CHAPTER-II

REVIEW OF LITERATURE

The mother Earth is a rich reservoir of natural resources particularly medicinal plants. Medicinal plants play a key role in human health care. A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic potential [Dahanukar et al., 2000; Agbar et al., 2008; Prusti et al., 2008]. There is an increasing interest in the use of herbs for the treatment of human diseases. Plants contain a wide variety of compounds that may have biological activities. Plants have been a prime source of highly effective conventional drugs for the treatment of many illnesses [Kaneshiro et al., 2005; Shoeb, 2006].

Over the past few years, the medicinal plants have required a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine in addition, the necessity of meeting the requirements of medicine for an increasing human population. Herbal drug product has a special place in the world of pharmaceuticals. The majority of the world’s population in developing countries still relies on herbal medicines to meet their health needs in cases when synthetic medicine could not relieve patients suffering from illnesses [Lee et al., 2000; Surendra et al., 2008].

In India, medicinal plants form the backbone of several indigenous traditional systems of medicine. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds [Prusti et al., 2008]. Phytochemicals from medicinal plants serve as lead compounds in drug
discovery and design [Ebi and Ofoefule, 2000]. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs [Ncube et al., 2008].

WHO, report depicts that more than 80% of world’s population rely on plants based products to meet their health care needs. Nearly, 25 to 45% of modern prescriptions contain plant derived lead molecules as a basic source in drug formulations. About 25% of top selling drugs world-wide are either directly obtained from natural sources or entities derived from plant products [Ramya et al., 2008].

In recent years, multiple drug resistance in pathogens has been developed due to indiscriminate use of synthetic drugs especially in the developing countries [Hart and Karriuri, 1998]. Thus, a diverse arsenal of new antibacterial agents is timely needed to combat the diminishing efficacy of existing antibiotics [Chopra et al., 1997]. To this emerging problem, phytochemicals obtained from medicinal plants are the sole remedy. This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have fewer side effects [Ramya et al., 2008].

T. chebula Retz. belongs to the family Combretaceae commonly known as black myrobalan. T. chebula is a native plant of India and Southeast Asia. T. chebula, is a deciduous tree growing upto 15-30m in height, with a trunk upto 1m in diameter. It is one of the most widely used plants in the traditional system of medicine. Its dried ripe fruits, has been traditionally used to treat various ailments in
Asia like, asthma, sore throat, vomiting, hiccough, bleeding, piles, diarrhoea, gout, heart and bladder diseases. In Myanmar, medicinal Terminalia fruit is used as a laxative and tonic agent. In China, it is applied as a carminative, deobstruent, astringent and expectorant agent, and also as a remedy for salivating and heartburn. In Indo-China, it is regarded as a purgative agent. In Malaysia, medicinal terminalia fruit is believed to exhibit anti-diarrheic, styptic, antibilious and anti-dysenteric activities.

T. chebula was reported to contain various biochemical compounds such as tannins, chebulinic acid, ellagic acid, gallic acid, punicalagin, flavonoids, etc. It has been reported to possess antioxidant, antidiabetic, antibacterial, antiviral, antifungal, anticancerous, antiulcer, antimutagenic, wound healing activities etc. [Suryaprakash, et al., 2012].

Maheswar et al., (2010) has studied anticonvulsant activity of petroleum ether, chloroform, ethanol and aqueous fruit extracts of T. chebula against Matsumoto Eosinophilia Shinshu (MES) and pentylenetetrazol (PTZ) induced seizures in rats. The ethanol and aqueous extracts showed significant activity in MES induced seizures by reducing tonic hind limb extension phase than compared with the other extracts and control. The ethanol extracts significantly delayed the onset of clonic convulsions induced by Pentylenetetrazol.

Rathore et al., (2004) determined the antiatherogenic activity of powdered myrobalan, the fruit of T. chebula on cholesterol fed mice. Male mice were fed a diet containing 1% cholesterol with or without myrobalan for 100 days. The cholesterol containing diet fed to mice caused increased food intake, body weight, serum cholesterol, triglyceride, thickening of the walls of aorta and shrinkage in its
lumen. The oral administration of myrobalan to mice on atherogenic diet successfully reversed those effects. The results suggest that myrobalan has hypcholesterolemic effect in animals fed with atherogenic diet.

Raja et al., (2011) examined the antimutagenicity activity of T. chebula on micronucleus formation and chromosomal aberration assay in bone marrow cells of Swiss albino mice. The protective effect of T. chebula extract is reported against cyclophosphamide induced micronuclei formation and chromosomal aberration in mouse bone marrow cells. A dose-dependent inhibition of micronuclei formation and chromosomal aberration was observed which was statistically significant as compared with the cyclophosphamide group. It was observed that T. chebula extract alone could not induce micronuclei formation and chromosomal aberrations at the test dose 50mg/kg b. wt. T. chebula extract showed protective potential against cyclophosphamide induced micronuclei formation and chromosomal aberration in mouse bone marrow cells.

Manohar et al., (2012) reported the preliminary phytochemical of ethanol and chloroform extract of fruits of T. belerica. The results of qualitative phytochemical screening showed the presence of phytosterols, carbohydrates, flavonoids, phenolic compounds and tannins. Antidiabetic potential, effect of the petroleum ether, methanol, and aqueous extracts of T. catappa fruit, on fasting blood sugar levels and serum biochemical analysis in alloxan-induced diabetic rats were investigated by Nagappa et al., (2003). All the extracts of T. catappa produced a significant antidiabetic activity at dose levels 1/5 of their lethal doses. Concurrent histological studies of the pancreas of these animals showed comparable regeneration by methanol and aqueous extracts which were earlier, necrosed by alloxan.
Vaibhav et al., (2010) concluded that alcoholic extract of T. chebula ripe fruits have potent immunomodulatory action. Oral administration of T. chebula alcoholic extract was found to increase the neutrophils and lymphocytes as compared to vehicle and cyclophosphamid treated groups. T. chebula alcoholic extract showed linear time dependent significant phagocytic activity as compared with sheep red blood cell (SRBC) sensitized and cyclophosphamide treated group. In zinc sulphate turbidity test, T. chebula treated rats serum showed more turbidity [cloudy] which indicate the increase in the immunoglobulin level as compared with vehicle, SRBC sensitized and cyclophosphamide treated group.

Priyadarsini et al., (2002) examined the aqueous extract of T. chebula for antioxidant activity and its ability to inhibit $\gamma$-radiation induced lipid peroxidation in rat liver microsomes and damage to SOD enzyme in rat liver mitochondria. HPLC analysis of the extract showed the presence of compounds such as ascorbate, gallic acid and ellagic acid. This was also confirmed by cyclic voltammetry. The extract inhibits xanthine oxidase enzyme activity and is also an excellent scavenger of DPPH radicals. The rate at which the extract and its constituents scavenge the DPPH radical was studied by using stopped-flow kinetic spectrometer. Based on all these results it is concluded that the aqueous extract of T. chebula acts as a potent antioxidant and since it is able to protect cellular organelles from the radiation induced damage, it may be considered as a provable radioprotector.

Walia et al., (2011) compared the antioxidant efficacy and the phenolic content of two hexane extracts viz. Hex 1 and Hex 2 of fruits of T. chebula prepared by maceration and sequential method respectively. The extracts were tested for their relative levels of antioxidant activity and the total phenolic content. The results
concluded that phenolic compound was predominant in the Hex 2 prepared by sequential extraction method. The antioxidative potential of Hex 2 was also far superior to the Hex 1 prepared by maceration method.

Itsarasook et al., (2012) investigated the antioxidant activity and cytotoxicity to primary human skin fibroblasts of T. chebula fruit extract. The fruits of this plant have been reported to contain high content of phenolic compounds, such as gallic acid, ellagic acid and corilagin, which possess strong antioxidant, anticancer, antimicrobial, and anti-inflammatory activities. Results revealed that the extract had antioxidant activity in a dose-dependent manner at the EC$_{50}$ of 0.61±0.02mg/ml. The extract at 50μg/ml showed the significantly increased percentage of primary human skin fibroblast cell viability.

The leaf gall of T. chebula is used widely as Karkatasringi in South Indian markets. Shankara et al., (2012) has evaluated antibacterial activity of aqueous ethanol extract of leaf gall of T. chebula against ten bacterial strains using the agar-well diffusion method. Ethanol extract presented the best results against all the bacteria while aqueous extract showed moderate inhibition of the microbial growth. Each extract is unique against different microorganisms; Staphylococcus aureus was more susceptible to both extracts among the tested organisms, whereas Serratia marcescens and Proteus mirabilis were less susceptible for ethanol and aqueous extract respectively. The inhibitory effect of the extracts was compared with standard antibiotic Ciprofloxacin. Likewise, Tariq and Reyaz, (2012) are tested antimicrobial activity of various fruit extracts of T. chebula against uropathogenic Escherichia coli. The fruit extracts of T. chebula showed good antibacterial activity on resistant uropathogenic Escherichia coli strains. The acetone and ethanol extracts
of fruit T. chebula showed superior antibacterial activity than the cold and hot water extracts. The minimal inhibitory concentration of acetone and ethanol extracts found in low concentration.

Kumar, et al., (2009) reported that anti-microbial activity of T. chebula fruit extract against Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Staphylococcus flexineria and Pseudomonas aeruginosa by disc diffusion method. The result revealed that the extract of fruit possess significant antimicrobial activity against both tested Gram-positive bacteria and Gram-negative bacteria.

Effects of methanol extract of T. chebula on the generation of superoxide radical, antibacterial activity against Bacillus subtilis, as well as on syncytium formation and cytopathic activity in virus-infected baby hamster kidney cells were examined by Lee et al., (2011). Methanol extract effectively inhibited syncytium formation in a concentration-dependent manner and infectious virus production was markedly reduced. However, glycoprotein synthesis was not affected. These results collectively indicate that methanol extract of T. chebula potentially inhibit glycosylation by acting as a suppressor of intracellular glycosylation trafficking.

Kannan, et al., (2009) studied the antibacterial potential of ethanol extract of T. chebula fruit against some clinically important standard reference bacterial strains using the disc diffusion method and the minimum inhibitory concentration. The results indicate that the T. chebula dry fruit possesses a potential broad spectrum of antimicrobial activity against Salmonella typhi, Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. The results
showed that fruit ethanol extract of T. chebula was active against most of tested bacteria. Similarly, Khan and Jain, (2009) evaluate the anti salmonellae efficacy of the aqueous extract of fruit of T. chebula in in vitro and in vivo. T. chebula exhibit anti salmonellae activity against Salmonella typhi and Salmonella typhimurium showing a clear zone of inhibition in vitro.

Huder et al., (2010) evaluated the antibacterial and antifungal activities of different fractions from fruits of T. chebula. Antimicrobial properties of ethyl acetate, chloroform, n-butanol and aqueous fractions of T. chebula exhibited different degree of inhibition activity. The result of microbial assay shows that ethyl acetate, aqueous and n-butanol fractions of T. chebula activity against Gram +ve organisms except chloroform fraction. Gram-negative organisms were found resistant to different fractions of T. chebula except Morexella catarrhalis. Candida species and Aspergillus species were found sensitive for antifungal activity of T. chebula.


Aruna et al., (2012) studied the antimicrobial and antioxidant activity of n-hexane and ethyl acetate extracts of seed of T. chebula. Phytochemical investigation of all these extracts showed the presence of steroids and flavonoids as major constituents. The antimicrobial activity of the extracts were carried out by disc
diffusion method using different Gram positive and Gram negative bacterial strains and fungal strains. The n-hexane and ethyl acetate extract of the plants showed significant antimicrobial activity when compared with standard drugs such as tetracycline and grieseofulvin respectively for antibacterial and antifungal activity. For antimicrobial activity, zone of inhibition were found to be in concentration of 5µg/ml for bacterial strains and 4µg/ml for fungal strains. Ethyl acetate extract of T. chebula showed more significant antioxidant activity than n-hexane extract.

Elizabeth, (2005) tested antimicrobial activity of aqueous and methanol extract of T. bellerica dry fruit by disc diffusion method, against 9 human microbial pathogens. Crude aqueous extract of dry fruit at 4mg concentration showed zone of inhibition ranging from 15-28mm. Staphylococcus aureus was found to be highly susceptible forming highest zone of inhibition, suggesting that T. bellerica was strongly inhibitory towards this organism. These pathogens were highly sensitive to the methanol extract forming 14 to 30mm zone of inhibition suggesting that the methanol extract of T. bellerica was more effective than crude extract against most of the microbes tested except Escherichia coli and Pseudomonasa aeruginosa. T. bellerica was highly effective against Staphylococcus aureus with lower minimum incubation concentration (MIC) values. These results indicate that T. bellerica dry fruit possesses potential broad spectrum antimicrobial activity.

Bag et al., (2009) screened the antibacterial potential of chebulic myrobalan extracts against multi-drug resistant bacterial pathogens like, methicillin resistant Staphylococcus aureus and trimethoprim sulphamethoxazole resistant uropathogenic Escherichia coli along with standard control strains. Antibacterial potency of the extracts was tested by standard growth inhibitory assay methods. All the tested
extracts showed to varying degrees of strain specific antibacterial potential against tested strains of which ethanol extract showed higher activity against Escherichia coli and hot aqueous extract against Staphylococcus aureus. Cold aqueous extract exhibited the least antibacterial activity against all the tested strains. These promising findings suggest to antibacterial activity of the plant material exhibited bioactive compounds against multi-drug resistant bacterial pathogens.

Muhammad et al., (2011) investigated the preliminary phytochemical analysis of leaf ethanol and partitioned into n-hexane, chloroform, ethyl acetate and aqueous methanol extracts of T. catappa. The results showed the presence of alkaloids, reducing sugars, saponins, tannins, resins and steroids in ethanol soluble fraction. The results of antimicrobial assay reveals that n-hexane, chloroform and ethyl acetate fractions activity against the bacterial tested.

Saheb et al., (2011) reported the antimicrobial activity of aqueous, alcoholic and ethyl acetate extracts of leaves of few T. species (T. alata, T. arjuna, T. bellerica, T. catappa, T. chebula) were tested against five plant pathogenic fungi like, Aspergillus flavus, Aspergillus niger, Alternaria brassicicola, Alternaria alternata and Helminthosporium tetramera by paper disc method. All the extracts were found effective against the tested fungi. The positive results of extracts were compared with the reference standard fungicide which was found to be more effective against fungi than the control fungicide.

The bioactive compounds were isolated from various Terminalia species such as, T. bellirica [Meena et al., 2010; Pfundstein et al., 2010], T. superba [Kuete et al., 2010; Tobopda et al., 2009; Eldeen et al., 2008], T. calamansanai [Tanaka et al., 1991; Chen et al., 2009], T. alata [Srivastava et al., 2010], T. stuhlmanii
[Katerere et al., 2003], T. tropophylla [Shugeng cao et al., 2010], T. horrida
T. horrida [Pfundstein et al., 2010; Lin et al., 1996], T. arjuna [Kalola and Rajani,
2006; Ali et al., 2003; Kruger, 2004] and T. chebula [Pfundstein et al., 2010; Lee et
al., 2007; Reddy et al., 2009] were studied.

The Ayurvedic systems of medicine recommended the medicinal use of the
pteridophytes. Ferns are also used in the Unani system of medicine [Uddin et al.,
1998]. In China, about 300 kinds of ferns were used as traditional medicinal herbs.
In addition to their antioxidant activity, the ferns showed bioactivities such as
antimicrobial, antiviral, antiinflammatory, antitussive, antitumor and anti-HIV
[Chang et al., 2011].

Antioxidant activities of frond and rhizome extracts of several genus such as
Davallia, Hypolepis, Pteridium, Cytominum, Dryopteris, Polystichum,
Dicranopteris, Lycopodium, Osmunda, Adiatum, ConioGramme, Polypodium,
Pyrrosia, Pteris, Lygodium, Selaginella, Thelypteris, Athyrium, Matteuccia, Onoclea
and Woodsia were analyzed. As a result, several ferns showed vigorous antioxidant
activities on scavenging of DPPH and ABTS radicals. Specially, Dryopteridaceae,
Osmundaceae, Woodsiaceae exhibit powerful antioxidant activities [Shin, 2010].
Some crude extracts obtained from ferns showed powerful antioxidant activities,
more powerful than those of vitamin C [Lee and Shin, 2011].

Ali et al., (2012) evaluated the antioxidant and cytotoxic activities of
methanol extract of leaves of D. filix-mas. The result reveals dose-dependent
scavenging activity of DPPH radical which was observed with good reducing power
of the extract. In DPPH radical scavenging assay, the IC\textsubscript{50} value of the extract was
275.50µg/ml and good reducing power activity was exhibited by the extract with

51
increasing concentrations. The results of total phenol and flavonoid contents were 238.10mg/gm and 126.80mg/gm respectively.

Socolsky et al., (2011) have isolated four new acylphlorogluclinols from a diethyl ether extract of the rhizomes and roots of the fern Elaphoglossum lindbergii. These compounds showed mild antibacterial activity and altered biofilm formation of the Gram (+ve) bacterium Staphylococcus aureus at 100µg/ml. Similarly, Bazzaz et al., (2008) evaluated the antimicrobial potential of D. filix rhizome essential oil against Staphylococcus aureus, Pseudomonas aeruginosa Escherichia coli and Bacillus cereus. The result reveals that MIC values of the tested essential oil are more than 1mg/ml. Likewise, Phatthalung et al., (2012) investigated antimicrobial ability of the stem ethanol extract of D. syrmatica which shows significant activity.

Lee et al., (2009) reported the antimicrobial effect of various solvent extracts from the rhizome of D. crassirhizoma and its phloroglucinol components, flavaspidic acids PB and AB. Flavaspidic acids PB and AB were isolated from the D. crassirhizoma rhizomes by methanol extraction. The antimicrobial activity of the extracts and compounds was tested by the paper disc method, the extracts and compounds were highly active against Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus, Streptococcus mutans and Bacillus subtilis. The extracts and compounds were not active against fungi and chlorella.

Voravuthikunchai et al., (2004) studied the antimicrobial activity of stem aqueous and ethanol extracts of D. syrmatica against some bacteria which reveals no antimicrobial activity. Similarly, Mandal and Mondal, (2011) has tested the antimicrobial capacity of the aqueous, ethanol methanol and acetone extracts of D. filix against Escherichia coli, Bacillus cereus, Vibrio cholarae and Klebsiella
pneumoniae. All the extracts showed significant antimicrobial results against tested pathogens. Likewise, Brijesh et al., (2012) stated that alcoholic extract of D. chrysocoma has potential antibacterial activity against both bacterial and fungal pathogens. Jahan et al., (2010) studied the root, leaves and stem extracts of D. chrysocoma against Pseudomonas aeruginosa, Citrobacter, Shigella flexneri, Yersinia aldogae Escherichia coli, Staphylococcus aureus, Saccharomyces cerevisiae, Candida albicans, Aspergillus parasiticus, Fusarium solani and Trichophyton rubrum. The extracts showed significant antimicrobial results.

Soare et al., (2012) reported the antioxidant activity, polyphenols content and antimicrobial activity of crude methanol leaf extract of some pteridophyete species. The oxygen radical absorbance capacity of the investigated ferns varied between 421.90μmol Trolox equivalent (TE) in Dryopteris filix-mas and 128.18μmol TE/g fresh weight (FW) in D. affinis. Polyphenols content in the leaves of ferns varies between 2340mg Gallic acid equivalents 100g FW in D. filix-mas and 887mg GAE/100g FW in D. affinis. The methanol extract obtained from ferns inhibits the growth of Gram negative Escherichia coli, Pseudomonas aeruginosa, Salmonella abony and Gram positive Staphylococcus aureus and Enterococcus faecalis. The highest antimicrobial activity was determined for the Dryopteris extract. The antimicrobial activity of methanol extracts obtained from leaves of D. filix-mas and D. affinis is better than the A. filix-femina in the case of Brevibacterium flavum, Sarcina sp., Bacillus cereus, Saccharomyces cerevisiae and Aspergillus niger.

Ban et al., (2012) reported the effect of a methanol extract of D. crassirhizoma on the viability, growth and virulence properties of Streptococcus mutans, in addition, the phytochemical composition. The extract showed bactericidal
and bacteriostatic activity against Streptococcus mutans. The extract significantly eliminated Streptococcus mutans up to 99.9% after 1h incubation. The extract also dose-dependently reduces growth rates of Streptococcus mutans at sub-MIC levels. GC-MS analysis revealed the presence of mono and disaccharides (44.9%), fatty acids (12.3%) and sugar alcohols (6.8%) in the extract.

Parihar et al., (2010) reported that crude methanol extracts of D. cochleata, have not inhibited the Staphylococcus aureus growth. Similarly, Parihar et al., (2010) investigated the antibacterial potential of aqueous and alcoholic extract of D. cochleata against Agrobacterium tumefaciens, Escherichia coli, Salmonella arizonae, Salmonella typhi and Staphylococcus aureus by disc diffusion method. The result reveals that the tested extracts poesses no significant antibacterial activity. Likewise, Thomas (2011) reported antimicrobial potential of petroleum ether, acetone, methanol and water extracts of leaves of D. cochleata towards some pathogenic bacterial strains by disc diffusion method. Both acetone and methanol extracts exhibited antibacterial activity; maximum activity was shown by acetone extract compared with others. Antibacterial activity was confirmed by MIC and MBC. MIC and MBC values of acetone extract of 12.5mg/ml and 25mg/ml were observed towards Staphyococcus albus while MIC and MBC values of 25mg/ml and 50mg/ml were observed towards Pseudomonas aeruginosa. Flavonoids, phenols, steroids were detected in acetone and methanol extracts.

Most pteridophytes are known to contain antimicrobial substances such as polyphenols and flavonoids [Francisco and Cooper-Driver, 1984]. Polyphenols are useful phytochemicals which provide health benefits such as antioxidants. Antioxidant is generally recognized to reduce the risk factors of chronic disease.
From experiments for screening of total polyphenol contents of 37 ferns and fern allies, Polystichum lepidocaulon and Polystichum polyblepharum were reported to have more than 13% of total polyphenols from dried materials of both fronds and rhizomes. In addition, fronds of Davallia mariesii and rhizomes of Cyrtomium fortune, Dicranopteris pedata, Athyrium niponicum and Dryopteris nipponensis showed more than 10% of total polyphenols from dried materials [Shin and Lee 2010; Shin, 2010].

Several compounds were reported from Dryopteris spp. with significant biological activity. Aspidinol B was reported from exudates of D. villarii [Wollenweber et al., 1998] desaspidinol was isolated from rhizomes of D. felix-mas [Schantz et al., 1964], Phloropyrone BB, PB and BP have been isolated from various Dryopteris spp. [Widen et al., 2010] Filicinic acid, isolated from Dryopteris ferns, possessed anthelmintic and bactericidal activity [Widen et al., 1985]. Similarly, another filicinic acid derivative, dryofragin has been isolated from Dryopteris fragrans [Arisawa et al., 1990]. Similar group of compounds, aspidins AA, AP, PP, AB, PB and BB, have been reported from various species of Dryopteris [Widen et al., 1975]. Aspidins are reported to possess various biological activities, including antihelmintic, ichthyotoxic and antitumourpromoting activities [Ito et al., 2000].

Desaspidins AA, AB, AP, PB, BB, PA and PP have been reported from Dryopteris species ferns [Jayasurya et al., 1989]. These have been used for treatment of tapeworm infections [Ostling, 1961]. Three paraaspidins, AA, AB and BB, have been reported from various Dryopteris species [Widen, 1974]. Margaspidins PP, BP, PB, BB, BV and VB were reported from ferns Dryopteris aemula, D. marginata,
D. inaequalis and D. pseudo-abbreviata. [Widen et al., 1974; Ostling 1961].
Abbreviatin BB, PB and AB were isolated from D. abbreviate [Coskun et al., 1982]
and aemulin BB from D. aemula and D. pseudo-abbreviata [Widen, 1976].

Similarly, methylene bis-desaspidinol BB was isolated from D. austriaca
and D. erythrosora [Widen et al., 1975] and methylene-bis-aspidinols VB and BB
were reported from several other Dryopteris spp [Widen et al., 2001]. Flavaspidic
acids AB, PB and BB have been reported from Dryopteris spp [Widen et al., 1985]
and norflavaspidic acid AB was reported from the fern D. dickinsii [Hisada et al.,
1972]. The effect of flavaspidic acids on cellular respiration and oxidative
phosphorylation in isolated hepatocytes has been studied [Burnett et al., 1979].

Atrataphloroglucinols A and B were reported from D. atrata [Fuchino et al.,
1979]. Triaspidin was isolated from D. austriaca [Penttila and Sundman, 1963] and
trisabbreviatin BBB was reported from D. abbreviate [Coskun et al., 1982].
Trisdesaspidin PBP was isolated from D. subimpressa [Lounasmaa, et al., 1974] and
its structural analogue trisdesaspidin BBB also isolated from rhizomes of the ferns
D. austriaca and D. caucasica [Widen et al., 1975]. Trisflavaspidic acids BBB and
ABB have been reported from the ferns D. austriaca, D. villarii and D. aitoniana
[Widen et al., 1975] and trisparaaspidins VBB, BBV, PBB and BBP were reported
from D. remota. [Widen et al., 1970]. Triaemulin BAB and triaemulin BBB have been
reported from D. aemula, D. inaequalis and D. erythrosora. [Widen et al., 1975].

2.1. Aim and scope of the study

Free radicals such as reactive oxygen species (ROS) and reactive
nitrogen species (RNS) are produced during metabolic processes and are
indispensable as mediators in many normal cellular processes. However, when produced excessively and/or when there is an inadequate antioxidant protection, it can lead to oxidative stress. Oxidative stress is considered as a major ethiological and/or pathogenic agent of most degenerative diseases such as cancer, Alzheimer’s disease, diabetes and aging. Several organic peroxides (e.g. benzyl peroxide) are tumour promoters in mouse skin; their conversion into free radicals is thought to be involved in their tumour-promoting effect [Jang et al., 2010].

In recent years, synthetic antioxidant additives such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate were used in the food industries. They are not easily soluble in water, causes some adverse effects to the consumers, low antioxidant efficiencies and also high cost. The adverse effects and cost of synthetic antioxidants stimulates researchers to find out the new antioxidant compounds which are much safer, effective and cheap. Nowadays, researchers focus on herbal and herbal preparations which can serve as high potent antioxidants and explore their leads.

At present, several phytochemicals are used in the treatment of several oxidative stress induced diseases in both traditional and modern medicine. Plants have provided a source of inspiration for novel drug compounds. Phytochemicals are natural blueprint for the development of new natural antioxidants.

Plant derived medicines are believed to be risk free and superior to chemically synthesized drugs for human health. Phytochemicals such as, phenols, tannins, terpenoids, alkaloids and flavonoids has proven with antioxidant properties. These phytoconstituents may provide long term physiological benefits without any detrimental side effects [Espin et al., 2007]. So, there is a renewed global interest
in the study and use of plants because such investigations provide important new lead on novel and active molecules of therapeutic importance.

Based on the active constituents present and the potent pharmaceutical property of T. chebula (leaves) and D. cochleata (leaves and rhizomes) it was aimed to study the followings,

2.2. Objectives of the investigation

< Preliminary phytochemical screening, determination of primary and secondary metabolites in different solvents crude extracts of T. chebula (leaves) and D. cochleata (leaves and rhizomes).

< To screen the in vitro antioxidant and antimicrobial potential in various solvent crude extracts of T. chebula and D. cochleata.

< Identification of essential elements present in these plant materials performed by Laser Induced Breakdown Spectroscopy (LIBS).

< To identify the active compounds from potent bioactive extracts using GC-MS analysis.

< Identification and isolation of bioactive compound(s) from the bioactive crude extracts of plants using chromatographic techniques (TLC and Column chromatography).

< Structural elucidation of the isolated bioactive compounds using spectral studies (LC-MS, element analysis, UV, FT-IR, $^1$H-NMR, $^{13}$C-NMR, HMBC, HSQC and $^1$H-$^1$H-COSY).

< Biological applications of isolated pure compounds.
2.3. Significance of the study

< To evaluate suitable solvent which would extract maximum phytochemical constituents and to rate their pharmacological behavior will be priceless in acting therapeutically against free radical mediated diseases.

< To identify, isolate and purify the active compounds might open up new avenues of potential therapeutic drugs. To determine and elucidate the chemical structure of active compounds by spectral studies will be the realistic picture of medicinal chemistry.

< To broaden the application spectrum on in vitro antibacterial, antimicrobial, antifungal and some free radical scavenging studies will contribute significantly to the health aspects of natural food products.