2. Review of Literature

2.1 Incidence of dermatophyte infections

The incidence of skin diseases has been reported by various authors from different parts of the world. A review of literature on the incidence of skin diseases in India and other countries revealed that among the fungal infections, dermatophytoses were the most predominant and *Trichophyton rubrum* was the most prevalent dermatophyte species (Prasad et al., 2005; Welsh et al., 2006; Sharma and Borthakur, 2007). A report on the incidence of dermatophytoses in Mexico, during 1978 and 1990 showed that out of the total 2397 reported cases *T. rubrum* was responsible for dermatophytoses in 45% cases, followed by *T. mentagrophytes* (23.7%), *T. tonsurans* (21%), *Microsporum canis* (7.1%) and *Epidermophyton floccosum* (2.5%) (Welsh et al., 2006). In the United States, the dermatophytic infection has been estimated to be up to 20% of the population (Straten et al., 2003). Nweze (2010) discussed the clinical signs and manifestations of dermatophytoses reported by various authors as well as the results of his own studies conducted in Western Africa. The epidemiological data of dermatomycosis presented a totally different pathogen spectrum and clinical manifestations in western Africa than those seen in other continents. The most common etiological agents of dermatophytoses in the West African sub-region are *T. soudanense* and *M. audouinii*. *T. rubrum, T. mentagrophytes, T. tonsurans* and *T. violaceum* played dominant role only in some locations in the region. As many patients could not afford the cost of conventional antifungal antibiotics they used local medicinal plants to treat the infections.
Clinical surveys carried out in India have shown dermatophytoses as one of the most common fungal diseases. Jain et al., (2008) reported that 58.33% cases of fungal infections were diagnosed as dermatophytoses in Jaipur, Rajasthan. Forty six percent cases of dermatophytoses were caused by *T. rubrum* followed by *T. mentagrophytes* (14.29%). *T. rubrum* was isolated in 66.3% clinical samples from patients in Madras Medical College, India (Kannan et al., 2006). A study of chronic dermatophyte infections revealed that the major cause of chronicity was onychomycosis which was primarily caused by dermatophytes. The other factors responsible for chronicity were infected site, prolonged sun exposure and diabetes mellitus (Prasad et al., 2005). They also reported that *T. rubrum* was the commonest isolate (17.3%). The findings of clinical studies by Gupta et al., (2000) also showed prevalence of dermatophyte infections in diabetic patients. They emphasized the need of development of an ideal antifungal agent having broad spectrum of activity and a favourable safety profile suitable for treatment of dermatophytoses in diabetic patients.

Incidence of skin diseases in Northeast India was also reported by some authors. Jaiswal (2002) reported that the incidence of fungal diseases in Northeast India accounted for almost fifty percent of the total infectious skin diseases. In another study, dermatophytes were found to be responsible for 78.6% cases of fungal skin infections (Sharma and Borthakur, 2007). The majority of skin infections in Imphal, Manipur (India) were caused due to the incidence of fungal infections (Devi and Zamzachin, 2006). Das (2003) studied 42198 cases of major skin diseases in the Guwahati Medical College and Hospital, Guwahati, Assam during the period of 1998 - 2000 and grouped them into eczema
(23.10%), pyoderma (14.29%), fungal infections (14.24%) and psoriasis (5.77%). In this study also the dermatophytosis was found to be the commonest (47.46%) fungal skin diseases. The review of literature on dermatophytoses occurring in India and other countries revealed that \textit{T. rubrum} was the predominant species causing skin diseases (Welsh et al., 2006; Prasad et al., 2005; Sharma and Borthakur, 2007).

2.2 Medicinal plants with antidermatophytic properties

Since the dawn of civilization, herbal products have been used in traditional medicines all over the world. WHO has estimated that perhaps 80\% of the more than 4000 million inhabitants of the world rely chiefly on traditional medicines for their primary health care needs and it can safely be presumed that a major part of traditional therapy involves the use of plant extracts or their active principles (Farnworth et al., 1985). Traditional medicines possess a spectrum of yet unknown properties, thus making them ideal candidates for novel drugs. Drug discovery from plants involves a multidisciplinary approach combining botanical, ethno botanical, biological and phytochemical techniques. Plants continue to provide us new chemical entities for the development of drugs against various pharmacological targets. Although plant-based drug discovery programmes continue to provide an important source of new drug leads, numerous challenges are encountered, including authentication of plant materials, implementation of high throughput screening bioassays and scale-up of bioactive compounds (Jachak and Saklani, 2007). The search for the development of novel drug molecules is very expensive and involves a significant time period of a decade or so. These can be
significantly cut down if a suitable lead is available from the plant kingdom. In India, traditional herbal remedies are still the backbone of medicines. Majority of healers rely mainly on locally available herbs. Although trade brings a few important herbs, the most of this information is outside current databases and remain unavailable to researchers. This has led the attention of the scientific community towards plant-based medicine, for new leads to develop better drugs against microbial infections. Considerable efforts are being made all over the world, to utilize more and more plant resources, as the medicine of today is shifting from synthetic molecules, to naturally occurring molecules as these molecules are biologically more compatible and less toxic to human system.

There are several reports on screening of active compounds from plants that has lead to the discovery of new efficient drugs against various diseases (Parekh and Chanda, 2007, Parekh and Chanda, 2008; Patel and Coogan, 2008; Sisti et al., 2008; Adigozel et al., 2005). As a consequence, in the recent years, there has been a rapid development in high throughput screening of plants for antimicrobial activity (Cowan, 1999; Phongpaichit et al., 2005; Katiyar and Sharma, 2005; Svetaz et al., 2010). These reports showed that there has been ample effort made all over the world on the evaluation of medicinal plant extracts for their antifungal activities. Results of this extensive research on herbs unfurled the potentiality of developing antimicrobial agents from plants. It is hoped that the therapeutic potential of herbs as reported by various authors (Farnworth et al., 1985; Phongpaichit et al., 2005; Svetaz et al., 2010) will open up a new vista in the future pharmacological research of herbal drug development.
Dubey et al., (2004) emphasized the importance of the criteria for collecting and processing medicinal herbs. The season and age of the plant is a matter of considerable importance, both in terms of quality and quantity of active constituent(s), because the amount and nature of active constituents vary with season and age of the plant. Proper drying and harvesting are other important parameters that ensure the quality and efficacy of active component. Abad et al., (2007) concluded that molecules with antimycotic activity obtained from the natural environment either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drugs. Antibacterial evaluation of aqueous and organic extracts of various parts of *Datura innoxia* indicated that the intensity of inhibition depended largely upon the plant part, extracting solvent and the test organism (Kaushik and Goyal, 2008). Kaushik and Goyal (2008) also observed that organic extracts showed more potent antibacterial activity than the aqueous extract. Ficker et al., (2003) selected 29 plant species using an ethno botanical approach and tested 36 numbers of extracts for antifungal activity. Among these, extracts of *Zingiber officinale* and *Juglans cinera* showed significant activity against a taxonomically diverse group of 13 human pathogenic fungi including strains that were highly resistant to amphotericin-B and ketoconazole. Interestingly, in many cases, the medicinal plants were found more effective than the commercial antimicrobial drugs. Ali-Shtayeh and Ghdeib (1999) reported the antidermatophytic activity of different plants such as *Anagalis arvensis, Capparis spinosa, Juglans regia, Pistacia lentiscus, Ruta calapensis, Innula viscosa, Asphodelus microcarpus, Clematis cirrhosa, Plumbago europea, Ruscus esculentus, Retema raetam* and *Salvia fruticosa* used in folk medicine in Palestine. The antifungal activity of ethanol extract of *Piper guineense, Ocimum gratissimum, Moringa*
oleifera and Erythrophleum suaveolens from south-eastern part of Nigeria was reported to be active against T. rubrum and T. mentagrophytes (Nwosu and Okafor, 1995). Mahmoudabadi and Nasery (2009) reported the antifungal activities of shallot (Allium ascalonicum) that has been used worldwide as a spice, food and folk medicine. The fresh juice of shallot showed remarkable antifungal activity against Candida species and dermatophytes. Hammer et al., (1999) reported in vitro antibacterial and antifungal activity of many essential oils and plant extracts. Njateng et al., (2010) tested the sensitivity of clinical isolates of M. gypseum and T. mentagrophytes towards essential oils extracted from Ageratum houstonianum leaves by agar dilution assay and found that lowest MIC value was shown by the oil and griseofulvin mixture at 10:1 ratio against both the test dermatophytes. The increased activity of 10:1 mixture of oil and griseofulvin as compared to other combination was attributed to presence of a little griseofulvin. LD_{50} value of the essential oil was determined as 5 g/kg body weight in guinea pigs. The findings demonstrated that the oil contains antidermatophytic compounds and may be safe for topical application. The results validated the traditional use of the A. houstonianum leaves against skin diseases in Cameroon. Jain and Sharma (2003) determined the potency of the essential oil extracted from the seeds of Trachyspermum ammi, both by in vitro as well as clinical trials against ringworm infections in human beings. The comparative in vivo study of ointment of T. ammi oil (1 and 2%) with different antimycotic drugs like griseofulvin, itraconazole, ketoconazole and zacon, tested on 30 patients of both the sexes and of different age groups (infant to 50 year-old) showed the better efficacy of T. ammi oil than standard drugs. Nalina and Rahim (2007) studied the influence of crude aqueous extract of Piper betle on Streptococcus mutans and
observed the damage of the plasma membrane and coagulation of the nucleoid in TEM micrograph.

Many medicinal plants have been studied by various authors (Sharma and Joshi 2004; Nayak et al., 2006; Braga et al., 2007; Das et al., 2008). Uses of *Cassia sophera* (seed), *Leucas aspera* (leaf), * Ocimum sanctum* and *Piper nigrum*, in treatment of skin diseases in veterinary, were reported by Sharma and Joshi (2004). Antimicrobial properties of neem (*Azadirachta indica*) leaf were reported by many workers (Ghorbanian et al., 2007; Natarajan et al., 2003). Antipyretic and analgesic effect of leaves of *Solanum melongena* in rodents was observed which may be attributed to the presence of alkaloids, flavonoids and tannins (Mutalik et al., 2003). * Ocimum gratissimum, Piper aduncum, Cajanus cajan* and *Schinus terebintifolius*, used in traditional medicine in Brazil, were reported to be antileishmanial and antifungal (Braga et al., 2007). The ethanol extract of *O. gratissimum* arrested the growth of *T. rubrum* and *T. mentagrophytes* (Nwosu and Okafor, 1995). Das et al., (2008) carried out a survey in different parts of Cachar district, Assam and gathered information on the medicinal uses of many plants e. g. *Alstonia scholaris, Azadirachta indica, Clitoria ternatea, Leucas aspera, Mimosa pudica, Dillenia indica, Ocimum sanctum, Vitex negundo* etc. by different tribes/communities of the region. The use of *Aloe vera* has been well known from ancient times for its marvelous healing properties. Today, *A. vera* is widely used in various cosmetics and pharmaceutical products. Commercially *A. vera* is found in the form of medicated cream, ointment, body lotion, moisturizer, gel, spray etc (Sharma, 2007). Masuduzzaman et al., (2008) reported the antifungal property of *Allamanda cathartica* leaf extract against some important plant
pathogenic fungi. The aqueous leaf extracts of *A. cathartica* and *Laurus nobilis* was tested by Nayak et al., (2006) for their wound healing activity using rat as test organism. They concluded that the leaf extract of *Allamanda* possessed better wound healing activity and it could be used in the treatment of different types of wounds in human beings. Adigozel et al., (2005) reported the antimicrobial effects of ethanol, methanol, and hexane extracts of *Ocimum basillicum* (aerial part) against a large number of bacteria, fungi and yeast species. The various pharmacological effects of *Camellia sinensis* was summarized by Bhatt et al., (2010).

The indigenous people of North East India use 10 species of *Piper* traditionally as insecticidal, larvicidal, fungicidal, carminative, antidote etc. (Gajurel et al., 2001). The uses of *Piper nigrum* in traditional medicines was reported by Parekh and Chanda, (2006). Extensive phytochemical investigations on 30 Indian *Piper* species and other medicinal plants have revealed the presence of a large number of novel compounds belonging to different classes (Prasad et al., 2005). Vaghasiya et al., (2007) opined that various biologically important species of Piper like *P. sarmentosum*, *P. argyrophyllum*, *P. longum*, *P. betle* and *P. chaba* should be explored to elucidate their active principle. Studies on extracts of *Piper longum* using different solvents against a wide variety of pathogenic bacteria and fungi, showed that chloroform extract was more effective in inhibiting fungi (Ali et al., 2007).

### 2.3 Phytochemical investigations

There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Jachak and Saklani, 2007). The active
components of many plants are secondary metabolites. Phytochemical analysis of the plant extract for their active compound, therefore, is vital.

Edeoga et al., (2005) reported the results of the phytochemical screening and quantitative estimation of the chemical constituents in ten medicinal plants, such as, *Cleome nutidosperma, Emilia coccinea, Euphorbia heterophylla, Physalis angulata, Richardia bransitensis, Scopania dulcis, Sida acuta, Spigelia anthelmia, Stachytarpheta cayennensis* and *Tridax procumbens* collected from South Nigeria. All the plants were found to contain alkaloids, tannins and flavonoids except for the absence of tannins in *S. acuta* and flavonoids in *S. cayennsis*. It has been found that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. Some of these plants contained steroidal compounds. *S. acuta* and *T. procumbens* possessed very high levels of alkaloids and flavonoids. The results established the significance of these plants in traditional medicine. Roy et al., (2010) studied the phytochemical profile and antimicrobial activity of *Andrographis paniculata*. Chloroform extract of this plant was more effective against five gram negative and four gram positive bacteria than chloroform + HCL extract. GC-MS analysis of chloroform extract showed the presence of phenols, aromatic carboxylic acids and esters, which might be responsible for the antimicrobial activity of *A. paniculata*. The results highlighted the need of developing a novel broad spectrum antimicrobial agent from this extract. The antimicrobial activity of crude ethanol extracts of *Acacia nilotica, Cinnamum zeylanicum* and *Syzygium aromaticum* against multidrug resistant microbial strains, isolated from nosocomial and community acquired infections, was reported and their activity was attributed to the presence of tannins, saponins,
phenolic compounds, essential oils and flavonoids (Khan et al., 2009). Sahare et al., (2008) observed the presence of alkaloids, saponins and flavonoids in the methanol roots extracts of *Vitex negundo* and coumarin in the leaves of *Aegle marmelos* using thin layer chromatography and determined the antifilarial activity of these extracts. Al-Adhroey et al., (2011) identified alkaloids, terpenes, anthraquinones, flavonoids, tannins, saponins and steroids in crude methanol extract of *Piper betle* leaves, which may be implicated in the antimalarial and antioxidant activity of the extract. The results of toxicological tests showed that the methanol extract of *Piper betle* leaves was safe by oral administration. The oil of *P. betle* has been used in the treatment of various diseases. The essential oils and extracts of leaves possess activity against several gram positive and gram negative bacteria and some fungi such as *Aspergillus niger*, *A. oryzae*, *Curvularia lunata* and *Fusarium oxysporum* (Anonymous, 1969). Investigation on chemical composition of methanol extracts of leaves from eight *Ocimum* species namely *O. gratissimum*, *O. americanum*, *O. minimum*, *O. citriodorum*, *O. kilimandscharicum*, *O. grandiflorum*, *O.lamiifolium*, and *O. selloi* showed the presence of phenolic acids, hydroxycinnamates and flavonoids (Hakkim et al., 2008).

### 2.4 Isolation of bioactive components from medicinal plants

Several workers have described the approaches that can be used for isolation of active principle from medicinal plants. A number of chemical compounds, particularly the secondary metabolites, produced by medicinal herbs are responsible for their biological activity. The identification of biologically active compounds is an essential requirement
for quality control and dose determination of plant-based drugs (Dubey et al., 2004). Bioassay followed by activity-guided fractionation has led to discovery of many bioactive compounds like antifungal, antibacterial, antimalarial etc (Hostettmann, 1999). Literature search in the area of discovery of antimicrobial agents, particularly antidermatophytic components in medicinal plants, revealed that although in some instances, the active ingredients of the plants have been identified, in many cases the active ingredients are yet to be explored.

An active fraction with remarkable antifungal activity against *Candida albicans* was separated from the crude extract of *Gracilaria changii* (alga) by various purification procedures such as thin layer chromatography, column chromatography, bioautography etc (Sasidharan 2008). Hassan et al., (2007) studied the antifungal activity of the hexane (4 fractions, HX1-HX4), petroleum ether (3 fractions, PE1-PE3) and chloroform (3 fractions, CHL1-CHL-3) fractions of stem bark of *Ficus sycomorus*, obtained using silica gel column chromatography. Out of these organic fractions, the hexane fraction exhibited the most significant inhibitory activity against different fungal isolates such as *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, *Aspergillus niger*, *A. flavus* and *Candida albicans*. Phytochemical studies of the active fractions revealed the presence of steroids, condensed tannins, cardiac glycosides and saponins. The principal antifungal constituent in the stem bark *F. sycomorus* might be anthroquinone glycosides, detected only in the most active hexane fraction, by TLC study. Antifungal fraction, purified by column chromatography from the ethanol extracts of *Galenia africana* obtained from sequential extraction with hexane, chloroform, ethanol and distilled water, on further spectroscopic
analysis showed the presence of flavonoids as major components of this fraction. The isolated compounds were identified as 1) 5-hydroxy-7-methoxy-flavanone or pinostrobin, 2) dihydroechinoidinin, 3) 2’, 4’-dihydroxychalcone, 4) 2’,4’dihydroxydihydrochalcone and 5) lupeone. The results suggested that antifungal activity probably requires a specific combination of these flavonoids rather than any single one of these compounds (Vries et al., 2005). An antibacterial compound, effective against *Xanthomous oryzae*, was isolated from dichloromethane extract of *Helianthus annus*, which was identified as unsaturated aliphatic aldehyde with OCH$_2$ groups based on spectra obtained from two dimensional $^1$H NMR recorded at 400MHz and $^{13}$C NMR recorded at 100 MHz (Sankanarayanan et al., 2008). Madan et al., (2008) isolated three compounds namely flemingiaflavanone (8, 3'-diprenyl-5, 7, 4'-tri hydroxy flavanone), genistin (5, 4-dihydroxy isoflavone 7-O-glucoside) and β - sitosterol-D glucoside from *Flemingia strobilifera* roots through bioactivity guided fractionation. Flemingiaflavanone showed significant antimicrobial activity against gram-positive (*Staphylococcus aureus*, *S. epidermidis*), gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and fungi (*C. albicans*), while genistin showed moderate activity. The isolation and antimicrobial activity of flemingiaflavanone is being reported for the first time. Martini et al., (2004) isolated seven antibacterial flavonoids i.e. apigenin, genkwanin, 5-hydroxy-7, 4’-dimethoxyflavone, rhamnocitrin, kaempferol, quercetin-5, 3’-dimethylether and rhamnazin through bioassay-guided fractionation, from *Combretum erythrophyllum*. All the isolated compounds had good activity against *Vibrio cholerae* and *Enterococcus faecalis*, with MIC values in the range of 25–50 μg/ml. Although these flavonoids were known, the biological activity of these compounds was reported for the first time. *Vismia*
rubescens a medicinal plant, popularly used in Cameroon and in several parts of Africa as febrifugal and for the treatment of various microbial infections including skin diseases. Evaluation of the crude methanol extract and five compounds namely 1) 1, 4, 8-trihydroxyxanthone, 2) 1, 7-dihydroxyxanthone, 3) physcion, 4) friedelin and 5) friedelanol, obtained after column chromatography of the methanol-soluble fraction from the stem bark of V. rubescens against four fungal species namely, Candida albicans, C. tropicalis, C. parapsilosis and Cryptococcus neoformans and three bacterial species-Salmonella typhi, Staphylococcus aureus and Pseudomonas aeruginosa revealed that the crude extract and compounds 1, 2 and 3 exhibited both antibacterial and antifungal activities showing MIC of 3.12 to 1000 μg/ml. The first time report on the antimicrobial activity of Vismia rubescens and two compounds namely 1, 4, 8-trihydroxyxanthone and 1, 7-dihydroxyxanthone provided the promising baseline information for their potential use in the treatment of skin diseases and diarrhoea (Tamokou et al., 2009). Sener, (1994) screened 102 aqueous and ethanolic extracts derived from Turkish medicinal plants with different pharmacological tests, such as antibacterial, antifungal, antiinflammatory, antiplatelet and analgesic activity. Bioassay-directed fractionation using different pharmacological tests with the extracts from Fumaria vaillantii, Fritillaria imperialis, Veratrum album, Symphytum officinale and Paeonia daurica has led to the determination of different bioactive compounds. A review on the above aspects with crude extracts, fractions as well as pure compounds indicated that ethanolic extracts were more effective than the aqueous extracts in inhibiting bacterial growth and identified 29 antifungal plants that might be related to their phenolic moieties. Chukwujekwu et al., (2011) determined the antibacterial activity of the stem bark of Erythrina caffra Thunb. and isolated four
known antibacterial flavonoids namely, abyssione-V 4′-O-methyl ether, 6, 8-
diprenylgenistein, alpinumisoflavone and burttinone. The antibacterial activity of
burttinone and the isolation of these compounds from *E. caffra* were reported for the first
time.

### 2.5 Compounds isolated from *Piper* species

The chemistry of members of the family piperaceae is of great interest owing its variety
of biological properties displayed. Many species of *Piper* are well known for their
therapeutic efficacy (Portet et al., 2007; Yang et al., 2002; Lee et al., 2005; Prasad et al.,
2005; Zaveri, 2010; Reddy et al., 2001). *Piper* species are a rich source of different
classes of secondary metabolites, particularly of biologically active amides, lignans and
neolignans (Prasad et al., 2005). Four new dihydrochalones as well as five known
compounds viz. 2′, 6′-dihydroxy-4′-methoxydihydrochalcone; linderatone; strobopinin;
adunctin E and (−)-methyllinderatin were isolated from n-hexane extract of *Piper
hostmannianum* from France, Guiana by bioassay-guided purification (Portet et al., 2007).
Nalina and Rahim (2003) described a fast and simplified TLC technique to separate
active antibacterial compound of *Piper betle* which can be used in bioautography. Rali et
al., (2007) analysed colourless oil and pale orange coloured oils extracted respectively
from leaves of *Piper aduncum* and fruits of *Piper gibbilimbum* by GC-MS. They found
that the volatile constituents of *P. aduncum* is composed of dill apiole (43.3 %), β-
caryophyllene (8.2 %), pipertione (6.7 %) and α-humulene (5.1 %), whilst the oil of *P.*
*gibbilimbum* is dominated by the gibbilimbols A, B, C, D (74.2%), with the remaining major constituents being the camphene (13.6%) and α-pinene (6.5%). Nakatani et al., (1986) studied chemical constituents of peppers (Piper species) and identified five phenolic amides from dry fruits of *P. nigrum*, seven compounds from *P. retrofractum* and two compounds from *P. baccatum*.

The chemistry and pharmacology of an important medicinal plant, *Piper longum* have been extensively reviewed by Zaveri et al., (2010). Uses of the roots, fruits and the whole plant in traditional remedy and Ayurveda were well reported. The fruit contains volatile oil, starch, protein and alkaloids, saponins, carbohydrates and amygdalin. Sylvatine and dieudesmin, fatty acids of crushed seeds were reported to be palmitic, hexadecenoic, stearic, linoleic, oleic, higher saturated acids, arachidic, and behenic acids. A large number of alkaloids and amides were isolated from the fruit and root. Lignans and esters were also detected in fruit. Piperine was reported to be the major constituent of *P. longum* (3-5%, which constitutes the active pungent principle (Zaveri, 2010; Kato 2007). Yang et al., (2002) reported characterisation of pipernonaline in crude methanol extract and hexane fraction of the methanol extract of *P. longum* fruit. Lee et al., (2005) isolated piperidine alkaloid, piperine, through bioassay-guided isolation of the ethanol extract of *P. longum* fruit, which showed an inhibitory effect against monoamine oxidase (MAO). Based on the results, he suggested that piperine possesses potent antidepressant-like properties that are mediated in part through the inhibition of MAO activity and therefore, represent a promising pharmacotherapeutic candidate as an antidepressant agent.
Through extensive phytochemical investigations on thirty *Piper* species growing in India and some other medicinal plants, Prasad et al., (2005) isolated a large number of novel compounds belonging to different classes. The antiviral activity of several lignans and neolignans belonging to different structural types has been evaluated against six different viral strains. Further, the effects of ethanol, chloroform and hexane extracts of *P. longum* and *P. galiatum* on cytokine intercellular adhesion molecule-1 (ICAM-1) expression on endothelial cells have been studied and a novel aromatic ester was isolated from the most active extract of *P. longum*. The results indicated that these extracts and the pure compounds derived from them could be used to develop anti-inflammatory agents. Reddy et al., (2001) described the antibacterial activity of the pure isolates from *P. longum* L. and *Taxus baccata* L. Of the four isolates of *P. longum*, except one isolate, 3-(3′-4′-5′-Trimethoxyphenyl) propionic acid, other three isolates, namely, piperlonguminine, piperine and pellitorine were highly active against gram-positive bacteria and moderately active against gram-negative bacteria. In case of *T. baccata*, rhododendrol inhibited *Salmonella typhimurium* and *Pseudomonas syringae*, while 4-(4′-hydroxyphenyl)-butan-2-one and 4-(4′-hydroxyphenyl)-trans-but-3-en-2-one inhibited *Pseudomonas syringae* and *Bacillus sphaericus*. Lee et al., (2001) isolated a piperidine alkaloid, pipernonaline, from hexane fraction of *P. longum* fruits and described its efficacy in controlling wheat leaf rust caused by *Puccinia recondita*. Parmer et al., (1998) isolated 38 compounds from 12 *Piper* species. Among these, some of the compounds were isolated for the first time from the genus *Piper*. They were 2, 6-dimethoxy-4-(2-propenyl) phenol (ether extract of *P. aduncum* leaves), β-sitosteryl palmitate (*P. betle*), nerolidol (*P. falconeri*), retrofractamide A (*P. longum*) and furacridone. Novel compounds, namely, 2-acetoxy-1,
3-dimethoxy-5-(2-propenyl) benzene from the ether extract of *P. aduncum* leaves, a long chain alcohol, 14-benzo[1, 3]dioxol-5-yl-tetradecan-2-ol from the petrol extract of *P. attenuatum* (stems and leaves) and an amide, 3-(3,4-dimethoxyphenyl) propanoyl pyrrole from *P. brachystachyum* were reported.

### 2.6 Authentication of medicinal plants using molecular tools

Accurate authentication of herbal medicines is always necessary to prevent the target herbs from intentional and inadvertent adulteration with other plant species. Conventional identification is not precise enough to authenticate those herbs which are morphologically indistinguishable and frequently substituted or adulterated by similar plants or plant materials. Therefore, it is necessary to develop a more effective, accurate, reliable and sensitive technology for the authentication of medicinal plants. Various molecular based markers have been developed in recent years that can be employed to analyze DNA for quality assurance, control and authentication of medicinal plant species. DNA-based methods for the authentication of plant species and adulteration detection in many medicinal plants, agricultural crops and genetically modified (GM) foods, have been published. Srivastava and Mishra (2009) gave a detail description of various genetic markers and their usefulness in various fields of herbal drug technology. Tewfik (2008) highlighted the useful analytical techniques that can be employed to analyse DNA for quality assurance and authentication of medicinal plant species. Bashalkhanov and Rajora (2008) developed high-throughput, fully automatable and cost effective system for DNA extraction from Conifers. Zhang et al., (2007) emphasized on construction of a
comprehensive database of DNA fingerprints and DNA sequences for a broad spectrum of medicinal species, along with voucher specimens, macro and microscopic data, chemical profiling for quality control of Chinese herbal materials.

In the current scenario, wild and cultivated plant varieties and their genes are being increasingly recognized as resources of high economic value. Consequently there is worldwide policy shift from free exchange and unhindered exploitation to controlled access to these resources. The World Trade Organization (WTO) agreement of which India is signatory, envisages adoption of either a patent system or some form of effective sui generis system or by combination thereof, for protecting the intellectual property rights of plant breeders. Considering this, a software system entitled ‘Crop DNA Fingerprint Database’ has been developed and implemented at the National Research Centre on DNA Fingerprinting (NRCDF), New Delhi, which provides huge molecular profile data on crop varieties, suitable for a perfect identification system to enforce the protection regimes for plant varieties and germplasm (Bala, 2007). Many authors studied RAPD analysis of different medicinal plants such as *Phyllanthus* species, *Palicourea coriacea* and *Trichodesma indicum* (Manissorn et al., 2010; Barbosa et al., 2010; Verma et al., 2010; Pirttila et al., 2001)

Joy et al., (2007) found considerable genetic variability among cultivars of *P. nigrum*. Fingerprinting analysis with AFLP showed the high level of polymorphism and the unique characterization of the major cultivars. RAPD analysis was conducted in 22 cultivars of *P. nigrum* from South India and one accession each of *P. longum* and *P.
The findings indicated the existence of wide genetic diversity in pepper cultivars from South India. Genetic proximity among *P. nigrum* cultivars could be related to their phenotypic similarities or geographical distribution. Greater divergence was observed among landraces than among advanced cultivars. Landraces grown in southern parts of coastal India and those grown in more northern parts were grouped in separate clusters (Pradeepkumar et al., 2003). Pradeepkumar and his coworkers, (2001) studied on the DNA fingerprinting of *P. nigrum* (13 land races and 9 advanced cultivars) using 34 primers and obtained cultivar-specific bands for all the varieties except Panniyur-3. Panniyur-1 and Panniyur-2 generated specific bands with OPA 08, while OPA10 were found efficient in differentiating three varieties, viz. Panniyur-4, Panniyur-5 and Panchami. Panniyur-1 is the most popular pepper variety grown in India, while land races Cheriakaniakadan and Karimunda are extensively used for pepper improvement work. On the basis of RAPD profiles, a clear distinction within and among the four groups of landraces of betel vine (*Piper betle*) namely Kapoori, Bangla, Sanchi and Others and gender-based distinction among all known male or female betel vine landraces was reported (Verma et al., 2004). The results indicated the ‘Kapoori’ group is the most diverse. Sen et al., (2010) analyzed the genetic diversity of eight species of *Piper* viz., *P. nigrum, P. longum, P. betle, P. chaba, P. argyrophyllum, P. trichostachyon, P. galeatum* and *P. hymenophyllum* from Kerala, India by RAPD. They reported the genetic variations and similarities of the *Piper* species by PCR-RAPD and finally selected 11 RAPD primers for comparative analysis of different species of *Piper*. The results of the study would be useful in facilitating germplasm identification, management, and conservation. Jain et al., 2007 reported that many species, which are frequently adulterated with other
plants, for example *Piper longum* fruiting spike with *P. chaba* fruiting spike and *P. nigrum* adulterated with *Carrica papaya* seeds, fruits of *Embelia ribes* and *Lantana camara*. Parani et al. (1997), based on RAPD fingerprints of twenty micro propagated plants and mother plant of *Piper longum*, by PCR of genomic DNA, showed that eighteen micro propagated plants formed a major cluster along with the mother plant. Thus the study established the potential use of RAPD fingerprinting in the selection of genotypes from micro propagated plants in order of preference to maintain maximum fidelity to the elite genotype (the mother plant) for conservation purpose. This would also be useful to manipulate the causes of variation between the mother plant and micro propagated plants as required. RAPD analysis on genotypic and morphogenetic differences among three female varieties of *P. longum*, one variety each from Assam and Calicut and one released variety, Viswam, revealed that all the varieties are genetically different. Compared to the Assam variety, Viswam and Calicut varieties are genetically closer (95% similarity) among themselves (Philip et al., 2000). Such effort might prove useful in fingerprinting the varieties of superior quality and also to conserve them for future need.