CHAPTER -2
REVIEW OF LITERATURE

2.1 MEDICINAL PLANTS AND AYURVEDA

Since ancient times plants play a significant role in existence of humans. World Health Organization (WHO, 1977) defined medicinal plants as beneficial plants that own active compounds. It is reported that 80% population in the world trusts traditional system of medicine for their health needs because of less side effects (Attisso, 1983). Along with providing oxygen and food, plants own significant medicinal properties (Lewis & Elvin-Lewis, 1995). Use of plants as herbal medicines have gained popularity. Vast documentation is available for herbal products all over the globe. Since then herbal plants are used to prepare medicines to cure diseases (Holm. et al., 1998).

Utilization of plants for medicinal purpose is as old as our history. Old records of more than 5000 years also documented 100 therapeutic plants. Earliest documents confirm the use of plants in countries like India, China, Egypt, Greece and Rome. Egyptians wrote Ebers papyrus in 1500 B.C and documented 850 herbal plants such as aloe, garlic, ginger (Sumner & Judith, 2000). Plants have become an inspiration for medical treatment and their compounds are proving utility in the same field. It is reported that medicinal plants have therapeutic properties to cure diseases and are utilized for beneficial uses (Anonymous, 2001). It is also documented that in ancient time therapeutic plants were frequently used by individuals and herbal products were consumed for medicinal purpose (Philipson, 2001, Halsberstein, 2005). As herbal plants show excellent inhibitory action against diseases and also provides defense to plants (Shariff, 2001). Indian people also rely on herbal plants for health needs (Kala, 2005). Various parts of therapeutic plants have been used by pharmaceutical companies for preparing drugs (Anonymous, 2007a). The active constituents in therapeutic plants have the property to combine with inactive compound. Researchers documented the presence of more than one valuable compound in a single plant. Important compounds such as amino acids, branched extended series of aldehyde, alkaloid elements, specific vital lubricants, H\textsubscript{2}O, ethanol, chloroform, methanol, butanol and soluble compounds also have been reported.

Since 3000 years India is utilizing plants as drugs and also documented plants in various medical systems like Ayurveda and Unani. Ayurveda is the most ancient and powerful medical system of the universe and it is in use since 5000 B.C. Ayurveda contain extensive information on therapeutics to cure diseases and it comprise of vast literature
written in Sanskrit (Dev, 1999). Ayurveda originates from vedas and documented texts called as samhitas. Various samhitas explains medicinal procedures to cure diseases. Atharva and Rigveda have documented therapeutic plants used for awakening of energy points and surgery (Ebadi, 2007). The leading reported treatise of “Charak Samhita (900 B.C) documented the basic concept of Ayurveda along with routine practice and therapeutic effects (Mehta, 1979) and mainly focused on digestive problems whereas Sushruta Samhita focused on surgical procedures (Majumdar, 1971; Singhal, 2009). Madhav nidanad (800-900 A.D) was a milestone work in Ayurveda for detection of diseases. Ayurveda’s basic concept states that each component is composed of five essentials things counting with earth, air, water, space, fire and on the basis of these five necessities the environment can be classified in three parts

1. The Vata (Wind): is the power of wind that regulate the functioning of nervous system
2. The Pitta (Energy of heat): is the heat energy of body that regulates biochemical and digestive function.
3. The Kapha (Water): is the power in body that regulates the liquid absorption.

A healthy person share balanced vata, pitta and kapha whereas its dis-balance represents state of disease (Thomas, 1977).

2.2 TRADITIONAL SYSTEM OF MEDICINE

2.2.1 Medical System in Asia

Ayurveda, Unani and Siddha are recognized as the most primitive medical system of India whereas kempo was established in Japan and WU hsing in China. Ayurveda was a comprehensive system of medicine that works on psychological, physical and spiritual system of human. Ayurvedic system of medicine includes plants and plant based drugs and work with an aim to study the active principle and its effects on human health.
2.2.2 Medical System in Ancient Europe

Europe was a much developed country as compared to India, but in ancient times people of Europe used plants to cure diseases. They used color and shape of the plants/parts to denote its beneficial uses like leaf of heart shape was used to cure heart disease. In mid nineteen century some of the plants of European system of medicine were adopted by homeopathy and allopathic system. But with time European medicine system has improved with medical system of America that choose herbal plants from pharmacopeias or imported from other countries and prepared single extract or mixture of different plant extracts for medicinal use, like Echinacea is therapeutic plant in Europe that have been used by north America for medicinal purpose (Sollar, 2000; Elvin-Lewis, 2001).

2.2.3 Indigenous System of Herbal Medicine

The indigenous system of herbal medicine has its own significance as it is very diverse and practiced till today. The information regarding the system is generally known to all. The system choose some specific plants that share different parameters for toxicity and efficacy and both of these parameters became ideal for selecting a specific plant for medical procedures.

2.2.4 Africa’s System of Traditional Medicine

Africa is recognized as cradle for humans as it has vast cultural and biological diversity and shares various curative procedures but regrettably till today this system is not appropriately documented (Gurib, 2006). Medicinal plants are practiced by man from old times and still in use (Kokwaro, 1993) but continuous deterioration in the knowledge of traditional medicinal plants needs renewal of interest in the field (Kala, 2005).

2.2.5 Status of India for Medicinal Plants

Asian continent is rich in biological values. Different regions of Asia have various wild/ medicinal plants. In India, rural people use plant extracts in crude form as medicine (Iwu et al. 1999; Srinivasan et al., 2001). Even WHO reported that herbal plant may be used in crude form and can be used as pure compound. These plant based medicines play a vital role in health care. Herbal plants works as a store house for medicines since so many centuries. There are around 250,000 to 500,000 species of plants on Earth (Borris, 1996). India secures its place in top 12 biodiversity countries of the world and has 10% of total world Biodiversity affluence. India have 16 regions for agro climate, 10 zones for vegetation and 15 biotic provinces, 426 biomes, 45000 different species of plants in which 15000 have
medicinal potential including 7000 plants species of Ayurveda, 600 of siddha, 700 of Unani medicine (Zeeshan et al. 2009). Still only 3000 plant species of therapeutic potential have been identified yet (Prakasha et al., 2010) and only 1-10% of plants have been utilized by mankind (Watkins, 2003). Comparatively a minor percentage (1 to 10%) is being utilized as food and edible content by both humans and animal. It is reported that remaining percent is used for medicinal purposes (Moerman, 1996).

India has so many medicinal plants that have low cost and safe health options. Ramar et al. (2008) documented the importance of bioactive compounds from medicinal plants and reported that herbal medicines have no side effects and this is why all pharmaceutical companies have decided to work on phyto pharmaceutical products. Though, acceptance of these raw medicines as drug is still lacking and there is a prerequisite to systematically appraise them for recognition of active components.

Government of India also showed keen interest in protection and promotion of medicinal plants. Thus Government set up specific organization that works for conservation of medicinal plants. The National Medicinal Plant Board (NMPB) was started by Government in year 2000 with an aim to preserve and protect the medicinal plants. NMPB regulate the overall matters of medicinal plants throughout the country including launch of new programs, schemes for marketing, in-situ and ex-situ conservation, harvesting, preserving the stock and development of drug from medicinal plants etc (Kala & Sajwan, 2007). Government also has taken some measures to ensure the quality of herbal medicine so as to certify the quality and manufacturing of herbal medicine. Government have established medicinal plant board at State as well as Centre levels so as to make farmers/common man more acquainted with rich medicinal system. Government have set up different educational and research institute like National Institute of Pharmaceutical Education and Research, Agriculture Universities, Center Institute for Medicinal and Aromatic Plants, Botanical Research Institute, Central Drug Research Institute for promoting valued plants of India (Singh et al., 2007).

Since thousands of years natural compounds have become the most significant component for drugs and it is estimated that about one third of pharmaceutical compounds are made up of natural constituents. Natural medicines are the safest and reliable source as compared to allopathic system as allopathic system has various side effects. Thus, people show more interest towards traditional medication (Parekh & Chanda, 2006). Various modern
allopathic medicines like Digoxin, Vitamin A, Penicillin G, Quinine, Morphine and Doxorubicin have natural component and these medicines have become milestone in modern system of medicine (Ebadi, 2007). Aspirin is harmless man-made drug from salicylic acid and is a secondary product of willow bark, which is administered for aches and fevers (Raskin et al., 2002). It is documented that plants and microbes have been used to prepare a range of anti-cancer medicines like vincristine, streptozocin, bleomycin, mitomycin, daunorubicin, etoposide, irontecan (Ebadi, 2007).

Therapeutic plants have always played a significant role in discovery and development of new drugs, in nineteenth century man began to isolate active extracts of therapeutic plants. In year 1890 two French researcher isolated quinine from bark of Chinchona plant . Gorman (1992) practiced on Chinese traditional drug for curing eczema and malaria, and reported active compounds from more than 5,000 plants including Quinghaosu and Chaihu. As further advancement a number of active principles were isolated from higher plants prior to Second World War, this has become a mile stone in this field as new extracts became basis for new pharmaceutical synthesis and a great number of products are still in use (Kong et al., 2003). Active principle of herbal plants become the key component in Indian system of medication because active principle have various significant chemical components, Still as compared to modern pharmaceutical system Indian system is less developed, the prime reason is poor documentation and lack of scientific awareness (Kalimuthu et al., 2010).

High demand of herbal drugs is reported in developed and developing nations, people are dependent on traditional medicine system for health care issues (Shoeb, 2006; Chinsembu & Hedimbi, 2010) because it shows less poisonousness, easy accessibility and affordability (Khaleeliah, 2001). For more than hundred decades people have used herbal plants to cure diseases (Sofowara, 1982; Hill, 1952). It is reported that natural compounds have been used as antimicrobials as they provide safe option and cannot be replaced by synthetic antimicrobial (Shah, 2005). Thomas (1995) reported that medicinal plants are always high in request as its active materials can’t be manufactured synthetically. WHO also documented that most of the population is not able to buy western pharmaceutical drugs and depend on traditional system of medicine, as western drugs have negative effects and lack safety (Rabe & Staden, 2000; Griggs et al., 2001). People in remote areas are dependent on traditional medicine where latest medical amenities are not accessible. According to a case study report
in India, a person in rural area spent 124 Rs in a year as expense for drug consumption and this marks the lowest amount spent on medical treatment in the world.

Furthermore, the concern for therapeutic plants have also been revealed by available datas that shows the significance of medicinal plants at international level, The estimated trading of medicinal plants is 60 billion US dollars in international market and every year an increase of 7% have been reported (Belt et al., 2003). Moreover the present data lack major amount of unrecorded trading and the reason includes: first, illegal trading at large level and second reason is of unrecorded use of medicinal plants in home land (India). So, in fact the entire trading is much greater than the mentioned data. Trading of medicinal plants at global level is of about 30% of the entire medicine market. This percentage does not including the plants that are used in preparation of essential oils, cosmetics and food items (Addae Mensah, 2000). Van et al. (1997) documented the use of herbal plants as tea and snuffs. Borris (1996) reported that every year these items add a good amount of money for the country.

India, China, Brazil, Singapore and Egypt are considered as the highest traders of medicinal plants throughout the world in which India along with China make more than 40% of the total plant biodiversity in the world. China leads the world in herbal trading with 5 billion US dollars; India secured Second position with 1.45 billion US dollars which has been increased to 2.2 billion US dollars in year 2010. (Anonymous, 2000). United States and Europe became the largest buyer of medicinal plants whereas Germany secured top position and became center for herbal trading (Belt et al, 2003). According to Farnsworth & Soejarto (1991) in United States on an average a medical prescription has recommendations from natural sources and seven percent are commercially manufactured.

2.3 OCCUPATION AND INCOME GENERATION WITH MEDICINAL PLANTS

Therapeutic plants have indicated fitness, source of income; cultural values, financial safety (Hamilton, 2004). The significance and trade estimations of medicinal plant have attracted small scale farmers so the cultivation and supervision of medicinal plants have become important. Report on Atis (from Aconitum species) has also supported the facts, Atis is grown in Himalayan area of north India by NGO (Society for Himalayan environmental research), whose main work is to protect and cultivate the medicinal plants in Utarkashi district in Uttaranchal state (Karki et al., 2003). The outcomes showed that traditional farming of potato gives 200 dollars per hectare whereas Atis species gives a net profit of 1600-6000 dollars per hectare.
Nautiyal et al. (2004) reported that in Chamoli district of North India farmers cultivated *Picrorhiza kurrooa* also recognized as kutki and the net profit with kutki was of 1961 US dollars that is far better than the traditional crop of potato that give 280 US doll. These reports on cultivation of medicinal plants brings the fact that cultivation and supervision of such significant medicinal plants not only conserves the biodiversity but also offers better option sustaining livelihood. Along with protection and cultivation of medicinal plants, appropriate information on medicinal and aromatic plants can also generate more jobs in villages of India. One such example is of ‘Jevani’ herbal medicine that was used by tribal people of kani community in Kerala, South India. Initially kani people used fruit of a wild tree for boosting energy level and later when the plant was tested for it medicinal properties, it was reported with glycolipids and non-steroids complexes that have anti-hepatotoxic, immune modulatory and anti-stress properties. The plant was recognized as *Trichopus zeylanicus*. The plant is used till today for preparing Jevani drug, The Govt. of India has given the license to a private company for manufacturing Jevani on a contract of one million Indian rupees for seven years and kani community of Kerala have received 50% of contract money along with royalty that comes with the sale of the drug. This has set an example for generating income with medicinal plants.
2.4 CONSERVATION OF BIODIVERSITY

Besides knowing the facts about the significance of medicinal plants, more than 90% of medicinal plants are picked up from wild sources and it is reported that sometime people tried to reseed them (Balick & Cox, 1996; Dhillion & Ampornpan, 2000). In more than 75% of reported cases a destructive harvesting was noticed as people need different parts of the plant like root, leaves, stem, bark and sometimes whole plant too. Whereas man also disturbs the natural environment by factors like suburbanization, development and habitat destruction that led to the destruction of wild plant life. According to a survey conducted by ICUN (International Union for Conservation of Nature) there are 33799 plant species and around 381 have extinct, 372 plant species are in danger zone, 6523 are in endangered list and remaining plants are in rare species (Pye, 1998). As reported by Shankar (1998), there are approximately 200 medicinal plants in Southern and Northern regions of India, Evergreen rainy forest of South India are rich in therapeutic plants and *Plumbago zeylanica* L and *Plumbago rosea* L are also recorded in this region but both the curative plants are on the edge of extinction, the prime reason is deforestation and adversarial conditions of environment (Ninan & Geethamma, 2009).

To protect these plants germplasm preservation could be the best option. As reported by International board of Plant genetic resources, main concern is of medicinal plants and their in-vitro cultivation (Staritsky, 1997). Now a day's plant tissue culture also provides an option for cultivating endangered medicinal plants. This novel practice offers a better option to grow the commercially significant medicinal plants.

2.5 PLANT EXTRACT AND PHYTOCHEMICALS

Extraction is a process of separating pharmaceutically active component of medicinal plant from its inactive state. In a basic extraction process selective solvents are used. The resultant extract of basic extraction procedure is crude extract or powder or semisolid content that can be applied externally as medicine. Fresh plant extract shows great results as compared to dried extract because loss of activity is reported in some dried plants. Eloff (1999) reported that chemical modification is the main reason for apparently lost activity in dried extracts (Eloff, 1999). Tucker (2002-2004) also reported that the loss of activity is due to storage of plant sample.

**Phytochemicals**: Phytochemicals are important chemicals components derived from plants and prevent diseases in human (Chung et al., 1998). Plant produces two types of metabolites
(a) Primary (b) Secondary metabolites. Since old times secondary metabolites are significant part of plant that have been used in more than 60% of drugs (Wang et al., 2008). Approximately 13,000 secondary metabolites have been extracted from therapeutic plants but still it counts only 10% of the total amount available in nature (Rubio et al., 1999).

Secondary metabolites like alkaloids, Phenol, Terpenoids, Flavonoids, Saponins, Xanthones and Polysaccharides etc are valuable metabolites present in plant. These secondary components works to protect plant or to accomplish specified function (Briskin, 2000). Kaufman et al. (1999) reported that active compound of any plant is responsible for therapeutic consequences along with antimicrobial, antipyretic and anti-inflammatory potentials (Cowan, 1999; Adesokan et al., 2008; Murugesan & Deviponnuswamy, 2014). Tyler (1999) compared secondary metabolites with man-made compounds and reported that secondary metabolites utilizes numerous chemical complexes working at single or multiple site where as manmade compound made up of synthetic chemical was not able to work at multiple site. As per a survey conducted by current Pharmaceutical reported that on an average, a medical prescription around 25% of medicines are manufactured by plant material (Fransworth & Morris, 1976; Ogundipe & Akinbiya, 1998). UNESCO also reported the use of therapeutic plants for medicinal purpose (UNESCO, 1996). It is documented that around 5-10% of higher plants have been investigated for Secondary metabolites (Ayensu & De Filipps, 1978; Balandrin et al., 1993). Approximately 100 small particles from plant are presently at clinical trials (Fowler, 2006). Eloff (2000) reported that activity of plant extract help in segregating the biological active constituents. Thus researchers started investigating medicinal plants for new studies (Aswal et al., 1996) According to Fabricant & Farnsworth (2001) the secondary metabolites of plants are used in:

1. Separation of bioactive compound that is used in research and drug manufacturing.
2. For identifying new compounds for semi synthesis reaction to produce some entities that have higher activity or lower toxicity.
3. For using some compound as agent for pharmacological fusion.

As reported by Harborne (1999) there are 3 major chemicals present in Plant are alkaloid, phenolic complexes, terpenoids and some plant components with nitrogen.

1. **Polyphenols**

This group is the largest group of plants secondary metabolites, as reported it is vast and highly distributed group. Paixao et al., (2007) reported that more than eight thousand
polyphenols have been identified. Poly phenolic components have been synthesized in two basic pathways that is acetate and shikimate pathway (Ross & Kusum, 2002). In environment phenolic can be found in simple to complex chemical form like phenolic acid (simple chemical) to tannis (complex polymerized component). Latest research on antioxidant and free radicals led researchers to study polyphenols because antioxidant and free radicals both are associated with polyphenol components. It is reported that poly phenol have anti-mutagenic (Liviero et al., 1994), antiulcer (Saito et al., 1998) and antitumor properties (Liu, 1991). Poly phenolics are able to suppress reactive oxygen and considered to have strong antioxidant power (Naczk & Shahidi, 2004).

Secondary metabolites have abundance of poly- phenolic component that plant exposes in stress, UV radiation and infections. Plants have various phenolic including tannis, phenolic acid, flavonoids, lignin and simple phenols (Naczk & Shahidi, 2004). Human take beneficial phenolic with food in legumes and cereals, oilseeds, beer, tea, juices, fruit, wine, vegetables (Naczk & Shahidi, 2004). Polyphenols plays dual role in maintaining human health as they maintain enzyme composition in fruits and vegetables and provide antioxidant to human (Naczk & Shahidi, 2004).

2. **Terpenoids**

Terpenoids is the next class of secondary metabolites found in green vegetables and grains, terpenoids are also called as iso- terpenoids (Harborne and Baxter 1993). Terpenoids is essential for plants as terpenoids help plant in photosynthesis with the aid of some specific photosensitive pigments. Terpenoids suppress antioxidant by separating its long carbon chain into fat membrane.

Terpenoids are present in citrus foods, vegetables, grains etc. Tocopherols and tocotrienols are terpenes present in grains. Limonoids are citrus fruit terpenes. Carotenoid is another class of terpenoids that is present in pigmented edibles of red, yellow and orange color. So it is present in high quantity in fruits and vegetables. Phyto-sterols are another significant class of terpenoids, which helps in regulating cholesterol in body. All terpenoids works as anti-inflammatory, immune modulatory and anti-neoplastic.

3. **Phenolic Components**

Phenolic acids are significant dietary phenolics, (it includes hydroxycinamic and hydroxybenzoic acid). It includes flavonoids and Polyphenol (it include condensed tannis).
Maximum studies and research are on flavonoid group of phenolic component (King & Young 1999). Phenol work as an anti-oxidant in plant and animals. Haslam (1998) reported importance of vitamin C, Carotenoid, β Caretenoid, Vitamin E in the food so as to protect body against diseases.

Flavonoids: Flavonoids help to suppress tumor, ulcer, virus, microbial infections, free radicals, allergies and inflammation (Kinsella et al., 1993). Flavonoids works on signaling molecules and metabolic enzyme so as to offer health benefits. Flavonoids reduces the risk of heart diseases (Hertog et al., 1993), cancer and platelet aggregation. Another important class of phenolic includes Gallic acid and catechins (source-green tea, grapes and berries), anthocyanidins (pigmented component in fruit and flower) and iso-falvenoids (source-soya beans).

4. **Alkaloid and Nitrogen Containing Metabolites**

It includes glucosinolates which is present in vegetables from cruciferous family and indoles. Glucosinolates and indole both work to suppress cancer, as reported by Telang et al.,(1997). Indole suppress carcinogenesis in rodent mouse whereas intake of cruciferous vegetables in diet suppress the risk of mutagenesis, carcinogenesis and provide immunity to body. Intake of sprouted vegetables of cruciferous family reduces the risk of cancer as compared to equal amount of vegetable from some other family (Fahey et al., 1997).

2.6 **ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS**

Antioxidant plays a major role in defense mechanism, it suppresses free radicals and available investigations state the significance of antioxidant in body's defense system. Free radical attacks the unsaturated fatty acids present in bio membrane, this cause membrane lipid per oxidation which is linked with aging, carcinogenesis and atherosclerosis (Sayanovo et al.,1997).The free radicals also outbreak DNA and leads to mutation and finally to cancer.

Antioxidant is molecule accomplished of inhibiting the oxidation of further molecules. They can defend cells from free radical mediated injury. Antioxidant eradicates free radical intermediates by dismissing the chain reactions, and constrains additional oxidation reactions by oxidizing themselves. Free radicals are basics of a biochemical progression and characterize vital measures for aerobic life and metabolism. The main cause of diseases is related to oxidative stress owing to free radicals (Velavan et al., 2007).
Human body is capable of producing antioxidant; these constituents have capability to halt the free radicals development or can edge the injury they create (Thomas, 1997). Irregularities in working mechanism of antioxidant have been a reason for most sicknesses counting with inflammation, diabetes, atherosclerosis, cancer and coronary heart disease. Drugs based on antioxidant are consumed for the inhibition and curing of diseases like atherosclerosis, stroke, diabetes, Alzheimer’s, malaria, dysentery, inflammations, ulcers, epilepsy and cancer (Van & Gericke., 2000; Von, 2001; Khaleeliah, 2001; Devasagayam et al., 2004).

Intake of antioxidants from external sources is a constructive process. Currently, research is ongoing to discover effective antioxidants for curing or inhibiting the injurious effects of free radical (Reiter & Robinson, 1995).

Curative plants and their filtered ingredients have shown valuable healing possibilities. Numerous herbs and spices have been described with antioxidant potential, counting with, Camellia sinensis Linn, Piper cubeba Linn, Terminalia bellerica, Allium sativum Linn, Ocimum sanctum, Zingiber officinale Roscoe. Anthocyanin, flavones, isocatechin, coumarin lignans, flavonoids, catechins and isoflavones are major anti-oxidant (Aqil et al., 2006).

Spices and herbs are documented as natural antioxidants and therefore they play a major role as chemo preventives (Nair et al., 1998), black pepper seeds (Piper nigrum Linn.) are reported to have antioxidant and radical scavenging actions (Gulcin, 2005). Water and ethanol extract of black pepper displayed robust antioxidant movement, it also exhibited antimicrobial (Dorman & Deans, 2000), larvicidal (Chaudhry & Tariq, 2006) and anti-cancer potential (Park et al., 2002).

The clove flower bud (Eugenia caryophyllus) has been used as anesthetic since ancient era Essential oil from plant has anesthetic properties, it also shows anticonvulsant, antimicrobial properties contrary to Pediculus capitis, clove is most used species in all the kitchens around the globe. It is reported that Piper cubeba Linn have antioxidant, superoxide dismutase and catalase activities (Karthikeyan & Rani., 2003;Aqil et al., 2006)

Green and black tea or fermented product from the same plant is administrated around the world and is reported to have high amount of natural therapeutic components that show
anti-cancer effects. Rooibos is therapeutic green tea, it is been used in South Africa and is also exported outside the country. The indigenous rooibos tea that produces fynbos vegetation in southwestern areas of Western Cape of Africa is cultured for manufacturing herbal tea. It has been reported that flavonol compounds epigallo and epicatechins are present in tea that accounts for biological effect (Yang & Wang, 1993).

Rabe & Staden (1997) reinvestigated the consumption of herbal tea rooibos and concluded that it decreases the amount of DNA aberrations in Chinese Hamster ovary (CHO) cells exposed with benzo pyren. The bark of the plant (Rhizophora mangle) is reported to have scavenging action against hydroxyl radicals, the bark extract of plant have antioxidants and is enclosed with polyphenols, carbohydrates and sterols (Sanchez & Melchior, 2006). *Asparagus racemosus* is reported to have saponins, alkaloids and flavonoids (Velavan *et al.*, 2007). The bark of *Diospyros malabarica* is also used for various diseases including fever. Fruit juice is used for curing wound ulcer (Mondal & Chakraborty, 2006). Extract from stem of the plant strives with oxygen to respond through nitric oxide and consequently, prevents the production of anions. The important phyto elements in the extract are phenolic compounds.

*Auricularia auricular* commonly recognized as tree ear or wood ear from Auriculaceae family, is rich in flavonoids and have strong hydroxyl radical scavenging and lipid per-oxidation inhibitory activity (Acharya *et al.*, 2004). *Eucalyptus globulus*, acknowledged as *Karpura maram* from Myrtaceae family. Eucalyptus oil is reported to have high antioxidant activity assessed by two in vitro assays viz. diphenyl picryl hydrazyl radical scavenging action and suppression of ascorbate induced lipid peroxidation technique (Kokate & Purohit, 2004). Therapeutic plant *Acacia arabica* was tested with in-vivo and in-vitro methods to prove its antioxidant activity, in vitro, lipid peroxidation was performed by tertiary butyl hydroperoxide (TBH). In vivo, tests were conceded out in CCl₄-induced hepatotoxicity in rats. The bark is reported to have epicatechin, quercetin, (+) catechin, (-) and Gallic acid. The bark is used to cure diabetes, asthma, dysentery, bronchitis, and skin ailments (Sundaram & Mitra, 2007). *Ligustrum vulgare* leaf and DPPH test was performed to investigate and it is reported that it have flavonoids, alkaloids, coumarins and essential oil, and flavonoid aglycones is accountable for free radical scavenging actions (Nagy & Sersen, 2006).
*Terminalia chebula* is rich in tannins, chebulinic and Gallic acids. The extract was examined for free radical scavenging action for investigating tannins, it constrains the growth of duodenal ulcer and the extract demonstrates a cyto-protective consequence on the gastric mucosa (Jagetia *et al.*, 2002). *Obelia nicotianaeefolia* is a member of family Campanulaceae. It has alkaloid as main phytochemical along with gum, volatile oil, resin and fixed oil, plant has been utilized to cure asthma and it works as respiratory stimulant (Kokate & Purohit, 2004). *Citrus lemon* of Rutaceae family is reported to have citral and limonene as antioxidant, Two *in-vitro* procedures were performed to detect the presence of antioxidant, it includes DPPH radical scavenging action and inhibition of ascorbate induced lipid peroxidation (LPO) method. The plant shows antioxidant properties because of the presence of citral (Kokate & Purohit,2004). *Decalepis hamiltonii* plant has 2-hydroxyl-4-methoxy benzaldehyde as an active component that work as antioxidant (Murthy & Rajasekaran, 2006).

*Origanum syriacum* L is used to cure various diseases in Arab, mainly leaves of plant has been used, leaves are also used as flavor or for fragrance and for aromatherapy along with water, it is also available for commercial teas, cooked or baked diets (Alma *et al.*, 2003). Oil from the leaf of the plant displays antioxidant potential. (Mehmet *et al.*, 2003)

*Majorana hortensis*, plant has strong aroma. The fresh extract from plant has various pharmacological uses like treating digestive disorders, fever. The new leaves of plant are used to investigate the free radical scavenging action (Radha & Padma., 2011).

*Rosmarinus officinalis* L is important herbal plant that has been used since old times; Rosemary has been used and refined in India, ancient Egypt and China. Rosemary is an extensively utilized aromatic plant which has high therapeutic value. This plant has great history that indicates its use as anti-phlogistic, antioxidant, antibacterial. Oil from rosemary has been used as antioxidant; Folk people believe that when flowers appear is the perfect time for oil extraction. Rosemary is used in drug and food industries because of phenolic and antioxidant component (Eva *et al.*, 2003).

*Cymbopogon citrates* is native to Africa, Australia, India and China. This plant is known to cure gastrointestinal problems. Tea made from lemon grass is used to cure fever, flu, pneumonia, and to resolve gastric and sudorific glitches. Researches indicated that lemon grass is an excellent antioxidant; it is used as antitussive, disinfectant, sudorific, and anti-
rheumatic, stomachic and to cure back pain, wrench and hemoptysis (Vanisha & Hema, 2012).

Thyme (*Thymus vulgaris*) is known for its medicinal belongings, since ancient times it is consumed in spice as household medicine, in fragrance and as pesticide. It is consumed as antimicrobial, antifungal, secretolytic, disinfectant, antihelmintic and antitussive. Fresh leaves of Thyme are rich in antioxidant, anti-spasmodic. The main phytochemical of thyme is flavonoid (Zeghad & Merghem, 2013).

*Aegle marmelos* (L) is found in India and it grows abundantly in deciduous forest, its fruit and leaves have therapeutic values. The plant have antifungal (Renu, 1983), antibacterial (Banerji & Kumar, 1980), anti-protozoan (Banerjee, 1980), anti-spermatogenic (Sur *et al.*, 1999) effects. *Ocimum sanctum* Linn found in South Asian region is also known as holy basil, a huge literature is available that states the uses of holy basil, oil of holy basil owns antibacterial, antifungal, antioxidant and radio protective possessions (Sharma *et al*., 2002). Earliest Hindu literature also states the usefulness of *Ocimum sanctum*. 
2.7 CHEMICAL FINGERPRINTING

Therapeutic plants and their products are extensively used throughout the globe for various remedies. Like as in China, Korea, Japan, etc (Liang et al., 2004). Till date most of the population prefers herbal medicine over other options. Medicinal plants are utilized individually and in clusters also but in both cases these plants encompass innumerable complexes, and multiple elements that characterize their beneficial effects.

The chemical composition of compounds in therapeutic plants products may have different effects due to their dissimilar process of handling at the time of harvesting, plant backgrounds, origins and other issues (Liang et al., 2004), this study was supported by so many researchers (Qiu et. al., 2007; Zeng, 2007; Zhang et al., 2008; Pan et al., 2011).

The procedures of validation are not adequately influential to recognize all the constituents of therapeutic plants so, the aim is to set the procedure to conclude the active element and the constituents that are considered as markers are often not active. This leads to a great difficulty in preparing a standard good quality drug, as a result low-grade drugs appeared in market place. To standardize the medicinal plants and its drugs the process of standardization has been followed, at first step, crude medicine was cut and perused further for its quality control because at some time the herbal drug could not pass the authentication process.

The microscopic examination and morphological recognition can only prove validation of therapeutic plant but this process is not sufficient to examine the quality of herbal medicine. Whereas the physiochemical characteristics are best to calculate the quality of therapeutic plants and its components. But some time these all methods increases the difficulty of validation of herbal drugs, so the chromatographic fingerprinting examination was introduced by the researchers, that can chemically characterize the individualities of the herbal drug (Liang et al., 2004). There are two main factors in the process of fingerprint technique: (i) how to prepare effective and constant information and (ii) how to estimate the resemblance and dissimilarity with chemometric technique.

So, a chromatographic fingerprint of a medicinal plant is a chromatographic design of that plant extract and its components (Liang et al., 2004). Fingerprints of therapeutic drug refers to the outlines that can demonstrate the specific properties of the analytic component.
counting with raw materials, semi-finished products and final outcomes after correct handling, and be acquired by assured investigation methods.

All drugs may be made up of a single molecule or may be a mixture of components, most of them are unidentified constituents, and mostly these unidentified constituents are present in small quantity, so to examine this minute quantity in extract, researchers applied fingerprinting. The fingerprint investigations have been acknowledged all over the world as one of the competent approaches to examine the superiority of herbal medicines (Liang et al., 2004). There are two methods of fingerprinting: Chemical and Biological fingerprinting patterns.

**Chemical fingerprint** is applicable to examine the chemical components present in a therapeutic plants and drugs including their chromatograms, like thin layer chromatography, high pressure liquid chromatography, gas chromatography, capillary electrophoresis and spectral fingerprints counting with UV, IR, MS.

**Biological fingerprint** includes fingerprints of the component at genomic level, the genomic arrangement is different in every singular plant and in case of medicinal plants and drugs their DNA content is not affected by environmental atmosphere, physiological situations, and by the age of that plant, storage, handing, etc. These days biological fingerprinting has been used to evaluate various parameter in plants like evaluation of adulterants, genus and similarities in two species or homogeneity analysis (Cheng et al., 1997; Wang et al., 2007).

### 2.7.1 Thin Layer Chromatography

TLC is the most popular method of chromatography that is used for herbal medicine and for detection of compound present in it. TLC is an old method that is worldwide accepted for analysis since it is simple, rapid and low-cost method.

TLC is able to distinguish between the dainty imageries and fluorescence imageries; this makes TLC a rapid and easy para meter for visual chromatograms. Various stages of images and consistent essential information through chromatography could be achieved. So far it is best method for daily analysis and on-site assessment of sample. TLC is used for organic as well as inorganic material. TLC provides a broad choice for mobile phase, easy in identification of dissimilar sample, bulk sample loading ability and low price.
TLC is an influential tool for selecting an unknown compound out of bulk medicines (Szepesi & Nyiredy, 1996). With so many advantages it has some disadvantages also. Like it resolves the compound at low level, lack of sensitivity and the trouble in analyzing trace elements. It offers a moderately high degree of declaration that all possible constituents of the medicine are parted. Innumerable sample and its components from pharmaceuticals have been recognized by TLC (White et al., 1992).

### 2.7.2 High Performance Thin Layer Chromatography

Advancement in this technique arose with thin layer chromatography in high performance. High Performance Thin Layer Chromatography (HPTLC) is imperative tool in pharmaceutical investigations. HPTLC is less time consuming process for separation and sufficiently bendable to examine a huge diversity of samples.

This method is beneficial in so many ways as it is easy to handle and needs less time to determine the compound present in crude drug or sample. HPTLC assesses the whole chromatogram with various parameters deprived of time restrictions.

Furthermore, there is instantaneous but independent expansion of several samples and standards on every plate, foremost to an amplified dependability of outcomes. HPTLC has been applied to quantify medicines such as ethinyl estradiol cyproterone (Pavic et al., 2003), alfuzosin (Fayed et al., 2006) and tramadol and pentazocine (Ebrahim et al., 2011).

### 2.7.3 High Pressure Liquid Chromatography (HPLC)

HPLC is progressive form of liquid chromatography that is applied to sort the compounds out of a sample; this method is used to determine the role of the molecule and to identify the characteristics of individual molecule at chemical and biological level. HPLC has been used widespread and the most common technique in the investigation arena of fingerprint examination of drugs, because HPLC is not complicated and is easy to perform and not restricted by the stability of sample compound (Liang et al., 2004). So, HPLC is frequently used method in every lab. First time HPLC was performed in 1980, for the analyses of bulk drug resources (United States Pharmacopoeia, 1980).

The biggest benefit of HPLC is that more than one detectors can be linked to it, like; UV, DAD, ELSD, FLD, RID, MS, and NMR, etc., and can attach to two or more of them at
the same time (Wang et al., 2010), which provides abundant options for perceiving diverse component (Qi et al., 2008). HPLC is the widely accepted system of chromatography.

The extensively applied detector in HPLC is UV detector which is accomplished of checking numerous wavelengths simultaneously; this is probable by giving more than one wavelength scanning program. UV detector guarantees altogether identification of UV-absorbing constituents. When a line of different photodiodes are set on an integrated circuit (IC) chip for spectroscopy is called as photodiode array, Situated at image appearance in spectrometer so as to allow detection of various wavelengths for detection at same time. When adaptable wavelength detector is applied then sample need to be injected abundant times, with varying wavelength, to be assured that all the mounts are identified.

If using photodiodes then a wavelength can be set and all the components in this range can be identified in single shoot and the refractive index detector is applied for particular sample. This detector consumes the lowermost sensitivity amongst all detectors but appropriate at great sample concentrations (Lakshmi & Rajesh, 2010). In all detectors, fluorescence detector is very sensitive. It is 10-1000 times more sensitive than UV detector for typical UV absorbing components.

One of the most sensitive detector among all is, LC detectors that has sensitivity that is 10–1000 times higher than that of the UV detector for strong UV absorbing materials and applied as benefit in the identification of fluorescent species. Detection of pharmaceuticals component is one of the biggest applications of fluorescence (Ulu & Tuncel, 2012).

Various medicines have been inspected in pharmacological preparations (Siddiqui et al., 2010, Tang et al., 2012) and in biological fluids (Samanidou et al., 2012) using HPLC. However, HPLC have some restrictions also like the cost of columns, solvents and absence of extended period, reproducibility owing to the exclusive habit of column packing etc.

2.8 PHARMACOLOGICAL ACTIVITY OF THERAPEUTIC PLANTS

2.8.1 Antimicrobial Activity of Medicinal Plants

Presence of various secondary metabolites in medicinal plants is responsible for therapeutic effects as plants are storage house of secondary metabolites and are used as raw drug (Srivastava et al., 1996). All secondary metabolites are significant for plant as well as for human. So, at present various investigations are ongoing on these natural compounds as
they provide safe medical option for man (Lewis & Elvin-Lewis, 1995). Plant extracts with known activity and identified phytochemicals are significant in the medical field for treatment (Nagesh & Shanthamma, 2009). These phytochemicals help researchers to extract new antibacterial and antifungal for beneficial uses (Kalimuthu et al., 2010). Cowan (1999) reported phytochemicals as an inhibitor of microorganisms in in-vitro conditions. Paiva et al. (2010) reported that secondary metabolites are present in plants which provide defense to plant. Indian system of medicine uses plants and its extract for curing several diseases, but proper documentation, scientific information and standardization of herbal plants are still lacking so this area is far behind than modern medicine system (Kalimuthu et al., 2010). So there is a need to assemble the evidences for keeping the data of such plants (Georges & Pandelai, 1949; Vanden et al., 1986; Silva et al., 1996; Rojas et al., 2003). A great number of scientists have studied the antimicrobial effects of herbal plants (Reddy et al., 2001; Erdo, 2002; Ateb & Erdo, 2003). Different approaches have also been applied to study the antibacterial activity of plant extracts (Caceres et al., 1993; Vlietinck et al., 1995, Cos et al., 2002 & Somchit et al., 2003). Mahesh & Satish (2008) and Nagesh & Shanthamma (2009) documented antimicrobial activity of medicinal plants and reported that plants with known medicinal effects are significant to treat diseases.

According to a study, approximately two or more than two medicines formulated from microorganisms are thrown every year in the commercial market (Clark, 1996). It is estimated that new vaccines and antibiotics has given rise to commercial market by up to 60% as compared to previous years (Alper, 1998). In 1990s a survey was conducted in United States and the outcome indicated that at least one third of people are using "unconventional" treatment (Eisenberg et al., 1993). Later in 1996 another survey was conducted and documented the sales of therapeutic drugs with an upsurge of 37% after 1995 (Klink, 1997).

McGee (1998), survey on the use of therapeutic plant species and its medicinal possessions, and concluded that therapeutic spices are powerful tool to minimize food borne disease. A study also revealed that therapeutic plant work well to preserve the food and hinders the growth of pathogenic microorganisms. In tropical countries around half of death befell due to Infectious diseases by pathogenic microorganisms (Iwu et al., 1999). *Staphylococcus* and *Streptococcus* are infectious microbes accountable for contaminations especially in wounds, boils, sores and swellings and can be suppressed by plant extracts
Enterobacter gains space within biological abdominal flora, but outside the intestine they might become a reason for wound contaminations (Kohler et al., 2001; Madigan et al., 2003). Plant extracts are documented to suppress such bacteria.

A great number of scientists have studied the antimicrobial effects of herbal plants. Osborn (1943) investigated twenty-three hundred plant species for antibacterial potential and then Nickell (1959) concluded the same work with 157 species of plant. Then Indian investigators also worked with the same aim with 880 species of plant (Dhar et al., 1968; Bhakuni et al., 1969, 1971). Plants with known antibacterial effect are of great importance. *Salvia santolinifolia* crude extract with ethanol is effective against gram negative and gram positive bacteria. The root was reported to be more effective against gram positive bacteria and aerial parts were effective against gram negative bacteria (Ahmed et al., 1994). *Juniperus communis* leaf extract is effective against a wide range of gram negative and gram positive bacteria (Chatterjee et al., 1993). *Acalypha torta* is used to cure skin diseases. Ethanol and methanol solvent of the plant were effective against anaerobic bacteria that cause infection in wound (Irobi & Banso, 1994). Hiremath et al., (1997) reported antimicrobial activity of *Hemidesmus indicus* along with *Striga sulphrea* and concluded that *Striga sulphrea* nighty five percent ethanol extract was effective than other extracts. It showed activity against all test microorganisms including *Aspergillus niger*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*. *Helianthemum glomeratum* is utilized as herbal medicine for diarrhea. Meckes et al., (1997) documented *Helianthemum glomeratum* for antibacterial activity against infectious bacteria of intestine. The methanol extract of leaf and acetone extract of root was found effective against *Shigella* and *Vibrio cholera*, the extract from leaf was found effective against *E. coli* and *Salmonella* species. Mehta et al., (1997) reported that *Abutilon indicum* root hexane extract was effective against *P. aeruginosa* while as benzene extract was found effective against *E. coli*. Mukherjee et al., (1997) reported that *Drymaria cordata* methanol extract was able to suppress *Bacillus subtilis*, *P. aeruginosa*, *S. aureus*, *B. pumilis* and *E. coli*.

*Rhynchostylis retusa* alcoholic extract was reported to have more antibacterial activity against *E. coli* and *Bacillus subtilis* as compared to *Staphylococcus auteur*, *Salmonella typhi* and *Klebsiella pneumoniae* (Ghanaksh & Kaushik, 1999). Singh & Nath (1999) investigated fruit of *Elaeocarpus sphaericus* with different solvent and found that acetone extract was
most effective and suppress the growth of 10 gram positive and gram negative bacteria and benzene extract suppress the growth of Morganella morganii, Salmonella typhi while ethanol extract was effective against Shigella sonneii, Shigella flexnerii and Plesiomonas shigelloides.

Arora & Kaur (1999) worked on several Indian spices and found that Indian spices have strong antimicrobial potential. Abo et al. (1999) investigated extract of Spondias mombin leaf and Croton zambesicus bark and found that both the extract were effective against a wide range of bacterial species and can compete with gentamycin and ampicillin. Lall & Meyer (2000) reported the antibacterial potential of Euclea natalensis acetonic and aqueous extract against a wide range of bacteria including Bacillus subtilis, Staphylococcus aureus, Bacillus cereus, Micrococcus kristinae and Bacillus pumilus and observed that aqueous extract was not effective to suppress the growth of the bacteria while acetonic extract showed excellent result against all the bacteria. Taddei & Rosas-Romero (2000) observed the antibacterial activity of Tridax procumbens with hexane, ethyl acetate and water and found that aqueous extract showed no activity against bacteria whereas aerial parts of the plant in hexane extract was effective against Salmonella paratyphi, E.coli and Mycobacterium smegastis. While Klebsiella species and Bacillus cereus can be suppressed with ethyl acetate extract with flowers of Tridax procumbens. Samy & Ignacimuthu (2000) reported thirty Indian therapeutic plants for antibacterial potential and found that Cassia corniculata and Cassia Occidentalis were effective against Staphylococcus aureus and Bacillus subtilis. Ebi (2001) reported that Alchornea cordifolia stem, root, leaf and bark methanol extract contain terpenoids and phenolics those show antimicrobial activity against Bacillus subtilis, E.coli and Pseudomonas aeruginosa.

Pichai et al., (2001) reported various extracts of Tabebuia rosea leaf and it showed antibacterial activity against various bacteria. Aqueous extract suppress Staphylococcus aureus, Salmonella typhi and E. coli while chloroform extract showed activity against Salmonella typhie whereas hexane extract failed to show antibacterial activity. Jatropha multifida root successive extract with ethyl acetate, methanol, hexane and chloroform was found effective against Bacillus subtilis and Staphylococcus aureus (Aiyelaagbe, 2001). Whereas Crataeva nurvala bark extract was found effective to suppress Pseudomonas species, E.coli and Klebsiella species, the extract showed maximum inhibition against E.coli then Pseudomonas followed by Klebsiella (Chandra & Gupta, 2001). Ahmad et al., (2001)
reported that *Apium graveolen* seeds extracts with methanol, pet ether and acetone were effective to suppress *Staphylococcus aureus* and *E. coli*, while the methanolic extract showed best activity when used at a concentration of 1 mg/ml that was comparable to chloramphenicol, a standard antibiotic.

Dash *et al.*, (2002) examined the antibacterial activity of *Evolvulus alsinoides* ethanol extract against *Pseudomonas aeruginosa* and *E.coli* and reported the effectiveness against *S.aureus* and *Candida albicans*. While studying the antibacterial effect of *Betula pendula* bark extract Mukhtar *et al.*, (2002) found that the extract was effective against *E. coli* and *Staphylococcus aureus*. Ates & Erdogrul (2003) examined the antimicrobial activity of five medicinal plants against 13 microbial species with chloroform, alcohol, acetone and ethyl acetate extract. Seed alcohol extract of *Pimpinella anisum*, ethyl acetate extract of bark of *Cinnamomum cassia*, chloroform, acetone extract and Seed extract from *Juniperus oxycedrus* and root extract of *Glycyrrhiza glabra* were found effective whereas *Coriandrum sativum* was not effective against test microbes.

*Semecarpus kathalekanensis* leaf ethanol, pet ether, chloroform and aqueous extract were tested for antibacterial potential against *Aspergillus nigar*, *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Klebsiella species*. All extract showed inhibitory effect against test microbes (Ramana *et al.*, 2005). Sarin & Khandelwal (2005) reported that *Ocimum sanctum* was effective to inhibit *Staphylococcus species*, *Escherichia coli* and *Pseudomonas species*. The methanolic extract of *Detarium microcarpum* was tested in *in-vitro* and *in-vivo* conditions and was found effective against *Escherichia coli*, *Proteus mirabilis*, *S. aureus*, *P. aeruginosa*, asin-vivo test animals were infected with these pathogens but showed no symptoms at clinical trial. Patel & Vimal (2007) reported *Neolamarkia kadamba* root alcoholic and aqueous extract were effective to suppress the growth of *Klebsiella pneumonia* and *Pseudomonas aeruginosa* whereas the leaf alcoholic and aqueous extract were reported to be more effective and showed ideal results as compare to ciprofloxacin antibiotic. Distill water extract of *Swetia chirata* was able to suppress the pathogenic growth of *K. pneumonia*, *S. aureus* and *E.coli*. But the same extract was not effective to suppress *B. subtilis* and *S. epidermis* (Bhargava *et al.*, 2007). Prusti *et al.*, (2008) reported 3 medicinal plants of Orissa for antibacterial potential. *Vitex peduncularis*, *Elephantopus scaber* and *Litseascaber* and the plants were tested against *Enterococcus faecalis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. 


coli. The results indicated that extract of *Elephantopus scaber* was more effective as compared with other 2 plant extract. Mahesh & Satish (2008) observed antimicrobial potential of *Tinospora cordifolia, Ziziphus mauritiana, S. cordifolia, Acacia nilotica* and *Withania somnifera* methanol leaf extract against *Staphylococcus aureus, Bacillus subtilis, Xanthomonas Axonopodis, Escherichia coli* and *pseudomonas fluorescens*.

Oluwafemi & Debiri (2008) examined economic plants of West Africa for antibacterial potential. *Phyllanthus amarus* and *Paraquatina nigrescens* ethanolic extract along with crude hot and cold aqueous extract were examined against *Salmonella typi, Phyllanthus amarus*, and reported that ethanolic extract was more effective. Hasan et al.,(2009) examined *Polygonum hydropiper* (root) chilled chloroform extract against *Enterobacter aerogenes, Bacillus subtilis, Staphylococcus aureus* and *Bacillus meaterium* (gram positive) and *Shigella sonnei, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* (gram negative). *Polygonum hydropiper* showed maximum activity against *Enterobacter aerogenes* and *Bacillus subtilis* that was comparable with Kanamycin (the reference antibiotic). Igbinosa et al.,(2009) reported *Jatropha curcas* with various extracts (distill water, methanol and ethanol) and the outcome indicated the distill water extract failed to give desired result and methanol extract was able to inhibit the pathogenic growth whereas ethanol was less effective. Methanol extract of *Abrus pulchellus* (leaf) was able to supress the pathogenic growth of *Clostridium perfrigens* and *S.aureus, E.coli* and *P.aeruginosa* (Vinayaka et al., 2009). Ogunfolakan et al.,(2010) examined leaf extract of *Tithonia diversifolia* and reported that it have broad spectrum antimicrobial potential against all human pathogenic microbes.

Prasannabalaji et al. (2012) reported antibacterial activity of *Adhatoda vasica, Ocimum sanctum, Aegle marmelos* and *Ocimum gratissimum* against 5 pathogenic microbes including *Salmonella paratyphi, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*. The outcome indicated that *Ocimum sanctum* and *Ocimum gratissimum* have strong antimicrobial potential against all the microbes whereas *Adhatoda vasica* have least activity against all microbes. Madikizela et al., (2013) examined 10 therapeutic plants with 4 different extracts for antimicrobial potential against pathogenic strains of *K. pneumoniae, S. aureus* and *Mycobacterium aurum*. The outcome indicated that in total of sixty eight test extracts seventeen have antibacterial activity against any one of the
microbe. Some plants were reported to have better activity includes *Pentanisia prunelloides*, *Abrus precatorius*, *Indigofera errecta* and *Terminalia phanerophlebia*.

Agarry *et al.*, (2005) reported the antibacterial potential of *Aloe vera* plant; gel from the plant is effective against a wide range of bacteria. Plants like garlic, tea tree and lemon grass are effective against a wide range of bacteria and juice from plants like bearberry and cranberry are effective to cure urinary tract infections (Rios & Recio, 2005). Mathabe *et al.*, (2006) documented the antibacterial activity of *Punica granatum*, *Ozoroa insignis*, *Ximenia caffra*, *Indigifera daleoides*, *Syzygium cordatum*, *Schiota brachypetala*, *Elephantorrhiza burkei*, *Spirostachys africana*, *Elephantorrhiza elephante* in hot water, ethanolic and acetonic extract and all extracts were found effective against *Staphylococcus aureus*, *Vibro cholera*, *Escherichia coli* and *Salmonella typhi*.

Girish & Satish (2008) documented the comparative study of methanol and aqueous extract of various medicinal plants and reported that methanol extract of selected medicinal plants showed higher antibacterial activity as compared to aqueous extract. Senthil & Reetha (2009) reported the methanol activity of *Aegle marmelos* along with *Cassia auriculata*. The methanol extract of plant showed higher antibacterial activity as compared to aqueous extract. Nikitina *et al.*, (2007) reported the polyphenol of some plants from *Rosaceae* family and *Geraniaceae* family for antibacterial potential, the polyphenol showed strong antibacterial activity. Toshitsugu *et al.*, (2004) reported the antibacterial activity of polyphenol from 10 plants against deadly food borne pathogens.

Uma & Sasikumar (2005) examined alcoholic extract of *Calotrops giganta*, *Piper betle*, *Justica adhotada* and *Moringa oleifera* and documented that all the extracts inhibit the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Aspergillus niger*. Leeja & Thoppil (2007) reported that *Origanum majorana* methanol extract was used against test fungi and bacteria and documented the plant as excellent natural protectant. Veeramuthu *et al.*, (2006) tested 18 therapeutic plants against 9 test bacteria and one fungal species. The outcome showed that 10 plants have therapeutic potential against every bacteria and fungi, when various concentrations of 1.25mg/disc, 2.5 mg/disc and 5mg/disc were applied. Erturk (2010) worked on 41 therapeutic plants from twenty six various species for examining the antimicrobial and antifungal potential, *C.albicans*, *A.niger* have been chosen for antifungal potential and *Pseudomonas aeruginosa* and *Staphylococcus aureus* were chosen as test bacteria. Thirty nine plants out of forty one plants displayed
activity against any of the microbe. *Cameli sinensis, Thymus capitatus, Cuminum cyminum, Achillea coarctata, Viscum album, Rhus coriaria, Pimental officinalis, Jasminum officinale, Pimpinella anisum, Galeba officinalis* and *Alnus glutinosa* showed better activity against the bacteria.

Dagmar et al. (2003) evaluated ten therapeutic plant’s alcoholic extract for antimicrobial potential against test microbes *Pseudomonas aeruginosa, Bacillus cereus, Escherichia coli* and *staphylococcus*. Five therapeutic plants were reported with antimicrobial potential to at least one test microbe. Ramasamy & Charles (2004) examined therapeutic potential of *Melia azadirachta, Anosomeles indica* and *Blumea lacera* for antimicrobial potential against *staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcesenes* and *Escherichia coli*. Ramasamy reported that acetonic and ethanol extracts of all the test plant displays excellent result whereas distill water and pet. ether extracts were failed to give desired outcomes.

In recent times the minimum inhibitory concentrations (MIC) of extracts have been detected by serial dilution methods (Fabry et. al., 1998). According to Eloff (2000), some experts were not able to calculate the MIC values due to some technical problems because when the method was practically performed on plant extracts, it did not give desired results may be because of extract color or the precipitation of compounds.

Most of these problems have been overwhelmed in a new micro-dilution technique using 96-well microtitre plates. For determining the activity of microorganism at biological level Tetrazolium salts are best option to be used, tetrazolium salt is a constituent without any color, this salt is capable of admitting an electron to reduce itself in a colored constituent by an active microorganism (Eloff, 2000). With the use of INT (*p*-iodo nitro tetrazolium violet) in the microtitre plate, bacterial growth is showed by red color when the INT is reduced to formazan (Eloff, 2000).

### 2.8.2 Antifungal Activity

Medicinal plants have become a substitute for preparing various drugs for infectious diseases. These medicines are a source of effective antibiotic against pathogenic strains (Fabricant & Farnsworth, 2001). The fungicide potential of medicinal plants came in light when Walker et al. (1929) reported the fungicidal activity of *Allium cepa* plant leaves in aqueous extract that suppress the growth of *Colletotrichum* spore. After this noted work by
Walker it took so many years to work again on fungicidal activity of medicinal plants. Then Abdullaeve (1959) reported the antifungal potential of garlic and onion. Ujevick & Stanek (1960) also documented garlic for its action against Colletotrichum lindemuthianum. In the same year Slavenas (1959) documented Brassica juncea and Sinapsis alba for its antifungal properties against Aspergillus niger. Ischenko (1961) worked on apple leaf and found it effective to suppress Venturia inequalis spore germination. Angalis arvensis (Nene & Thapliyal, 1965), Clematis gouria (Mishra & Dixit, 1977), Solanum nigrum (Singh, 1977), Rauwolfia serpentine and Datura Alba (Dwivedi et al., 1978), Aegle marmelos, Mentha arvensis and Citrus aurantifolia (Pandey et al., 1983) were reported for antifungal activity.

Abrus precatorius, Brassica juncea, Aporosa lindleyana and Areca catechu were tested for anti-fungicidal potential against Aspergillus niger, all the plants showed excellent result but Aporosa lindleyana failed to give desired results (Raji & Raveendran, 2013). Fungal growth of Alternaria brassicae was suppressed by organic extract of Allium sativum, Withania somnifera and Azadirachta indica. Alternaria brassicae is a pathogenic fungus in mustard blight (Singh et al., 2013). For suppressing the growth of Sclerotinia sclerotiorum, Allium sativum is used with Potato dextrose agar in aqueous extract. These inhibit fungal inhibition for up to two days after inoculation (Yadav et al., 2011). Nashwa et al., (2012) reported in-vitro and in-vivo studies against Alternaria solani with 6 plant species. The maximum inhibition was recorded with Datura stramonium, Allium sativum, Azadirachta indica. Leaves and seed of papaya showed antifungal activity against Rhizopus stolonifer, Collectotrichum gloeosporioides and Fusarium species. The ripped seeds and leaves and unripe seeds and leaves of the plant were used and the leaves extract showed more antifungal activity as compared to seeds (Chavez et al., 2011). Twenty six plants were used to examine the antifungal potential against Phytophthora infestans. In all the plants Styrax officinalis, Xanthium strumarium and Lauris nobilis showed maximum antifungal activity against the pathogen (Yanar et al., 2011).

Mahalingam et al.,(2011) reported Setophaeria rostrata is a pathogenic bacterium that causes seedling blight in sugarcane. 10 plants were used to examine the antifungal activity against Setophaeria rostrata. The methanolic extract of all the plants showed the highest activity against fungus. Ethanolic extract of all the plants showed moderate results whereas the water extract failed to give desired outcomes.
Raja (2010) reported 7 plants and examined for antifungal activity against *Alternaria tenuissima* that causes spots on leaves of eggplant. Leaf and bulb of the plants were used to make an extract in which maximum efficacy was shown by garlic, neem and mesquite plant leaves and bulb extract. For protecting the eggplant the leaves can be sprayed by above 3 plant extract. Raghavendra *et al.*, (2009) reported that *Alternaria alternate* infects tobacco leaf with brown spots can be controlled by *Prosopis juliflora* organic extract in methanol and ethanol, the plant extract is reported to reduce the infection of fungus in tobacco leaves.

*Fusarium solani* in addition with *Pythium aphanidermantum* causes storage rot in ginger. Ram & Thakore (2009) reported nineteen plant extract including, *Lawsonia inermis*, bulb of ginger, neem, *Datura stramonium*, *Lantana camara* that reduces the growth of pathogen in ginger. Sengupta *et al.*, (2008) reported *Myrothecium roridum*, *Rhizoctonia aolani*, *Acremonium kiliense* and *Penicillium expansum* growth can be suppressed by *plumeria acutifolia* plant latex. Aloe vera alcoholic extract suppresses the fungal growth of *Penicillium gladioli*, *Botrytis gladiolorum*, *Heterosporium pruneti* and *Fusarium oxysporum* (Rosca *et al.*, 2007). *Ixora brachiate* leaves and roots were tested against Epidermophyton, Microsporum and Trichophyton where root extracts were found to be more effective against all pathogens (Sadeghi & Deokule, 2007).

*Diopyros anisandra* bark hexane extract show antifungal activity against *Colletotrichum gleosporioides*, *Candida albicans* and *Aspergillus niger* (Borges *et al.*, 2007). Elizabeth & Jayalaxmi (2006) reported plant *Michelia champace* for its antifungal activities against *Mucor luteus*. The crude extract of the plant is effective against rhizopus and methalioic extract was effective against *Mucor luteus*.

Sabovljevic *et al.*, (2006) reported the antifungal activity of *Bryum argenteum* ethanolic extract against *Trichophyton mentagrophytes*, *Aspergillus niger* and *Candida albicans*. Becker *et al.*, (2005) reported the antifungal potential of *Lythrum salicaria* against fungal growth of *Cladosporium cucumerinum*. Elizabeth *et al.*, (2006) reported *Acacia nilotica* bark was not effective as antifungal against *Aspergillus niger* but reported to show excellent activity against *Streptococcus aureus*. Prasad *et al.*, (2002) reported *Pyricularia grisea* is responsible for blast disease in rice, can be suppressed by *Pinus kesiya*, Ginger rhizome and Garlic bulb and turmeric rhizome. Petroleum and chloroform extract of *Heliopropium subulatum* showed excellent antifungal activities against *Penicillium chrysogenum* (Singh *et al.*, 2002). *Solanum chrysotrichum* methanolic leaf extract can
suppress the growth of *Microsporum gypsum*, *Trichophyton mentagrophytes* and *Trichophyton indicum*. Abou *et al.*, (2002) investigated various plant species of wild area for antifungal activities in Lebanon and reported that *Origanum syriacum* pet ether extract was most effective against all fungal pathogens. As reported by Glowniak *et al.*, (2000) *Peucedanum verticillare* plant extract was tested against *Epidermophyton floccosum*, *Candida albicans*, *Trichophyton species*, *Geotrichum candidum* and *Aspergillus fumigates*. Dubey *et al.*, (2000) documented *Sphaeranthus indicus* for its antifungal activities in ethanol and aqueous extract against *Penicillium pinophilum*, *Alternaria solani* and *Fusarium oxysporum*. Ristic *et al.*, (2000) reported *Phlomis fructicosa* showed antifungal activity against *Phomopsis helianthi*, *Aspergillus niger*, *Fusarium tricinctum*, *Aspergillus ochraceus* and *Cladosporium cladosporiodes* in ethanol extract.

Growth of *Fusarium avenaceum*, *Alternaria alternate*, *Fusarium culmorum* and *Cladosporium oxysporum* can be suppressed with organic extract of *Cupressocyparis leylandii* (Baranowska *et al.*, 1999). Cavin *et al.*, (1999) reported antifungal activity of two hundred four plants from Indonesia belonging to seventy seven species from forty three families. Series of all test plants were examined against pathogenic fungus *C. cucumerinum* and *C. albicans* and the outcome showed that 20 plants were able to inhibit the fungal growth. Adedayo *et al.*, (1999) examined *Senna alata* methanolic extract for its antifungal activity, the methanolic extract of flower showed best results against *Penicillium species*, *Aspergillus niger*, *Aspergillus brevipes*, *Geotrichum candidum* and *Candida utilis*. Radha *et al.*, (1999) reported various organic extracts of *Syzygium travancorium* were effective to suppress *Pestalotiopsis palmarum*, *Candida albicans* and *Fusarium oxysporum* but hexane extract showed best results as compared to ethyl acetate and chloroform extract.

Baykal (1999) reported *Nitraria schoberia* plant with its methanolic extract of leaf and stem and reported that it was effective against *Pleurotus ostreatus*, *Microsporum canis* and *Epidermaphyton floccusum*. While the extract showed toxic reactions with *Curvularia lunata*. Ozcan (1999) reported the antifungal activities of *Prangos uechtritzii*, *Micromeria myrtifolia* decoctions against *Penicillium digitatum*, *Alternaria alternate*, *Fusarium oxysporum*, *Aspergillus niger* and *Botrytis cinerea* and documented *Prangos uechtritzii* for more antifungal potential than *Micromeria myrtifolia*. Gangopadhyay (1998) worked on seed protection powder made up of turmeric that is helpful in suppressing rice disease and seed borne fungal infections. Amadioha & Obi (1998) reported that hot water of neem and neem
oil and *Xylopia aethiopica* extract from fruit is helpful to suppress germination of *Colletotrichum lindemuthianum*. Amadioha also reported that papaya leaves hot and cold water extract is helpful to suppress the mycelial growth of powdery mildew on pepper plant. *Rhizoctonia solani* is a pathogenic fungus that destroy rice crop. Kurucheve *et al.* (1997) examined 13 plant species with cold and hot aqueous extract against the pathogenic fungus and found *Prosopis juliflora* cold extract was capable to suppress the growth of the fungus whereas *Lawsonia inermis, Caesalpinia pulcherrima* and *Eucalyptus globosus* can inhibit the production of sclerotial. Kapoor (1997) reported that *Cucurma longa* and *Zingiber officinale* can suppress the growth of *Aspergillus niger* and *Penicillium digitatum*. The fresh juice and water extract of both the plant can suppress the growth but when compared it was found that fresh juice is more capable to suppress pathogenic fungi. The different extract (chlorofomic extract, acetonic extract, ethanolic extract and pet ether extract) of *Cassia alata* leaf exhibited antifungal action when exposed to different fungi viz. *Aspergillus niger, R. Japonicum, Candida albicans, C.tropiathis and R. glutinis* (Shankar, 1998). *Trachyspermum ammi* seed extract can suppress the growth of *Rhizoctonia solani, Pythium aphanidermatum* and *Macrophomina phaseolone* whereas *Coriandrum sativum, Foeniculum vulgare* and *Cominum cyminum* can only suppress the fungal growth of *Pythium aphanidermatum* (Pandey & Pant, 1997). Palanakumbura *et al.*, (1997) reported that *Barringtonia ceylanica* bark’s methanol extract can inhibit the fungal growth of plant pathogens like *Rhizoctonia solani, Rigiodiporus lignosus, Curvularia species, Cylindrocladium quinquesptatum* and *Colletotrichum gleosporiodes* where as for *Colletotrichum leosporiodes* and *Curvularia species* excellent results were observed. Hidalgo & Fernandez (1996) documented *Parthenium hysterophorus, Annona muricana, Swietenia mahagoni, Leucaena leucoceohala, Annona glabra* and *Citrus reticulata* for antifungal potential against *Fusarium subglutinans, Alternaria solani* and *Fusarium oxysporum*. All plant extract show different inhibition results on every pathogen. Mahasneh *et al.*, (1996) reported *Suaeda vermiculata* and *Salsola villosa* pet. ether and butanol extract inhibit the fungal growth of *Candida albicans* and *Miconazole nitrate* and *Fusarium oxysporum*. Yagi *et al.*, (1993) reported plant extract from Sophora flavescens suppress pathogenic growth of *Pythium vanterpooli* and *Pythium graminicola* at small concentration. Sharma & Jandaik (1994) documented antifungal activity of *Eichhornia crassipes, Garlic bulb, Azadirachta indica, Tegetes erecta* and *Eucalyptus tereticarnis* against some fungal species that blights *Agaricus bisporus*. Bambawale *et al.* (1995) reported plant extract of *Datura metel, Datura stramonium* and *Lawsonia alba* can inhibit *Alternaria macrospora*, a cotton pathogen. Tiwari *et al.*, (1987) detected antifungal activity of
Eupatorium capillifolium and Aegle marmelos leaf extract and found it effective against Aspergillus flavus and Penicillium oxalicum. Whereas Clementis gouriana leaf extract inhibit Aspergillus niger growth (Mishra et al., 1988). Eswaramurthy et al. (1989) reported the fungicidal potential of Ipomea species, Acacia prosopis and Azadirachta indica against Sarocladium oryzae and Fusarium oxysporum. Tiwari et al. (1990) reported that the pathogenic growth of Aspergillus flavus and Penicillium oxalicum can be suppresses by Citrusmedica leaf extract and Cleome viscosa leaf extract.

Four siddha medicines Nandhi mezhugh, Parangi pattai choornam, Erasa kenthi mezhugu and Vaan mezhugu (in order of effectiveness) have been found effective as antifungal against 14 strains of Candida albicans (Suresh et al., 1994). The filtrate of Neem leaf in ethanol and distill water showed potent anti-microbial activity against dermal problems, the extracts were applied to 88 persons with dermatophytic problems and the maximum outcome was reported with ethanolic leaf extract. (Venugopal & Venugopal, 1994)

Rai (1996) studied anti-mycotic movements of Pestalotiopsis mangiferae in 14 therapeutic plants and reported Eucalyptus globulus (88%) and Catharanthus roseus (88%) with best results and moderate results were observed in Ocimum sanctu (85.50%), Azadirachta indica (84.66%), Ricinus communis (75%) and Lawsonia inermis (74.33%) and least action was logged with jatropha curcas (10%). Santolina chamaecyparissus was tested for its antimycotic action in in-vivo (in mice) and in-vitro conditions (in 13 strains Candida albicans) (Suresh et al., 1997). Santolina chamaecyparissus was reported to suppress antifungal activity in guinea pigs (Suresh et al., 1995).

2.9 Plumbago zeylanica L

2.9.1 Chemical Composition

Plumbago zeylanica L is a member of plumbaginaceae family and therapeutically significant plant. The plant is widely used in Indian system of medicine. Plumbago zeylanica is documented for its pharmaceutical activities with imperious biochemical complexes (Modi, 1961; Kiritkar & Basu,1975; Krishnaswamy & Purushottamam 1980, Pillai et al., 1981), Plumbago zeylanica is reported with phenolic composites, tri-terpenoids, steroids, coumarins glycosides, starches, saponins, tannins, alkaloids, naphthaquinones, flavonoids, fatty acids and amino acids (Van Der Vijver, 1974; Ravikumar, 2011; Kodati et al., 2011; Ming, 2011). Seselin (Kostova et al., 2001), 5-methoxyseselin (Kofinas et al., 1998), suberosin (Uchiyama
Xanthyletin and xanthoxyletin is present in roots (Lin et al., 2003). Among all ‘plumbagin’ is active chemical of the plant that mainly accumulates in root. Muthukumarasamy et al. (2015) reported presence of L-dopa, Plumbagin (naphthoquinone), droseron, chitranone, triterpenoid, anthraquinone in the plant.

Different parts of the plant are reported with various chemicals, like flower have Plumbagin along with zeylanone and glucose, Leaf has Chitranone and plumbagin. Leaf is also reported for the presence of terpenoids, alkaloids and flavonoids (Tyagi & Menghani, 2014). Stem of the plant is rich in various chemical including dihydroflavonol plumbagin, zeylanone, sitosterol, campesterol, stigmasterol and isozeylanone, bark too have plumbagin, some pigments have also been isolated from the roots and recognizes as droserone, zeylanone, isozeylanone and elliptinone. Along with these pigments, root are reported to have 3-chloroplumbagin and 3,6-biplumbagin (Satyawati et al., 1987; Handa et al., 1995). As per the latest finding Plumbago zeylanica is reported to have gibberellic acid, quinol R. Acetonic extract of roots showed maximum amount of gibberellic acid followed by methanolic extract (Mohanty et al., 2014).

Plumbagin

‘Plumbagin’ (5-hydroxy-2-methyl-1, 4- naphthoquinone- C_{11}H_{8}O_{3}) is a chemical that accumulates in roots (Van der Vijver, 1974). Approx. 2% plumbagin is present in whole plant; plumbagin is a yellow colored pigment that is limited to Plumbaginaceae family (Van Der Vijver, 1974). Stems are reported with less amount of plumbagin whereas leaf has no plumbagin. Presence of naphthoquinone gives yellow color to plumbagin, it appear like needles. It is extremely toxic having corrosive assets. Plumbagin is soluble in alcoholic, acetonic, chloroform and benzoic solvent.

![Plumbagin Structure](image)

2.9.2 Therapeutic Uses

Since ancient time P. zeylanica has been utilized for skin diseases (leprosy, scabies, dermatitis, pimples, lesions and pustules) contaminations and abdominal worm, many scientists believed that malaria, rheumatism, abdominal worm, anemia, shock, toxic inflammation and furunculous scabies can be cured with the plant (Jiangsu New Medical
College. Zhonyao Dictionary, 1979; Dai et al., 2004; Olagunju et al., 2006; Jeyachandran et al., 2009; Ankita & Nimal, 2015). In Africa it is used to cure flu and dark water fever. Outer shell of the plant is applied to stop hemorrhage, to treat wound, gonorrhea, syphilis, tuberculosis, reports suggested that the plant is effective against obesity.

Ayurveda mentioned Chitrak as rejuvenator and it is acclaimed for its bitter taste. Chitrak is also used in combination with vata, sunthi, kutaja and is very inclusive medicine against diarrhea, associated with abdominal pain. The decoction of plant is helpful to recover abdominal sicknesses like splenomegaly, hepatomegaly and ascites. It increases digestion and appetite (Nadkarni & Nadkarni, 1954).

Different parts of the plant are applied in diversity of alignments. Stem of P. zeylanica was reported with beneficial phytochemicals that boost immune system (Tyagi & Menghani, 2014). Flower of this therapeutic plant is used to cure digestive problems (Paiva et al. 2003). Leaf is used to cure scabies, swelling and sore as they are vesicant and caustic (Sharma et al., 2001). Leaf paste is applied to cure painful rheumatic zone and to cure skin problems (Mukherjee, 2002). It is recommended to take great care before taking the dose of Chitrak, as it is very hot in its properties. So small doses are favored. Ladies take Chitrak to correct menstrual disorders but large dose may cause abortion in pregnant ladies (Premakumari et al., 1977; Bhargava, 1984), it protects uterus by enhancing the contraction and cleanses it. Chitrak is useful against liver diseases, used to correct enlarge liver and spleen; it relieves the obstructed phlegm in chronic colds and cough, used to suppress the disease that originates from loss of appetite. It works well in ano-rectal swellings. (Nadkarni & Nadkarni, 1954), according to Paiva et al. (2003) stem extract in methanol is helpful to suppress Leishmania amazonensis up to 88% at a concentration of 100mg/ml, it can also help to prevent cancer (Teshome et al., 2008) anti-atherogenic (Gupta et al., 1999), heart diseases (Bopaiah & Pradhan, 2001), also work as memory enhancer (Tilak et al., 2004) and stimulant for nervous system (Gou et al., 2009). Root paste with Abrus precatorius is applied to cure leukoderma (Kaushik & Dhiman, 2000). The filtrate from garden fresh roots remains operative against bleeding piles, root is used to upsurge ingestion and to uphold craving, minor amounts encourage central nervous organization. Roots have been used for abortions in rural areas. Fresh paste of root is smeared on skin to cure pustules, and other skin ailments counting with boils and scabies (Olagunju et al. 2006; Arunachalam et al., 2010). Dried root powder is consumed with butter milk to cure piles and if taken with honey then cures high cholesterol.
and increase the amount of blood to cure anemia and upsurge the production of blood cells. The root methanol extract was reported to suppress proliferation of Ehrlich Ascites carcinoma in Swiss albino mice to suppress cancer, the root induced apoptosis in Ehrlich Ascites carcinoma cells (Raihan et al., 2012). Root paste is operative to suppress chicken pox and acne, filarial limb and is also consumed to cure dysentery, diarrhea, peptic ulcers and duodenal parasite (Chiu & Chang, 2003).

As the roots of plant are highly useful so, for therapeutic purpose the outer bark of the roots are dipped in lime water then later filtrate for medicinal purposes. Paste from this soaked roots can be applied for filariasis, depigmentation of dermis. The same is beneficial in colitis when used for at least 6 months. The detoxed root extract with ethanol showed anti-Helicobacter pylori and cytotoxicity activity and reported to possess cytotoxic effect against HE-17 cell lines (Paul et al., 2013). The hydro alcoholic extract of Plumbago zeylanica L root bark showed anti-inflammatory and analgesic properties. 85% alcohol is used with 15% of water to make test medicine and applied to test on mouse and the outcome reveals excellent result (Thanigvelan et al., 2014). As reported the root bark of P. zeylanica has strong analgesic and anti-inflammatory action which has been used in various formulations of Indian system of medicine for treating Cancer (Thanigavelan et al., 2014).

Countless medicinal verdicts have specified that filtrate of the plant works against plasmodial cells (Simonsen et al., 2001), infectious microorganism (Ahmad et al., 2000, Sao & Dubey, 2015), fungus (Mehmood et al., 1999), suppresses inflammation (Oyedapo, 1996) and works to low high lipid (Sharma et al., 1991). It also prevent diarrhea (The Wealth of India, 2003), allergies (Dai et al., 2004), diabetes (Olagunju et al., 1999) and possess hepato-protective properties (Gupta et al., 1999; Kanchana & Sadiq, 2011)

Plumbagin is documented for its antimicrobial activities (Gujar, 1990, Durga et al., 1990), plumbagin’s organic extract with methanol suppress Staphylococcus aureus, Escherichia coli and Salmonella typhi, Klebsiella pneumonia, Serratia marcescens, Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa (Beg & Ahmad, 2000). Mohanty et al. (2014) reported that ethanol, chloroform and acetone extract of root, stem and leaves have antimicrobial activity and root extract shows maximum inhibition zone and least inhibitory concentration against E.coli. Even the root and leaf extract of acetone, chloroform and ethanol showed higher antimicrobial activity than kanamycin (Standard drug) against E.coli
Aqueous extract of *Plumbago zeylanica* L is not as effective as other solvents for antimicrobial activities. Plumbagin shows anticancer (Melo *et al.*, 1974), antifungal (Gujar, 1990), antibiotic effects (Durga *et al*., 1990). *Plumbago zeylanica* L is known for its excellent antibacterial activities. Extract of methanol, petroleum ether, and dichloromethane were found effective to cure *Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Staphylococcus aureus, Salmonella gallinarum*. Root alcohol extract showed strong antibacterial potential against *Escherichia coli, Staphylococcus aureus, Salmonella paratyphi, Shigella dysenteriar*. When resistance strains of *Escherichia coli, Staphylococcus aureus* were inoculated with streptomycin and rifampicin in media, exhibited deferred growth but when microbes were grown with plumbagin the growth was completely wiped out (Durga *et al*., 1990). The ethanolic, acetonic, ethyl acetate and water extract of *Plumbago zeylanica* L was tested against *Helicobacter pylori* where extract of ethyl acetate showed least inhibitory concentration against all the strains followed by acetonic extract and then ethanolic extract (Wang & Huang, 2005). *Plumbago zeylanica* L alcoholic extract was found effective against infective yeast *Candida albicans* and *Trichophyton indicum, Epidermophyton floccosum, Microsporum gypseum*. Where 4mg/ml was minimum inhibitory concentration. (Mehmood *et al*., 1999).

*Plumbago zeylanica* L is effective against almost all deadly viruses. *Plumbago zeylanica* L methanolic extract was tested against influenza A virus, Herpes simplex virus type 1, Coxsackie virus B3 using plaque reduction assay and the plant showed excellent results against all the species (Mariam *et al*., 2006). Plumbagin is an excellent source to suppress malaria as it suppress succinate dehydrogenase enzyme of *Plasmodium falciparum*. Latest findings showed that plumbagin can overpower the growth of the parasite in *in-vitro* conditions if used at 5mM concentration. (Paiva *et al*., 2003). Plumbagin is reported to have anti-leishmanicidal activity contrary to *Leishmania donovani* and *Leishmania amazonesis*. Plumbagin and 3, 3’ bis- plumbagin and 8, 8’-bis plumbagin is reported to be effective against leishmanicidal activity at Amazonian bolokia (Paiva *et al*.,2003).

Davender *et al.* (2011) reported the wound healing activity of *Plumbago zeylanica* methanolic extract in wistar albino rats. Kakjing *et al.* (2012) reported anti-ulcer (*indomethacin* gastric ulcer in acute phase) activity in wistar albino rats induced with aspirin. Kanchana& Sadiq (2011) reported that root extract in petroleum ether showed significant hepatoprotective action against paracetamol induced liver damage in mice. Goyal & Sharma
(2012) also induced wistar mice with paracetamol, chloroform and alcohol and later methanolic extract of *Plumbago zeylanica* L was introduced in mice and it showed excellent recovery, that indicates that plant have strong hepato-protective ability. Plumbagin derived 1,4-naphthoquinone, help to suppress human prostate cancer in xenograft mice (Bilal *et al.*, 2013). *Plumbago zeylanica* L is reported to have strong anti-carcinogenic potential, Nguyen *et al.*, (2004) reported that male mice fed with plumbagin and azoxymethane shows more positive results as compared to those fed with azoxymethane alone. In second condition the mice developed tumor in small intestine. This showed that plumbagin is an excellent neoplastic. In hepatoma bearing mice the level of Hexokinase, aldolase and phosphoglucoisomerse was found to be increased but when plumbagin is supplemented to these mice the level comes to normal. In normal condition individual with hepatoma have low level of glucose-6-phosphate, gluconeogenic enzyme, but when administrated with plumbagin they showed positive results and increased levels. Plumbagin is also used to suppress the activation of Nf-kB that is encouraged by tumor necrosis factor, carcinogenic components etc. Plumbagin is reported to suppress the Nf-kB activation in some carcinogenic growth (Sandur *et al.*, 2006).

**Side Effects:** As chitrak is very hot and sharp in its activities so excess dose may result in irritation, vomiting, diarrhea and burning sensation in urinary track. Excess of chitrak may cause abortion and ulcer.

### 2.10 Dyerophytum indicum (Gibs. Ex Wt) Kuntze

The great status of herbs in human health cannot be overlooked. Plant kingdom has infinite source of active compound that are used for preparing medicines. Ayurveda is a system that uses plants to cure various alignment and diseases. Instead of having so many therapeutic options, plants remains the best option for all. These plants are used as a starting material for the production of medicine. Since last so many years a great number of medicines have been innovated but the practical utility of these drugs is still lacking (Cohen, 1992). *Dyerophytum indicum* is one such plant that was used for various diseases in old times.

#### 2.10.1 Therapeutic Uses

*Dyerophytum indicum* is used in rural areas to cure different alignments. The fresh stem and shoots of plant contain maximum amount of beneficial components. Bark from fresh stem is munched to suppress mouth ulcers and constipation. The inner side of the bark
is dried in shade and grounded into fine powder. A small quantity of powder is taken orally with milk twice a day to cure pimples. People use *Dyerophytum indicum* to cure asthma, chest infections. Paste from *Dyerophytum indicum*’s root is applied to cure skin contaminations, scabies and moles. Root paste is applied on forehead to cure headache as well. As reported early shoots and stems are maximum utilized for therapeutic uses, these parts are used as dried powder for different breathing alignments. The early shoots and branches of this plant are highly salty and is used in cooking in rural areas at the time of salt shortage.

In Oman’s dhofar place *Dyerophytum indicum* dried stem parts and powder is used with tobacco for smoking to cure cheat pain and smoke help to prevent breathing problems (Miller & Morris, 1988). *Dyerophytum indicum* is reported to be used in different medicine in Africa and Asia.

Prospecting of minerals in *Dyerophytum indicum* showed significant amount of macro and micro nutrients in leaf and stem including zinc, lead, cadmium and copper. The results revealed that the quantity of copper is remarkable, whereas lead, zinc and cadmium were found in high quantity. Then the quantity was measured in different parts of *Dyerophytum indicum* and it was reported that the stem has maximum quantity of copper whereas leaf has maximum quantity of zinc and iron. Lead was minimum in leaf and iron, zinc were marked minimum in stem of the plant. Lead, cadmium and copper were in significant quantity in the leaf whereas lead, cadmium, zinc were in significant quantity in stem of *Dyerophytum indicum*. When grown in mineralized area quantity of nutrients were recorded with high amount. (Tiagi & Aery, 1981)