyl acetate extracts of Morus alba root showed cytotoxic activity against hepatoma cells in rats (Kofujita et al. 2004). Methanolic extract obtained from Morus alba and its sub fractions obtained from aqueous butanol and chloroform fractions inhibited the NO production and significantly reduced the formation of tumor necrosis factor-α (TNF-α) in macrophages (Choi et al. 2005). Takashi Kikuchi et al. (2010) worked on Albanol A, isolated from the root bark of Morus alba L. and concluded that, albanol A may be a promising lead compound for developing an effective drug for treatment of leukemia. Stem bark of Morus wittiorum exhibited selective cytotoxicity against human ovarian cancer and human gastric cancer (Tan et al. 2010). Anthocyanins isolated from
Morus alba fruit showed inhibitory effect on invasion and migration of highly metastatic human lung carcinoma cells in dose-dependent manner (Colonna et al. 2012).

ANALGESIC ACTIVITY

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

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

Evaluation of analgesic agents is done by several methods some prominent ones are -physical stimulus (tail-flip method), thermal stimulus, hot plate method, tail flick method, tail immersion method, chemical stimulus, writhing test, writhing induced by 4% NaCl Solution and writhing induced by aconitine etc.

In view of the side effects of the synthetic analgesic drugs, investigators on the lookout for the safer ways in plants have been well documented viz. Caesalpinia ferrea (Carvalho et al. 1996); Psidium guajava (Kulkarni et al. 1999); Piperomia pellucid (Peter et al. 2001); Carthamus lanatus (Bocheva et al. 2003); Euphorbia decipiens (Ahmad et al. 2005); Euphorbia tirucalli L (Prabha et al. 2008); Mollugo pentaphylla Linn (Prabhat Kumar and Bhabani Shankar, 2009); Commiphor acaudata (Mohan et al. 2009); Taxus baccata L (Dutta et al. 2010); Boswellia serrata (Sharma et al. 2010); Trichosanthes bracteata (Verma et al. 2010); Terminalia arjuna (Biswas et al. 2011); Moringa oleifera (Kumbhare and Sivakumar et al. 2011); Jasminum sambac L (Rahman et al. 2011); Pinus roxburghii (Kaushik et al. 2012); Tamarindus indica (Suralkar et al. 2012); Camellia oleifera (Yong et al. 2012); Nyctanthes arbor-tristis (Kakoti et al. 2013); Vetiveria zezanuioides (Kamble et al. 2013); Nelsonia canescens Lam (Mohaddesi et al. 2013); Sarcochlamys pulcherrima (Ibrahim et al. 2014); Fumaria officinalis Linn (Sharma et al. 2014); Grewia crenata (Ukwuani et al. 2014); Acacia hydaspica R (Afsar et al. 2015); Ocimum Suave (Tesema et al. 2015); Cistus ladanifer L (Youbi et al. 2016).

Even though the mulberry has been used in herbal medicine for analgesic purpose (Fukai et al. 2003), previous work on analgesic activity is extremely scanty. Yamatake et al. (1976) reported the analgesic and anticonvulsive activities of water and butanol fractions of Morus alba root bark and found significant inhibition in writhing responses in tested animals.
ANTI-INFLAMMATORY ACTIVITY

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

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

Inflammation research involves a number of experimental models to study the anti-inflammatory activity. According to Lewis, (1989) there are two models viz. acute and chronic anti-inflammatory models. Acute models are designed to test drugs that modulate erythema, changes in vascular permeability, leukocyte migration and chemotaxis, phagocytosis-polymorphonuclear leucocytes and other phagocytic cells, measurement of local pain, antipyretic activity and local analgesic action and rat paw edema (Barbosa-Filho et al. 2006) while, chronic models are designed to find drugs that may modulate the disease process and these include sponge and pellet implants and granulama pouches which deposit granulation tissue, adjuvant induced arthritis and rabbit monoarticular arthritis which have an immune etiology (Lewis, 1989).
Natural products have long been recognized as an important source of therapeutically effective medicines for anti-inflammation (Cragg et al. 2003). Different approaches used to analyze the anti-inflammatory potential of plant and plant derived compounds have been developed in the past years. Further, traditional herbal medicines like Commiphora mukul, Boswellia serrata, Harpagophytum procumbens and Pluchea indica have been used for anti-inflammatory effect with success (Vohara and Dandiya, 1992).

Plants as anti-inflammatory agents

Practitioners of traditional Indian medicine use formulation for anti-inflammatory action with considerable success. Dashmoola (combination of roots of ten plants) is standard Ayurvedic remedy for anti-inflammatory diseases (Sharma et al. 1973). The anti-inflammatory activity of many medicinal plants have been scientifically evaluated viz. Curcuma amada (Mujumdar et al. 2000); Goniothalamus andersonii (Shigeo et al. 2001); Leucas aspera (Goudgaon et al. 2003); Calendula officinalis; Erigeron floribundus (Asongalem, 2004); Securidaca vitex negundo (Rasadah et al. 2005); Ruta graveolens (Ratheesh and Helen, 2007); Aloe buettneri (Metowogo, 2008); Bambusa vulgaris (Carey et al. 2009); Ocimum sanctum L (Thakur and pitre 2009); Rubia cordifolia Linn (Tailor et al. 2010); Barleria prionitis L (Khadse et al. 2011); Murraya koenigii (Darvekar et al. 2011); Quisqualis indica Linn (Yadav et al. 2011); Boswellia serrata (Afsar et al. 2012); Coffea arabica (Chandra et al. 2012); Ajuga bracteosa Wall (Singh et al. 2012); Nymphaea alba (Jacob et al. 2013); Brachystegi aeurycoma (Okenwa et al. 2013); Tecomastans (Prasanna et al. 2013); Garcinia pedunculata (Mundugaru et al. 2014); Senecio flammeus (Xiao et al. 2014); Crotalaria burhia buch.-ham (Talaviya et al. 2014); Artemisia maritima L (Irum et al. 2015); Achyranthes aspera Linn (Ndhlala et al. 2015); Allium sativum and Tagetes erecta (Palacios et al.2015); Bauhinia pulchella (Lopes et al.2016) etc.
Mulberry plants have a long history in traditional medicine as anti-inflammatory agents (Kim et al. 2003; Renu Sharma et al. 2008; Jiang Hao et al. 2011). The root epidermis of *M. alba* shown to have anti-inflammatory effects (Wang et al. 2002). Butanol extract of *M. alba* significantly reduced LPS-induced PGE2 production, TNF-alpha and COX-2 expression in RAW264.7 macrophages (Choi et al. 2005). Jiang Hao et al. (2011) studied the research progress on active ingredients and pharmacological functions of black mulberry (*Morus nigra* L.) and concluded the pharmacological functions including reducing blood sugar level, anti-tumor, anti-oxidation and anti-inflammation.

**WOUND HEALING ACTIVITY**

A wound is a disruption in the continuity of cells - anything that causes cells that would normally be connected to become separated. It is an intricate process in which the skin repairs itself after injury. In normal skin, the epidermis and dermis exists in steady-state equilibrium, forming a protective barrier against the external environment (Joan et al. 2003).

Wound healing or wound repair is an intricate process in which the skin or organ or tissue repairs itself after injury. Wound caused can be healed by a spontaneous process in the organism through a cascade of events, which starts by switching on various chemical signals in the body; this facilitates the restoration of anatomical continuity and function (Joan et al. 2003).

While partial thickness wound heals by mere epithelialization, the healing of full thickness wound which extends through the entire dermis involves more complex well-regulated biological events. Healing of wound follows a predictable chain of events. This chain of events occurs in a carefully regulated fashion that is reproducible from wound to wound. The phases of wound healing are overlapping, but are described in a linear fashion for the purpose of clarity. The five phases that characterize wound healing include; (1) Haemostasis, (2) Inflammation, (3) Cellular migration and proliferation,
(4) Protein synthesis and wound contraction, and (5) Remodeling to form scar (Joan et al. 2003).

In the traditional systems of medicine, various plants have been used to promote wound healing. Many investigators reported the wound healing effect of the various plant extracts such as Aloe vera (Udupa, et al. 1994); Trigonella foenum graecum (Taranalli and Kuppast, 1996); Hypericum mysorens (Mukherjee and Suresh, 2000); Nelumbo nucifera (Mukherjee, et al. 2000); Ginkgo biloba (Bairy and Rao, 2001); Bryophyllum pinnatum (Mahamood and Patil, 2002); Gmelina arborea Roxb (Shirwaikar et al. 2002); Terminalia arjuna (Madhura and Sushma, 2003); Eucalyptus globulus (Kusum et al. 2004); Sauussurea lappa (Ganachari et al. 2005); Diospyros cordifolia (Mankani et al. 2005); Madhu ghrita (Charde et al. 2006); Plagiochasma appendiculatum (Meenakshi et al. 2006); Embelia ribes (Kumara Swamy et al. 2007); Ocimum sanctum (Somashekar Shetty et al. 2008); Abutilon indicum (Roshan et al. 2008); Lantana camara (Mahmood et al. 2009); Malva sylvestris and Punica granatum (Pirbalouti et al. 2010); Bacopa monnieri (Sharath et al. 2010); Scorzonera species (Akkol et al. 2011); Vitis vinifera and Vaccinium macrocarpon (Nayak et al. 2011); Ranunculus pedatus and Ranunculus constantinopolitanus (Akkol et al. 2012); Solanum xanthocarpum (Dewangan et al. 2012); Albizzi alebbeck (Joshi et al. 2013); Ophioglos sumvulgatum (Clericuzio et al. 2014); Terminalia catappa (Khan et al. 2014); Argyreia speciosa (Yadav et al. 2014); Malva sylvestris and Solanum nigrum (Fahima et al. 2015) and Echium species (Eruygur et al. 2016).

Morus alba plant has been used by tribes for ailments such as asthma, cough, bronchitis, edema, insomnia, wound healing, diabetes, influenza, eye infections and nosebleeds (Anonymous, 1952). Manish Kaushik et al. (2013) evaluated the healing promoting potentials of leaves of Morus alba L. in albino rats using incision and excision model. From the observation it was found that the aqueous extract of Morus alba possess better healing ability than the ethanol extract. Wound healing potential of the Morus alba extract attributed to
the anti-oxidative potential of its major components such as oxyresveratrol and resveratrol or other flavonoids (Bhatia et al. 2014).

**CNS DEPRESSANT ACTIVITY**

The central nervous system (CNS) comprises of brain and spinal cord, in which the process information mediates with the help of chemical messenger viz. neurotransmitter, neuromodulators, neuroregulators, neuromediators and neurotropic (are the various factor which act via precise mechanism to mediate neurotransmission) and neurotransmitter viz. nor adrenaline, adrenaline, dopamine, Gamma Amino Butyric Acid (GABA), glutamate, acetylcholine, 5 hydroxyl tryptamine (5 HT) etc. and neuromodulator viz. prostaglandins (PGs), purines and neuropeptides interact with their respective receptor and control the various functions of central nervous functions (Seth, 2005).

According to the World health organization report (WHO, 2001) about 450 million people experience from a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment, so global burden of disease will rise to 15% by 2020 (Ruiz et al. 2006). Hence primitive human was among the first to be discovered the drug acting in the central nervous system. As drugs acting on CNS produces specific physiological and psychological effects they are not useful therapeutically, and from the indigenous system of medicine too many plants have been reported to have activity against CNS disorders and hence act as very useful remedies for the alleviation of human distress (Suba et al. 2002). Worldwide plant research for search of new therapeutic product in the treatment of neurological disorder has been progressed constantly, signifying the pharmacological effectiveness of different plant species in a variety of animal models (Ruiz et al. 2006). Many standard animal models are there for testing the preliminary CNS related pharmacological activities, which afford information about action of constituents present in the plants upon psychomotor performance, motor behaviour and neuro-toxicity. The depression activity gives an indication of the
excitability of the CNS and this decrease may be related to sedation resulting from depression of CNS (Franco et al. 2005).

Basic neuroscience offers the promise of improving our understanding of disease patho-physiology, identifying novel mechanisms that can be targeted by more effective pharmaco-therapies and screening of herbal sources of drugs. These considerations implicate the search for new CNS depressant and antidepressant agents that have a fast onset of action, with less side effects and a wider safety margin. Various plants are being used in complementary and alternative medicines for management of mood disorders (Santosh et al. 2011). Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders (Sembulingam et al. 1997; Tripathi, 2008).

Several herbal products are available all over the world with an acclaimed CNS depressant and antidepressant activity, which are considered to be less toxic and free from side effects like Barringtonia acutangula Linn (Balaji et al. 2008); Nyctanthes bortristis Linn (Das et al. 2008); Sterculia guttata (Katade et al. 2009); Nardostachys jatamansi DC and Coscinium fenestratum Colebr (Prashith et al. 2009); Lawsonia inermis (Luthfun et al. 2010); Momordica dioica Roxb (Maharudra et al. 2010); Acalypa indica Linn (Ramakrishnan et al. 2011); Cocos nucifera (Dilipkumar et al. 2011); Ficus bengalensis (Rahman et al. 2011b); Mimuso pselengi (Kasimala et al. 2012); Derris trifoliate (Mamoon et al. 2012); Sesbania grandiflora (Sutradhar et al. 2012); Kalanchoe pinnata Lam (Matthew et al. 2013); Piper methysticum (Selvan et al. 2013); Typha angustata (Ashok et al. 2014); Erythrina variegata (Murugalakshmi et al. 2014); Alpinia oxyphylla (Chauhan and Swapna, 2015).

The work on CNS activity in mulberry is very scarce. The sedative and hypotensive effects of the mulberry root found in animal experiments are by the prevention of palsy accompanying cerebral apoplexy (Yamatake et al, 1976). Anand Sohit (2011) evaluated the antidepressant potential of aqueous extract of leaves of Morus alba and reported that, decrease in the levels of
monoamine neurotransmitters and oxidative stress are important factors involved in pathogenesis of depression. The report attributes *Morus alba* (white mulberry) leaves contains flavonoids which are known to inhibit catechol-o-methyl transferase (COMT) and mono amine oxidase (MAO) and have free radical scavenging property and may prove beneficial for the treatment of depression.

**ANTHELMINTIC ACTIVITY**

Helminthiasis is prevalent globally, but is more common in developing countries with poor personal and environmental hygiene. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm multiple infestations in the same individual are not infrequent. In the human body, gastro intestinal tract is the abode of many helminthes, but some also live in tissues. They harm the host by depriving food, causing blood loss, injury to organs intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of ill health (Tripathi, 2003). The main reasons responsible for the widespread nature of this disease in the developing countries are the lack of adequate sanitary facilities and supply of water, coupled with poverty and illiteracy. The helminth infection can be acquired by contact with infected animal, ingestion of infected meat, animal or human excreta *via* ground water, by means of certain mosquitoes.

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

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

*In vitro* anthelmintic activity is matter of several investigations in various plants *viz.* *Zingiber zerumbet*; *Allium sativum*; *Alpinia calcarata*; *Citrus acida*; *Citrus aromatica*; *Citrus medica*; *Cucuruma aromatica* and *Punica granatum* (Kalesaraj, 1962); *Ananas sativus*, *Embellia ribes*, *Macuna prurita* and *Melia azedarach* has significant activity against *Taenia canina* and *Paramphistomum cervi*; *Macuna prurita* was especially quite active against trematodes (Neogi *et al.* 1964); Anacardic acid form *Semecarpus anacardium* and its sodium salt have been found to be potent anthelmintic agent (Chattopadhyaya and Khare, 1969); *Balanites roxburghii* (Basavaraj Padmashali *et al.* 2001); *Cordia dichotoma* (Kuppasta and Nayak, 2003); *Bacopa monnieri* (Ghosh *et al.* 2005), *Flemingia vestita* (Tandon and Das, 2007); *Carthamus tinctorious* (Paramesha *et al.* 2009); *Chlorophytum borivilianum* (Deore and Khadabade, 2010); *Tamarindus indica* Linn (Das *et*
al. 2011); Cassia auriculata L (Gaikwad et al. 2011); Saraca Indica (Sarojini et al. 2011); Coldenia procumbens (Aleemuddin et al. 2012); Luffacyl indrical (Partap et al. 2012); Hibiscus rosasinensi (Pekamwar et al. 2013); Acorusc alamus (Prashanta et al. 2013); Cassia auriculata Linn (Sachin Chaudhary and Amit Kumar, 2014); Cassia occidentalis Linn (Sayyad et al. 2014); Artemisia vestita Wall and Artemisia maritima L (Irum et al. 2015) etc.

The indigenous system of medicine reports the anthelmintic effects of different Morus species which has been validated by few workers viz. in M. alba (Hogade et al. 2010), F. bengalensis, F. religiosa, F. glomerata, M. indica (Bandna Devi et al. 2013) and reported anthelmintic effect of tannins which binds to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite causing death (Mughal et al. 2013). Nawaz et al. (2014) reported the anthelmintic activities of Morus alba leaf by in vitro and in vivo methods.

ANTIMICROBIAL ACTIVITY

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

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

The efficacy of plant extracts against microorganisms is of considerable interest among various investigators. Many plant species have shown antimicrobial activities like *Mitracarpus scaber* (Ekpendu et al. 1994); *Landolphia owrrrience* (Ebi and Ofoefule, 1997); *Bixa orellina* (Castello et al. 2002); *Solanum stramoenifolium* Jacq, *S. seaforthianum* Andr. and *S. violaceum* Ortg (Manjunatha et al. 2004); *Bacopa monnieri* (Ghosh et al. 2005); weeds of Euphorbia family *Euphorbia tirucalli* (Asha et al. 2009) and *Carthamus tinctorious* (Paramesha et al. 2009); *Cycleapeltata Lam* (Abraham et al. 2010); fresh green tea, commercial green tea and black tea (Archana and Jayanthi Abraham, 2011); *Memecylon malabaricum, Andrographis serpyllifolia, Cochlospermum religiosum* and *Cochlospermum religiosum* (Jamuna et al. 2011); *pomegranate rind* (Yehia et al. 2011); *Cannabis sativa* L (Ali et al. 2012); *Schinus lentiscifolius* (Gehrke et al. 2013); *Callistemon lanceolatus* DC (Kavitha and satish 2014); *Careya arborea* Roxb (Prabhakaran et al. 2014); *Litchichinensis* and *Nephelium lappaceum* (Ramesa Shafi Bhat and Sooad Al-daihan, 2014); *Artemisia annua* (Tajehmiri et al. 2014); *Momordica charantia* L (Filho et al. 2015); *Azadirachta indica* and *Minusops elengi* (Mistry et al. 2015); *Nigella sativa* L (Emeka et al. 2015) etc.

Mulberry leaves containing phytochemicals have been proved to possess the pharmacological activities like reducing blood glucose, anti-hyperlipidemia, hypertensive, bacteriostatic and antivirus (Zou Sheng-qin and Chen Wu, 2003). *Morus nigra* L (Black mulberry), found to possess antioxidative and anti-bacterial activities against *Staphylococcus aureus, Bacillus subtilis, Micrococcus flavus, Streptococcus faecalis, Salmonella abony, Pseudomonas aeruginosa* (Ofentse Mazimba et al. 2011). Antimicrobial endophytic strain, ME-2, isolated and characterized from fresh mulberry twig tissues in China were evaluated against *Bacillus thuringiensis, Escherichia*
coli, Staphylococcus aureus, Beauveria bassiana and Mycoid bacillus and reported that its antimicrobial materials might be considered as a potential antimicrobial agents (Qiong-Ying Wu et al. 2012). Experiment on the biological activity of extracts from Morus alba L, Albizia lebbeck (L) Benth and Casuarina glauca Sieber against the growth of some pathogenic bacteria was conducted and reported that the ethyl acetate fraction of M. alba wood and bark exhibited the strongest antibacterial activity against the bacterial strains studied (Salem et al. 2013). The bioactive constituents isolated from M. alba such as leachianone and kuwanon G showed antibacterial activities and 1-deoxynojirimycin (DNJ) showed α-glycosidase inhibitors activity. Further, M. alba extract and its other compounds usually flavonoids have antioxidant properties by scavenging free radicals and protect many organs from oxidative stress. Anti HIV and chemo-protective activities have also been reported (Shoaib Zafar et al. 2013).

IN SILICO STUDIES

Computational tools are routinely used for characterization of genes, determining structural and physiochemical properties of proteins, phylogenetic analyses, and performing simulations to study how biomolecule interact in a living cell (Mehmood et al. 2014). Although these tools cannot generate information as reliable as experimentation, which is expensive, time consuming and tedious, however, the in silico analyses can still facilitate to reach an informed decision for conducting a costly experiment. in silico methods include databases, quantitative structure-activity relationships, similarity searching, pharmacophores, homology models and other molecular modeling, machine learning, data mining, network analysis tools and data analysis tools that use a computer. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization (Ekins et al. 2007).
**IN SILECO TOXICOLOGY**

With more than 70,000 chemicals in use today and many more being synthesized, it is vital that there are effective methods to assess the effect of these compounds on the environment and on human health (Smith *et al.* 2000; Mwense *et al.* 2004). In the development of pharmaceuticals, many potential leads are dropped due to their toxicity after millions of dollars have been invested (Escher *et al.* 2002). Experimental testing is both time-consuming and expensive, and accordingly, there is a pressing requirement for accurate *in silico* methods to provide an initial screen that generates alerts for toxicity (Klopman *et al.* 2004). Often the strategy to develop these predictors follows a more general approach to derive qualitative/quantitative structure activity relationships (SAR) (Smiesko *et al.* 2004). Thus many of the toxicity prediction methods are based on regression from chemical properties, advanced machine learning, or expert-derived rule-based systems (Helma *et al.* 2003; Dourson *et al.* 1997).

The prediction of properties and molecular features necessary for drug-like effects is of obvious interest in early preclinical development for pharmacophore identification. A widely recognized and condensed example is the “Lipinski rule of 5”, which predicts absorption or permeation of a drug if it has a molecular weight of 500 or less, a calculated Log P under 5, five or fewer hydrogen-bond donor sites and fewer than 10 hydrogen-bond acceptor sites (eg. N and O atoms) (Lipinski *et al.* 2001). Clearly there are many other important general considerations related to properties that need to be taken into account during preclinical development for a drug’s intended therapeutic use (eg. blood–brain barrier partitioning for neurologic drug products).

The use of ADME-Tox software for individual compounds and classes of compounds analyzed through virtual screening producing large libraries can be informative. ADME-Tox considerations cover metabolic, pharmacokinetic and toxicological issues related to drug disposition and fate. Its role in assessing preclinical and clinical pharmacology and the toxicity of new drugs
in development and regulatory review is well recognized (Yu and Adedoyin, 2003; Hou and Xu, 2004; Segall et al. 2006; Hilmer, 2008; Hop et al. 2008; Zhang et al. 2008).

*In silico* toxicology tools are now designed to assist in predictive toxicological and pharmacological profiling of pharmaceutical substances for understanding drug safety liabilities (Durham and Pearl, 2001; Ekins et al. 2007; Jacobson Kram and Contrera, 2007; Muster et al. 2008) supporting regulatory decision making on chemical safety and risk of toxicity (Durham and Pearl, 2001; Greene, 2002; van de Waterbeemd, 2002; Veith, 2004; Helma, 2005; Simon-Hettich et al. 2006; Kavlock et al. 2008; Merlot, 2008; Greene and Naven, 2009; Nigsch et al. 2009).

Efforts in drug toxicity database development have passed milestones demonstrating a limited utility for assessing carcinogenic potential (Contrera et al. 2003; Matthews et al. 2008), genetic toxicity (Yang et al. 2008), reproductive and developmental toxicity (Matthews et al. 2006; Matthews et al. 2007), hepatotoxicity (Clark et al. 2004), cardiotoxicity (Chekmarev et al. 2008), phospholipidosis (Kruhlak et al. 2008) and allergic contact dermatitis of xenobiotics (Roberts et al. 2007).


**IN SILICO PHARMACOLOGY**

Drug discovery and development is a complex, lengthy process and failure of a candidate molecule can occur as a result of combination of reasons such as poor pharmacokinetics, lack of efficacy, side effect and commercial reasons. Most drugs are discovered by either modifying the structure of known drugs, by screening compound libraries or by developing
proteins as therapeutic agents. With the advent of genomics, proteomics, bioinformatics and technologies like crystallography, NMR, the structures of more and more protein targets are becoming available. So there is a need for computational tools that can identify and analyze active sites and suggest potential drug molecule that can bind to these sites.

Target identification and validation is the first key stage in the drug discovery pipeline. However, identification and validation of druggable targets from among thousands of macromolecules is still a challenging task. Numerous technologies for addressing the targets have been developed recently. Genomic and proteomic approaches are the major tools for target identification.

Computational methods developed for virtual screening of therapeutic protein targets such as HIV integrase, protein kinase C, Janus kinase 3 and many others involve target- and ligand-based approaches covered under the field known as in silico pharmacology and computational therapeutics (Schneider and Fechner, 2005; Ekins et al. 2007; Chen et al. 2008). These techniques are important and have lead to successful results in identification of novel compounds in therapeutic target development (Madeswaran et al. 2011; Chian et al. 2012; Patil et al. 2012; Kumar et al. 2013; Rajesh et al. 2013; Jagadeesh et al. 2014; Shah et al. 2014).

The literature survey supports the selected target molecules for in silico docking studies for different activities. Cells respond to changes in the physical and chemical properties of the environment by altering many cellular functions such as survival, proliferative potential, metabolism rate, interaction with other cells, and numerous cellular processes involved in the homeostasis and health of the organism. In response to those changes, mammalian cells activate four well characterized subfamilies of mitogen-activated protein kinases (MAPKs). The p38 MAP kinase inhibitors are efficacious in several disease models, including inflammation, arthritis and other joint diseases, septic shock, and myocardial injury. Treatment with p38 MAP kinase inhibitors attenuated both p38 activation and disease severity (Lee et al. 2002). Several attempts have
been made in the identification of potent drug against p38 MAP kinase (Ensen et al. 2000; Enslen et al. 2001; Chang et al. 2002; Weston et al. 2002; Biondi et al. 2003; Petri et al. 2010; Youn et al. 2013).

GABA (γ-aminobutyric acid) receptors are among the most ubiquitous in the brain, having been identified (neurophysiologically) in all regions and at all levels (Iversen, 1978). All neuronal cells respond to the iontophoretic application of GABA, usually with a decrease in firing rate associated with hyperpolarization (Curtis, 1979). However, well-known instances of GABA-induced membrane depolarization occur, and the same cells may respond with a local depolarization or hyperpolarization, depending on where the GABA is applied (Andersen et al. 1980). This electrophysiological evidence indicates that all cells possess GABA receptors (Dilipkumar et al. 2011; Mamoon et al. 2012; Parimala et al. 2013; Vijusha et al. 2013; Murugalakshmi et al. 2014; Ashok et al. 2014; Chauhan and Swapna, 2015).

Cyclooxygenase (COX) is an endogenous enzyme which catalyses the conversion of arachidonic acid into Prostaglandins (PGs) and thromboxane. PGs (prostaglandin) are a kind of inflammatory endogenous mediator but also maintenance of the lining of the stomach and prevention of ulceration. The enzyme exists in two isoforms, constitutive enzyme COX-1 which responsible to the supply of prostaglandins to maintain the gastric mucosa and stabilize adequate vascular homeostatis, and inducible enzyme COX-2 which is induced by inflammatory factors. COX-1 is found mainly in the gastrointestinal lining, and COX-2 at sites of inflammation (Kurumbail et al. 1996; Smith et al. 2000; Blobaum and Lawrence, 2007; Musfiroh et al. 2013; Rauf et al. 2014; Yaksh et al. 2015).

Wound healing is a highly ordered and well-coordinated process that involves inflammation, cell proliferation, matrix deposition, tissue remodeling, collagenation and epithelialization. Many investigators evaluated the wound
healing properties of many of the medicinal herbs, clinically on animal models using excision, incision and dead space models (Nasrabadi et al. 2011). GSK3-β protein is an important regulatory enzyme whose inhibition promotes wound healing through β-catenin dependent Wnt signalling pathway (Zhang et al. 2009; Vidya et al. 2012a; Paramesha et al. 2014; Hemmati et al. 2015).

Some of the anthelmintic drugs showed activity by binding selectively to β-Tubulin of nematodes, cestodes and fluke, a protein subunit of microtubule and thereby disrupting microtubule structure and function (Friedman and Platzer, 1978). Microtubules are highly dynamic, ubiquitous cellular organelles serving a variety of vital functions including mitosis, motility and transport, in all eukaryotes. In view of the crucial roles, that microtubules play in many cellular processes, their drug-induced destruction eventually leads to the death of the organism (Satyendra et al. 2011; Shruthi et al. 2013; Sravani et al. 2014).

Bacterial proteins are the ultimate target to inhibit their growth since these are involved in many cellular functions. The key enzyme L-glutamine: D-fructose-6-phosphate amidotransferase, generally known as glucosamine-6-phosphate synthase is responsible for the synthesis of glucosamine-6-phosphate (GlcN-6-P) from D-fructose-6-phosphate and L-glutamine. In bacteria this enzyme is concerned to build peptidoglycan, a macromolecule important for the cell wall assembly. In mammals, UDP-GlcNAc is utilized for biosynthesis of glycoproteins and mucopolysaccharides (Marek et al. 2005). In spite of the fact, glucosamine-6-phosphate synthase (GlcN-6-P synthase) may be exploited as a target for potential antibacterial drug, since it is an important life sustaining enzyme present in all kinds of cells. GlcN-6-P, the product of this enzyme is very crucial for bacteria as well for human, but the significance of its deficiency is very different in both species. It has been shown that even a short-time inactivation of GlcN-6-P synthase is lethal for the pathogenic microorganisms by inducing morphological changes, agglutination and lysis, while in mammals depletion of the aminosugar pool for a short time is not lethal, because of the much longer lifespan of mammalian cells, long half
lifetime of GlcN-6-P synthase, and rapid expression of the mammalian gene which encodes the GlcN-6-P synthase (Bates et al. 1966; Chmara and Borowski, 1986; Milewski et al. 1986; Shruthi et al. 2013).

In fungi, lanosterol 14α-demethylase (P45014DM, CYP51), member of cytochrome P450 superfamily, is essential requirement for fungal viability (Bossche and Koymans, 1998). Lanosterol 14α-demethylase catalyzes removal of a methyl group at position C14 in the sterol molecule, which is a key step in ergosterol synthesis in fungi (Yoshida et al. 2000). This cytochrome p450 14 alpha-sterol demethylase can be used as a target to study the effect of drug molecules on fungal defense mechanism. Shinde et al. 2011; Ayati et al. 2012; Vidya et al. 2012b; Rodrigo et al. 2013).