# A STUDY ON THE ANTIDERMATOPHYTIC ACTIVITY OF SOME ETHNO-MEDICINAL PLANTS OF NORTH EAST REGION IN RELATION TO INTELLECTUAL PROPERTY RIGHTS

## THESIS

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**IN**
**HORTICULTURE, AROMATIC AND MEDICINAL PLANTS**

**By**

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# DECLARATION BY THE CANDIDATE

## Mizoram University

## May, 2013

I, Rita Gupta, hereby declare that the subject matter of the thesis entitled ***"A study on the Antidermatophytic Activity of Some Ethno-medicinal Plants of North East Region in Relation to Intellectual Property Rights"*** is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge, to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institutes.

This is being submitted to the Mizoram University for the Degree of Doctor of Philosophy in Horticulture, Aromatic and Medicinal Plants.

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# Abbreviations

| | |
|---|---|
| CBD: | Convention on Biological Diversity |
| CCNSC: | Cancer Chemotherapy National Service Center |
| CGPDTM: | Controller General of Patents, Designs & Trade Marks |
| CLSI: | Clinical Laboratory Standards Institute |
| DMSO: | Dimethyl Sulphoxide |
| E1: | Electron Impact Ionisation |
| EPC: | European Patent Convention |
| EPO: | European Patent Office (EPO) |
| GC-MS: | Gas-Chromatographic-Mass Spectrometry |
| IPR: | Intellectual Property Right |
| IR: | Retention Indices |
| JPO: | Japan Patent Office |
| KIPO: | Korean Intellectual Property Office |
| MFC: | Minimum Fungicidal Concentration |
| MIC: | Minimum Inhibitory Concentration |
| MS: | Mass Spectroscopy |
| MSC: | Minimum Static Concentration |
| MSD: | Mass Selective Detector |
| NE Region: | North East Region |
| PTO: | Patent Cooperation Treaty |
| RT: | Retention Time |
| SIPO: | State Intellectual Property Office of the People's Republic of China (SIPO) |
| TDW: | Triple Distilled Water |
| TRIPS: | Trade Related Intellectual Property Rights |
| USPTO: | United States Patent and Trademark Office |
| WIPO: | World Intellectual Property Organization |
| WTO: | World Trade Organization |

# 1. Introduction

## 1.1 Dermatophytes

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair, and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm. Infection is generally cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts. Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors.

Raimond Sabouraud, one of the best known and most influential of the early medical mycologists, began his scientific studies of the dermatophytes around 1890, culminating in the publication of his classic volume, Les Teignes, in 1910 (Sabouraud, 1910). Sabouraud's contributions included his studies on the taxonomy, morphology, and methods of culturing the dermatophytes and the therapy of the dermatophytoses. Sabourad classified the dermatophytes into four genera, *Achorion*, *Epidermophyton*, *Microsporum*, and *Trichophyton*, primarily on the basis of the clinical aspects of the disease, combined with cultural and microscopic observations. The medium that he developed is in use today for culturing fungi (although the ingredients are modified) and is named in his honor, Sabouraud glucose (dextrose) agar. Sabouraud's treatment of tinea capitis by a one-dose, single-point roentgenologic epilation achieved cures in 3 months as opposed to the then current therapy of manual epilation and topical application of medications.

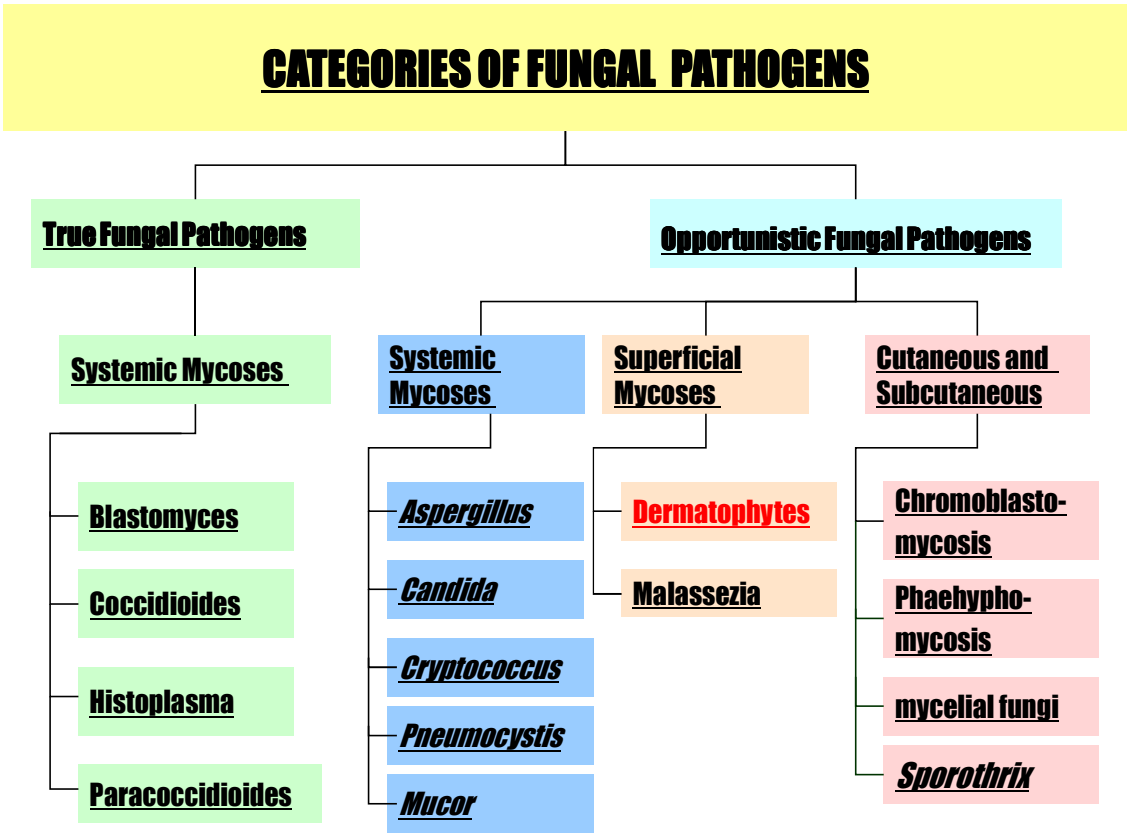



**Figure 1.1: A Schematic Representation of the Fungal Pathogens in Human beings**

In 1934, Chester Emmons modernized the taxonomic scheme of Sabouraud and others and established the current classification of the dermatophytes on the bases of spore morphology and accessory organs. Chester Emmons (1934) eliminated the genus *Achorion* and recognized only the three genera *Microsporum*, *Trichophyton*, and *Epidermophyton* on the basis of mycological principles.

### 1.1.1 Etiologic Agents

#### 1.1.1.1 Anamorphs

The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, of anamorphic class Hyphomycetes of the Deuteromycota (Fungi Imperfecti).

The genera and their descriptions are as follows.

#### 1.1.1.1.1 *Epidermophyton* spp.

The type species is *E. floccosum*. The macroconidia are broadly clavate with typically smooth, thin to moderately thick walls and one to nine septa, 20 to 60 by 4 to 13 mm in size. They are usually abundant and borne singly or in clusters. Microconidia are absent. This genus has only two known species to date, and only *E. floccosum* is pathogenic.

#### 1.1.1.1.2 *Microsporum* spp.

The type species is *Microsporum audouinii*. Macroconidia are characterized by the presence of rough walls which may be asperulate, echinulate, or verrucose. Originally, the macroconidia were described by Emmons as spindle shaped or fusiform, but the discovery of new species extended the range from obovate (egg shaped) as in *M. nanum* to cylindrofusiform as in *M. vanbreuseghemii*. The macroconidia may have thin, moderately thick to thick walls and 1 to 15 septa and range in size from 6 to 160 by 6 to 25 mm. Microconidia are sessile or stalked and clavate and usually arranged singly along the hyphae or in racemes as in *M. racemosum*, a rare pathogen.

#### 1.1.1.1.3 *Trichophyton spp*.

The type species is *Trichophyton tonsurans*. Macroconidia, when present, have smooth, usually thin walls and one to 12 septa, are borne singly or in clusters, and may be elongate and pencil shaped, clavate, fusiform, or cylindrical. They range in size from 8 to 86 by 4 to 14 mm. Microconidia, usually more abundant than macroconidia, may be globose, pyriform or clavate, or sessile or stalked, and are borne singly along the sides of the hyphae or in grape-likeclusters. The anamorphic species of the dermatophytes are listed in Table 1. Descriptions of the species and related keratinophilic fungi may be found in several publications (Rippon, 1974).

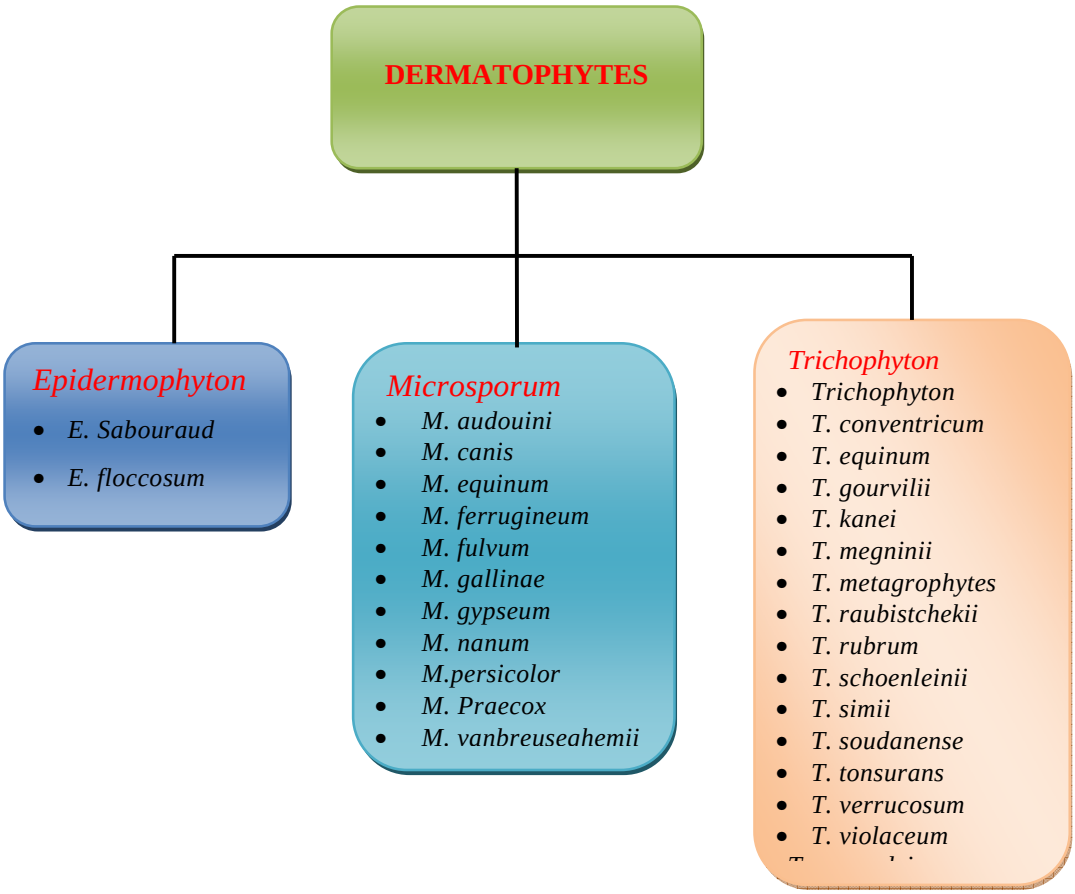



**Figure 1.2: A Schematic Representation of the Dermatophytes**

**Table 1.1: Anamorph Genera and Species of Dermatophytes**.

**Species of Dermatophytes**

- *Epidermophyton Sabouraud* 1907
- *E. floccosum* (Harz) Langeron et Milochevitch 1930
- *Microsporum* Gruby 1984
- *M. audouini* Gruby 1843
- *M. canis* Bodin 1902
- *M. equinum* (Delacroix et Bodin) Gueguen 1904
- *M. ferrugineum* Ota 1921
- *M. fulvum* Uriburu 1909
- *M. gallinae* (Megnin) Grigorakis 1929
- *M. gypseum* (Bodin) Guiart et Grigorakis 1928
- *M. nanum* Fuentes 1956
- *M. persicolor* (Sabouraud) Guiart et Grigorakis 1928
- *M. praecox* Rivalier, ex Padhye, Ajello, et McGinnis 1987
- *M. racemosum* Borelli 1965
- *M. vanbreuseghemii* Geort, Ajello, Friedman et Brinkman 1962
- *Trichophyton* Malmsten 1845
- *T. conventricum* Blanchard 1895
- *T. equinum* (Matruchot et Dassonville) Gedoelst 1902
- *T. gourvilii* Catane 1933
- *T. kanei* Summerbell 1989[a]
- *T. megninii* Blanchard 1896
- *T. metagrophytes* (Robin) Blanchard 1896
- *T. raubistchekii* Kane, Salkin, Weitzman, Smitka 1981[a]
- *T. rubrum* (Castellani) Sabouraud 1911
- *T. schoenleinii* (Lebert) Langeron et Milochevitch 1930
- *T. simii* (Pinoy) Stockdale, Mackenzie et Austwick 1965
- *T. soudanense* Joyeus 1912
- *T. tonsurans* Malmsten 1845
- *T. verrucosum* Bodin 1902
- *T. violaceum* Bodin 1902
- *T. yaoundei* Cochet et Doby Dubois 1957 (not validly published).

[a] Some mycologists consider *T. kanei* and *T. rubitschekii* to fall within the circumscription of *T. rubrum.*

### 1.1.2 Epidemiology and Ecology

Dermatophytes are among the few fungi causing communicable disease, that is, diseases acquired from infected animals or birds or from the fomites they have engendered. Some *Trichophyton*, *Epidermophyton,* and *Microsporum* species closely related to the dermatophytes appear to be exclusively saprobic or nearly so. The members of these three genera have no collective designation. The term dermatophytes should be restricted to designate infectious organisms and will be referred to below as dermatophytes and their congeners.

Dermatophytes and their congeners have long been divided into anthropophilic, zoophilic, and geophilic species on the basis of their primary habitat associations. Anthropophilic dermatophytes are primarily associated with humans and rarely infect other animals (Mayer, 1989). Zoophilic dermatophytes usually infect animals or are associated with animals but occasionally infect humans. Geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves, and horns after these materials have been dissociated from living animals and are in the process of decomposition. These species may cause human and animal infection. Geophilic species are thought to have been ancestral to the pathogenic dermatophytes, preadapted to cutaneous pathogenesis by their ability to decompose keratin and their consequent close association with animals living in hair and feather-lined nests in contact with soil. The distinction between geophilic and zoophilic dermatophytes is based on detailed ecological analysis and may not be obvious in small-scale studies. Many infections by zoophilic dermatophytes appear to be acquired indirectly from keratinous fomites, often deriving from apparently healthy animal carriers. Potentially infectious geophilic dermatophytes such as members of the *M. gypseum* complex, growing on similar keratinous debris, overlap in ecology with these zoophiles. They differ mainly by their greater persistence in soil and are found regularly in habitats not strongly modified by the constant presence of animal associates. A synopsis of dermatophyte species and congeners, ecological and host preferences, and endemicity may be found in Table 1.2.

**Table 1.2 : Current synopsis of dermatophyte species and congeners: ecological classification, host preference, and endemicity.**

| Anthrophilic species (area of endemicity) | Zoophilic species (typical host) | Geophilic species |
|---|---|---|
| *E. floccosum* | *M. canis* (cat, dog) | *E. stockdaleae* |
| *M. audouinii* (Africa) | *M. equinum* (horse) | *M. amazonicum* |
| *M. ferrugineum* (East Asia, East Europe) | *M. gallinae* (fowl) | *Microsporum anamorph of A. cookiellum* |
| *T. concentricum* (Southeast Asia, Melanesia, Amazone area, Central American, Mexico) | *M. persicolor* (vole) | *M. boullardii* |
| *T. gourvilii* (Central Africa) | *T. equinum* (horse) | *M. cookie* |
| *T. kanei* | *T. mentagrophytes* (two sibling species and variants; rodents, rabbit, hedgehog) | *M. gypseum* (complex of three species) |
| *T. megninii* (Portugal, Sardinia) | *T. sarkisorii* (Bactrian camel) | *M. nanum* |
| *T. mentagrophyes* (compelx of two species) | *T. simii* (monkey, flow) | *M. praecox* |
| *T. raubitschekii* (Asia, Africa, Mediterranean) | *T. verrucosum* (cattle, sheep, dromedary) | *M. racemosum* |
| *T. rubrum* | | *M. ripariae* |
| *T. schoenleinii* | | *M. vanbreuseghemii* |
| *T. Soudanense* (Subsabaran Africa) | | *T. ajelloi* |
| *T. tonsuruns* | | *T. flavescens* |
| *T. violaceum* (North Africa, Middle East, Mediterranean) | | *T.gloriae, T.longifusum* |
| *T. yaoundei* (Central Africa) | | *T. phaseolifurme,* *T. terrestre* (complex of three species). *T. Vanbreuseghemii* |

Rippon (1974) has pointed out a correspondence between soil association and conidial production in dermatophytes, the less significant the growth on dissociated keratin in the ecology of a dermatophyte, the less likely is the dermatophyte to produce conidia abundantly. Soil association also tends to correlate with the ability to form heterothallic teleomorphs in nature, ability not found in most anthropophilic dermatophytes and some zoophiles.

The dermatophyte structure most commonly associated with contagion, especially in the poorly conidial anthropophilic dermatophytes, is the oblong to rounded, persistent ‘‘spore,’’ ‘‘arthroconidium,’’ or ‘‘chlamydospore’’ found within or attached to the exterior of infected hairs and within skin scales. These structures, particularly in certain species, may persist for years in the environment and are highly heat resistant, particularly when embedded in hair or skin scales. In some anthropophilic species studied in detail, arthroconidia have a tendency to adhere to adhere in vitro to corneocytes derived from particular body sites. It is possible that they may dissociate from skin cells in the environment and come in contact with new potential hosts as disseminated arthroconidia. Their persistence as an environmental source of contagion may lead to recurrent outbreaks of dermatophytosis in individuals and in institutions. According to Rippon the arthroconidia of *T. rubrum* do not survive as long as do those of other species, e.g., *E. floccosum* (Rippon, 1974). The transition from potentially sexual to asexual life histories in the non-soil-associated dermatophytes appears to have led to adaptive radiation, at least in the anthropophilic dermatophytes. By most estimates, approximately two-thirds of the recognized dermatophyte species primarily associated with mammalian pathogenesis are anthropophiles. Within the anthropo philes, polymorphous morphological variation is common, and numerous atypical and variant types are recognized (Ribell and Taplin, 1970), probably indicating further genetic drift. Allopatric speciation appears to have been common in anthropophilic dermatophytes but rare in zoophiles, and several anthropophilic species have well-defined areas of endemicity (Rippon, 1974), while others, such as *T. rubrum* and *T. tonsurans*, are now cosmopolitan but appear to have had a more restricted distribution in the past, having been transported widely as a result of human migration (the anthropophiles travel with their human hosts) (Rippon, 1974). Also, spatial and ecological sympatric

isolation appears to have been a predisposer to speciation in the anthropophiles human-associated dermatophytes, unlike zoophiles, often have marked affinities for particular body sites. Most recognized asexual anthropophilic dermatophyte species are distinctive in morphology, physiology, and body site preference.

Recognition of dermatophytic taxa is clinically relevant. The need for species identification of dermatophytes in clinical settings is often related to epidemiological concerns. Especially relevant is the identification of dermatophytes that,

(i) may have animal carriers;

(ii) are linked to recurrent institutional or family outbreaks, such as *T. tonsurans* and *Trichophyton violaceum*;

(iii) may cause rapidly progressing epidemics, such as *M. audouinii* and *T. tonsurans* (Bronson *et al.*, 1983); and

(iv) are geographically endemic, reflecting exposure during travel or residence in the area of endemicity or contact with a person with such a history (Badillet, 1988).

Epidemiology is important in infection control and public health issues related to the different types of dermatophytoses. In tinea capitis, the predominant agents in North America are *T. tonsurans* and *M. canis*. The former is usually acquired from infected humans or their fomites and has caused a progressive, continent-wide epidemic now of some 40 years in duration. Urban areas and their communities of minorities have been particularly strongly affected. *M. canis* is usually acquired from infected cats or dogs, although limited human to human transfer leading to outbreaks can occur (Shah *et al.*, 1988).

Zoophilic and geophilic dermatophytes in general tend to form lesions that are more inflammatory than those formed by anthropophilic dermatophytes but are also more likely to resolve spontaneously (Rippon, 1974). This pattern is seen in tinea capitis caused by *M. canis* (Rippon, 1974). The closely related anthropophile *M. audouinii*, once common in North America but now mainly restricted to parts of Africa and Asia (Rippon, 1974), appears particularly specialized as an agent of juvenile tinea capitis (Rippon, 1974).

Adult infections are rare, and spontaneous resolution usually occurs upon attainment of puberty.

Tinea other than tinea capitis, when caused by anthropophilic fungi, tends to be associated with adults and adolescents, although infection of children may occur. *T. rubrum*, *E. floccosum*, and the anthropophilic *T. mentagrophytes* (i.e., cottony and velvety forms known as *T. mentagrophytes var. interdigitale*) show a common pattern of association with tinea corporis, tinea cruris, and tinea pedis (English, 1980). In addition, *T. rubrum* and *T. mentagrophytes* are associated with tinea manuum and onychomycosis. It is likely that exposure to these dermatophytes is a common occurrence. Although the ecological and host factors involved in developing symptomatic infection are poorly known, known risk factors include foot dampness and abrasion combined with likely exposure to high fungal inoculum in communal aquatic facilities, such as swimming pools and showers. Exchange of clothing, towels, and linen, either directly or via substandard communal laundering, is another recognized risk (Rippon, 1974) which may lead to outbreaks. Damp foot conditions may lead to aggravated symptoms due to mixed infection by dermatophytes and bacteria.

Zoophilic dermatophytes, apart from causing tinea capitis, most commonly cause tinea corporis (including tinea faciei) in persons of any age group. Tinea of the extremities, tinea cruris, and onychomycosis caused by zoophiles are uncommon to rare.

### 1.1.3 Clinical Manifestations

Traditionally, infections caused by dermatophytes (ringworm) have been named according to the anatomic locations involved by appending the Latin term designating the body si te after the word tinea, e.g., tinea capitis for ringworm of the scalp. The clinical manifestations are as follows: (i) tinea barbae (ringworm of the beard and mustache); (ii) tinea capitis (scalp, eyebrows, and eyelashes); (iii) tinea corporis (glabrous skin); (iv) tinea cruris (groin); (v) tinea favosa (favus); (vi) tinea imbricata (ringworm caused by *T. concentricum*); (vii) tinea manuum (hand); (viii) tinea pedis (feet); and (ix) tinea unguium (nails). Several anatomic sites may be infected by a single dermatophyte species, and different species may produce clinically identical lesions. The major etiologic agents may be global, such as *T. rubrum*, while the distribution of others may vary geographically.

The clinical conditions and their major etiologic agents are described briefly; more detailed information may be found in the texts by Rippon (1988).

#### 1.1.3.1 Tinea Barbae

Tinea barbae, an infection of the bearded area, may be mild and superficial or a severe inflammatory pustular folliculitis, the latter form more commonly caused by the zoophilic dermatophytes *T. verrucosum, T. mentagrophytes var. mentagrophytes*, and *T. mentagrophytes var. erinacei*.

#### 1.1.3.2 Tinea Capitis

Tinea capitis, an infection commonly involving the scalp, is usually caused by members of the genera *Microsporum* and *T.*. The infection may range from mild, almost subclinical, with slight erythema and a few patchy areas of scaling with dull gray hair stumps to a highly inflammatory reaction with folliculitis, kerion formation, and extensive areas of scarring and alopecia, sometimes accompanied by fever, malaise, and regional lymphadenopathy. Both the skin surface and hairs are involved. Infection of the hair may be described as ectothrix (sheath of arthroconidia formed on the outside of the hair shaft) or endothrix (arthroconidia formed within the hair shaft). The current predominant cause of tinea capitis in most of North, Central, and South America is *T. tonsurans* (endo-thrix) replacing *M. audouinii* (ectothrix) (Rippon, 1985).

#### 1.1.3.3 Tinea Corporis

Ringworm of the body, usually involving the trunk, shoulders, or limbs, and occasionally the face (excluding the bearded area), may be caused by any dermatophyte. The infection may range from mild to severe, commonly appearing as annular, scaly patches with sharply marginated, raised erythematous vesicular borders.

#### 1.1.3.4 Tinea Cruris (‘‘Jock Itch’’)

Infection of the groin, perianal, and perineal areas, and occasionally the upper thighs, is usually seen in adult men. *T. rubrum* and *E. floccosum* are the most frequent etiologic agents. Lesions are erythematous to tawny brown and covered with thin, dry scales. They are usually bilateral and often asymmetric, extending down the sides of the inner thigh and exhibiting a raised, sharply marginated border that is frequently studded with small vesicles.

### 1.1.3.5 Tinea Favosa

Tinea favosa, usually caused by *Trichophyton schoenleinii,* is severe and chronic, characterized by the presence on the scalp and glabrous skin of yellowish, cup-shaped crusts called scutula, which is composed of epithelial debris and dense masses of mycelium. The disease is most common in Eurasia and Africa.

### 1.1.3.6 Tinea Imbricata

Tinea imbricata, the chronic infection which is a specialized manifestation of tinea corporis, is characterized by concentric rings of overlapping scales scattered throughout the body. It is geographically restricted to certain of the Pacific islands of Oceana, Southeast Asia, Mexico, and Central and South America. *T. concentricum,* a strictly anthropophilic dermatophyte, is the only etiologic agent.

### 1.1.3.7 Tinea Manuum

The palmar and interdigital areas of the hand are usually involved in tinea manuum, most frequently presenting as unilateral diffuse hyperkeratosis with accentuation of the flexural creases. Most infections are caused by *T. rubrum*.

### 1.1.3.8 Tinea Pedis (‘‘Athlete’s Foot’’)

The feet, especially the soles and toe webs, are most frequently involved in tinea pedis. The most common clinical manifestation is the intertriginous form, which presents with maceration, peeling, and fissuring, mainly in the spaces between the fourth and fifth toes. Another common presentation is the chronic, squamous, hyperkeratotic type in which fine silvery scales cover pinkish skin of the soles, heels, and sides of the foot (moccasin foot). An acute inflammatory condition, characterized by the formation of vesicles, pustules, and sometimes bullae, is most frequently caused by *T. mentagrophytes*. The more chronic agents of tinea pedis are *T. rubrum*, *T. mentagrophytes var. interdigitale,* and *E. floccosum.*

### 1.1.3.9 Tinea Unguium

Invasion of the nail plate by a dermatophyte is referred to as tinea unguium, infection of the nail by nondermatophytic fungi is called onychomycosis. The latter word is often used as a general term for a nail infection. There are two main types of nail involvement: invasive subungual (distal and proximal) and superficial white mycotic infection (leukonychia trichophytica). *T. rubrum* and *T. mentagrophytes,* respectively, are the most common

dermatophytes of this infection.

### 1.1.4 Laboratory diagnosis

#### 1.1.4.1 Clinical material

Skin scrapings, nail scrapings and epilated hairs are the clinical materials. For a laboratory diagnosis, clinicians should be aware of the need to generate an adequate amount of suitable clinical material. Unfortunately many specimens submitted are either of an inadequate amount or are not appropriate to make a definitive diagnosis. The laboratory needs enough specimens to perform both microscopy and culture. Routine turn around times for direct microscopy should be less than 24 hours; however culture may take several weeks.

In patients with suspected dermatophytoses of skin [tinea or ringworm] any ointments or other local applications present should first be removed with an alcowipe. Using a blunt scalpel, tweezers, or a bone curette, firmly scrape the lesion, particularly at the advancing border. In cases of vesicular tinea pedis, the tops of any fresh vesicles should be removed as the fungus is often plentiful in the roof of the vesicle.

In patients with suspected dermatophytoses of nails (onychomycosis) the nail should be pared and scraped using a blunt scalpel until the crumbling white degenerating portion is reached. Any white keratin debris beneath the free edge of the nail should also be collected.

Skin and nail specimens may be scraped directly onto special black cards which make it easier to see how much material has been collected and provide ideal conditions for transportation to the laboratory.

It must be stressed that up to 30% of suspicious material collected from nail specimens may be negative by either direct microscopy or culture. A positive microscopy result showing fungal hyphae and/or arthroconidia is generally sufficient for the diagnosis of dermatophytosis, but gives no indication as to the species of fungus involved. Culture is often more reliable and permits the species of fungus involved to be accurately identified. Repeated collections should always be considered in cases of suspected dermatophytosis with negative laboratory reports.

#### 1.1.4.2 Direct Microscopy

Superficial scrapings from spreading border of the lesion is recommended. If vesicular lesion oredominate, the roof of the blister should be taken. These samples are mounted in 10% KOH and septate, branching hyphae with or without arthospores should be seen.

#### 1.1.4.3 Culture

Specimens should be inoculated onto primary isolation media, like Sabouraud's dextrose agar containing cycloheximide (actidione) and incubated at 25-30$^{o}$C for 1-4 weeks. The growth of any dermatophyte is significant.

#### 1.1.4.4 Identification

Characteristic clinical, microscopic and culture features.

## 1.2 Biodiversity in India

India's rich biodiversity is well known. By an estimate, India is endowed with about 47,000 species of plants and around 8000 plants having medicinal properties. The Indian system of medicine is traditionally based on plants materials or their extracts as active ingredient for preparation of drugs. These plants are exploited either legally or illegally by traditional healers and for trade purposes. This warrants that the plant and its use be conserved and the communities or the holders of the knowledge of the use of these plants receive due recognition.

### 1.2.1 Biodiversity in NE States:

North Eastern region is one of the hotspots of the world biodiversity. The landlocked northeast (NE) region of the country flanked by Himalayas comprises seven states of Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Tripura and a separate state of Sikkim. The region has a geographical area of 263179 km$^2$ and the total population of this region is around 38.8 million (3.77% of the country, 2001 census). The region has cold snowy winters and mild summers It has In this region, the average annual rainfall is about 3000 mm/year The variations in the temperature pattern exist from below

0°C to high upto 30°C. All these have great influence over the rich diversity of vegetation in thisregion (Dutta and Dutta, 2006).

The area is the home land of large number of indigenous and immigrant ethnic and tribal groups The tribal people of NE region from the anthropological point of view comprise the Khasis and Jaintia tribe of Meghalaya, and Mongoloids (Dutta and Dutta, 2006)

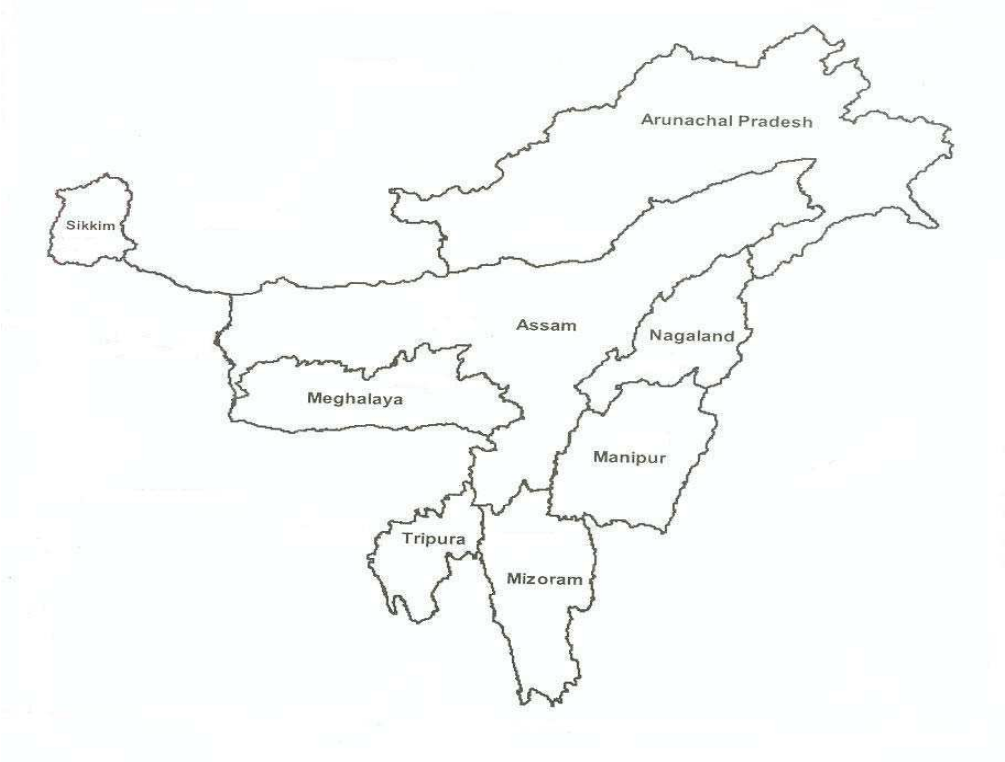



**Figure 1.3**

### 1.2.1.1 Arunachal Pradesh

The climate varies from an alpine or Tundra climate to temperate. Arunachal Pradesh receives heavy rainfall of 80 to 160. The mountain slopes and hills are covered with alpine, temperate, and subtropical forests of dwarf rhododendron, oak, pine, maple, fir, and juniper; sal (Shorea) and teak are the main economic species. Gigantic *Dipterocarpus* forms a very characteristics species of the evergreen forests of Tirap,*Terminalia*, *Duabanga*,

*Dalbergia, Abies,Rhododendron*, Oak and Bamboo species are abundant in the state. http://ignca.nic.in/craft004.htm).

### 1.2.1.2 Assam

Assam located south of the eastern Himalayas Assam is known for its rich biodiversity. Assam was also known for its Sal tree forests and forest products which are much depleted now.(http://ignca.nic.in/craft004.htm). Assam is one of the richest biodiversity zones in the world and consists of Evergreen forest, Semievergreen forest, Moist deciduous forest,, Degraded forest, Sal Forest, Chirpine forest, , Hollong forest. . It forms part of a global bio-diversity "**hotspot**",

### 1.2.1.3 Manipur

Manipur, covering an area of 22,327 sq. km, is conspicuous with regard to various floras and faunas. The climate of Manipur is largely influenced by the topography of this hilly region which defines thegeography of Manipur. (http://ignca.nic.in/craft004.htm). The Kukis and Nagas live in the hills of the state The vegetation consists of a large variety of plants ranging from short and tall grasses, reeds and bamboos to trees of various species. Broadly, there are four types of forests below Tropical Semi-ever Green, Dry Temperate Forest, Sub-Tropical Pine, Tropical Moist Deciduous, Teak, Pine, Oak, Uningthou, Leihao, Bamboo, Cane, etc. are important forest resources growing in plenty.

### 1.2.1.4 Meghalaya

Meghalaya is a hilly about 300 km long (east-west) and 100 km wide, with a total area of about 22,429 km². The climate of Meghalaya is moderate but humid. With average annual rainfall as high as 1200 cm in some areas, The main tribes in Meghalaya are the Khasis ,the Garos and the Jaintias, The forest types found in Meghalaya are Tropical wet evergreen forest, Tropical Semi evergreen forests, Tropical moist deciduous, Subtropical pine, East Himalayan moist deciduous forests. Due to the diverse climatic and topographic conditions, Meghalayan forests support a vast floral diversity, including a large variety of Parasites and Epiphytes, Succulent plants and Shrubs. Two of the most important tree varieties include: Shorea robusta or Sal and the Tectona grandis or teak. Meghalaya is also the home to large variety medicinal plants.

### 1.2.1.5 Nagaland

With a population of 1990036 people, it has a total area of 16,579 km. Nagaland is largely a mountainous state. Nagaland has a largely monsoon climate with high humidity levels. Annual rainfall averages around, 70-100 inches Temperatures range from 70 degrees to 104 degrees Fahrenheit. Nagaland is rich in flora and fauna. About one-sixth of Nagaland is under the cover of tropical and sub-tropical evergreen forests - including palms, bamboo and rattan as well as timber and mahogany forests. The major forest types in the state are: Tropical wet evergreen, Semi evergreen forests, Moist deciduous forests, Himalayan subtropical pine forests.

### 1.2.1.6 Sikkim

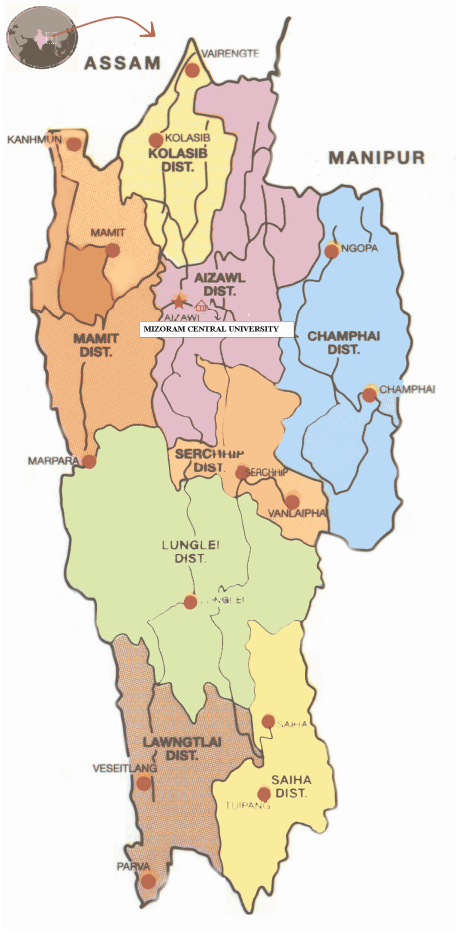
Sikkim is a landlocked Indian state nestled in the Himalayas. The climate ranges from subtropical to high alpine having a total geographical area 7096 sq. km The State receives an annual Rainfall of 2000mm to 4000mm. The State is bestowed with abundant natural resources. Covering just 0.2% of the geographical area of the country, it has tremendous biodiversity and has been identified as one of the Hot Spot for biodiversity. The Sikkim Himalayas harbors more than 26 % of the flowering plants reported in the country and known to be an important phytogeographical reserve of the country. Species wise, it has approx. 5000 Flowering plants, 515 Orchids, 36 Rhododendrons, 16 Conifers, 23 Bamboos, 362 Ferns and Ferns allies, 8 Tree Ferns, 60 Primulas, 11 Oaks, over 424 medicinal plants, (Dept of information and public relations, Govt. of Sikkim, http://sikkimipr.org/ INDEX.HTM)

### 1.2.1.7 Tripura

Tripura is a landlocked hilly state in NE India with altitudes varying from 50 to 3080 ft above sea level, though the majority of the population lives in the plains. with an area of 10,492 km² Tripura is the third smallest state of the country. Tripura has a tropical climate and receives rainfall during the monsoons

### 1.2.1.8 Mizoram

Mizoram is one of the mountainous regions of great strategic importance in this region. Situated between Myanmar in the east and south and Bangladesh in the west, it has a total of 404 km boundary with Myanmar and 318 kms with Bangladesh. It is located at Latitudes of 21$^{o}$ 58' & 24$^{o}$ 35' N, Longitudes of 92$^{o}$ 15' & 93 $^{o}$ 29' E and occupies an area of




21,087 sq kms (North South-277 kms and East West-121 kms). It shares inter state border with Assam (123 kms), Tripura (277kms) and Manipur (95 kms) (http://www.mizoram.gov.in; http://www.zoram.com; http://www.mizoram.nic.in).

**Fig.2.1: Geographical View of Mizoram (not in scale)**

Mizoram has the most variegated hilly terrain. The hills are green, steep and are separated by rivers which flow to the north or south creating deep gorges between the hill ranges. The average height of the hill is about 1000 meters. Blue Mountain (Phawngpui) with a height of 2210 meters is the highest peak in Mizoram. Mizoram has a pleasant climate. Temperature varies from $11^{o}$-$20^{o}$C in winter and $20^{o}$-$30^{o}$ C in summer. The average rainfall is 254 cm per annum. It has great natural beauty and endless variety of landscape and is also very rich in flora and fauna. Almost all kinds of tropical tress and plants thrive in Mizoram.

Mizoram has eight districts, 22 Blocks, 23 Sub-divisions and three autonomous district Councils. District wise area is as follows:

| State/District.... | Total Area covered (Sq kms)* |
|---|---|
| ➢ Mamit | 3,025.75 |
| ➢ Kolasib | 1,382.51 |
| ➢ Aizawl | 1,382.51 |
| ➢ Champhai | 3,185.83 |
| ➢ Serchhip | 1,421.60 |
| ➢ Lunglei | 1,421.60 |
| ➢ Lawngtlai | 4,538.00 |
| ➢ Saiha | 1,399.90 |

(*According to the Mizoram Census 2001 Provisional Figures; http://www.mizoram.gov.in; http://www.zoram.com; http://www.mizoram.nic.in).

Mizoram has a population of about 9.0 lakhs and a literacy rate of about 89%. Chakmas, Pawi, Hmar, Ralte, and Kuki are the major tribes. Agriculture is the mainstay of the people. More than 70% of the total population is engaged in Agriculture practicing Jhum cultivation.

The climatic condition in the state with various soil types have contributed to the occurrence of a wide spectrum of rich and varied flora and fauna. The socio-economic life of the rural people depends on their ambient vegetation from where they derive all their material requirements – timber, food, fuel wood, medicinal plants etc. Some of the medicinal plants found in Mizoram are : *Achyranthes aspera* (Buchhawl), *Actephila exelsa* (Maiteleng), *Altstonia scholaris* (Thuamriat), *Aporusa octandra* (Chhawntual), *Berginak ciliate* (Khamdamdawi), *Blumea lanceolaris* (Buaro), *Catharanthus roseus* (Kumtluang), *Curcuma zedaoria* (Ai disung), *Cynocardia odorata* (Sai thei), *Lonicera macrantha* (Leihruisen), *Martynia annua* (Vatelu), *Phyllanthus fraternus* (Mitthisulhlu), *Stemona tuberose*(Kaimam), *Zanonia indica* (Lalruanga daibur), *Holarrhena antidysenterica* (Thlengpa).

About 80% of the interior population depends on herbal medicine and nearly 95% of raw materials are harvested from the wild plant resources without replenishing the growing stocks. The village herbal preparation includes uprooting of the plants which is detrimental to the individuals or sub-populations. And as a result, commonly used and effective herbal plants became rare and endangered species, and some plants are on the verge of extinction unless conservation measures are taken up for revival (Lalramnghinglova, 2003).

## 1.3 Ethno-botanical aspects

Ethnobotany is “the study of the relationship which exists between people of primitive societies and their plant environment” Schultes (1962), Ethnobotany and ethnopharmacology have variously been seen as a tool for drug discovery (Schultes, 1962), a mode of ascertaining conservation as threat to the integrity of indigenous cultures or as a field of research which will require the development of novel forms of partnership between

indigenous peoples and researchers (Laird, 2002). While these approaches are highly diverse, they are united by a relatively static view of local and traditional medicinal plant use.

A large population of the world (about 80%) especially in the developing countries depends on traditional medicines to meet their health requirements. In recent years the popularity of the traditional system of medicine in developed countries has also grown enormously.

Ethno-botany has emerged as a new branch of study which acknowledges age old wisdom and knowledge of the ancient societies and seeks to emphasize on the conservation efforts for the traditional culture and medical knowledge of the region. The renewed interest and activity in the medicinal and aromatic plants is also justified in the context of the Convention on Biological Diversity (CBD) and Trade Related Intellectual Property Rights (TRIPS) - the international agreements to which majority of the countries including India, are signatories.

The subject is not alien even to India. Kirtikar and Basu (1935) stated that "The ancient Hindus should be given the credit for cultivating what is now called ethnobotany". India has a long tradition of ethno biological studies, and efforts to conduct these have intensified during the post-independence period (Geeta *et al.,* 1996). The first major step in this direction was taken by the Economic Botany section of the Botanical Survey of India, in the mid 1950s, when it took a number of initiatives. The volume of research has seen a steady increase since then. The 1980s saw a particularly large increase in interest in these studies. According to a review of ethno botanical literature published during 1982-2000, about 1250 publications appeared in India during this period (Ghose *et. al.,* 1996). Most Indian ethno botanical studies deal with tribal people, who account for about 19% of the population. Twenty seven indigenous knowledge associated with health care has been the major focus of these studies. An analysis of major journals published during 1995-2004 shows that almost half of the ethno biological studies during this period focused on the indigenous knowledge related to the medicinal use of plants (Hosagoudar *et al*., 1996).

Though ethnobotany provides several approaches in plant researches, those relevant to medicinal plants include archaeological, search in literature, herbaria and the field studies

### 1.3.1 Archaeological resources

India has a rich treasure of archaeological sculptures of antiquity, which can be valuable in identifying the plants being used during early civilization. Bas reliefs on the gateways of the Great Stupa at Sanchi and the railing of Bharhut stupa, belonging to the first and second century B.C., respectively, are some of the examples from which about 40 plants have been reported to have been described.

### 1.3.2 Literature resources

Our ancient literature is another significant resource that can be tapped for information on medicinal plants. Though prevedic period offers no authentic record from any source excepting from a few archaeological sculptures of Mohenjo-Daro, our ancient Vedic literary resources such as Rigveda and Atharvaveda, which date back to 2000 to 1000 B.C., provide useful information on medicinal plants of that period. Recently, checklists of Ayurvedic and Yunani treatises have been published (Tripathi *et al.,* 1978). Using the literature, Cancer Chemotherapy National Service Center (CCNSC) has compiled a huge documentation of all anti-tumour plants, cited in old texts and local folk medicine from all over the world for screening purpose.

### 1.3.3 Herbarium Resources

Herbarium sheets and field notes have also proved to be a good source of ethnobotanical data. Worth mentioning is the search of about 2.5 million plant specimens in Harvard University Herbarium, of which 5,178 useful notes of drugs and food value were recorded by Altschul 1973.

### 1.3.4 Field Resources

Ethnobotanist gets clues from tribals on the useful raw plant material to be tapped for its potential therapeutic value. Recently, the Central Councils for Research in Ayurveda, Siddha and Yunani conducted several medicobotanical surveys in some important ethnic and tribal regions of the country. It was found that the Nicobaris use the resinous wood of Canarium and Dipterocarpus spp. for repelling mosquitoes and as a torch. In the Nilgiris, the decoction of *Bambusa arundinacea* is used as an abortifacient (Ragunathan, 1976).

### 1.3.5 Comparative study of the Ethnobotanical Resources

Ethnobotany becomes a more important and interesting subject when its study reaches a point when the results are studied comparatively. For example, *Ficus religiosa* and *Ficus racemosa* are among the most important sacred as well as medicinal plants of antiquity. In Atharvaveda, Ficus racemosa is attributed the property of increasing the number of domestic cattle, giving virility and strength of its wearer, add to the fertility of his land and growth of the fruits. In Charak Samhita there are about 23 references of Ficus religiosa corresponding to medicinal and other properties. A few therapeutic uses described there are: in fever, in rheumatism, in urinary troubles, in spermatorrhoea, in pile and in dysentery. Schultes (1962) rightly stated, "Our challenge is to salvage some of the modern medico-botanical lore before it becomes for ever entombed with the cultures that give it birth". Kirtikar and Basu (1935) stated, "The only way to illumine the whole field of native therapeutics is to survey it in small tracts and sift the value of those drugs peculiar to each province… There is wide feeling that there is beneficence in the scheme of nature which provides in every country, suitable remedies on the spot for the ill to which humanity is locally most prone. Very little has been done so far to incorporate in the practice of physicians in the country, the medicines, which in India nature scatters broadcast from her lap".

## 1.4 Significance of herbal medicine

Herbal medicine are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability, and lesser side effects. The chemicals present in the herbals constitute physiological functions of the living flora and hence providing better compatibility with human body. These drugs are also being made from renewable resources and by eco- friendly processes and bring economic prosperity to the masses growing these raw materials (Kamboj, 2000).

The traditional medicines have been attracting researchers from all parts of the globe. Hence a worldwide interest is created in the field of ethno-medicines for finding some new and effective herbal drugs. Intensive research in scientific disciplines like ethno medicines, ethno pharmacology and ethno psychiatry is needed.(Brahma, 1992).

Increasing awareness among public at large and dermatologists in particular could enforce development of new techniques for diagnosis and treatment of skin diseases. (Scher and Coopa 1988). Much of our efforts are being wasted in routine testing of the standard fungicides, often there is a pressing need to investigate new sources of effective fungicides. As such there is an urgent need to develop new, effective, biodegradable agent which could be free from side ill effects.

The sources of the most of the synthetic antifungal drugs are largely petrochemicals, which are exhaustible and also a case of major concern in terms of drain on foreign exchange. Therefore, hunt for inexhaustible sources of such chemotherapeutics is highly desirable. Green plants appear to be reservoir of effective chemotherapeutants and can provide renewable sources of useful fungicides (Swaminathan, 1978) and thereby reducing pressure on foreign exchange (Shahi, 1997).

Ethnobotany and ethnomedicine , in sum, are important field of research, and if investigated thoroughly and systematically, will yield results of great value to the ethnologists, archaeologists, anthropologists, plant-geographers and pharmacologists.

# 2. Review of Literature

## 2.1 Research on ethno-medicinal plants

Although, a number of researches have already been made on ethno medicinal plants of North Eastern Region of India, investigations up to their IPR issues have not been paid much attention, so far. However, ethno-botanical studies carried out in some north eastern states especially in the state of Mizoram have already been reported. The usage of wild plants by the native people for the cure of cuts and wounds is described. The use of 17 species, belonging to 14 families together with their local names and other uses have been enumerated. The plants not only contain antiseptic value but also have regenerative and healing properties. Sticking property of paste of bark was also observed in *Laki* tree. In addition, blood-clotting properties of some plants have also been reported. A wide range of plants with ethno-medicinal value against some common diseases have been reported but much larger numbers of folk medicines have remained endemic to certain tribal pockets in North East India. Various works have been undertaken to document different types of medicinal plants used by various ethnic groups all over India including the North Eastern States (Alcorn, 1995; Kothari, 1999; Huntington, 2000; Anonymous, 2001 & 2004; Jain and Srivastava 2001; Jain, 2003; Negi *et. al*., 2002; Shankar, 2002; Sharma, 2002; Chandrasekar and Srivastava, 2003; Gurmet, 2004; Dhyani and Kala, 2005; Patil and Bhaskar, 2006; Prakasha and Krishnappa, 2006; Shukla *et al.*, 2011; Kumar *et al.*, 2011; Kumar *et al.*, 2012) and also in North East India (Lalramnghinglova, 1996; 1997; 1999 & 2003; Lalramnghinglova and Jha, 1999; Dutta and Dutta, 2005; Bhardwaj and Gakhar, 2005; Kala, 1998; 2000; 2002; 2003; 2004 and 2005; Das and Tag, 2006; Shukla *et al.*, 2011) to mention a few.

In continuation with this it is also essential to quote the statement of some pioneering workers of this field:

"The usual criteria for recognizing IPR, novelty and non-obviousness, generally tend to ignore the knowledge systems of rural and tribal families, although they are often characterized by a high degree of inventiveness". …… *Swaminathan (1996)*

"From earliest times, herbs have been used for pain-relieving and health care needs. They have provided all the medicament to man and his domestic animals for a wide spectrum of ailments and to soothe his aches and pains. According to an estimate of the World Health Organization, approximately 80 per cent of the people in developing countries rely chiefly on traditional medicines for primary health care needs; a major portion of these involves the use of medicinal plants. Amongst the ancient civilizations, India has been known to be a rich repository of medicinal plants, the *Rig Veda, Yajur Veda, Atharva Veda, Charaka Samhita, Sushruta Samhita* described the properties and uses of plants".

*… Trivedi (2009).*

## 2.2 Antidermatophytic activity of some important medicinal plants

Several natural antimicrobial substances (essential oils, extract, and isolated chemicals) have been reported to possess antifungal activity. Important findings of these investigations have been presented in chronological order for selection of active secondary plant metabolites. Following these investigations, through literature survey, will definitely be helpful for exploring the potential antidermatophytic plants of North Eastern Region as well as their IPR concern.(Table 2.1)

**Table 2.1: Medicinal plants and their antidermatophytic activity**

| Workers | Plants | Plant part(s)/ Seconadry metabolites | Pathogens |
|---|---|---|---|
| Arnold (1958) | *Oenothera argentenea* | Seed extract | *A. niger* |
| Salvenas (1959) | *Sinapsis alba, Brassica juncea* | Leaf extract | *A. niger* |
| Sriubaite (1960) | *Ranunculus polyanthemos, R. lanuginosum, R. Acer* | Extract | *F. oxysporum, A. niger* |
| Salvenas and Razinskarte (1962) | *Juniperus communis* | Essential oil | *A. niger* |

| | | | |
|---|---|---|---|
| Korta and Staryzk (1963) | *Saureja hortensis, S. dalmalica, Origanum vulgare, Nepeta nuda* | Essential oil | *C. albicans, C. tropicalis, C. parakrusei, C. krusei* |
| Davis (1964) | *Black mustard* | α-phenyle ethyl-isothiocyanate | *F. oxysporum* |
| Kovacs (1964) | *Allium sativum, A. cepa, Cochlearia armoracia* | Bulb and root extract | *A. niger* |
| Tschesche and Wulff (1964) | *Aesculus hippocastanum, Centauria diffusa* | Aesein, asiaticoride | *T. mentagrophytes* |
| Lalita *et al.,* (1964) | *Acacia catechu* | Nut extract | *C. albicans, T. rubrum* |
| Kahl *et al.,* (1966) | *Aesculus hippocastanum* | extract | *T. rubrum* |
| Hashem and Rehim (1967) | *Vicia faba* | β-alanine | *F. oxysporum* |
| Imai *et al.,* (1967) | *Dioscorea tokora* | Dioscin, gracillin prosapogenin-B | *T. mentagrophytes* |
| Khovanskaya (1967) | *Populus and Betula* | Bud extract | *C. albicans* |
| Tetsuro *et al.,* (1967) | *Juglans regia, J. seilodiana* | Walnut shell extract, 5-hydroxy-1,4 napthoquinone | *T. mentagrophytes* |
| Sinha *et al.,* (1969) | *Artemisia vestita, A. vulgaris, Aster mulliusculus, Erigeron* | Essential oil | *T. mentagrophytes* |

| | | | |
|---|---|---|---|
| | *linifolius, Mentha sylvastris, Salvia leucantha* | | |
| Gupta and Banerjee (1970) | *Curcuma zeodaria, Brassica sp.* | Extract | *T. rubrum* |
| Desai (1971) | *Vallaris heyni* | Root extract | *M. canis, T. interdigitale* |
| Dayal and Purohit (1971) | *Andropogon livarancusa, Anethum sowa, Justicia procumbens, Kaempblena galanga, Ophiorrhiza munjos, Pavonia odorata, Xanthium strumarium* | Essential oil | *M. canis T. mentagrophytes* |
| Kokata and Verma (1971) | *Cymbopogon nardus, C. citrates* | Essential oil | *M. gypseum* |
| Korbely and Florian (1971) | *Cinnamom sp.* | Essential oil | *T. mentagrophytes* |
| Rao and Joseph (1971) | *Apium graveolens, Atalant monophylla, Citrus aurantium, Lantana aculeata, L. indica, Leucas aspera, Ocimum canum, Polyathia longifolia* | Essential oil | *E. floccosum, T. mentagrophytes T. rubrum* |
| Band Cirenko *et al.,* (1972) | *Psoralea drupacea* | Essentail oil | *T. rubrum* |
| Komissarov and | *Allium sativum* | Extract | *A. niger* |

| | | | |
|---|---|---|---|
| Andreeva (1972) | | | |
| Ahmad *et al.*, (1973) | *Juglans regia* | Bark extract | *M. gypseum* |
| Cullen *et al.*, (1973) | *Nuphar luteum* | 6-6-dihydroxy thiobinupharidine | *M. gypseum* |
| Fukui *et al.*, (1973) | *Lupinus luteus* | Lutenne | *T. mentagrophytes, T. rubrum* |
| Hejtmankova *et al.*, (1973) | *Thiyopsis dolabrata* | Extract | *C. krussei, T. mentagrophytes, T. rubrum* |
| Wellmann *et al.*, (1973) | *Pelargonium roseum* | Essential oil | *E. floccosum, T. mentagrophytes* |
| Anonymous (1974) | *Pelargonium roseum* | Essential oil | *T. mentagrophytes* |
| Disalvo (1974) | *Baccharis glutinosa* | Leaf extract | *E. floccosum, M. audouinii, T. mentagrophytes, T. rubrum, T. Tonsurans* |
| El-Hissey (1974) | *Helianthus annuus, Chrysanthemum coronarium, Nigella sativa, Dathura innoxia* | Root and stem extract | *A. niger* |
| Holz and Knox-Davis (1974) | *Allium cepa* | Extract | *F. oxysporum* |
| Afifi (1975) | *Origanum majorana, O. basilicum* | Leaf extract | *A. niger* |
| Lindsey and Turner (1975) | *Arachis hypogea* | Embryo extract | *A. flavus* |
| Pfleger and | *Pisum sativum* | Seed extract | *F. oxysporum* |

| | | | |
|---|---|---|---|
| Harman (1975) | | | |
| Tansey and Appleton (1975) | *Allium sativum* | Bulb extract | *E. floccosum, M. gypseum, T. Rubrum* |
| Turner *et al.,* (1975) | *Arachis hypogea* | 5-7-dimethoxy isoflavone | *A. flavus* |
| Gupta *et al.,* (1976) | *Curcuma zedoaria* | Ethyl-*p*-methoxy cinnamate | *T. rubrum* |
| Thind and Dahiya (1976) | *Allium cepa, A. sativum, Azadirachta indica, Coriandrum sativum, Ruta graveolens* | Essential oil | *M. gypseum T. rubrum* |
| Chakravarty and Pariya (1977) | *Achyranthus aspera* | Root and leaf extract | *A. niger* |
| Damjanic *et al.,* (1977) | *Rosmarinus officinalis* | Essential oil | *C. albicans* |
| Jain (1977) | *Aegle marmelos* | Essential oil | *C. albicans* |
| Mukharya and Dahia (1977) | *Plumbago sp.* | Root extract | *M. gypseum* |
| Rojashenandez and Diazperez (1977) | *Catharanthus roseus* | Alkaloides | *A. fumigatus, C. albicans* |
| Sawhney *et al.,* (1977) | *Cymbopogon citratus, C. martini, C. winterianis, Mentha citrata, O. basilicum, O. citriodorum, O. gratissimum* | Essential oil | *M. gypseum* |

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| Yamada and Azuma (1977) | *Allium sativum* | Allicin | *A. fumigatus, C. albicans, E. floccosum, M. gypseum, T. mentagrophytes, T.rubrum* |
| Avadhoot and Varma (1978) | *Lantana camara* | Essential oil | *C. albicans, F. oxysporum* |
| Banerjee and Nigam (1978) | *Curcuma longa* | Essential oil | *M. gypseum, T. mentagrophytes* |
| Chaurasia and Kher (1978) | *Piper nigrum, Ayapana triplinerve* | Essential oil | *A. fumigatus* |
| Dwivedi *et al.,* (1978) | *Datura alba, Rauvolfia serpentine* | Leaf extract | *A. flavus* |
| Komatsu *et al.,* (1978) | *Sophora tomentosa* | Extract | *C. albicans, A. fumigatus* |
| Petricic *et al.,* (1978) | *Allium sativum* | Essential oil | *C. albicans* |
| Tripathi *et al.,* (1978) | *Inula recemosa* | Root extract, Alantolactone, isoalaniolactone | *M. canis, T. mentagrophytes* |
| Bhatt and Saxena (1979) | *Anogeissus leiocarpa* | Seed extract | *M. gypseum* |
| Geda and Bokadia (1979) | *Lumea membranacea* | Essential oil | *F. oxysporum* |
| Kuntze *et al.,* (1979) | *Strobilanthes cusia* | Leaf extract | *T. mentagrophytes* |
| Lahariya and Rao (1979) | *Cyperus scariosus, O. basilicum* | Essential oil | *M. gypseum* |
| Sampurna and Nigam (1979) | *Skimmia laureole, Cymbopogon* | Essential oil | *T. mentagrophytes* |

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| | *flexuosus, Cinnamomum zeylanicum, Geranium sp., Eucalyptus citriodora* | | |
| Singh and Agrawal (1979) | *Ipomoea palmata, Saraca indica* | Seed extract | *M. gypseum* |
| Sharma and Singh (1979a) | *Oenanthe javanica* | Essential oil | *A. fumigatus, M. gypseum, T. mentagrophytes T. rubrum* |
| Sharma and Singh (1979b) | *Eupatorium avapana* | Essential oil | *M. gypseum, T. mentagrophytes T. rubrum* |
| Suri *et al.,* (1979) | *Eucalyptus citriodora* | Essential oil | *A niger, M. gypseum* |
| Bhatt and Saxsena (1980) | *Amoora rohituka* | Seed extract | *M. gypseum* |
| Honda *et al.,* (1980) | *Isatis tinctoria, Polygonum tinctorium* | Tryptanthrium | *T. mentagrophytes* |
| Kim and Kwang (1980) | *Polygonum ariculare* | Leaf extract | *E. floccosum, M. canis, T. mentagrophytes, T. Rubrum* |
| Krisnaswamy and Purushothaman (1980) | *Plumbago zeylanica* | Pulumbagin | *E. floccosum, M. nanum* |
| Mosca and | *Arnica Montana* | Extract | *C. albicans,* |

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| Castazo (1980) | | | *A. flavus* |
| Renu *et al.,* (1980) | *Cestrum diurnum* | Essential oil | *A. flavus, A. fumigatus, A. terrius, A. versicolor, F. Oxysporum* |
| Timon *et al.,* (1980) | *Hedera helix* | Saponin | *M. canis, E. floccosum, T. Rubrum* |
| Chile *et al.,* (1981) | *Vinca rosea* | Leaf, stem, root and flower extract | *T. rubrum* |
| Clark *et al.,* (1981) | *Magnolia grandiflora* | Phenolic compounds | *A. niger, C. albicans, T. mentagrophytes* |
| Deshmukh and Agrawal (1981) | *Angelica archangelia, Artemisia vestita, Ferula jaeschkeana, Mentha arvensis, M. Piperita* | Essential oil | *M. gypseum, T. equinum,* |
| Grover and Rao (1981) | *Vateria indica* | Essential oil | *A. flavus, A. fumigatus, A. niger, C. albicans* |
| Garg and Oswal (1981) | *Buddleia asiatica* | Essential oil | *T. rubrum* |
| Gutkind *et al.,* (1981) | *Prosopis ruscifolia, Usnea compestris* | Extract | *C. albicans* |
| Kishore *et al.,* (1981) | *Chenopodium ambrosioides, Citrus medica , Cedrus deodara, Lippia alba, Mentha arvensis, Ocimum canum* | Essential oil | *M. gypseum T. mentagrophytes* |
| Wahab *et al.,* (1981) | *Inula racemosa* | Alantolactone | *M. canis, T. mentagrophytes* |

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| Asthana *et al.*, (1982) | *Ageratum conyzoides, Cymbopogon martinii, Eupatorium capillifolium, Ocimum adscendens* | Essential oil | *A. flavus, A. fumigatus, A. niger, F. oxisporum* |
| Ceruti *et al.*, (1982) | *Mentha arvensis, Eucalyptus citriodora, Thymus serphyllum* | Essential oil | *A. flavus, A. fumigatus* |
| Deshmukh and Chile (1982) | *Arachis hypogaea, Chenopodium album, Cuminum cyminum* | Essential oil | *M. gypseum, T. equinum, T. Rubrum* |
| Deshmukh *et al.*, (1982) | *Cymbopogon martini, Eucalyptus globules, Thuja orientalis* | Essential oil | *M. gypseum, T. rubrum* |
| Dikshit and Dixit (1982) | *Cedrus deodara* | Essential oil | *A. flavus, A. fumigatus, A. niger, A. versicolor* |
| Dubey *et al.*, (1982) | *Citrus midica and Erigeron bonariensis* | Leaves extract | *T. mentagrophytes* |
| Garg and Oswal (1982) | *Chloroxylon swietenia* | Essential oil | *T. rubrum* |
| Mc Callion *et al.*, (1982) | *Pseudowintera colorata* | Polygodial | *C. albicans* |
| Muir *et al.*, (1982) | *Schefflera digitata* | Falcarindiol | *E. floccosum, M. canis, M. nanum, T. mentagrophytes, T. interdigitale* |
| Pandey *et al.*, (1982) | *Caesulia axillaries* | Essential oil | *M. gypseum, T. equinum, T. Mentagrophytes* |
| Srivastava and | *Allium sativum, A.* | Essential oil | *M. gypseum* |

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| Singh (1982) | *cepa, Azadirachta indica* | | |
| Takazawa *et al.,* (1982) | *Lutinus edodes* | Extract | *T. rubrum* |
| Dubey *et al.,* (1983) | *Melaleuca leucadendron* | Essential oil | *A. flavus, A. fumigatus, A. terreus, A. versicolor, M. gypseum T. mentagrophytes* |
| Pandey *et al.,* (1983) | *Ageratum houstonianum* | Essential oil | *E. floccosum, M. canis, M. gypseum* |
| Singh *et al.,* (1983) | *Coleookia oppositifolia, Hyptis suaveolens, Leonotis nepetaefolia, Ocimum americanum, O. basilicum, O.gratissimum, O. sanctum, Pogostemon benghalense* | Essential oil | *E. floccosum, M. canis T. mentagrophytes* |
| Staib *et al.,* (1983) | *Sanseviera trifasciata* | Polyene | *C. albicans, C. tropicalis, E. floccosum, M. canis, T. mentagrophytes, T. rubrum* |
| Tripathi *et al.,*(1983) | *Iberis amara* | Seed extract | *T. mentagrophytes* |
| Chile and Vayas (1984) | *Vinca rosea* | Root leaves, flower extract | *T. rubrum* |
| Dikshit and Husain (1984) | *Anethum graveolens, Cymbopogon* | Essential oil | *M. gypseum, T. equinum, T. rubrum* |

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| | *flexuosus, Trachyspermum ammi, Vetiveria zizanioides* | | |
| Honda *et al.,* (1984) | *Penilla frutescens* | Penillaldehyde, citral | *E. floccosum, M. canis, M. gypseum, T. rubrum, T. mentagrophytes, T. tonsurans* |
| Sahai and Srivastava (1984) | *Heteropharagma guadriloculare* | Essential oil | *M. gypseum* |
| Saxena (1984) | *Carum capticum, Cumin cyminum, Germanium sp., Cymbopogon flexuosus, Cinnimomum zeylanicum* | Essential oil | *M. gypseum, T. equinum, T. rubrum* |
| Saxena *et al.,* (1984) | *Anaphalis contorta* | Essential oil | *M. gypseum* |
| Chauhan and Saxena (1985) | *Inula caspidata* | Essential oil | *A. flavus, A. fumigatus.* |
| Mall *et al.,* (1985) | *Juniperus verginiana* | Essential oil | *E. floccosum T. rubrum* |
| Rao and Rao (1985) | *Adenocalyma allicea* | Leaf extract | *T. mentagrophytes* |
| Singh and Deshmukh (1985) | *Allium cepa, A. sativum, Asparagus racemosus* | Essential oil | *M. gypseum* |
| Deshmukh *et al.,* (1986) | *Cyperus scarious* | Essential oil | *M. gypseum, T. equinum, T. mentagrophytes, T. rubrum* |

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| Dikshit *et al.*, (1986) | *Schinus molle* | Essential oil | *A. flavus, E. floccosum, M. canis, M. gypseum, T. mentagrophytes, T. rubrum, T. violaceum, T. tonsurans* |
| Neerja *et al.*, (1986) | *Azadirachta indica* | Cyclic tri and tetra sulphides | *T. mentagrophytes* |
| Singh *et al.*, (1986) | *Trachyspermum ammi* | Essential oil | *E. floccosum, M. canis T. mentagrophytes* |
| Bader *et al.*, (1987) | *Salidago vigaurea* | Saponin | *C. albicans* |
| Kusano *et al.*, (1987) | *Solanum sp.* | Alkaloides | *C. albicans, T. rubrum, T. mentagrophytes* |
| Mall (1987) | *Eupatorium copillifolium, E. cannabinum* | Essential oil | *M. canis, M. gypseum, T. mentagrophytes, T. rubrum* |
| Garg and Dengre (1988) | *Capillipedium foctidum, Tagetes erecta* | Essential oil | *A. niger, C. albicans, T. rubrum* |
| Gupta (1988) | *Acorus calamus* | Essential oil | *E. floccosum, T. mentagrophytes T. tonsurans* |
| Honda *et al.*, (1988) | *Lithospermum erythrorhizon* | Deoxyshikonin | *E. floccosum, M. gypseum, T. rubrum* |
| Hufford *et al.*, (1988) | *Trillium grandiflorum* | Rhizome extract | *C. albicans* |
| Steinmetz *et al.*, (1988) | *Salvia officinalis, Rosmarinus officinalis* | Essential oil | *C. albicans* |
| Singh and Pandey | *Lawsonia inermis* | Extract | *M. gypseum, T.* |

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| (1989) | | | *mentagrophytes* |
| Clark *et al.,* (1990) | *Juglans nigra* | Unripe hulls extract | *T. mentagrophytes, M. gypseum* |
| Fun and Svendsen (1990) | *Lippia alba* | Essential oil | *T. mentagrophytes, C. albicans* |
| Mishra and Dubey (1990) | *Prunus persica* | Essential oil | *M. gypseum, T. mentagrophytes* |
| Bader (1991) | *Sanicula europaea, Astrantia major, Salidago canadensis, Bellis perennis* | Triterpene glycosides | *C. albicans, M. gypseum, T. mentagrophytes, T. rubrum, T. tonsurans* |
| Garg and Jain (1991) | *Capillipedium foetidium* | Essential oil | *M. gypseum T. mentagrophytes* |
| Mishra (1991) | *Nepeta hindostana, Vitex negundo* | Essential oil | *E. floccosum T. mentagrophytes* |
| Qamar and Chaudary (1991) | *Acorus calamus, Callistemon lanceolatus, Laurus nobilis, Cymbopogon martini* | Essential oil | *C. albicans, T. tonsurans* |
| Alkiewiez and Lutomski (1992) | *Allium sativum* | Bulb extract | *C. albicans* |
| Willigmann *et al.,* (1992) | *Bellis perennis* | Saponin esters, saponins | *C. albicans, M. canis, M. gypseum, T. rubrum, T. mentagrophytes* |
| Hajji *et al.,* (1993) | *Eucalyptus sp.* | Essential oil | *A. niger, C. albicans* |
| Kishore *et al.,* (1993) | *Artemissia nelagrica, Casesula axillaris,* | Essential oil | *M. gypseum, T. rubrum* |

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| | *Chenopodium ambrosioides, Cymbopogon citratus, Mentha arvensis* | | |
| Mehrotra *et al.,* (1993) | *Artemisia sp.* | Essential oil | *C. albicans* |
| Fournier *et al.,* (1994) | *Xylopia aromatica* | Essential oil | *C. albicans* |
| Gundidza *et al.,* (1994) | *Clausena anisata* | Essential oil | *C. albicans* |
| Perry and Foster (1994) | *Hebe cupressoides* | Flavonoides, triterpene | *T. mentagrophytes* |
| Yadav and Dubey (1994) | *Cinnamomum tamola, Citrus maxima, Eupatorium cannabium, Nepeta hindustana, Ocimum canum* | Essential oil | *T. mentagrophytes M. audouinii* |
| De Pooter *et al.,* (1995) | *Alpinia speciosa* | Essential oil | *C. albicans* |
| Iyengar *et al.,* (1995) | *Cassia alata* | Extract | *E. floccosum, M. gypseum, T. rubrum* |
| Rath *et al.,* (1995) | *Moriuda lucid* | Anthraquinone | *C. albicans* |
| Steinmetz *et al.,* (1995) | *Pycnoporellus fulgens* | Essential oil | *E. floccosum, M. canis, M. gypseum, T. mentagrophytes, T. rubrum* |
| Yadav (1995) | *Cinnamomum tamola, Citrus maxima* | Essential oil | *T. mentagrophytes* |
| Mukherjee *et al.,* | *Cassia tora* | Extract | *A. niger,* |

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| (1996) | | | *T. mentagrophytes* |
| Mahasneh *et al.*, (1996) | *Capparis spinosa, Prosopsis farcta, Salsola villosa, Suaeda vermiculata* | Extracts | *C. albicans, F. oxysporium* |
| Nenoff *et al.*, (1996) | *Melaleuca alternifolia* | Essential oil | *C. albicans, T. rubrum, T. mentagrophutes* |
| Pandey *et al.*, (1996) | *Cymbopogon pendulus* | Essential oil | *M. gypseum T. mentagrophytes* |
| Shahi *et al.*, (1996a) | *Eucalyptus citriodora* | Essential oil | *Aspergillus flavus* |
| Shahi *et al.*, (1996b) | *Trachyspermum ammi* | Essential oil | *E. floccosum, T. rubrum* |
| Shimoyamada *et al.*, (1996) | *Asparagus officinalis* | Extract | *C. albicans* |
| Wannissorn *et al.*, (1996) | *Cymbopogon citrates* | Essential oil | *E. floccosum, M. gypseum, T. mentagrophytes, T. Rubrum* |
| Pandey (1997) | *Cymbopogon pendulus* | Essential oil | *E. floccosum, T. mentagrophytes* |
| Pandey *et al.*, (1997) | *Cymbopogon pendulus* | Essential oil | *M. nanum, T. Mentagrophytes, T. rubrum* |
| Shahi (1997) | *Trachyspermum ammi, Cymbopogon flexuosus.* | Essential oil | *E. floccosum, M. gypseum, M. canis, M. nanum, T. mentagrophytes, T. rubrum, T. violaceum* |

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| Shahi *et al.,* (1997a) | *Cymbopogon flexuosus* | Essential oil | *T. mentagrophytes, M. gypseum* |
| Shahi *et al.,* (1997b) | *Eucalyptus spp.* | Essential oil | *T. rubrum, T. mentagrophytes* |
| Shahi *et al.,* (1997c) | *Citrus sinensis* | Essential oil | *E. floccosum, M. gypseum T. Rubrum* |
| Amvam Zolla *et al.,* (1998) | *Bixa orellana, Hoslundia opposite, Hyptis lanceolata, H. suaveolens, Ocimum basilacum, O. canum, Piper capense, P. ghineense, Plectranthus glandulosus* | Essential oil | *A. flavus, C. albicans, M. gypseum T. rubrum* |
| Concha *et al.,* (1998) | *Melaleuca alternifolia* | Essential oil | *C. albicans, T. rubrum* |
| Kirmizigul *et al.,* (1998) | *Cephalaria transsylvanica* | Triterpenoid glycosides | *A. flavus* |
| Shahi *et al.,* (1998a) | *Eucalyptus citriodora* | Essential oil | *M. nanum, T. Mentagrophytes, T. Rubrum* |
| Shahi *et al.,* (1998b) | *Eucalyptus pauciflora* | Essential oil | *A.flavus, A. fumigatus* |
| Shahi *et al.,* (1998c) | *Cladonia furcata, Heterodermia leucomela, Leptogeum trichophorum, Lobaria retigera, Stereocaulon* | Extract | *E. floccosum, M. gypseum, T. mentagrophytes,* |

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| | *foliosum* | | |
| Kawai *et al.*, (1998) | *Aloe arboorescens* | Extracts | *T. mentagrophytes* |
| Ali *et al.,* (1999) | *Aloe era, A. berger, A. vera, A. arborescens* | Extracts | *A. niger* |
| Haraguchi *et al.*, (1999) | *Ilex intergra* | Triterpenes | *C. albicans* |
| Shahi *et al.*, (1999a) | *Cymbopogon flexuosus* | Essential oil | *E. floccosum, M. canis, M. nanum T. rubrum, T. mentagrophytes, T. violacium, T. Tonsurance* |
| Shahi *et al.*, (1999b) | *Eucalyptus laveopenia* | Essential oil | *E. floccosum, M. Gypseum, T. rubrum* |
| Shahi *et al.*, (1999c) | *Eucalyptus citriodora* | Essential oil | *E floccosum, M. nanum, T. rubrum* |
| Shahi *et al.*, (1999d) | *Eucalyptus laveopenia, E. dalrampleana* | Essential oil | *E. floccosum, M. canis, M. nanum T. rubrum, T. mentagrophytes, T. violacium, T. tonsurance* |
| Aiyelaagbe *et al.*, (2000) | *Jatropha podagrica* | Extract | *C. albicans* |
| Patra *et al.,* (2000) | *Foeniculum vulgare* | Essential oil | *S. dimidiatum, T. mentagrophytes, T. rubrum* |
| Singh *et al.*, (2000) | *Homalomena aromatica , Odontotermus obesces* | Essential oil | *A. niger* |

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| Shahi *et al.,* (2000a) | *Eucalyptus pauciflora* | Essential oil | *E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.,* (2000b) | *Everniastrum cirrhatum* | Extract | *E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.,* (2000c) | *Cetraria pallescens, Cladonia furcata, Leptogeum trichophorum, Lobaria retigera, Stereocaulon foliosum, Sticta henryana* | Extract | *E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| D' Auria *et al.,* (2001) | *Melaleuca alternifolia* | Essential oil | *C. albicans, T. rubrum, T. mentagrophytes,* |
| Gadhi *et al.,* (2001) | *Aristolochia paucinevis* | Extract | *E. floccosum, T. rubrum, T. mentagrophytes, T. violaceum* |
| Diallo *et al.,* (2001) | *Cussonia barteri, Glinus oppastiflius, Launea velutina* | Extract | *Candida albicans* |
| Patra *et al.,* (2001) | *Eugenia caryophyllata* | Essential oil | *A. flavus, A. fumigatus, A. niger, A. ustus, E. floccosum, M. gypseum,* |

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| | | | *M. nanum, M. canis, S. dimidiatum T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.*, (2001a) | *Hetrodermia leucomela* | Extract | *A. flavus, A. fumigatus, A. niger, A. ustus, E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.*, (2001b) | *Parmelia cirrhatum* | Extract | *A. flavus, A. fumigatus, A. niger, A. ustus, E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.*, (2001c) | *Peltigera paratextala* | Extract | *E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.*, (2001d) | *Eucalyptus dalrympleana* | Essential oil | *E. floccosum, M. gypseum, T. rubrum,* |
| Shahi *et al.*, | *Eucalyptus* | Essential oil | *E. floccosum, M.* |

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| (2001e) | *dalrympleana* | | *gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.,* (2001f) | *Rabdosia millisoides* | Essential oil | *E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Atindehou *et al.,* (2002) | *Discorea minutiflora, Erythrina vogelli* | Extract | *C. albicans* |
| Kalemba *et al.,* (2002) | *Artemissia asiatica* | Extract | *C. albicans, A. fumigatus* |
| Pandey *et al.,* (2002) | *Mentha spicata, Taxodium distichum* | Essential oil | *E. floccosum, M. gypseum, M. nanum* |
| Pandey K P (2002) | *Curcuma longa* , | Essenrial oil | *M. gypseum, T. mentagrophytes* |
| Patra *et al.,* (2002) | *Foeniculum vulgare* | Essential oil | *A. flavus, A. fumigatus, A. niger, A. ustus, E. floccosum, M. gypseum, M. nanum, M. canis, S. dimidiatum T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.,* (2002a) | *Eucalyptus pauciflora* | Essential oil | *A. flavus, F. oxysporum* |
| Shahi *et al.,* (2002b) | *Cymbopogon flexuosus* | Essential oil | *A. flavus, A. fumigatus, A. niger, A. ustus, F. oxysporum* |

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| Shahi *et al.*, (2002c) | *Everniastrum cirrihatum* | Extract | *E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.*, (2002d) | *Peltigra paratextala* | Extract | *A. flavus, A. fumigatus, A. niger, A. ustus, E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| Shahi *et al.*, (2002e) | *Usnea longisimma* | Extract | *A. flavus, A. fumigatus, A. niger, A. ustus, E. floccosum, M. gypseum, M. nanum, M. canis, T. rubrum, T. mentagrophytes, T. violaceum, T. tonsurans* |
| VillaSenor *et al.*, (2002) | *Cassia alata* | Extracts | *C. albicans, T. mentagrophyte* |
| Rao *et al.*, (2005) | Citral, geraniol, citronellal, eugenol, menthol, lemongrass oil etc | 26 Chemical constituents | *Epidermophyton floccosum, Trichophyton mentagrophytes, T. rubrum* and *Microsporum gypseum* |
| Silva *et al.*, (2005) | *Ocimum gratissimum* | Hexane, chloroform fractions, the | *Microsporum canis, M. gypseum, Trichophyton rubrum* |

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| | | essential oil and eugenol | *T.mentagrophytes, Trichophyton rubrum* |
| Inouye S. *et al.* (2006) | 72 plants | Essential oil | *T. mentagrophytes* |
| Pyun M. S. *et al.*,(2006) | *Allium sativum for. pekinense, A. cepa,* and *A. fistulosum* | Essential oil | Three *Trichophyton* species |
| Cavaleiro *et al.*,(2006) | Juniperus | Essential oils | Dermatophyte, *Aspergillus* and *Candida* |
| Chuang *et al.*,(2007) | *Moringa oleifera* | Crude extracts and essential oil | *Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum,* and *M. canis.* |
| Hadad *et al.*, (2007) | *Baccharis grisebachii* hieron | Essential oil | *Cryptococcus neoformans, Aspergillus* spp., and particulary against *Trichophyton mentagrophytes* and *T. rubrum* |
| Inouye S. *et al.*, (2007) | *Cinnamomum zeylanicum, Eugenia aromatica, Geranium maculatum, Lavandula angustifolia, Cymbopogon citratus, C. martinii Origanum vulgare, palmarosa,* | Essential oil | *T. mentagrophytes* & *T. rubrum,* |

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|---|---|---|---|
| | *Mentha x piperita, Melaleuca alternifolia,* and *Thymus vulgaris* | | |
| Tullio A. *et al.,* (2007) | Various | Essential oil | Dermatophytes |
| Inouye S. *et al.,* (2007) | Various | Essential oil | Dermatophytes |
| Inouye S. *et al.,* (2007) | Oregano, perilla, tea tree, lavender, clove, and geranium oils | Essential oil | *Trichophyton mentagrophytes* |
| Park *et al.,*(2007) | Leptospermum petersonii and Syzygium aromaticum | Essential oil | *Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum* and |
| Luqman *et al.,* (2008) | *Eucalyptus citriodora* | Essential oil | *Trichophyton rubrum, Histoplasma capsulatum, Candida albicans* |
| Sim and Shin (2008) | *Ligusticum chuanxiong* | Essential oil | *Trichophyton species, T. erinacei, T. mentagrophytes, T. rubrum, T. schoenleinii, T. tonsurans and T. soudanense* |
| Mishra *et al.,* (2009) | *Cinnamomum zeylanicum* | Bark and Leaves extracts | *Alternaria solani* and *Curvularia lunata* |

| | | | |
|---|---|---|---|
| Sokovic, *et al*., (2009) | *Thymus vulgaris* L., *Thymus tosevii* L., *Mentha spicata* L., and *Mentha piperita* L. | Essential oils | *Alternaria alternata, Aspergillus niger, Penicillium funiculosum, Trichosporon mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Microsporon canis, Microsporon gypseum, Epidermophyton floccosum.* |
| Zuzarte1 et al., (2011) | *Origanum vulgare, Thymus serpyllum, Eugenia caryophyllata, Cymbopogon nardus, Pelargonium roseum, Lindera umbellata, Aniba rosaeodora, Thymus vulgaris, Lavandula latifolia, L. angustifolia* and *Melaleuca alternifolia.* | Essential oils | *Microsporum canis, Trichophyton mentagrophytes, T. equinum T. verrucosum* and *M. gypseum* |
| Bhadauria and Kumar (2011) | *Allium sativum, Cymbopogon martinii* and *Catharanthus roseus* | Extracts | *Candida albicans* |

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|---|---|---|---|
| Shukla, *et al.*, , (2011) | *Curcuma domestica* | Essential oil | *Epidermophyton floccosum, Trichophyton rubrum* and *Microsporum gypseum* |
| Shukla, *et al.*, , (2011) | *Curcuma aromatica* | Essential oil | *Epidermophyton floccosum, Trichophyton rubrum* and *Microsporum gypseum* |
| Shukla, *et al.*, (2012) | *Eucalyptus citriodora* Hook | Essential oil | *Trichophyton rubrum, Microsporum gypseum* and *Epidermophyton floccosum* |
| Duraipandiyan *et al.*, (2012) | *Costus speciosus* | Esential oils and extracts | *Trichophyton mentagrophytes, T. rubrum Epidermophyton floccosum* and *Aspergillus niger* |

**Table 2.2 Ethnomedicinal plants of North Eastern states and their use against different ailments**

| Plant and the parts used | Diseases in which used and specific part of the plant used | states where found in abundance | References |
|---|---|---|---|
| *Abroma augusta* (L.) Willd. Sterculiaceae Bark, Seed, Leaves, Flower, Root | Menstrual disorder, Uterine tonic (Bark), Fever, Cold, Ringworm, Scabies (Seed), | Arunachal Pradesh, Meghalaya, Sikkim | Rajendran (2003), Maikhuri and Gangwar (1993), Chhetri *et al.,* (2005), Tag (2006), |
| *Albizia chinensis* (Osb.) Merr. Mimosaceae Bark, Gum | Ringworm, Insect bite (Bark), Headache (Gum), Skin burn, Scabies (Bark),*Snake bite | Meghalaya, Mizoram | Chhetri (1994), Lalramnghinglova (2003) |
| *Allium cepa* L. Liliaceae Bulb, Whole plant | Urticaria, Leprosy, Ringworm, Scabies, Abscess, Fever, | Assam, Meghalaya, Manipur | Saikia *et al.,* (2006); Sharma (2004), |
| *Allium sativum* L. Liliaceae Bulb | Ringworm, Scabies, Abscess, Ascites, Hepatitis (associated with dyspepsia and loss of appetite), Whooping cough | Assam, Manipur, Meghalaya, Nagaland, Tripura, Arunachal Pradesh | Saikia *et al.,* (2006), Chaturvedi and Jamir (2007), Chaturvedi and Jamir (2007) |
| *Alpinia allughas* Retz. (Rose) Zingiberaceae Fruit, Rhizome, *Stem | Ring worm (Fruit), Fever (Rhizome), | Arunachal Pradesh, Assam, Tripura | Sarmah *et al.,* (2006) |

| | | | |
|---|---|---|---|
| *Anacardium occidentale* L. Anacardiaceae Bark, Fruit, Leaves | High blood pressure, Blotch (Bark), Ringworm, Leprosy, Warts (Bark), Antiscorbutic (Fruits), Pharyngitis (Leaves) | Assam, Mizoram, Tripura | Das and Sharma (2003), Lalramnghinglova (2003) |
| *Arisaema jacquemontii* Bl. Araceae Tuber | Ringworms, Skin diseases (Tuber) | Meghalaya | Rao (1981) |
| *Bambusa oliveriana* Gamble Poaceae Shoot | Nail injury, Ring worm, Tumours, meningitis, Alopecia (Shoot) | Manipur | Sinha (1996) |
| *Bonnaya reptans* Spreng. Scrophulariaceae Leaves, Root, Whole Plant | Snake Bite (Leaves, Root), Ringworm (Whole Plant), Urinary disorders (Leaves) | Arunachal Pradesh, Meghalaya | Maikhuri and Gangwar (1993), Tiwari and Tiwari (1996), |
| *Brassica juncea* (L.) Czern. Brassicaceae Seeds | Scabies, Pimples, Boils, Ringworm, Dyspepsia, Flatulence, Carminative, Digestive (Seeds) | Assam | Saikia *et al.*, (2006), Sharma (2004) |
| *Butea monosperma* (Lam.) O. Ktze. Fabaceae Leaves, Flower, Seeds, Bark | Abortifacient, Cooling, Ophthalmic, Anthelmintic, Skin Diseases, Ringworm (Seeds), | Assam, Meghalaya | Purkayastha and Nath (2006), Saikia & Nath (2003), Rao (1981), Sharma (2004), |
| *Callicarpa arborea* Roxb. Verbenaceae Leaves, Bark, Shoot, Buds, Root, | Scabies, Ringworm, Tonic, Carminative (Bark), Gastric trouble (Young Shoot/ Stem | Arunachal Pradesh, Assam, Manipur, Mizoram, Nagaland, | Jamir and Rao (1990), Das and Sharma (2003), Chhetri (2005), Sarmah *et al.*, (2006), |

| | | | |
|---|---|---|---|
| Stem, *White powder of young stem (1996) | bark), Skin diseases (Stem Bark), | Sikkim | Bhardwaj and Gakhar (2005), Chaturvedi and Jamir (2007) |
| *Calotropis gigantea* (L.) Ait. f. Asclepiadaceae Leaves, Root, Latex, whole plant, *Bark, Stem | Ringworm, Leprosy, Leucoderma (Leaves, Root), Hepatomegaly, Skin diseases, | Assam, Meghalaya, Sikkim, Tripura | Majumder *et al.,* (1978), Saikia & Nath (2003), Das and Sharma (2003), Purkayastha and Nath (2006) |
| *Cannabis sativa* L. Cannabinaceae Leaves, Inflorescence, Bark, Seeds, Fruits | Diabetes, Pimples, Boils, Cuts and Wounds, Aphrodisiac, Ringworm, Cattle Flatulence, | Arunachal Pradesh, Assam, Meghalaya, Sikkim, Nagaland | Chhetri *et al.,* (2005), Das *et al.,*, (2003), Saikia & Nath (2003), Tiwari *et al*., Purkayastha and Nath (2006), Gurung (2002) |
| *Carica papaya* L. Caricaceae Fruit, Root, Latex, Bark (root), Root, seeds, Buds, *Leaves | Snake Bite, Galactogogue (Root), Liver disorders, Gingivitis, Ringworm, Skin Burns, | Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim | Saikia *et al.,* (2006), *Et al.,* (1999), Das and Sharma (2003), Yonggam (2005), Chaturvedi and Jamir (2007), |
| *Cassia alata* L. Caesalpiniaceae Leaves, Whole plant, Flower, Seed | Eczema, Itch, Ringworm, Scabies (Leaves), Asthma, Bronchitis, | Arunachal Pradesh, Assam, Tripura, Nagaland, Mizoram | Rawat and Shankar (2005), Das and Sharma (2003), Purkayastha and Nath (2006), Sharma (2004), Majumdar *et al.,* (2006), Chaturvedi and Jamir (2007) |
| *Cassia fistula* L. Caesalpiniaceae Fruit, Leaves, Bark, Root, Seed | Jaundice, Laxative, Diuretic, Chronic fever, Difficulty in Urination (Fruit), Ringworm (Leaves), Leprosy (Leaves/Root) | Arunachal Pradesh, Assam, Sikkim, *Tripura | Purkayastha *et al.,* (2005), Srivastava et al (1987), Tomar *et al.,* , (2003), Saikia et al. (2006), Purkayastha and Nath (2006) |

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|---|---|---|---|
| *Cassia occidentalis* L. Caesalpiniaceae Leaves, Root, Whole Plant | Alexiteric, Cough, Asthma (Leaves), Pneumonia, Malaria, Aphonia, Dysentery, Ring worm | Assam, Nagaland, Sikkim, Arunachal Pradesh | Gogoi and Das (2003), Gurung (2002) |
| *Cassia occidentalis* L. Caesalpiniaceae Leaves, Root, Whole Plant | Stomachache, Flatulence, Skin Diseases, Dysentery, Ring worm, Elephantiasis, Scorpion sting (Root) | Nagaland, Sikkim, Arunachal Pradesh | Jamir and Rao (1990), Bora (1999), Choudhury and Neogi (1999), Gogoi and Das (2003), Gurung (2002) |
| *Cassia sophera* L.Caesalpiniaceae Root, Whole Plant, Bark, eavesHydrophobia (Root), | Expectorant (Whole Plant), Diabetes (Bark), Ringworm (Leaves), *Elephantiasis (Root) | Assam, Tripura | Sarma *et al.,* (2001), Gogoi and Das (2003), Das *et al.,* (2006), *NS-f |
| *Cassia tora* L. Caesalpiniaceae Leaves, Seed, Twigs | Skin diseases (Leaves/ Leaves and Seed), Low Blood pressure during pregnancy, Laxative, Ringworm (Leaves and Seeds) | Arunachal Pradesh, Assam, Manipur, Meghalaya | Purkayastha and Nath (2006), Nath & Maiti (2003), Gogoi and Das (2003), Yonggam (2005) |
| *Citrus latipes* (Swingle) Tanaka Rutaceae Fruit, Leaves | Appetiser, Rashes, Ringworm (Fruit), Gout, Rheumatism (Leaves), Body ache, Vomiting, Tonic, Cold, Fever (Fruit, Leaves) | Meghalaya, Manipur | Chhetri (1994), Upadhaya *et al.,* (2005) |
| *Clerodendrum indicum* (L.) O. Ktze. Verbenaceae Root, Leaves | Ringworm, Eczema, Anthelmintic, Cholera, Fever | Assam, Tripura, Manipur | Pandey *et al.,* (1996), Sharma (2004), Maiti & Nath (2003), Majumdar *et al.,* (2006) |

| | | | |
|---|---|---|---|
| *Curcuma longa* L. Zingiberaceae Rhizome, Flower, Root | Inflammation, Elephantiasis, Ringworm, Snakebite, Leech bite, Small Pox, Swelling, Boils, Bruises, Sprain | Assam, Manipur, Meghalaya, Sikkim | Saikia *et al.,* (2006), Purkayastha and Nath (2006), Tripathi and Borthakur *et al.,* (2004), Sarma *et al.,* (2002), Saikia & Nath (2003). |
| *Desmodium gyrans* DC. Fabaceae *Fresh root and Leaves with flower | *Fungal skin infection (Fresh root and Leaves with flower) | Assam | |
| *Drymaria cordata* (L.) Willd. ex Schult. Caryophyllaceae Apical portion, Whole plant, Leaves | Tonsilitis, Epistaxis, Bone fracture (Leaf), Skin diseases (Whole Plant/ Leaves), Ringworm | Arunachal Pradesh, Assam, Manipur, Meghalaya, Sikkim, Nagaland | Singh *et al.,* (1996), Chhetri (2005), Pfoze and Chhetry (2004), Sharma (2004), ., |
| *Drymaria cordata* Willd./(L.) Roem. ex Schult. Caryophyllaceae Whole plant, Shoot, Leaves | Vomiting due to fever, Diarrhoea, Urinary Trouble, Snake Bite, Laxative, Ringworm | Arunachal Pradesh, Assam, Manipur, Meghalaya, Sikkim | Purkayastha *et al.,* (2005), Purkayastha and Nath (2006), Neogi *et al.,* (1989), Upadhaya *et al.,* (2005), Yonggam (2005), Khumbongmayum et al. (2005) |
| *Drymaria diandra* Bl. Caryophyllaceae Whole plant, *Stems, Leave | Abscess, Allergy (Whole plant), Ringworm | Arunachal Pradesh, Nagaland, Sikkim | Murtem and Das (2005), Rao and Jamir (1982), |
| *Emblica officinalis* Gaertn. Euphorbiaceae Fruit, Bark, Leaves, Seeds | Constipation, Bleeding gums, Piles, Diuretic, Laxative, Purgative, improvement, Ring worm | Arunachal Pradesh, Assam, Manipur, Mizoram, Nagaland, Sikkim | Saikia *et al.,* (2006), Das and Sharma (2003), Purkayastha and Nath (2006), |

| | | | |
|---|---|---|---|
| *Enhydra fluctuans* Lour. Asteraceae Whole plant, Shoot, Leaves, Aerial parts | Antibilious, Demulcent, Ring worm (Leaves), Pimples (Aerial parts) | Arunachal Pradesh, Assam, Meghalaya | Kalita *et al.*, (2005), Das *et al.*, (2006), Islam and Hasin (2003), Das and Sharma (2003)- Paper. |
| *Eucalyptus* spp | Antidermatophytic activity against human pathogens | Mizoram | Shukla *et al.*, (2012) |
| *Euphorbia hirta* L. Euphorbiaceae Latex, Whole plant, Leaves, Root | Itching, Skin disorders, Pimples (Latex), Hypolectemia, Dysentry, Acute abdominal Pain, Cough, Asthma, Skin Diseases | Arunachal Pradesh, Assam, Tripura, Meghalaya, Sikkim, Manipur | Rawat and Shankar (2005), Srivastava *et al.*, (1987), Maiti & Nath (2003), Gogoi and Borthakur (2001), |
| *Euphorbia neriifolia* L. Euphorbiaceae Branches, Leaves, Whole Plant,Latex, Pith, Soft Stem | Carbuncle, Abscess (Branches), Cold, Cough (Leaves), Anthelmintic, Cuts, Burns, Astringent, Ringworm (Latex) | Arunachal Pradesh, Assam, Nagaland | Saikia *et al.*, (2006), Sharma (2004), Rao and Jamir (1982), Bhuyan (2003), Yonggam (2005) |
| Ficus benjamina L. Moraceae Leaves, Tender Shoot | Sores, Scabies, Ringworm (Leaves), Ulcer, Dysentery, Cough (Leaves, Tender Shoot) | Manipur, Sikkim | Chhetri (2005), Khumbongmayum *et al.*, (2005) |
| *Ficus hispida* L. f. Moraceae Roots, Fruit, Latex (stem), Bark, Leaves | Emetic, Astringent (Fruit, Seed, Bark), Ringworm (Leaves), Tuberculosis (Bark, Root) | Arunachal Pradesh, Assam, Manipur, Meghalaya | Purkayastha *et al.*, (2005), Nath & Maiti (2003),Sharma (2004), Das and Tag (2006), Khumbongmayum *et al.*, (2005) |
| *Flemingia strobilifera* (L.) R. Br. | Scabies, Induce sleeping, Relieve Pain | Assam, Sikkim | Dutta and Nath (1999) |

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|---|---|---|---|
| Leguminosae<br>Root, Leaves | (Root), Anthelmintic (Leaves), *Ringworm (Root) | | |
| *Fimbristylis falcata* (Vahl) Kunth<br>Cyperaceae<br>Rhizome | Skin Diseases, Ring worm (Rhizome) | Nagaland | Rao and Jamir (1982) |
| *Garuga pinnata* Roxb.<br>Burseraceae<br>Leaves, Fruit, Stem | Ringworm, Asthma, Indigestion, Conjuctivitis | Assam, Meghalaya | Saikia *et al.,* (2006), Chhetri (1994) |
| *Gelsemium elegans* Benth.<br>Loganiaceae<br>Leaves, Root | Wounds (Leaves), Stomach Ulcer, Ringworm (Root) | Mizoram | Bhardwaj and Gakhar (2005) |
| *Gonatanthus pumulus* D. Don.<br>Araceae<br>Bulb | Snake bite, Dog bite, Ringworm, Mumps, Scabies (Bulb) | Sikkim | Janmeda *et al.,* (2006) |
| *Gongronema nepalense* (Wallich) Decne<br>Asclepiadaceae<br>Leaves | Boils, Ringworm (Leaves) | Meghalaya | Singh *et al.,* (2003), Kumar *et al.,* (1987) |
| *Gynocardia odorata* R.Br.<br>Flacourtiaceae<br>Fruit, Seeds, Oil | Diabetes, Anthelmintic, Ulcer, inflammation, *Ringworms, Scabies (Oil) | Arunachal Pradesh, Meghalaya, Sikkim | Chhetri *et al.*, (2005), Chhetri (2005), Sarmah *et al.,* (2006), Rai *et al.,* (1998), Ahmed & Borthakur (2005) |
| *Holarrhena pubescens* (Buch.-Ham.) Wall. ex D. Don<br>Apocynaceae<br>Bark,Seed, Latex, Flower | Dysentry, Diarrhoea, Antitetanic (Bark), Fever, Bilious problems (Bark and Seed), Ringworm, Leprosy, (Bark) | Assam, Sikkim, Tripura | Chhetri (2005), Sharma (2004), Srivastava *et al.*, (2003), Singh *et al.*, (2003), |

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|---|---|---|---|
| *Jasminum angustifolium* (L.)Willd.Oleaceae Root | Ringworm, Leprosy, Wounds (Roots) | Assam | Sharma (2004) |
| *Jasminum lanceolaris* Roxb. Oleaceae Root, Leaves | Ringworm (Root, Leaves) | Meghalaya | Maikhuri and Gangwar (1993) |
| *Leea macrophylla* Hornemann Leeaceae *Tubers, Root, Leaves | *Ringworm Guinea worm (Tubers), Sores (Root), Cuts, Wounds (Leaves) *Sikkim *NS-k | Sikkim | |
| *Mallotus philippinensis* (Lamk.) Muell. Arg Euphorbiaceae Fruit, Bark | Anthelmintic, Constipation, Skin diseases, Scabies, Ringworm, Herpes, Oral | Arunachal Pradesh, Assam, Manipur, Meghalaya, Sikkim | Khumbongmayum *et al.*, (2005), Tiwari and Tiwari (1996), Gurung (2002) |
| *Micromeria biflora* Benth and *Citrus reticulata* | Antibacterial activity | Mizoram | Kumar *et al.,* (2011) |
| *Mikania micrantha* H. B. K. Asteraceae Leaves, Whole Plant, Shoots (Tender), *Aerial parts | Cuts, Wounds, Ringworm, Skin diseases (Leaves), Stomach Trouble (Whole Plant), Dysentery (Tender shoots, leaves) | Assam, Meghalaya, Mizoram, Nagaland, Arunachal Pradesh , | Khumbongmayum *et al.*, (2005), Bhardwaj and Gakhar (2005), Chaturvedi and Jamir (2007), Ahmed & Borthakur (2005), *NS-d,g,e, |
| *Moghania strobilifera* Jaume St.Hil. Leguminosae Root | Ringworm (Root) | Assam | Saikia *et al.,* (2006) |
| *Myrica esculenta* Ham. ex Don. Myricaceae Fruit, Bark (stem/root) Indigestion (Fruit) | Dysentry, Diarrhoea, Stomachache, Ringworm (Bark), Fever | Manipur, Meghalaya, Sikkim, *Assam | Samati (2004), Pfoze and Chhetry (2004), Upadhaya *et al.*, (2005), |

| | | | |
|---|---|---|---|
| *Nelumbium speciosum* Willd. Nymphaeaceae Root (From red flower Variety), Whole Plant | Tendency of abortion (Root From red flower variety), Ring worm, Dysentery, Dyspepsia, Diuretic, Antifungal, Fever | Assam | Tiwari *et al.,* Sharma (2004) |
| *O. basilicum* L. Lamiaceae Leaves, Seed, Root, Aerial part, *Fruit | Headache, Common Fever, High blood pressure, Indigestion, Fever, Diarrhoea, Dysentery, Ringworm (Leaves), Cooling, Gonorrhoea (Seed) | Arunachal Pradesh, Assam, Manipur, Meghalaya, Sikkim, Nagaland | Nath and Bordoloi (1989), Sarmah *et al.,* (2006), Chaturvedi and Jamir (2007) |
| *Ocimum sanctum* L. Lamiaceae Shoot (Tender), Leaves, Inflorescence, Root, Rhizome | High Blood Pressure, Otorrhoea, Cough, Cold, Fever, Stomach Trouble, Hepatic Infection, Bronchitis, Stimulant, Cuts, Wounds, Urticaria, Ringworm | Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Tripura, *Sikkim | Purkayastha *et al.,* ,(2005), Saikia *et al.,* ,(2006), Purkayastha and Nath (2006), Sharma (2004), Sarmah *et al.,* , (2006) |
| *Polygonum hydropiper* L. Polygonaceae Whole plant, Leaves, Twigs, Shoot, *Aerial parts | Fungal infection, Itching, Cuts, Wounds, Dog Bite, Skin diseases, Skin diseases (Whole Plant/Shoot) | Assam, Nagaland, Sikkim, *Arunachal Pradesh | Nath & Maiti (2003), Srivastava *et al.,* (1987), Sharma (2004), Das et al. (2006) |
| *Pouzolzia hirta* Hassk. Urticaceae Leaves, Whole plant, Root | Ring worm (Leaves), Body pain, Stiffness of muscle (Whole plant) | Assam, Meghalaya, Sikkim, *Arunachal Pradesh | Singh *et al.,* (1996), Srivastava *et al.,* (1987), Rao (1981), Upadhaya *et al.,* (2005) |

| | | | |
|---|---|---|---|
| *Raphanus sativus* L. Brassicaceae Seeds, Leaves, *Root, Flower | Ringworm (Seeds), Acidity (Leaves) | Meghalaya, *Assam | Samati (2004) |
| *Rhinacanthus nasuta* Kurz Acanthaceae Root | Ringworms (Root) | Assam | Borthakur *et al.*, (1996), |
| *Rumex maritimus* L. Polygonaceae *Leaves | Ring worm (Leaves) | *Manipur | Chhetri (2005), |
| *Rumex nepalensis* Spreng. Polygonaceae Root, Young shoot, Whole plant | Hepatits, Liver Tonic, Jaundice, Food poison (Root), Stomach colic (Roots), Ringworm, Scabies, Skin diseases (Leaves or young shoot) | Assam, Sikkim | Chhetri (2005), Dash *et al.*, (2003), Khumbongmayum *et al.*, (2005) |
| *Siegesbeckia orientalis* L. Asteraceae Leaves, whole plant | Injuries, Wounds (Leaves), Skin diseases, Sores, Ringworm & other allied diseases | Assam, Meghalaya | Neogi *et al.*, (1989), Islam & Hasin (2003), |
| *Solanum nigrum* L. Solanaceae Fruit, Root, Whole plant, Leaves, Shoot | Emollient, Dysentery, Pustules, Anaemia of infants, High Blood Pressure, Diuretic, Alterative, Ringworm, Skin diseases | Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, *Mizoram | Purkayastha and Nath (2006), Samati (2004), Sarmah *et al.*, (2006), |
| *Vernonia cinerea* L. Asteraceae Whole Plant, Leaves, Root | To Promote perspiration, Piles, Tonic, Stomachic, Ringworm | Assam, Arunachal Pradesh, Manipur, *Tripura | Gogoi and Das (2003), Sarmah *et al.*, (2006), Sinha (1996) |
| *Vernonia roxburghii* Less. Asteraceae Leaves | Ringworm, Cough, Colic, Diarrhoea (Leaves) | Manipur | Sinha (1996)-Book |

# 3. IPR in relation to antidermatophytic plants

## 3.1 Intellectual Property

Intellectual Property (IP) relates to legal rights which emanate from intellectual activities in the industrial, scientific, literary and artistic fields. IP laws provide protection for a limited period, to the moral and economic rights of creators for their creations and also ensure rights of the public in access to those creations. Such protection promotes creativity, enables dissemination of the creative work and application of its results thereby contributing to social and economic progress. (WIPO)

'In a society which can be described as being saturated with a large amount of information, technological reforms can progress very quickly. Today, this progress involves an international society, and in recent years, this progress has been based on what is known as intellectual rights such as patent rights, trademark rights, confidential business information, copyright, and other rights related to intellectual property (referred to as intellectual property rights).'

Patents, trademarks, and copyrights are the principal means for establishing ownership rights to inventions and ideas, and provide a legal foundation by which intangible ideas and creations generate tangible benefits to businesses and employees. Intellectual property (IP) protection affects commerce throughout the economy by: providing incentives to invent and create; protecting innovators from unauthorized copying; facilitating vertical specialization in technology markets; creating a platform for financial investments in innovation; supporting startup liquidity and growth through mergers, acquisitions, and IPOs; making licensing-based technology business models possible; and, enabling a more efficient market for technology transfer and trading in technology and ideas.(uspto.gov).

### 3.1.1 Types of Intellectual Property

Intellectual property is divided into two broad groups;

(i) "**Industrial property"** (**includes inventions**, industrial designs, trademarks, service marks, commercial names & designations, protection against unfair competition)

(ii) "**Copyright"** and related rights (includes literary, artistic and scientific works including performances of performing artists, phonograms and broadcasts)

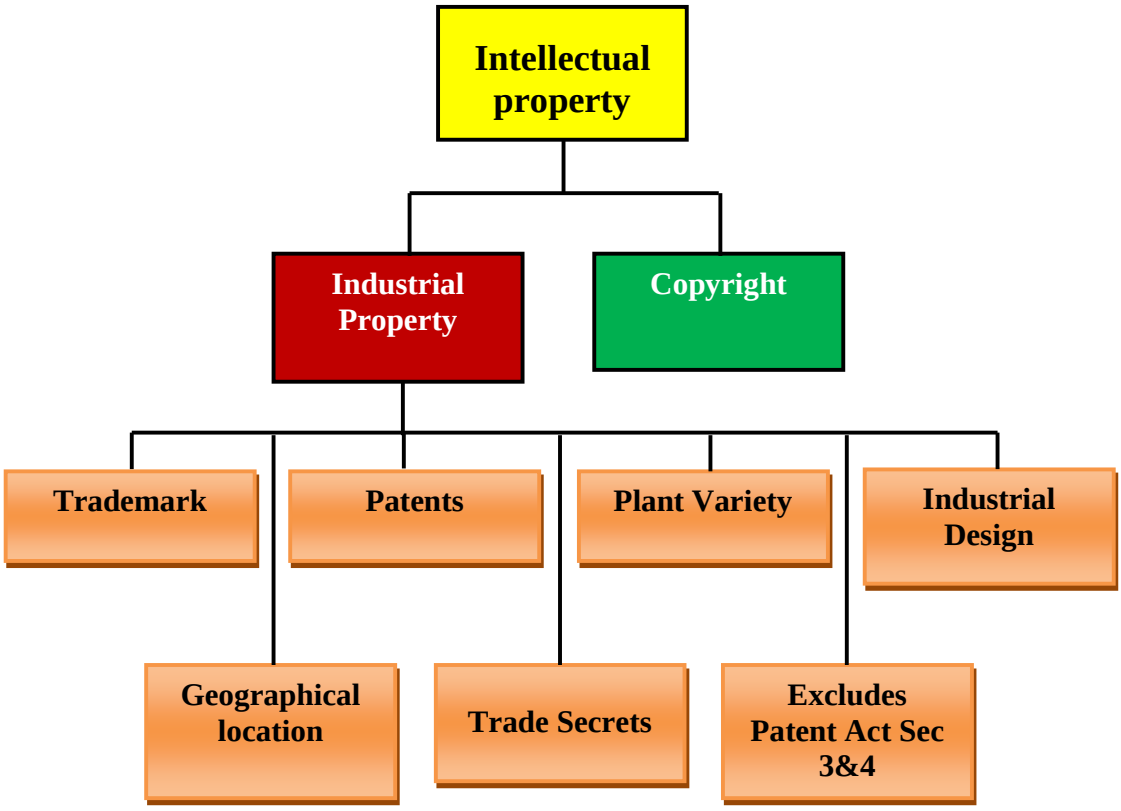


**Figure 3.1 : A Schematic Representation of Intellectual Property**

### 3.1.2 Intellectual Property Right (IPR) relevant to present investigation (Invention/patent)

As per the WIPO rules and regulations (1979), the "invention is a thought" which solves the critical problems in area of technology.

The word "invention" originated from the Latin word "Invenire", which means: 'to get something by chance'.Moreover, an **'invention'** is new solutions to a specific problem in the field of technology and is protected by grant of a **'Patent'** by a governmental or regional organization. A Patent is a document which describes an invention and provides a legal right to the owner of the patent to prevent others from commercially exploiting the invention, without the authorization of the owner of the patent.

Patent is given for a product or a process for a limited period, generally not exceeding 20 years and is granted in return for the disclosure of the invention so that others may derive benefits from the invention.

The procedures for acquiring patent rights are governed by the rules and regulations of national and regional patent offices. These offices are responsible for issuing patents, and the rights are limited to the jurisdiction of the issuing authority. To obtain patent rights, applicants must file an application describing the invention with a national or regional office. They can also file an "international application" through the Patent Cooperation Treaty (PCT), an international treaty administered by WIPO, that facilitates the acquisition of patent rights in multiple jurisdictions. The PCT system simplifies the process of multiple national patent filings by delaying the requirement to file a separate application in each jurisdiction in which protection is sought. However, the decision of whether or not to grant patents remains the prerogative of national or regional patent offices, and patent rights are limited to the jurisdiction of the patent granting authority

(World Economics and Statistics Series 2012, World Intellectual Property Series).

### 3.1.3 Possibilities of Patenting

Presently based on inventor's inventions thepatent can be grantedeither on any product or any process; separately called as (a) product patent and (b) process patent.

**a. Product patent** includes the following:

•Genetic material,

•Unicellular organisms,

•Non human multi cellular organisms,

•Human body parts,

•Human body.

**b. Process patent** includes the following:

•Non biological and essentially biological processes,

•Gene therapies,

•Cloning,

•Methods of medical treatments.

**Patent Law- Salient Features:**

- Both product and process patent provided.
- Examination on request.
- Both pre-grant and post-grant opposition.
- Fast track mechanism for disposal of appeals.
- Provision for protection of bio-diversity and traditional knowledge.
- Publication of applications after 18 months with facility for early publication.
- Substantially reduced time-lines.
- Compulsory license to ensure availability of drugs at reasonable prices.
- Provision to deal with public health emergency.
- Revocation of patent in public interest and also on security considerations.
- Term of patent – 20 years.

**From 1.1.1995**

- *Mail-Box for pharmaceutical and agrochemicals products* (TRIPS requires that countries, not providing product patents in respect of pharmaceuticals and chemical inventions have to put in a mechanism for accepting product patent applications w.e.f. 1st January 1995. Such applications will only be examined for grant of patents, after suitable amendments in the national patent law have been made. This mechanism of accepting product patent applications is called the "mail box" mechanism).
- *Exclusive Marketing Rights*

**From 1.1.2000**

- Patent term increased to 20 years
- Definition of invention – inclusion of inventive step
- Reversal of burden of proof – on the infringer
- Mandatory compulsory license provision for food, drugs and chemicals removed
- Right of patentee (importation also included)

**From 1.1.2005**

- Product patents for food, chemical and pharmaceutical.

### 3.1.4 Conditions of Patentability

An invention becomes patentable only if it:

(i) Consists of a patentable matter;

(ii) is non-obvious to the person skilled in the art;

(iii) is novel;

(iv) has industrial use;

## 3.2 Major world patent infrastructures

Following are some important infrastrures/ organizations working at the world level, for intellectual properties/ patents

### 3.2.1 World Intellectual Property Organization (WIPO)

'The World Intellectual Property Organization (WIPO) is one of the specialized agencies of the United Nations (UN) system of organizations. The "Convention Establishing the World Intellectual Property Organization" was signed at Stockholm in 1967 and entered into force in 1970. However, the origins of WIPO go back to 1883 and 1886, with the adoption of the Paris Convention and the Berne Convention respectively. The agreement between the United Nations and WIPO recognizes that WIPO is, subject to the competence of the United Nations and its organs, responsible for taking appropriate action in accordance with its basic instrument and the treaties and agreements administered by it, *inter alia*, for promoting creative intellectual activity and for facilitating the transfer of technology related to industrial property to developing countries in order to accelerate economic, social and cultural development.'

'The mission of WIPO is to promote through international cooperation the creation, dissemination, use and protection of works of the human mind for the economic, cultural and social progress of all mankind.' 'Its effect is to contribute to a balance between the stimulation of creativity worldwide, by sufficiently protecting the moral and material interests of creators on the one hand, and providing access to the socio-economic and cultural benefits of such creativity worldwide on the other'. 'An outstanding example of the

expansion of WIPO's work is creation of the facility of a single procedure to apply for patents, valid in up to all States party to those treaties. The Patent Cooperation Treaty (PCT) has given rise to an increased volume of registration activities.' 'To strengthen this aspect of WIPO's work, a new international treaty, namely, the Patent Law Treaty, came into existence in June 2000: its purpose is to streamline application procedures and to reduce the cost of obtaining simultaneous patent protection in several countries'. 'WIPO's cooperation for development program is closely interwoven with governmental and intergovernmental cooperation, including WIPO's agreement with the World Trade Organization (WTO), whereby WIPO assists developing countries in the implementation of WTO's Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS).' 'WIPO is increasingly adopting a global approach not only to intellectual property in itself, but to the place of intellectual property in the wider framework of emerging issues such as traditional knowledge, folklore, biological diversity, environmental protection and human rights'. (WIPO Intellectual Property Handbook, WIPO Publication, No. 489 (E), ISBN 978-92-805-1291-5, WIPO 2004, Second Edition, Reprinted 2008**)**

### 3.2.2 The United States Patent and Trademark Office (USPTO)

The United States Patent and Trademark Office (USPTO) is the Federal agency for granting U.S. patents and registering trademarks. In doing this, the USPTO fulfills the mandate of Article I, Section 8, Clause 8, of the Constitution that the Executive branch "promote the progress of science and the useful arts by securing for limited times to inventors the exclusive right to their respective discoveries." Under this system of protection, American industry has flourished. New products have been invented, new uses for old ones discovered, and employment opportunities created for millions of Americans. The strength and vitality of the U.S. economy depends directly on effective mechanisms that protect new ideas and investments in innovation and creativity. The continued demand for patents and trademarks underscores the ingenuity of American inventors and entrepreneurs. The USPTO is at the cutting edge of the Nation's technological progress and achievement. The USPTO advises the President of the United States, the Secretary of Commerce, and U.S. Government agencies on intellectual property (IP) policy, protection, and enforcement;

and promotes the stronger and more effective IP protection around the world. The USPTO furthers effective IP protection for U.S. innovators and entrepreneurs worldwide by working with other agencies to secure strong IP provisions in free trade and other international agreements. It also provides training, education, and capacity building programs designed to foster respect for IP and encourage the development of strong IP enforcement regimes by U.S. trading partners. (uspto.gov)

### 3.2.3 The European Patent Office (EPO)

The European Patent Organisation is an intergovernmental organisation that was set up on 7 October 1977 on the basis of the European Patent Convention (EPC) signed in Munich in 1973. It has two bodies, the European Patent Office (EPO) and the Administrative Council, which supervises the Office's activities. The Organisation currently has 38 member states. European patent applications refer to applications filed directly under the EPC or to applications filed under the Patent Cooperation Treaty (PCT) and designated to the EPO (Euro-PCT). Patent applications are counted according to the year in which they are filed and are assigned to a country according to the inventor's place of residence, using fractional counting if there are multiple inventors

(Eurostatsite: *epp.eurostat.ec.europa.**eu**/statistics_explained/index/**Patent**_statistics).*

### 3.2.4 Japan Patent Office (JPO)

The Japan Patent Office is engaged in administering the industrial property rights system—the collective name for the patent, utility model, design and trademark systems—in its quest to promote the progress of industrial technology and improve its people's lives in the 21st century. JPO is active in its efforts to boost Japanese industry, including: 1) appropriate granting of industrial property rights, 2) drafting of industrial property-related measures, 3) promotion of international harmonization and assistance to developing nations, 4) reviews of the industrial property rights system, 5) implementation of support measures designed for SMEs and universities, and 6) improvement in industrial property-related information services. JPO is actively involved in international activities, such as trilateral co-operation with the USPTO and EPO, IP5 co-operation together with China and South Korea, cooperation with developing nations in the areas of examination and human

resources development, promotion of the Patent Prosecution Highway (PPH) program, and tougher implementation of anti-counterfeiting and piracy measures.

### 3.2.5 Korean Intellectual Property Office (KIPO)

The Korean Intellectual Property Office (KIPO) is the governmental authority in charge of intellectual property in Korea. KIPO is responsible for affairs regarding patents, utility models, industrial designs, trademarks, and the related examinations and trials. The main functions of KIPO are as follows: the examination and registration of intellectual property rights (for patents, utility models, trademarks, and industrial designs); the conducting of trials on intellectual property disputes; the management and dissemination of information on intellectual property rights; the promotion and public awareness of invention activities; the promotion of international cooperation on intellectual property rights; and the training of experts on intellectual property rights.

### 3.2.6 The State Intellectual Property Office of the People's Republic of China (SIPO)

The State Intellectual Property Office of the People's Republic of China (SIPO), also known as the Chinese Patent Office, is the *patent office* of the *People's Republic of China* (PRC). It was founded on 1980, as the Patent Office of the People's Republic of China, the predecessor of SIPO. It is responsible "for patent work and comprehensively coordination of the foreign related affairs in the field of intellectual property".(SIPO)

#### 3.2.6.1 Chinese Patent system and Trends in Chinese patent activities

The first patent law in China came into being in 1889. Several new laws and revisions have been introduced since then. With the “opening up” in 1978, a patent system and a patent law, was finally approved in 1984. In the first patent law that took effect in 1985, chemicals and pharmaceuticals were not patentable and a revision in 1992 introduced chemical and pharmaceutical patents and it extended the maximum duration of patent protection to 20 years. China joined the WTO in 2001 and also adopted the TRIPS agreement. The Paris Convention was already adopted earlier (in 1985) and the Patent Cooperation Treaty (PCT) was signed in 1994. The intellectual property (IP) offices of China became the largest in the world, as measured by the number of applications received

for patents, utility models (UMs), trademarks and industrial designs. China's patent office overtook the United States Patent and Trademark Office (USPTO) in 2011 to become the largest in the world, after having surpassed the Japan Patent Office (JPO) in 2010. (WIPO 2011). Between 2008 and 2011, both SIPO and the USPTO saw filing growth in patents.. However, filings at SIPO increased at a faster rate than at the USPTO.

The Chinese patent applications have been growing considerably in the recent decade, both on the national and the transnational level. This is the result of enormous investments in R&D, but also of a political willingness to internationalise and to improve the role of technology and innovation in general. The Chinese patent system underwent several reforms in the past 20 years where the last two – one in 2000 and another one in 2009 – had the strongest impact and aimed to shift the Chinese system to international standards. In 2009, absolute novelty has been introduced while before only novelty to the Chinese market was examined.

The Chinese patent profile at the SIPO is distinct from the profile on the transnational level. On the national level it is mainly chemistry and pharmaceuticals including biotechnology, where comparative advantages occur. On the transnational level it is by far communication engineering and related fields that play major role, while the contribution of chemistry in relation to this clearly diminished in the recent decade. (Frietsch et all (2011). In the 1990s the Chinese patent profile on the transnational level was dominated by chemistry patents and the like. It decreased from almost 40% in the 1990s to a level below 20% since the mid 2000s. (Frietsch/Wang 2009). At the same time the increase of electrical engineering patents is evident.

Until the priority year 2005, the majority of patent applications in China were filed by foreign companies and inventors, while since then Chinese applicants are responsible for the largest share of applications. China accounts for about 53% of all patent applications to the SIPO in 2008, Japan accounts for 12%, the USA for 6% and Germany for about 3%.(EPO – PATSTAT; Fraunhofer ISI calculations). The total number of invention patent applications has steadily grown since 2000 from a level of 51,747 to 391,177 applications in the year 2010, according to SIPO's official statistics.

(http://english.sipo.gov.cn/statistics/index.html)

The Chinese positive specialisation therefore, is visible in the areas of chemistry, pharmaceuticals, biotechnology, and some mechanical engineering fields, even though the index values are not very high, reflecting not outstanding comparative advantages in patenting.

### 3.2.7 The Five IP Offices (IP5)

The Five IP Offices (IP5) is the name given to a forum of the five largest intellectual property offices in the world, set up to improve the efficiency of the examination process for patents worldwide. The members of IP5 are: the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), and the United States Patent and Trademark Office (USPTO). The IP5 Offices account for about 90% of all patent applications filed worldwide and for 93% of all work carried out under the Patent Cooperation Treaty (PCT).The vision of the IP5 Offices is global co-operation, which has been defined as "the elimination of unnecessary duplication of work among the IP5 Offices, the enhancement of patent examination efficiency and quality and guarantee of the stability of patent right". The objective is to address the ever-increasing backlog at the world's five biggest intellectual property offices. As the world sees economic barriers between nations fade away, innovators want their intellectual creations protected concurrently in multiple major markets. Hence, applications for the same technology are filed at more than one patent office. The solution to the backlog problem is to reduce, to the maximum extent possible, the duplication of work which takes place at each office for a family of patent applications. (www.ip5offices.com)

## 3.3 Trends in patent filing and grant across the world and fields

During the last decade the world trend of patent activities was as follows:

### 3.3.1 World Trends in patent filing and grant

The World trends indicate that over the last many years there is a tremendous increase in the patent activities across the world. According to the WIPO statistics database

as on November 2012, number of patent grants in the world shows an increase from 535100 in the year 2001 to 996800 in the year 2011 (Table 3.1).

In comparison, the European Patent filing shows an increase from 164,144 in the year 2002 to a total of 244,437 in the year 2011 (Table: 3.2). Top five European patent filing countries are the United States, Japan, Germany, China and Korea (Table: 3.3). However European patent office **granted** a total of 62112 patents in the year 2011 as against a total of 47380 in the year 2002, the top patentees being the US, Japan and Germany (Table: 3.4)

**Table 3.1: Trends of Patent 'Grants' in the World (2002-2011)**

| | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Granted** | 558900 | 618300 | 621300 | 630400 | 751300 | 770700 | 772100 | 809000 | 908600 | 996800 |

(Source: WIPO statistics database as updated: November 2012)

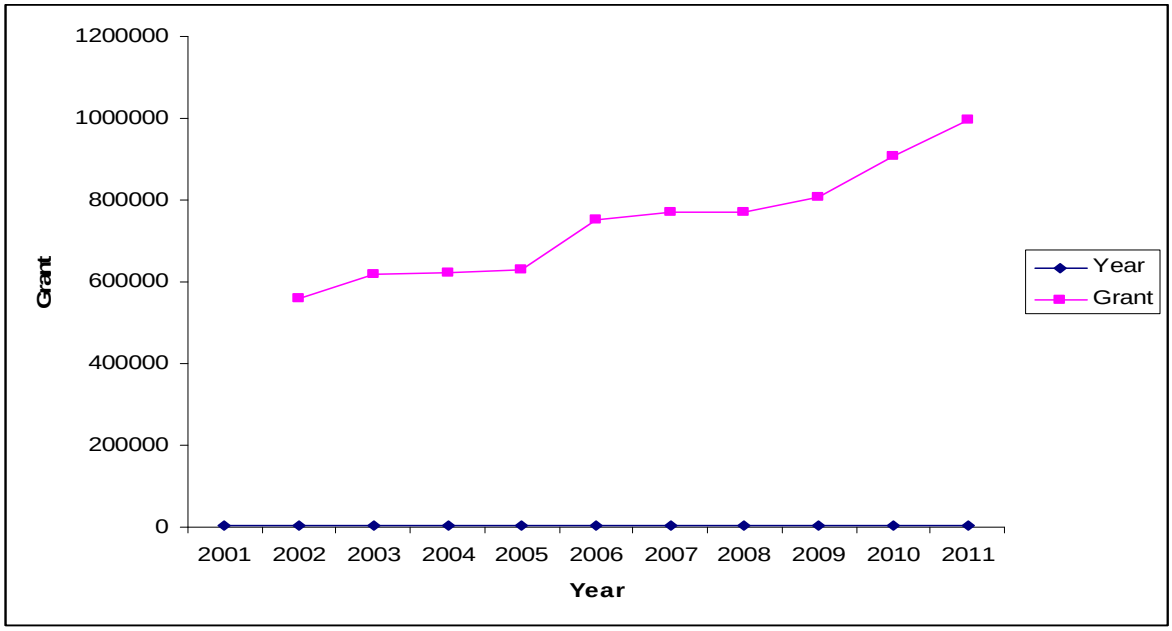


**Figure 3.2: Trends of Patent Grants in the World (2002-2011) (Graphical)**

**Table 3.2 : European Patent 'Filing' (EPO) as per the applicant's residence country (2002-2011)**

| Country of residence | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|
| Australia | 1,810 | 1,745 | 1,912 | 2,105 | 2,106 | 2,159 | 2,066 | 1,824 | 2,002 | 1,873 |
| Brazil | 216 | 244 | 294 | 309 | 370 | 441 | 530 | 539 | 522 | 605 |
| Canada | 2,613 | 2,715 | 2,698 | 2,966 | 3,323 | 3,670 | 3,651 | 3,374 | 4,171 | 4,065 |
| Switzerland | 5,159 | 5,326 | 5,609 | 6,240 | 6,892 | 7,160 | 7,072 | 6,882 | 7,861 | 7,786 |
| China, People's Republic of | 1,137 | 1,455 | 1,881 | 2,687 | 4,213 | 5,835 | 6,490 | 8,270 | 12,750 | 16,946 |
| Germany | 26,507 | 27,211 | 28,227 | 29,152 | 30,670 | 32,128 | 33,405 | 30,486 | 33,146 | 33,181 |
| France | 9,044 | 9,174 | 9,654 | 10,129 | 10,460 | 10,800 | 11,503 | 11,605 | 11,718 | 12,107 |
| United Kingdom | 6,887 | 6,631 | 6,463 | 6,594 | 6,727 | 7,256 | 7,178 | 6,565 | 7,137 | 6,464 |
| India | 572 | 788 | 762 | 741 | 887 | 965 | 1,125 | 1,000 | 1,378 | 1,530 |
| Italy | 4,400 | 4,739 | 4,877 | 5,129 | 5,371 | 5,628 | 5,437 | 4,806 | 4,946 | 4,879 |
| Japan | 25,859 | 28,534 | 32,267 | 36,469 | 37,930 | 38,643 | 39,676 | 38,268 | 41,869 | 47,404 |
| Korea, Republic Of | 3,563 | 4,412 | 5,723 | 7,679 | 9,325 | 10,327 | 10,252 | 10,217 | 12,352 | 13,254 |
| Russian Federation | 546 | 577 | 527 | 656 | 699 | 701 | 776 | 742 | 836 | 999 |
| United States | 51,012 | 51,517 | 53,482 | 57,273 | 60,966 | 63,331 | 61,184 | 53,892 | 60,762 | 59,688 |
| South Africa | 396 | 360 | 419 | 368 | 425 | 417 | 404 | 383 | 307 | 317 |
| All origin total | 164,144 | 170,530 | 181,162 | 197,539 | 210,782 | 222,572 | 225,975 | 211,354 | 235,700 | 244,437 |

**Table 3.3: European Patent 'Applications' (EPO) as per the applicant's residence country (2002-2011)**

| Country of residence | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|
| ustralia | 874 | 841 | 864 | 859 | 974 | 984 | 1,036 | 836 | 979 | 837 |
| Brazil | *86* | *106* | *97* | *129* | 150 | 153 | 180 | 182 | 191 | 208 |
| Canada | *1,438* | *1,576* | *1,704* | *1,738* | 1,884 | 2,033 | 1,873 | 2,036 | 2,675 | 2,348 |
| Switzerland | *3,973* | *4,241* | *4,734* | *5,137* | 5,559 | 5,859 | 5,901 | 5,829 | 6,767 | 6,409 |
| China, People's Republic of | *226* | *340* | *449* | *563* | 728 | 1,122 | 1,496 | 1,608 | 2,040 | 2,548 |
| Germany | 20,904 | 22,636 | 22,937 | 23,648 | 24,802 | 25,208 | 26,672 | 25,125 | 27,354 | 26,234 |
| France | 6,764 | 7,389 | 8,101 | 8,020 | 8,070 | 8,356 | 9,068 | 8,949 | 9,569 | 9,633 |
| United Kingdom | 4,619 | 4,767 | 4,708 | 4,592 | 4,691 | 4,922 | 4,992 | 4,806 | 5,376 | 4,765 |
| India | 134 | 161 | 267 | 390 | 368 | 393 | 439 | 325 | 425 | 474 |
| Italy | 3,329 | 3,673 | 3,971 | 4,177 | 4,149 | 4,387 | 4,341 | 3,885 | 4,082 | 3,982 |
| Japan | 15,952 | 18,563 | 20,643 | 21,482 | 22,202 | 22,938 | 22,993 | 19,893 | 21,792 | 20,568 |
| Korea, Republic Of | 1,427 | 2,083 | 2,882 | 3,871 | 4,627 | 4,945 | 4,325 | 4,187 | 4,723 | 4,889 |
| Russian Federation | 83 | 111 | 123 | 89 | 142 | 129 | 155 | 164 | 175 | 169 |
| United States | 30,026 | 31,735 | 32,361 | 32,525 | 34,525 | 35,349 | 37,026 | 32,889 | 39,466 | 34,993 |
| South Africa | 99 | 128 | 104 | 116 | 116 | 120 | 132 | 131 | 102 | 115 |
| All origin total | 89,934 | 98,350 | 103,945 | 107,336 | 112,987 | 116,898 | 120,629 | 110,845 | 125,716 | 118,172 |

**Table 3.4 : European Patent 'Grants' (EPO) 2002-2011**

| Country of residence | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|
| Australia | 173 | 229 | 235 | 245 | 302 | 300 | 336 | 240 | 295 | 308 |
| Brazil | 27 | 44 | 33 | 50 | 61 | 43 | 57 | 43 | 47 | 52 |
| Canada | 568 | 743 | 680 | 630 | 791 | 770 | 768 | 666 | 730 | 738 |
| Switzerland | 1726 | 2400 | 2120 | 1920 | 2216 | 1985 | 2421 | 2221 | 2389 | 2531 |
| China, | 26 | 50 | 66 | 80 | 115 | 136 | 270 | 351 | 431 | 515 |
| Germany | 11246 | 13407 | 13607 | 12487 | 14275 | 11929 | 13496 | 11375 | 12552 | 13583 |
| France | 3795 | 4810 | 4364 | 3738 | 4499 | 3980 | 4801 | 4029 | 4540 | 4799 |
| United Kingdom | 2129 | 2668 | 2504 | 2144 | 2241 | 1900 | 1969 | 1647 | 1851 | 1948 |
| India | 18 | 56 | 70 | 76 | 106 | 113 | 126 | 124 | 121 | 117 |
| Italy | 1613 | 2213 | 2219 | 1864 | 2314 | 1966 | 2254 | 1992 | 2286 | 2289 |
| Japan | 8250 | 10294 | 10441 | 9549 | 12044 | 10651 | 10915 | 9436 | 10587 | 11649 |
| Korea, Republic Of | 250 | 367 | 460 | 486 | 787 | 858 | 1201 | 1095 | 1390 | 1427 |
| Russian Federation | 30 | 45 | 50 | 25 | 34 | 34 | 43 | 58 | 69 | 40 |
| United States | 11843 | 15090 | 14204 | 13004 | 14833 | 12505 | 12730 | 11347 | 12512 | 13382 |
| South Africa | 35 | 55 | 56 | 55 | 59 | 58 | 53 | 49 | 53 | 53 |
| All origin total | 47380 | 59989 | 58725 | 53255 | 62777 | 54700 | 59801 | 51957 | 58119 | 62112 |

### 3.3.2 Trends in 'patent filing and grant' across fields of technology

Search for patent application in a select patent office i.e. European Patent Office (EPO) indicate that the patents are classified in broad technology categories viz. Chemistry, Electrical Engineering, Instruments, Mechanical Engineering and Others (Civil engineering, furniture, other consumer goods). During the year 2011, field of Instrument shows maximum number of application being filed in the sub-field of Medical Technology : 10534 applications with patent grants of 4384. (Table 3.5 and 3.6) The field of Electrical engineering indicates the maximum applications filed in the sub field of electrical machinery and energy : 8963 applications with total patent grant of 3816.(Table 3.5 and 3.6)

**Table 3.5: European patent 'Applications' across fields of technology (2002-2011)**

| Field Of technology | | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemistry | Basic materials chemistry | 2,999 | 3,003 | 3,214 | 3,269 | 3,597 | 3,856 | 4,215 | 3,936 | 4,740 | 4,253 |
| | Biotechnology | 5,656 | 5,389 | 5,175 | 5,303 | 5,222 | 5,459 | 5,596 | 5,210 | 7,681 | 5,865 |
| | Organic fine chemistry | 4,843 | 5,357 | 5,556 | 6,117 | 7,207 | 7,499 | 7,493 | 7,064 | 7,615 | 6,887 |
| | Pharmaceuticals | 4,710 | 5,579 | 6,050 | 6,013 | 6,097 | 6,286 | 6,142 | 5,542 | 6,879 | 5,759 |
| Electrical engineering | Audio-visual technology | 3,724 | 4,441 | 5,001 | 5,539 | 5,401 | 4,819 | 4,506 | 3,627 | 4,099 | 3,568 |
| | Computer technology | 7,609 | 8,331 | 8,873 | 9,017 | 8,793 | 8,688 | 9,161 | 7,989 | 8,819 | 8,197 |
| | Digital communication | 3,287 | 3,966 | 4,459 | 4,947 | 5,539 | 6,053 | 6,565 | 7,188 | 8,323 | 7,843 |
| | Electrical machinery, apparatus, energy | 5,794 | 6,198 | 6,332 | 6,590 | 7,048 | 7,280 | 8,110 | 7,784 | 8,737 | 8,963 |
| Instruments | Analysis of biological materials | 1,081 | 1,364 | 1,427 | 1,233 | 1,382 | 1,366 | 1,401 | 1,333 | 1,629 | 1,403 |
| | Medical technology | 5,386 | 6,248 | 7,185 | 7,843 | 8,525 | 9,425 | 9,704 | 9,893 | 11,072 | 10,534 |

(Source: www.wipo.int/ipstats/en/statistics/patents/pdf/wipo_ipc_technology.pdf)

**Table 3.6: European Patents 'Grants' across fields of technology (2002-2011)**

| Field of technology | | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemistry | Basic materials chemistry | 1502 | 2031 | 2179 | 1525 | 1732 | 1365 | 1315 | 1169 | 1281 | 1506 |
| | Biotechnology | 983 | 1309 | 1532 | 1642 | 2366 | 1915 | 2214 | 2078 | 2194 | 2139 |
| | Organic fine chemistry | 2696 | 3720 | 2905 | 2235 | 2580 | 2384 | 2576 | 2387 | 2562 | 2660 |
| | Pharmaceuticals | 1961 | 2369 | 2268 | 2237 | 1890 | 1685 | 1928 | 1688 | 1695 | 1704 |
| Electrical engineering | Audio-visual technology | 1482 | 1572 | 1445 | 1203 | 1710 | 1478 | 1741 | 1520 | 1656 | 1812 |
| | Computer technology | 2294 | 2920 | 2045 | 1833 | 2360 | 2361 | 2555 | 2284 | 2397 | 2525 |
| | Digital communication | 395 | 573 | 996 | 1744 | 2108 | 2236 | 2133 | 2493 | 2753 | 3097 |
| | Electrical machinery, apparatus, energy | 2717 | 3048 | 2877 | 2667 | 3409 | 3356 | 3151 | 2844 | 3521 | 3816 |
| Instruments | Analysis of biological materials | 351 | 354 | 348 | 328 | 413 | 400 | 462 | 508 | 547 | 514 |
| | Medical technology | 1988 | 3193 | 3749 | 3625 | 3380 | 3076 | 3480 | 3628 | 3842 | 4384 |

(Source:www.wipo.int/ipstats/en/statistics/patents/pdf/wipo_ipc_technology.pdf )

### 3.3.2.1 Trends in 'patent filing and grant' in field of Pharmaceuticals, (2002-2011)

However, Patent applications filed during 2011 in the field relevant to the present study classified under the broad field of Chemistry and in the sub field of Organic Fine Chemistry are: 6887 applications with patent grant of 2660; Biotechnology :5865 applications with patent grant of 2139 and Pharmaceuticals :5759 applications with patent grant of 1704. (Table 3.5 and 3.6). The field of pharmaceuticals shows increases in the applications from the year 2002 to the year 2012 in which 5759 applications for patents are filed. There is however slight decrease in the number of patent grants in the field of pharmaceuticals from a total of 1961 in the year 2002 to 1704 in the year 2011. Status of patent applications and grants in the fields of technology relevant directly to the present investigations is given at tables 3.7 and 3.8.

**Table 3.7: European Patent 'Applications' in the field of Pharmaceuticals (2002-2011)**

| Field of technology[1] | | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemistry | Basic materials chemistry | 2,999 | 3,003 | 3,214 | 3,269 | 3,597 | 3,856 | 4,215 | 3,936 | 4,740 | 4,253 |
| | Biotechnology | 5,656 | 5,389 | 5,175 | 5,303 | 5,222 | 5,459 | 5,596 | 5,210 | 7,681 | 5,865 |
| | Chemical engineering | 2,568 | 2,842 | 3,002 | 3,001 | 3,201 | 3,416 | 3,451 | 3,264 | 3,524 | 3,402 |
| | Environmental technology | 1,248 | 1,367 | 1,352 | 1,366 | 1,516 | 1,625 | 1,757 | 1,778 | 1,821 | 1,868 |
| | Food chemistry | 945 | 1,033 | 1,138 | 1,204 | 1,334 | 1,485 | 1,442 | 1,436 | 1,495 | 1,443 |
| | Macromolecular chemistry, polymers | 2,711 | 2,815 | 2,743 | 2,938 | 3,201 | 3,350 | 3,538 | 3,238 | 3,621 | 3,266 |
| | Materials, metallurgy | 1,880 | 2,069 | 2,103 | 2,136 | 2,225 | 2,337 | 2,488 | 2,412 | 2,647 | 2,707 |
| | Micro-structural and nano-technology | 71 | 118 | 115 | 189 | 153 | 138 | 207 | 171 | 171 | 176 |
| | Organic fine chemistry | 4,843 | 5,357 | 5,556 | 6,117 | 7,207 | 7,499 | 7,493 | 7,064 | 7,615 | 6,887 |
| | Pharmaceuticals | 4,710 | 5,579 | 6,050 | 6,013 | 6,097 | 6,286 | 6,142 | 5,542 | 6,879 | 5,759 |
| | Surface technology, coating | 1,607 | 1,796 | 1,920 | 2,057 | 2,094 | 2,193 | 2,330 | 1,992 | 2,147 | 2,102 |

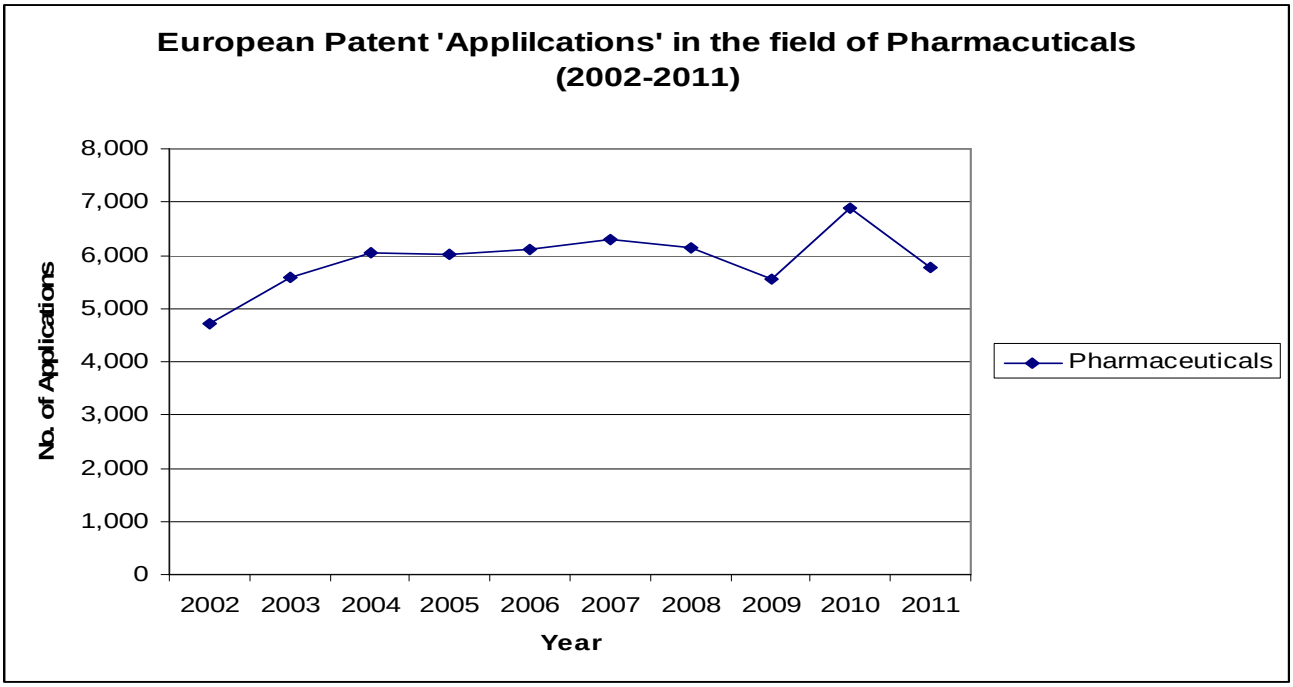


**Figure 3.3: European Patent 'applications' in the field of Pharmaceuticals (2002-2011)(Graphical)**

**Table 3.8: European Patent 'Grants' in the field of Pharmaceuticals (2002-2011)**

| Field of technology[1] | | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemistry | Basic materials chemistry | 1502 | 2031 | 2179 | 1525 | 1732 | 1365 | 1315 | 1169 | 1281 | 1506 |
| | Biotechnology | 983 | 1309 | 1532 | 1642 | 2366 | 1915 | 2214 | 2078 | 2194 | 2139 |
| | Chemical engineering | 1275 | 1800 | 1600 | 1385 | 1530 | 1312 | 1325 | 1205 | 1443 | 1463 |
| | Environmental Tech | 664 | 838 | 773 | 734 | 820 | 665 | 585 | 549 | 801 | 789 |
| | Food chemistry | 485 | 541 | 478 | 497 | 527 | 501 | 505 | 457 | 448 | 527 |
| | Macromolecular chemistry, polymers | 1411 | 1843 | 2227 | 1671 | 1830 | 1437 | 1604 | 1479 | 1503 | 1628 |
| | Materials, metallurgy | 1239 | 1500 | 1277 | 1061 | 1004 | 1001 | 863 | 807 | 902 | 994 |
| | Micro-structural and nano-technology | 0 | 2 | 9 | 7 | 37 | 64 | 52 | 34 | 27 | 59 |
| | Organic fine chemistry | 2696 | 3720 | 2905 | 2235 | 2580 | 2384 | 2576 | 2387 | 2562 | 2660 |
| | Pharmaceuticals | 1961 | 2369 | 2268 | 2237 | 1890 | 1685 | 1928 | 1688 | 1695 | 1704 |
| | Surface technology, coating | 871 | 969 | 1014 | 725 | 889 | 695 | 793 | 673 | 854 | 956 |

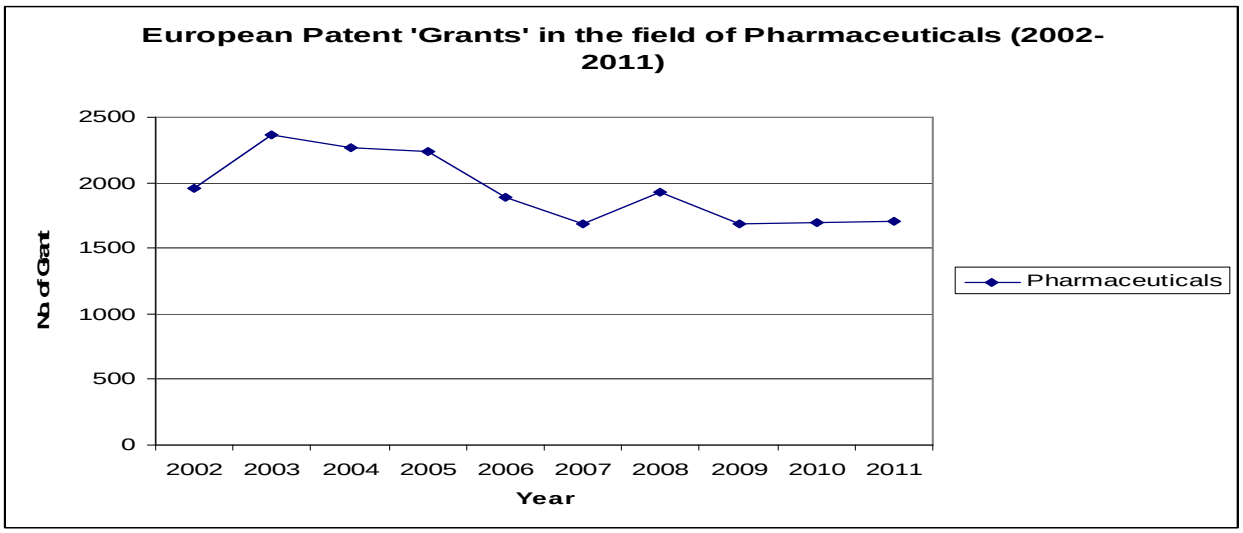


**Figure 3.4: European Patent 'Grants' in the field of Pharmaceuticals (2002-2011) (Graphical)**

### 3.3.3 Patent Activities on Antidermatophytic plants

Patent search , with the key words *antidermatophytic, dermis* and *skin,* on various sites viz., Web of Knowledge, US Patent site, European Patent Office site, Chinese Patent site and Indian Patent site show that some of the patents have been recorded but not any patent have been recorded on the plants as *'antidermatophytic'* agent (Table 3.9).

**Table 3.9: Patent search on 'Antidermatophytic activity of plants' using relevant key words**

| SOURCE | Key word | No. of patents | Field in which patent granted | Remarks |
|---|---|---|---|---|
| **Web of knowledge** | Skin, Dermis | 4 | Whitening agent, Grey hair , pain, herbal bath liquid, Inflammation | No patent found on selected plant/ metabolite showing antidermatophytic activity |
| **Web of knowledge** | Antidermatophytic | 5 | Agent containing ortho-methoxy cinnamaldehyde | No patent found on selected plant /metabolite showing antidermatophytic activity |

**(Source**:http://apps.webofknowledge.com/summary.do?product=DIIDW&searchmode=CombineSearches&qid=5&SID=U1PpkK16eapejiL4eEG&&page=2-)

### 3.3.4 Patent search on selected plants showing Antidermatophytic ativity

Search for grant of patents was conducted on various patent sites viz., USPTO, EPO, IPO as well as on Google; using the key words = the selected plant's name, i.e. *E. odoratum* L, *M. piperita* L. *O. basilicum, C. aurantifolia* L.and *M. micrantha*as an *antidermatophyte.* Besides this, patent search using the key words= selected plant name + dermatophyte's name, i.e. *Epidermophyton floccosum, Microsporum gypsum, Trichophyton mentagrophytes* and *T. rubrum;* were also made for updated information as well as their future prospects.

The observation shows some indicative results showing patent grants on pharmaceutical compositions and method for treating dermatophytes; but (excepting *E. odoratum*) no patent grant was found on the plants as selected in the present study. Moreover, some patents were

found on anti-fungal formulations containing mixtures of oils from two or more plants, such formulation(s) contains:

- Single O**ils: (**Oils of *Rabdosia melissoides*; *Aframomum aulocacarpus, Aframomun danellii, Dracaena arborea, E. odoratum, Glossocalyx brevipes, Napoleonaea imperialis)*
- O**il mixtures: (**mixtures of oils of *Eugenia caryophyllata, Myroxyon pereira, Eucalyptus globulus, Lavandula augustifolia, Mentha piperita, and Mentha spicata;*
- S**elective mixtures: (**Selective mixtures of *Origanum vulgare L., Thymus vulgaris L., Cinnamomum zeylanicum Nees, Rosmarinus officinalis L., Lavandula officinalis L., Mentha piperita L., Citrus Limon L., Hydrastis Canadensis L. and Olea europaea* L,
  (selective mixtures of Origanum oil, menthol, and Atlantic cedarwood oil, thuja oil, cedarwood oil, cinnamon oil, clove oil, cumin oil, fennel oil, peppermint oil, or rosemary)
- A**t least two essential oils: (**derived from a plant genus *Pelargoniu, Cymbopogon, Mentha, Aniba, Lavandula, Origanum, Litsea, Citrus, Melissa, Pogostemon, Santalum,; Valeriana, Styrax, Cinnamonum, and Rosa).*
- Besides, patent was also granted on **high essential oil yielding variety** of *O. basilicum*.

### 3.3.5 An illustrative list of patents granted to the broad field of the study area

USPTO No. 4202877 date 13 May 1980:

**Title : Antidermato-mycotic agent:**

**Patentee:** Masaki Sato et al

**Abstrac**t: The invention relates to Pharmaceutical compositions and a method for treating dermatophytes are based upon the antidermatophytic activity of o-methoxycinnamaldehyde.

USPTO No. 6312698 date Nov 6, 2001

**Title: Anti-fungal formulation active against a broad spectrum of dermatophytoses**

**Patentee:** Shahi et al

**Abstract:** The invention provides a novel anti-fungal formulation active against a broad spectrum of dermatophytoses, said formulation comprising at least about 1% by weight of oil extracted from *Rabdosia melissoides* and one or more vegetable oils, solvents and additives

EP 1143986 A3 / WO2000024411A2

**Title: Plant-derived anti-parasitic and antifungal compounds and methods of extracting the compounds**

**Abstract:** Provided are biologically active extracts from *Aframomum aulocacarpus, Aframomun danellii, Dracaena arborea, Eupatorium odoratum, Glossocalyx brevipes and Napoleonaea imperialis* which are suitable for use in **treating fungal** and protozoa diseases.

EP 1023436 A4 (text from WO1999019460A1) date *16 oct 1997*

**Title: Geranyl diphosphate synthase from mint (*Mentha piperita*)**

**Patentee**: C. Charles Burke et all

**Abstract:** Systems and methods are provided for the recombinant expression of geranyl diphosphate synthase that may be used to facilitate the production, isolation and purification of significant quantities of recombinant geranyl diphosphate synthase for subsequent use, to obtain expression or enhanced expression of geranyl diphosphate synthase in plants in order to enhance the production of monoterpenoids, to produce geranyl diphosphate in cancerous cells as a precursor to monoterpenoids having anti-cancer properties or may be otherwise employed for the regulation or expression of geranyl diphosphate synthase or the production of geranyl diphosphate.

US 6582736 date 24 Jun 2003

**Title** : **A therapeutic oil composition for topical application to painful areas of the human body.**

**Patentee**: Richard S. Quezada

**Abstract**: The therapeutic oil composition is created by mixing *Eugenia caryophyllata, Myroxyon pereira, Eucalyptus globulus, Lavandula augustifolia, Mentha piperita,* and *Mentha spicata*.

No. 20040096418 , Chinese 1489454 date 14.4.2004

**Title : Cosmetically effective composition containing *Malva sylvestris* and *Mentha piperita* extracts**

**Patentee:** Gafner, Thomas

**Abstract:** The invention relates to a concentrate for the production of cosmetically effective compositions that lighten the skin, wherein said concentrate contains at least one extract (i) of mallow (*Malva sylvestris)* and (ii*) peppermint (Mentha piperita)*

USPTO no. 6008043 date 28.12.1999

**Title : Isolation and bacterial expression of a sesquiterpene synthase CDNA clone from peppermint(M*entha χ piperita*,** L.) that produces the aphid alarm pheromone (E)-β-farnesene Croteau;

Patentee: Rodney Bruce, Washington state research foundation

**Abstract:** An isolated DNA sequence (SEQ ID NO:1) is provided which codes for the expression of (E)-β-farnesene synthase (SEQ ID NO:2), from peppermint (***Mentha piperita).***

No. 2006/0073218 A1,US7429396 date Sep 30, 2008

**Title : Antifungal composition, its fungicidal effect on pathogenic dermatophytes**

**Patentee**: Frank S. D'Amelio, Youssef W. Mirhom

**Abstract**: A natural preservative composition obtained from plant materials provides antimicrobial activity for use as an antifungal agent. The antifungal agent is effective in inhibiting the growth of *E. floccosum, T. mentagrophytes and Microsporum canis.* The antimicrobial agent has a MIC as low as 0.03 µl/ml capable of inhibiting and/or killing these organisms. The antimicrobial agent includes selective mixtures of *Origanum vulgare L., Thymus vulgaris L., Cinnamomum zeylanicum Nees, Rosmarinus officinalis L., Lavandula officinalis L., Mentha piperita L., Citrus Limon L., Hydrastis Canadensis L. and Olea europaea L.*

No.. WO/2005/087244, WO/2011/110793 JP2001151689

**Title; Antimicrobial composition for treating microbial infections and preventing** microbial contamination of surfaces and devices

**Patentee**: Stringer, Jacqueline, Unversity of Manchester

**Abstract:** Compositions comprising at least two essential oils derived from a plant genus *Pelargonium; Cymbopogon; Mentha; Aniba; Lavandula; Origanum; Litsea;* ***Citrus;*** *Melissa; Pogostemon; Santalum; Valeriana; Styrax; Cinnamonum; and Rosa,.*

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No. 10255 1235A 11.7.2012

**Title: anti cold mask impregnated with wild *Ocimum bascillicum* essential oil**

Inventor; Dong Shixian

US7435877 date Oct 14, 2008

**Title: Distinct type cultivar of *Ocimum basilicum* "CIM-SAUMYA"**

**Patentee:** Khanuja et al

**Abstract**: The present invention relates to the development of an early, short duration, dwarf, high essential oil, methyl chavicol and linalool yielding variety of Indian basil (*O. basilicum*), Family—Lamiaceae) named as 'CIM-SAUMYA' through open pollination in the germplasm followed by half-sib progeny selection and evaluation for the yield characters of selected population for 3 years in field conditions. The new cultivar possesses better growth and vegetative growth.

USPTO 8333981 B2 18.Dec 2012

**Title : Antifungal treatment of nails**

**Patentee:** John Olin Trimble, Humco Holding Group, Inc

**Abstract:** A **fungus treatment composition** used to deliver active drugs trans-nail as well as a method for producing the fungus treatment composition, which may contain up to 50% additive ingredients. Preferred embodiments of the invention may include fungus treatment compositions which provide high nail penetrating power, essential oils, with optimum drying and barrier properties, pharmaceutically elegant properties, or having a total combination thereof.

EP 2451468 A2 date 8 July 2009 Also US20110008474

**Title: Topical antifungal composition**

**Patentee**: J. Charles Boegli Onikolabs

**Abstract:** A composition obtained primarily from plant materials provides antimicrobial activity for use as an anti-fungal agent. The anti-fungal agent is effective in inhibiting the growth of *Trichophyton rubrum,* the fungus that is the most common cause of *Tinea pedis*. The composition includes selective mixtures of the origanum oil, menthol, and Atlantic cedarwood oil, thuja oil, cedarwood oil, cinnamon oil, clove oil, cumin oil, fennel oil, peppermint oil, or rosemary.

USPTO N0 6887869 date 3 May 2005

**Title: Mikanolide derivatives, their preparation and therapeutic uses**

**Patentee**: Olivier Lavergne et al

**Abstract:** Novel *Mikanolide* derivatives, their preparation method and their therapeutic uses, in particular as anti-cancer and anti-viral agents

## 3.4 The Indian Patent System

### 3.4.1 The Indian Patent History

The history of Indian Patent System dates back to the year 1856. The Act of 1856 on the Protection of Inventions sought to provide certain exclusive privileges to the inventors of new and useful manufacturers for a duration of 14 years. The Act was subsequently replaced in 1859. Based on the British Patent Law of 1852, the Act of 1859 granted exclusive rights to useful inventions only. The Act of 1859 was amended in the year 1872 to include protection to Designs, further in 1883 when it introduced the provision of protection to the novelty of the invention and again in 1888 to make it conform to certain

amendments made in the UK law. The Indian Patents and Designs Act, 1911 repealed all previous acts. The Amendments to the Act of 1911 were also made in the years 1920, 1930 and 1945 by including various provisions. After independence, in view of economic and political scenario in the country and with the view to make the patent system conducive to the national interests, the 1911 Act was amended in 1950 in relation to compulsory license and revocation of patents. Another amendment In 1952 made provision for compulsory license in relation to patents in respect of food and medicines, insecticide, germicide, and fungicide and a process for producing substance or any invention relating to 'surgical or curative devices'.

The Patents Act of 1970 repealed and replaced the Act of 1911 as far as patents were concerned. The Act remained in force till December 1994. In 1999, the patent Act of 1970 was amended (with effect from 1.1.1995) to include filing of applications for 'product patents' in the areas of drugs, pharmaceuticals and agro chemicals. The second amendment to the 1970 Act through Patents (Amendments) Act 2002 introduced new Patent Rules with effect from 20$^{th}$ May 2003 and the third amendment to the Patents Act 1970 led to Patents (Amendment) Act 2005 which came into force from 1$^{st}$ January 2005.

### 3.4.2 The Indian Patent Office

The Office of the Controller General of Patents, Designs & Trade Marks (**CGPDTM**) is located at Mumbai. The Head Office of the Patent office is at Kolkata and its Branch offices are located at Chennai, New Delhi and Mumbai. The Trade Marks registry is at Mumbai and its Branches are located in Kolkata, Chennai, Ahmedabad and New Delhi. The Design Office is located at Kolkata in the Patent Office. The Offices of The Patent Information System (PIS) and National Institute of Intellectual Property Management (NIIPM) are at Nagpur. The Controller General supervises the working of the Patents Act, 1970, as amended, the Designs Act, 2000 and the Trade Marks Act, 1999 and also renders advice to the Government on matters relating to these subjects. In order to protect the Geographical Indications of goods a Geographical Indications Registry has been established in Chennai to administer the Geographical Indications of Goods (Registration and Protection) Act, 1999 under the CGPDTM.

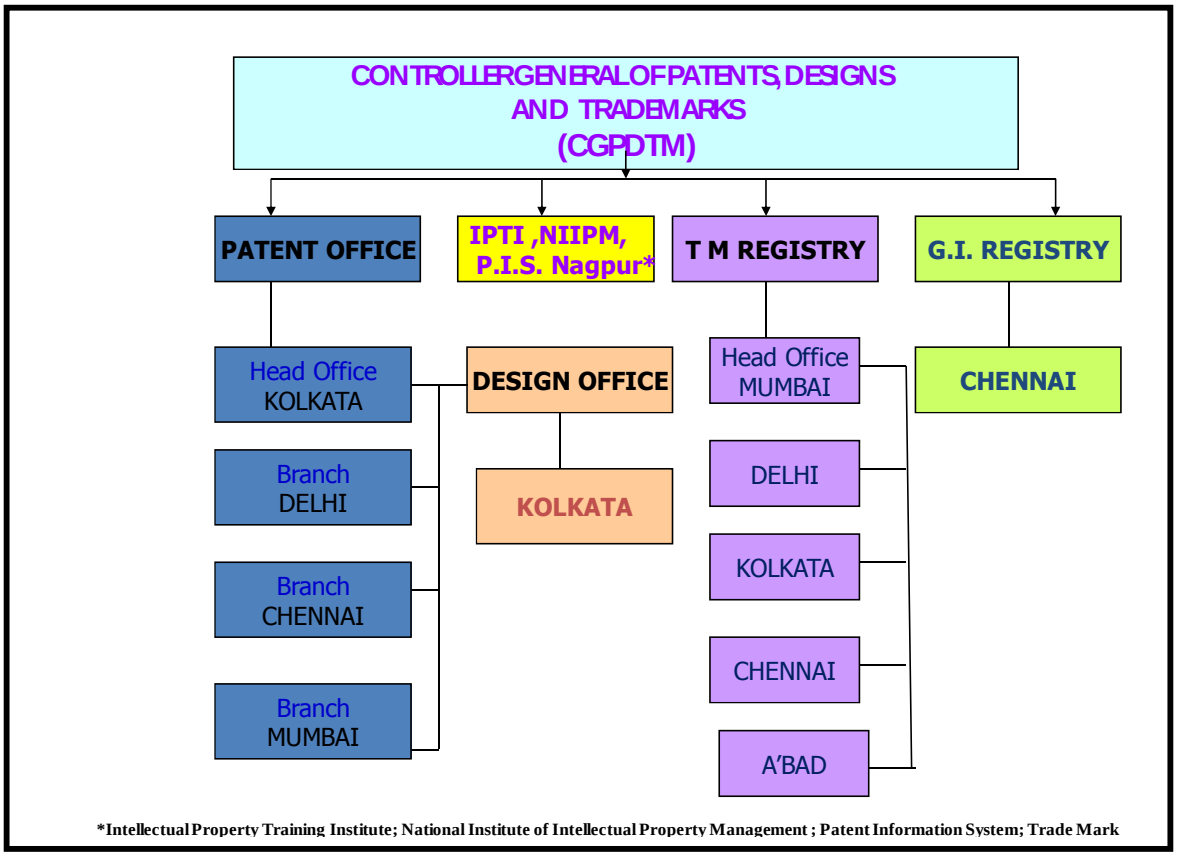



**Figure 3.5 Patent in India- A Schematic Representation**

### 3.4.3 Trends of patent filing and grant in India

The Indian patent office indicates tremendous increase in patent filing from 10592 in the year 2001-02 to 39400 in the year 2010-11. The patent grants in the Indian Patent office for the corresponding period show an increase from 1591 in the year 2001-02 to 7509 in the year 2010-11. (Table 3.10)

**Table 3.10: Trends of filing / grant of patents in the Indian Patent Office 2001-02 to 2010-11**

| | 2001-02 | 2002-03 | 2003-04 | 2004-05 | 2005 06 | 2006-07 | 2007-08 | 2008-09 | 2009-10 | 2010-11 |
|---|---|---|---|---|---|---|---|---|---|---|
| Filed | 10592 | 11466 | 12613 | 17466 | 24505 | 28940 | 35218 | 36812 | 34287 | 39400 |
| Granted | 1591 | 1379 | 2469 | 1911 | 4320 | 7539 | 15316 | 16061 | 6168 | 7509 |

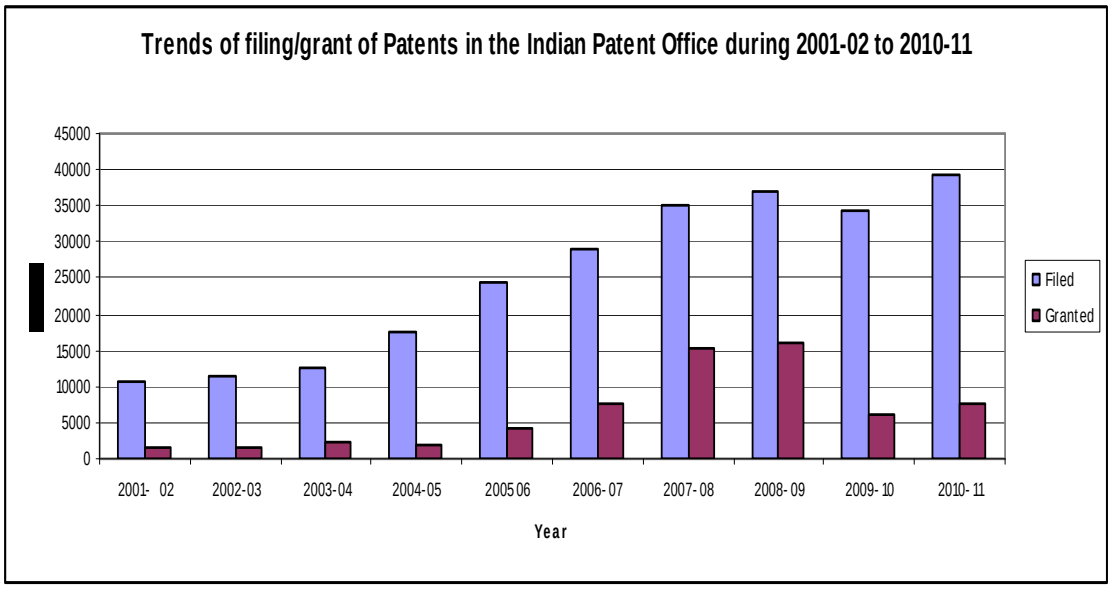



**Figure 3.6: Trends of filing / grant of patents in the Indian Patent Office 2001-02 to 2010-11 (Graphical)**

### 3.4.4 Trends in patent grant in India in the field of 'Drugs' And 'Pharma'

As far as field of drugs and pharma, relevant to the present study, is concerned the patent filing in India shows an increase in number of from a mere 879 in the year 2001-02 to 3526 in the year 2010-11. Against this, number of patent grants shows an increase from 320 in the year 2001-02 to 596 in the year 2010-11 (Table 3.11)

**Table 3.11: Trends of filing / grant of patents in the field of 'drugs' at the Indian Patent Office (2001-11)**

| Status | 2001-02 | 2002-03 | 2003-04 | 2004-05 | 2005 06 | 2006-07 | 2007-08 | 2008-09 | 2009-10 | 2010-11 |
|---|---|---|---|---|---|---|---|---|---|---|
| Filed | 879 | 966 | 2525 | 2316 | 2211 | 3239 | 4267 | 3672 | 3070 | 3526 |
| Granted | 320 | 312 | 419 | 192 | 457 | 798 | 905 | 1207 | 530 | 596 |

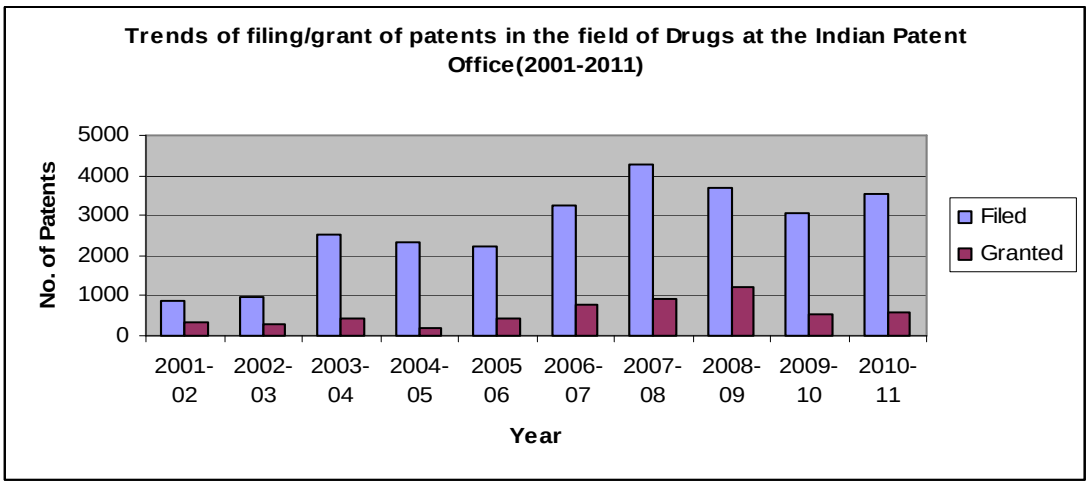



**Figure 3.7: Trends of filing / grant of patents in the field of 'drugs' at the Indian Patent Office (2001-11) (Graphical)**

### 3.4.5. Status of IPR (Patent activity) from North Eastern Region of India

The searches of patent grants from the applicants of North East India indicate sparse patenting activity. Out of the total 8 sister states, only the state of Assam and Meghalaya shows grant of a few patents on varied topics. However, no patent grants were found in the field of drugs/pharma/antidermatophytes (Table 3.12)

**Table 3.12 Status of patent activity in the states of North-East India**

| **KEY WORD** | No. of Patents | Field of patent | Remarks |
|---|---|---|---|
| **Arunachal Pradesh** | 0 | --- | No patent found on plant metabolite showing anti-dermatophytic activity |
| **Assam** | 125 | On varied aspects | No patent found on plant metabolite showing anti-dermatophytic activity |
| **Manipur** | 0 | --- | No patent found on plant metabolite showing anti-dermatophytic activity |
| **Meghalaya** | 2 | Cancer Zoonosis | No patent found on plant metabolite showing anti-dermatophytic activity |
| **Mizoram** | 0 | --- | No patent found on plant metabolite showing anti-dermatophytic activity |
| **Nagaland** | 0 | --- | No patent found on plant metabolite showing anti-dermatophytic activity |
| **Sikkim** | 0 | --- | No product /process patent found on plant metabolite showing anti-dermatophytic activity |
| **Tripura** | 0 | --- | No product /process patent found on plant metabolite showing anti-dermatophytic activity |

(Source:http://apps.webofknowledge.com/summary.do?product=DIIDW&searchmode=CombineSearches&qid=5&SID=U1PpkK16eapejiL4eEG&&page=2)

## 3.5 Significance of IPR and patents

'Patent applications and grants, as output indicators for R&D processes, are the most common and widely used indicators for the measurement of the technological performance of countries or innovation systems in general' (Freeman 1982; Grupp 1998).

'A count of patents shows a country's capacity to exploit knowledge and translate it into potential economic gains; in this context, patent statistics are widely used to assess the inventive and innovative performance of countries.' (EPO- patent context).

A U.S. Commerce Department comprehensive report finds that intellectual property (IP)-intensive industries support at least 40 million jobs and contribute more than $5 trillion dollars to, or 34.8 percent of, U.S. Gross Domestic Product (GDP). Some of the most IP-intensive industries include: computer and peripheral equipment, audio and video equipment manufacturing, newspaper and book publishers, pharmaceutical and medicines, semiconductor and other electronic components, and the medical equipment space. The entire U.S. economy relies on some form of IP, because virtually every industry either produces or uses it. IP-intensive industries directly accounted for 27.1 million American jobs, or 18.8% of all employment in the economy, in 2010. A substantial share of IP-intensive employment in the U.S. was in the 60 trademark-intensive industries, with 22.6 million jobs in 2010. The 26 patent-intensive industries accounted for 3.9 million jobs in 2010, while the 13 copyright-intensive industries provided 5.1 million jobs. (figure 3.8) ("Intellectual Property and the U.S. Economy: Industries in Focus," April 11,2012)

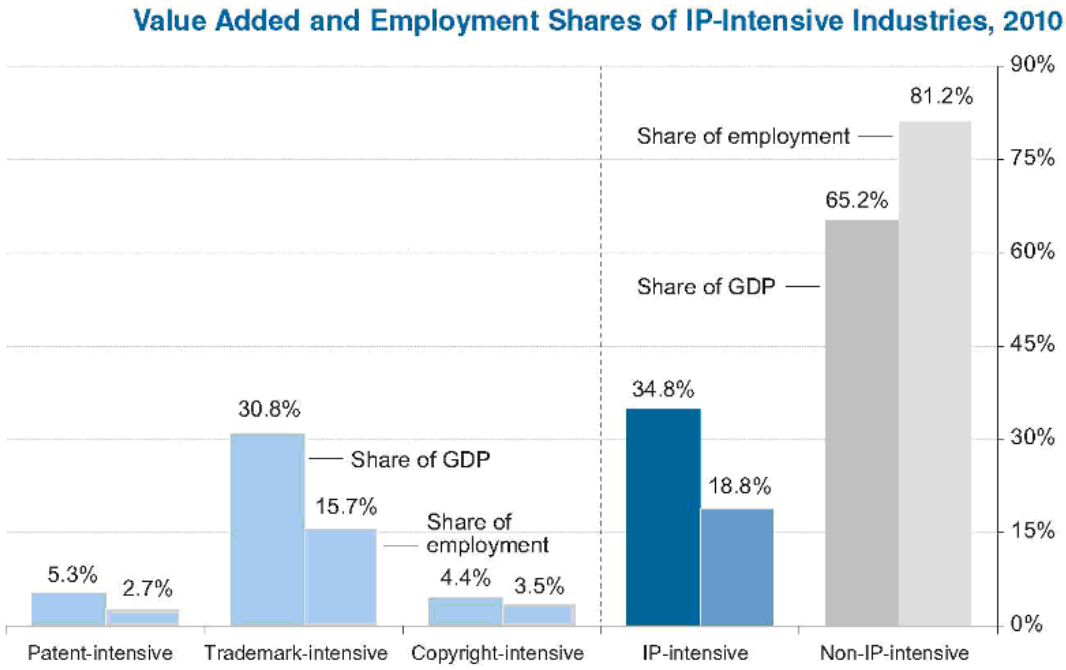



**Figure 3.8 : Value added employment shares of IP industries**

Patents are an important instrument to secure market shares in international technology markets. A substantial growth of patent applications in a certain technological field in the last decade indicates that relevant market returns were generated in that field. Additionally, it can be stated that the competition in this market is largely based on technological innovation increasing the probability that the relevance of this field will persist at least in the next decade (Frietsch *et al.*, 2011).

'Traditional medicine is an important part of human health care in many developing countries and also in developed countries, increasing their commercial value. Although the use of medicinal plants in therapy has been known for centuries in all parts of the world, the demand for herbal medicines has grown dramatically in recent years. The world market for such medicines has reached US $ 60 billion, with annual growth rates of between 5% and 15%. Researchers or companies may also claim intellectual property rights over biological resources and/or traditional knowledge, after slightly modifying them. The fast growth of patent applications related to herbal medicine shows this trend clearly'. (Kartal, M. 2007).

# 4. Materials and Methods

The present research work was conducted in the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl (with the supervisor) and in the Biological Product Laboratory, Department of Botany, University of Allahabad (with the joint supervisor), during June 2009 - December 2012.

The research work was carried out as follows:

- Collection, categorization, identification and documentation of common ethnomedicinal plants, used against skin ailments among the tribal communities;
- Preliminary *in vitro* investigations
    - Extraction of the plant secondary metabolites in the form of essential oil/ extract;
    - Procurment of the test pathogens/ culture collection (dermatophytes causing skin infection in human beings), revival and multiplication;
    - Antimicrobial screening of the extracted essential oil/ extract, against the dermatophytic pathogens;
- General description of the five most potent antidermatophytic plants;
- Detailed *in vitro* anti-dermatophytic investigations;
- Physico-chemical characterization of the selected plant metabolites;
- Identification of the scopes and issues related to Intellectual Property Rights.

## 4.4 Collection, categorization and identification of some common ethno-medicinal plants of North Eastern region, specially in mizoram

### 4.1.1 The Study area

Mizoram popularly known as the "Scotland of India" is bounded by Assam in the north, Manipur in the northeast, Myanmar in the east and south, Bangladesh in the west and Tripura in the northwest. Mizoram has a beautifully mountainous topography. Its steep

slopes form deep gorges through which Mizoram's many streams and 15 major rivers flow. The state enjoys a pleasant climate. Summers are cool and winters are not bitterly cold. The temperature in winter usually ranges from 11°C to 25°C and in summer it varies from 20°C to32°C. The state of Mizoram lies between 21° 58′ and 24°35′ north latitude and 92°15′ and 93° 29′ east longitude.

### 4.1.2 Ethno medicinal survey

The state of Mizoram is one of the seven sister states of the North Eastern region of India. Its unique ethnic culture and diverse vegetation make Mizoram an excellent study region. The present ethnomedicinal survey was started in August 2009 and carried out periodically in all the three seasons (winter, summer and monsoon) till early 2011.Data were collected in different parts of the state (Plate 1). Qualitatively, primary data was collected through interviews, discussions with the villagers after obtaining oral prior informed consent, and personal observations. The study is based on extensive field work, secondary information from locally available literature and personal interviews with local practitioners. (Plate 2). In Mizoram, phytotherapy (the treatment of illness using medicines derived from plants) forms an integral part of the local culture, and information about plants and their uses is passed on through oral folklore. Transmission of knowledge occurs primarily amongst the elderly; they are the natural retainers of traditional knowledge in their communities. Information on the use of medicinal plants was obtained through structured and semi structured questionnaires, complemented by free interviews and informal conversations.

During the field survey, local practitioners and others with knowledge of plants were consulted. Interviews were conducted in the field during collection trips and by examination of freshly collected specimens with informants, after seeking oral consent. Inquiries on the prevalence, types, mode of transmission and symptoms of skin ailments were made by interviewing local people.

Further, the selected plants were identified with the help of floras (Hooker, 1872-1892; Duthie, 1903-1929; Maheshwari, 1963; Santapau, 1967 and Gupta, 1968), and the authentic herbarium/ specimens lodged in the Duthie herbarium of the Department of

Botany, University of Allahabad as well as their confirmations were made with the Botanical Survey of India, Allahabad. The plants thus identified were deposited in the herbarium of the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl for future references, and the same were selected for the present investigation (Table 5.1).

## 4.5 Preliminary *in- vitro* investigations

### 4.5.1 Extraction of the plant secondary metabolites in the form of the essential oil/ extracts

The selected plants were subjected to extraction of their secondary metabolites (extract/essential oil). The *solvent extraction method*, using different solvents (viz., alcohol, acetone, benzene, chloroform, hexane etc), was used for extraction of the extract however, hydro-distillation method, using Clevenger's apparatus (Clevenger 1928), was used for extraction of the essential oil. (Plate 3 and 4)

Specific plant parts used for extraction of secondary metabolites were leaves, fruits, flowers, and in some cases, the whole plants.

#### 4.5.1.1 Washing/ Sterilization of the Plant Material

The various parts were washed separately with water and then rewashed with sterile distilled water. After washing they were dried for further careful extraction of the secondary metabolites.

#### 4.5.1.2 Solvent Extraction Method

In the present investigation, methanol and ethanol were used in combination with distilled water in the ratio of 50-50% for the extraction of the plant constituents and as per the following protocol. (Plate 3)

- 1.0 to 5.0 g sample of a collected plant material (leaves, stem, bark, roots and inflorescence) was weighed,
- Powder of the above sample was made with a ceramic mortar and a ceramic pestle or cut into small pieces by the scissors,

- Powder was placed into above mentioned organic solvents (generally treated with 50% alcohol),
- Kept over night for proper extraction,
- The supernatant was filtered using a Watman filter paper (No. 1), making sure that no plant particulates remain in the filtrate,
- Finally for extraction of crude extract the Rotatory evapoter equipment was used.

#### 4.5.1.3 Hydro-distillation Method

In hydro-distillation, Clevenger apparatus was commonly used for the extraction of secondary metabolites. Clevenger apparatus is a glass apparatus invented by Clevenger (1928) and commonly used for extraction of the volatile plant secondary metabolites/ essential oil (Figure 4.1). The technique behind hydro-distillation and the steps followed are summarized as follows: (Plate 4)

1. Collected the fresh plant material (leaves, stems, seeds and roots etc.) & washed with tap water.
2. Chopped & weighed the plant material
3. Loaded the plant material into the flask & filled the water upto 50% of the total volume of the flask.
4. Adjusted the temperature at 30-40 degress C to minimize the chances of degrading the compound.
5. The apparatus was run continuously, for 4-6 hrs for proper extraction of the volatile constituents.
6. Collected the sample (essential oil) in sterilized airtight voil. Any droplet was removed by using sodium anhydrous sulphate.

**Fig.4.1 Clevenger Apparatus**

### 4.5.2 Procurement of test organism from IMTECH Chandigarh

Four common dermatophytes viz., *Trichophyton rubrum*; *T. mentagrophytes*; *E. floccosum* and *M. gypseum* which cause the ring worm infections in human beings, were selected for the present investigation. The authentic cultures (MTCC- Microbial type culture collection) of these dermatophytes were procured from the Institute of the Microbial Technology (IMTECH), Chandigarh, India. The strains of the cultures were:

i) *Trichophyton rubrum* (Castellani) Sabouraud (MTCC-3272)
ii) *Trichophyton mentagrophytes* (Robin) Blanchard (MTCC-8476)
iii) *Epidermophyton floccosum* (Hartz) Langeron et Mitochevitch (MTCC-7880)
iv) *Microsporum gypseum* (Bodin) Guiart and Grigorakis (MTCC-2830)

#### 4.5.2.1 Protocol for Revival of Pathogens

The cultures thus procured were subjected for revival of the pathogens, using following protocols:

- Carefully opened the ampoule as the contents are in a vacuum.
- Marked the ampoule near the middle of the cotton wool with a sharp file.
- Disinfected the surface around the mark with alcohol.
- Wraped thick cotton around the ampoule and broke at the marked area.
- Gently removed the pointed top of the ampoule. Snap opening could draw the cotton plug to one end; hasty opening could release fine particles of dried organisms in to the air of the laboratory.
- Carefully removed the cotton plug and added about 0.3 to 0.4 ml of SDA medium to make a suspension of the culture. Avoided frothing or creating aerosols.
- Streaked a few drops of the suspension on to medium (solidified with agar) in a petriplate.

- Incubated at recommended temperature (25±2$^{o}$C) and conditions for the proper culture.
- Proper condition followed, showed the good results, growth of the culture could be visible within a 5-7 days.
- All the remains of the original ampoule treated as infected and autoclaved before discarding.

#### 4.2.2.2 Multiplication of fungal pathogens

The revived cultures were multiplied on the Sabouraud Dextrose Agar (SDA) for further investigations, and the routine subculturing was applied for purification and maintaining the pure culture through out the study.

### 4.2.3 Microscopic and Taxonomical studies of the test pathogens (dermatophytes)

#### 4.2.3.1 *Trichophyton rubrum* (Castellani) Sabouraud

The colony on Sabouraud dextrose agar at an early stage is white, fluffy, and hemispheric, later becoming velvety to powdery with a central umbo and some times showing radial folds; on the undersurface there is a characteristic reddish-purple pigment **(Plate-5)**.

**Microscopic morphology:** Microscopically, long, slender, thin walled multiseptale macroconidia (2-3 X 3-5 µ) with parallel sides and large number of pyriform microconidia borne from sides of hyphae are found.

#### 4.2.3.2 *Trichophyton mentagrophytes* (Robin) Blanchard

The anthrophilic form grows as flat, downy thallus with white edges and a cream tinted central area. Zoophilic isolates produce a flate, rapidly growing, granular colony that is cream, yellowish, buff to tan or reddish brown in coloar. Mycelium is usually sparse, and the powdery appearance is due to quantities of microconidia. The edges are often ray like. The colonies having aerial mycelium ranging from cottony to powdery, of white to cream to tan or pinkish colour with cream to tan to reddish-brown pigment on the reverse surface. **(Plate-5)**.

**Microscopic morphology:** Microscopically, there are found in the downy and granular types numerous clavate to pyriform microconidia borne singly along the side of hyphae and in cluster; typical, long, clavate, thin walled, multiseptate macroconidia (4-8 X 20-50 µ) with constriction at septa. Only few microconidia may be found.

#### 4.2.3.3 *Epidermophyton floccosum* (Hartz) Langeron et Mitochevitch

The colony on Sabouraud dextrose agar( SDA) medium is moderately fast growing at room temperature (26 ± 1°C); the surface is velvety or felt-like, often with radial furrows and an irregular, folded centre, or the surface may be smooth, the colour is pinkish white in beginning turning to dull-white with age. The reverse of colony is yellow-tan **(Plate-5)**.

**Microscopic morphology:** Microscopically, there will be seen the distinctive clavate macroconidia (7-12 X 12-40 µ) with blunt ends; they are smooth, thin walled, and multiseptate, and are frequently borne in banana-like clusters; numerous intercalary and terminal chlamydospores are also characteristic; no microconidia are produced.

#### 4.2.3.4 *Microsporum gypseum* (Bodin) Guiart and Grigorakis

Colonies are fast growing, producing a flat, spreading, powdery surface that is rich cinnamon buff to brown, occasionally with overtones of violet. The powder consists of masses of macroconidia. The edges of the colony are entire to scalloped or ragged. Diffuse pleomorphism rapidly develops **(Plate-5)**

**Microscopic morphology:** Macroconidia are produced in great abundance. They are thin walled, 8 to 16 x 20 to 60µ, roughened, and have 4 to 6 septa. Other usual variety of conidia including microconadia is seen. *M. gypseum* is geophilic and abundant in soil throughout the world. It is ectothrix but produces few arthroconidia. It is usually associated with an inflammatory disease and may cause a kerion formation. Fluorescence is absent or dull in infected hair.

### 4.2.4 Classification/Taxonomical position

Three genera, *Trichophyton, Microsporum* and *Epidermophyton* are collectively known as dermatophytes. Dermatophytes belonging to the

**Systematic Position**

| | | | |
|---|---|---|---|
| **1.** | **Kingdom** | : | Mycota |
| | **Division** | : | Eumycota |
| | **Sub division** | : | Duteromycotina |
| | **Class** | : | Hypomycetes |
| | **Order** | : | Hypomycetales (Moniliales) |
| | **Family** | : | Moniliaceae |
| | **Genus** | : | *Epidermophyton* |
| | **Species** | : | *floccosum* |
| **2.** | **Kingdom** | : | Mycota |
| | **Division** | : | Eumycota |
| | **Sub division** | : | Duteromycotina |
| | **Class** | : | Hypomycetes |
| | **Order** | : | Hypomycetales (Moniliales) |
| | **Family** | : | Moniliaceae |
| | **Genus** | : | *Microsporum* |
| | **Species** | : | *gypseum* |
| **3.** | **Kingdom** | : | Mycota |
| | **Division** | : | Eumycota |
| | **Sub division** | : | Duteromycotina |
| | **Class** | : | Hypomycetes |
| | **Order** | : | Hypomycetales (Moniliales) |
| | **Family** | : | Moniliaceae |
| | **Genus** | : | *Trichophyton* |
| | **Species** | : | *rubrum* |
| **4.** | **Kingdom** | : | Mycota |
| | **Division** | : | Eumycota |
| | **Sub division** | : | Duteromycotina |
| | **Class** | : | Hypomycetes |
| | **Order** | : | Hypomycetales (Moniliales) |
| | **Family** | : | Moniliaceae |
| | **Genus** | : | *Trichophyton* |
| | **Species** | : | *mentagrophytes* |

### 4.2.5 Antimicrobial screening against the dermatophytic fungi

The antimicrobial screening of the above listed fifteen plants (table 5.1) against the dermatophytic pathogens viz., *Trichophyton rubrum* (Castellani) Sabouraud; *T. mentagrophytes* (Robin) Blanchard; *Epidermophyton floccosum (Hartz)* Langeron et Mitochevitch and *Microsporum gypseum* (Bodin) Guiart and Grigorakis; was done separately, using disc diffusion method. **(Plate 9)**

The protocol followed was as follows:

- Cleaned the surface of the inoculation chamber with 95% alcohol then sterilized with UV light for given time before proceeding to the experiment.
- Wore the gloves in both the hands.
- Labeled at the bottom of SDA plate- label the name of the test pathogen (dermatophytes), date and maked the codes about 20 mm away from the plate edge.
- With the fresh culture plate, inoculated the dermatophytes inoculums, into the SD broth in order to obtain a concentration of $1.5 \times 10^6$ CFU/ml (0.5 McFarland used for the turbidity match).
- Held the tube of sterilized swabs, with the non-dominant hand curled the little finger around the cotton plug and removed it with dominant hand.
- From swabs tube took one swab, taking care not to touch the end of other. Replaced the plug and put it into tube stand without putting the plug down.
- Similarly, picked up the SD broth tube removed the plug and quickly passed the opening of the tube through the flame three times, to avoid any contamination.
- Cotton swab inserted into broth culture and removed excess medium.
- Re-placed the plug and put the tube into stand.
- Using these inocula in swab, inoculated the entire surface of SD agar plate in back-and-forth motion.
- Again dipped the swab in the broth culture and inoculate on agar surface in second direction.
- Discarded the contaminated swab in the biohazard container.
- Kept the plate lid on dry agar surface for 5 minutes.
- Picked the sterile paper discs from its container with sterilized forceps.

- Dipped the edge of the paper discs into the solution of plant secondary metabolites (containing different concentrations viz., 20mg/ml, 30mg/ml and 50mg/ml, prepared in DMSO) and saturated the discs through capillary action.
- Placed the saturated discs over its letter code by applying some light pressure, to ensure it prevented the discs from falling off when the plate is inverted during incubation.
- Repeated same process for remaining plates with different concentrations of plant secondary metabolites, flaming the forceps for each new solution.
- After completion of the experiment placed all the inoculated plate upside-down in the incubator and adjusted the temperature at 25± $2^0$C for 36-72 hours.
- Measured the diameter (in mm) of any zone of growth inhibition around a disc after incubation and recorded the observations.
- Finally, discarded the plate into biohazard container for proper sterilization.

## 4.6 Description of the five most potent antidermatophytic plants

Out of the 15 screened antimicrobial plants, five plants: ***Eupatorium odoratum*** L., ***Mentha piperita*** L. ***Ocimum basilicum*** L**.,** ***C. aurantifolia*** L. and ***Mikania micrantha*** Kunth ex H.B.K. were selected for further detailed *in vitro* investigation, using Broth micro dilution method on spectrophotometer, as they were found to be the most effective against test pathogens. **(Plate 6)**

The description of these selected plants follows:

### 4.3.1 *Eupatorium odoratum* L.

**Classification/Taxonomical position**

| | | |
|---|---|---|
| **Kingdom** | : | Plantae |
| **Class** | : | Dicotyledons |
| **Order** | : | Asterales |
| **Family** | : | Asteraceae |
| **Genus** | : | ***Eupatorium*** |
| ***Species*** | : | ***odoratum*** |

**Common name:** Asamlata (Beng.);
Bitter bush (Eng.).

**Fig. 4.2 *Eupatorium odoratum* L.**

**Habitat and Distribution:**

*E. odoratum* grows in human disturbed areas as well as Pine Woodlands, and the edges of Dry Broadleaf Evergreen Formation – Woodland/Shrubland (low coppice/scrubland). *E. odoratum* is now found all over tropical Asia. It is sometimes grown as a medicinal and ornamental plant. It is native to North America, from Florida and Texas to Mexico and the Caribbean and has been introduced to tropical Asia, West Africa, and parts of Australia. *E. odoratum* occurs on all island groups in the Bahamian Archipelago as well as throughout tropical and subtropical regions of the world.

**Habit and Life cycle:**

*E. odoratum* is a woody herbaceous perennial growing as a climbing shrub to 3 meters in height, typically shorter. The leaves are arranged oppositely, to 15 cm in length, triangular to ovate with an acuminate leaf apex and dentate leaf margin with large teeth. The vegetative structures are covered with articulate hairs throughout. The actinomorphic flowers are arranged in corymbs of heads subtended an involucre made of 4 series of phyllaries. The calyx is modified as hairs forming a pappus. The corolla has 5 fused white to lavender petals. There are 5 stamens fused to the base of the corolla. The ovary is

inferior with a single locule. The fruit is an achene at maturity that retains the modified calyx (pappus).

**Medicinal/Cultural/Economic usage:**

*E. odoratum* is commonly used medicinally in the North East India. *E. odoratum* tea helps to stop smoking and relieves coughing. It is extensively used traditionally by the tribal people as anti-inflammatory, analgesic, antiprotozoal agent's e.t.c. Extraction of the leaves of the plant *E. odoratum* using water and ethanol and evaluation of their antioxidant activity. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds.

*Chromolaena odorata* (L.) King & Robinson (Asteraceae: Eupatorieae), commonly called chromolaena, is a sprawling shrub that has become one of the worst invasive terrestrial weeds in the humid tropics and subtropics of the Old World (Figure 1). Until the early 1970s, chromolaena was known as *E. odoratum* (L.). *Chromolaena* is known by a large number of mostly local vernacular names, in both native and invasive ranges. In English, it is mostly commonly known as Siam weed, while in French, it is often called l'herbe du Laos.

### 4.3.2 *Mentha piperita* L.

(White Peppermint)

**Classification/Taxonomical position**

| | | |
|---|---|---|
| **Kingdom** | : | Plantae |
| **Class** | : | Dicotyledons |
| **Sub class** | : | Gamopetalae |
| **Order** | : | Lamiales |
| **Family** | : | Lamiaceae |
| **Genus** | : | *Mentha* |
| **Species** | : | *piperita* |

**Fig. 4.3 *Mentha piperita* L.**

**Historical background:**

*Mentha piperita* L. is a native of Europe. It is highly aromatic. The ancient Egyptians, Greeks and Romans knew it as flavouring for food and as medicine. It was first cultivated in England commercially around

1750 while its aerial parts have been widely used for their medicinal effects. Peppermint is widely known to relieve digestive ailments, being a popular remedy for at least two centuries.

In the Indian Materia Medica, leaves of *M. piperita* L., in infusion, are used in cases of vomiting, gastric colic, cholera, diarrhoea, flatulence, weak digestion, hiccup and palpitation of the heart.

**Physical characteristics:**

*M. piperita* L. (peppermint) is a medicinally important plant that belongs to the family Labiate. It is hardy to zone 3 and was not frost tender. Peppermint is a non-native herbaceous plant, it is a perennial, which can reach 100 cm in height (40 inches) has four-sided stem. The flowering time is from August to October. The flowers are hermaphrodite (have both male and female organs) and pollinated by Insects. The leaves are stalked opposite and toothed. The flower are irregular in shape, they are pinkish or purplish.

**Cultivation**

The plant fully succeeds in moist soils and situations so long as the soil is not too dry. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and grows in heavy clay soil. It can be grow in semi-shade (light woodland) or no shade and generally requires moist soil. The plant prefers acid, neutral and basic (alkaline) soils. A sunny position is best for the production of essential oils, but the plant also succeeds in partial shade. It is often grown in the herb garden and also commercially for its essential oil. The whole plant is a pleasant aroma of peppermint. Most mints have fairly aggressive spreading roots and, unless there is space to let them roam, they need to be restrained by some means such as planting them in containers that are buried in the soil. They can hybridize freely with other members of this genus. The flowers are very attractive to bees and butterflies. It is a good companion for growing near cabbages and tomatoes, helping to keep them free of insect pests. It produces a better quality essential oil if the plant is grown in dry ground.

**Edible uses**

An essential oil from the leaves and flowers is used as flavouring in sweets, chewing gum, ice cream etc.

**Medicinal uses**

White peppermint is a very important and commonly used remedy, being employed by allopathic doctors as well as herbalists. It is also widely used as a domestic remedy. This cultivar is considered to be milder acting than black peppermint (*Mentha piperita vulgaris*). A tea made from the leaves is used traditionally in the treatment of fevers, headaches, digestive disorders (especially flatulence) and various minor ailments. The herb is abortifacient, anodyne, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, refrigerant, antistomachic, tonic and vasodilator. An infusion is used in the treatment of irritable bowel syndrome, digestive problems, spastic colon *etc.* Externally a lotion was applied to the skin to relieve pain and reduce sensitivity. The leaves and stems can be used fresh or dried; they are harvested for drying in August as the flowers start to open. The essential oil in the leaves is antiseptic and strongly antimicrobial, though it is toxic in large doses. When diluted it is used as an inhalant and chest rub for respiratory infections. The essential oil is used in aromatherapy.

**Other uses**

The essential oil obtained from the whole plant used in perfumery. It is also a constituent of oral hygiene preparations, toiletries etc. Peppermint leaves are used as a component of pot-pourri. They were formerly used as a strewing herb. The plant repels insects, rats etc. Rats and mice intensely dislike the smell of mint. The plant is therefore used in homes as a strewing herb and had also been spread in granaries to keep the rodents off the grain (Phillips and Gentry, 1993).

### 4.3.3 *Ocimum basilicum* L.

**Classification/Taxonomical position**

| | | |
|---|---|---|
| **Kingdom** | : | Plantae |
| **Class** | : | Dicotyledons |
| Series | : | Gamopetalae |
| Order | : | Lamiales |
| Family | : | Lamiaceae |
| Genus | : | ***Ocimum*** |
| *Species* | : | ***basilicum*** |

**Fig. 4.4 *Ocimum basilicum* L.**

**Common name:** Kali Tulsi (Hin.) Saint Joseph's Wort in some English-speaking countries.

**Habitat:**

Basil, originally from India, is a half-hardy annual plant, best known as a culinary herb prominently featured in Italian cuisine, and also plays a major role in the Northeast Asian cuisine of Taiwan and the Southeast Asian cuisines of Indonesia, Thailand, Vietnam, Cambodia, and Laos. Depending on the species and cultivar, the leaves may taste somewhat like anise, with a strong, pungent, often sweet smell. Basil is originally native to India and other tropical regions of Asia, having been cultivated there for more than 5,000 years.

There are many varieties of *O. basilicum,* as well as several related species or species hybrids also called basil. The type used in Italian food is typically called sweet basil, as opposed to Thai basil (O. basilicum var. thyrsiflora), lemon basil (O. *citriodorum*) and holy basil (*Ocimum tenuiflorum*), which are used in Asia. While most common varieties of basil are treated as annuals, some are perennial in warm, tropical climates, including holy basil and a cultivar known as 'African Blue'.

**Description:**

Sweet basil (*O. basilicum*) will grow to a size of 1-2 feet in height. Basil will prolifically produce large green leaves, measuring around 2 inches in length, throughout the summer. Basil flowers are white, and are commonly removed to increase yield of leaves.

Cultivars of sweet basil include Lemon Basil, Italian or Curly Basil, and Lettuce-leaf Basil; the names of these cultivars give way to their variances.

**Medicinal Uses:**

*O. basilicum*, popularly known as "Sweet Basil" is used in both Ayurvedic and Unani systems of Medicine. It is a small perennial, tropically growing shrub of Asian origin (Dhar, 1968).

Despite continuing advances in understanding the basic pharmacology of cardiac glycosides, digitalis intoxication remains a common clinical problem. It necessitates research for new nature based drugs which increase cardiac muscle contractility with a broad therapeutic index. As a part of the screening for a suitable natural drug we have chosen *O. basilicum* Linn. and evaluated its cardio active potential and its mechanism of action.

### 4.3.4 *Citrus aurantifolia* L.

**Classification/Taxonomical position**

| | | |
|---|---|---|
| **Kingdom** | : | Plantae |
| **Class** | : | Dicotyledons |
| **Sub-class** | : | Polypetalae |
| **Order** | : | Geranials |
| **Family** | : | Rutacese |
| **Genus** | : | ***Citrus*** |
| ***Species*** | : | ***aurantifolia*** |

Common name: Nimbu (Hin), Lemon (Eng.)

**Fig. 4.5 *Citrus aurantifolia* L**

**Habitat and Occurance:**

Key lime is believed to be native of eastern Malaysia. It was introduced to the Asian mainland early in historical times and carried by Arab traders to the Middle East and eventually came to Europe during the Crusades. The species was introduced to the West Indies by Columbus during his second voyage. Key lime has been planted throughout the tropics and has naturalized in at least Puerto Rico, the Virgin Islands.

*C. aurantifolia* is native to Southeast Asia. Its apparent path of introduction was through the Middle East to North Africa, then to Sicily and Andalusia and via Spanish explorers to the West Indies, including the Florida Keys. From the Caribbean, lime cultivation spread to tropical and subtropical North America, including Mexico, Florida, and later California. Since the North American Free Trade Agreement came into effect, many Key limes on the US market are grown in Mexico and Central America. They are also grown in Texas and California. This particular cultivar might be a hybrid.

**Description:**

Key lime is the name used most often to refer to a primitive race of *Citrus aurantiifolia* cultivated and naturalized in the West Indies. It is also referred to as Mexican lime, West Indian lime, lima, limón criollo, limón agria, limón boba, and citron. Key lime is an evergreen, spiny shrub or small tree to 6 m in height. The plant has single or multiple stems and irregular branches covered with smoothish brown to gray bark. The twigs are quadrangular (when young), green, and bare sharp axillary spines 3 to 17 mm long. The leaves are yellow-green to dark green, with 5- to 28-mm winged petioles and elliptic to oval leathery 4- to 13-cm long blades with edges that have minute rounded teeth. The crushed foliage has a strong, distinct, spicy (citrus) odor and taste. The four- to five-petaled white flowers occur in few-flowered axillary clusters. The fruits (hesperidiums) are ellipsoidal, 3 to 5 cm in diameter, have juicy, greenish-yellow flesh, and are yellow at maturity. They contain a few white, pointed seeds about 1 cm long (Liogier 1988).

*C. aurantifolia* Swingle, including herbaceous plant that has many branches and twigs. Woody trunk and tough. Dull colored outer skin. Lime - *C. aurantifolia*, Swingle at age 2 1/2 years has begun to bear fruit. The flowers are small white colored fruit is round and colored (outer skin) or yellowish green. Orange juice sour old. Citrus plants generally

**Medicinal Uses:**

It is used for Diseases which can be treated like- tonsillitis, malaria, hemorrhoid, shortness of breath, influenza, cough, fever, constipation, Late menstruation, abdominal pain during menstruation, dysentery, abdominal pain, nauseous, fatigue, body odor, facial wrinkles.

### 4.3.5 *Mikania micrantha* Kunth ex H.B.K.

**Classification/Taxonomical position**

| | | |
|---|---|---|
| **Kingdom** | : | Plantae |
| **Class** | : | Dicotyledons |
| **Sub-class** | : | Gamopetalae |
| **Order** | : | Asterales |
| **Family** | : | Asteraceae |
| **Genus** | : | ***Mikania*** |
| ***Species*** | **:** | ***micrantha*** |

**Fig. 4.6 *Mikania micrantha* Kunth ex H.B.K**

Common name: · titaiya baur (Hindi), mile-a-minute, Chinese creeper, climbing hempvine, bittervine (English).

**Habit:**

Mikania vine is a perennial creeper that will climb trees, shrubs and any other structure in the area. It is a herbaceous, sprawling and twining vine that smothers other vegetation and infrastructure. Mikania vine has a rapid growth rate and has been observed to grow 9 cm per day under ideal conditions. The lateral shoots will twine around its own main stem until other support is found. In the absence of support, mikania vine is prostrate and will form roots along any stems touching the ground.

**Habitat and distribution:**

Mikania vine is native to Central and South America, and has become a serious weed in West Africa through to India, South-East Asia, Indonesia and the Pacific Islands. Mikania vine thrives in humid areas where rainfall exceeds 1500 mm per annum. It prefers areas with rich damp soils. The potential distribution in Australia includes the coastal regions of the Northern Territory and northern Western Australia, and much of eastern Queensland, extending into north-eastern New South Wales.

*M. micrantha*is a rapidly growing, scrambling perennial vine with many branches. It has the potential to spread throughout humid regions of northern Australia, and poses a

major threat to agricultural production and the environment in these areas. The weed has the ability to spread rapidly and smother native vegetation, crops and agricultural infrastructure.

**Leaves and stems**

The leaves are smooth and heart-shaped. They are 4–13 cm in length and taper to an acute point. The leaves have three main veins that arise from the base of the leaf. The leaf stalk is 2–8 cm long and the leaves are arranged in opposite pairs along the stem. The stems of the mikania vine are slender, ribbed and bear fine, white hairs. The lateral stems are as vigorous as the main stem and it is often difficult to distinguish between the two.

**Flowers and seeds**

The flowers of mikania vine are white to greenish-white and are produced in clusters mainly at the ends of stems growing in full sunlight. Each flower head is 4–6 mm long and contains four individual flowers. Mikania vine produces tufted seeds which are equipped for wind dispersal. The seeds are black, 1.5–2 mm long, thin and five-angled. The seed tufts (pappus) consist of over 30 fine white hairs or bristles. The pappus is longer than the seed itself. Mikania vine generally flowers during winter; however, plants will also flower heavily out of season in areas exposed to full sunlight.

The leaves are opposite to one another and heart-shaped, 4-13cm in length and 2-9cm in width. The petiole of each leaf is 2-8cm long. Both surfaces of the leaf are glabrous (hairless). The flowers are found in flowerheads (4 flowers/ head) and are white.

**Medicinal Properties**

Albert C. Smith in his Flora Vitiensis Nova (1991) notes that "the macerated plant is used to apply to new wounds, insect stings and other skin irritations, and the leaves after being boiled in saltwater and cooled are applied to the skin to relieve itching" Recent biochemical research has shown that it has antimicrobial (germ killing) properties.

**Allelopathic Properties**

Mikania has also been shown to have allelopathic properties i.e. release chemicals that can inhibit the growth of plants. Recent studies have isolated these allelopathic chemicals and demonstrated their growth-inhibition properties on young seedlings. Mikania debris incorporated into soil was also shown to inhibit germination and seedling growth in some crops.

## 4.4 Detailed *in-vitro* antidermatophytic investigations

Fungicidal properties of the plant secondary metabolites were determined, using Broth microdilution method.

### 4.4.1 Broth Microdilution Method

Broth microdilution method is currently the most widely using method, standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 2002); now known as Clinical Laboratory Standards Institute (CLSI). It was found very sensitive, modern, rapid, automated, reproducible economical and quantitative *in-vitro* fungicidal testing method. As per the protocols, broth microdilution method involved the following steps (Plate-10).

- The experiment was conducted in a flat bottom 96 well Microtitre culture plates.
- Eacch wells of the cuture plate were filled with 100µl of RPMI-1640 sterilized media.
- Further, in $2^{nd}$ column, additionally added 100µl media; in $3^{rd}$ column, 90µl more media and in the $4^{th}$ column, 80µl of culture media was added.
- Furthermore, 10µl of the test sample was added in the wells of $3^{rd}$ column (Drug Control), and 20µl of the test sample was added in $4^{th}$ column, respectively.
- Mixed the test sample thoroughly, using multichannel micropipette.
- Now, transferred 100µl test samples from $4^{th}$ column to $5^{th}$ column; and repeated the same process from $5^{th}$ to $6^{th}$; $6^{th}$ to $7^{th}$ and so on till the $11^{th}$ column was reached.
- 100µl inoculum (CFU 1 x $10^3$ CFU/ml) was added to $1^{st}$ to $12^{th}$ column except $2^{nd}$ and $3^{rd}$ column.
- Plates were then incubated in a moist chamber at $25\pm2^0$C for 48-72 hrs.
- Absorbance was recorded at 530 nm using a Microtitre plate reader.

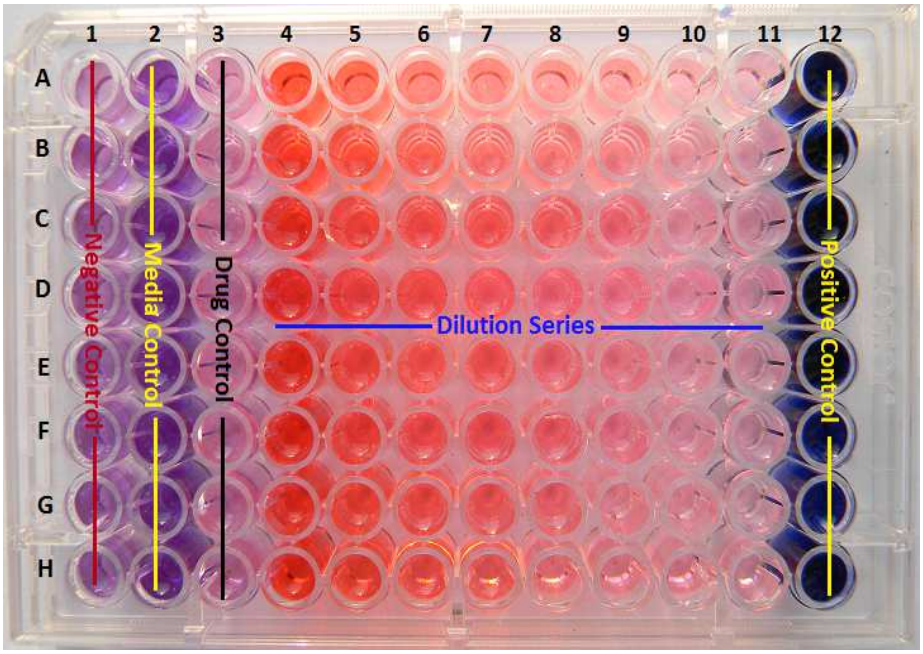



**Figure 4.7 : Format for testing the sample(s) in 96 well plate**

### 4.4.2 Testing Format

**i. Negative control**

The test organism was added to the media at a CFU of 1 x $10^6$ CFU/ml, and 100µl of 40% formaldehyde was added to kill the cells. 100µl of this culture suspension was added to each well. This served as a negative control.

**ii. Media control**

200µl of media was added to each well in media/ broth control lane per well. No organism and no 'test sample(s)' were added. This was done to check the contamination in the media.

**iii. Sample control**

For sample control highest testing concentration of the 'test sample' was used along with media. Since, some 'test sample' absorb at the test wavelength and some 'test sample' are turbid, hence, in such cases it is difficult to say that the given 'test sample' under consideration is active or not. Therefore, the O.D. values of the sample control were subtracted from the O.D. values obtained by culturing the test organism at different

concentrations of the 'test sample' so that, the exact per cent inhibition by the 'test sample' could be observed.

**iv. Growth control**

100µl of culture suspension at a CFU of $1 \times 10^6$ CFU/ml and 100µl of media was added to each well. This serves as a positive control.

**4.4.3 Plant Secondary Metabolite(s) used as sample**

The following plant secondary metabolites in form of essential oils and 50 % ethanolic extract were used for *in vitro* study:

**(i). Essential oils**

1. *Eupatorium odoratum* Linn.
2. *Ocimum basilicum* L.
3. *Mentha piperita* L.
4. *C. aurantifolia* L.

**(ii). 50 % ethanolic extract**

5. *M. micrantha*

**4.4.4 Media used for testing of the sample**

RPMI 1640, buffered to a pH 7.0 with MOPS (morpholine propane sulfonic acid) was used as the medium for testing the 'sample' (NCCLS, 2002).

**i. Preparation of Medium for Testing the 'Sample'**

- 7.2gm of RPMI 1640 and 34.72 gm of MOPS were weighed and dissolved in1000ml SDW (Single Distilled Water).
- The pH of the medium was maintained at 7.0 using HCl or NaOH.
- After adjusting the pH media was filtered by 0.2 µm millipore filter paper for sterilization. Since, RPMI 1640 is a heat sensitive medium hence, it can't be sterilized through autoclaving.

**ii. Preparation of Normal Saline with tween 20**

NaCl - 8.5gm
Tween 20 - 0.25ml
Distilled water - 1000ml

The contents were mixed well and then autoclaved at $121^0$C, 15 lbs for 15 min.

### 4.4.5 Preparation of Stock Solution of the 'Test Sample'

Since, the entire 'test sample' used in the present study were insoluble in water, but soluble in organic solvents, hence, DMSO (dimethyl sulphoxide - an organic solvent) was used for preparing their stock solution.

**(i) Preparation of stock solution for natural 'test sample'**

10mg or higher concentration of essential oil/ active constituent was weighed and dissolved in 1 ml DMSO. The stock solution was aliquoted and stored at $-20^0$C.

**(ii) Preparation of stock solution for synthetic 'test sample'**

1mg of synthetic 'test sample' was weighed and dissolved in 1 ml DMSO. The stock solution was aliquoted and stored at $-20^0$C.

**Table 4.2: Protocol for different concentration of the 'test sample' in 96 well plate**

| Stock solution of the 'test sample' | CLSI Broth microdilution method Concentration of the 'test sample' from 4th well to 11th well (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4th | 5th | 6th | 7th | 8th | 9th | 10th | 11th |
| 1mg/ml | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.563 | 0.781 | 0.391 |
| 10mg/ml | 500 | 250 | 125 | 62.5 | 31.25 | 15.625 | 7.8125 | 3.90625 |
| 20mg/ml | 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 15.625 | 7.8125 |
| 30mg/ml | 1500 | 750 | 375 | 187.5 | 93.75 | 46.875 | 23.4375 | 11.718 |
| 40mg/ml | 2000 | 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 15.625 |
| 50mg/ml | 2500 | 1250 | 625 | 312.5 | 156.25 | 78.125 | 39.062 | 19.5312 |

### 4.4.6 Mc Farland Standard

As per the NCCLS norms; an inoculum ranges from $1 \times 10^3$ CFU/ml is the standard inoculum for fungal pest. To prepare an inoculum with this range, the best method is- matching of turbidity of the inoculum suspension at 530 nm with 0.5 McFarland standards. Because, the the absorbance of 0.5 McFarland is equal to the absorbance of inoculum suspension containing 1x $10^3$ CFU/ml.

#### 4.4.6.1 Preparation of McFarland Standard Stocks:

1. 1% $H_2SO_4$: 2.04ml dissolved in 197.96 ml TDW (Triple Distilled Water)
2. 1% $BaCl_2$: 0.1 gm dissolved in 10ml TDW.

Table-4.3 **Protocol for preparation of Standard McFarland solution**

| McFarland | 1% $H_2SO_4$ | 1% $BaCl_2$ |
|---|---|---|
| 1 | 9.9 | 0.1 |
| 2 | 9.8 | 0.2 |
| 3 | 9.7 | 0.3 |
| 4 | 9.6 | 0.4 |
| 5 | 9.5 | 0.5 |
| 6 | 9.4 | 0.6 |
| 7 | 9.3 | 0.7 |
| 8 | 9.2 | 0.8 |
| 9 | 9.1 | 0.9 |
| 10 | 9.0 | 1.0 |

Optical density was recorded at 530 nm. McFarland was diluted 10 times and corresponding O.D was recorded.

#### 4.4.6.2 CFU Counting

**Protocols for CFU counting:**

- PDA plates were prepared.
- Normal saline was poured into a culture tube in which selected dermatophytes were grown and transferred into a centrifuge tube.
- The content of the tube was vortexed thoroughly.
- Serial 10 fold dilutions of the cell suspension were prepared.
- From each dilution 200µl of inoculum was taken and spread on SDA plates. Two plates were used for each dilution.
- Incubated the plates at $30\pm2^0$C for 48 hrs in an inverted position.

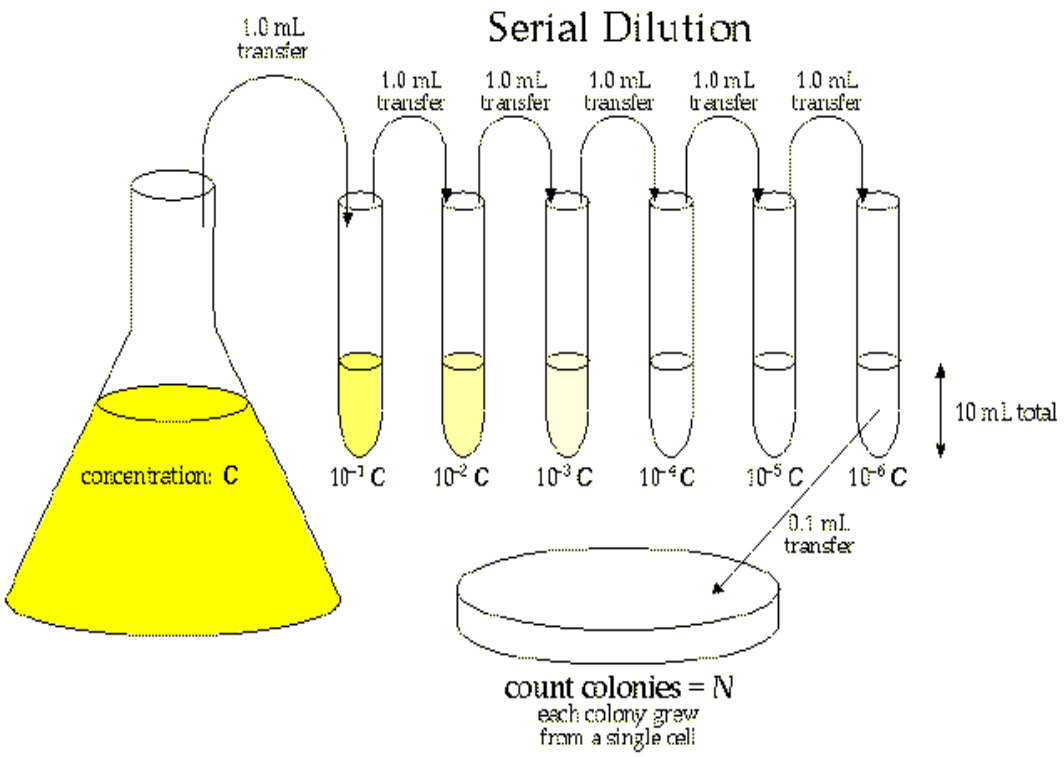



**Figure 4.8 Schematic representations, serial dilution method for fungal inoculum preparation**

### 4.4.7 Minimum Inhibitory Concentration $IC_{50}$, using Broth Microdilution Method

The antifungal activity of the active constituent(s) was determined using broth microdilution method as described by Clinical Laboratory Standards Institute (CLSI) (NCCLS 2002) in RPMI 1640. **(Plate 10).** The 96-well tissue culture plates were used for two fold serial dilution. The proper growth control, sample control and the blank was adjusted into the plate. Constituents were dissolved in 5-10% DMSO at the concentration of 1mg/ml or higher in case of synthetic antifungals, and 10mg/ml or higher in case of natural antifungals. 20 µl of 'test sample' was added to 96-well tissue culture plate having 180µl RPMI-1640, so that, the maximum concentration of the 'test sample' could reach 50mg/ml to 500mg/ml, and higher respectively. Further, the solution was serially diluted resulting into the half of the concentration of 'test sample' and then fungal inoculum was added. Incubation was made for 48-72hrs in a moist, dark chamber at 25±2$^{o}$C. After the incubation, minimum inhibitory concentration (MIC) and $IC_{50}$ values were recorded

spectrophotometrically at 530 nm (NCCLS, 2002) (**Tables 5.3 to 5.16 and figures 5.1 to 5.10)**

### 4.4.8 Nature of toxicity, Fungistatic/Fungicidal

To determine Minimum Static Concentration (MSC) and Minimum Fungicidal Concentration (MFC), 100 µl aliquots of inoculum was taken aseptically from tube that did not show turbidity, and plated on to agar by the pour-plate method using agar plate count and incubated for 24 hours at 25±2°C. MFC was defined as the lowest concentration of the essential oil at which no fungal growth was observed. If there was growth, it means the concentration was static (MSC means the pathogens are not killed but only their growth was inhibited). All tests were performed in triplicate.

### 4.4.9 Inoculum density, using McFarland

As per the NCCLS norms, an inoculum ranges from 1 x $10^3$ CFU/ml is the standard inoculum for fungal pathogen. Therefore, to prepare an inoculum with this range, the method used was matching of turbidity of the inoculum suspension at 530 nm with 0.5 McFarland standards. Because, the absorbance of 0.5 McFarland is equal to the absorbance of inoculum suspension containing 1x $10^3$ CFU/ml. However, the inoculum density of the test pathogen was increased in a multiple series viz., 1 x $10^6$, 1 x $10^9$, 1 x $10^{12}$ and 1 x $10^{18}$ CFU/ml, respectively **(Table- 5.14).**

**Table-4.4: O.D. values recorded spectrophotometrically following McFarland preparation**

| McFarland | 1% $H_2SO_4$ | 1% $BaCl_2$ | O.D-1 | O.D-2 | Avg.O.D. |
|---|---|---|---|---|---|
| 1 | 9.9 | 0.1 | 0.082 | 0.083 | 0.0825 |
| 2 | 9.8 | 0.2 | 0.101 | 0.124 | 0.112 |
| 3 | 9.7 | 0.3 | 0.249 | 0.289 | 0.269 |
| 4 | 9.6 | 0.4 | 0.381 | 0.367 | 0.374 |
| 5 | 9.5 | 0.5 | 0.463 | 0.450 | 0.456 |
| 6 | 9.4 | 0.6 | 0.560 | 0.570 | 0.565 |
| 7 | 9.3 | 0.7 | 0.597 | 0.588 | 0.592 |
| 8 | 9.2 | 0.8 | 0.671 | 0.679 | 0.675 |
| 9 | 9.1 | 0.9 | 0.753 | 0.717 | 0.735 |
| 10 | 9.0 | 1.0 | 0.824 | 0.841 | 0.832 |

**Table 4.5: Corresponding O.D values of McFarland diluted 10 times**

| McFarland | O.D-1 | O.D-2 |
|---|---|---|
| 0.1 | 0.002 | 0.025 |
| 0.2 | 0.014 | 0.022 |
| 0.3 | 0.027 | 0.033 |
| 0.4 | 0.039 | 0.041 |
| 0.5 | 0.055 | 0.056 |

**Table 4.6: Prepartion of dilutions of inoculum, plating & evaluation at McFarlands**

| Dilution | No of colonies in plate-1 | No of colonies in plate-2 |
|---|---|---|
| $1^{st}$ | Infinite | Infinite |
| $2^{nd}$ | Infinite | Infinite |
| $3^{rd}$ | Infinite | Infinite |
| $4^{th}$ | Infinite | Infinite |
| $5^{th}$ | 346 | 335 |
| $6^{th}$ | 33 | 31 |
| $7^{th}$ | 3 | 1 |
| $8^{th}$ | No colonies | No colonies |
| $9^{th}$ | No colonies | No colonies |
| $10^{th}$ | No colonies | No colonies |

Countable colonies were obtained for 5th, 6th and 7th dilutions.

Formula for calculation of CFU using $7^{th}$ dilution:

Average no of colonies = $\frac{3 + 1}{2}$ = 2

No. of colonies in 200µl = 2

No. of colonies in 1ml = 5 x 2 = 10

Hence, colonies comes out to be 10 CFU/ ml

- Initial inoculum suspension= 1 x $10^3$ CFU/ ml. Further, its multiple inoculum suspension were made in the following series:
- For $2^{nd}$ inoculum suspension = 1 x $10^6$ CFU/ ml
- For $3^{rd}$ inoculum suspension = 1 x $10^9$ CFU/ ml

- For $4^{th}$ inoculum suspension =1 x $10^{12}$ CFU/ ml
- For $5^{th}$ inoculum suspension = 1 x $10^{15}$ CFU/ ml
- For $6^{th}$ inoculum suspension = 1 x $10^{18}$ CFU/ ml
- For $7^{th}$ inoculum suspension = 1 x $10^{21}$ CFU/ ml

The observations recorded are given in (Table 5.14).

### 4.4.10 Comparison with some Synthetic Fungicides/ Antifungal drugs

The efficacy of these plant constituents (essential oils/ extract) was also compaired with some synthetic antifungal drugs, available in the market. This was determined by comparing their minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs), respectively **(Table 5.15).**

## 4.5 Characterization of the essential oils

### 4.5.1 Identification of the active constituents of the tested essential oils

The oils thus recorded potential anti-dermatophytic efficacy; were subjected for standardization and characterization on various physico chemical properties, using following techniques (Table 5.17- 5.21)

### 4.5.2 Gas-Chromatographic analysis

Gas-Chromatographic (GC) analysis of the oil was performed on a Perkin-Elmer GC 8500, using a fused silica capillary column (25 m X 0.55 mm, film thickness 0.245 µm), coated with dimethyl siloxane (BP-1). The oven temperature was programmed from $60^0$ C to $220^0$ C at $5^0$ C/min, then held isothermally at $220^0$ C; detector temperature, $300^0$ C; carrier gas-nitrogen at a inlet pressure of psi; split, 1: 80.

### 4.5.3 Gas-Chromatographic-Mass Spectrometry

GC-MS data were obtained on a Shimadzu QP-2000 Mass Spectrometer instrument at 70 eV and $250^0$ C. GC column: Ulbon HR- 1 (equivalent to OV –1), fused silica capillary column (0.25 mm X 50m, film thickness 0.25 µm). The initial temperature was $100^0$C for 7mm, and then heated at $5^0$C/ min to $250^0$ C. Carrier gas, Helium was used at flow rate of 2 ml /min.

### 4.5.4 Quantitative analysis by GC

Quantitative GC analysis showed the number of components in sample, their retention time and approximate boiling points. The time taken for a particular component to pass through the column was called the compounds Retention Time (RT). The RT was a function of the physical properties of the compound, the rate of gas flow, the temperature, the liquid phase and the length and diameter of the column. Retention Time was measured from the point of injection of the sample to the top of the compound peak and was usually reported in minutes.

### 4.5.5 Calculation of Kovats Retention Indices (IR)

The oils were separately spiked with a standard mixture of homologous n-alkane series ($C_9$-$C_{28}$) and then analyzed by GC under the above-mentioned conditions. Retention indices were directly obtained by applying Kovats procedure (Kovats, 1965; Jennings and Shibamoto, 1980).

### 4.5.6 Mass Spectroscopy (MS)

Mass spectral analysis was run by E1 (Electron Impact Ionwasation) at 70ev by MSD (Mass Selective Detector). MS is a technique of separating the ions in accordance with their masses. Mass spectrometer separates the individual atoms or molecules on the basis of the difference in their masses. Mass spectroscopy is used to characterize organic molecules in two principal ways:

- To measure exact molecular weights and from that exact molecular formulae can be determined.
- To indicate with a molecule the points at which it prefers to fragment; from that the presence of certain structural units in the organic compound can be recognized.

### 4.5.7 Identification of compounds

Compounds were identified by comparing the retention indices of the peaks on the BP-1 column with literature values, computer matching against the library spectra built up using pure substances and components of known essential oils, and finally confirmed by comparison of mass spectra with published data. The relative amounts of individual components were based on peak areas obtained without FID response factor correction.

### 4.5.8 Physico-chemical properties of the selected plant metabolites

Various physico-chemical properties of the essential oil of *E. odoratum, M. piperita*, *O. basilicum* and *C. aurantifolia* were determined following the techniques of Langenau (1948) (**Table- 5.22).**

#### 4.5.8.1 Specific Gravity

It is the ratio of the weight of the oil to the weight of an equal volume of water. The pycnometer was cleaned with chromic acid, water, alcohol and finally with ether and then dried in hot air oven. It was filled with double distilled water and weighed. The pycnometer was then emptied, rinsed several times with alcohol and finally with ether. The ether fumes were removed by putting the pycnometer in hot air oven. The weight of the emptied pycnometer was recorded. It was then filled with oil and weighed. The weights of water and oil were determined. The specific gravity of oil was calculated by the following formula:

$$SG = \frac{\text{Weight of the oil}}{\text{Weight of an equal volume of water}}$$

According to "United States Pharmacopoeia" and the "National Formulary" the specific gravity is represented at $25^0$C. To convert specific gravity determined at room temperature to $25^0$C, a correction factor of 0.008 per $^0$C was used. If the room temperature was higher than $25^0$C, the correction factor was added. On the other hand, if the room temperature was lower than $25^0$C, the correction factor was subtracted from the original value (Table-5.22).

#### 4.5.8.2 Optical rotation

When the solution of an essential oil is placed in a beam of polarized light it possesses the property of rotating its plane. This property is known as specific rotation. Rotation due to pure oil is known as optical rotation. Specific rotation is temperature dependent **(Table-5.22).**

10 ml of absolute alcohol was pipetted to a flask containing weighed amount of essential oil and swirled properly. The percentage of the solution was calculated. Now a polarimeter tube (10 cm) containing the known concentration of the oil solution was placed in a trough of the polarimeter (Lippich type) between polarizer and analyzer. The analyzer was slowly turned until both the halves of the field viewed through the telescope showed equal intensities of illumination. At the proper setting, a small rotation to right or left caused

a pronounced inequality in the intensities of illumination of the two halves of the field. Direction of rotation was determined. If the analyzer was turned counter clockwise from zero position to obtain the final reading, the rotation was laevorotatory (-); if clockwise dextrorotatory (+).

The eye piece of the telescope was adjusted to give a clear sharpline between the two halves of the field. Rotation was determined by means of protractor by reading the degree directly and the minutes with the aid of either of the two fixed verniers.

The movable magnifying glasses help in obtaining accuracy. Specific rotation was calculated by following formula:

$$[\alpha]_{t^0}^{D} = \frac{\alpha \times 100}{l \times c}$$

Where,

$[\alpha]_{t^0}^{D}$ = specific rotation at temperature ($28\pm2^0$C) using sodium light.

(D = sodium lamp for monochromatic light; t = room temperature = $34^0$C).

$\alpha$ = observed rotation

$l$ = length of the column (Polarimeter tube) in decimeter (10 cm = 1 decimeter)

c = concentration of the solution in per centage.

**4.5.8.3 Refractive index**

When a beam of light enters a denser medium, it bends toward the normal.

According to law of refraction: $\frac{Sin\, i}{Sin\, r} = \frac{N}{n}$

i = angle of incidence

r = angle of refraction

n = index of refraction of the less dense medium

N = index of refraction of the more dense medium

Refractometer was used for determining the refractive index. Refractometer (Abb'e type) was placed in such a position that day light can readily be obtained for illumination. The prisms of refractometer were cleaned with alcohol and then with ether. To charge the instrument, the double prism was opened by means of the screw head (clamp) and one drop of the oil was placed on the prism. The prism was then closed firmly by clamp. The alidade was moved backward or forward until the field of vision was divided into a light and dark zone. The dividing line (border line) would not be a sharp line but a band of colour- the solar

spectrum. The colours are eliminated by rotating the screw head of the compensator until a sharp colourless line was obtained. The border line was adjusted so that falls on the point of intersection of the cross hairs. The refractive index was read directly on the scale of the sector.

The refractive index is represented at $25^0$C. To convert refractive index from room temperature to $25^0$C a correction factor of 0.00045 per degree increase or decrease of temperature was used **(Table-5.22).**

#### 4.5.8.4 Solubility in water

This property plays a significant role in determining the quality of oil. 1ml of the oil was introduced into 10 ml glass stoppered cylinder (calibrated to 0.1 ml). Water was then pipetted into it drop by drop with concurrent shaking of the cylinder after each addition. The volume of water used to obtain an uncleared solution was recorded (**Table-5.22).**

#### 4.5.8.5 Solubility in different organic solvents

Besides the earlier described physico-chemical properties, the solubility of the oils in different organic solvents was also determined. 1 ml of each the oil was introduced separately to a glass stoppered tube (10 ml) so as to prepare a set of 12 tubes for the each oil. In this way the solubility of each the oil in 1:1 ratio with respect to the following 13 different organic solvents was observed and recorded in **Table-5.23.**

## 4.6 Identification of the scope and issues related to intellectual property rights (IPR) pertaining to antidermatophytic activity of selected medicinal plants

The value of plants for medicines is more widely recognized and the "intellectual property rights" (IPR) connected with their use have been debated worldwide. Traditional medicine is an important part of human health care in many developing countries and also in developed countries, increasing their commercial value. The fast growth of patent applications related to herbal medicine shows this trend clearly. India is home to a reservoir of medicinal plants and traditional knowledge and its North East area a hot spot of biodiversity. The vast resources face the threat of piracy and infringement of IPR which need to be protected under the patents systems.

Search for IPR (Patent) on plants investigated in the present study for their antidermatophytic activity was conducted to establish scope for future *in- vivo* investigations, pre clinical trials, multilocational clinical trials as well as isolation and synthesis of the bioactive molecule(s) for drug formulation (i.e. product patent)/ technology transfer (i.e. process patent) to the pharmaceutical companies.

Search on patent activity was conducted on various patent sites viz., WIPO, USPTO, EPO, IPO as well as on Google. Trends in patent filing and grant across the world and fields of technologies especially in the field of 'pharmaceuticals', during the last decade (2001-11) were mapped. Patent Search on selected plants showing antidermatophytic activity was conducted using the key words = the selected plant's name, i.e. *E. odoratum* L, *M. piperita* L. *O. basilicum, C. aurantifolia* L.and *M. micrantha*as an *antidermatophyte.* Besides this, patent search using the key words= selected plant name + dermatophyte's name, i.e. *Epidermophyton floccosum, Microsporum gypsum, T. mentagrophytes* and *T. rubrum;* were also made. Trends of Patent Filing, and Grant in India during 2001-2011; especially in the field of 'Drug' were also mapped and compared with the trends at the world. Further, the search for IPR (Patent activity) from North East India was conducted on significant patent office sites including on the Indian Patent Office site (IPO) by using the key words = name of the state i.e. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura as well as the key words = selected plant's name and/or dermatophyte's name. Finally, patents grants for North East India in the field of pharmaceuticals and drugs were searched.

# 5. Results

The present research entitled *' A study on the Antidermatophytic Activity of Some Ethno-medicinal Plants of North East Region in relation to Intellectual Property Rights (IPR)'* was conducted during June 2009- December 2012. The survey relating to ethnomedicinal plants as well as IPR scope and issues was conducted at the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl. However, detailed *in vitro* investigation with the selected potential plants was carried out at Biological Product Laboratory, Department of Botany, University of Allahabad, with the joint supervisor. The results are summarized as follows:

## 5.1 Collection, categorization, identification and documentation of common ethnomedicinal plants, used against skin ailments

Based on the literature survey as well as local field visits, information about some important ethno medicinal plants, used for skin ailments in human beings, were collected and utilized for making a priority based list of fifteen most potent plants. These were the plants which showed the maximum frequency of use, against skin ailments, among the local communities (Table-5.1).

**Table 5.0 List of common ethno-medicinal plants, collected at the grass root level**

| SN | Name of Plants | Common name | Family |
|---|---|---|---|
| **1.** | *Eupatorium odoratum* L. | Dettol Plant | Asteraceae |
| **2.** | *Cinnamomum tamala* (Buch.-Ham) Nees & Eberm | Tejpatta | Lauraceae |
| **3.** | *Achyranthes aspera* L. | Buchhawl | Amaranthaceae |
| **4.** | *Cymbopogon flexuosus* (Steud.) Watson | Lemongrass | Poaceae |
| **5.** | *Foeniculum vulgare* Miller | Souf | Apiaceae |
| **6.** | *Euphorbia hirta* L. | Bhangaria | Euphorbiaceae |
| **7.** | *Hedyotis scandens* Roxb. | Kel-hnamtur | Rubiaceae |
| **8.** | *Mentha piperita* L. | Peppermint | Lamiaceae |
| **9.** | *Ocimum basilicum* L. | Tulsi | Lamiaceae |
| **10.** | *Phyllanthus niruri* L. | Bhuiaonla | Eupheribaceae |
| **11.** | *Moringa oleifera* Lam. | Drumstick | Moringaceae |
| **12.** | *Citrus aurantifolia* L. | Wild basil | Lamiaceae |
| **13.** | *Ageratum conyzoides* L. | Vaihlenhlo | Asteraceae |

| | | | |
|---|---|---|---|
| **14.** | *Mikania micrantha* Kunth ex H.B.K. | Japanhlo | Asteraceae |
| **15.** | *Colebrookea oppositifolia* Smith. | Squirrel Tail | Lamiaceae |

The plants thus identified were deposited in the herbarium of the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl for future references, and the same were selected for the present investigation.

## 5.2 Extraction of the plant secondary metabolites in the form of essential oil/ extract

In the present investigation, 15 most frequent ethno-medicinal plants (as listed in table 5.1 were selected for extraction of the essential oil/ solvent extract, from different parts of plant.

**Table 5.1 Collected plants and their parts used for extraction of the essential oil/ extract**

| SN | Name of Plants | Common name | Family | Oil + Extract Code | Extraction of essential oil from |
|---|---|---|---|---|---|
| **1.** | *Eupatorium odoratum* | Dettol Plant | Asteraceae | *E-Eo-1 | Leaves |
| **2.** | *Cinnamomum tamala* | Tejpatta | Lauraceae | E-Ct-2 | Leaves |
| **3.** | *Achyranthes aspera* | Buchhawl | Amaranthaceae | **X-Aa-1 | Leaves |
| **4.** | *Cymbopogon flexuosus* | Lemongrass | Poaceae | E-Cf-3 | Leaves |
| **5.** | *Foeniculum vulgare* | Souf | Apiaceae | E-Fv-4 | Seed |
| **6.** | *Euphorbia hirta* | Dudhi | Euphorbiaceae | X-Eh-2 | Leaves |
| **7.** | *Hedyotis scandens* | Kel-hnamtur | Rubiaceae | X-Hs-3 | Fruit peels |
| **8.** | *Mentha piperita* | Peppermint | Lamiaceae | E-Mp-5 | Leaves |
| **9.** | *Ocimum basilicum* | Tulsi | Lamiaceae | E-Ob-6 | Leaves |
| **10.** | *Phyllanthus niruri* | Bhuiaonla | Eupheribaceae | X-Pn-4 | Leaves |
| **11.** | *Moringa oleifera* | Drumstick | Moringaceae | X-Mo-5 | Leaves & Seed |
| **12.** | *Citrus aurantifolia* | Wild basil | Lamiaceae | E-Ca-7 | Leaves |
| **13.** | *Ageratum conyzoides* | Vaihlenhlo | Asteraceae | E-Ac-8 | Leaves |
| **14.** | *Mikania micrantha* | Japanhlo | Asteraceae | X-Mm-6 | Leaves |
| **15.** | *Colebrookea oppositifolia* | Squirrel Tail | Lamiaceae | X-Co-7 | Leaves |

***E = Essential oil; **X = Extract***

## 5.3 Antimicrobial screening of the extracted essential oil/ extract, against the dermatophytic pathogens

Further, the preliminary antimicrobial screening of these essential oils/ extracts (test sample) was carried out using disk diffusion method. **(Plate 9).** The stock solution of each test samples (at 50mg/ml conc) were prepared separately and subjected against the dermatophytic pathogens *Epidermophyton floccosum* (MTCC-7880), *Microsporum gypseum* (MTCC-2830),

*Trichophyton mentagrophytes* (MTCC-8476) and *T. rubrum* (MTCC-3272). The observations were recorded in the Table-5.2

**Table 5.2 Screening efficacy of the test samples against the dermatophytes**

| SN | Name of Plants | Extracted Form | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|---|
| | | | ***E. floccosum* (MTCC-7880)** | ***M. gypseum* (MTCC-2830)** | ***T. mentagrophytes* (MTCC-8476)** | ***T. rubrum* (MTCC-3272)** |
| **1** | *Achyranthes aspera* L. | Extract | NA | NA | NA | 2±2 |
| **2** | *Ageratum conyzoides* L. | Essential oil | NA | NA | 1±2 | 3±2 |
| **3** | *Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm. | Essential oil | 1±2 | 3±2 | 3±2 | 3±2 |
| **4** | *Citrus aurantifolia* L. | Essential oil | 17±2 | 19±2 | 20±2 | 21±2 |
| **5** | *Colebrookea oppositifolia* Smith. | Extract | NA | NA | NA | 1±2 |
| **6** | *Cymbopogon flexuosus* (Steud.) Watson | Essential oil | 2±2 | 3±2 | 4±2 | 3±2 |
| **7** | *Eupatorium odoratum* L. | Essential oil | 21±2 | 27±2 | 26±2 | 26±2 |
| **8** | *Euphorbia hirta* L. | Extract | NA | NA | NA | NA |
| **9** | *Foeniculum vulgare* Miller | Essential oil | NA | NA | 2±2 | 3±2 |
| **10** | *Hedyotis scandens* Roxb. | Extract | NA | NA | 1±2 | 1±2 |
| **11** | *Mentha piperita* L. | Essential oil | 14±2 | 16±2 | 18±2 | 17±2 |
| **12** | *Mikania micrantha* Kunth ex H.B.K. | Extract | 8±2 | 11±2 | 12±2 | 12±2 |
| **13** | *Moringa oleifera* Lam. | Extract | NA | NA | 1±2 | 1±2 |
| **14** | *Ocimum basilicum* L. | Essential oil | 17±2 | 19±2 | 21±2 | 22±2 |
| **15** | *Phyllanthus niruri* L. | Extract | NA | NA | NA | 1±2 |

Out of the 15 selected plants, 4 essential oil bearing plants viz., *E. odoratum* L., *M. piperita* L. *O. basilicum,* and *C. aurantifolia* L. as well as 50 % ethanolic extract of *M. micrantha* were found to be more effective against all the test pathogens. The maximum inhibition zone was recorded in case of *E. odoratum* essential oil (27 mm); followed by *O. basilicum* essential oil (22 mm), *C. aurantifolia* (21 mm) and *M. piperita* (18 mm) and the ethanolic extract of *M. micrantha* (12 mm).

Therefore, these five potential plants (which show the maximum zone of inhibition) were selected for detailed *in vitro* investigations, and characterized under the following heads:

## 5.4 Detailed *in-vitro* study of the selected essential oils as well as plant extracts against dermatophytess

### 5.4.1 Antidermatophytic activity of *E. odoratum* against *Tr, Tm, Ef & Mg*

The efficacy of the oil of *E. odoratum* against four main dermatophytes *T. rubrum; T. mentagrophytes*; *E. floccosum* and *M. gypseum* recorded are shown in table- 5.3 to 5.6 as well as Fig- 5.1 & 5.2.

**Plate-1 O.D. of 96 well plate: *Eupatorium odoratum* gainst Tr, Tm, Ef & Mg**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.009 | 0.007 | 0.040 | 0.138 | 0.116 | 0.045 | 0.034 | 0.026 | 0.022 | 0.040 | 0.326 | 0.293 |
| B | -0.005 | -0.005 | 0.039 | 0.130 | 0.118 | 0.065 | 0.031 | 0.731 | 0.001 | 0.017 | 0.075 | 0.252 |
| C | 0.003 | -0.005 | 0.039 | 0.153 | 0.134 | 0.069 | 0.055 | 0.041 | 0.021 | 0.028 | 0.007 | 0.005 |
| D | -0.001 | -0.004 | 0.038 | 0.143 | 0.121 | 0.076 | 0.065 | 0.035 | 0.023 | 0.018 | 0.009 | 0.001 |
| E | 0.003 | -0.006 | 0.038 | 0.035 | 0.178 | 0.154 | 0.176 | 0.179 | 0.197 | 0.201 | 0.236 | 0.203 |
| F | -0.001 | -0.003 | 0.091 | -0.003 | 0.172 | 0.273 | 0.303 | 0.209 | 0.153 | 0.214 | 0.173 | 0.199 |
| G | 0.002 | -0.004 | 0.038 | 0.114 | 0.320 | 0.464 | 0.179 | 0.255 | 1.036 | 1.228 | 0.173 | 0.219 |
| H | -0.010 | -0.004 | 0.025 | 0.110 | 0.247 | 0.178 | 0.253 | 0.506 | 1.109 | 0.218 | 0.885 | 0.209 |

Endpoint
Lm1 530
Automix: Once
Calibrate: Once
Start Read:
2:01 AM 12/29/2011

Wavelength Combination: !Lm1
Temperature Set Point: 31.0 Mean 31.0
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.257
Reader: PLUS384 ROM v1.23 Jun 19 2008

**Figure 5.1:** O.D. of 96 well plate: *E. odoratum* against *Tr, Tm, Ef* & *Mg* where the column 1 showed Negative Control Column 2 Media Control Column 3 Drug Control Column 4-11 Serial dilution of drug and Column 12 Positive Control

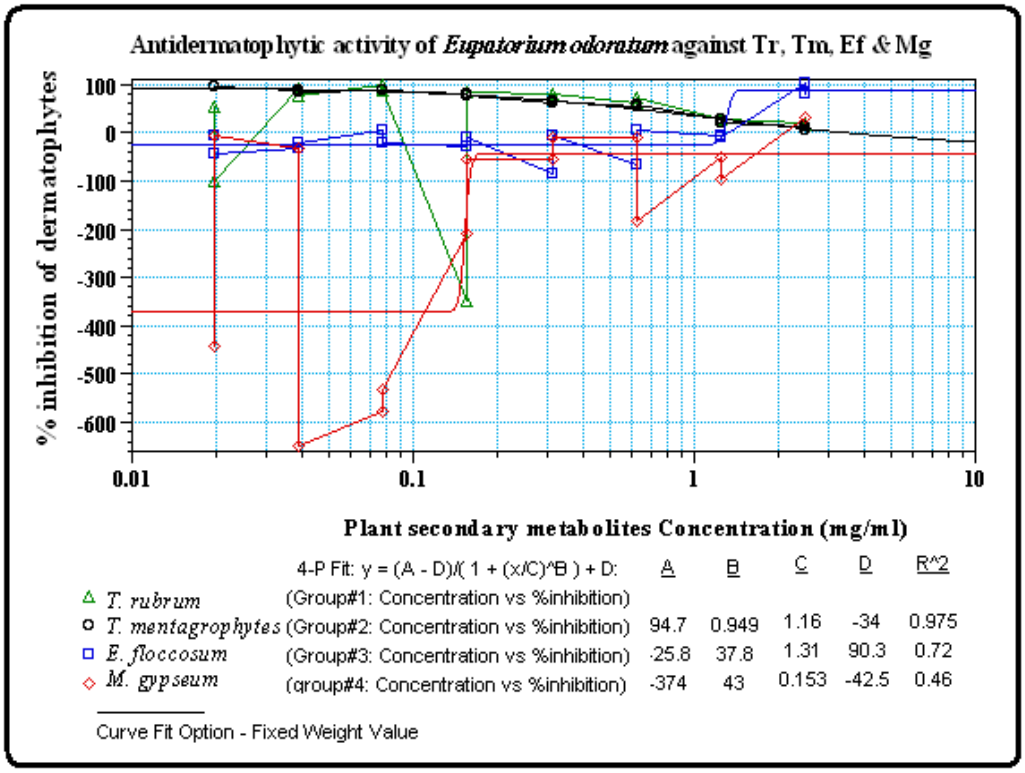



**Figure 5.2: Percentage inhibition of dermatophytes against *E. odoratum* Linn.**

**Table-5.3: Antidermatophytic activity of *E. odoratum* essential oil against *T. rubrum***

| Sample | Conc. (mg/ml) | Wells | Values | Mean Values | Std. Dev. | CV% | Inhibition |
|---|---|---|---|---|---|---|---|
| EO1 | 2.500 | A4 | 0.138 | 0.134 | 0.006 | 4.5 | 15.56% |
| | | B4 | 0.130 | | | | 20.82% |
| EO 2 | 1.250 | A5 | 0.116 | 0.117 | 0.002 | 1.4 | 29.13% |
| | | B5 | 0.118 | | | | 27.72% |
| EO 3 | 0.625 | A6 | 0.045 | 0.055 | 0.014 | 25.4 | 72.22% |
| | | B6 | 0.065 | | | | 60.06% |
| EO 4 | 0.313 | A7 | 0.034 | 0.032 | 0.002 | 6.4 | 79.37% |
| | | B7 | 0.031 | | | | 81.15% |
| EO 5 | 0.156 | A8 | 0.026 | 0.379 | 0.498 | 131.6 | 83.90% |
| | | B8 | 0.731 | | | | -346.66% |
| EO 6 | 0.078 | A9 | 0.022 | 0.012 | 0.015 | 128.9 | 86.34% |
| | | B9 | 0.001 | | | | 99.36% |
| EO 7 | 0.039 | A10 | 0.040 | 0.028 | 0.016 | 58.2 | 75.77% |
| | | B10 | 0.017 | | | | 89.89% |
| EO 8 | 0.020 | A11 | 0.326 | 0.200 | 0.178 | 88.7 | -99.28% |
| | | B11 | 0.075 | | | | 54.37% |

Based on the observations of table-5.3 as well as figures 5.1 and 5.2, the Minimum Inhibitory Concentration (MIC) of the oil of *E. odoratum* against *T. rubrum* was recorded 0.68 mg/ml but it was fungicidal at 1.25 mg/ml. However, the $IC_{50}$ value was recorded as 0.17.

**Table-5.4: Antidermatophytic activity of *E. odoratum* essential oil against *T. mentagrophytes***

| Sample | Conc. (mg/ml) | Wells | Values | Mean Values | Std. Dev. | CV% | Inhibition |
|---|---|---|---|---|---|---|---|
| EO1 | 2.500 | C4 | 0.153 | 0.148 | 0.007 | 4.9 | 6.45 |
| | | D4 | 0.143 | | | | 12.75 |
| EO 2 | 1.250 | C5 | 0.134 | 0.127 | 0.009 | 7.2 | 18.19 |
| | | D5 | 0.121 | | | | 26.07 |
| EO 3 | 0.625 | C6 | 0.069 | 0.073 | 0.005 | 7.2 | 57.92 |
| | | D6 | 0.076 | | | | 53.40 |
| EO 4 | 0.313 | C7 | 0.055 | 0.060 | 0.006 | 10.7 | 66.11 |
| | | D7 | 0.065 | | | | 60.55 |
| EO 5 | 0.156 | C8 | 0.041 | 0.038 | 0.004 | 10.3 | 75.22 |
| | | D8 | 0.035 | | | | 78.58 |
| EO 6 | 0.078 | C9 | 0.021 | 0.022 | 0.002 | 8.3 | 87.26 |
| | | D9 | 0.023 | | | | 85.67% |
| EO 7 | 0.039 | C10 | 0.028 | 0.023 | 0.007 | 31.4 | 82.86% |
| | | D10 | 0.018 | | | | 89.09% |
| EO 8 | 0.020 | C11 | 0.007 | 0.008 | 0.001 | 17.0 | 95.75% |
| | | D11 | 0.009 | | | | 94.59% |

As per the observations of table-5.4 as well as figures 5.1 and 5.2, the minimum inhibitory concentration of the oil of *E. odoratum* against *T. mentagrophytes* was recorded 0.59 mg/ml but it was fungicidal at 1.25 mg/ml. However, the $IC_{50}$ value was recorded as 0.13.

**Table-5.5: Antidermatophytic activity of *E. odoratum* essential oil against *E. floccosum***

| Sample | Conc. (mg/ml) | Wells | Values | Mean Values | Std. Dev. | CV% | Inhibition |
|---|---|---|---|---|---|---|---|
| EO1 | 2.500 | E4 | 0.035 | 0.016 | 0.027 | 167.7 | 78.70 |
| | | F4 | -0.003 | | | | 101.81% |
| EO 2 | 1.250 | E5 | 0.178 | 0.175 | 0.005 | 2.6 | -8.88% |
| | | F5 | 0.172 | | | | -4.97% |
| EO 3 | 0.625 | E6 | 0.154 | 0.214 | 0.084 | 39.4 | 5.72% |
| | | F6 | 0.273 | | | | -67.13% |
| EO 4 | 0.313 | E7 | 0.176 | 0.239 | 0.090 | 37.6 | -7.48% |
| | | F7 | 0.303 | | | | -85.29% |
| EO 5 | 0.156 | E8 | 0.179 | 0.194 | 0.021 | 10.8 | -9.31% |
| | | F8 | 0.209 | | | | -27.46% |
| EO 6 | 0.078 | E9 | 0.197 | 0.175 | 0.031 | 17.7 | -20.68% |
| | | F9 | 0.153 | | | | 6.21% |
| EO 7 | 0.039 | E10 | 0.201 | 0.208 | 0.009 | 4.5 | -22.82% |
| | | F10 | 0.214 | | | | -30.89% |
| EO 8 | 0.020 | E11 | 0.236 | 0.205 | 0.045 | 21.9 | -44.46% |
| | | F11 | 0.173 | | | | -5.76% |

Based on the observations recorded in table-5.5 as well as figures 5.1 and 5.2; the minimum inhibitory concentration of the oil of *E. odoratum* against *E. floccosum* was 1.38 mg/ml but it was fungicidal at 2.50 mg/ml. However, the $IC_{50}$ value was recorded as 1.32.

**Table-5.6: Antidermatophytic activity of *E. odoratum* essential oil against *M. gypseum***

| Sample | Conc. (mg/ml) | Wells | Values | Mean Values | Std. Dev. | CV% | Inhibition |
|---|---|---|---|---|---|---|---|
| EO1 | 2.500 | G4 | 0.114 | 0.112 | 0.003 | 2.5 | 30.60% |
| | | H4 | 0.110 | | | | 32.98% |
| EO 2 | 1.250 | G5 | 0.320 | 0.284 | 0.052 | 18.3 | -95.86% |
| | | H5 | 0.247 | | | | -51.06% |
| EO 3 | 0.625 | G6 | 0.464 | 0.321 | 0.203 | 63.1 | -183.82% |
| | | H6 | 0.178 | | | | -8.76% |
| EO 4 | 0.313 | G7 | 0.179 | 0.216 | 0.052 | 24.2 | -9.37% |
| | | H7 | 0.253 | | | | -54.42% |
| EO 5 | 0.156 | G8 | 0.255 | 0.380 | 0.178 | 46.8 | -55.64% |
| | | H8 | 0.506 | | | | -209.49% |
| EO 6 | 0.078 | G9 | 1.036 | 1.072 | 0.051 | 4.8 | -533.15% |
| | | H9 | 1.109 | | | | -577.65% |
| EO 7 | 0.039 | G10 | 1.228 | 0.723 | 0.714 | 98.7 | -650.51% |
| | | H10 | 0.218 | | | | -33.39% |
| EO 8 | 0.020 | G11 | 0.173 | 0.529 | 0.504 | 95.2 | -5.76% |
| | | H11 | 0.885 | | | | -441.09% |

Based on the observations recorded in table-5.6 as well as figures 5.1 and 5.2; the minimum inhibitory concentration of the oil of *E. odoratum* against *M. gypseum* was 0.96 mg/ml but it was fungicidal at 1.25 mg/ml. However, the $IC_{50}$ value was recorded as 0.54.

### 5.4.2 Antidermatophytic activity of *O. basilicum* oil against *Tr, Tm, Ef & Mg*

The efficacy of the oil of *O. basilicum* against the against four main dermatophytes *T. rubrum; T. mentagrophytes*; *E. floccosum* and *M. gypseum* recorded are shown in table- 5.7 to 5.10 as well as Fig- 5.3 and 5.4.

**Plate-2 O.D. of 96 well plate: *Ocimum basilicum* against Tr, Tm, Ef & Mg**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.009 | 0.020 | 0.147 | 0.209 | 0.290 | 0.333 | 0.411 | 0.683 | 0.811 | 0.938 | 0.640 | 0.352 |
| B | -0.005 | -0.002 | 0.110 | 0.143 | 0.285 | 0.329 | 0.320 | 0.398 | 0.456 | 0.416 | 0.492 | 0.628 |
| C | 0.016 | -0.002 | 0.724 | 0.079 | 0.036 | 0.024 | 0.007 | 0.003 | 0.001 | -0.009 | -0.008 | 0.049 |
| D | -0.005 | 0.001 | 0.150 | 0.133 | 0.079 | 0.046 | 0.398 | 0.020 | 0.014 | 0.131 | 0.010 | 0.006 |
| E | -0.005 | -0.006 | 0.123 | 0.143 | 0.116 | 0.126 | 0.123 | 0.124 | 0.113 | 0.119 | 0.130 | 0.182 |
| F | -0.004 | 0.000 | 0.199 | 0.138 | 0.130 | 0.506 | 0.130 | 0.108 | 0.109 | 0.158 | 0.162 | 0.149 |
| G | -0.003 | -0.001 | 0.108 | 0.593 | 0.730 | 0.361 | 0.301 | 0.298 | 0.455 | 0.342 | 0.300 | 0.313 |
| H | -0.004 | 0.001 | 0.117 | 0.372 | 0.318 | 1.238 | 0.373 | 0.331 | 0.339 | 0.339 | 0.782 | 0.359 |

Endpoint
Lm1 530
Automix: Once
Calibrate: Once
Start Read:
1:55 AM 12/29/2011

Wavelength Combination: !Lm1
Temperature Set Point: 31.0 Mean 30.8
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.262
Reader: PLUS384 ROM v1.23 Jun 19 2008

**Figure 5.3:** O.D. of 96 well plate: *O. basilicum* against Tr, Tm, Ef & Mg where the column 1 showed Negative Control Column 2 Media Control Column 3 Drug Control Column 4-11 Serial dilution of drug and Column 12 Positive Control

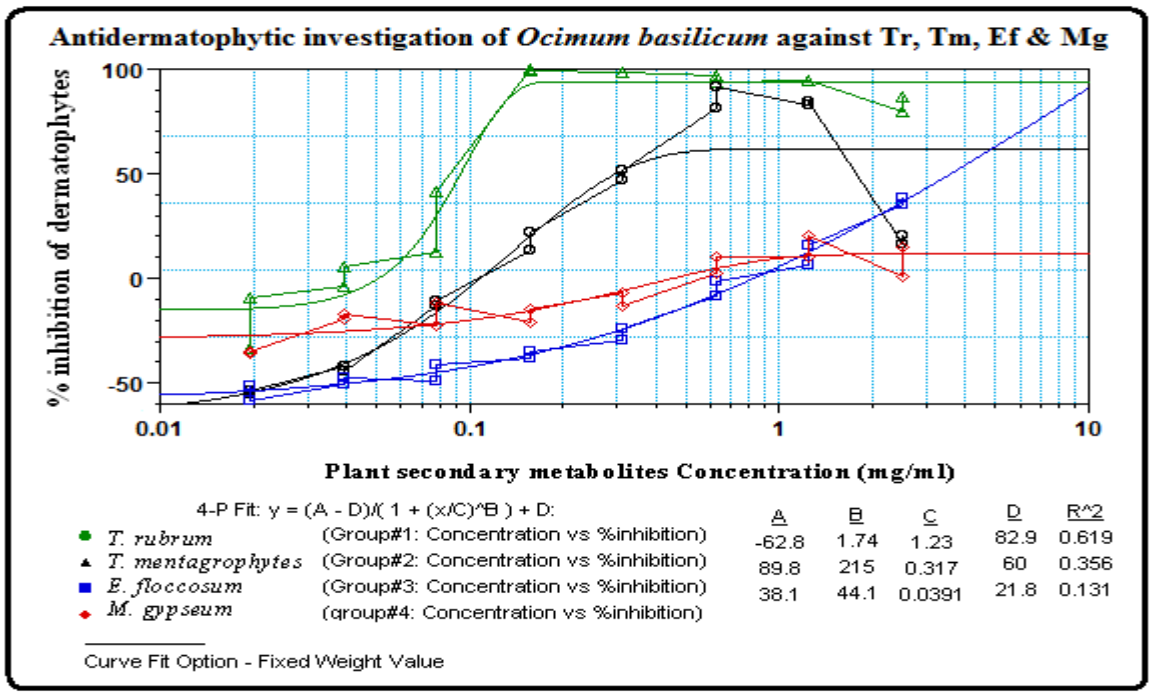



**Figure 5.4: Percentage inhibition of dermatophytes against *O. basilicum***

**Table-5.7: Antidermatophytic activity of *O. basilicum* essential oil against *T. rubrum***

| Sample | Concentration | Wells | Values | Mean Values | Std.Dev. | CV% | %inhibition |
|---|---|---|---|---|---|---|---|
| BL1 | 2.500 | A4 | 0.130 | 0.235 | 0.150 | 63.6 | 82.334 |
| | | B4 | 0.341 | | | | 53.459 |
| BL2 | 1.250 | A5 | 0.068 | 0.057 | 0.016 | 28.1 | 90.745 |
| | | B5 | 0.045 | | | | 93.812 |
| BL3 | 0.625 | A6 | 0.029 | 0.026 | 0.005 | 18.5 | 96.048 |
| | | B6 | 0.022 | | | | 96.962 |
| BL4 | 0.313 | A7 | 0.021 | 0.018 | 0.004 | 22.3 | 97.098 |
| | | B7 | 0.015 | | | | 97.889 |
| BL5 | 0.156 | A8 | 0.081 | 0.117 | 0.050 | 43.1 | 88.932 |
| | | B8 | 0.152 | | | | 79.225 |
| BL6 | 0.078 | A9 | 0.355 | 0.391 | 0.052 | 13.2 | 51.660 |
| | | B9 | 0.427 | | | | 41.721 |
| BL7 | 0.039 | A10 | 0.614 | 0.670 | 0.079 | 11.8 | 16.242 |
| | | B10 | 0.726 | | | | 1.028 |
| BL8 | 0.020 | A11 | 0.892 | 0.978 | 0.122 | 12.5 | -21.589 |
| | | B11 | 1.065 | | | | -45.201 |

Based on the observations recorded in table-5.7 as well as figure 5.3 and 5.4; the minimum inhibitory concentration of the essential oil of *O. basilicum* against *T. rubrum* was 1.71 mg/ml but it was fungicidal at 2.5 mg/ml. However, the $IC_{50}$ value was recorded as 0.90

**Table-5.8: Antidermatophytic activity of *O. basilicum* essential oil against *T. mentagrophytes***

| Sample | Concentration | Wells | Values | Mean Values | Std.Dev. | CV% | %inhibition |
|---|---|---|---|---|---|---|---|
| **BL1** | 2.500 | C4 | 0.578 | 0.552 | 0.037 | 6.6 | 21.150 |
| | | D4 | 0.527 | | | | 28.211 |
| **BL2** | 1.250 | C5 | 0.166 | 0.117 | 0.069 | 58.4 | 77.385 |
| | | D5 | 0.069 | | | | 90.609 |
| **BL3** | 0.625 | C6 | 0.060 | 0.107 | 0.067 | 62.3 | 91.836 |
| | | D6 | 0.154 | | | | 78.994 |
| **BL4** | 0.313 | C7 | 0.178 | 0.251 | 0.102 | 40.8 | 75.681 |
| | | D7 | 0.323 | | | | 55.954 |
| **BL5** | 0.156 | C8 | 0.418 | 0.443 | 0.035 | 7.9 | 43.017 |
| | | D8 | 0.468 | | | | 36.241 |
| **BL6** | 0.078 | C9 | 0.458 | 0.622 | 0.232 | 37.2 | 37.495 |
| | | D9 | 0.786 | | | | -7.179 |
| **BL7** | 0.039 | C10 | 0.764 | 0.904 | 0.198 | 21.9 | -4.167 |
| | | D10 | 1.045 | | | | -42.434 |
| **BL8** | 0.020 | C11 | 0.991 | 1.012 | 0.030 | 2.9 | -35.154 |
| | | D11 | 1.033 | | | | -40.852 |

As per the observations inferred with table-5.8 as well as figures 5.3 and 5.4; the minimum inhibitory concentration of the oil of *O. basilicum* against *T. mentagrophytes* was recorded 1.89 mg/ml but it was fungicidal at 2.5 mg/ml. However, the $IC_{50}$ value was recorded as 0.97.

**Table-5.9: Antidermatophytic activity of *O. basilicum* essential oil against *E. floccosum***

| Sample | Concentration | Wells | Values | Mean Values | Std.Dev. | CV% | %inhibition |
|---|---|---|---|---|---|---|---|
| **BL1** | 2.500 | E4 | 0.262 | 0.276 | 0.019 | 6.8 | 66.807 |
| | | F4 | 0.289 | | | | 63.467 |
| **BL2** | 1.250 | E5 | 0.395 | 0.369 | 0.037 | 10.0 | 49.968 |
| | | F5 | 0.343 | | | | 56.547 |
| **BL3** | 0.625 | E6 | 0.338 | 0.291 | 0.067 | 22.9 | 57.230 |
| | | F6 | 0.244 | | | | 69.173 |
| **BL4** | 0.313 | E7 | 0.436 | 0.395 | 0.058 | 14.7 | 44.845 |
| | | F7 | 0.354 | | | | 55.206 |
| **BL5** | 0.156 | E8 | 0.502 | 0.577 | 0.106 | 18.3 | 36.457 |
| | | F8 | 0.651 | | | | 17.582 |
| **BL6** | 0.078 | E9 | 0.809 | 0.819 | 0.013 | 1.6 | -2.407 |
| | | F9 | 0.828 | | | | -4.747 |
| **BL7** | 0.039 | E10 | 0.877 | 0.955 | 0.110 | 11.5 | -10.946 |
| | | F10 | 1.033 | | | | -30.631 |
| **BL8** | 0.020 | E11 | 0.944 | 1.023 | 0.111 | 10.9 | -19.460 |
| | | F11 | 1.101 | | | | -39.335 |

Based on the observations recorded in table-5.9 as well as figures 5.3 and 5.4; the minimum inhibitory concentration of the oil of *O. basilicum* against *E. floccosum* was 2.48 mg/ml but it was fungicidal at 3.2 mg/ml. However, the $IC_{50}$ value was recorded as 1.18.

**Table-5.10: Antidermatophytic activity of *O. basilicum* essential oil against *M. gypseum***

| Sample | Concentration | Wells | Values | Mean Values | Std.Dev. | CV% | %inhibition |
|---|---|---|---|---|---|---|---|
| **BL1** | 2.500 | C4 | 0.239 | 0.259 | 0.028 | 11.0 | 69.742 |
| | | D4 | 0.279 | | | | 64.656 |
| **BL2** | 1.250 | C5 | 0.250 | 0.261 | 0.015 | 5.9 | 68.376 |
| | | D5 | 0.272 | | | | 65.643 |
| **BL3** | 0.625 | C6 | 0.207 | 0.195 | 0.017 | 8.8 | 73.841 |
| | | D6 | 0.182 | | | | 76.915 |
| **BL4** | 0.313 | C7 | 0.149 | 0.142 | 0.010 | 6.8 | 81.153 |
| | | D7 | 0.135 | | | | 82.874 |
| **BL5** | 0.156 | C8 | 0.335 | 0.308 | 0.039 | 12.7 | 57.572 |
| | | D8 | 0.280 | | | | 64.568 |
| **BL6** | 0.078 | C9 | 0.724 | 0.541 | 0.258 | 47.7 | 8.435 |
| | | D9 | 0.358 | | | | 54.649 |
| **BL7** | 0.039 | C10 | 0.438 | 0.395 | 0.061 | 15.6 | 44.566 |
| | | D10 | 0.351 | | | | 55.560 |
| **BL8** | 0.020 | C11 | 0.300 | 0.287 | 0.018 | 6.3 | 62.050 |
| | | D11 | 0.275 | | | | 65.263 |

Based on the observations recorded in table-5.10 as well as figures 5.3 and 5.4 the minimum inhibitory concentration of the oil of *O. basilicum* against *M. gypseum* was 1.24 mg/ml but it was fungicidal at 2.5 mg/ ml. However, the $IC_{50}$ value was recorded as 0.66

### 5.4.3 Antidermatophytic activity of *M. piperita* oil against *Tr, Tm, Ef & Mg*

The efficacy of essential oil of *M. piperita* against the against four main dermatophytes *T. rubrum; T. mentagrophytes*; *E. floccosum* and *M. gypseum* recorded are shown in table- 5.11 as well as Figures 5.5 & 5.6.

**Plate-3 O.D. of 96 well plate: *Mentha piperita* against Tr, Tm, Ef & Mg**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | -0.006 | 0.006 | 0.044 | 0.033 | 0.020 | 0.003 | 0.080 | 0.267 | 0.550 | 0.771 | 0.899 | 0.886 |
| B | -0.006 | -0.008 | 0.030 | 0.026 | 0.021 | 0.012 | 0.080 | 0.281 | 0.569 | 0.796 | 0.884 | 0.859 |
| C | -0.005 | -0.009 | 0.333 | 0.221 | 0.044 | 0.017 | 0.442 | 0.456 | 0.628 | 0.754 | 0.816 | 0.845 |
| D | -0.005 | -0.021 | 0.284 | 0.200 | 0.042 | 0.339 | 0.544 | 0.539 | 0.693 | 0.808 | 0.900 | 0.965 |
| E | 0.004 | 0.002 | 0.104 | 0.415 | 0.598 | 0.640 | 0.682 | 0.778 | 0.826 | 0.802 | 0.833 | 0.955 |
| F | 0.008 | 0.005 | 0.062 | 0.546 | 0.605 | 0.653 | 0.674 | 0.833 | 0.877 | 0.816 | 0.911 | 0.973 |
| G | 0.005 | 0.005 | 0.020 | 0.604 | 0.680 | 0.681 | 0.812 | 0.913 | 0.901 | 0.929 | 0.909 | 1.002 |
| H | 0.005 | 0.006 | 0.207 | 0.581 | 0.710 | 1.014 | 1.010 | 1.013 | 1.015 | 1.049 | 1.038 | 1.008 |

Endpoint
Lm1 530
Automix: Once
Calibrate: Once
Start Read:
1:20 AM 12/29/2011

Wavelength Combination: !Lm1
Mean Temperature: 29.9
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.095
Reader: PLUS384 ROM v1.23 Jun 19 2008

**Figu re5.5: O.D. of 96 well plate: *M. piperita* against Tr, Tm, Ef & Mg where the column 1 showed Negative Control Column 2 Media Control Column 3 Drug Control Column 4-11 Serial dilution of drug and Column 12 Positive Control.**

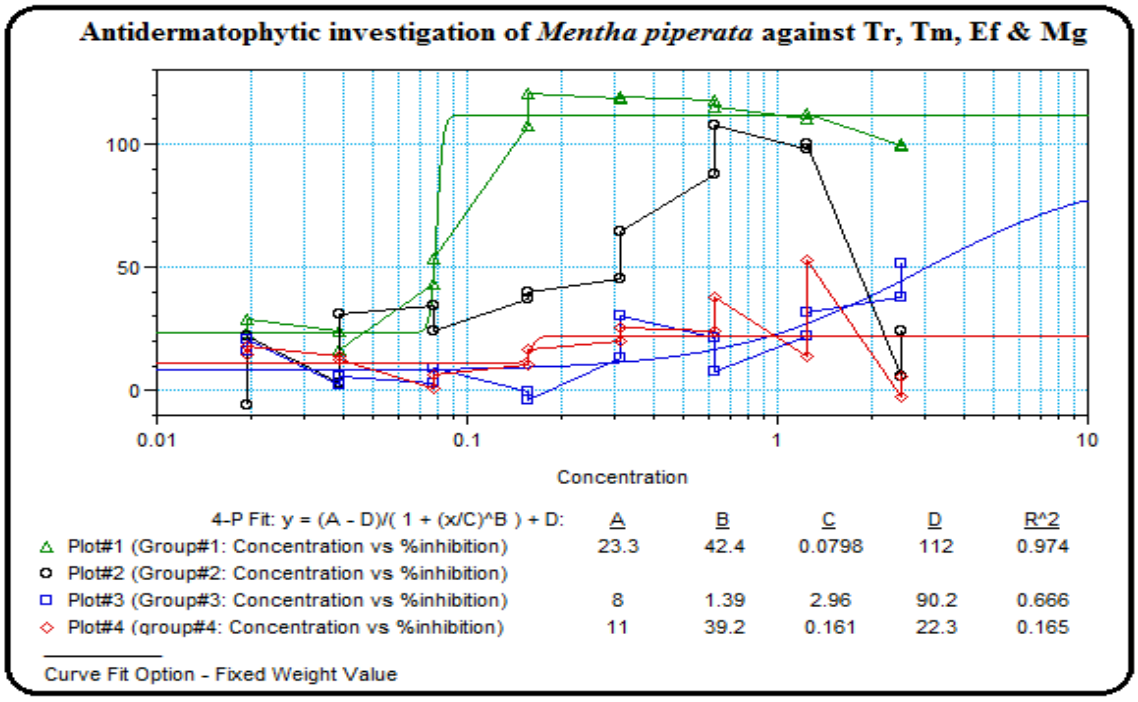



**Figure 5.6: Percentage inhibition of dermatophytes against *M.piperita***

**Table-5.11: Antidermatophytic activities *M. piperita* essential oil against *Tr, Tm, Ef & Mg***

| S. No. | Dermatophytes | Antidermatophytic activities of *M. piperita* (mg/ml) | | |
|---|---|---|---|---|
| | | **MIC** | **$IC_{50}$** | **MFC** |
| **1.** | ***T. rubrum*** | 2.43 | 1.27 | 2.50 |
| **2.** | ***T. mentagrophytes*** | 2.51 | 1.34 | 3.21 |
| **3.** | ***E. floccosum*** | 2.89 | 1.56 | 5.70 |
| **4.** | ***M. gypseum*** | 1.99 | 0.95 | 3.87 |

As per the observations recorded in table-5.11 as well as figures 5.5 and 5.6; the MIC of the oil of *M. piperita* was ranges from 1.99 to 2.89 mg/ ml, and MFC was 2.50 to 5.70 mg/ ml; however, $IC_{50}$ value was ranges from 0.95 to 1.56 mg/ ml against all the test pathogens, respectively.

### 5.4.4 Antidermatophytic activity of *C. aurantifolia* oil against *Tr, Tm, Ef & Mg*

The efficacy of essential oil of *C. aurantifolia* against the against four main dermatophytes *T. rubrum; T. mentagrophytes*; *E. floccosum* and *M. gypseum* recorded are shown in table- 5.12 and Figures 5.7 and 5.8.

**Plate - 4 O.D. of 96 well plate: *Citrus aurantifolia* against Tr, Tm, Ef & Mg**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.001 | 0.000 | 0.024 | 0.034 | 0.012 | 0.027 | 0.426 | 0.589 | 0.648 | 0.657 | 0.714 | 0.716 |
| B | 0.001 | -0.002 | 0.034 | 0.036 | 0.020 | 0.011 | 0.424 | 0.553 | 0.628 | 0.660 | 0.678 | 0.642 |
| C | -0.001 | -0.001 | 0.372 | 0.299 | 0.182 | 0.423 | 0.555 | 0.601 | 0.668 | 0.716 | 0.718 | 0.705 |
| D | 0.000 | -0.005 | 0.275 | 0.221 | 0.302 | 0.516 | 0.544 | 0.633 | 0.693 | 0.735 | 0.697 | 0.752 |
| E | -0.003 | -0.006 | 0.038 | 0.369 | 0.472 | 0.561 | 0.581 | 0.588 | 0.678 | 0.703 | 0.672 | 0.751 |
| F | -0.000 | -0.002 | 0.028 | 0.404 | 0.531 | 0.572 | 0.635 | 0.666 | 0.656 | 0.756 | 0.672 | 0.719 |
| G | -0.002 | -0.001 | 0.278 | 0.491 | 0.634 | 0.697 | 0.672 | 0.689 | 0.682 | 0.662 | 0.665 | 0.777 |
| H | 0.004 | -0.000 | 0.135 | 0.482 | 0.609 | 0.665 | 0.648 | 0.650 | 0.666 | 0.725 | 0.710 | 0.714 |

Endpoint
Lm1 530
Automix: Once
Calibrate: Once
Start Read:
1:52 AM 12/29/2011

Wavelength Combination: !Lm1
Temperature Set Point: 31.0 Mean 28.9
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.261
Reader: PLUS384 ROM v1.23 Jun 19 2008

**Figure 5.7: O.D. of 96 well plate: *C. aurantifolia* against *Tr, Tm, Ef* & *Mg* where the column 1 showed Negative Control Column 2 Media Control Column 3 Drug Control Column 4-11 Serial dilution of drug and Column 12 Positive Control**

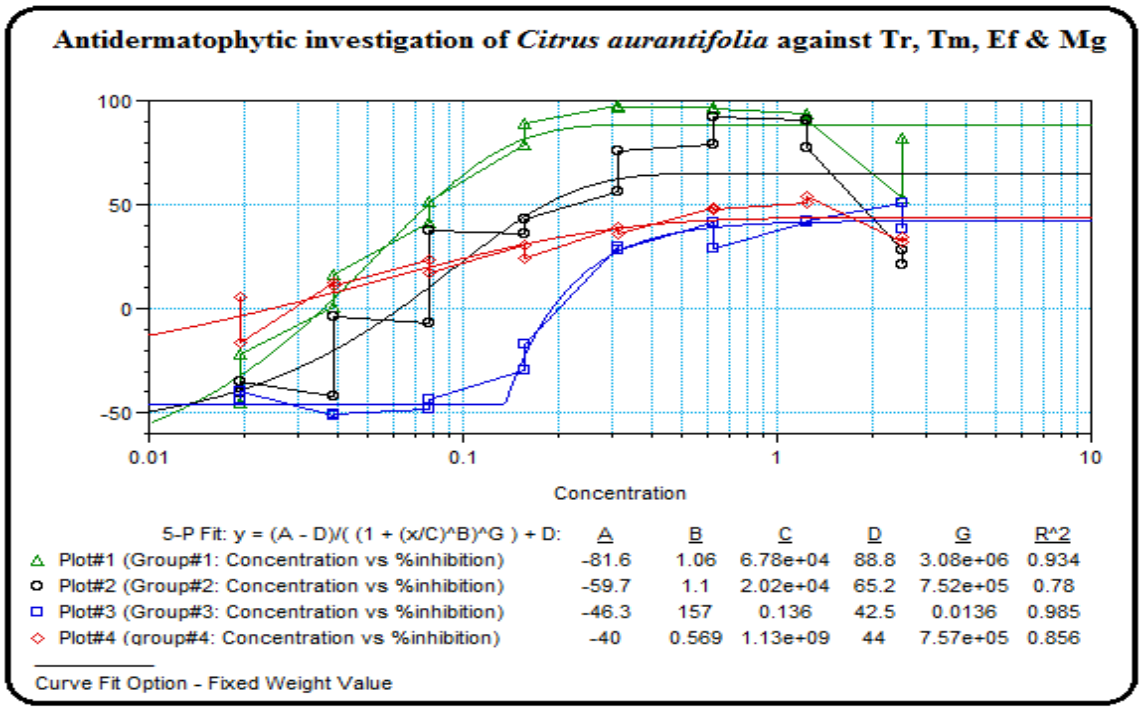



**Figure 5.8: Percentage inhibition of dermatophytes against *C. aurantifolia***

**Table-5.12: Antidermatophytic activities of *C. aurantifolia* essential oil against *Tr, Tm, Ef & Mg***

| S. No. | Dermatophytes | Antidermatophytic activities of *C. aurantifolia* (mg/ml) | | |
|---|---|---|---|---|
| | | **MIC** | **$IC_{50}$** | **MFC** |
| **1.** | ***T. rubrum*** | 2.33 | 1.00 | 2.5 |
| **2.** | ***T. mentagrophytes*** | 1.09 | 0.51 | 2.5 |
| **3.** | ***E. floccosum*** | 1.87 | 0.92 | 2.5 |
| **4.** | ***M. gypseum*** | 1.97 | 0.98 | 2.5 |

As per the observations recorded in table-5.12 as well as figures 5.7 and 5.8; the MIC of the oil of *C. aurantifolia* was ranges from 1.09 to 2.33 mg/ ml, and MFC was 2.5 mg/ ml; however, $IC_{50}$ values were recorded in between 0.95 to 1.56 mg/ ml against the test pathogens, respectively.

### 5.4.5 Antidermatophytic activity of 50% ethanolic leaf extract of *M. micrantha* against *Tr, Tm, Ef & Mg*

The efficacy of 50% ethanolic leaf extract of *M. micrantha* against the against four main dermatophytes *T. rubrum; T. mentagrophytes*; *E. floccosum* and *M. gypseum* recorded are shown in table- 5.13 and Figures 5.9 and 5.10.

**Plate- 5 O.D. of 96 well plate: *Mikania micrantha* against Tr, Tm, Ef & Mg**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.020 | 0.017 | 0.093 | 0.069 | 0.148 | 0.192 | 0.686 | 0.669 | 0.394 | 0.316 | 0.782 | 0.623 |
| B | 0.002 | -0.005 | 0.098 | 0.092 | 0.364 | 0.193 | 0.664 | 0.604 | 0.339 | 1.498 | 2.053 | 0.510 |
| C | 0.000 | 0.028 | 0.090 | 0.062 | 0.039 | 0.027 | 0.018 | 0.025 | 0.079 | 0.022 | 0.018 | 0.009 |
| D | -0.005 | -0.001 | 0.102 | 0.071 | 0.035 | 0.029 | 0.031 | 0.033 | 0.041 | 0.021 | 0.028 | 0.022 |
| E | -0.007 | 0.002 | 0.085 | 0.049 | 0.126 | 0.118 | 0.153 | 0.139 | 0.149 | 0.129 | 0.144 | 0.188 |
| F | -0.006 | -0.004 | 0.090 | 0.089 | 0.125 | 0.137 | 0.130 | 0.147 | 0.134 | 0.145 | 0.116 | 0.164 |
| G | -0.001 | -0.000 | 0.095 | 0.236 | 0.322 | 0.356 | 0.339 | 0.357 | 0.359 | 0.360 | 0.369 | 0.337 |
| H | -0.001 | 0.000 | 0.081 | 0.230 | 0.316 | 0.342 | 0.523 | 0.345 | 0.299 | 0.313 | 0.319 | 0.371 |

Endpoint
Lm1 530
Automix: Once
Calibrate: Once

Start Read:
1:43 AM 12/29/2011

Wavelength Combination: !Lm1
Temperature Set Point: 31.0 Mean 19.2
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.252
Reader: PLUS384 ROM v1.23 Jun 19 2008

**Figure 5.9: O.D. of 96 well plate: *M. micrantha* against Tr, Tm, Ef & Mg where the column 1 showed Negative Control Column 2 Media Control Column 3 Drug Control Column 4-11 Serial dilution of drug and Column 12 Positive Control**

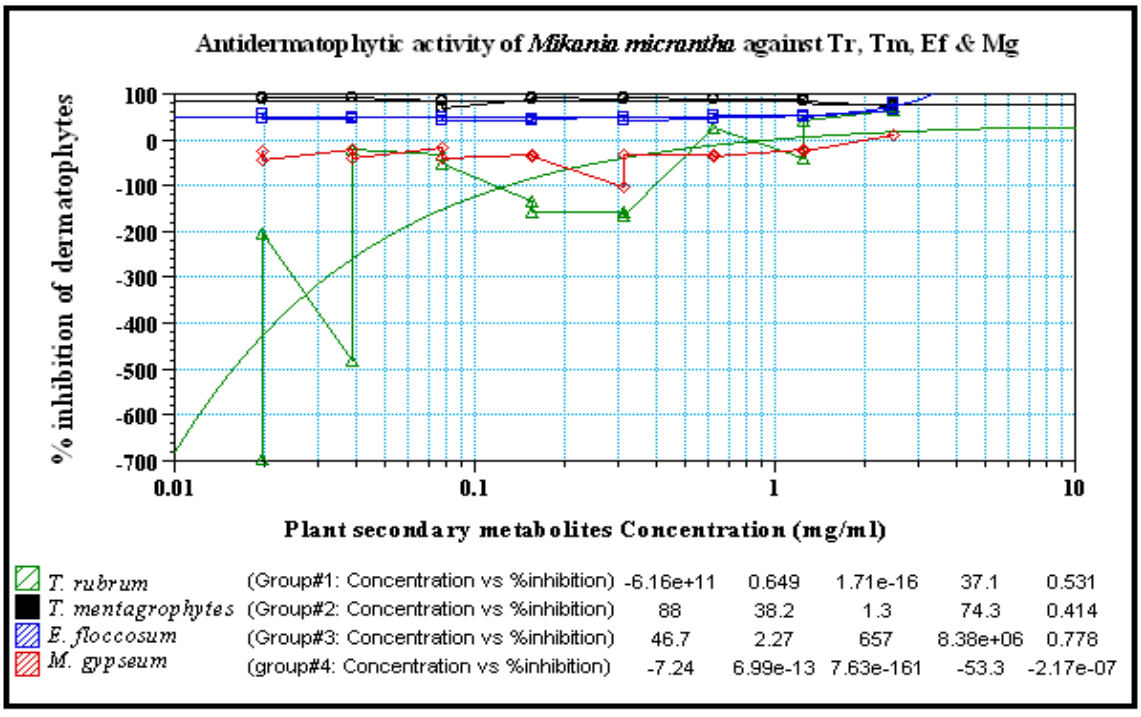



Figure 5.10: Percentage inhibition of dermatophytes against *M. micrantha*

**Table-5.13: Antidermatophytic activities of 50% ethanolic leaf extract of *M. micrantha* (mg/ml)**

| S. No. | Dermatophytes | Antidermatophytic activities of *M. micrantha*(mg/ml) | | |
|---|---|---|---|---|
| | | MIC | $IC_{50}$ | MFC |
| 1. | ***T. rubrum*** | 2.57 | 1.00 | 5.00 |
| 2. | ***T. mentagrophytes*** | 1.31 | 1.00 | 2.50 |
| 3. | ***E. floccosum*** | 2.75 | 0.99 | 5.00 |
| 4. | ***M. gypseum*** | 2.21 | 0.86 | 4.65 |

As per the observations recorded in table-5.13 as well as figures 5.9 and 5.10; the MIC of the 50% ethanolic leaf extract of *M. micrantha*ranges from 1.31 to 2.75 mg/ ml, and MFC was 2.5 to 5.00 mg/ ml; however, $IC_{50}$ values were recorded in between 0.86 to 1.00 mg/ ml against the test pathogens, respectively.

## 5.5 Inoculum Density vis-v-vis Fungicidal Activity

The efficacy of the essential oil of *E. odoratum, O. basilicum, M. piperita, C. aurantifolia; and ethnolic extract of Mikania micrantha*, on inoculum density of the test pathogens- E. floccosum, M. gypseum, Trichophyton mentagrophytes and T. rubrum; was determined, and recorded in table-5.14.

**Table 5.14: Efficacy of the oil/ extract on inoculum density of dermatophytes**

| Essential oils/ active constituents at their MFC | Fungi | Negative C | Media Control | Drug Control | Mycelial Growth Inhibition (MGI) Inoculum as per McFarland Standard (0.5) Serial Dilution | | | | | | | | Positive Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| *E. odoratum* | *E.f.* | - | - | - | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | # |
| | *M.g.* | - | - | - | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | # |
| | *T.m.* | - | - | - | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | # |
| | *T.r.* | - | - | - | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | # |
| *O. basilicum* | *E.f.* | - | - | - | +++ | +++ | +++ | +++ | ++ | ++ | ++ | ++ | # |
| | *M.g.* | - | - | - | +++ | +++ | +++ | +++ | +++ | ++ | + | + | # |
| | *T.m.* | - | - | - | +++ | +++ | +++ | ++ | +++ | ++ | + | - | # |
| | *T.r.* | - | - | - | +++ | +++ | +++ | ++ | ++ | + | + | - | # |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *M. piperita* | *E.f.* | - | - | - | +++ | +++ | +++ | ++ | ++ | + | | - | # |
| | *M.g.* | - | - | - | +++ | +++ | +++ | +++ | +++ | ++ | + | + | # |
| | *T.m.* | - | - | - | +++ | +++ | +++ | +++ | +++ | ++ | ++ | + | # |
| | *T.r.* | - | - | - | +++ | +++ | +++ | +++ | +++ | + | + | + | # |
| *C. aurantifolia* | *E.f.* | - | - | - | +++ | +++ | +++ | +++ | + | + | - | - | # |
| | *M.g.* | - | - | - | +++ | +++ | +++ | +++ | ++ | ++ | - | - | # |
| | *T.m.* | - | - | - | +++ | +++ | +++ | +++ | + | + | - | - | # |
| | *T.r.* | - | - | - | +++ | +++ | +++ | +++ | + | + | - | - | # |
| *M. micrantha* | *E.f.* | - | - | - | + | - | - | - | - | - | - | - | # |
| | *M.g.* | - | - | - | + | + | + | - | - | - | - | - | # |
| | *T.m.* | - | - | - | ++ | + | - | - | - | - | - | - | # |
| | *T.r.* | - | - | - | + | + | - | - | - | - | - | - | # |

- Negative Control = only Formaldehyde
- Media Control = only media
- Drug Control = Drug+ Media
- Positive Control = Media+ Culture
- *E.f. = E. floccosum*
- *M.g. = M. gypseum*
- *T.m.= T. mentagrophytes*
- *T.r.= T. rubrum*
- - = No fungal growth inhibition
- + = 25% growth inhibition
- ++ = 50% growth inhibition
- +++ = 100% growth inhibition
- # = fungal growth observed

As per the observations made from table-5.14, the minimum fungicidal concentration (MFC) of the test samples persisted heavy inoculum density of the test pathogens (dermatophytes) against the tested essential oil; however, in case of *M. micrantha*, it was recorded only 25% growth inhibition at $4^{th}$ & $5^{th}$ wells.

## 5.6 Comparison with some Synthetic Fungicides/ Antifungal drugs

The efficacy of these plant constituents (essential oils/ extract) was also compaired with some synthetic antifungal drugs, available in the market such as Dactrine; Nizaral and Tenderm. This was determined by comparing their minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs), respectively **(Table 5.15).**

**Table- 5.15: Comparative MICs of the test samples with some Synthetic Antifungal Drugs**

| Oil & Trade Name of Antifungal Drugs | **Active Ingredients** | **Minimum Inhibitory Concentration (mg/ml)** | | | |
|---|---|---|---|---|---|
| | | *E. floccosum* | *M. gypseum* | *T. mentagrophytes* | *T. rubrum* |
| ***E. odoratum*** | E.O. | 1.38 | 0.96 | 0.59 | 0.68 |
| ***O. basilicum*** | E.O. | 2.48 | 1.24 | 1.89 | 1.71 |
| ***C. aurantifolia*** | E.O. | 1.87 | 1.97 | 1.09 | 2.33 |

| | | | | | |
|---|---|---|---|---|---|
| ***M. piperita*** | E.O. | 2.89 | 2.21 | 1.31 | 2.57 |
| **Dactrine** | Miconazole Nitrate | 6.0 | 6.0 | 6.0 | 6.0 |
| **Nizaral** | Ketoconazole | 6.0 | 0.5 | 5.0 | 5.0 |
| **Tenaderm** | Tolnaftate | 2.0 | 1.5 | 0.8 | 0.8 |

As per the observation recorded in table 5.15; the MIC of *E. odoratum* have the strongest toxicity against all the test pathogens (ranges from 0.59-1.38 mg/ml) followed by *C. aurantifolia* (ranges from 1.09-2.33 mg/ml); which shows an edge over the synthetic antifungal (Dactrine, Nizaral and Tenaderm), where the efficacy ranges from 0.5- 6.0 mg/ml.

## 5.7 Fungicidal activity of the selected plants: an over view

The comparative analysis (MIC, $IC_{50}$ and MFC) of the oils of *E. odoratum* L., *O. basilicum; M. piperita* L., *C. aurantifolia* L. and 50% leaf extract of *M. micrantha* Kunth ex H.B.K against the test pathogerns viz., *E. floccosum ;M. gypseum; T. mentagrophytes and T. rubrum*; are summarized **(Table-5.16)**

**Table: 5.16 Antidermatophytic activities of selected plant secondary metabolites against four dermatophytes reported as MIC, $IC_{50}$ and MFC in (mg/ml)**

| S N | Samples | Antiderma-tophytic Parameters | Antidermatophytic activity of selected plant secondary metabolites against four dermatophytes | | | | |
|---|---|---|---|---|---|---|---|
| | | | Essential oils | | | | 50 % ethanolic extract |
| | | | ***E. odoratum*** | ***O. basilicum*** | ***C. aurantifolia*** | ***M. piperita*** | ***Mikania micrantha*** |
| **1.** | ***E. floccosum*** | MIC | 1.38 | 2.48 | 1.87 | 2.89 | 2.75 |
| | | $IC_{50}$ | 1.32 | 1.18 | 0.92 | 1.56 | 0.99 |
| | | MFC | 2.5 | 3.2 | 2.5 | 5.70 | 5.00 |
| **2.** | ***M. gypseum*** | MIC | 0.96 | 1.24 | 1.97 | 1.99 | 2.21 |
| | | $IC_{50}$ | 0.54 | 0.66 | 0.98 | 0.95 | 0.86 |
| | | MFC | 1.25 | 2.5 | 2.5 | 3.87 | 4.65 |
| **3.** | ***T. mentagrophytes*** | MIC | 0.59 | 1.89 | 1.09 | 2.51 | 1.31 |
| | | $IC_{50}$ | 0.13 | 0.97 | 0.51 | 1.34 | 1.00 |
| | | MFC | 1.25 | 2.5 | 2.5 | 3.21 | 2.5 |
| **4.** | ***T. rubrum*** | MIC | 0.68 | 1.71 | 2.33 | 2.43 | 2.57 |
| | | $IC_{50}$ | 0.17 | 0.90 | 1.00 | 1.27 | 1.00 |
| | | MFC | 1.25 | 2.5 | 2.5 | 2.5 | 5.00 |

As per the observation recorded in table 5.16; two of the test pathogens viz., *E. floccosum* and *T. mentagrophytes*, show similar sequence of plant's efficacy i.e. *E. odoratum > C. aurantifolia > O. basilicum> M. piperita*; however *M. gypseum* and *T. rubrum* shows similar sequence of plant's efficacy i.e. *E. odoratum > O. basilicum > C. aurantifolia > M. piperita.*

Moreover, *E. odoratum* shows the strongest potency (ranges from 0.59-1.38 mg/ml) and *M. piperita* shows the weakest potency (ranges from 1.99-2.89 mg/ml) against all the four dermatophytes.

## 5.8 Identification of active constituents from selected plant essential oils

The essential oils of *E. odoratum, M. piperita, O. basilicum* and *C. aurantifolia* were further subjected to identification of their active constituents.

### 5.8.1 Identification of constituents of *E. odoratum,* using GC MS analysis

The GC-MS analysis of *E. odoratum* oil shows **α-pinene** (27.50%) **pregeijerene** (14.50%) and **Geijerene** (12.20%) as the major constituents (Table 5.17).

**Table-5.17: Constituents from *E. odoratum* essential oil**

| **Constituents** | **RI** | **% of constituents** |
|---|---|---|
| Sabinene | 934 | 1.52 |
| β-pinene | 968 | 10.20 |
| Myrcene | 979 | 2.20 |
| **α-pinene** | **986** | **27.50** |
| α-Terpinene | 995 | 0.40 |
| Limonene | 1024 | 1.10 |
| γ-terpinene | 1035 | 3.21 |
| (Z)- β-Ocimene | 1050 | 0.23 |
| Myrtenol | 1137 | 0.53 |
| Geijerene | 1148 | 12.20 |
| isogeijerene C isomere | 1241 | 0.10 |
| Pregeijerene | 1247 | 14.50 |
| Isogeijerene | 1265 | 1.00 |
| pregeijerene isomere | 1277 | 1.20 |
| α-copaene | 1339 | 1.30 |
| trans-β-caryophyllene | 1465 | 6.60 |
| α-humulene | 1485 | 2.10 |
| β-cubebene | 1495 | 1.20 |
| germacrene-D | 1499 | 9.80 |
| δ-cadinene | 1534 | 2.80 |

### 5.8.2 Identification of constituents of *M. piperita* oil, using GC MS analysis

The GC-MS analysis of *M. piperita* essential oil resulted in total of 14 components (Table 5.18). Out of these, 2 Constituents i.e. Menthol 37.20% and Menthone 22.52% was the major Constituents

**Table 5.18 Constituents from *M. piperita* essential oil**

| Constituents | RI | % of constituents |
|---|---|---|
| α- pinene | 938 | 0.68 |
| β- pinene | 944 | 1.12 |
| d- limonene | 956 | 3.31 |
| Sabinene | 965 | 0.38 |
| Isomenthol | 979 | 0.44 |
| **Menthol** | 1002 | **37.20** |
| Neomenthol | 1029 | 3.56 |
| Isomenthone | 1048 | 4.70 |
| Isopulegone | 1079 | 0.09 |
| Menthone | 1121 | 22.52 |
| Pulegone | 1149 | 3.70 |
| Isomethyl acetate | 1176 | 0.06 |
| methyl acetate | 1237 | 4.18 |
| 1,8- cineole | 1261 | 4.68 |

### 5.8.3 Identification of constituents of *Oscimum bascilicum* oil, using GC MS analysis

The GC- MS analysis of the essential oil of *Oscimum bascilicum* shows methyl chevicol (71.25%) and linalyl acetate (19.32%) detected as the major **Constituents** followed by α-cubabene (5.19).

**Table 5.19 Constituents from *O. bascilicum* essential oil**

| Constituents | RI | % of constituents |
|---|---|---|
| camphene | 933 | 0.02 |
| α-pinene | 946 | 0.05 |
| β-pinene | 977 | 0.10 |
| Limonene | 1037 | 0.19 |
| 1,8-cineole | 1043 | 0.09 |
| cis-ocimene | 1049 | 0.23 |
| β-ocimene | 1059 | 0.08 |
| linalool | 1097 | 0.31 |
| **methyl chevicol** | **1168** | **71.25** |
| (Z)-citral | 1259 | 0.31 |
| linalyl acetate | 1286 | 19.32 |
| (E)-citral | 1247 | 0.52 |
| trans-caryophyllene | 1298 | 0.27 |
| carvacrol | 1309 | 0.44 |
| α-cubabene | 1355 | 6.22 |
| α-humulene | 1460 | 0.12 |
| β -farnesene | 1482 | 0.20 |
| β -selinene | 1494 | 0.25 |

### 5.8.4 Identification of constituents of *C. aurantifolia* oil, using GC MS analysis

The GC-MS analysis of *Citrus auranifolia* essential oil shows 17 Constituents. Among these, limonene (54.20%), γ-terpinene (14.23%) and terpinolene (11.02%) were the major constituents (Table 5.20).

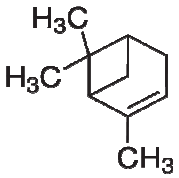
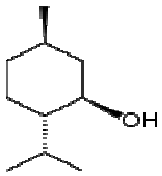
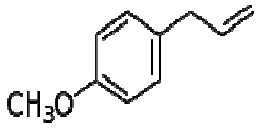
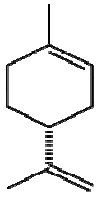
**Table-5.20: Constituents from *C. aurantifolia* essential oil**

| **Constituents** | **RI** | **Constituents (%)** |
|---|---|---|
| α-thujene | 923 | 0.32 |
| α-pinene | 930 | 0.60 |
| β-pinene | 969 | 1.10 |
| Sabinene | 972 | 0.27 |
| Myrcene | 988 | 0.94 |
| 3-Carene | 1006 | 1.62 |
| αTerpinene | 1013 | 2.57 |
| ρ-Cymene | 1018 | 1.69 |
| **D-Limonene** | **1025** | **54.20** |
| α-Terpineol | 1051 | 6.20 |
| Trans-α-Bergamotene | 1096 | 1.41 |
| γ-Terpinene | 1135 | 14.23 |
| Terpinolene | 1167 | 11.02 |
| Benzoic acid | 1181 | 1.42 |
| β-Caryophyllene | 1357 | 0.78 |
| α-Cedrene | 1442 | 1.15 |
| α-Bisabolene | 1543 | 0.48 |

## 5.9 Description of major compounds from bioactive essential oil

Constituents were identified by comparing the retention indices of the peaks on the BP-1 column with literature values, computer matching against the library spectra built up using pure substances and components of known essential oils, and finally confirmed by comparison of mass spectra with published data. Further, the general properties of these selected essential oils are recorded as follows (Table-5.21):

**Table-5.21: General properties of the selected plants**

| **Bioactive essential oils** | **Code** | **Name of Major Compound** | **Mol. formula** | **Molar mass ( g/mol)** | **Composition (%)** | **Chemical struchure** |
|---|---|---|---|---|---|---|
| ***E. odoratum*** | E-Eo-1 | α-Pinene | $C_{10}H_{16}$ | 136.23 | 27.50 |  |
| ***M. piperita*** | E-Mp-5 | Menthol | $C_{10}H_{20}O$ | 156.27 | 37.20 |  |
| ***O. basilicum*** | E-Ob-6 | methyl chevicol | $C_{10}H_{12}O$ | 148.20 | 71.25 |  |
| ***C. aurantifolia*** | E-Ca-7 | D-Limonene | $C_{10}H_{16}$ | 136.24 | 54.20 |  |

## 5.10 Physico-chemical properties of the selected essential oils

The selected essential oils were also studied on some common physio-chemical properties viz., appearance, boiling point, odor, specific gravity, optical rotation, refractive index and solubility in water; and recorded in table-5.22.

**Table-5.22: Physico-chemical properties of selected essential oils**

| Parameter studies | *E. odoratum* | *O. basilicum* | *M. piperita* | *C. aurantifolia* |
|---|---|---|---|---|
| **Plant height** | 1-3 M | 1-1.5 M | 30-95 Cm | 2 to 6 M |
| **Appearance** | Light yellow liquid | Pale yellow to dark yellow | Light yellow to clear in colour | Light olive to pale yellow in colour |
| **Odur** | Floral | Characteristic of basil, spicy | Menthol smell | Bitter orange odor |
| **Boiling Point** | 85°C | 95°C | 90°C | 85°C |
| **Specific gravity at $20^0$C** | 0.860 - 0.885 | 0.905 - 0.955 | 0.900 - 0.912 | 0.845-0.855 |
| **Optical rotation** | -3.00 to -2.00 | +1.00 to -18 | -16 to -30 | $-4^0$50' to $-5^0$40' |
| **Refractive index at $20^0$C** | 1.475 – 1.490 | 1.500 – 1.520 | 1.487 - 1.515 | 1.465 - 1.485 |
| **Solubility in water** | Insoluble | Insoluble | Insoluble | Insoluble |

## 5.11 Solubility in various organic solvents

The solubility of all selected secondary metabolites in various organic solvents was also investigated and it was found that the oils were soluble in 13 different organic solvents viz., acetone, alcohols, benzene, chloroform, carbon tetrachloride, dimethyl sulphoxide (DMSO), ethanol, hexane, methanol, n-butanol, petroleum ether and propanol solvent ether; used for testing. However, the oils were insoluble in water (Table- 5.23).

**Table-5.23: Solubility of plant metabolites in different organic solvents (1:1) ratio**

| S. No. | Organic Solvents | Selected Plant Essential oils and 50 % ethanolic extract | | | | |
|---|---|---|---|---|---|---|
| | | ***E. odoratum*** | ***O. basilicum*** | ***M. piperita*** | ***C. aurantifolia*** | ***Mikania micrantha*** |
| 1. | Hexane | + | + | + | + | + |
| 2. | Petroleum ether | + | + | + | + | + |
| 3. | Benzene | + | + | + | + | + |
| 4. | Chloroform | + | + | + | + | + |
| 5. | Carbon tetrachloride | + | + | + | + | + |
| 6. | Solvent ether | + | + | + | + | + |
| 7. | N-Butanol | + | + | + | + | + |
| 8. | Propanol | + | + | + | + | + |
| 9. | Methanol | + | + | + | + | + |
| 10. | Ethanol | + | + | + | + | + |
| 11. | Acetone | + | + | + | + | + |
| 12. | Alcohols | + | + | + | + | + |
| 13. | DMSO ( dimethyl sulphoxide) | + | + | + | + | + |

+ indicate solubility; - indicate insolubility

## 5.12 Scopes and issues related to Intellectual Property Rights

The present research works was aimed to collect, categorize and identify some commonly known plants from the North Eastern part of India, validate their anti-dermatophytic activity and identifies the scope and issues related to Intellectual Property Rights. Preliminary screening and antidermatophytic investigations on the selected plants was followed by search on IPR (patent) activity which was conducted on various patent sites viz., WIPO, USPTO, EPO, IPO as well as on by Google search.

Trends in (i) patent filing and grant across the world; (ii) patent grant in the fields of technologies especially in the field of 'pharmaceuticals'; (iii) patents on selected plants showing antidermatophytic activity; (iv) patent activities in India and its North East region specially in the area of drugs and pharma were mapped by using keywords = selected plant's name i.e. *E. odoratum* L, *M. piperita* L. *O. basilicum, C. aurantifolia*

L.and *M. micranthaa*s an *antidermatophyte; keyword*= selected plant name + dermatophyte's name, i.e. *E. floccosum, M. gypsum, T. mentagrophytes* and *T. rubrum; keyword= India/ North Eastern states of India.* IPR (Patent activity) from North East India was conducted on significant patent office sites including on the Indian Patent Office site (IPO) by using the key words = name of the state i.e. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura as well as the key words = selected plant's name and/or dermatophyte's name. Patents grants for North East India in the field of pharmaceuticals and drugs were searched.

However patent search broadly related to present investigations indicated grant of few patents on the products/processes on : antidermatophytic agents/ antifungal or antimicrobial agents/ or on other features of the plants selected in the present study, as follows:

**Illustrations of Patents on Antidermatophytes**

- A US patent (USPTO No. 4202877 date 13 May 1980) entitled "**Antidermato-**mycotic agent" was granted to Masaki Sato *et al.,* for invention on Pharmaceutical compositions and a method for **treating dermatophytes** based upon the antidermatophytic activity of o-methoxycinnamaldehyde.

- Patent (No. 2006/0073218 A1,US7429396 date Sep 30, 2008) on "Antifungal composition, its fungicidal effect on **pathogenic dermatophytes** was also granted to Frank S. D'Amelio, Youssef W. Mirhom for a natural preservative composition obtained from plant materials including **selective mixtures** of *Origanum vulgare L., Thymus vulgaris L., Cinnamomum zeylanicum Nees, Rosmarinus officinalis L., Lavandula officinalis L., **M. piperita** L., Citrus Limon L., Hydrastis Canadensis L. and Olea europaea L.* that provides antimicrobial activity for use as an antifungal agent effective in inhibiting the growth of ***E. floccosum**, T. mentagrophytes and M. canis.*

- Another US patent ( USPTO No. 6312698 date Nov 6, 2001) entitled "Anti-**fungal formulation** active against a broad spectrum of dermatophytoses" was granted to Shahi *et al.,* for the invention that provides a novel anti-fungal formulation active against a broad spectrum of **dermatophytoses,** said formulation comprising at least about 1% by weight of oil extracted from *Rabdosia melissoides* and one or more vegetable oils, solvents and additives.

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**Illustrations of Patents on Antifungal/ antimicrobial agents**

- A Patent (US USPTO 8333981 B2 18.Dec 2012) on "Antifungal treatment of nails Granted to John Olin Trimble, Humco Holding Group, Inc provided invention on **fungus** treatment composition to deliver active drugs trans-nail as well as a method for producing the **fungus treatment** composition, which may contain up to 50% additive ingredients.

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- European/US patent was granted (EP 2451468 A2 date 8 July 2009/ US20110008474) for invention on "**Topical antifungal composition**" to J. Charles Boegli Onikolabs, for a composition obtained primarily from plant materials and provides antimicrobial activity for use as an anti-fungal agent. The anti-fungal agent is effective in inhibiting the growth of *T. rubrum*, the fungus that is the most common cause of *Tinea pedis*. The composition includes selective mixtures of the origanum oil, menthol, and Atlantic cedarwood oil, thuja oil, cedarwood oil, cinnamon oil, clove oil, cumin oil, fennel oil, peppermint oil, or rosemary.

- A European patent (EP 1143986 A3 / WO2000024411A2) entitled "Plant-derived anti-parasitic and **antifungal compounds** and methods of extracting the compounds" provided biologically active **extracts** from *Aframomum aulocacarpus, Aframomun danellii, Dracaena arborea,* ***E. odoratum****, Glossocalyx brevipes and Napoleonaea imperialis* which are suitable for use in treating fungal and protozoa diseases.

- Patent (No.. WO/2005/087244, WO/2011/110793 JP2001151689) entitled "Antimicrobial composition for treating **microbial infections** and preventing microbial contamination of surfaces and devices" issued to Stringer, Jacqueline, Unversity of Manchester, provided compositions comprising **at least two essential oils** derived from a plant genus *Pelargonium; Cymbopogon;* ***Mentha;*** *Aniba; Lavandula; Origanum; Litsea;* ***Citrus****; Melissa; Pogostemon; Santalum; Valeriana; Styrax; Cinnamonu; and Rosa,.*

**Illustrations of Patents on other features of the plants selected in the present study**

- A European patent (EP 1023436 A4 date *16 oct 1997* ) entitled Geranyl diphosphate synthase from mint (***M. piperita***) was granted to C. Charles Burke *et al.,* for providing **system and method** for recombinant expression of geranyl diphosphate synthase, to enhance the production of monoterpenoids, to produce geranyl diphosphate in cancerous cells .

- A US patent (US 6582736 date 24 Jun 2003) entitled 'A therapeutic oil composition for topical application to **painful areas** of the human body was granted to Richard S. Quezada, for creation of therapeutic oil composition **by mixing** *Eugenia caryophyllata, Myroxyon pereira, Eucalyptus globulus, Lavandula augustifolia, M. piperita,* **and** ***Mentha*** *spicata*

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- Chinese patent (No. 20040096418 , Chinese 1489454 date 14.4.2004) titled Cosmetically effective composition containing *Malva sylvestris* and *Mentha piperita* extracts granted to Gafner, Thomas relates to invention for the production of **cosmetically effective** compositions that lighten the skin, wherein said concentrate contains at least one extract (i) of mallow (*Malva sylvestris)* and (ii*)* ***peppermint (Mentha*** *piperita)*

- Patent No. 10255 1235A 11.7.2012 on "**Anti cold** mask impregnated with wild ***Ocimum bascillicum*** essential oil" was issued to Inventor Dong Shixian.

- US patent (US7435877 date Oct 14, 2008) entitled "Distinct type cultivar of ***Ocimum basilicum*** "CIM-SAUMYA" patented to Khanuja *et al.,* for invention relating to the development of an early, short duration, dwarf, **high essential oil,** methyl chavicol and linalool yielding variety of Indian basil (*O. basilicum*), through open pollination in the germplasm followed by half-sib progeny selection and evaluation for the yield characters of selected population for 3 years in field conditions. The new cultivar possesses better growth and vegetative growth.

Patent search also revealed that some product/process patents in the broad field relating to "skin" are available which pertained to: whitening agent; greying of hair; pain & inflammation relief. It is therefore evident from the patent search that few patents were available on the products/processes on antidermatophytic agents but no specific patents

were found on plant products with antidermatophytic activity, selected for the present study.

Search for Patents awarded to the Applicants/ inventor from the State of North East Region and those having a mention in the topic/ abstract of the patent showed number of patents in the name of the state of Assam(121), Meghalaya(2), Sikkim and Tripura but no patent was granted in the field of drugs and pharma and on the selected plant metabolites showing antidermatophytic activity. Moreover there was no patent filed/ granted in the name of state of Mizoram.

Non-existence of IPR (Patent) pertaining to antidermatophytic activity of the investigated plants imply that there is great scope for further *in- vivo* investigations, pre clinical trials, multilocational clinical trials as well as isolation and synthesis of the bioactive molecule(s) for drug formulation (i.e. product patent)/ technology transfer (i.e. process patent) to the pharmaceutical companies; so that, the validated information can be converted into the technological information for health and welfare of the society.

# 6. Discussion

Most of the prevalent synthetic antifungal drugs are known to cause various side effects. Moreover, the increasing resistance to synthetic antifungal compounds and the reduced number of available drugs demand search for therapeutic alternatives among aromatic plants and their essential oils. The present investigation was therefore undertaken with the objectives to investigate the *in vitro* antifungal activity from the secondary metabolites of some traditionally used ethnomedicinal plants against dermatophytes viz.,*E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum* in order to contribute in the future towards development of potential, indigenous antidermatophytic entity; scopes related to intellectual property right as well as their proper documentation before they are lost foreover. In the light of above, the findings of the present study have been discussed as follows:

## 6.1 Collection, categorization, identification and documentation of common ethnomedicinal plants, used against skin ailments among the tribal communities

Literatures reveal that there are various methods for selection of plants for investigating their bioefficacy. It may be based on extensive field survey or based on thorough antimicrobial screening. However, the findings of the present investigations are based on extensive field work, secondary information from locally available literatures and personal interviews with local practitioners followed by their screening.

In Mizoram, treatment of illness using medicines derived from plants is an integral part of the local culture, and information about plants and their uses is passed on through oral folklore. Transmission of knowledge occurs primarily amongst the elderly; they are the natural retainers of traditional knowledge in their communities. Information on the use of medicinal plants was obtained through structured and semi structured questionnaires, complemented by free interviews and informal conversations. Moreover, during the field survey, local practitioners and others with knowledge of plants were consulted. Interviews were conducted in the field during collection trips and by examination of freshly collected specimens with informants, after seeking their oral consent. Inquiries on the prevalence, types, mode of transmission and symptoms of skin ailments were made by interviewing local people.

Further, based on these findings; a priority based list of fifteen most frequently used plants against skin ailments were shortlisted, and identified with the help of floras (Hooker, 1872-1892; Duthie, 1903-1929; Maheshwari, 1963; Santapau, 1967 and Gupta, 1968), and the authentic herbarium/ specimens lodged in the Duthie herbarium of the Department of Botany, University of Allahabad as well as their confirmations were made with the Botanical Survey of India, Allahabad. The plants thus identified were deposited to the herbarium of the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl; for future references, and the same were selected for further investigations (table 5.1).

## 6.2 Selection and Screening of the Plants

Literature reveals that large number of plants belonging to different families, genera and species have been screened mostly against plant pathogens, many of those pathogens were reported as opportunistic fungal pathogens (non-dermatophytic moulds) causing dermatophytosis (Gupta and Banerjee (1970); Mishra *et al.,* (1974); Dixit *et al.,* (1978); Dikshit (1980); Pandey *et al.,* (1983a); Tripathi *et al.,* (1985); Antonio and Mantilla (1986); Deshmukh *et al.* (1986); Dikshit *et al.,* (1986); Mall (1987); Mishra (1991), Shahi *et al.,* (1996a); Amvam Zolla *et al.,* (1998); Shahi *et al.,* (2001a, b), Rajendra *et al.,* (2004); Premshankar *et al.,* (2005), Singh *et al.,* (2007), Shukla *et al.,* (2011).

However, in the present investigation, based on the literature survey as well as local field visits, a list of fifteen ethnomedicinal plants were selected for screening against the test pathogen *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum;* the dermatophytes causing ringworm infection in human beings.

**6.2.1** The secondary metabolites from plants of different families show different level of antifungal activity and differ from family to family. Gilliver (1947) found strong antifungal activity in the members of Chenopodiaceae, Ranunculaceae, Myrsinaceae, Primulaceae, Sapotaceae, Ebenaceae, Solanaceae, Asteraceae, Liliaceae, Dioscoreaceae, Brassicaceae, Saxifragaceae, Hamamelidaceae, Pittosporaceae, Araliaceae, Cornaceae, Apiaceae and Theophrastaceae. Petrushova (1960) screened 184 families and reported that the members of Anacardiaceae, Asteraceae, Brassicaceae, Lamiaceae, Liliaceae, Ranunculaceae, Rosaceae and Solanaceae possess greater antifungal activity than the others. Abdullaeva (1959) screened 70 families and reported Poaceae, Araceae, Liliaceae and Papilionaceae to

be more activity than other families. Hajek (1961) found legumes to contain stronger antifungal activity than grasses. Bhakuni *et al.,* (1971) tested 297 species of 86 families and found the member of Boraginaceae to be more effective. Dhar *et al.,* (1973) screened 287 species of 54 families and recorded the members of family Polygonaceae, Theaceae and Liliaceae to contain strong antifungal activity. Later, Dhar *et al.,* (1973) reported that the members of family Guttiferae were more fungitoxic than other investigated families. Mishra (1975) reported Acanthaceae, Apocynaceae, Combretaceae, Liliaceae, and Ranunculaceae have strong antifungal activity. Euphorbiaceae, Papilionaceae, Lythraceae, Anacardiaceae and Combretaceae have stronger fungitoxic properties as reported by Tripathi (1977). Chaturvedi (1979) observed the Bignoniaceae to be the most effective, among the families studied. Dikshit (1980) screened 49 families, out of which only the members of family Rutaceae and Lamiaceae exhibited strong fungitoxicity. Renu (1981) tested 56 families and recorded only the member of Rutaceae and Solanaceae to extibit strong fungitoxicity. Asthana (1984) reported Lamiaceae, and Chandra (1984) found Asteraceae to be more fungitoxic than other families tested. Kishore (1985) reported the family Chenopodiaceae to contain the maximum fungitoxicity. Mall (1987) and Gupta (1988) reported Asteraceae and Arecaceae respectively to contain strong fungitoxicity. Mishra (1991) reported that out of 72 families screened only the members of family Lamiaceae and Verbinaceae possessed strong antifungal properties. Yadav (1995) observed that Rutaceae, Myrtaceae, Poaceae, Lamiaceae are the most potent families against fungi. Shahi (1997) investigated the potency of Poaceae and Apiaceae against dermatophytes, Ali *et al.,* (1999); Shahi *et al.,* (1999 a,b,c) reported the potentiality of Myrtaceae family against dermatophytes. The bioactivity of Liliaceae family was investigated by Kawai *et al.,* (1998) and Ali *et al.,* (1999). Shahi *et al.,* (2002b) reported the potentiality of Poaceae family against non-dermatophytic moulds. Fabaceae family was also found to be active against yeast and dermatophytes according to Villa Senor *et al.,* (2002) and also by Rajendra Prasad (2004). Shukla *et al.,* (2011) reported Plants from *Zingiberaceae* to be strong toxicant against *E. floccosum, M. gypseum* and *T. rubrum*.

However, in the present investigation out of selected plant families viz., Asteraceae, Lauraceae, Amaranthaceae, Poaceae, Apiaceae, Euphorbiaceae, Rubiaceae, Lamiaceae, Myrtaceae, Fabaceae, and Moringaceae; the plants belonging

to families Asteraceae and Lamiaceae show strong antidermatophytic activity against the test pathogens.

**6.2.2** Various workers observed the variations in the antifungal activity from genus to genus within a family. Mishra (1975) reported *Allamanda* of Apocyanaceae, *Terminalia* of Combretaceae, *Allium* of Liliaceae and *Clematis* of Ranunculaceae to possess strong antifungal activity while other genera of respective families were less effective. Similarly out of three tested genera of the family Rutaceae only *Aegle* showed strong antifungal activity (Renu 1981). *Ocimum* of family Lamiaceae exhibited strong fungitoxicity while 12 other genera of the family showed moderate activity as reported by Asthana (1984). Dikshit and Husain (1984) observed that out of 4 genera of family Apiaceae only *Anethum* showed strong antifungal activity. *Melissia* of the family Lamiaceae have strong antifungal activity than other genera *Lavandula* and *Mentha* of the same family. Out of 11 genera belonging to the family Verbenaceae, only *Vitex* extibited strong fungitoxicity while the remaining ones showed poor activity, similarly out of six genera of family Lamiaceae screened only *Nepeta* exhibited strong antifungal activity while other showed moderate activity (Mishra 1991). Hajji *et al.,* (1993) reported the activity of Mrytaceae family against yeast and non-dermatophytic moulds, Kishore *et al.,* (1993) showed the bioactivity of Asteraceae, Chenopodiaceae, Poaceae and Lamiaceae against dermatophytes. Yadav and Dubey (1994) reported the activity of Rutaceae and Lamiaceae against dermatophytes. Fabaceae family found to be bioactive according to the study of Iyenger *et al.,* (1995); Mukherjee *et al.,* (1996) and Villa Senor *et al.,* (2002b). Pandey *et al.,* (1996); Pandey (1997); Pandey *et al.,* (1997); Shahi (1997); Shahi *et al.,* (1997a, 2002b) reported Poaceae family found to be effective against fungal pathogens. Nenoff *et al.,* (1996); Shahi *et al.,* (1997b); Shahi *et al.,* (1998a); Ali *et al.,* (1999); Shahi *et al.,* (1999a, b); reported the bioactive potentiality of Mrytaceae family. However In the present investigation, **Asteraceae** (*E. odoratum; Mikania micrantha)* and **Lamiaceae** *(O. basilicum; C. aurantifolia; Mentha piperita)* show the strong efficacy.

**6.2.3** Variation in antifungal activity among different species of the same genus has also been observed by several workers (Mishra 1975; Chaturvedi 1979; Dikshit 1980; Singh 1980; Asthana 1984; Dikshit and Husain 1984; Kishore 1985; Mishra 1991, Shahi 1997; Ali *et al.,* 1999; Shahi *et al.,* 1999c). Shukla *et al.,* (2011) screened the essential oil(s) of some spp. of *Curcuma* (Family- *Zingiberaceae*) viz., *Curcuma angustifolia, C. aromatica, C. domestica* and *C. zedoaria* against three common

dermatophytic fungi causing ringworm infection in human beings and reported the essential oil of *Curcuma domestica* Valet as the strongest toxicant against *E. floccosum, M. gypseum* and *T. rubrum.* However, in the present investigation, variations in antifungal activity among different plant species have been investigated. It can therefore be concluded that the plants exhibiting antifungal activity are distributed throughout the groups of flowering and non flowering plants and their activities are quite unrelated to their taxonomic positions.

**6.2.4** The antifungal activity may be restricted to certain parts of a plant or may be distributed uniformly throughout the plant. It has been found to be confined to **roots** (Hajek 1961; Bhakuni *et al.,* 1969; El-Hissey 1974; Narain and Satapathy 1977; Singh 1977; Dikshit 1980), stems (Southam and Ehrlic 1943; Nene and Thaplial 1965; Misra *et al.,* 1974; El-Hissey 1974; Singh 1977), **leaves** (Salvenas 1959; Dhar *et al.,* 1973; Disalvo 1974; Afifi 1975; Mishra 1975; Chakravarty and Pariya 1977; Egwa *et al.,* 1977; Dikshit 1980; Dubey 1981; Mishra 1991; Pandey *et al.,* 1996; Shahi 1997; Shahi *et al.,* 1997a 99b, 02b; Shukla *et al.,* 1997), Premshankar (2005), **flowers** (Nahrash 1961; Mishra *et al.,* 1974; Tripathi 1976) and **seeds and fruits** (Arnold 1958; Garber and Houston 1959; Zemenek and Bartos 1961; Kaul *et al.,* 1966; Pfleger and Harman 1975; Afifi 1977; Dikshit and Husain 1984; Alkiewiez and Lutomski 1992, Iyenger *et al.,* 1995; Mahasneh *et al.,* 1996; Shahi 1997; Kawai *et al.,* 1998; Ali *et al.,* 1999; Gadhi *et al.,* 2001; Shahi *et al.,* 2001c; Atindehou *et al.,* 2002; Villasenor *et al.,* 2002). Moreover several workers reported uniformly distributed antifungal activity throughout the plant (Gupta and Banerjee 1970; Dhar *et al.,* 1973; Dikshit 1980). However, in the present investigation, antifungal activity was investigated from the leaves of the ethnomedicinal plants.

**6.2.5** It is worth noting, that all the parts of a particular plant may not always be active. To have complete picture of distribution of antifungal principle in a plant, different parts such as stems, leaves flowers and seeds etc. of each plant have been screened. Earlier the plants were screened for their antifungal activity using their expressed juices or aqueous extracts by various workers (Southam and Ehrlich 1943; Gilliver 1947; Arnold 1958; Abdullaeva 1959; Petrushova 1960; Mishra 1975; Singh 1977; Mishra 1991; Alkiewiez and Lutomski 1992; Iyenger *et al.,* 1995; Mahasneh *et al.,* 1996; Shahi 1997; Kawai *et al.,* 1998; Ali *et al.,* 1999; Shahi *et al.,* 2000b; Gadhi *et al.,* 2001; Shahi *et al.,* 2001a,b; Atindehou *et al.,* 2002; Villasenor *et al.,* 2002). However, some workers used different organic solvents as extractives (Skinner 1955; Dhar *et al.,* 1968; 1973; Bhakuni *et al.,*

1969, 1971; Gupta and Banerjee 1970; Tripathi 1976; Tripathi 1977; Chaturvedi 1979; Dikshit 1980; Dubey 1981) and other used the secondary metabolities (essential oils), for screening of plants. (Garg 1974; Kaul *et al.,* 1976; Egwa *et al.,* 1977; Sawhney *et al.,* 1977; Banerjee and Nigam 1978; Chaturvedi 1979; Dikshit *et al.,* 1981; Dubey 1981; Singh *et al.,* 1983; Dikshit and Husain 1984; Qamar and Chaudhary 1991; Garg and Jain 1992; Kishore *et al.,* 1993; Fournier *et al.,* 1994; De Pooter 1995; Mukherjee *et al.,* 1996; Shahi *et al.,* 1996a,b; Wannissorn *et al.,* 1996; Pandey *et al.,* 1997; Shahi 1997; Shahi *et al.,* 1997a,b,c; Amvam Zolla *et al.,* 1998; Shahl *et al.,* 1999a,b,c; Singh *et al.,* 2000; Marshell *et al.,* 2001; Shahi *et al.,* 2002a,b,c). Silva *et al*., 2005; Mishra *et al*., in 2009, used the extracts (hexane, chloroform fractions, the essential oil and eugenol) which showed antifungal activities against *M. canis*, *M. gypseum*, *T. rubrum*, *T. mentagrophytes* and dematious molds. Chuang *et al.,* (2007) used Ethanol extracts of seeds and leaves of *Moringa oleifera* Lam showed anti-fungal activities *in vitro* against dermatophytes such as *T. rubrum*, *T. mentagrophytes*, *E. floccosum*, and *M. canis*. Sokovic *et al.,* (2009) used essential oils of *Thymus vulgaris, T. tosevii and Mentha piperita* and *M. spicata* as fungitoxicants and could safely use as natural preservatives to replace synthetic fungicides in the prevention and cure of some plant, human and animal fungal disease. However, in the present investigation, both the ethonolic extract as well as essential oil were investigated against the test dermatophytes.

**6.2.6** Different methods such as 'Inverted petri plate method' (Böcher, 1938), 'Slide germination technique' as recommended by American Phytopathological Society; Modified paper disc technique (Sharvelle and Peletier, 1956); 'Hanging drop technique' (Hoffmann, 1960); Double petri-plate method' (Latham and Linn, 1965); Poisoned food technique (Grover and Moore, 1962); Modified spore germination Inhibition technique (Shahi *et al.,* 1996a) and Modified broth microdilution method (Shukla, 2010) were followed during antifungal investigation of plant constituents. However, in the present investigation, disc diffusion method (also k/a Kirby-Bauer Technique) as well as 'broth micro dilution method' (NCCLS, 2002) were used for investigating the antidermatophytic activities of *E. odoratum*; *O. basilicum*; *M. piperita*; *C. aurantifolia* and *Mikania micrantha.*

## 6.3 *In-vitro* Antidermatophytic Investigations

As per the literature records: Rao and Joseph (1971); Deshmukh *et al.,* (1986); Mall *et al.,* (1985); Dikshit *et al.,* (1986); Steinmetz *et al.,* (1995); Yadav

(1995); Nenoff *et al.,* (1996); Pandey *et al.,* (1997); Wannissorn *et al.,* (1996); Shahi *et al.,* (1997a,b); Pandey (1997); Shahi *et al.,* (1998a); Amvam Zolla *et al.,* (1998); .Ali *et al.,* (1999); Singh *et al.,* (2000); Shahi *et al.,* (1999c); Shahi *et al.,* (2000a,b); Premshankar *et al.,* (2005); Singh *et al.,* (2007); Sokovic *et al.,* and Shukla *et al.,* (2011) screened antifungal activity of different essential oils against prominent dermatophytes, *T. rubrum*, *T. mentagrophytes M. canis* and *E. floccosum* causing tinea corporis infection. While, Qamar and Chaudhary (1991); Fun and Svendsen (1990); Garg and Dengre (1988) worked on essential oil against yeast and dermatophytes. Garg and Dengre (1988), Dikshit *et al.,* (1986), Sharma and Singh (1979a) reported essential oil against dermatophytic and non-dermatophytic moulds both. Further, Garg and Degnre (1988), worked on *Tagetus erecta* and *Capillipedium foectidum* oil against the fungal pathogen- *T. rubrum, Candida albicans* and *Aspergillus niger;* and Rajendra *et al., (*2004) reported the extract of the seeds of *Psoralea corylifolia* against the dermatophyte. However, in the present investigation, secondary metabolites (essential oil of *E. odoratum, O. basilicum, M. piperita, C. aurantifolia;* and ethnolic extract of *Mikania micrantha*) were used for detailed *in vitro* investigations against the dermatophytes- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum.* Minimum inhibitory concentrations, minimum fungicidal concentrations, inoculum density, $IC_{50}$, comparison with some synthetic antifungals and their physico-chemical characterization were the important parameters of investigation.

### 6.3.1 Minimum Inhibitory Concentrations (MICs)

A number of synthetic chemicals are shown to inhibit the growth of pathogens, but at high concentrations, resulting in wastages as well as residual toxicity after application. Thus, development of bioactive chemicals inhibiting the growth of test pathogens at low concentration is desirable. The Minimum Inhibitory Concentration (MIC) is the minimum amount of a bioactive chemical which is required for complete growth inhibition of tested pathogens making determination of Minimum Inhibitory Concentrations (MICs) a significant requirement for any antifungal development. Determination of the MICs makes it feasible to find the potentiality of the bioactive chemical compound and arrive at the cost benefit ratio.

Literature on MICs indicates that very few workers investigated essential oils against dermatophytic fungi involved in tinea corporis and reported the MICs at different ppm. Maruzzella (1963) reported the MICs ranges from 500-1300 ppm;

Suri *et al.,* (1979) 1000 ppm; Dikshit and Husain (1984) 400 ppm. Nenoff *et al.,* (1996) tested essential oil of *Melaleuca alternifolia* against 26 strain of *T.* spp. and *Candida albicaus* strains and found effective ranges from 50-500 ppm. Further, Shukla *et al.,* (2011) reported the minimum inhibitory concentration of the essential oil of *Curcuma aromatica* Salisb at 1.8µl/ml against *E. floccosum* and *T. rubrum,* and 1.6µl/ml against *M. gypseum*; however, it was fungicidal at 2.0 µl/ml against *E. floccosum* and *T. rubrum,* and 1.8 µl/ml against *M. gypseum,* respectively.

**6.3.1.1** In the present investigation, the essential oil of ***E. odoratum*** shows the minimum inhibitory concentration against *T. rubrum* was 0.68 mg/ml and $IC_{50}$ value was 0.17 mg/ml; against *T. mentagrophytes* the MIC was 0.59 mg/ml and $IC_{50}$ value was 0.13 mg/ml; against *E. floccosum* the MIC was 1.38 mg/ml and $IC_{50}$ value was 1.32 mg/ml, while, against *M. gypseum* the MIC was 0.96 mg/ml and the $IC_{50}$ value was recorded as 0.54 mg/ml. (**Table-5.16 & 6.1)**.

**6.3.1.2** The minimum inhibitory concentration of the essential oil of ***O. basilicum*** against *T. rubrum* was 1.71 mg/ml and $IC_{50}$ value was 0.90; against *T. mentagrophytes* the MIC was found 1.89 mg/ml and $IC_{50}$ value was 0.97; against *E. floccosum* the MIC was 2.48 mg/ml and $IC_{50}$ value was 1.18; while, against *M. gypseum,* MIC was 1.24 mg/ml and $IC_{50}$ value was 0.66 **(Table-5.16 & 6.1).**

**6.3.1.3** The minimum inhibitory concentration of the essential oil of ***M. piperita*** against *T. rubrum* was 2.43 mg/ ml and $IC_{50}$ value was 1.27; against *T. mentagrophytes* the MIC was 2.51mg/ ml and $IC_{50}$ value was 1.34; against *E. floccosum* the MIC was 2.89 mg/ ml and the $IC_{50}$ value was 1.56; while, against *M. gypseum* the MIC was 1.99 mg/ ml and $IC_{50}$ value was recorded as 0.95 **(Table-5.16 & 6.1).**

**6.3.1.4** The minimum inhibitory concentration of the essential oil of ***C. aurantifolia*** against *T. rubrum* shows the the MIC was 2.33 mg/ml and the $IC_{50}$ value was 1.00; against *T. mentagrophytes* the MIC was 1.09 mg/ml and $IC_{50}$ value was 0.51; against *E. floccosum* the MIC was 1.87 mg/ml and $IC_{50}$ value was 0.92, while in case of *M. gypseum,* the MIC was 1.97 mg/ml and the $IC_{50}$ value was 0.98 **(Table-5.16 & 6.1).**

**6.3.1.5** However, in case of extracts, Premshankar *et al.,* (2005) reported *in-vitro* antidermatophytic activity of methanolic extract of *Pistia stratiotes* (leaves) was most effective against *T. mentagrophytes, T. rubrum,* and *E. floccosum,* with MIC value 250 µg/ml while against *M. gypseum* the MIC value was 125 µg/ml. Rajendra *et al.,* (2004) reported that the methanol extract of the seeds of *Psoralea*

*corylifolia* at 250 µg shows the maximum inhibition against *T. rubrum, T. mentagrophytes, E. floccosum* and *M. gypseum.* In another study, Winkelhousen *et al.,* (2005) reported that the methanol extract of *Lantana* (leaves and flowers) shows antifungal activity (20 mm) against *M. gypseum, T. mentagrophytes, M. canis,* and *T. gypseum* while, the olive extract shows the lowest activity against all tested dermatophytes (8-10 mm).

However, in the present investigation, antidermatophytic activities of ***M. micrantha***leaf extract against *T. rubrum* show the minimum inhibitory concentration as 2.57 mg/ml and the $IC_{50}$ value as 1.00; against *T. mentagrophytes* the MIC was 1.31 mg/ml and $IC_{50}$ value was 1.00; against *E. floccosum* the MIC was 2.75 mg/ ml and $IC_{50}$ value was 0.99, and against *E. gypseum,* the minimum inhibitory concentration was 2.21 mg/ml and the $IC_{50}$ value was recorded as 0.86. **(Table-5.16 & 6.1).**

### 6.3.2 Nature of Toxicity

Nature of toxicity plays a significant role in selection of bioactive compound. A fungistatic bioactive compound is not found suitable for antifungal study and for subsequent antifungal development owing to the fungistatic nature of the bioactive compound leading to reoccurrence of the condition and due to inability of the compounds to conclusively kill the pathogen involved in the studied disease. It is therefore important to follow the investigations based on the fungicidal nature of the bioactive compound.

A number of workers have reported the nature of toxicity of the selected oil viz., *Cedrus deodara* (Dikshit 1980); *Ocimum canum, Citrus medica* (Dubey 1981); *Cymbopogon martini* (Singh *et al.,* 1980); *Ageratum houstonianum* (Pandey *et al.,* 1983b); *Alpinia galanga* (Tripathi *et al.,* 1983); *Eupatorium cannabinum* and *E. capillifolium* (Mall 1987); *Nepetha hindostana* and *Vitex negundo* (Mishra 1991) showing fungistatic nature of the oil at their minimum inhibitory concentrations (MICs). On the other hand the oils of *Adenocalyma allicea* (Chaturvedi 1979); *Pepromia pellucida* (Singh 1980); *Cestrum diuranum* (Renu *et al.,* 1980); *Caesulia axillaries, Hyptis suaveolens* (Pandey *et al.,* 1982), *Ocimum adscendens* (Asthana *et al.,* 1982); *Iberis amara* (Tripathi *et al.,* 1983); *Chenopodium ambrosioides* (Kishore 1985); *Cymbopogon pendulus* (Pandey *et al.,* 1996,97); *Trachyspermum ammi* and *Cymbopogon flexuosus* (Shahi 97); *Eucalyptus pauciflora* (Shahi *et al.,* 1999a); *Cymbopogon flexuosus* (Shahi *et al.,* 2002c) exhibited fungicidal nature at their respective minimum inhibitory concentrations (MICs). However, in the present study the oils of *E. odoratum, O. basilicum, M. piperita,* and *C. aurantifolia*

shows fungicidal activity (between 1.25 to 5.0 mg/ ml), against all the test pathogens- *E. floccosum*, *M. gypseum*, *T. mentagrophytes* and *T. rubrum*, respectively (Table-5.16 & 6.1). Thus, the finding indicates that the nature of toxicity of the oil/ extract is either dose dependent or pathogen dependent.

**Table: 6.1 Antidermatophytic activities of the oil/ extract against the dermatophytes [MIC, $IC_{50}$ and MFC (mg/ml)], an overview**

| SN | Samples | Anti-dermatophytic Parameter | Antidermatophytic activity of selected plant secondary metabolites against four dermatophytes | | | | |
|---|---|---|---|---|---|---|---|
| | | | Essential oils (mg/ml) | | | | 50 % ethanolic extract |
| | | | ***E. odoratum*** | ***O. basilicum*** | ***C. aurantifolia*** | ***M. piperita*** | ***Mikania micrantha*** |
| **1** | ***E. floccosum*** | MIC | 1.38 | 2.48 | 1.87 | 2.89 | 2.75 |
| | | $IC_{50}$ | 1.32 | 1.18 | 0.92 | 1.56 | 0.99 |
| | | MFC | 2.5 | 3.2 | 2.5 | 5.70 | 5.00 |
| **2** | ***M. gypseum*** | MIC | 0.96 | 1.24 | 1.97 | 1.99 | 2.21 |
| | | $IC_{50}$ | 0.54 | 0.66 | 0.98 | 0.95 | 0.86 |
| | | MFC | 1.25 | 2.5 | 2.5 | 3.87 | 4.65 |
| **3** | ***T. mentagrophytes*** | MIC | 0.59 | 1.89 | 1.09 | 2.51 | 1.31 |
| | | $IC_{50}$ | 0.13 | 0.97 | 0.51 | 1.34 | 1.00 |
| | | MFC | 1.25 | 2.5 | 2.5 | 3.21 | 2.5 |
| **4** | ***T. rubrum*** | MIC | 0.68 | 1.71 | 2.33 | 2.43 | 2.57 |
| | | $IC_{50}$ | 0.17 | 0.90 | 1.00 | 1.27 | 1.00 |
| | | MFC | 1.25 | 2.5 | 2.5 | 2.5 | 5.00 |

### 6.3.3. Inoculum Density against Antifungal Activity

Inoculum is the infectious material instrumental in causing disease after coming in contact with the host. According to Garrett (1965), Inoculum potential has, "the energy of a fungal parasite available for infection of a host at the surface of the host organ to be affected." Mishra (1975) stated that, antifungal activity of the *Allium sativum* was decreased on the increase of the inoculum density. While, Dikshit (1980) recorded that, there is no effect on antifungal activity on increase of the inoculum density of *Cedrus deodara* oil. The oils of *Pepromia pellucida* (Singh *et al.,* 1983), *Nepeta hindustana* and *Vitex negundo* (Mishra 1991) *Cymbopogon pendulus* (Pandey *et al.,* 1996), *Eucalyptus laveopinea* and *E. dalyrampleana* (Shahi *et al.,* 1997b), *Trachyspermum ammi* and *Cymbopogon flexuosus* (Shahi 1997), *Eucalyptus pauciflora* (Shahi *et al.,* 2000a), *Cymbopogon flexuosus* (Shahi *et al.,* 2002b) reported the same observation that, increase of inoculum density does not bring about a change in the antifungal activity of the bioactive compound (essential

oil). Shukla *et al.*, (2011) reported that *Curcuma aromatica* oil efficacy contains heavy doses of inoculums (25 discs of 5 mm each.)

However, while the previous investigations were made using traditional methods, the present finding was determined using broth micro dilution method (NCCLS 2002). The effect of inoculum density of the fungal pests (matched with 0.5 McFarland contained 1x $10^3$ CFU/ml) against the respective MFCs of the essential oil of *E. odoratum*, *O. basilicum*, *M. piperita*, *C. aurantifolia;* and ethnolic extract of *M. micranth*awas determined. The observation shows that the fungicidal activity persisted even at heavy doses of inoculum, thereby indicating its potential as a natural antifungal (Table- 5.14).

### 6.3.4 Comparison with some Synthetic Fungicides/ Antifungal drugs

The efficacy of the plant constituents (essential oils/ extract) was also compared with some synthetic antifungal drugs, available in the market. This was determined by comparing their minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs), respectively (Table 5.15 & 6.2).

**Table- 6.2: Comparative MICs of the test samples with some Synthetic Antifungal Drugs**

| Oil & Trade Name of Antifungal Drugs | **Active Ingredients** | **Minimum Inhibitory Concentration (mg/ml)** | | | |
|---|---|---|---|---|---|
| | | *E. floccosum* | *M. gypseum* | *T. mentagrophytes* | *T. rubrum* |
| ***E. odoratum*** | E.O. | 1.38 | 0.96 | 0.59 | 0.68 |
| ***O. basilicum*** | E.O. | 2.48 | 1.24 | 1.89 | 1.71 |
| ***C. aurantifolia*** | E.O. | 1.87 | 1.97 | 1.09 | 2.33 |
| ***M. piperita*** | E.O. | 2.89 | 2.21 | 1.31 | 2.57 |
| **Dactrine** | Miconazole Nitrate | 6.0 | 6.0 | 6.0 | 6.0 |
| **Nizaral** | Ketoconazole | 6.0 | 0.5 | 5.0 | 5.0 |
| **Tenaderm** | Tolnaftate | 2.0 | 1.5 | 0.8 | 0.8 |

The observations shows that the MIC of *E. odoratum* have the strongest toxicity against all the test pathogens (ranges from 0.59-1.38 mg/ml) followed by *C. aurantifolia* (ranges from 1.09-2.33 mg/ml); which shows an edge over the synthetic antifungal (Dactrine, Nizaral and Tenaderm), where the efficacy ranges from 0.5- 6.0 mg/ml.

## 6.4 Identification of Active Constituents

### 6.4.1 Identification of constituents of *E. odoratum*, using GC MS analysis

The GC-MS analysis of *E. odoratum* oil shows **α-pinene** (27.50%) **pregeijerene** (14.50%) and **Geijerene** (12.20%) as the major cpnstituents (Table 5.17).

### 6.4.2 Identification of constituents of *M. piperita* oil, using GC MS analysis

The GC-MS analysis of *M. piperita* essential oil resulted in total of 14 components. Out of 14 components 2 compounds i.e. Menthol 37.20% and Menthone 22.52% was the major compounds (Table 5.18).

### 6.4.3 Identification of constituents of *Oscimum bascilicum* oil, using GC MS analysis

The GC- MS analysis of the essential oil of *Oscimum bascilicum* shows methyl chevicol (71.25%) and linalyl acetate (19.32%) detected as the major component, followed by α-cubabene (5.19).

### 6.4.4 Identification of constituents of *C. aurantifolia* oil, using GC MS analysis

The GC-MS analysis of *Citrus auranifolia* essential oil shows 17 compounds. Among the identified compounds, limonene (54.20%), γ-terpinene (14.23%) and terpinolene (11.02%) were the major constituents (Table 5.20).

## 6.5 Significance and Scope of IPR on Selected Plants Showing Antidermatophytic Activity

Protection of indigenous knowledge from misappropriation by others (those not belonging to the community which is the source of the indigenous knowledge) is of great significance. With the increased role of IPR, the threat of misappropriation by commercial interests has become particularly serious. It is therefore important that:

**a)** The patenting (or any other form of IPRs) of inventions based primarily on indigenous knowledge is avoided and

**b)** The rights of communities over their indigenous knowledge are established.

The strategy favoured by most observers is to place this information in the public domain so that it can be used to challenge patent (and other IPR applications, such as

Plant Breeder's Rights) applications involving indigenous knowledge based inventions. This can be done by preparing comprehensive and searchable databases of indigenous knowledge, which can be made available to patent offices for use while assessing patent applications. Most of the ethno-medicinal information is also freely published in academic journals. As a result a large amount of indigenous knowledge is now available in the public domain. However, as this information is not arranged in a searchable format, it has only a limited role in preventing the patenting of indigenous knowledge. Only if it is put in databases, can this information become a useful tool in defense of indigenous knowledge, preventing it from being patented by outside interests. Indian TKDL initiative in this regard is a significant step. TKDL is an electronic database of traditional knowledge in the field of medicinal plants. It aims to prevent the patenting of existing knowledge. TKDL database would enable the patent officers all over the world to search and examine any prevalent use and thereby prevent grant of patent based on knowledge in public domain.

The value of plants for medicines is more widely recognized and the "intellectual property rights" (IPR) connected with their use have been debated worldwide. "Convention on Biological Diversity" (CBD) which was signed in Rio in 1992, enforces protection of the rights of local people and local knowledge as well as conservation of the biological resources which forms the basis of all those health systems. India is home to a reservoir of medicinal plants and traditional knowledge and its North East area a hot spot of biodiversity. The vast resources face the threat of piracy and infringement of IPR which need to be protected under the patents systems. Intellectual Property Rights(Patents) provide protection for a limited period, to the moral and economic rights of creators for their creations and also ensure rights of the public in access to those creations. Patents thus, are the principal means for establishing ownership rights to inventions and ideas, and provide a legal foundation by which intangible ideas and creations generate tangible benefits.

### 6.5.1 Growth in the patenting activity

6.5.1.1 Over the last many years there is a tremendous increase in the patent activities across the world. According to the WIPO statistics database as on November 2012, number of **patent grants** in the world shows an increase from 535100 in the year 2001 to 996800 in the year 2011. In comparison, the European Patent filing shows an increase from 164,144 in the year 2002 to a total of 244,437 in the year 2011 (table: 3.2). Top five European patent filing countries are the United States, Japan, Germany, China and Korea

(table: 3.3). However European patent office granted a total of 62112 patents in the year 2011 as against a total of 47380 in the year 2002, the top patentees being the US, Japan and Germany (Table: 3.4).

6.5.1.2 Patent search across **fields of technologies** in the European Patent office(EPO) shows that while the sub-field of Medical Technology shows great activity in EPO :10534 applications with patent grants of 4384. The **field of pharmaceuticals** shows increase in the applications and grants from the year 2002 (4710 applications/ 1961 grants ) to the year 2012 (5759 applications/ 1704 grants).

### 6.5.2 Patent scenario pertaining to present investigation.

**6.5.2.1** Patent search broadly and specifically related to present investigations indicated grant of very few patents **on the products/processes on different features of the plants** selected for the present study, such as :

- A European patent on mint (***Mentha piperita***) to 'enhance the production of monoterpenoids, to produce geranyl diphosphate in cancerous cells';
- A US patent on 'therapeutic oil composition for topical application to **painful areas** of the human body' b**y mixing** *Eugenia caryophyllata, Myroxyon pereira, Eucalyptus globulus, Lavandula augustifolia, M. piperita,* **and** ***Mentha*** *spicata;*
- *A* Chinese patent for 'production of **cosmetically effective** compositions that lighten the skin', containing at least one extract (i) of mallow (*Malva sylvestris)* and (ii*)* ***peppermint (M. piperita****);*
- *A* Patent No. **'**Anti cold mask impregnated with wild ***Ocimum bascillicum*** essential oil';
- A US patent on 'Distinct type cultivar of ***O. basilicum'*** *on* 'an early, short duration, dwarf, high essential oil, methyl chavicol and linalool yielding variety of Indian basil (*O. basilicum*)'.

**6.5.2.2** Search for patents as '**antifungal agents'** indicated grant of patents such as:

- A US patent on 'Antifungal treatment of nails' for composition to deliver active drugs trans-nail as well as a method for producing the **fungus treatment** composition, which may contain up to 50% additive ingredients;
- A European/US patent for '**Topical antifungal composition'** inhibiting the growth of *T. rubrum* that includes selective mixtures of the origanum oil,

menthol, and Atlantic cedarwood oil, thuja oil, cedarwood oil, cinnamon oil, clove oil, cumin oil, fennel oil, peppermint oil, or rosemary;

- A European patent on 'Plant-derived anti-parasitic and **antifungal compounds and methods of extracting** the compounds' / extracts from *Aframomum aulocacarpus, Aframomun danellii, Dracaena arborea, E. odoratum, Glossocalyx brevipes and Napoleonaea imperialis;*
- A world/ Japanese on 'Antimicrobial composition for treating **microbial infections and preventing microbial contamination'** comprising at least two essential oils derived from genus *Pelargonium; Cymbopogon; Mentha**;** Aniba; Lavandula; Origanum; Litsea; **Citrus**; Melissa; Pogostemon; Santalum; Valeriana; Styrax; Cinnamonu; and Rosa.*

**6.5.2.3.** However, search for patents, as '**antidermatophytic agents'**, on the plants/products including on the plants selected in the present studies indicate grant of patents such as;

- A US patent on '**Antidermatomycotic** pharmaceutical compositions and method' based upon the antidermatophytic activity of o-methoxycinnamaldehyde.
- A Patent on 'Antifungal composition, its fungicidal effect on **pathogenic dermatophytes'** as a natural preservative composition from plant materials including **selective mixtures** of *Origanum vulgare L., Thymus vulgaris L., Cinnamomum zeylanicum Nees, Rosmarinus officinalis L., Lavandula officinalis L., **M. piperita L.,** Citrus Limon L., Hydrastis Canadensis L., Olea europaea L***.**
- A US patent on '**Anti-fungal formulation** active **against a broad spectrum of dermatophytoses'** comprising at least about 1% by weight of oil extracted from *Rabdosia melissoides* and one or more vegetable oils, solvents and additives.

It is evident from the forgoing that although a few patent pertaining to plants/constituents selected for the present study viz. *E. odoratum*; *O. basilicum*; *M. piperita*; *C. aurantifolia* and *Mikania micranth*aes are available; however there is no evidence of any patent on the selected plant/ constituent as an 'antidermatophyte'. Patent search also reveal that while some product/process patents in the broad field relating to "skin" are available on: whitening agent; greying of hair; pain & inflammation relief, but no specific patents were found on plant products with antidermatophytic activity, selected for our study (**Table 3.9).**

### 6.5.3 The Indian patent scenario

**6.5.3.1** The Indian patent office (IPO) indicates tremendous increase in patent filing from 10592 in the year 2001-02 to 39400 in the year 2010-11. The patent grants in the Indian Patent office for the corresponding period show an increase in number from 1591 in the year 2001-02 to 7509 in the year 2010-11 **(Table 3.11).**

**6.5.3.2** However, in the **field of drugs and pharma,** the patent filing in India shows an increase in number from a mere 879 in the year 2001-02 to 3526 in the year 2010-11. Against this, number of patent grants shows an increase from 320 in the year 2001-02 to 596 in the year 2010-11 **(Table 3.12).**

**6.5.3.3** Search on various patent sites viz. WIPO, USPTO, EPO, IPO, for Patents awarded to the Applicants/ inventor from the State of North East Region and those having a mention in the topic/ abstract of the patent showed number of patents in the name of the State of Assam(121), Meghalaya(2), Sikkim and Tripura but no specific patents were found on the selected plants/ metabolites showing antidermatophytic activity**.(Table 3.13)** Moreover no patent grant was made to any applicant/ inventor from the state of Mizoram.

## 6.6 Legislative provision and future scope of IPR related to present study

Indian Patent Act 1970 as amended in 2005 introduced the provision of 'product patent on food, chemicals and pharmaceuticals'. Section 3 of the act, however, provides for some exclusion. The sub-section 3 (p) of section 3 excludes from the purview of patents the "Inventions which are Traditional Knowledge or an aggregation or duplication of known properties of traditionally known component or components" (Examples: Traditional Knowledge already in public domain; Wound healing property of Haldi). However, "Any value-addition using Traditional Knowledge leading to a new process or product, which is novel with inventive step and industrial applicability" is patentable.

The present study contains the scientific validation of the traditional information (i.e. "tradition to technology"). It includes the collection and categorization of some common ethno-medicinal plants used against the skin ailments, their screening against the dermatophytic pathogens causing the ringworm infections in human beings. Furthermore, the potential antidermatophytic plants were subjected to detailed

phytochemical characterization as well as *in vitro* investigations against *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum,* thus resulting in lot of value addition to the existing knowledge on the subject.

Thus, in view of search for IPR (Patent) on novel antidermatophytic product/process from *E. odoratum*; *O. basilicum*; *M. piperita*; *C. aurantifolia* and *M. micrantha* or their metabolites investigated in the present study for their antidermatophytic activity and consequent lack of evidence of patent on the aspect implies that there is great scope for further *in- vivo* investigations, pre clinical trials, multilocational clinical trials as well as isolation and synthesis of the bioactive molecule(s) for drug formulation (i.e. product patent)/ technology transfer (i.e. process patent) to the pharmaceutical companies; so that, the available information can be converted into the technological information for the health and economic welfare of the society/community with appropriate documentation / conservation/ IPR protection , before they are lost forever.

# 7. Abstract

The present research entitled "*A study on the Antidermatophytic activity of some Ethno-medicinal Plants of North East Region in Relation to Intellectual Property Rights*" was carried out in the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl (with the supervisor) as well as in the Biological Product Laboratory, Department of Botany, University of Allahabad (with the joint supervisor), during June 2009 - December 2012.

The abstract of the research work follows:

## 7.1 Dermatophytes

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair, and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm. The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton, Microsporum,* and *Trichophyton,* on the basis of conidial morphology and formation of conidia. Infection is generally cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent host. Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors.The clinical manifestations are as follows: (i) tinea barbae (ringworm of the beard and mustache); (ii) tinea capitis (scalp, eyebrows, and eyelashes); (iii) tinea corporis (glabrous skin); (iv) tinea cruris (groin); (v) tinea favosa (favus); (vi) tinea imbricata (ringworm caused by *T. concentricum*); (vii) tinea manuum (hand); (viii) tinea pedis (feet); and (ix) tinea unguium (nails). The major etiologic agents may be global, such as *T. rubrum,* while the distribution of others may vary geographically.

## 7.2 Biodiversity in India

**A** large population of the world (about 80%) especially in the developing countries depends on traditional medicines to meet their health requirements. In recent years the popularity of the traditional system of medicine in developed countries has also grown enormously. The renewed interest and activity in the medicinal and aromatic

plants is also justified in the context of the Convention on Biological Diversity (CBD) and Trade Related Intellectual Property Rights (TRIPS) - the international agreements to which majority of the countries including India, are signatories. North Eastern region is one of the hotspots of the world biodiversity and homeland of large number of indigenous and immigrant ethnic and tribal groups. The variations in the temperature pattern exist from below 0°C to high upto 30°C. All these have great influence over the rich diversity of vegetation in this region.

## 7.3 Ethno-botany and significance of herbal medicine

India's rich biodiversity is well known. By an estimate, India is endowed with about 47,000 species of plants and around 8000 plants having medicinal properties. The Indian system of medicine is traditionally based on plants materials or their extracts as active ingredient for preparation of drugs. These plants are exploited either legally or illegally by traditional healers and for trade purposes. The traditional medicines have been attracting researchers from all parts of the globe. Hence a worldwide interest is created in the field of ethno-medicines for finding some new and effective herbal drugs.This warrants that the plant and its use be conserved and the communities or the holders of the knowledge of the use of these plants receive due recognition.

## 7.4 Collection, identification and preliminary antimicrobial screening of some plants from NE region

Although, a number of researches have already been made on ethno medicinal plants of North Eastern Region of India, investigations up to their IPR issues have not been paid much attention.

### 7.4.1 Collection of plants used in skin ailments

Based on the literature survey as well as local field visits and discussions with local practioners, information about some important ethno medicinal plants, used for skin ailments in human beings, were collected and utilized for drawing a list of fifteen most potent plants. These were the plants which showed the maximum frequency of use, against skin ailments, among the local communities (**Table-5.1).**

### 7.4.2 Extraction of the plant secondary metabolites in the form of the essential oil/ extracts

The selected plants were subjected to extraction of their secondary metabolites (extract/essential oil). The *solvent extraction method*, using different solvents (viz.,

alcohol, acetone, benzene, chloroform, hexane etc), was used for extraction of the extract however, *hydro distillation method*, using Clevenger′s apparatus, was used for extraction of the essential oil**. (Plate 3 and 4)**

### 7.4.3 Procurement of test organism

The authentic cultures (MTCC- Microbial type culture collection) of these dermatophytes were procured from the Institute of the Microbial Technology (IMTECH), Chandigarh, India. The strains of the cultures were: (i) *Trichophyton rubrum* (Castellani) Sabouraud (MTCC-3272); (ii) *Trichophyton mentagrophytes* (Robin) Blanchard (MTCC-8476); (iii) *Epidermophyton floccosum* (Hartz) Langeron et Mitochevitch (MTCC-7880) and (iv) *Microsporum gypseum* (Bodin) Guiart and Grigorakis (MTCC-2830).

### 7.4.4 Antimicrobial screening against the dermatophytic fungi

The antimicrobial screening of the shortlisted fifteen plants (table 5.0) against the dermatophytic pathogens viz., *Trichophyton rubrum* (Castellani) Sabouraud; *Trichophyton mentagrophytes* (Robin) Blanchard; *Epidermophyton floccosum* (Hartz) Langeron et Mitochevitch and *Microsporum gypseum* (Bodin) Guiart and Grigorakis; was done using disc diffusion method**. (Plate 9).** Out of the 15 selected plants, 4 essential oil bearing plants viz., *E. odoratum* L., *M. piperita* L., *O. basilicum*, and *C. aurantifolia* L. as well as 50 % ethanolic extract of *M. micrantha* were found to be more effective against all the test pathogens. The maximum inhibition zone was recorded in case of *E. odoratum* essential oil (27 mm); followed by *O. basilicum* essential oil (22 mm), *C. aurantifolia* (21 mm) and *M. piperita* (18 mm) and the ethanolic extract of *M. micrantha* (12 mm). Therefore, these five potential plants showing maximum zone of inhibition were selected for detailed *in vitro* investigations, and characterization.

## 7.5 Detailed *in-vitro* study of the selected essential oils as well as plant extracts against dermatophytes

### 7.5.1 Fungicidal properties of the plant secondary metabolites

Fungicidal properties of the plant secondary metabolites were determined, using Broth microdilution method standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 2002); now known as Clinical Laboratory Standards Institute (CLSI). The minimum inhibitory concentration (MIC) and $IC_{50}$ values were recorded spectrophotometrically with the following observations.

**7.5.1.1 T**he essential oil of ***E. odoratum*** shows the minimum inhibitory concentration against *T. rubrum* was 0.68 mg/ml and $IC_{50}$ value was 0.17 mg/ml; against *T. mentagrophytes* the MIC was 0.59 mg/ml and $IC_{50}$ value was 0.13 mg/ml; against *E. floccosum* the MIC was 1.38 mg/ml and $IC_{50}$ value was 1.32 mg/ml, while, against *M. gypseum* the MIC was 0.96 mg/ml and the $IC_{50}$ value was recorded as 0.54 mg/ml. (**Table-5.16 & 6.1)**.

**7.5.1.2.** The minimum inhibitory concentration of the essential oil of ***O. basilicum*** against *T. rubrum* was 1.71 mg/ml and $IC_{50}$ value was 0.90; against *T. mentagrophytes* the MIC was found 1.89 mg/ml and $IC_{50}$ value was 0.97; against *E. floccosum* the MIC was 2.48 mg/ml and $IC_{50}$ value was 1.18; while, against *M. gypseum,* MIC was 1.24 mg/ml and $IC_{50}$ value was 0.66 **(Table-5.16 & 6.1).**

**7.5.1.3** The minimum inhibitory concentration of the essential oil of ***Mentha piperita*** against *T. rubrum* was 2.43 mg/ ml and $IC_{50}$ value was 1.27; against *T. mentagrophytes* the MIC was 2.51mg/ ml and $IC_{50}$ value was 1.34; against *E. floccosum* the MIC was 2.89 mg/ ml and the $IC_{50}$ value was 1.56; while, against *M. gypseum* the MIC was 1.99 mg/ ml and $IC_{50}$ value was recorded as 0.95 **(Table-5.16 & 6.1).**

**7.5.1.4** The minimum inhibitory concentration of the essential oil of ***Citrus aurantifolia*** against *T. rubrum* shows the the MIC was 2.33 mg/ml and the $IC_{50}$ value was 1.00; against *T. mentagrophytes* the MIC was 1.09 mg/ml and $IC_{50}$ value was 0.51; against *E. floccosum* the MIC was 1.87 mg/ml and $IC_{50}$ value was 0.92, while in case of *M. gypseum,* the MIC was 1.97 mg/ml and the $IC_{50}$ value was 0.98 (**Table-5.16 & 6.1).**

**7.5.1.5** The antidermatophytic activities of ***Mikania micrantha*** leaf extract against *T. rubrum* show the minimum inhibitory concentration as 2.57 mg/ml and the $IC_{50}$ value as 1.00; against *T. mentagrophytes* the MIC was 1.31 mg/ml and $IC_{50}$ value was 1.00; against *E. floccosum* the MIC was 2.75 mg/ ml and $IC_{50}$ value was 0.99, and against *E. gypseum*, the minimum inhibitory concentration was 2.21 mg/ml and the $IC_{50}$ value was recorded as 0.86. **(Table-5.16 & 6.1).**

### 7.5.2 Inoculum Density vis-v-vis Fungicidal Activity

The efficacy of the essential oil of Eupatorium odoratum, Ocimum basilicum, Mentha piperita, Citrus aurantifolia; and ethnolic extract of Mikania micrantha, on inoculum density of the test pathogens- Epidermophyton floccosum, Microsporum gypseum, Trichophyton mentagrophytes and Trichophyton rubrum was determined

using the protocols recommended by NCCLS-2002. The observations showed that the minimum fungicidal concentration (MFC) of the test samples persisted heavy inoculum density of the test pathogens (dermatophytes)

The comparative analysis (MIC, $IC_{50}$ and MFC) of the oils of *E. odoratum* L., *M. piperita* L. *O. basilicum, C. aurantifolia* L. and 50% leaf extract of *M. micrantha* Kunth ex H.B.K against the test pathogerns viz., *T. rubrum*; *T. mentagrophytes*; *E. floccosum* and *M. gypseum* are summarized below **(Table-5.16).**

| SN | Samples | Antiderma-tophytic Parameters | Antidermatophytic activity of selected plant secondary metabolites against four dermatophytes | | | | |
|---|---|---|---|---|---|---|---|
| | | | Essential oils | | | | 50 % ethanolic extract |
| | | | *Eupatorium odoratum* | *Ocimum basilicum* | *Citrus aurantifolia* | *Mentha piperita* | *Mikania micrantha* |
| 1. | ***Epidermophyton floccosum*** | MIC | 1.38 | 2.48 | 1.87 | 2.89 | 2.75 |
| | | $IC_{50}$ | 1.32 | 1.18 | 0.92 | 1.56 | 0.99 |
| | | MFC | 2.5 | 3.2 | 2.5 | 5.70 | 5.00 |
| 2. | ***Microsporum gypseum*** | MIC | 0.96 | 1.24 | 1.97 | 1.99 | 2.21 |
| | | $IC_{50}$ | 0.54 | 0.66 | 0.98 | 0.95 | 0.86 |
| | | MFC | 1.25 | 2.5 | 2.5 | 3.87 | 4.65 |
| 3. | ***Trichophyton mentagrophytes*** | MIC | 0.59 | 1.89 | 1.09 | 2.51 | 1.31 |
| | | $IC_{50}$ | 0.13 | 0.97 | 0.51 | 1.34 | 1.00 |
| | | MFC | 1.25 | 2.5 | 2.5 | 3.21 | 2.5 |
| 4. | ***Trichophyton rubrum*** | MIC | 0.68 | 1.71 | 2.33 | 2.43 | 2.57 |
| | | $IC_{50}$ | 0.17 | 0.90 | 1.00 | 1.27 | 1.00 |
| | | MFC | 1.25 | 2.5 | 2.5 | 2.5 | 5.00 |

The observations indicate that *E. odoratum* shows the strongest potency (ranges from 0.59-1.38 mg/ml) and *M. piperita* shows the weakest potency (ranges from 1.99-2.89 mg/ml) against all the four dermatophytes.

### 7.5.3 Comparison with some synthetic fungicides/ antifungal drugs

The efficacy of these plant constituents (essential oils/ extract) was also compaired with some synthetic antifungal drugs, available in the market such as Dactrine; Nizaral and Tenderm. This was determined by comparing their minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs), **respectively (Table 5.15).** As per the observations, the MIC of *E. odoratum* have the strongest toxicity against all the test pathogens (ranges from 0.59-1.38 mg/ml) followed by *C. aurantifolia* (ranges from 1.09-2.33 mg/ml); which shows an edge over the synthetic antifungal (Dactrine, Nizaral and Tenaderm), where the efficacy ranges from 0.5- 6.0 mg/ml.

### 7.5.4 Identification of active constituents of the tested essential oils

The essential oils extracted from *E. odoratum, M. piperita, O. basilicum,* and *C. aurantifolia* were further subjected to GC-MS for identification of their active constituents, and the observations are in tables 5.17-5.21.

#### 7.5.4.1 Identification of constituents of *E. odoratum*, using GC MS analysis

The GC-MS analysis of *E. odoratum* oil shows **α-pinene** (27.50%) **pregeijerene** (14.50%) and **Geijerene** (12.20%) as the major constituents **(Table 5.17).**

#### 7.5.4.2 Identification of constituents of *M. piperita* oil, using GC MS analysis

The GC-MS analysis of *M. piperita* essential oil shows total of 14 components (**Table 5.18).** Out of 14 components, 2 compounds i.e. Menthol 37.20% and Menthone 22.52% are the major compounds.

#### 7.5.4.3 Identification of constituents of *Oscimum bascilicum* oil, using GC MS analysis

The GC- MS analysis of the essential oil of *Oscimum bascilicum* shows methyl chevicol (71.25%) and linalyl acetate (19.32%) as the major component, followed by α-cubabene **(5.19).**

#### 7.5.4.4 Identification of constituents of *C. aurantifolia* oil, using GC MS analysis

The GC-MS analysis of *Citrus auranifolia* essential oil shows 17 compounds. Among the identified compounds, limonene (54.20%), is the most abundant compound and γ-terpinene (14.23%) and terpinolene (11.02%) the major constituents (**Table 5.20).**

## 7.6 Physico-chemical properties of the selected essential oils

The selected essential oils were also studied for some common physio-chemical properties viz., appearance, boiling point, odor, specific gravity, optical rotation, refractive index and solubility in water; and are recorded in table-5.22.

## 7.7 Solubility in various organic solvents

Further, the solubility of all selected secondary metabolites in various organic solvents was also investigated and it was found that the oils were soluble in 13 different organic solvents viz., acetone, alcohols, benzene, chloroform, carbon tetrachloride,

dimethyl sulphoxide (DMSO), ethanol, hexane, methanol, n-butanol, petroleum ether and propanol solvent ether; used for testing (Table- 5.23).

## 7.8 Scope and issues related to Intellectual Property Rights (IPR)

### 7.8.1 Significance of IPR on medicinal plants

The value of plants for medicines is more widely recognized and the "intellectual property rights" (IPR) connected with their use have been debated worldwide. "Convention on Biological Diversity" (CBD) which was signed in Rio in 1992, enforces protection of the rights of local people and local knowledge as well as conservation of the biological resources which forms the basis of all those health systems. Protection of indigenous knowledge from misappropriation by others (those not belonging to the community which is the source of the indigenous knowledge) is of great significance. With the increased role of IPR, the threat of misappropriation by commercial interests has become particularly serious. It is therefore important that:

India is home to a reservoir of medicinal plants and traditional knowledge and its North East area a hot spot of biodiversity. The vast resources face the threat of piracy and infringement of IPR which need to be protected under the patents systems. Intellectual Property Rights(Patents) provide protection for a limited period, to the moral and economic rights of creators for their creations and also ensure rights of the public in access to those creations. Patents thus, are the principal means for establishing ownership rights to inventions and ideas, and provide a legal foundation by which intangible ideas and creations generate tangible benefits.

### 7.8.2 World-wide growth in the patenting activity

Over the last many years there is a tremendous increase in the patent activities across the world. According to the WIPO statistics database as on November 2012, number of **patent grants** in the world shows an increase from 535100 in the year 2001 to 996800 in the year 2011. In comparison, the European Patent filing shows an increase from 164,144 in the year 2002 to a total of 244,437 in the year 2011 (table: 3.2). Top five European patent filing countries are the United States, Japan, Germany, China and Korea (table: 3.3). However European patent office granted a total of 62112 patents in the year 2011 as against a total of 47380 in the year 2002, the top patentees being the US, Japan and Germany (table: 3.4). Patent search across **fields of technologies** shows that while the sub-field of Medical Technology shows great activity in EPO :10534 applications with patent grants of 4384. The **field of pharmaceuticals** shows increase in the

applications and grants from the year 2002 (4710 applications/ 1961 grants ) to the year 2012 (5759 applications/ 1704 grants).

### 7.8.3 Patent scenario pertaining to present investigation.

Patent search broadly and specifically related to present investigations indicated grant of few patents **on the products/processes on different features of the plants** selected for the present study, such as :

- A European patent on mint (***Mentha piperita***) to 'enhance the production of monoterpenoids, to produce geranyl diphosphate in cancerous cells';
- A US patent on 'therapeutic oil composition for topical application to **painful areas** of the human body' b**y mixing** *Eugenia caryophyllata, Myroxyon pereira, Eucalyptus globulus, Lavandula augustifolia, Mentha* ***piperita,*** **and** ***Mentha spicata;***
- *A* Chinese patent for 'production of **cosmetically effective** compositions that lighten the skin', containing at least one extract (i) of mallow (*Malva sylvestris)* and (ii*)* ***peppermint (Mentha*** *piperita);*
- *A* Patent No. **'**Anti cold mask impregnated with wild ***Ocimum bascillicum*** essential oil';
- A US patent on 'Distinct type cultivar of ***Ocimum basilicum'*** *on* 'an early, short duration, dwarf, high essential oil, methyl chavicol and linalool yielding variety of Indian basil (*Ocimum basilicum*)'.

Whereas, search for patents as '**antifungal agents'** indicated grant of patents such as:

- A US patent on 'Antifungal treatment of nails' for composition to deliver active drugs trans-nail as well as a method for producing the **fungus treatment** composition, which may contain up to 50% additive ingredients;
- A European/US patent for '**Topical antifungal composition'** inhibiting the growth of *Trichophyton rubrum* that includes selective mixtures of the origanum oil, menthol, and Atlantic cedarwood oil, thuja oil, cedarwood oil, cinnamon oil, clove oil, cumin oil, fennel oil, peppermint oil, or rosemary;
- A European patent on 'Plant-derived anti-parasitic and **antifungal compounds and methods of extracting** the compounds' / extracts from *Aframomum aulocacarpus, Aframomun danellii, Dracaena arborea, Eupatorium odoratum, Glossocalyx brevipes and Napoleonaea imperialis;*

- A world/ Japanese on 'Antimicrobial composition for treating **microbial infections and preventing microbial contamination'** comprising at least two essential oils derived from genus *Pelargonium; Cymbopogon; Mentha**;** Aniba; Lavandula; Origanum; Litsea; **Citrus**; Melissa; Pogostemon; Santalum; Valeriana; Styrax; Cinnamonu;* and *Rosa.*

However, search for patents, as '**antidermatophytic agents'**, on the plants/products including on the plants selected in the present studies indicate grant of patents such as;

- A US patent on '**Antidermatomycotic** pharmaceutical compositions and method' based upon the antidermatophytic activity of o-methoxycinnamaldehyde.
- A Patent on 'Antifungal composition, its fungicidal effect on **pathogenic dermatophytes'** as a natural preservative composition from plant materials including **selective mixtures** of *Origanum vulgare L., Thymus vulgaris L., Cinnamomum zeylanicum Nees, Rosmarinus officinalis L., Lavandula officinalis L., **Mentha piperita L.,** Citrus Limon L., Hydrastis Canadensis L. and Olea europaea L.*
- A US patent on '**Anti-fungal formulation** active **against a broad spectrum of dermatophytoses'** comprising at least about 1% by weight of oil extracted from *Rabdosia melissoides* and one or more vegetable oils, solvents and additives.

It is observed from the forgoing that there is no evidence of any patent on the selected plant/ constituent as an 'antidermatophytic plant agent'. (Table 3.9)

### 7.8.4 The Indian Patent Scenario

The Indian patent office (IPO) indicates tremendous increase in patent filing from 10592 in the year 2001-02 to 39400 in the year 2010-11. The patent grants in the Indian Patent office for the corresponding period show an increase in number from 1591 in the year 2001-02 to 7509 in the year 2010-11 **(Table 3.11).** However, in the **field of drugs and pharma,** the patent filing in India shows an increase in number from a mere 879 in the year 2001-02 to 3526 in the year 2010-11. Against this, number of patent grants shows an increase from 320 in the year 2001-02 to 596 in the year 2010-11 (**Table 3.12).**

Search on various patent sites viz. WIPO, USPTO, EPO, IPO, for Patents awarded to the Applicants/ inventor from the State of North East Region showed few patents in the name of the State of Assam (121), Meghalaya (2), Sikkim and Tripura but

no specific patents were found on the selected plants/ metabolites showing antidermatophytic activity.(**Table 3.13)** Moreover no patent grant was made to any applicant/ inventor from the state of Mizoram.

### 7.8.5 Legislative provision and future scope of IPR related to present study

Indian Patent Act 1970 as amended in 2005 introduced the provision of 'product patent on food, chemicals and pharmaceuticals'. Section 3 of the act, however, provides for some exclusions. The sub-section 3 (p) of section 3 excludes from the purview of patents the "Inventions which are Traditional Knowledge or an aggregation or duplication of known properties of traditionally known component or components" (Examples: Traditional Knowledge already in public domain; Wound healing property of Haldi). However, "Any value-addition using Traditional Knowledge leading to a new process or product, which is novel with inventive step and industrial applicability" is patentable.

The present study contains the scientific validation of the traditional information (i.e."tradition to technology"). It includes the collection and categorization of some common ethno-medicinal plants used against the skin ailments, their screening against the dermatophytic pathogens causing the ringworm infections in human beings. Furthermore, the potential antidermatophytic plants were subjected to detailed phytochemical characterization as well as *in vitro* investigations against *Epidermophyton floccosum, Microsporum gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum,* thus resulting in lot of value addition to the existing knowledge on the subject.

Thus, in view of search for IPR (Patent) on novel antidermatophytic product/process from *Eupatorium odoratum*; *Ocimum basilicum*; *Mentha piperita*; *Citrus aurantifolia* and *Mikania micrantha* or their metabolites investigated in the present study for their antidermatophytic activity and consequent lack of evidence of patent on the aspect implies that there is great scope for 'IPR & Patenting' after detail *in- vivo* investigations, pre clinical trials, multilocational clinical trials as well as synthesis of the bioactive molecule(s); which can be used for drug development (i.e. **product patent**) or for technology transfer (i.e. **process patent**) to the pharmaceutical companies, before they are lost forever.

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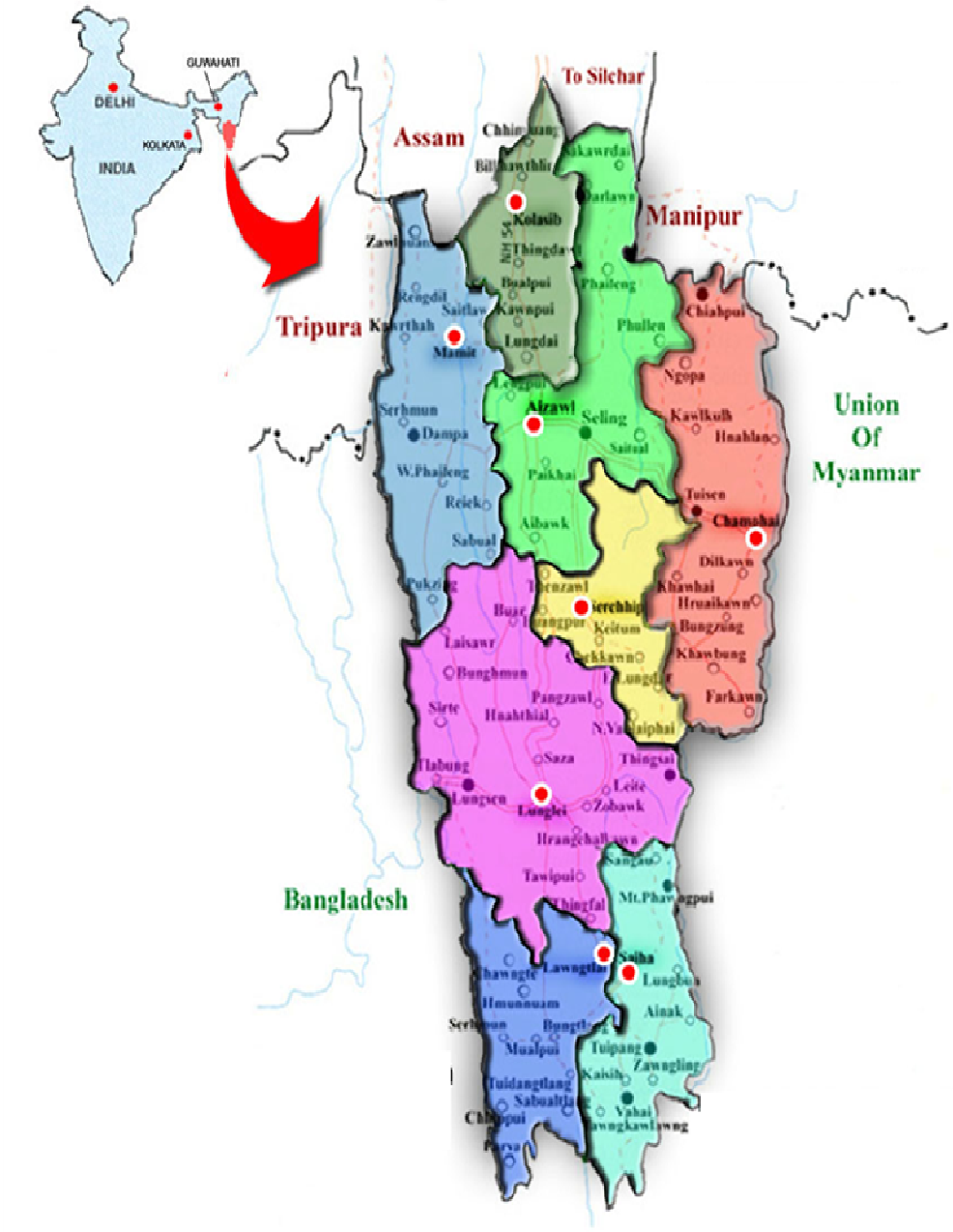


**Plate-1: The Study Area/ Mizoram**






**Plate- 2: Ethno botanical survey & Plant collection from different locations in Mizoram**


**Plate- 3(a-d): Processing of the collected plants/ the Solvent Extraction**

**Plate- 4(a-d): Processing of the collected plants & extraction of the essential oil**


**Plate- 5 (a-d): Test Pathogens/ the Dermatophytes- *T.m., T.r., M.g.* & *E.f.***





**Plate- 6(a-e): The Selected Plants**

a) ***Mentha piperita*** L.
b) ***Citrus aurantifolia*** L.
c) ***Eupatorium odoratum*** L.,
d) ***Ocimum basilicum*** L. and
e) ***Mikania micrantha*** Kunth ex HBK


**Plate- 7 (a-d): Preparation of the Culture Media & Inoculation**




**Plate- 8 (a-d): Preparation of the Culture Media & Inoculation**

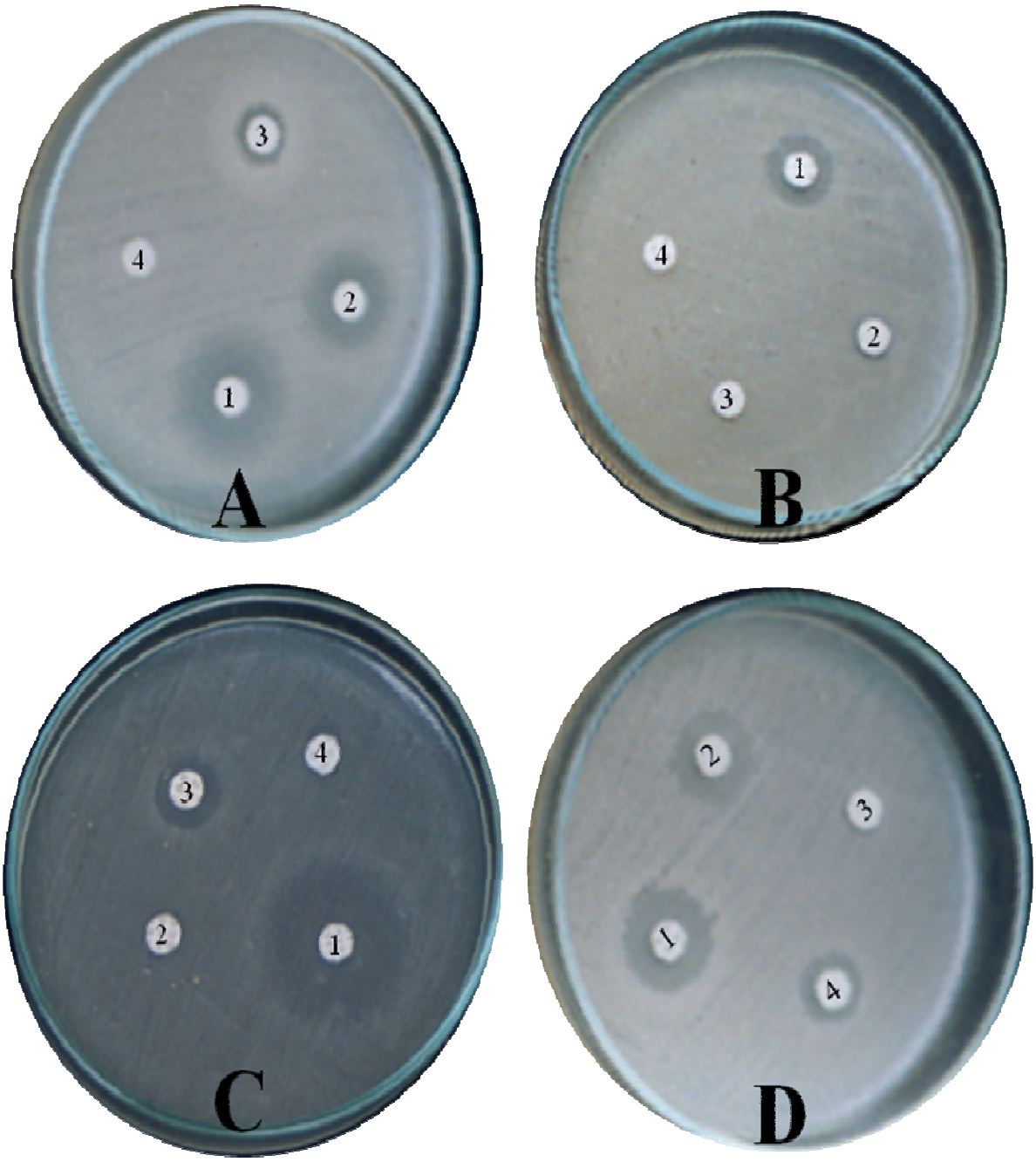


**Plate- 9 (a-d): Antidermatophytic Screening against *T.m., T.r., M.g. & E.f.*, Using Disc Diffusion Method**

**Plate- 10 (a-d): Antidermatophytic investigation of the test samples against *T.m.*, *T.r.*, *M.g.* & *E.f.*, using Broth Microdilution Method (NCCLS, 2002)**

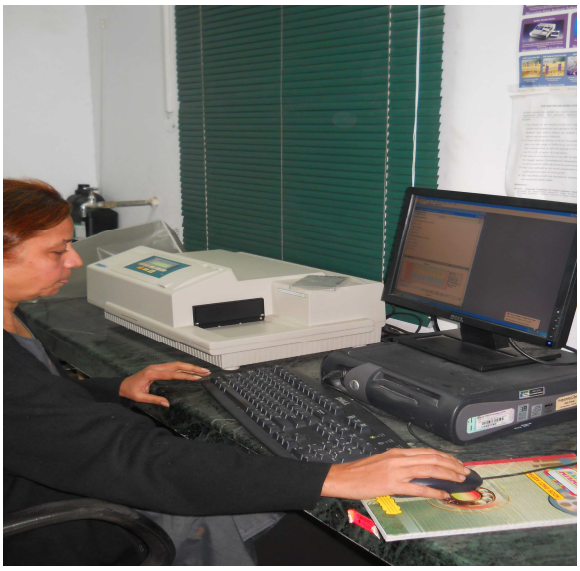

**Plate- 11 (a-c): Antidermatophytic investigation/ observation of the test samples against *T.m., T.r., M.g.* & *E.f.*, using Spectramax plus[384]**

**Appendix (i)**

## BIO DATA

| | | |
|---|---|---|
| 1. **Name** | **:** | Rita Gupta |
| **2. Date of Birth** | **:** | 31.10.1960 |
| **3. Father's name** | **:** | Shri R L Gupta |
| **4. Mother's Name** | **:** | Smt. Vira Gupta |
| **5. Nationality** | **:** | Indian |
| **6. Residential Address** | **:** | 2500 Sector D 2<br>Vasant Kunj, New Delhi 110070<br>Email Address: : ritagupta@nic.in |
| **7. Educational Qualifications:** | **:** | M.Sc. (Botany); LL. B<br>Ph.D (Thesis being submitted ) |
| **8. Experience :** | **:** | Working as Principal Scientist with the Dept of Science and Technology(GOI), New Delhi and have been involved in the<br>- Management/coordination of S&T Activities<br>- R& D Promotion programme in the area of life sciences<br>- S&T Manpower/ Infrastructure development<br>- Forging International S&T Co operation |

9. **Professional Trainings relevant to Academic and professional Interest:**

9.1 Foundation Training programme for Scientists and Technologist conducted by the Indian institute of Public Administration(IIPA) N Delhi

9.2 Basic Management tools and Techniques conducted by Institute of Training and Management N Delhi.

9.3 Training Programme in " Research Methodologies" organized by AIIMS N Delhi during August 23-28, 2010

9.4 IPR and WTO issues conducted by Patent Facilitation Centre (PFC), TIFAC during September 6-10, 2010.

9.5 International Training programme on "Contemporary management strategies in IPR relevant to NAM and Other developing Countries " conducted by NAM S&T Centre.

## 10. Recent Seminar/ Symposium/ Conference Attended

10.1 National Conference on Natural Resources Management at Dept. of Horticulture & Medicinal and Aromatic Plants, Mizoram University, Aizawl on 25 March 2009

10.2 'Science and Laws' organized by ASCI , Hyderabad.

10.2 31st IBS Conference & International Conference, Department of Botany, University of Allahabad, Allahabad-211002 during 10. 17-19 Dec. 2008

## 11. Research Publications: two

Kamran, A., Mishra, RK., **Gupta, R.,** Kumar, A., Bajaj, A.K. and Dikshit, A. (2012). Therapeutic Effects of Essential Oil fromWaste Leaves of *Psidium guajava* L. against Cosmetic Embarrassment Using Phylogenetic Appproach. American Journal of Plant Sciences, 2012, **3**: 745-752.

Kumar, A., **Gupta, R**., Mishra, R.K., Shukla, A.C., and Dikshit, A. (2012). Pharmaco-Phylogenetic Investigation of Micromeria biflora Benth and Citrus reticulata Blanco. National Academy of Science Letters, (ISSN 0250-541X), Springer Publication DOI: 10.1007/s40009-012-0029-7.

## 12. Awards/ membership of societies:

- Secured 1st position in the management programme conducted by Instt of Training and management.
- Secured 1st position and DST Medallion in the training programme for Scientists and Technologist conducted by IIPA N Delhi
- Member of Indian Science congress Association
- Member of Indian Women Scientists Association.