INVESTIGATION OF ANTI-DERMATOPHYTIC ACTIVITY FROM SELECTED MEDICINAL PLANTS OF MIZORAM

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By

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I Fanai Lalsangluaii, hereby declare that the subject matter of the thesis entitled ***"Investigation of Anti-dermatophytic Activity from selected Medicinal Plants of Mizoram"*** is the record of work done by me, 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.

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Abbreviations

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DMSO	:	Dimethyl Sulphoxide
GC-MS:		Gas Chromatographic-Mass Spectrometry
IR	:	Retention Indices
MFC	:	Minimum Fungicidal Concentration
MIC	:	Minimum Inhibitory Concentration
MS	:	Mass Spectroscopy
MSC	:	Minimum Static Concentration
NE Region	:	North East Region
RT	:	Retention Time
SDS	:	Sodium dodecyl sulfate
ATCC	:	American Type Culture Collection
MTCC	:	Microbial type culture collection
NA	:	Nutrient agar
NB	:	Nutrient broth
D.W	:	Distilled water
CFU	:	Colony forming units
MAP	:	Medicinal and Aromatic plant
IC50	:	Inhibition concentration at 50% concentration
NCCLS:	:	National Committee for Clinical Lab. Standards
CLSI	:	Clinical Laboratory Standards Institute
Ml	;	Micro litre
WHO	:	World Health Organisation
MOEF	:	Ministry of Environment & Forest
GOI	:	Government of India
g	:	Microgram
g	:	Gram
l	:	Liter
mg	:	Milligram
ml	:	Millilitre
ppm	:	Parts per million
h	:	Hours
lbs	:	Pounds
No.	:	Number
pH -	:	log10 hydrogen ion concentration
sp.	:	Species
spp.	:	More than one species
viz.	:	Namely
wt	:	Weight
yr	:	Year
%	:	Percentage
/	:	Per
<	:	Less than
>	:	Greater than

1 Introduction

In general, all humans are dependent on plants in order to meet various requirements for their survival (Phillips and Meilleur, 1998). Based on our traditional knowledge of herbal medicines and several new investigations, nearly 1,800 plant species are put under the category of medicinal plants and 7,600 plants that find their use in tribal and local health care systems all over the country. Nearly 6,780 pharmacies (Handa, 1992) are dealing with preparation of herbal based drugs resulting in an annual turnover of Rs 400 billion. In addition, approximately 10000 units of ISM including 7 lakh licensed practitioners and 10 lakh traditional healers in 6 lakh villages of India are involved in preparation, processing and use of herbal medicines. The need for revitalization of the Indian system of medicine, based primarily on herbs, has now been recognized all over the world.

According to WHO estimate, approximately four billion people all over world rely on herbs for their primary health care and 25% prescription drugs are also plant derived chemicals (Rai *et al.,*2000). Medicinal plants have always been greatly considered by rural communities, as they improve the economy of rural people. Our traditional system of medicine is related to the wealth of herbal biodiversity and cultural biodiversity. Indigenous knowledge on natural resources utilization of medicinal plants not exceeding the resilience of the surrounding environment is regarded as an important measure of sustainable plant biodiversity conservation. If such indigenous knowledge is being lost, people are forced to change their livelihood which serves as environmental degradation. Ethnobotanists, all over the world, have been actively working to collect, document and conserve the indigenous medicinal plants of different areas.

According to ethnobotanical survey carried out by MOEF, GOI approximately 8000 species come under the group of medicinal plants, which account for around 50% of all the higher flowering plant species of India. Millions of rural households use medicinal plants in a self-help mode to meet their health care requirements. Over one and a half million practitioners of the Indian System of Medicine in the oral and codified streams use medicinal plants for preventive, promotive and curative applications. Approximately

more than 7800 units in India manufacture plant based medicines, and the growing demand for herbal products has led to a quantum jump in volume of trade in plant based materials within and across the globe recently. An estimate of the EXIM Bank puts the international market of medicinal plants related trade at US$ 60 billion per year with a conservative growth rate of 7% approx. Even though India has a rich biodiversity; the growing demand is putting a heavy strain on the existing resources of medicinal plants.

Moreover, in Indian society, traditional medicine plays a dominant role. In the last two decades, many reports have been published on medicinal plants of the states of Bihar, Madhya Pradesh, the North eastern hill states, Tamil Nadu and Orissa reports reflect the intense plant use of the large tribal populations of the regions [Patil and Bhaskar (2006)]. Among all resources available to rural communities, medicinal plants have been of great importance as they improve the economy of rural people [Ojha (2000); Ticktin *et al.,* (2002)].

Herbal based health care traditions in India are as old as our civilization, and NE region in India is known for rich traditions of utilizing herbal formulations. Even today, herbal medicines are in daily use and each person at least knows the uses of one or few plants as medicine. Most of the village population is totally dependent on these plants for primary health care. Various serious diseases are also diagnosed and there are formulations available for many serious diseases with local vaidhayas and elders. However, unwilling to disclose such formulations due to fear of over utilization by others and increased awareness of local people about their IPRs, the proper documentation of this information is lacking. Much of this information available with local inhabitants is believed to be ruined and several informations are on the verge of disintegration. This fact is understandable especially when most of the local vaidhayas are only known to diagnose primary health problems and there are very few vaidhayas / ethnic groups who have rich knowledge and tradition of using Ayurvedic and ancient local health care formulations. Therefore, identification of these vaidhayas and documentation of important formulations having potential use in past and equally important for modern medical system as a source of new drug discovery/ formulations should be a main priorities to conserve our traditional knowledge of utilizing medicinal plants.

The NE region of country is well known for various forms of non-formal (traditional) medical treatments. The inhabitants of this region have gradually become familiar with the healing properties of the available plants. This has resulted in a traditional system of cure, which has remained non-formal and undocumented. Some literature is available on MPs diversity and some ethno botanical uses of the region including Borthakur (1976); Hajra (1977); Bhuyan (1989); Devi (1990); Chhetri *et al.,* . (1992); Jamir (1997); Kumar (2002); Lalramnghinglova (2003); Dutta and Dutta (2005) and Rao *et al.,* . (2006). However, a consolidated account and analysis of the total use pattern with regard to skin ailment and traditional treatments by different communities is not available and therefore considered in the present study.

Since the NE States, including Mizoram; are inhabited by tribal populations, beside common health concerns, many people are suffering from skin diseases due to adverse climate, hard work, unhygienic condition and poor livelihood options. In a study, it has also been estimated that skin diseases accounts for 34% of all occupational diseases (Spiewak, 2000). Infectious diseases, particularly skin and mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits (Ram *et al.,* (2004). An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida* spp., besides certain pathogenic bacteria are the most frequent .[Desta B (1993)].

However, on the other hand, medical facilities even for primary health care are not available near the vicinity of populations; several poor people still depend on vaidhayas even for the treatment of serious ailments. However, our experiences based on field trips for documentation of traditional knowledge of primary health care system and ethnobotanical heritage of traditional practitioners/ Vaidhays suggested that most of these people treat common diseases viz., Jwara (fever), Kas (Cough), Pratisyaya (cold) Ajirna (indigestion), Sirsula (headache), Naitra and Danta Rogas etc. using locally available plants/herbs. A few vaidhayas are also known to treat Amavat (Rheumatoid arthritis), Twak Roga (Skin diseases) and burns. However, most of the practitioners did not have any formulations for the treatments of serious ailments. Besides, for skin related problems (especially ring worm infections) there are well known and equally effective

herbal remedies which needed to be documented. Furthermore, proper identification, documentation and conservation of such ethno medicinally important plants can be made as well as their scientific validation; especially exploration of plant secondary metabolites against the pathogens causing skin diseases in human beings (i.e. dermatophytes), their biochemical analysis is needed much more, so that, after detailed investigations some bioactive constituents can be used for drug development, before they lost forever.

Although, in North eastern states, some researches on ethno medicinal plants have already been made e.g., in the states of Assam [Hajra and Baishya (1981); Das *et al.,* (2008); Sajem and Gosai (2006)]; Meghalaya [Rao (1981);Chetri (1994)]; Nagaland [Rao and Jamir (1982)]; Arunachal Pradesh [Tiwari (1980); Dam and Hajra (1981)] and in Mizoram [Fischer (1938); Mahanti (1994); Singh (1996); Lalramnghinglova and Jha (2000); Sharma *et al.,* (2001); Lalramnghinglova (2003); Rai *et al.,* (2007); Lalfakzuala *et al.,* (2007); Rai and Lalramnghinglova (2010 a); Rai and Lalramnghinglova (2010 b)]. But, there were no definite reports on ethno medicinal plants used against skin infections. Keeping all these views in mind, in the present investigation, an attempt has been made to analyze the traditional information available at the grass root level among the tribal communities of Mizoram, and their scientific validation.

1.1 Geographical Background of Mizoram

Mizoram is a beautiful state knows as the “Scotland of India”. It 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.

In the local language, Mizoram means "Land of the Highlanders". The Mizo Hills, which dominate the state's topography, rise to more than 6560 ft near the Myanmar border. Aizawl, the state capital, is 4000 ft above sea level.

Mizoram is a land of rolling hills, valleys, rivers and lakes. As many as 21 major hills ranges or peaks of different heights run through the length and breadth of the state, with plains scattered here and there. The average height of the hills to the west of the state is about 1,000 meters (3,300 feet). These gradually rise up to 1,300 meters (4,300 feet) to the east. Some areas, however, have higher ranges which go up to a height of over 2,000 meters (6,600 feet). Phawngpui Tlang also known as the Blue Mountain, situated in south-eastern part of state, is the highest peak in Mizoram at 2,210 meters (7,250 feet).

About three-fourths of the population earns their livelihood from agriculture. Paddy, maize, mustard, sugarcane, sesame, fiber less ginger and potatoes are the other prominent crops grown in this area. Small-scale irrigation projects are being developed to increase the crop yield. There are no major industries in the state. Small-scale industries include sericulture, handloom and handicrafts industries, sawmills and furniture workshops, oil refining, grain milling, and ginger processing. The service sector comprises of Tourism, Real Estate and Insurance.

The biggest river in Mizoram is Chhimtuipui, also known as Kaladan. It originates in Chin State in Burma and passes through Saiha and Lawngtlai districts in the Southern tip of Mizoram, goes back to Burma's Rakhine state, and finally enters the Bay of Bengal at Akyab, which is a very popular port in Sittwe, Burma. The Indian government has invested millions of rupees to set up inland water ways along this river to trade with Burma. The project is known as the Kaladan Multi-modal Transit Transport Project. Although many more rivers and streams drain the hill ranges, the most important and useful rivers are the Tlawng, Tut, Tuirial and Tuivawl which flow through the northern territory and eventually join the Barak River in Cachar District. The Chhimtuipui which originates in Burma is an important river in the south of Mizoram. It has four tributaries and the river is in patches. The western part is drained by river Karnaphuli (Khawthlang tuipui) and its tributaries. A number of important towns, including Chittagong in Bangladesh, are situated at the mouth of the river. Before Independence, access to other parts of the country was only possible through the river routes via Cachar in the north, and via Chittagong in the south. Entry through the latter

was cut off when the subcontinent was partitioned and ceded to East Pakistan (now Bangladesh) in 1947.

The Palak lake, the biggest in Mizoram is situated in Saiha District which is part of southern Mizoram covering 30 hectares (74 acres). It is believed that the lake was created as a result of an earthquake or a flood. The local people believe that a village which was submerged still remains intact deep under the waters. The Tamdil Lake is a natural lake situated 85 km (53 miles) from Aizawl. Legend has it that a huge mustard plant once stood in this place. When the plant was cut down, jets of water sprayed from the plant and created a pool of water, thus the lake was named Ţamdil which means of 'Lake of Mustard Plant'. Today the lake is an important tourist attraction and a holiday resort. The most significant lake in Mizo history, Rih Dil, is ironically located in Burma, a few kilometers from the India-Burma border. It was believed that the departed souls pass through this lake before making their way to Pialral or heaven.

1.2 Tribal communities of Mizoram

In Mizoram, 16 Scheduled castes, 14 Scheduled Tribes and 37 Sub- tribes have been recognized. (Anonymous, 1991). Lalramnghinglova (2003) so far traced 15 ethnic groups or population in Mizoram, such as *Lusei (Mizo), Paihte, Hualngo, Tlau, Thadou, Ralte, Hmar, Mara, Pawi, Bawm, Pang, Chakma, Riang, Biate and Mog.*

These ethnic groups are a very distinctive people rich in old- age traditions, cultures and customs, and continue to retain their respective dialects and ethnic identity. Out of the fifteen people groups, the Mizos, Maras, Pangs and Lais are gradually losing their valuable indigenous knowledge of traditional medicines, particularly among young generations, where as the Chakmas, Brus, Bawms and the Mogs are ethno-bio-culturally rich in their folklore knowledge. In most cases, older people in the community have more knowledge in the field of ethnobotany and they preserve it traditionally. Therefore, in the present study, frequent ethnobotanical surveys were made, so that, the actual information at the grass root level can be collected and explored scientifically.

1.3 Ethnobotanical Survey

The term "Ethnobotany" was first coined by Dr. John W. Harshberger in 1895. He described ethnobotany as the 'study of plants used by primitive and aboriginal people' (Harshberger, 1896). Since then, it has been amplified and defined in the following manner:

1) The study of the relationship which exists between people of a primitive society and their plant environment (Schultes, 1962).

2) The study of the past and present interrelations of primitive or aboriginal human societies with the ambient vegetation (Maheshwari, 1987).

3) The study of the relationship between the inhabitants and the habitats (Sarin, 1989).

4) The study of useful plants prior to their commercial exploitation and eventually domestication, including the use of plants by tribal and non-tribal communities without any complications of primitive or developed communities (Wickens, 1990).

5) The study of the total direct relationship between humans and plants (Jain, 1994).

6) The study of the interactions between people and plants (Martin, 1995).

According to Balick (1996), the new ethnobotany links diverse disciplines, such as anthropology, botany, nutrition, ecology, conservation, economics and pharmacology. The Inter- disciplinary nature of ethnobotany has been explained by Maheswari (1987).

It has been found that most of the plant species having medicinal value grow in the forests. About 95% of the medicinal plants used by herbal pharmaceuticals and for export are collected from the wild with no parallel regeneration programme to replenish medicinal plants stock (Anonymous, 1995). It is the general concept that 80% of the rural

population in developing countries depend upon medicinal plants for their primary health care needs. A scientific survey of these medicinal plants and their ethnobotanical studies has been done by Botanical Survey of India, Research Institutions and Laboratories, Universities and Non- Government Organizations in India. A number of ethnobotanic exploitations were conducted in different tribal areas of the country.

Out of 15,000 species of higher plants recorded so far in India, All India Coordinated Research Project has recorded over 9, 500 wild plants species used by tribals for meeting their varied requirements. Of these, approximately 7, 500 species are used for medicinal purposes, about 800 species for food, over 525 species for fibers and cordages, about 400 species for fodder, and about 300 species for piscicides and pesticides, of which at least 175 are promising for safe boipesticides (Anonymous, 1994).

It is reported that more than 800 plant species of ethnobotanical interest were collected at different centers (Anonymous, 1990). These plant species were over exploited for a very long time and many of them have become endangered species. On the basis of new IUCN (International Union for Conservation of Nature) Red List Categories, a first Red Data List of Indian Medicinal Plants was brought out as per the assessment of Conservation Assessment and Management Plan (CAMP) workshops held during 1995- 1997. The first Red Data List of Indian Medicinal Plants include 36 medicinal plant species of South India and about 75 species from rest of the country, including North- East India. (Anonymous, 1995).

Mizoram- the state of Mizoram receives little attention as far as the survey of medicinal plants is concerned. Like other tribes, the tribes in Mizoram practice traditional herbal medicines, the local knowledge of which has been descending through generations since time immemorial. Ethnomedicinal plants are still widely used for curing different diseases both in urban and rural areas. There is a need for documentation of such valuable indigenous knowledge and domestication of economically important medicinal plants to decrease pressure over natural resources and to fulfill the requirements of national and local needs.

Ethno- medicinal survey for various plants, used against dermatophytes, was carried out to collect information from traditional use of the medicinal plants. It a fact that folk

medicine has been used for thousands of years among the native people with significant contributions made by its practitioners to human health, especially as primary health care providers at the community level. Traditional folk medicine uses the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to its cultures, for maintenance of health. It is solely based on the expertise gained by local practitioners over a long period of time. It has been estimated that folk healers in India employed approximately about 2500 species of plants, which serves as a regular and effective source of medicine. Although lacking in scientific validation, ethno-medicinal use of plants and its extracts is still prevalent in many parts of the country till date mostly due to its affordability, availability, accessibility and acceptability.

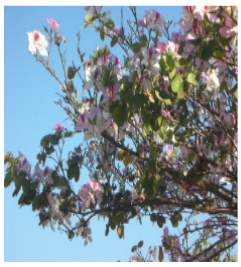
Various plants have been used in traditional medicine for thousands of years and the knowledge and importance of medicinal plants have been known in the course of many centuries based on many medicinal systems such as Unani, Ayurveda, Siddha, etc. On continuation, during the last few decades there has been increased interest in the field of medicinal plants and their traditional use in different parts of the world. Hence, documenting the indigenous knowledge through ethno botanical studies is important for the conservation and utilization of plant resources. Keeping these views in mind, the present study aims to prepare an inventory of ethno medicinal plants used by different ethnic groups of Mizoram to treat different kinds of skin ailments. Beside, informations have also been collected from traditional healers of rural areas as well as through various secondary informations, available in Mizoram. Further, the findings are categorized and organized in tabular form containing their local name, botanical name, family and a brief note on the parts of plants used method of administration and dosages.

Furthermore, a comprehensive list of plant species used for the treatment of skin diseases is summarized in table- 1.1.

Table-1.1: Ethno medicinal plants used against skin ailments among the tribal communities in Mizoram:

Picture of the Plant	Local name	Botanical and Family name	Part used	Ethno- medicinal use(as anti-dermatophytes)
	Anchiri	*Homalomena aromatica* Schott Araceae	Petiole and Rhizome	Leaves, rhizome or juice of whole plant is used as lotion for treatment of skin diseases.
	Japanhlo ral/ bawrai ral	*Cuscuta reflexa* (i) Roxb. Convolvulaceae	Whole plant	Juice of crushed plant is used against various skin diseases.
	Phuihnamchhia	*Clerodendrum viscoscum.* Vent. Verbanaceae	Rhizome leaves, roots.	The rhizome is boiled and the warm water is dabbed on the infected scabies skin. Juice of crushed leaves is also used for the same.
	Thasuih	*Lindernia ruellioides* (Colsm.) Linderneaceae	Whole plant.	Decoction of whole plant is rubbed against ringworm infected skin.
	Dawng	*Commelina nudiflora* Linn. Commellinaceae	Leaves and stem.	Juice extract from crushed leaves and tender stem are used for the treatment of different types of skin diseases.
	Tlawr Thing.	*Tectona grandis* Linn. Verbenacaeae	Bark, flower, heart of wood, saw dust and fruit.	Decoction of bark and crushed/ powdered bark mixed with oil is used against various skin diseases.

	Buarze	*Blumea lanceolaria* Roxb. Compositae/Asteraceae	Leaves.	Juice of crushed leaves applied on various skin diseases.
	Vang	*Albizia chinensis* Merr. Leguminosae	Bark, latex.	Skin rashes, itching or various kinds of skin infections.
	Rûnhmui	*Ocimum americanum* Linn. Lamiaceae	Leaves, seed.	Juice of crushed leaves is mainly used in external application of various skin diseases, itch, tick bites etc.
	Rawl-damdawi	*Calotropis gigantia* Linn. Asclepiadaceae	Leaves.	The latex is applied externally on various other skin diseases.
	Kawrthin-deng	*Dillenia indica* L. Dilleniaceae	Leaves & barks	The blaze is grounded to powder and is applied externally on various skin infections
	Fartuah	*Erythrina stricta* Roxb. Fabaceae	Bark and leaves	Fresh barks and leaves applied on sores.
	Hmawng	*Ficus bengalensis* Linn Moraceae	Bark, fruit, leaves, latex and root.	Decoction of bark is given orally for treatment of various skin diseases

	Kangdam	*Jatropa curcas* Linn Euphorbiaceae	Latex, leaves, seed.	Watery juice (latex) from stem is applied directly to –Scabies, Eczema, Skin sores, Ringworm & Boils, etc
	Vau/Vau bê	*Bauhima variegata* Linn Caesalpiniaceae	Bark, flower, root, leaves.	Decoction of the bark is used in Sores, itches and various skin diseases. **Note:** Based on nature of disease, it is taking orally or applied on affected parts.
	Makpazangkang/Luahmur	*Cassia nodosa* Ham. Caesalpiniaceae	Bark, leaves, fruit, root.	Juice of crushed leaves mixed with lemon juice is used in treatment of certain skin diseases.
	Tuihlo/Dadu-hlo/Kel-be-pui	*Cassia alata* Linn. Caesalpiniaceae	Leaves.	Juice of crushed leaves is applied externally as a remedy for various skin diseases like - Ringworm, Gonorrhoea, Scabies, Eczema & Snake-bite etc.
	Kêl-bê	*Cassia tora* Linn. Caesalpiniaceae	Root, leaves, fruit	Crushed leaves and fruits are often applied on skin for treatment of ringworm, itching, swelling, scabies etc.
	Ngaingaw/ Phungril	*Cassia fistula* Linn Caesalpiniaceae	Root, leaves, fruit.	Juice of crushed leaves used on various skin diseases like ringworm or any kind of skin itches, applied externally or crushed leaves bandaged onto affected skin.

	Sazupumpui thei	*Anacardium occidentale* Linn. Anacardiaceae.	Bark, nut.	Juice of cruhsed bark applied directly on ringworm.
	Buchhawl	*Achyranthes aspera* L. Amaranthaceae	Leaves	Juice extracted from crushed leaves used direcly on cuts and wounds and other skin ailments.
	Lenhmui	*Syzygium cumini* Linn. Myrtaceae	Seed, bark, leaves, fruit.	Decoction of bark (20gm in 10 lits of H_2O) is used for treating skin diseases
	Sarawn/Ka-nân-par	*Nerium indicum* Mill. Apocynaceae	Root, leaves.	Crushed root mixed with water is used against ringworm.Decoction of leaves is used for treatment of skin diseases like ringworm, leprosy, itches etc
	Ngaihhih	*Linostoma decandrum* Thymeleaceae	Root.	The roots are boiled in water and the water is used for dressing scabies and other skin disorders.
	Nawhalh Thing	*Eucalyptus citriodora* Labil. Myrtaceae	Leaves, oil (from seed), root.	Oil obtained from seed is applied externally on all kinds of skin diseases.

	Hnahbial/Darbengbur/Lambak	*Centella asiatica* Linn Umbelliferae	Whole plant, leaves.	The juice of crushed leaves is applied on various skin diseases such as itches, ulcers, sores, etc
	Purunvar	*Allium sativum* Linn Liliaceae	Bulb.	The bulb is cut and the juice is applied to all kinds of skin diseases including ringworms, scabies, itches, acne, leucoderma etc.
	Pudina	*Mentha arvensis* L. Lamiaceae	Whole plant	Decoction of leaves used directly against itching
	Thuamriat	*Alstonis scholaris R. Br.* Apocynaceae	Leaves, bark, latex, root	Latex from plant is used against skin diseases like leprosy, ringworm, etc
	Zawngtei	*Chukrassia tabularis* Andr. Juss. Meliaceae	Bark	Juice extracted from bark is applied on skin for cuts and wounds.
	Saisiak	*Securinega virosa* Euphorbiaceae	Leaves	Decoction of leaves used for bathing or juice from crushed leaves applied on skin for treatment of various diseases like measles, chicken pox, itches, etc

	Tlangsam	*Eupatorium odoratum* Linn. Compositae	Leaves	Paste made from crushed leaves is applied on skin for treatment of cuts, wounds, etc
	Japanhlo	*Mikania micrantha* Kunth. Asteraceae	Leaves	Fresh juice from crushed leaves is exracted and applied directly on skin for various skin ailments.
	Ru- teng	*Milletia pachyacarpa Benth.* Papillionaceae	Root	Juice from crushed root is used in scabies and other skin diseases.
	Vai- umkhal	*Terminalia catappa Linn.* Combretaceae	Bark, leaves.	Juice of young leaves used to prepare ointment for leprosy, scabies and other skin diseases.
	Khawitur	*Hydnocorpus kurzi(King)Warb.* *Flacourtiaceae*	Seed, fruit	Oil extracted from seeds used in leprosy and other skin diseases.
	Vaithinthang	*Houttuyia cordata* Thumb. Saururaceae	Rhizomes, leaves, root	Leaves are used for various skin diseases.
	Buarzo	*Gynura conyza. Sp.* Compositae	Leaves	Juice of leaves used externally in scabies, fresh wounds and skin diseases.

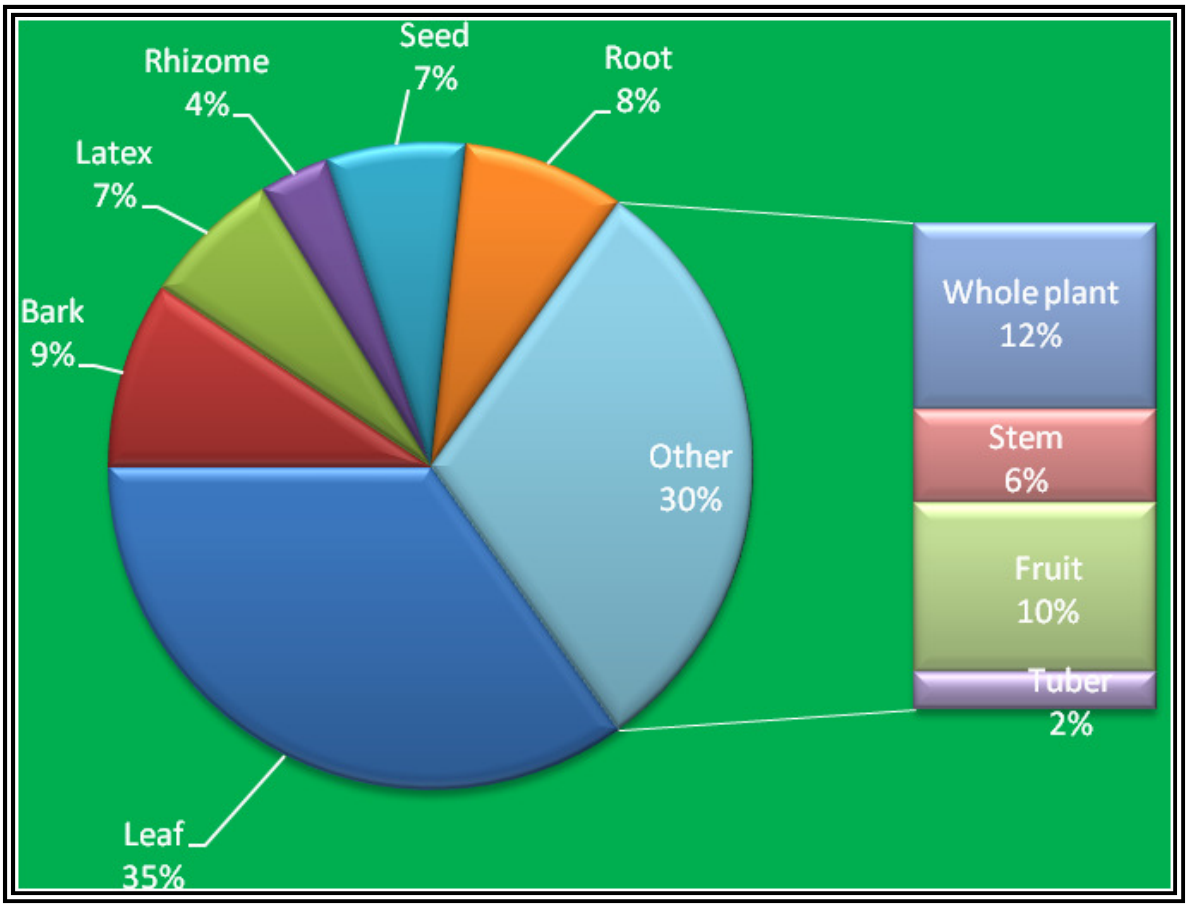
	Hnamtur	*Gelsemium elegans.* Benth. Logniaceae	Root	Juice from crushed root is used in external application of ringworm.
	Lawngthing	*Dipterocarpus turbinatus* Gaerta. Dipterocarpaceae	Resin	Oleo-resins used against ringworm.
	Sairial	*Buddleia asiatica* Lour. Loganiaceae	Leaves	Crushed leaves used against skin diseases.
	Chul- be-raw	*Lobelia pyramidalis* Campanulaceae	Latex, leaves	The milky juice is applied over warts, boils and various skin diseases.
	Lamkhuang	*Artocarpus heterophyllus* Lam. Moraceae	Root, latex, seed.	Latex obtained from plant is appied onto skin for itches, boils, insect bite, wounds and many skin infections.
	Sunhlu/Sinhlu	*Phyllanthus emblica* Linn. Euphorbiaceae.	Leaves, Fruits & Bark	Powered seed is used as anti-dandruff. Bark juice is applied on cut and wounds, ring worm and cutaneous diseases.
	Kumtluang	*Catharanthus reseus* G. Bom. Apocynaceae	Leaves, root, flower.	Juice extracted from crushed leaves is applied directly on skin for various skin ailments.

	Khiang	*Schima wallichii* Theaceae	Leaves, Latex	Crushed leaves juice as well as latex directly applied to skin sores and skin ulcers.
	Theihai	*Mangifera indica Linn.* Anacardiaceae	Bark, root, dried leaves, gums.	Resinous gum is used for treatment of scabies and other skin diseases.
	Thingfang-hma	*Carica papya* Linn. Caricaeae	Fruit, latex/gum, root, leaves.	Latex/ gum is used for treatment of various skin diseases.
	Zawngtah	*Parkia timoriana* Merill. Mimosaceae	Fruit, bark, seed, tender leaves	Green covering of fruit is scraped off, soaked in water and applied on skin for treatment of rabies, scabies, boils, etc

As far as plant parts are concerned, the tribals employ almost all parts of the plant in ethno medicine. In terms of plant parts used, 35% of medicines employed the leaves, 12% the whole plant, fruit 10%, 9% bark, 8% root 7% latex, 7% seed, 6% stem, 4% rhizome, and 2% tubers (Figure 1.1). The prevalence of the use of leaves for the preparation of traditional herbal remedies as shown in this study corresponds with earlier reports in other studies [Anitha et al, (2008); Yineger and Yewhalaw (2007); Pradhan and Badola (2008); Zainol et al., (2008)]. Leaves are known to accumulate alkaloids, tannins and inulins which are the active components of most herbal preparations [Okoegwale and

Omefezi (2000); Focho et al., (2009)]. The most prevalent forms of administration of medicine were topical.

Fig- 1.2: Plant parts & their percent use



Plant parts are prepared as medicine using fresh material, dried plant material (powder), in the form of juice, decoction and infusion. Most of the plant remedies are prepared as juice form (30%) or as a paste in water (29%) followed by the decoction (14%), Resin /Latex (9%), infusion (4%), poultice (4%) and dried powder form (4%) and oil of plant part (6%) (Figure 1.1).

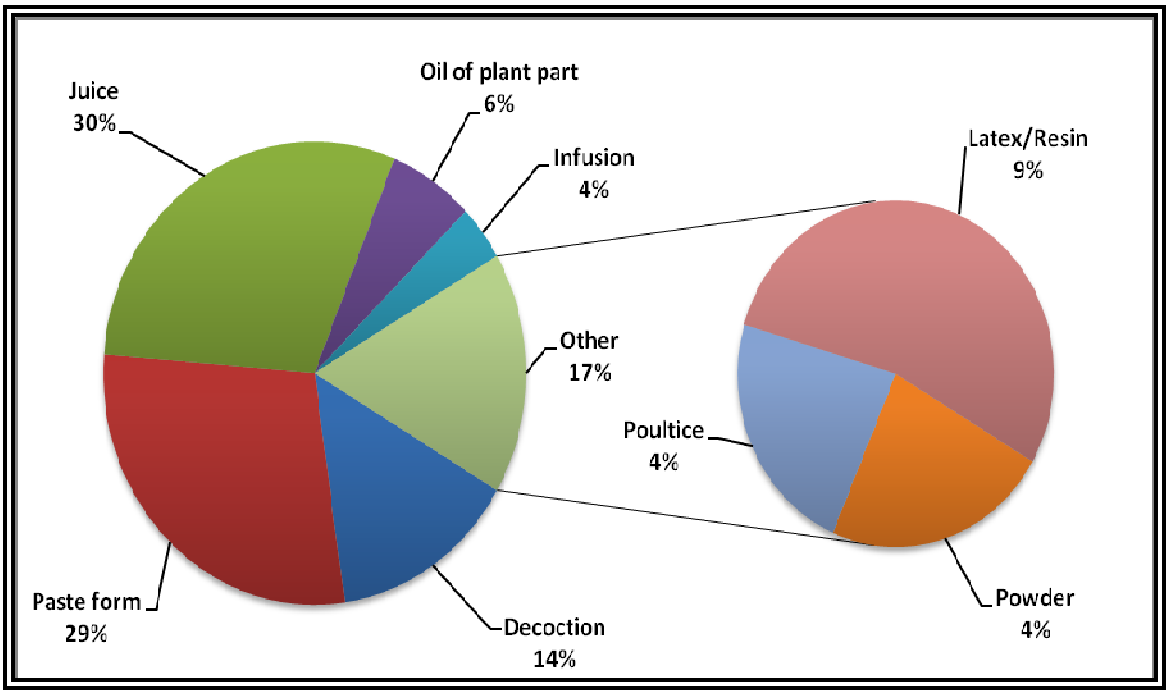


Fig- 1.3: Mode of applications &their percent

The use of the various plants could be attributed to cultural acceptability, efficacy, physical accessibility and economic affordability as well as playing a major role in the treatment of various kinds of skin ailments in comparison to modern medicine. Based on the difficulty in identifying types of skin problems such as, ringworm, scabies, itching, herpes, measles, skin ulcer etc by the local people, many of the plants have been used in treating either of these infections unknowingly, thereby, indirectly showing their multipurpose efficacies

2. The Dermatophytes

2.1 Dermatophytes

The history of human medical mycology started with discovery and incrimination of aetiologic agents of dermatophytosis (Emmons *et al*, 1977).

Dermatophytosis - "ringworm" disease of the nails, hair, and/or stratum corneum of the skin caused by fungi called dermatophytes.

Dermatophytes are keratinophilic - "keratin loving" microorganism. Keratin is a major protein found in horns, hooves, nails, hair, and skin.

Dermatomycosis - more general name for any skin disease caused by a fungus.

Ringworm - Growth of dermatophyte in the skin and scalp is more or less equal in all directions and lesions produced tend to creep in a circular or ring form. For this reason, the Greeks named the disease Herpes. The **Romans** associated the lesions with insects and named the disease **tinea** meaning any small insect larva (Rippon, 1974).

2.2 Mycology

Emmons (1934) classified dermatophytes, into three genera and these genera have many species included in them.

2.2.1 *Microsporum* (Skin and Hair)

1. *Microsporum audouinii*
2. *Microsporum canis*
3. *Microsporum gypseum*
4. *Microsporum ferrugineum*
5. *Microsporum cookei*

2.2.2 *Trichophyton* (Skin, Hair and Nail)

1. *Trichophyton mentagrophytes*
2. *Trichophyton rubrum*
3. *Trichophyton tonsurans*
4. *Trichophyton violaceum*
5. *Trichophyton schoenleinii*
6. *Trichophyton verrucosum*

2.2.3 *Epidermophyton* (Sabouraud, 1910) (Skin and Nail)

1. *Epidermophyton floccosum*

The identification of dermatophytes is based on morphology of fungal colony and also on microscopic appearance of conidia and accessory structures in culture.

2.3 Macroscopic and microscopic features of each species

2.3.1 *Microsporum* spp.

Members of this genus affect skin and hair. This genus is characterized by fusiform or spindle shaped multiseptate macroconidia usually thick and rough walled. Microconidia are club shaped.

2.3.1.1 *Microsporum audouinii*

Macroscopic features: Colony is slowly growing, forming a flat or gray tan with radiating edges. Reverse is salmon, rust or peach.

Microscopic features: Mycelium is usually sterile with many terminal or intercalary chlamydospores. Macroconidia are usually absent. Microconidia are rare, if present, are club shaped borne singly along sides of hyphae.

2.3.1.2 *Microsporum canis*

Macroscopic features: White woolly colony with a buff to brown center and bright yellow periphery is formed within one week. The reverse is bright yellow to yellow orange.

Microscopic features: There are numerous rough, thick-walled, spindle shaped macroconidia containing 6 to 15 cells. A few club shaped microconidia are borne singly along sides of the hyphae. Racquet hyphae, nodular bodies and chlamydoconidia may be present.

2.3.1.3 *Microsporum gypseum*

Macroscopic features: Rapidly growing, powdery colony with surface tan, buff to cinnamon. Reverse is buff or reddish brown.

Microscopic features: There are numerous, rough, thin walled elliptical macroconidia containing 4 to 6 cells. A few club shaped microconidia are borne singly along the hyphae.

2.3.1.4 *Microsporum ferrugineum*

Macroscopic features: The growth is slow, forming a heaped, folded reddish-yellow with waxy surface.

Microscopic features: Irregular hyphae lacking conidia or coarse hyphae with prominent septa (Bamboo hyphae).

2.3.1.5 *Microsporum cookie*

Macroscopic features: This produces moderately growing yellowish, reddish, tan powdery or granular colony with reverse deep wine red.

Microscopic features: Numerous thick walled ellipsoidal macroconidia. Abundant microconidia.

2.3.2 *Trichophyton* spp.

Members of this genus affect skin, hair and nail. This genus is characterized by smooth thin walled pencil shaped macroconidia. Microconidia are spherical or club shaped.

2.3.2.1 *Trichophyton mentagrophytes*

Macroscopic features: The growth is rapid, forming creamy tan to pink surface, with powdery to granular consistency. Reverse is buff to red or reddish brown.

Microscopic features: Smooth walled, pencil-shaped macroconidia. Abundant spherical microconidia arranged in grape like clusters.

2.3.2.2 *Trichophyton rubrum*

Macroscopic features: A granular or fluffy white colony with a pink periphery and deep wine red reverse forms in two weeks.

Microscopic features: Numerous or few smooth walled pencil-shaped macroconidia. Numerous club shaped microconidia are borne singly along the sides of the hyphae.

2.3.2.3 *Trichophyton tonsurans*

Macroscopic features: Fairly fast growing, surface white, cream pale yellow powdery, suede like with flat or raised and folded edges. Reverse is mahogany reddish brown.

Microscopic features: Rare smooth walled club shaped or aborted macroconidia. Numerous microconidia with a great size and shape variation. Chlamydoconidia and racquet hyphae may be present.

2.3.2.4 *Trichophyton violaceum*

Macroscopic features: A waxy or suede, violet coloured heaped colony with lavender reverse forms in 2 to 3 weeks.

Microscopic features: Conidia are typically absent. Irregular hyphae and chlamydospores are present.

2.3.2.5 *Trichophyton schoenleinii*

Macroscopic features: A waxy, heaped light yellow to buff colony with colourless to yellow-orange reverse forms in 2 to 3 weeks.

Microscopic features: Conidia usually absent, antler like hyphae (favic chandeliers) chlamydoconidia and hyphal swelling may be present.

2.3.2.6 *Trichophyton verrucosum*

Macroscopic features: A heaped, waxy, white to bright yellow colony is formed on SABHI with yeast extract.

Microscopic features: On thiamine enriched media rare 3 to 5 celled macroconidia with an elongated rat-tail end. Few club shaped microconidia borne along the hyphae are observed.

2.3.3 *Epidermophyton* spp.

Members of this genus affect skin and nails. This genus is characterized by club shaped, smooth walled macroconidia. Microconidia are not produced.

2.3.3.1 *Epidermophyton floccosum*

Macroscopic features: A velvety, khaki-yellow colony with tan reverse forms within 10 days.

Microscopic features: Numerous club-shaped, smooth,thin-walled macroconidia containing 2 to 4 cells borne singly or in clusters, no microconidia [Emmons *et al*., (1977); Rippon (1974); Chung and Bennet (1992); Collier *et al* (1998); Monica (2000)].

2.4 Ecology and Epidemiology

Dermatophytes have been grouped into geophilic, zoophilic and anthropophilic species based on their ecology and host preference. Geophilic species are considered ancestral to pathogenic dermatophytes. The natural habitat of these species is the soil. Exposure to soil is the main source of infection for humans and lower animals. Ex: *M. gypseum, M. fulvum, T. ajelloi.* Zoophilic species have gradually evolved from soil to parasitize animals. These fungi are primarily animal parasites. Human infections are acquired either by direct contact with an infected animal or indirectly by contact with fomites.

Ex: *M. canis, M. gallinae, T. mentagrophytes* var *mentagrophytes*, *T. verrucosum*. Anthropophilic species have evolved from zoophilic species. Humans are normal hosts for this species and transmission may occur directly or indirectly.

Ex: *E. floccosum, M. audouinii, T. mentagrophytes var interdigitale, T. rubrum, T. schoenleinii, T. tonsurans, T. violaceum* [Rippon (1974); Chung and Bennet (1992)].

2.4.1 Epidemiology

Age, sex, race and occupation have little recognized influences upon the frequency of dermatophytosis.

Age: Tinea capitis is common in children and rarely seen in adults.

Cultural factors: In extremely hot humid climates, tinea corporis occurs readily under occlusive garments. Ex: tinea corporis around the waistline of Indian women who wear sarees.

Tinea pedis is more common among people who wear occlusive footwear, frequenting swimming pools or among individuals who use community washing facilities such as those in armycamps, boarding schools, etc.

In India, tinea capitis is frequently seen in south compared to north due to the poor scalp hygiene, abstinence from application of vegetable oil to hair which has inhibitory effect on fungus [Rippon (1974); Chung and Bennet (1992)].

2.5 Geographic Distribution

Dermatophytes vary from one geographic region to other depending on passage of time, living conditions, migration of population, environmental status of surroundings, personal hygiene and variable climatic conditions.

Before 1900, in Western Europe tinea capitis was rare and was caused mostly by *M. canis,* from 1900 to mid 1950's a grey patch ectothrix type of ringworm in children caused by *M. audouinii* replaced *M. canis* due to improved standard of living and spread over the USA and Canada. This inturn is replaced by T. tonsurans.

In 1900, Dr. Powell reported first case of dermatophytosis in India. He showed prevalence of ringworm disease in Assam with complete absence of tinea capitis, in marked contrast to the then published records from Britain. He suggested anatomical

distribution was influenced by dress worn by patients in Assam and discussed rarity of ringworm infection before puberty, while it was common in infants and children in Europe.

The prevalence of dermatophytosis varies in India. Most Indian studies indicate its prevalence in southern and eastern region than the northern regions of the country. In India the commonest species isolated are *T. rubrum* followed by *T. mentagrophytes* and *E. floccosum.* is commonest species causing tinea capitis. Tinea cruris and tinea corporis are the commonest varieties seen in India followed by tinea capitis, tinea pedis, tinea barbae, tinea unguium and tinea manuum in a descending order of frequency [Emmons *et al*., (1977); Rippon (1974); Chung and Bennet (1992); Collier *et al* (1998); Monica (2000)].

2.6 Source of Infection and Transmission

Ringworm infection is transmitted indirectly via fallen infected hairs and desquamated epithelium more often than by direct contact. Fomites play an important role in transmission, but host factors such as immunological status and local factors such as trauma, excessive moisture or occlusive clothing may constitute risk factors when combined with exposure to the fungus. Use of contaminated combs, hairbrushes and caps are the common methods of indirect transmission.

In tinea pedis, exogenous exposure to the fungus in showers, swimming pools, etc. as well as host factors play an important role in causing clinical disease. Infection by T. concentricum is transmitted from diseased mother to child after birth by contact [Emmons *et al*., (1977); Rippon (1974); Chung and Bennet (1992); Collier *et al*(1998); Monica (2000)].

2.7 Pathogenesis

Several factors, which can account for natural resistance to dermatophytosis in humans and animals, are known. Natural resistance to M. audouinii in the human clearly appears after puberty. Rothman *et al*. believed that the resistance is due to increase in fungistatic long chain fatty acids in the sebum at puberty. Internal organs of humans and animals are

naturally resistant to dermatophytosis. A fungistatic substance called “serum factor” of normal individuals and animals is believed to limit the growth of dermatophytes to the stratum corneum. The “serum factor” is an unstable, dializable, heat labile component of fresh serum and tissue fluid. Colonization of dermatophytes begins in the horny layer of the skin, hair and nail and ultimate outcome of the disease depends on host, strain, species variation and anatomic site. On the glabrous skin, the Infection spreads centrifugally, showing the classic ringworm pattern.

Infection with geophilic and zoophilic organisms characteristically invoke an intense inflammatory reaction and almost never cause chronic infections, anthropophilic organisms elicit less inflammation.

Klingman divides the infection into several stages: a period of incubation, enlargement, spread, a refractory period and stage of involution.

In the incubation period, hyphae grow in the stratum corneum of the scalp with minimal clinical signs of infection. A carrier state has been postulated when presence of dermatophyte is detected on seemingly normal skin by KOH examination or culture.

During the period of spread, the fungus grows radially from the point of origin in stratum corneum. Once the infection is established in the stratum corneum, two factors are important in determining the size and duration of the lesion. These are the rate of growth of organism and rate of epidermal turn over.

Inflammatory response at the rim of lesion stimulates an increased epidermal turnover in an effort to shed the organisms. However there is undoubtedly lag periods between the initiation of infection, the host inflammatory response and increased epidermal turnover. Therefore probably only the organisms at the inflammatory rim are being shed, while just ahead maintain infection. The annular appearance of most dermatophyte infections is compatible with these findings.

2.7.1 Virulence factors

1. Certain dermatophytes produce substance that diminishes the immune response. Foremost among these are mannans, glycoprotein constituent of cell wall that are

released from fungi during growth, they probably interfere with RNA synthesis or RNA functions that are necessary for presentation of antigen to appropriate T-cells.

2. Dermatophytes produce catalase which may act as defense against phagocyte myeloperoxidase system.

3. Dermatophytes produce enzymes such as chitinase or proteinase to derive nutrition from surrounding epidermal structures [Emmons *et al*., (1977); Rippon (1974); Chung and Bennet (1992); Collier *et al* (1998); Monica (2000) and Weissfeld (2002)]

2.8 Clinical Features

Infections caused by dermatophyte (ringworm or tinea) are clinically classified on the basis of location of the lesions on the body. Although different body sites may be affected, each focus of infection is generally a result of local inoculation. The invading dermatophyte grows in centrifugal manner, forming irregular rings with inflammatory borders with some clearing in central area of lesion.

2.8.1 The clinical types are classified as follows:

1. Tinea capitis: Ringworm of scalp
2. Tinea corporis: Ringworm of glabrous skin
3. Tinea cruris: Ringworm of groin
4. Tinea pedis: Ringworm of feet
5. Tinea manuum: Ringworm of hand
6. Tinea barbae: Ringworm ofbeard and moustache
7. Tinea unguium: Ringworm of nail
8. Tinea faciei: Ringworm of face
9. Tinea imbricata: variant of tinea corporis caused by *T. concentricum*

2.8.1.1 Tinea capitis

Ringworm of scalp involves infection of hair and scalp and presents as following two clinical types

a) Inflammatory: Kerion, Favus

b) Non-inflammatory: Black dot, Seborrheic dermatitis like

Hair invasion are of two types:

a) Ectothrix type

The invading fungus infects the hair shaft at mid follicle and forms a sheath of hyphae and arthroconidia 2-3 µm in diameter that surrounds the shaft. The infected hair become lustreless, brittle and hair filaments break off at the level of scalp to give an appearance of partial alopecia.

Common species: *M. audouinii, M. canis, T. mentagrophytes, M. gypseum, M. ferrugineum.*

b) Endothrix type

The hyphae invade hair follicle and then the hair shaft and form numerous arthroconidia within the hair shaft. The infected hair becomes grayish white, breaks off easily to give "black-dot" appearance.

Common species: *T. violaceum, T. tonsurans*

2.8.1.2 Tinea corporis

Ringworm of glabrous skin. The lesions are well marginated with raised erythematous borders. The annular, scaly patches may coalesce to form large area of chronic infection.

Common species: *T. rubrum, T. mentagrophytes, T. tonsurans*

2.8.1.3 Tinea cruris (Jock itch)

Ringworm of inguinale area involving the groin, perianal, perineal areas often involving the upper thigh.

Common species: *T. rubrum, T.mentagrophytes, E. floccosum*

2.8.1.4 Tinea pedis (Athlete's foot)

Ringworm infection of feetinvolving interdigital webs and sole. The most common clinicalmanifestation is intertriginous form associated with maceration, scaling, fissuring and erythema which presents with itching and burning.

Common species: *E. floccosum,T. rubrum, T. mentagrophytes*

2.8.1.5 Tinea manuum

Ringworm infection of palms and interdigital areas of hands and lesions present as diffuse hyperkeratotic areas.

Common species: *T. rubrum*

2.8.1.6 Tinea barbae

Ringworm infection of coarse hair of beard and moustache. The lesions are inflammatory and pustular.

Common species: *T. verrucosum, T. mentagrophytes*

2.8.1.7 Tinea unguium

Ringworm infection of nail plate. Distal subungual infection is the commonest pattern and involves nail bed and underside of nail in distal portion. The nail plate is brittle, friable, thickened and may crack because of piling up of subungual debris. The colour of nail is often brown or black.

Common species: *T. rubrum, T.mentagrophytes, E. floccosum*

2.8.1.8 Tinea faciei

Ringworm infection of glabrous skin of face.

2.8.1.9 Tinea imbricata

It is a geographically restricted form of tinea corporis caused by *T. concentricum*. It is characterized by polycyclic, concentrically arranged rings of papulosquamous patches of scales scattered over and often covering most of the body.

2.9 Laboratory Diagnosis

Detailed clinical examination of lesions can be done in bright light. Clinical lesions produced by these fungi are highly variable and closely resemble other skin diseases, making laboratory diagnosis and confirmation necessary. Though various species of dermatophytes produce clinically characteristic lesions, a single species may produce variety of lesions depending upon site of infection.

2.9.1 Specimen collection

2.9.1.1 Skin

Disposable scalpel blade of solid type is used to obtain skin scrapings. First the affected part of the skin is cleaned with 70% alcohol and the material is taken from active margin. The scrapings are collected in a clean, white paper packet to prevent contamination.

2.9.1.2 Hair

The infected hairs are removed by plucking with the roots intact using epilating forceps. Brush samples are excellent method for culture of scalp infections. A sterile plastic scalp massager or disposable plastic hair brush is brushed firmly over the scalp and then pressed against culture media.

2.9.1.3 Nail

The affected nail is cleaned with 70% alcohol. Nail clippings of the infected part and scrapings beneath the nail are collected in a clean white paper packet.

2.10 Direct Microscopy

2.10.1 Potassium Hydroxide (KOH) Preparation

2.10.1.1 Skin

The material is placed on a microscope slide and covered with a drop of 10% KOH solution. A cover-slip is placed with a little pressure. The excess of solution is blotted with blotting paper to make even preparation. The slide is gently heated, taking care not to boil, to hasten digestion.

2.10.1.2 Nail

Nail specimen take longer time to clear, but if small pieces and debris are taken, they will usually soften within short time. 40% KOH is used for nail samples.

2.10.1.3 Hair

Hair is examined as soon as possible after mounting in10% KOH solution since the infected hairs are very delicate. The base of the hairshaft and follicular debris are the areas where fungi are mostly seen.

The KOH clears the keratinaceous material by digesting proteinaceous debris, bleaching the pigment and loosening the sclerotic material without damaging the fungus. The slides are examined under the microscope with low power objective, first and then under high power.

The dermatophytes in skin and nail samples are seen as highly refractile, long undulating branched or unbranched septate hyphae, breaking at the septa, to form arthrospores.

Arthrospores are seen in hairs, and it is noted whether they are outside the hair shaft (ectothrix invasion) or inside the hair shaft (endothrix invasion).

2.11 Culture methods

2.11.1 Sabouraud's dextrose agar with chloramphenicol

Sabouraud's dextrose agar with chloramphenicol (0.05%) inhibits the growth of bacteria without deterring the growth of dermatophytes.

2.11.2 Sabouraud's dextrose agar with chloramphenicol and cycloheximide

The standard medium for growing dermatophyte is Sabouraud's dextrose agar containing chloramphenicol and cycloheximide, which inhibit bacteria and saprophytic fungi respectively. The cycloheximide (Actidione) in a concentration of 0.1 to 0.4 mg per ml suppresses the growth of most saprophytic fungi such as *Scytalidium, Hendersonula*, *Aspergillus, Candida* species without deterring the growth of dermatophytes. The various

antibacterial antibiotics used include chloramphenicol (0.05 mg/ml) or gentamicin (0.1 mg/ml).

2.11.3 Dermatophyte test medium (DTM)

This was developed by Taplin and colleagues, which is a selective media with a phenol red pH indicator that changes colour from yellow to red in the presence of dermatophytes. It inhibits most bacteria and many contaminating fungi. The medium contains chlortetracycline, gentamicin and cycloheximide. Dermatophytes release alkaline metabolites into the medium, which results in an increase in pH within 10 days. This medium is used for screening and not for routine use.

2.11.4 Corn meal agar

It is a nutritionally deficient media which suppresses the vegetative growth and induces sporulation and hence can be used in slide culture. When corn meal agar is used with dextrose it can be used to differentiate Trichophyton rubrum from Trichophyton mentagrophytes based on pigment production.

2.11.5 Potato dextrose agar

It induces sporulation in dermatophytesand can be used in slide cultures.

2.11.6 Rapid sporulating medium (RSM)

This works on the same principle as DTM. It contains an indicator system that changes colour from yellow to blue green. The advantages of RSM are that it stimulates conidial production, and the bluecolour does not mask the red pigment often produced by T. rubrum. RSM is commercially available.

2.11.7 Trichophyton agars (available commercially)

These are useful for culturing several dermatophyte species, some of which have nutritional requirements (either essential or partial) for growth.

Ex: *T. verrucosum* which requires thiamine and inositol, *T. tonsurans* which requires thiamine, can be correctly identified with the use of Trichophyton agars.

2.11.8 Media for the maintenance of stock culture

Most dermatophytes are unstable if they are maintained on Sabouraud's dextrose agar. Takashio used a 1:10 dilution of neutral Sabouraud's agar (Emmons modification) enriched with mineral salts and found that this medium was superior to other conventional media for preservation of dermatophytes.

2.12 Incubation

The media is inoculated with the material and incubated at room temperature i.e., 25-30°C for 1-4 weeks. In majority of dermatophytes, growth and sporulation occurs in 3 to 10 days. In some it may take longer time. *T. verrucosum* and some strains of *T. tonsurans* grow better at 37°C. At the onset of sporulation and pigment production, growth is examined by lactophenol cotton blue preparation (LPCB).

2.13 Dermatophytes are identified based on the following features

(i) Colony obverse: The colour (white, pearl, ivory etc), texture of the surface (glabrous or waxy, powdery, granular, suede-like velvety, downy or fluffy).

(ii) Colony reverse: Presence or absence of pigment, whether diffusing into the medium or not and topography (flat, raised, heaped) and rate of growth.

(iii) Microscopic morphology: Studied in tease mounts, slide cultures and pressure-sensitive tape preparation.

2.13.1 Tease-mounts (Lactophenol Cotton Blue):

Cultures were examined microscopically by removing a portion of aerial mycelium with a spud. The material was placed on a glass slide in a drop of Lacto phenol cotton blue and the matted mycelium was gently teased with a pair of teasing needles. A cover-slip was then placed and excess of stain removed with blotting paper. The morphology was then observed under microscope.

2.13.2 Slide culture (Riddell's Method)

Preparation of the petridish for slide culture sterile petridish was taken. Piece of filter paper cut according to the shape of petridish was placed inside the petridish. A 'V' or 'U' tube placed on the filter paper.

A clean glass slide was placed over the V tube and 1-2 cover-slips were placed inside. Petridish was closed with the lid, wrapped with kraft paper and sterilized using hot-air oven.

2.13.2.1 Procedure

Blocks of (1 x 2 cms) Sabouraud's dextrose agar with chloramphenicol and cycloheximide and corn meal agar were cut to a depth of 4 mm using sterile scalpel blade. One such block from both was transferred onto the glass slide supported on V-tube in a petridish. Corners of agar block were inoculated with the fungal colony using spud. A cover-slip is placed on surface of agar block using sterile forceps. Little amount of sterile distilled water is added to the petridish and the lid closed and incubated at room temperature. After each day or two the slide was taken out of petridish and examined under microscope without disturbing the cover-slip. When the sporulation was well evident, cover-slip was carefully removed from the agar block and placed on a drop of Lactophenol Cotton Blue polyvinyl alcohol stain taken on a separate glass slide taking care to avoid trapping of air bubbles. Agar block from the original slide was carefully removed and decanted into 5% phenol solution.

One or two drops of Lactophenol Cotton Blue Polyvinyl alcohol stain placed on the slide with fungal growth and cover-slip placed on it. Both the slides were examined under the microscope. Character of hyphae, size, shape and arrangement of macroconidia, microconidia and other structures were studied.

2.13.2.2 Advantage

Advantage of slide culture over tease mount technique is that it allows the study of microscopic features of fungus preserving the continuity between the hyphae, microconidia, macroconidia.

2.13.3 Cellophane Tape Method

A microscope slide with a drop of Lactophenol cotton blue was prepared and set aside. A 2 inch piece of transparent cellophane tape was taken, held between the fingers with sticky side out, and the sticky side was touched to the top of fungal colony. Sporulation structures should stick tothe tape. Tape was then placed with sticky side down, on the slide over the Lacto phenol cotton blue, while stretching the tape and pressing both endsto attach it firmly to the slide. The tape acts as a cover-glass. The advantage of this method is that it doesn't disturb the position of structures.

An improved method uses a device which dispenses a thin layer of transparent adhesive to the surface of a cover glass. Touch the sticky side of the cover glass to the top of fungal colony, and then place the cover glass over a drop of Lacto phenol cotton blue on slide.

All these preparations are examined for presence of hyphae, microconidia, macroconidia, their size, shape and arrangement and other structures such as:

Non-reproductive vegetative hyphae

1) Nodular organs: These are knots of twisted hyphae.
2) Racquet hyphae: The name racquet hyphae are given because the hyphae resemble tennis racquets placed end to end.
3) Spiral hyphae: These are corkscrew like twisted hyphae.
4) Favic chandeliers: Favic chandeliers resemble the antlers of a buck deer, seen at the ends of the hyphae. These are diagnostic of T. schoenleinii.
5) Pectinate bodies: These are unilateral digitate comb-like parallel extensions projecting at right angle to main hyphal branch like the teeth of a comb.

2.14 Classification/Taxonomical position of the Test Pathogens

Three genera, *Epidermophyton, Microsporum* and *Trichophyton* are collectively known as dermatophytes, and their systematic positions are as follows:

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	:	*mentagrophytes*
4.	**Kingdom**	:	Mycota
	Division	:	Eumycota
	Sub division	:	Duteromycotina
	Class	:	Hypomycetes
	Order	:	Hypomycetales (Moniliales)
	Family	:	Moniliaceae
	Genus	:	*Trichophyton*
	Species	:	*rubrum*

3. Review of Literature

"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)

Literature reveals that wide range of plants with ethno-medicinal value against some common diseases have already 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; 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 [(Fischer (1938); Mahanti (1994); Singh (1996); Sharma *et al.,* (2001); Lalramnghinglova, 1996; 1997; 1999 & 2003; Lalramnghinglova and Jha, 1999; Lalramnghinglova and Jha (2000); Dutta and Dutta, 2005; Bhardwaj and Gakhar, 2005; Kala, 1998; 2000; 2002; 2003; 2004 & 2005; Das and Tag, 2006; Rai *et al.,* (2007); Lalfakzuala *et al.,* (2007); Rai and Lalramnghinglova (2010 a); Rai and Lalramnghinglova (2010 b); Shukla *et al*., 2011)] to mention a few.

3.1 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. The following investigations, through literature survey, will definitely be helpful for exploring the potential antidermatophytic plants of North Eastern Region.

Table-3.1: Medicinal Plants and their Activity against Pathogenic Fungi

Workers	**Plants**	**Nature of Seconadry metabolites**	**Pathogens**
Anand and Johar	*Cinnamomum zelanicum*	Extract	*A. niger*
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-	*F. oxysporum*

		isothiocyanate	
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*
Nene and Thapliyal (1965)	*Anagallis arvensis*	Root, stem, leaves extract	*A. niger*
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 linifolius, Mentha sylvastris, Salvia leucantha*	Essential oil	*T. mentagrophytes*
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*	Essential oil	*E. floccosum, T. mentagrophytes T. rubrum*

	longifolia		
Band Cirenko *et al*., (1972)	*Psoralea drupacea*	Essentail oil	*T. rubrum*
Komissarov and Andreeva (1972)	*Allium sativum*	Extract	*A. niger*
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-	*Allium cepa*	Extract	*F. oxysporum*

Davis (1974)			
Afifi (1975)	*Origanum majorana, Ocimum basilicum*	Leaf extract	*A. niger*
Lindsey and Turner (1975)	*Arachis hypogeal*	Embryo extract	*A. flavus*
Pfleger and Harman (1975)	*Pisum sativum*	Seed extract	*F. oxysporum*
Tansey and Appleton (1975)	*Allium sativum*	Bulb extract	*E. floccosum, M. gypseum, T. Rubrum*
Turner *et al*., (1975)	*Arachis hypogeal*	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 mormelos*	Essential oil	*C. albicans*
Mukharya and Dahia (1977)	*Plumbago sp.*	Root extract	*M. gypseum*
Naraian and	*Vinica rosea*	Root, stem,	*A. niger, F. oxysporum*

Satapathy (1977)		flower extract	
Rojashenandez and Diazperez (1977)	*Catharanthus roseus*	Alkaloides	*A. fumigatus, C. albicans*
Sawhney *et al*., (1977)	*Cymbopogon citratus, C. martini, C. winterianis, Mentha citrata, Ocimum basilicum, O. citriodorum, O. gratissimum*	Essential oil	*M. gypseum*
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*.,	*Inula recemosa*	Root extract,	*M. canis, T. mentagrophytes*

(1978)		Alantolactone, isoalaniolactone	
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, Ocimum basilicum*	Essential oil	*M. gypseum*
Sampurna and Nigam (1979)	*Skimmia laureole, Cymbopogon flexuosus, Cinnamomum zeylanicum, Geranium sp., Eucalyptus citriodora*	Essential oil	*T. mentagrophytes*
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 Castazo (1980)	*Arnica Montana*	Extract	*C. albicans,* *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*	Alantolacton e	*M. canis, T. mentagrophytes*
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 Singh (1982)	*Allium sativum, A. cepa, Azadirachta indica*	Essential oil	*M. gypseum*
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 flexuosus, Trachyspermum ammi, Vetiveria*	Essential oil	*M. gypseum, T. equinum, T. rubrum*

	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*
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*., (1986b)	*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*	Deoxyshikon in	*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*

Rai and Upadhaya (1989)	*Parthenium hysterosphorus*	Extract	*T. mentagrophytes*
Singh and Pandey (1989)	*Lawsonia inermis*	Extract	*M. gypseum, T. 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, Chenopodium ambrosioides, Cymbopogon citratus, Mentha arvensis*	Essential oil	*M. gypseum, T. rubrum*
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*.,	*Pycnoporellus*	Essential oil	*E. floccosum, M. canis, M.*

(1995)	*fulgens*		*gypseum, T. mentagrophytes, T. rubrum*
Yadav (1995)	*Cinnamomum tamola, Citrus maxima*	Essential oil	*T. mentagrophytes*
Mukherjee *et al*., (1996)	*Cassia tora*	Extract	*A. niger, 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)	*Eucalyptuc 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*
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*
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*.,	*Cladonia furcata,*	Extract	*E. floccosum,*

(1998c)	*Heterodermia leucomela, Leptogeum trichophorum, Lobaria retigera, Stereocaulon foliosum*		*M. gypseum, T. mentagrophytes,*
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*.,	*Foeniculum vulgare*	Essential oil	*S. dimidiatum,*

(2000)			*T. mentagrophytes, T. rubrum*
Singh *et al*., (2000)	*Homalomena aromatica , Odontotermus obesces*	Essential oil	*A. niger*
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,*	Extract	*Candida albicans*

	Launea velutina		
Patra *et al*., (2001)	*Eugenia caryophyllata*	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*., (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)	*Eucalyptus dalrympleana*	Essential oil	*E. floccosum, M. gypseum, T. rubrum,*
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)	*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*.,	*Mentha spicata,*	Essential oil	*E. floccosum, M. gypseum,*

(2002)	*Taxodium distichum*		*M. nanum*
Pandey K P (2002)	*Curcuma longa* ,	Essential 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*
Shahi *et al*., (2002c)	*Everniastrum cirrihatum*	Extract	*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*
Shahi *et al*., (2003)	*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*
Rao *et al.,* (2005)	Citral, geraniol,	26 Chemical	*Epidermophyton floccosum,*

	citronellal, eugenol, menthol, lemongrass oil etc	constituents	*Trichophyton mentagrophytes, T. rubrum* and *Microsporum gypseum*
Silva *et al*., (2005)	*Ocimum gratissimum*	Hexane, chloroform fractions, the essential oil and eugenol	*Microsporum canis, M. gypseum, Trichophyton rubrum* and *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*
Inouye S. *et al*., (2007)	*Cinnamomum zeylanicum, Eugenia aromatica, Geranium maculatum, Lavandula angustifolia, Cymbopogon citratus, Origanum vulgare, palmarosa Cymbopogon martinii, Mentha x piperita, Melaleuca alternifolia*, and *Thymus vulgaris*	Essential oil	*T. mentagrophytes* & *T. rubrum,*

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, Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum* and *Microsporum gypseum*
Luqman *et al*., (2008)	*Eucalyptus citriodora*	Essential oil	*Trichophyton rubrum, Histoplasma capsulatum, Candida albicans*
Sim Y. *et al*., (2008)	*Ligusticum chuanxiong*	Essential oil	*Trichophyton species, T. erinacei, T. mentagrophytes, T. rubrum, T. schoenleinii, T. tonsurans and T. soudanense*
Tavares *et al*., (2008)	*Daucus carota* subsp. *Halophilus*	Essential oil	Yeasts, dermatophyte and *Aspergillus* strains
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*

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*
Shukla *et al*., (2013)	*Lantana camara*	Essential oil	*Trichophyton mentagrophytes, T. rubrum*

Table-3.2 Some Important Ethnomedicinal Plants of North Eastern Regions and their use against different ailments

Plant/ 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	Maikhuri and Gangwar (1993), Kumar *et al*., (1994)
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	Assam, Manipur, Meghalaya, Nagaland, Tripura,	Saikia *et al*., (2006), Chaturvedi and Jamir (2007), Chaturvedi and Jamir (2007)

	appetite), Whooping cough	Arunachal Pradesh	
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,	Snake Bite (Leaves, Root), Ringworm (Whole Plant), Urinary disorders	Arunachal Pradesh, Meghalaya	Maikhuri and Gangwar (1993), Tiwari and Tiwari (1996)

Whole Plant	(Leaves)		
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), Ahmed & Borthakur (2005)
Callicarpa arborea Roxb. Verbenaceae Leaves, Bark, Shoot, Buds, Root, Stem, White powder of young stem (1996)	Scabies, Ringworm, Tonic, Carminative (Bark), Gastric trouble (Young Shoot/ Stem bark), Skin diseases (Stem Bark),	Arunachal Pradesh, Assam, Manipur, Mizoram, Nagaland, Sikkim	Jamir and Rao (1990), Das and Sharma (2003), Sarmah *et al*., (2006), Bhardwaj and Gakhar (2005), Chaturvedi and Jamir (2007)
Calotropis gigantea (L.) Ait. f. Asclepiadaceae Leaves, Root, Latex, whole plant,	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).

Bark, Stem			
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	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), Sharma *et al.,* (1999), Das and Sharma (2003), Yonggam (2005), Chaturvedi and Jamir (2007), Ahmed & Borthakur (2005)
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	Arunachal Pradesh, Assam, Sikkim, Tripura	Purkayastha *et al*., (2005), Srivastava et al (1987), Tomar *et al.,* (2003), Saikia et al. (2006), Purkayastha and

	(Leaves), Leprosy (Leaves/Root)		Nath (2006)
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), Barua *et al.,* (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	Appetiser, Rashes,	Meghalaya,	Chhetri (1994), Upadhaya *et*

(Swingle) Tanaka Rutaceae Fruit, Leaves	Ringworm (Fruit), Gout, Rheumatism (Leaves), Body ache, Vomiting, Tonic, Cold, Fever (Fruit, Leaves),	Manipur	*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), 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,	Tonsilitis, Epistaxis, Bone fracture (Leaf), Skin diseases (Whole Plant/ Leaves), Ringworm	Arunachal Pradesh, Assam, Manipur, Meghalaya, Sikkim,	Singh *et al.,* (1996),Pfoze and Chhetry (2004), Sharma (2004)

Leaves		Nagaland	
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)
Eucalyptus spp	Antidermatophytic activity against human	Mizoram	Shukla *et al.,* (2012)

	pathogens		
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	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	Scabies, Induce	Assam,	Dutta and Nath (1999),

strobilifera (L.) R. Br. Leguminosae Root, Leaves	sleeping, Relieve Pain (Root), Anthelmintic (Leaves), Ringworm (Root)	Sikkim	Baruah and Sharma (1987)
Fimbristylis falcata (Vahl) Kunth Cyperaceae Rhizome	Skin Diseases, Ring worm (Rhizome)	Nagaland	Rao and Jamir (1982)
Garuga pinnata Roxb. Burseraceae Leaves, Fruit, Stem	Ringworm, Asthma, Indigestion, Conjuctivitis	Assam, Meghalaya	Saikia *et al*., (2006), Chhetri (1994)
Gelsemium elegans Benth. Loganiaceae Leaves, Root	Wounds (Leaves), Stomach Ulcer, Ringworm (Root)	Mizoram	Bhardwaj and Gakhar (2005)
Gonatanthus pumulus D. Don. Araceae Bulb	Snake bite, Dog bite, Ringworm, Mumps, Scabies (Bulb)	Sikkim	Janmeda *et al*., (2006)
Gongronema nepalense (Wallich) Decne Asclepiadaceae Leaves	Boils, Ringworm (Leaves)	Meghalaya	Singh *et al*., (2003), Kumar *et al*., (1987)

Gynocardia odorata R.Br. Flacourtiaceae Fruit, Seeds, Oil	Diabetes, Anthelmintic, Ulcer, inflammation, Ringworms, Scabies (Oil)	Arunachal Pradesh, Meghalaya, Sikkim	Sarmah *et al*., (2006), Rai *et al*., (1998)
Holarrhena pubescens (Buch.-Ham.) Wall. ex D. Don Apocynaceae Bark,Seed, Latex, Flower	Dysentry, Diarrhoea, Antitetanic (Bark), Fever, Bilious problems (Bark and Seed), Ringworm, Leprosy, (Bark)	Assam, Sikkim, Tripura	Sharma (2004), Srivastava *et al.,* (2003), Singh *et al*., (2003),
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.)	Anthelmintic, Constipation, Skin diseases,	Arunachal Pradesh, Assam,	Khumbongmayum *et al*., (2005), Tiwari and Tiwari (1996), Gurung

Muell. Arg Euphorbiaceae Fruit, Bark	Scabies, Ringworm, Herpes, Oral	Manipur, Meghalaya, Sikkim	(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),
Myrsine semiserrata Wall. Myrsinaceae Fruit, Seed Laxative (Fruit),	Ringworm, Skin diseases (Seed)		Baruah and Sharma (1987)

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)
Ocimum 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,	Fungal infection, Itching, Cuts, Wounds, Dog Bite, Skin diseases,	Assam, Nagaland, Sikkim, Arunachal Pradesh	Nath & Maiti (2003), Srivastava *et al.,* (1987), Sharma (2004), Das *et al.,* (2006)

Shoot, Aerial parts	Skin diseases (Whole Plant/Shoot),		
Polygonum sarbhanganicum Subha Rao Polygonaceae Root	Ringworm, eczema (Root)	Assam	Barua *et al.,* (1999),
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)
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	Dash *et al.,* (2003), Khumbongmayum *et al*., (2005)
Siegesbeckia orientalis L. Asteraceae Leaves, whole	Injuries, Wounds (Leaves), Skin diseases, Sores, Ringworm &	Assam, Meghalaya	Neogi *et al*., (1989), Islam & Hasin (2003),

plant	other allied diseases		
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

4. Materials and Methods

The present study entitled *'Investigation of Anti-dermatophytic Activity from selected Medicinal Plants of Mizoram'* was carried out during January 2009- December 2012, at the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl. However, some of the part of the *in vitro* investigation was carried out at the Biological Product Laboratory, Department of Botany, University of Allahabad, with proper permission.

The research work was designed and carried out as per the following sequences:

- Collection of plants traditionally used against skin diseases/ fungal diseases, and their identification.
- Extraction of the plant secondary metabolites and their antidermatophytic screening.
- Selection of 4-5 most potent plants for detailed *in vitro* investigations against the common dermatophytes causing ringworm infection in human beings.
- Physico-chemical characterization of the most efficacious plant metabolite(s).

4.1 Collection of plants traditionally used against skin diseases/ fungal diseases, and their identification

4.1.1 The Study area

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, also known as the "Scotland of India". It 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 botanical survey

In Mizoram, so far 15 ethnic groups/ population have been recognized viz., Lusei (Mizo), Paihte, Hualngo, Tlau, Thadou, Ralte, Hmar, Mara, Pawi, Bawm, Pang, Chakma, Riang, Biate and Mog (Lalramnghinglova, 2003).

These ethnic groups are a very distinctive people rich in old- age traditions, cultures and customs, and continue to retain their respective dialects and ethnic identity. Out of the fifteen people groups, the Mizos, Maras, Pangs and Lais are gradually losing their valuable indigenous knowledge of traditional medicines, particularly among young generations, where as the Chakmas, Brus, Bawms and the Mogs are ethno-bio-culturally rich in their folklore knowledge. In most cases, older people in the community have more knowledge in the field of ethno-botany and they preserve it traditionally. Therefore, in the present study, frequent ethno-botanical surveys (during Aug 2009- Jan 2011) were conducted, so that, the actual information at the grass root level was able to collect and explored scientifically (Table 3.1).

During ethno-botanical survey, information on the use of medicinal plants was obtained through structured and semi structured questionnaires, complemented by free interviews and informal conversations. Local practitioners and others with knowledge of plants were also consulted. Inquiries on the prevalence, types, transmission and symptoms of skin diseases along with traditional treatment methods, mode of application, dose etc were recorded properly, and plants were collected for investigations (table 5.1).

Further, the plants thus collected were categorized and the most frequently used plants were selected for identification. This was done 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 the School of Earth Sciences & Natural Resources Management, Mizoram University; State Medicinal Plants Board and State Forest Department, Aizawl. Further, their confirmations were made with the help of experts at the Department of Botany, University of Allahabad as well as 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 the present investigation (table 5.2).

4.2 Extraction of the plant secondary metabolites and their antidermatophytic screening

4.2.1 Extraction of the plant secondary metabolites

The plants thus collected, were subjected for extraction of their secondary metabolites (extract/ essential oil). The *solvent extraction method*, using different solvent (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.

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

4.2.1.1 Cleaning/ Sterilization of the Plant samples

The collected plant parts were washed separately with water and then sterilized with distilled water. After proper cleaning they were dried in the shed for the next step/ extraction.

4.2.1.2 Protocols for Solvent Extraction Method

The plant constituents were extracted using methanol and ethanol, in combination with distilled water (50:50 ratios), and the protocols were as follows:

- 1.0 to 5.0 g sample of a collected plant material (leaves, stem, bark, roots and inflorescence) was weighed,

- The samples were chopped into small pieces and/ or powdered using pestle and mortar,
- The powder was placed into organic solvents (as mentioned above),and kept over night for proper extraction,
- The supernatant was filtered using a Whatman filter paper (No. 1), making sure that no plant particulates remain in the filtrate,
- Finally for extraction of crude extract the Rotatory evaporator equipment was used.

4.2.1.3 Protocols for Hydro-distillation Method

In hydro-distillation, Clevenger apparatus, a glass apparatus invented by Clevenger (1928); is commonly used for extraction of the volatile plant secondary metabolites/ essential oil (Fig.4.1). It includes the following steps:

1. The fresh plant material (leaves, stems, seeds and roots etc.) were collected and washed with tap water.
2. The plant materials were chopped and weighed.
3. The plant materials were loaded into the flask & filled with water upto 50% of the total volume of the flask.
4. The temperature was adjusted at 30-40 ^{0}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. The extracted essential oil was collected in a sterilized airtight vial. Any water droplet was removed by using sodium anhydrous sulphate.

Fig.4.1 Clevenger Apparatus

4.2.2 Procurement of Test organism

The present investigation was targeted on four dermatophytes viz., *Trichophyton rubrum*; *Trichophyton mentagrophytes*; *Epidermophyton floccosum* and *Microsporum gypseum;* which cause the ringworm infestations in human beings. The authentic cultures (Microbial type culture collection) of these dermatophytes were procured from the Institute of 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)**

4.2.2.1 Protocol for Revival of the Test organism

The cultures thus procured were revived for the present investigation. It includes the following protocols:

- The ampoules were opened with proper care as the contents were in a vacuum.
- The ampoule was marked near the middle of the cotton wool with a sharp file.
- The surface around the mark was disinfected with alcohol.
- The ampoule was wrapped with a thick cotton and was broken at the marked area.
- The pointed top of the ampoule was gently removed. Snap opening could draw the cotton plug to one end; hasty opening could release fine particles of dried organisms in the air.
- The cotton plug was carefully removed and about 0.3 to 0.4 ml of SDA medium was added to make a suspension of the culture, avoiding frothing or creating aerosols.

- Few drops of the suspension was streaked on to the medium (solidified with agar) in a petri plate and incubated at the recommended temperature (25±2^{o}C) and conditions for proper culture.
- The culture gives good result and could be visible within 5-7 days.
- All the remains of the original ampoule were treated as infected and autoclaved before discarding.

4.2.2.2 Multiplication of the test organism

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 throughout the study.

4.2.3 Antidermatophytic screening

The antidermatophytic screening of the plant secondary metabolites (as listed in table 5.2 & 5.3) against the test pathogen- *Trichophyton rubrum, T. mentagrophytes*; *E. floccosum* and *M. gypseum*; was determined using **disc diffusion method.**

4.2.3.1 Protocols for disc diffusion method

- Before preceding to the experiment the hands and the surface of the inoculation chamber was cleaned with 95% alcohol then the inoculation chamber was sterilized with UV light for a given time.
- The bottom of the SDA plate was tagged, which contains the name of the test pathogen (dermatophytes), date of inoculation and the codes marked. (It should be about 20 mm away from the plate edge).
- With the fresh culture plate, the test pathogen inoculum was transferred to the SDA broth in order to obtain a concentration of 1.5×10^6 CFU/ml (0.5 McFarland used for the turbidity match).
- Using these inocula, with a cotton swab, the entire surface of SD agar plate was inoculated in back-and-forth motion.
- The cotton swab was again dipped in the broth culture and inoculated on agar

surface in second direction.

- The contaminated swab was discarded in the biohazard container.
- The plate lid was placed on dry agar surface for 5 minutes.
- The sterile paper discs were picked up from its container with sterilized forceps.
- The edge of the paper discs was dipped into the solution of plant secondary metabolites (stock solution at different conc 20mg/ml, 30mg/ml and 50mg/ml, were prepared in DMSO), and the discs were saturated through capillary action.
- The saturated discs was placed over its letter code with gentle pressure, to avoid the discs falling off, when the plate is inverted during incubation.
- Similarly, the same process was repeated with different concentrations of plant secondary metabolites, flaming the forceps for each new solution.
- Further, the inoculated plates were placed upside-down in the incubator and the temperature was adjusted at 25± 2^0C for 36-72 hours.
- After the incubation period, the diameter (in mm) of any zone of growth inhibition around a disc was measured and the observations recorded.
- The experiment was repeated twice to get the final observation.
- Finally, the plate was discarded into autoclave for proper sterilization.

4.3 Description of the Most Potent Plants

During antidermatophytic screening of 20 ethno medicinal plants; the essential oil of *Homalomena aromatica* Schott., *Eucalyptus citriodora* Hook., *Ocimum americanum* Linn and *Mentha arvensis* Linn and the 50 % ethanolic extract of *Dillenia indica* Linn were recorded as the most effective plants. The plant that shows the most potent efficacy against the test pathogens, were selected for detailed *in vitro* investigations, using Broth micro dilution method (NCCLS, 2002).

The detailed descriptions of these selected plants are as follows:

1. *Homalomena aromatica* Schott

Classification/Taxonomical position

Kingdom	:	Plantae
Class	:	Monocotyledonae
Series	:	Nudiflorae
Family	:	Araceae
Genus	:	*Homalomena*
Species	:	*aromatica*

Historical background:

Homalomena aromatica Schott is a rhizomatous aromatic perennial herb found in Assam, lower altitudes of Arunachal, Nagaland, Tripura, Mizoram, etc. The dry rhizomes are known in trade as 'Sugandhmantri'Stem short, slow growing, leaves radical with sheathing bases, long petioled, sagittate - cordate.The large rhizomes bearing withered leaf scales and numerous white rootlets are esteemed as an aromatic stimulant. Its aromatic rhizomes contain an essential oil used for blending of most oriental perfume. On steam distillation of rhizome yields yellow coloured essential oil about 1.0% on air-dry basis. The essential oil has a very good demand in perfumery and cosmetic industries.The spent material after extraction of essential oil is largely used in *Dhup* manufacturing. More than 400 MT of dry rhizomes are collected and transported to outside the state mainly to Kannauj, Kanpur, Delhi, Kolkata, Mumbai etc from Barak Valley of Assam and other parts of the region every year. It is being exploited as a minor forest product since long back without conservation and / or cultivation measures. If this is continued unabated very soon the unique and valuable species will be extinct from its place of origin.

Ecology

Hill slopes (tilla land) and foothill areas covered by forests and other vegetation. It is moisture and shade loving species and grows best under 40-60% shade.

Soil

Sandy and sandy loam to clay loam with organic litter found suitable. Temporary water logging for 24-48 hours is beneficial for quick rhizome elongation. Prefer acidic soil of pH range 4.9-5.5.

Climate

Being a sub-tropical species it loves warm and humid climate with annual rainfall ranging from 2000-3000 mm.

Plant height

Plant height varies from 0.45-0.75 m, slightly more in shady areas.

Variety

No named variety as yet available. Commercially acceptable local material should be used.

Stock Nursery

A stock nursery is important to start with a commercial cultivation. Generally 1/5th of the target area is brought under this nursery one year ahead of plantation. This is well nursed with adequate manuring and fertilization, mulching and other intercultural operations. By doing so, cost of planting material can be reduced by about 40%.

Propagation

The individual seed material is the piece of rhizome with active buds of 2.5-3 cm size. Fresh rhizome pieces with active buds may be sprouted in sand beds before planting in main field.

Land Preparation

Land is prepared by deep ploughing or hoeing with addition of sufficient quantity of organic matter.

Planting time

Best time of planting is April to June with pre-monsoon shower to onset of monsoon.

Planting method

Appropriate method is ridge and furrow method. 15 cm deep furrows are opened at 30 cm apart. Rhizome pieces after sprouting are planted in the furrows at 45 cm apart and covered with soil up to 5-7 cm. Rest part is filled up by rice husk or other available mulch material. Rice husk is more beneficial as it takes longer time to decompose and thus helps in rhizome elongation. When fresh cuttings are planted sprouting started within 30 days of planting.

Spacing

45 x 30 cm in plains or 60 x 30 on slopes.

Requirement of planting material

The 20-22 q of fresh rhizome / ha to get a plant population of 75000.

Fertilizer application

The 40:50:60 kg N, P2O5 and K2O/ha per year may be applied. Entire quantity of P and K are applied along with organic matter as basal. N is applied in 3 equal splits starting from 40 days after planting.

Interculture

It is weed-suppressing crop and one or two light hoeing in the first and second month of planting is enough to keep down the weed population. Removal of old and dry leaf and sheath is beneficial for rhizome elongation.

Shade management

Sugandhmantri prefers 40-60 % shade, therefore, it can be ideally fitted with some plantation crops or it can be mixed with some other tall perennial crops.

Pests and Diseases

No pests and diseases have been reported so far.

Harvesting

Sugandhmantri becomes ready for rhizome harvest on 3rd year of planting. However, the crop can also be harvested in the second year. But three years rotation period has been observed to be more profitable from both yield and quality of the

products. It is harvested during winter rain free period (November-April). During this period it is observed that the vegetative growth as well as moisture content of rhizome is minimum and the oil accumulation is maximum. The rhizomes attain a length of 20-30 cm or more in three years. Rhizome exceeding 15 cm long is chosen for harvesting. Harvesting is done using hand *Chiprang* or garden hoe that may require digging out the rhizome from 5-10 cm deep. During harvest the side suckers may be left undisturbed for ratoon crops. Harvest may be taken every year by selection of well-grown rhizome starting from third year upto 10th year and then terminated or one time harvesting after 30 - 32 months may also be done.For ratoon crop manuring & fertilization should be done every year with pre monsoon shower.

Rhizome processing

After harvest leaves, stalks with leaf sheath and roots are removed and cleaned and then spread on floor. Clean rhizomes are then cut into 2.5-3 cm pieces and dried by using deemed fire and smoke. When about 50% drying takes place then they are taken out and dried under sun till a rattling sound on handling is obtained. The fresh and dry ratio of about 5.5: 1 is safe for storing or distillation.

Yield of rhizome

Fresh yield is about 10-12 times of planting material and thus average fresh yield after 3 years is about 240 q / ha. On drying it yields about 50 q dry rhizomes, which on steam distillation may yield 45 - 48 kg of essential oil at 1.0% recovery.

Distillation

After drying, distillation should be done within one month to get maximum recovery (1% or more). With ageing, oil recovery goes down to 0.8 % if distilled after 3 months of storing. Since the oil glands are situated inside the rhizome, which contains lot of starch, it has to be disintegrated or crushed to expose the oil glands in such way that during the process heat is not generated. Heat generation will destroy considerable quantity of oil. Distillation process takes about 10-12 hours to exhaust one batch. At the end of distillation, oil is taken out from the receiver and is treated with anhydrous sodium sulphate salt @ 15 - 20 g /litre of oil. After making water free and filtering the oil is

stored in aluminum container. The spent material obtained after oil extraction is utilized as organic manure or as a raw material for *Dhup* or other incense sticks manufacturing. Distillation plant used for patchouli can be employed for Sugandhmantri as well. Fresh rhizomes can also be distilled after crushing in SS Roller mill. After disintegration or crushing the material should be put into the distillation tank immediately to avoid loss of oil.

Essential oil content in different plant parts

Plant parts Oil content (%)

- Fresh leaves traces
- Petioles 0.01
- Roots 0.03
- Spadix 0.05
- Dried rhizomes 2.00-2.70
- Oil content as affected by age of the plant
- Less than one year 0.08 -1.00 % oil
- More than 32 months old 1.50 – 2.70 % oil

Oil content decreases when the dry rhizomes stored for more than 3 months. Drying under sun without application of heat takes more time and such rhizomes cannot store for longer period and also not preferred in trade but can be used for oil extraction.

Ethno - medicinal uses:

Decoction of leaves is given to pregnant women for easy delivery. Rhizome is used for treating jaundice and also as aromatic stimulant. Burnt smoke of tender rhizome is inhaled for treating influenza. Leaves, rhizome or juice of whole plant is used as lotion in skin diseases. Stem/rhizome is crushed and bandaged onto fractured bones. Juice of crushed rhizome is used in steatorrhoea. Juice of crushed stem is also used against tick-bites.

2. *Ocimum americanum* L.

Classification/Taxonomical position

Kingdom	:	Plantae
Phylum	:	magnoliophyta
Class	:	magnoloipsida
Order	:	lamiales
Family	:	lamiacaea
Genus	:	*Ocimum*
Species	:	*americanum.*

Common name: Ban Tulsi. (Beng.); Hoary Basil, Rosary Basil. (Eng.).

Description of the plant:

An annual aromatic much-branched herb, 15 - 60 cm high. Leaves 2.5-3.8 cm long, elliptic-lanceolate, acute at both ends. Flowers small, white, in rather close whorls, in spiciform racemes, up to 20 cm long. Nutlets 1.25 mm long, ellipsoid, black.

Geographical Distributions:

Ocimum americanum occurs wild and cultivated throughout tropical Africa and tropical Asia. Its exact origin is unknown. In Southeast Asia, it has been reported from the continental parts of Indonesia and Papua New Guinea. Its occurrence in the Philippines is doubtful. It has also been introduced into tropical America and some islands of the West Indies.

Plant Description:

O. americanum is an erect, much-branched, annual, aromatic herb that can reach up to 0.3-1 m tall. The stem and branches are quadrangular, yellowish-green and densely white-pilose in the young parts but less when older. The leaves are simple, decussate and with petiolate. The petiole is up to 2.5 cm long. The leaf-blade is lance-shaped to elliptical, measuring 2.5-5 cm x 1-2.5 cm, wedge-shaped at the base, entire at the margin, acute at the apex, hairless and gland-dotted on both surfaces.

The inflorescence is up to 15 cm long, composed of decussate, with 3-flowered cymes and appears as 6-flowered whorls (verticillasters), which is up to 3 cm apart,

terminal, simple or branched. The peduncle and axis are quadrangular. The 2-3 mm long bracts are elliptical-lance-shaped, hairy and persistent. The pedicle is up to 4 mm long and strongly recurved at the top. The sepal is bilobed where in the flower it is 2-2.5 mm long while in the fruit is 3-4.5 mm long. It is villous inside and pubescent with long white hairs outside. The upper lobe is flat and suborbicular while the lower lobe is canaliculated and sharply 4-toothed at the top. The petal is tubular, 2-lipped, measures 4-6 mm long and white. The upper lip is strongly recurved at the top and crenately 4-lobed while the lower lip is entire and smaller than the upper lip. There are 4 didynamous stamens, which are slender and exserted. The pistil is with 4-ovuled and 4-lobed ovary, slender style and with 2-lobed stigma.

The fruit is composed of 4 distinct nutlets that are enclosed within the tube of the persistent sepal. The black nutlets are ovoid, measuring upto 1.25 mm x 1 mm. The nutlet-wall produces a thick white cover of slimy threads when soaked for several minutes in water. The seed is free within the nutlet.

Ecology / Cultivation

O. americanum is often found growing on roadsides, in fields, in teak forests, and in open waste places close to settlements. It prefers sunny and wind-sheltered spots. It grows well from the plains up to 500(-2000) m altitude, preferably on upland soils, but it is also planted on dikelets of paddy fields.

Ethno- medicinal uses:

The plant is carminative, diaphoretic and stimulant; used in cold, coughs, catarrh and bronchitis, Leaf juice is used for dysentery and as a mouth-wash for relieving toothache; poured into nostrils for migraine. Decoction of the leaf is used for checking nose bleeding and malarial fever. Leaf paste is used as a cure for parasitic skin diseases. Decoction of the leaf is used in fever, indigestion and diarrhoea. Dried plant is burnt as mosquito repellant. Essential oil of the leaves and inflorescences possesses strong antifungal and antibacterial properties.

3. *Mentha arvensis* L.

(Mint, Pudina in Hindi)

Classification/Taxonomical position

Kingdom	:	Plantae
Class	:	Dicotyledons
Sub class	:	Gamopetalae
Series	:	Bicarpellatae
Order	:	Lamiales
Family	:	Lamiaceae
Genus	:	*Mentha*
Species	:	*arvensis*
Range	:	Asia, U.S.A

Habitat

Plant easily grows on sandy and clay loams with periodic water-soaking indicator of damp soil conditions with moist to wet ground. It froms fibrous roots, often with hollow adventitious roots from lower nodes. The plant is well distributed in Western Europe, the Himalayas, India and China. European part of the former USSR, the Caucasus, Central Asia, Western Siberia, Eastern Siberia (Shiskin,1954).

Morphology

Aromatic perennial from creeping rhizomes with fleshy surface and underground shoots. Stem dull green, somewhat purple-splotched, prostrate to erect, square, often ramified, 15-60 cm high. Cotyledons broad, rounded triangular. Leaves horizontally opposed, ovate or elliptical, petiolate, slightly serrate to notch along margin. Flowering time is June to September; Flowers are lilac or rose-lilac (Nikitin, 1983).

Edible Uses:

The leaves of wild mint are edible, raw or cooked. Having a pleasantly strong minty flavor with a slight bitterness, they are used as flavorings in salads or cooked

foods. A herb tea can be made from the fresh or dried leaves. The North American tribes used the leaves to make tea or beverages, to spice pemmican and soups, and to add flavor to certain meats in cooking. An essential oil from the plant is used as a flavoring in sweets and beverages (Nikitin, 1983).

Medicinal Uses:

Mint is often used as a domestic herbal remedy, being valued especially for its antiseptic properties and its beneficial effect in digestion. The whole plant is anaesthetic, antispasmodic, antiseptic, aromatic, and has agents that counteract inflammation, that relieve and remove gas from the digestive system, promote secretion of milk, relieve fever and thirst, give strength and tone to the stomach, and is a stimulant.

A decoction of plant parts was taken for stomach pain, colds, swellings, headaches, diarrhea, and fevers. Dried leaves were chewed and swallowed for chest pains and heart ailments. Fresh leaves were put in the nostrils for colds. An infusion of leaves and stems was taken for vomiting, colds, pains, swellings, fevers, headaches, to prevent influenza, for stomach troubles and indigestion. Leaves were used for carious teeth and in the sweat bath for rheumatism.

Other Uses:

The plant is used as an insect repellent. Rats and mice intensely dislike the smell of mint. The plant was therefore used in homes as a strewing herb and has also been spread in granaries to keep the rodents off the grain. The leaves also repel various insects. Native people used leaves and stems as perfume to deodorize houses. Leaves were powdered and sprinkled on meat and berries as a bug repellant. Plants were boiled with traps to destroy the human scent.

Economic significance

Weed of grain and tilled crops; occurs in kitchen gardens, fallow lands, along roads and irrigation ditches. Control measures include multiple-field crop rotation, removing stubble, pre-winter plowing, chemical weeding (Agaev, 1988).

4. *Eucalyptus citriodora* Hook

Classification/Taxonomical position

Kingdom	:	Plantae
Class	:	Dicotyledonae
Sub class	:	Polypetalae
Series	:	Calyciflorae
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	*Eucalyptus*
Species	:	*citriodora*

Historical background

Native to Queensland, Australia, *E. citriodora* is commonly planted throughout the tropics, in the Mediterranean area and in the Malesian area, mainly in Malaysia. In Victoria, Australia, it has been planted as an ornamental plant in northern and western plains. It was tried in Cornwall, UK, but was killed at an early age by frost. A most successful introduction of eucalypts is the afforestation of the 'pantana' grasslands of Sri Lanka. E. citriodora was recently introduced to Cyprus for trials in coastal plains and foothills. It was introduced to Fiji in 1939, and so far it is the most promising of the eucalypts tried. It was tried and established in Ghana, and was widely planted in Madras, India, between 1937 and 1955.

Plant Description

Evergreen tree 24–40 m high with tall straight trunk 0.6–1.3 m in diameter, and thin, graceful crown of drooping foliage. Bark smooth, gray, peeling off in thin irregular scales or patches and becoming mottled, exposing whitish or faintly bluish inner layer with powdery surfaces appearing dimpled. Twigs slender, slightly flattened, light green, tinged with brown. Leaves alternate, narrowly lance-shaped, 10–20 cm long, 1–2.5 cm wide, apically acuminate, basally acute, entire, glabrous, thin, light green on both surfaces, with many fine parallel straight veins and with vein inside edge. Corymbs terminate at leaf base, to 6 cm long, branched. Flowers many, 3–5 on equal short stalks

(umbels) from ovoid buds 8–12 mm long, 5–8 mm wide. Stamens many, threadlikes, white, 6 mm long, spreading ca 12 mm across, anthers with long gland. Pistil inferior 3-celled ovary and long, stout style. Capsules few, urn-shaped or ovoid, narrowed into short neck, 10–12 mm long, 8–10 mm wide, brown with scattered raised dots. Seeds few, irregularly ellipsoid, 4–5 mm long, shiny black.

Ecology

Said to grow where the rainfall, mostly during summer, is 6 to 13 cm, with 5–7 month dry season, withstanding high temperatures (29–35°C mean monthly maximum) and light frosts. In tropical and subtropical arid to semi-arid zones, in infertile clays, laterites, poor and gravelly soils and podzols, preferrably well drained.

Cultivation

Eucalyptus thrives both in the tropics and subtropics. High humidity and plenty of rainfall are conducive to its luxuriant growth. It can be grown in various types of soil. In Zimbabwe, seeds are broadcast successfully on the ashes of recently burned tracts. More usually seedlings are transplanted from the nursery. Seed require no special treatment. Though needing protection from frost and weeds when young, older saplings show more tolerance to both.

Harvesting

For oil extraction, trees are not allowed to mature to the timber stage. Instead, they are lopped for the foliage; sucker shoots produce copious foliage.

Preparation of land

Clear the land of jungle growth. Take pits of size 45 x 45 x 45 cm at a spacing 2 x 2 m at least one month prior to planting and allow to weather. Fill up the pits with soil completely so as to prevent water stagnation.

Planting

Nursery is raised and 4-5 month old seedlings are planted with the commencement of southwest monsoon. After planting, press the soil around the seedling and form mound to prevent stagnation of water.

Manuring

Manuring is not usually done. However, application of 400 g ammonium sulphate, 60 g superphosphate and 25 g muriate of potash per plant per year during August from third year onwards is found to be useful in increasing leaf yield.

After cultivation

During first year, cultivate the rows in both directions to prevent weed growth. Hand weeding is done around the seedlings. Fire belts are to be provided all around.

Intercropping

Eucalyptus can be grown along with coffee, lemongrass and palmarosa. In the first four years, intercropping with pineapple, yam and vegetables can be done.

Harvest and curing

Pruning of side branches may be started from second year onwards. Lopping at a height of 2 m is- done during third or fourth year and there after lopping is resorted to at half-yearly intervals leaving only one branch. For extracting oil, steam distillation is often employed. Optimum time for distillation is two hours and the average recovery of oil is 1.5-1.8% of the net weight of leaves. Wilting of the cut leaves under shade for 24 hours before distillation will increase the oil recovery percentage.

Chemistry

Glabrous leaves may contain oil with 65.5% citronellal, 12.2% citronellol, and 3.6% isopulegol; hairy leaves contain more oil with 86.6–90.1% citronellal, 4.6–6.0% citronellol, and 0.7–0.8% isopulegol, 1-pinene, b-pinene, and isovaleric aldehyde are also recovered (Hussain *et al,* 1988).

Ethno- Medicinal use

Essential oil extracted from leaves is used as antiseptic, infections of upper respiratory tract and certain skin diseases. It is also mixed with equal quantity of olive oil and used as rubifacient for rheumatism. The oil is also used in ointments for burns, mosquito repellent and internally used as a stimulating expectorant in chronic bronchitis and asthma.

Other uses

The essential oil is used in the preparation of cosmetics, hair oil and soap and forms a raw material for menthol manufacture. Root is used as purgative. Decoction of leaves is also used as insect and vermint repellent. Leaves yield a lemon-scented oil rich in citronellal, and favored in perfumery. The wood is good for saw-timber, used for general construction, poles, railroad ties, and tool handles. Bark may contain up to 12% tannin. Kenyans favor the honey produced by this species.

5. *Dillenia indica* Linn.

Classification/Taxonomical position

Kingdom	:	Plantae
Class	:	Dicotyledons
Sub class	:	Polypetalae
Series	:	Thalamiflorae
Order	:	Ranales
Family	:	Dilleniaceae
Genus	:	*Dillenia*
Species	:	*indica*

Historical background:

This plant is native to China (Yunnan), India, Bangladesh, Indonesia, Malaysia, Sri Lanka, Thailand and Vietnam, where it grows in the humid evergreen forests mainly along the banks of the rivers. The genus is honoured to the German botanist Johann Jacob Dillen (Latinized in Dillenius), (1687-1747); the Latin name of the species "indica" = of India, refers to one of its origin places.

Plant Description

The ***Dillenia indica*** L. (1753) is an evergreen or semi-evergreen tree with a roundish top, tall up to about 25 m with a reddish brown smooth bark which tends to flake off; the young branches are tomentose. It has intense green coloured leaves, glossy on the upper side, alternate, usually grouped at the apex of the branches, of elliptic-

oblong or obovate-oblong shape, 15-40 cm long and 7-14 cm broad, with prominent parallel lateral nervations and indented margins; the 2-6 cm long petioles are grooved and pubescent at the base.

The flowers are solitary, terminal, hanging on an approx. 8 cm long peduncle, grows upto approx. 20 cm diameter, with five roundish sepals, concave, thick and fleshy, 4-6 cm long, of a pale green colour. The corolla, formed by five white obovate petals, 7-10 cm long and 6-8 cm broad, surrounds several yellow stamina which form a compact mass on which the white lanceolate styles extends out, radially placed and about 2.5 cm long.

The fruit is aggregate with a diameter of 5 to 15 cm which comes from the enlargement of more ovaries, 15-20, with persistent fleshy sepals, indehiscent (it does not open when ripe), with 5 or more, reniform, of brown colour, per ovary. It easily reproduces by seed, which germinates in about one month at 20-25 °C, and by cutting; when by seed reproduction, the first fructification takes place after 8-10 years. A popular ornamental plant for its foliage, perfumed flowers and globular fruits, it is often utilized as shade tree in parks and gardens.

Chemical constituents

Previous phytochemical studies with *D. indica* species revealed the occurrences of a number of triterpenes and flavonoids (Pavanasasivam *et al*., 1975). We, herein, report the isolation of lupeol, betulinaldehyde, betulinic acid and stigmasterol as well as the antimicrobial activity, cytotoxicity and antioxidant activity of extracts from *D. indica*.

Cultivation

It may be cultivated in the tropical and subtropical climates zones, with low temperatures which are not to be less than +10 °C, even though it may survive, for a short time, at temperatures close to the -2 °C. It prefers sandy, neutral or acidic, maintained wet soils; if kept dry in winter, it may loose the leaves.

Ethno-Medicinal uses

The leaves, bark and fruits are utilized in traditional medicine especially as anti-inflammatory; the juice of the fruit is utilized for preparing cough syrups and, blended with water and sugar, for reducing fever, the bark for poultices for arthritis. Decoction of the bark, leaves or fruit is taken orally for diarrhoea and dysentery (@50 ml twice daily). The fruit is used as tonic, laxative and in abdominal pain. The blaze is ground to powder and is applied externally on ulcers and sores. Laboratory studies have shown some evidences of an anti-leukaemic activity of extracts from the fruits, probably due to the presence of betulinic acid.

Other uses

The fleshy sepals, with a pleasant acidulous flavour, are consumed raw or cooked, or utilized for preparing preserves and jellies and for aromatizing food preparations; the fruits are cooked and consumed in small quantities as they have some laxative properties. The wood is compact and durable and has moderate utilization in buildings and for fabricating cages, plywood and other objects.

4.4 Detail *In-Vitro* Investigations against the Test Pathogens

The antidermatophytic activity of the selected plant secondary metabolites were investigated against the test fungi with special reference to minimum inhibitory concentration, IC_{50} value, nature of toxicity, killing time assay, effect of inoculum density, effect of temperature on toxicity, range of spectrum and comparison with some synthetic antifungals. These parameters were determined using the Broth microdilution method.

4.4.1 Broth Microdilution Method

Broth microdilution method is currently the most widely used method, standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 2002); now known as Clinical Laboratory Standards Institute (CLSI). It was found to be very sensitive, modern, rapid, automated, reproducible, economical and quantitative *in-vitro* fungicidal testing method.

It includes the following steps (Fig. 4.7):

Protocols for Broth Microdilution Method:

- Flat bottom 96 well Microtitre culture plates were used. All the wells of the plate were filled with 100µl of RPMI-1640 sterilized media.
- In the 2nd column 100µl medium was added again, in 3rd column, 90µl more medium was added, while the wells of the 4th column were supplied additionally with 80µl of medium.
- 10µl of the test sample was added to the wells in 3rd column (Drug Control).
- 20µl of the test sample was added to the wells of 4th column.
- The test sample was mixed thoroughly using a multichannel micropipette.
- 100µl was transferred to 5th column. This process was repeated from 5th to 6th; 6th to 7th and so on till the 11th column was reached.
- Then 100µl inoculum (CFU 1 x 10^3 CFU/ml) was added from 1st to 12th column except 2nd and 3rd column.
- Plates were then incubated in a moist chamber at 25±2°C for 48-72 hrs.
- Absorbance was recorded at 530 nm using a Microtitre plate reader.

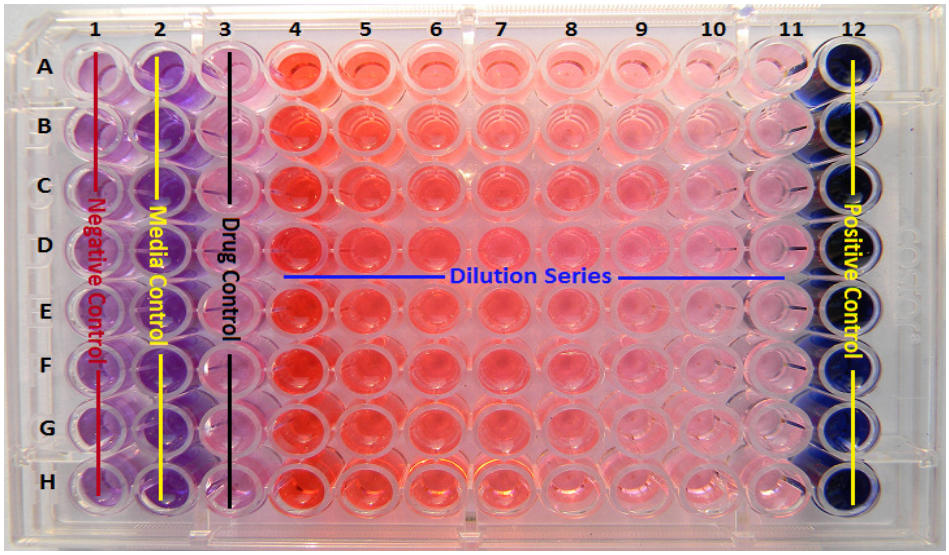


Fig-4.1: Format for testing the sample(s) in 96 well plate

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. 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 × 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. *Homalomena aromatica schott.*
2. *Ocimum americanum* L.
3. *Mentha arvensis* L.
4. *Eucalyptus citriodora* L.

(ii). 50 % ethanolic extract

5. *Dillenia indica.*

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 it cannot 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 121^0C, 15 lbs for 15 min.

4.4.5 Preparation of Stock Solution of the 'Test Sample'

Since, all the 'test sample' used in the present research were insoluble in water, but soluble in organic solvents,an organic solvent DMSO (dimethyl sulphoxide) was used for preparing their stock solutions.

(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 -20^0C.

(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 -20^0C.

Table- 4.1: 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

According to the NCCLS/ CLSI norms an inoculum ranges from 1×10^3 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, since 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-4.2 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 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.
- The plates were incubated at 25±2^{0}C for 48 hrs in an inverted position.

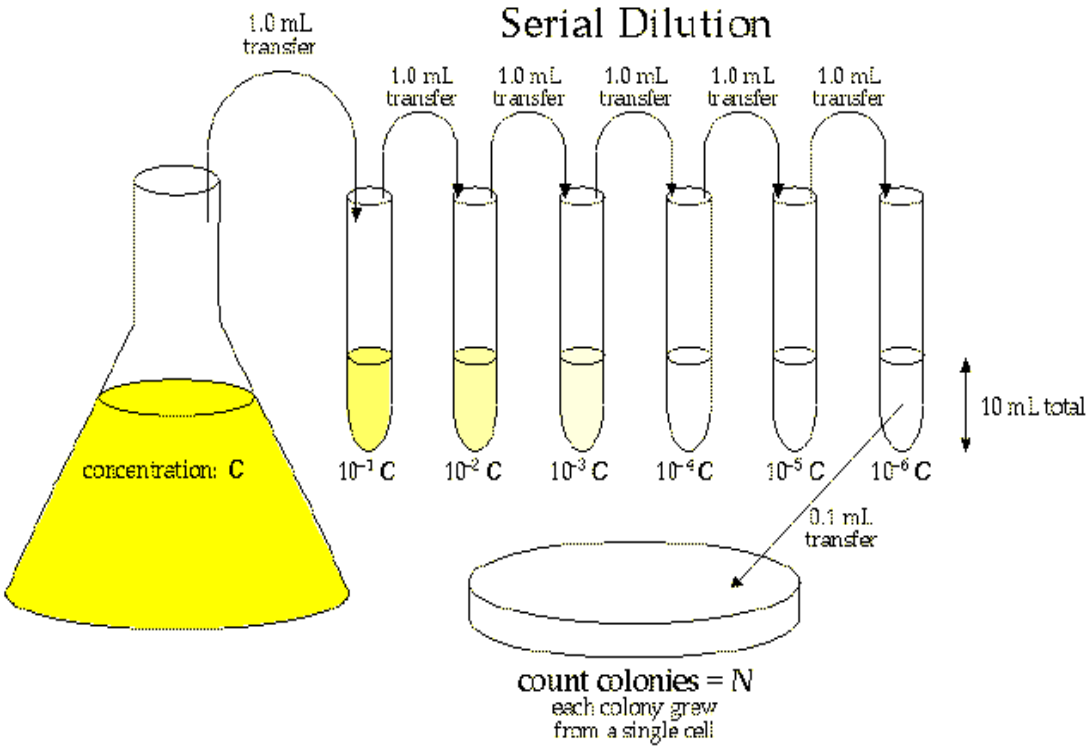


Fig-4.2 Schematic representations, serial dilution method for fungal inoculum preparation

4.4.7 Minimum Inhibitory Concentration and IC_{50}, using NCCLS, 2002

The minimum inhibitory concentration (MIC) of the plant secondary metabolites (oil/ extract) against the test pathogen was determined using NCCLS (2002). The 96-well tissue culture plates were used for two fold serial dilution. The proper growth control, sample control and 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 48-72 hrs in a moist, dark chamber at 25±2^{o}C. Further, minimum inhibitory concentration (MIC) values were recorded spectrophotometrically at 530 nm [Table-5.4-5.20; 5.29 & Fig-5.1-5.10].

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 (minimum static concentration means the pathogens was not killed but only their growth was inhibited). All tests were performed in triplicate [Table- 5.4- 5.20; Fig- 5.1 and 5.10].

4.4.9 Inoculum density, using McFarland

As per the NCCLS/ CLSI 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. The absorbance of 0.5 McFarland is equal to the

absorbance of inoculum suspension containing 1x 10^3 CFU/ml. While increasing the inoculum density of the test pathogen, the CFU/ml was 1 x 10^6, 1 x 10^9, 1 x 10^{12} and 1 x 10^{18} CFU/ml, respectively.

Table-4.3: O.D. values recorded spectrophotometrically, using McFarland

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-4.4: 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-4.5: Prepartion of dilutions of inoculum and plating and its evaluation at McFarlands

Dilution	No of colonies in plate-1	No of colonies in plate-2
1st	Infinite	Infinite
2nd	Infinite	Infinite
3rd	Infinite	Infinite
4th	Infinite	Infinite
5th	346	335
6th	33	31
7th	3	1
8th	No colonies	No colonies
9th	No colonies	No colonies
10th	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-5.21.

4.4.10 Effect of temperature on the efficacy of oil/ extract:

Effect of temperature on the efficacy of oil *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, was also determined. Samples of oil in small vials, each contains 01ml, were exposed at 40, 60 and 80^0 C in hot water bath, separately. Further, the oil's efficacy was tested against the test fungi- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum* at their respective minimum fungicidal concentration (MFCs). This was made as per the NCCLS-2002 method, and the observations are recorded in table-5.22.

4.4.11 Killing Time of the Essential oil/ Extract, against the Test Pathogens

The minimum killing time (MKT) of the oil, *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, against the test fungi- *E. floccosum,*

M. gypseum, T. mentagrophytes and *T. rubrum* was determined by using the following protocols and the observations are recorded in table-5.23 to 5.26.

Protocols for MKT

The killing time assay using Microtiter plates was determined using the MIC, 2MIC and the pure oil. All the wells were filled with 100µl medium RPMI- 1640. 80µl more medium was added to the wells of the 4th column. Test samples/ plant constituents (20µl) were added to the wells of 4th column and serially diluted up to the 11th column using a multichannel micropipette. Then 100µl of inoculum was added to all the wells except 1st 2nd and3rd column. The wells corresponding to the MIC, 2MIC and the pure oil were marked. Prior to incubation, touch the loop over the wells containing MIC, 2MIC and pure oil and drag over the SDA plates, gently. The Microtiter plate and the SDA plates were kept for incubation. Further, after 6 min, 1hr, 6 hrs, 12 hrs, 18 hrs, 24 hrs and 30 hrs; the same process was repeated. The SDA plates were kept for incubation at 25±2°C up to 48-72 hrs. Observations were made after the incubation period and recorded in Table-5.23- 5.26.

4.4.12 Fungi-toxic Spectrum of the Essential oil/ Extract

The range of spectrum of the oil, *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, against some other human pathogenic fungi, available in the lab, viz., *Microsporum auddouinii, M. canis, M. nanum, T. tonsurans* and *T. violaceum* was also determined. This was done as per usual method (NCCLS 2002), and the observations are recorded in table-5.27.

4.4.13 Comparison with some Synthetic Fungicides/ Antifungal drugs

The efficacy of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica* was also compared with some synthetic antifungal drugs, available in the market viz., Dactrine; Nizaral and Tenderm. The observations were recorded by comparing their minimum inhibitory concentrations (Table 5.28).

4.5 Characterization of the Essential Oil

4.5.1 Physico-chemical properties of the most effecicious plant metabolites (i.e. *H. aromatica, E. citriodora, O. americanum* and *M. arvensis*), was determined using the methods of Langenau (1948) and Shukla (1998), and recorded in table-5.30- 5.31.

4.5.1.1 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 25^0C. To convert specific gravity determined at room temperature to 25^0C, a correction factor of 0.008 per ^{0}C was used. If the room temperature was higher than 25^0C, the correction factor was added. On the other hand, if the room temperature was lower than 25^0C, the correction factor was subtracted from the original value (Table-5.30).

4.5.1.2 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-5.30).

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±2^{0}C) using sodium light.

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

α = observed rotation

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

c = concentration of the solution in per centage.

4.5.1.3 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 25^0C. To convert refractive index from room temperature to 25^0C a correction factor of 0.00045 per degree increase or decrease of temperature was used (Table-5.30).

4.5.1.4 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 (Table-5.30).

4.5.1.5 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 each of 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-5.31.

4.5.2 Identification of the active constituents from selected plant essential oils

The essential oils of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* were subjected to identification of their active constituents. This was made using the following methods and their observations were recorded (table 5.32-5.36):

4.5.2.1 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.

4.5.2.2 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 100^0C for 7mm, and then heated at 5^0C/ min to 250^0 C. Carrier gas, Helium was used at flow rate of 2 ml /min.

4.5.2.3 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.

4.5.2.4 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).

4.5.2.5 Mass Spectroscopy (MS)

Mass spectral analysis was run by E1 (Electron Impact Ionisation) 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 from which the 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.

4.5.2.6 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.

5. Results

The findings of the present investigation entitled *'Investigation of Anti-dermatophytic Activity from selected Medicinal Plants of Mizoram'* are summarizing under the following heads:

5.1 Collection of plants traditionally used against skin diseases/ fungal diseases, and their identification

Based on frequent field visits as well as literature survey, information about some important traditionally used plants were collected and utilized for making a priority based list of twenty most frequently used plants against skin ailments (table-5.1).

Table 5.1 List of common ethno-medicinal plants, collected at the grass root level

S.No.	Local name	Botanical and Family name	Family name
1.	Anchiri	*Homalomena aromatica*	Araceae
2.	Rûnhmui	*Ocimum americanum* Linn.	Lamiaceae
3.	Tilduh Par	*Lantana camara* Linn	Verbenaceae
4.	Nawhalh Thing	*Eucalyptus citriodora* Hook.	Myrtaceae
5.	Pudina	*Mentha arvensis* L	Lamiaceae
6.	Thakthing	*Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm	Lauraceae
7.	Derhken	*Tagetes erecta* Linn., Asteraceae	Asteraceae
8.	Vailenhlo	*Ageratum conyzoides* Linn.	Asteraceae
9.	Lawngthing	*Dipterocarpus turbinatus* Gaerta.	Dipterocarpaceae
10.	Sairial	*Buddleia asiatica* Lour	Loganiaceae
11.	Chul- be- raw	*Lobelia pyramidalis* L.	Campanulaceae
12.	Japanhlo ral/ bawrai ral	*Cuscuta reflexa* Roxb.	Convolvulaceae

13.	Phuihnamchhia	*Clerodendrum viscoscum* Vent.	Verbanaceae
14.	Vang	*Albizia chinensis* Merr.	Leguminosae
15.	Kawrthindeng	*Dillenia indica* L., Dilleniaceae	Dilleniaceae
16.	Ngaingaw/ Phungril	*Cassia fistula* Linn.,	Caesalpiniaceae
17.	Hnahbial /Lambak	*Centella asiatica* Linn.	Umbelliferae
18.	Ru- teng	*Milletia pachyacarpa* Benth.	Papillionaceae
19.	Vaithinthang	*Houttuyia cordata* Thumb.	Saururaceae
20.	Lamkhuang	*Artocarpus heterophyllus* Lam.	Moraceae

The plants thus collected were categorized 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 the School of Earth Sciences & Natural Resources Management, Mizoram University; State Medicinal Plants Board and State Forest Department, Aizawl. Furthermore, their confirmations were made with the help of experts at the Department of Botany, University of Allahabad as well as Botanical Survey of India, Allahabad.

The identified plants were then 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 the present investigation (table 5.2).

5.2 Extraction of plant secondary metabolites (essential oil/ extract)

Out of the total plants; 20 most frequently used ethno-medicinal plants were selected for extraction of the secondary metabolites (extract/ essential oil). The *solvent extraction method*, using different solvent (viz., alcohol, acetone, benzene, chloroform, hexane etc), was used for extraction of the secondary metabolites and Clevenger′s apparatus (Clevenger 1928), was used for hydro-distillation of the volatile constituents/ essential oil.

Table 5.2 Collected plants and their parts used for extraction of the essential oil/ extract

Local name	Botanical and Family name	Part used	Oil + Extract Code
Anchiri	*Homalomena aromatica*	Petiole and Rhizome	EHA-1
Rûnhmui	*Ocimum americanum*	Leaves, seed.	EOA-2
Tilduh Par	*Lantana camara*	Leaves	ELC-3
Nawhalh Thing	*Eucalyptus citriodora*	Leaves, oil (from seed), root.	EEC-4
Pudina	*Mentha arvensis*	Whole plant	EMA-5
Thakthing	*Cinnamomum tamala*	Bark, leaves,	ECT-6
Derhken	*Tagetes erecta*	Leaves, Flowers	ETE-7
Vailenhlo	*Ageratum conyzoides*	Leaves	EAC-8
Lawngthing	*Dipterocarpus turbinatus*	Resin	XDT-1
Sairial	*Buddleia asiatica*	Leaves	XBA-2
Chul- be- raw	*Lobelia pyramidalis*	Latex, leaves	XLP-3
Japanhlo ral/ bawrai ral	*Cuscuta reflexa*	Whole plant	XCR-4
Phuihnamchhia	*Clerodendrum viscoscum*	Rhizome, leaves & root.	XCV-5
Vang	*Albizia chinensis*	Bark, latex.	XAC-6
Kawrthindeng	*Dillenia indica*	Leaves & barks	XDI-7
Ngaingaw/ Phungril	*Cassia fistula*	Root, leaves, fruit.	XCF-8
Hnahbial /Lambak	*Centella asiatica*	Whole plant, leaves.	XCA-9
Ru- teng	*Milletia pachyacarpa*	Root	XMP-10
Vaithinthang	*Houttuyia cordata*	Rhizomes, leaves, root	XHC-11
Lamkhuang	*Artocarpus heterophyllus*	Root, latex, seed.	XAH-12

E = Essential oil; **X = Extract

5.3 Antidermatophytic screening

The essential oil/ extracts thus collected were subjected for antidermatophytic screening against the test pathogens *E. floccosum* (MTCC-7880), *M. gypseum* (MTCC-2830), *T. mentagrophytes* (MTCC-8476) and *T. rubrum* (MTCC-3272), using disc diffusion method; and the observations are recorded as follows (table-5.2).

Table 5.3 Screening efficacy of the test samples against the dermatophytes

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)**
Homalomena aromatica Schott.	Essential oil	21±2	25±2	27±2	30±2
Ocimum americanum Linn.	Essential oil	17±2	19±2	20±2	24±2
Lantana camara Linn.	Essential oil	1±2	3±2	3±2	3±2
Eucalyptus citriodora Hook.	Essential oil	24±2	22±2	22±2	25±2
Cinnamomum tamala (Buch.-Ham.) Nees & Eber	Essential oil	2±2	3±2	3±2	4±2
Tagetes erecta Linn.	Essential oil	2±2	3±2	4±2	3±2
Mentha arvensis L.	Essential oil	16±2	19±2	23±2	23±2
Ageratum conyzoides Linn.	Essential oil	2±2	3±2	3±2	4±2
Dipterocarpus turbinatus Gaerta.	Extract	NA	NA	2±2	3±2

Buddleia asiatica Lour.	Extract	NA	NA	1±2	1±2
Lobelia pyramidalis L.	Extract	NA	NA	1±2	1±2
Cuscuta reflexa Roxb.	Extract	NA	1±2	1±2	1±2
Clerodendrum viscoscum Vent.	Extract	NA	1±2	1±2	1±2
Albizia chinensis Merr.	Extract	NA	1±2	1±2	1±2
Dillenia indica Linn.	Extract	4±2	6±2	8±2	12±2
Cassia fistula Linn.	Extract	NA	1±2	1±2	1±2
Centella asiatica Linn.	Extract	NA	2±2	1±2	1±2
Milletia pachyacarpa Benth.	Extract	NA	1±2	1±2	1±2
Houttuyia cordata Thumb.	Extract	17±2	1±2	1±2	1±2
Artocarpus heterophyllus Lam.	Extract	NA	NA	NA	1±2

As per the observations made over table 5.3; out of 20 plants, only 4 essential oil bearing plants viz., *Homalomena aromatica, Eucalyptus citriodora, Ocimum americanum* and *Mentha arvensis* and 50 % ethanolic extract of *Dillenia indica* were recorded as strongest toxicant, against all the test pathogens. The maximum inhibition zone was observed in the essential oil of *H. aromatica* (30 mm); followed by *E. citriodora* essential oil (25 mm), *O. americanum* essential oil (24 mm), *M. arvensis* (23 mm) and extract of *D.indica* (12 mm).

Further, 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* Investigations against the Test Pathogens

5.4.1 Antidermatophytic activity of *H. aromatica* essential oil against *Ef, Mg, Tm* and *Tr*

The efficacy of the oil of *H. aromatica* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* were recorded in table-5.4- 5.7 as well as Fig- 5.1 and 5.2.

Plate-1 O.D. of 96 well plate: *Homalomena aromatica* against Tr, Tm, Ef & Mg

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.016	0.020	0.021	0.014	0.002	0.012	0.013	0.067	0.077	0.097	0.126	0.335
B	-0.003	0.004	0.069	-0.003	0.010	-0.004	-0.002	0.127	0.068	0.091	0.130	0.385
C	-0.003	0.005	0.011	0.005	0.009	0.010	0.003	0.013	0.024	0.006	0.001	0.002
D	-0.002	0.000	0.117	0.015	0.029	0.001	0.006	0.008	0.007	0.005	0.007	0.004
E	-0.002	0.005	0.092	0.020	0.080	0.107	0.140	0.150	0.167	0.249	0.255	0.189
F	-0.001	0.001	0.033	0.014	0.086	0.105	0.155	0.174	0.119	0.133	0.195	0.220
G	-0.001	0.004	0.032	0.017	0.016	0.015	0.079	0.075	0.103	0.097	0.131	0.152
H	-0.004	0.002	0.060	0.020	0.013	0.014	0.087	0.134	0.106	0.119	0.137	0.179

Endpoint
Lm1 530
Automix: Once
Calibrate: Once

Start Read:
2:06 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

Fig-5.1: O.D. of 96 well plate: *H. aromatica* against *Ef, Mg, Tm* and *Tr;* where column 1 shows Negative Control, Column 2 Media Control, Column 3 Drug Control, Column 4-11 Serial dilution of drug and Column 12 Positive Control.

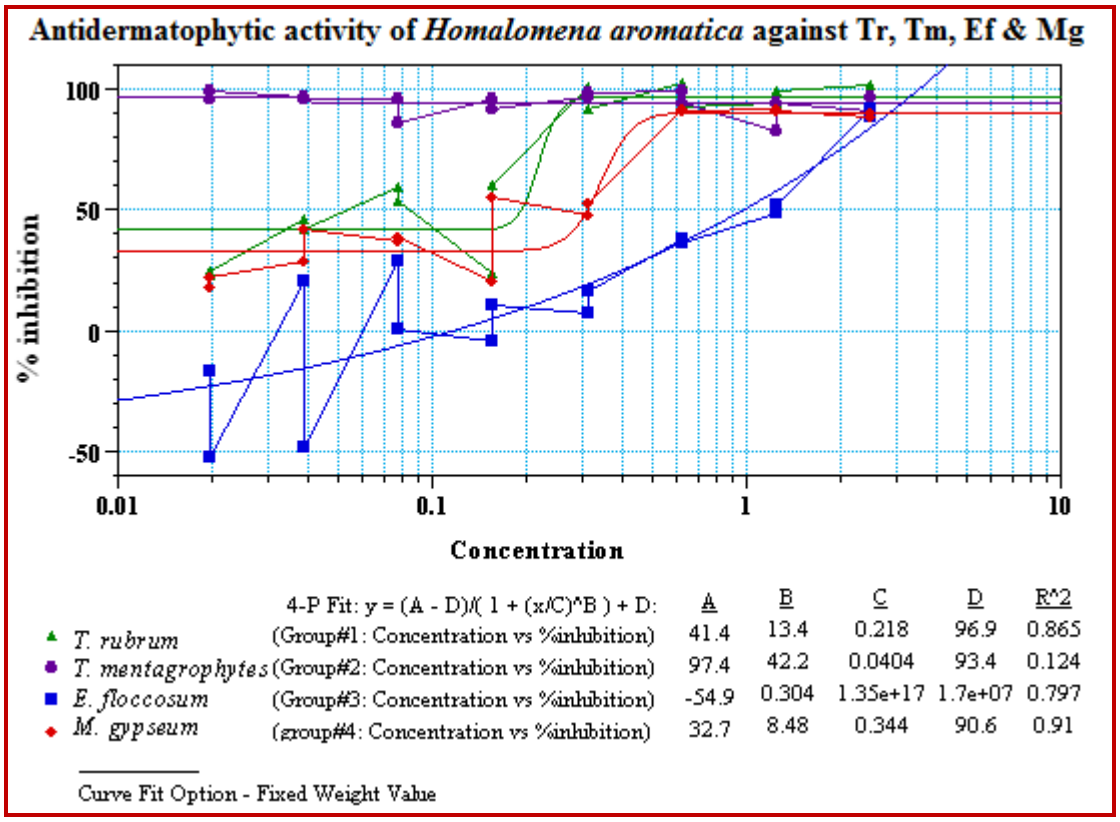


Fig- 5.2: Percentage inhibition of dermatophytes against *H. aromatica*

Table-5.4: Antidermatophytic activity of *H. aromatica* essential oil against *E. floccosum*

Sample	Conc. (mg/ml)	Wells	Values	Mean Values	Std. Dev.	CV%	Inhibition
HA1	2.500	E4	0.020	0.017	0.004	24.0	88.26%
		F4	0.014				91.67%
HA 2	1.250	E5	0.080	0.083	0.004	5.0	51.94%
		F5	0.086				48.42%
HA 3	0.625	E6	0.107	0.106	0.001	1.3	36.35%
		F6	0.105				37.49%
HA 4	0.313	E7	0.140	0.148	0.011	7.3	16.40%
		F7	0.155				7.32%
HA 5	0.156	E8	0.150	0.162	0.017	10.5	10.31%
		F8	0.174				-4.02%
HA 6	0.078	E9	0.167	0.143	0.034	23.8	0.15%
		F9	0.119				28.89%
HA 7	0.039	E10	0.249	0.191	0.082	43.0	-48.70%
		F10	0.133				20.64%
HA 8	0.020	E11	0.255	0.225	0.042	18.8	-52.47%
		F11	0.195				-16.74%

Based on the observations recorded in table-5.4 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the oil of *H. aromatica* against *E. floccosum* was recorded 2.25 mg/ml but it was fungicidal at 2.5 mg/ml. However, the IC_{50} value was recorded as 0.98 mg/ml.

Table-5.5: Antidermatophytic activity of *H. aromatica* essential oil against *M. gypseum*

Sample	Conc. (mg/ml)	Wells	Values	Mean Values	Std. Dev.	CV%	Inhibition
HA1	2.500	G4	0.017	0.018	0.002	10.4	89.82%
		H4	0.020				88.20%
HA 2	1.250	G5	0.016	0.015	0.002	10.7	90.65%
		H5	0.013				91.97%
HA 3	0.625	G6	0.015	0.015	0.000	3.4	91.01%
		H6	0.014				91.43%
HA 4	0.313	G7	0.079	0.083	0.006	7.2	52.90%
		H7	0.087				47.82%
HA 5	0.156	G8	0.075	0.104	0.041	39.5	54.99%
		H8	0.134				20.16%
HA 6	0.078	G9	0.103	0.104	0.002	1.8	38.44%
		H9	0.106				36.83%
HA 7	0.039	G10	0.097	0.108	0.015	13.9	41.79%
		H10	0.119				29.12%
HA 8	0.020	G11	0.131	0.134	0.004	3.1	21.84%
		H11	0.137				18.31%

Based on the observations recorded in table-5.5 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the oil of *H. aromatica* against *M. gypseum* was recorded 0.41 mg/ml but it was fungicidal at 0.62 mg/ ml. However, the IC_{50} value was recorded as 0.31 mg/ml.

Table-5.6: Antidermatophytic activity of *H. aromatica* essential oil against *T. mentagrophytes*

Sample	Conc. (mg/ml)	Wells	Values	Mean Values	Std. Dev.	CV%	Inhibition
HA1	2.500	C4	0.005	0.010	0.007	70.5	96.93%
		D4	0.015				90.83%
HA 2	1.250	C5	0.009	0.019	0.014	73.7	94.54%
		D5	0.029				82.65%
HA 3	0.625	C6	0.010	0.006	0.006	106.3	93.94%
		D6	0.001				99.1%
HA 4	0.313	C7	0.003	0.005	0.002	38.5	98.00%
		D7	0.006				96.51%
HA 5	0.156	C8	0.013	0.010	0.004	39.3	92.03%
		D8	0.008				95.49%
HA 6	0.078	C9	0.024	0.016	0.012	75.5	85.58%
		D9	0.007				95.61%
HA 7	0.039	C10	0.006	0.006	0.001	16.5	96.27%
		D10	0.005				97.05%
HA 8	0.020	C11	0.001	0.004	0.004	103.9	99.32%
		D11	0.007				95.55%

Based on the observations recorded in table-5.6 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the oil of *H. aromatica* against *T. mentagrophytes* was recorded 0.20 mg/ml but it was fungicidal at 0.62 mg/ml. However, the IC_{50} value was recorded as 0.12 mg/ml.

Table-5.7: Antidermatophytic activity of *H. aromatica* essential oil against *T. rubrum*

Sample	Conc. (mg/ml)	Wells	Values	Mean Values	Std. Dev.	CV%	Inhibition
HA1	2.500	A4	0.014	0.006	0.012	205.1	91.67%
		B4	-0.003				101.53%
HA 2	1.250	A5	0.002	0.006	0.006	94.9	98.84%
		B5	0.010				94.12%
HA 3	0.625	A6	0.012	0.004	0.012	302.2	92.80%
		B6	-0.004				102.60%
HA 4	0.313	A7	0.013	0.006	0.011	181.4	91.97%
		B7	-0.002				100.99%
HA 5	0.156	A8	0.067	0.097	0.042	43.8	59.95%
		B8	0.127				24.05%
HA 6	0.078	A9	0.077	0.073	0.007	8.9	53.80%
		B9	0.068				59.29%
HA 7	0.039	A10	0.097	0.094	0.005	5.1	41.79%
		B10	0.091				45.85%
HA 8	0.020	A11	0.126	0.128	0.003	2.2	24.88%
		B11	0.130				22.55%

Based on the observations recorded in table-5.7 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the essential oil of *H. aromatica* against *T. rubrum* was recorded 0.23 mg/ml but it was fungicidal at 0.62 mg/ml. However, the IC_{50} value was recorded as 0.19 mg/ml.

5.4.2 Antidermatophytic activity of *O. americanum* oil against *Ef, Mg, Tm* and *Tr*

The efficacy of the oil of *O. americanum* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table- 5.8- 5.11 as well as Fig- 5.3 and 5.4.

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.004	0.643	0.673	1.153	1.082	1.202	1.236	1.020	1.137	1.354	1.056	1.153
B	0.001	1.090	0.545	1.144	1.219	1.236	1.215	1.232	1.269	1.305	1.173	0.919
C	0.005	0.683	1.269	1.636	1.428	1.419	1.404	1.360	1.412	1.175	1.241	1.037
D	0.011	0.632	1.726	1.582	1.379	1.341	1.344	1.360	1.378	1.208	1.257	0.996
E	0.002	1.013	1.413	1.620	1.395	1.329	1.355	1.331	1.398	1.367	1.237	1.064
F	-0.002	0.598	1.291	1.254	1.387	1.253	1.296	1.326	1.384	1.322	1.271	1.133
G	-0.003	0.385	0.202	0.561	1.254	1.500	1.534	1.267	1.251	1.171	1.283	1.097
H	-0.008	0.330	0.600	0.609	0.976	1.293	1.051	1.037	1.055	0.952	1.069	1.125

Endpoint
Lm1 530
Automix: Once
Calibrate: Once

Start Read:
1:59 AM 12/25/2012

Wavelength Combination: !Lm1
Mean Temperature: 29.8
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.091
Reader: PLUS384 ROM v1.23 Jun 19 2008

Fig-5.3: O.D. of 96 well plate: *O. americanum* against *Ef, Mg, Tm* and *Tr;* where column 1 shows Negative Control, Column 2 Media Control, Column 3 Drug Control, Column 4-11 Serial dilution of drug and Column 12 Positive Control.

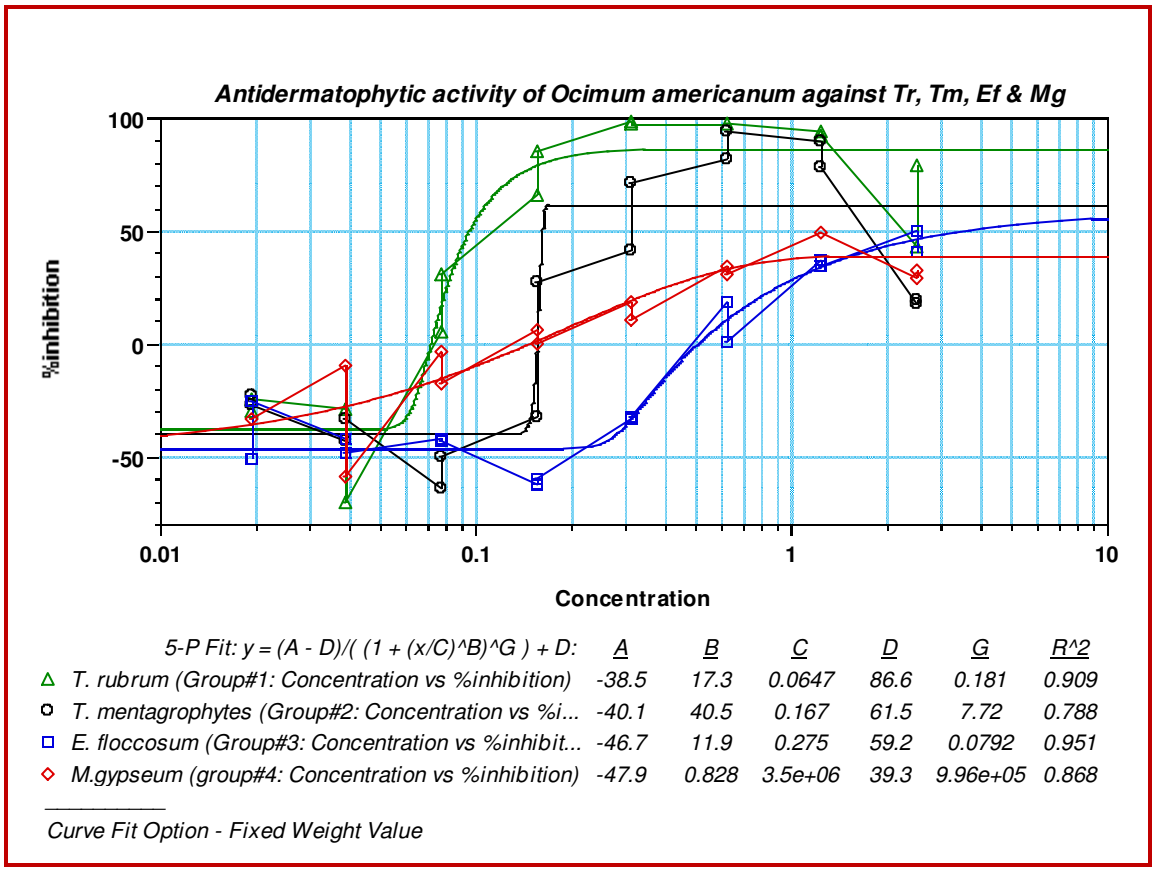


Fig-5.4: Percentage inhibition of dermatophytes against *O. americanum*

Table-5.8: Antidermatophytic activity of *O. americanum* essential oil against *E. floccosum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	G4	0.257	0.306	0.069	22.5	74.746
		H4	0.354				65.191
BL2	1.250	G5	0.305	0.265	0.057	21.5	70.027
		H5	0.224				77.941
BL3	0.625	G6	0.613	0.635	0.032	5.1	39.789
		H6	0.658				35.326
BL4	0.313	G7	0.762	0.748	0.020	2.7	25.103
		H7	0.734				27.885
BL5	0.156	G8	0.872	0.932	0.086	9.2	14.319
		H8	0.993				2.405
BL6	0.078	G9	1.004	1.051	0.067	6.3	1.284
		H9	1.099				-7.986
BL7	0.039	G10	0.971	0.967	0.006	0.6	4.518
		H10	0.963				5.344
BL8	0.020	G11	1.047	1.018	0.041	4.1	-2.894
		H11	0.988				2.847

Based on the observations recorded in table- 5.8 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the oil of *O. americanum* against *E. floccosum* was recorded at 2.40 mg/ml but it was fungicidal at 3.32 mg/ml. However, the IC_{50} value was recorded as 1.22 mg/ml.

Table-5.9: Antidermatophytic activity of *O. americanum* essential oil against *M. gypseum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	A4	0.282	0.203	0.112	55.1	71.201
		B4	0.124				87.342
BL2	1.250	A5	0.164	0.122	0.060	49.1	83.236
		B5	0.080				91.878
BL3	0.625	A6	0.488	0.488	0.000	0.1	50.177
		B6	0.487				50.218
BL4	0.313	A7	0.794	0.845	0.073	8.6	18.906
		B7	0.897				8.394
BL5	0.156	A8	0.761	0.762	0.003	0.3	22.308
		B8	0.764				21.940
BL6	0.078	A9	0.828	0.836	0.011	1.4	15.412
		B9	0.844				13.777
BL7	0.039	A10	0.859	0.837	0.030	3.6	12.245
		B10	0.816				16.648
BL8	0.020	A11	0.922	0.891	0.044	4.9	5.778
		B11	0.860				12.102

Based on the observations recorded in table- 5.9 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the oil of *O. americanum* against *M. gypseum* was recorded as 1.24 mg/ml but it was fungicidal at 2.50 mg/ ml. However, the IC_{50} value was recorded as 0.68 mg/ml.

Table-5.10: Antidermatophytic activity of *O. americanum* essential oil against *T. mentagrophytes*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	E4	0.044	0.039	0.008	19.8	67.102
		F4	0.034				75.174
BL2	1.250	E5	0.016	0.017	0.001	8.2	87.911
		F5	0.018				86.430
BL3	0.625	E6	0.011	0.011	0.001	5.9	91.687
		F6	0.010				92.354
BL4	0.313	E7	0.070	0.041	0.042	101.9	47.922
		F7	0.011				91.539
BL5	0.156	E8	0.130	0.118	0.017	14.6	4.008
		F8	0.105				21.929
BL6	0.078	E9	0.148	0.130	0.026	20.4	-9.914
		F9	0.111				17.782
BL7	0.039	E10	0.136	0.140	0.007	4.7	-0.361
		F10	0.145				-7.248
BL8	0.020	E11	0.148	0.152	0.006	3.9	-9.766
		F11	0.157				-16.060

Based on the observations recorded in table- 5.10 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the oil of *O. americanum* against *T. mentagrophytes* was recorded as 1.56 mg/ml but it was fungicidal at 2.50 mg/ml. However, the IC_{50} value was recorded as 0.97.

Table-5.11: Antidermatophytic activity of *O. americanum* essential oil against *T. rubrum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	A4	0.118	0.119	0.001	0.8	83.464
		B4	0.120				83.283
BL2	1.250	A5	0.065	0.067	0.002	3.3	90.868
		B5	0.068				90.435
BL3	0.625	A6	0.039	0.039	0.001	2.5	94.598
		B6	0.040				94.402
BL4	0.313	A7	0.028	0.033	0.007	21.3	96.092
		B7	0.038				94.709
BL5	0.156	A8	0.057	0.070	0.018	25.9	92.027
		B8	0.083				88.451
BL6	0.078	A9	0.604	0.608	0.006	0.9	15.561
		B9	0.612				14.458
BL7	0.039	A10	0.806	0.793	0.018	2.3	-12.544
		B10	0.780				-8.968
BL8	0.020	A11	0.905	0.876	0.041	4.7	-26.415
		B11	0.847				-18.355

Based on the observations recorded in table- 5.11 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the essential oil of *O. americanum* against *T. rubrum* was recorded as 1.60 mg/ml but it was fungicidal at 2.50 mg/ml. However, the IC_{50} value was recorded as 0.88 mg/ml.

5.4.3 Antidermatophytic activity of *M. arvensis* essential oil against *Ef, Mg, Tm* and *Tr*

The efficacy of essential oil of *M. arvensis* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table-5.12- 5.15 as well as Fig- 5.5 and 5.6.

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.002	0.042	0.301	0.329	0.360	0.360	0.551	0.486	0.721	0.603	0.642	0.694
B	-0.003	0.064	0.228	0.356	0.374	0.362	0.248	0.394	0.531	0.695	0.736	0.751
C	0.015	0.129	0.262	0.239	0.250	0.207	0.149	0.335	0.724	0.438	0.300	0.289
D	0.003	0.067	0.234	0.279	0.272	0.182	0.135	0.280	0.358	0.351	0.275	0.291
E	-0.005	0.085	0.258	0.262	0.395	0.338	0.436	0.502	0.809	0.877	0.944	1.106
F	-0.006	0.064	0.257	0.289	0.343	0.244	0.354	0.651	0.828	1.033	1.101	1.164
G	-0.003	0.036	0.270	0.306	0.347	0.374	0.392	0.574	0.700	0.799	0.924	0.979
H	0.001	0.045	0.046	0.433	0.328	0.423	0.583	0.729	0.734	0.865	0.963	1.033

Endpoint
Lm1 530
Automix: Once
Calibrate: Once

Start Read:
1:50 AM 12/25/2012

Wavelength Combination: !Lm1
Mean Temperature: 29.8
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.091
Reader: PLUS384 ROM v1.23 Jun 19 2008

Fig-5.5: O.D. of 96 well plate: *M. arvensis* against *Ef, Mg, Tm* and *Tr;* where column 1 shows Negative Control, Column 2 Media Control, Column 3 Drug Control, Column 4-11 Serial dilution of drug and Column 12 Positive Control.

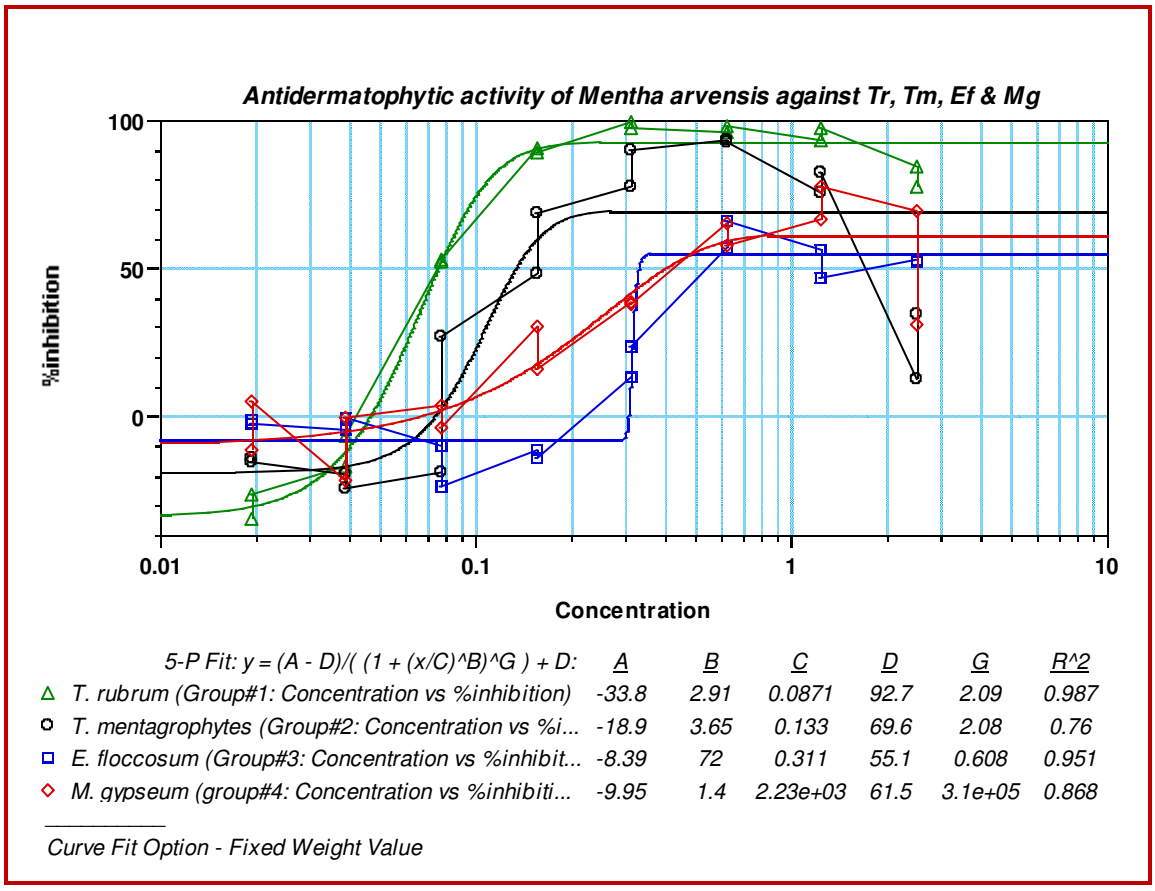


Fig-5.6: Percentage inhibition of dermatophytes against *M. arvensis*

Table-5.12: Antidermatophytic activities *M. arvensis* essential oil against *E. floccosum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	C4	0.472	0.493	0.030	6.1	51.791
		D4	0.514				47.470
BL2	1.250	C5	0.466	0.434	0.045	10.4	52.394
		D5	0.402				58.891
BL3	0.625	C6	0.495	0.563	0.096	17.1	49.431
		D6	0.631				35.558
BL4	0.313	C7	0.596	0.631	0.049	7.8	39.123
		D7	0.665				32.054
BL5	0.156	C8	0.735	0.748	0.018	2.4	24.882
		D8	0.760				22.328
BL6	0.078	C9	0.775	0.792	0.024	3.1	20.867
		D9	0.809				17.333
BL7	0.039	C10	0.823	0.826	0.004	0.5	15.892
		D10	0.830				15.249
BL8	0.020	C11	0.797	0.802	0.007	0.9	18.579
		D11	0.807				17.517

Based on the observations recorded in table- 5.12 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of *M. arvensis* against *E. floccosum* was recorded as 2.48 mg/ml but it was fungicidal at 3.82 mg/ ml. However, the IC_{50} value was recorded as 1.26 mg/ml.

Table-5.13: Antidermatophytic activities *M. arvensis* essential oil against *M. gypseum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	G4	0.257	0.306	0.069	22.5	74.746
		H4	0.354				65.191
BL2	1.250	G5	0.305	0.265	0.057	21.5	70.027
		H5	0.224				77.941
BL3	0.625	G6	0.613	0.635	0.032	5.1	39.789
		H6	0.658				35.326
BL4	0.313	G7	0.762	0.748	0.020	2.7	25.103
		H7	0.734				27.885
BL5	0.156	G8	0.872	0.932	0.086	9.2	14.319
		H8	0.993				2.405
BL6	0.078	G9	1.004	1.051	0.067	6.3	1.284
		H9	1.099				-7.986
BL7	0.039	G10	0.971	0.967	0.006	0.6	4.518
		H10	0.963				5.344
BL8	0.020	G11	1.047	1.018	0.041	4.1	-2.894
		H11	0.988				2.847

Based on the observations recorded in table- 5.13 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of *M. arvensis* against *M. gypseum* was recorded as 2.01 mg/ml but it was fungicidal at 3.60 mg/ ml. However, the IC_{50} value was recorded as 0.98 mg/ml.

Table-5.14: Antidermatophytic activities *M. arvensis* essential oil against *T. mentagrophytes*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	E4	0.502	0.469	0.046	9.9	29.633
		F4	0.436				38.839
BL2	1.250	E5	0.154	0.126	0.040	31.9	78.368
		F5	0.098				86.327
BL3	0.625	E6	0.100	0.084	0.022	26.5	85.963
		F6	0.069				90.391
BL4	0.313	E7	0.113	0.101	0.017	17.0	84.211
		F7	0.088				87.602
BL5	0.156	E8	0.667	0.586	0.115	19.6	6.540
		F8	0.504				29.339
BL6	0.078	E9	0.681	0.659	0.032	4.9	4.537
		F9	0.636				10.870
BL7	0.039	E10	0.748	0.732	0.023	3.1	-4.796
		F10	0.716				-0.326
BL8	0.020	E11	0.698	0.689	0.013	1.9	2.140
		F11	0.680				4.691

Based on the observations recorded in table- 5.14 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of *M. arvensis* against *T. mentagrophytes* was recorded as 1.83 mg/ml but it was fungicidal at 2.50 mg/ml. However, the IC_{50} value was recorded as 0.96 mg/ml.

Table-5.15: Antidermatophytic activities *M. arvensis* essential oil against *T. rubrum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	E4	0.443	0.415	0.041	9.8	33.896
		F4	0.386				42.438
BL2	1.250	E5	0.117	0.112	0.008	6.8	82.525
		F5	0.106				84.135
BL3	0.625	E6	0.049	0.047	0.003	5.4	92.677
		F6	0.046				93.213
BL4	0.313	E7	0.029	0.032	0.003	9.6	95.613
		F7	0.034				94.972
BL5	0.156	E8	0.468	0.392	0.108	27.6	30.199
		F8	0.315				53.008
BL6	0.078	E9	0.604	0.549	0.076	13.9	10.029
		F9	0.495				26.144
BL7	0.039	E10	0.674	0.622	0.074	11.9	-0.436
		F10	0.569				15.113
BL8	0.020	E11	0.712	0.679	0.046	6.8	-6.071
		F11	0.646				3.634

Based on the observations recorded in table- 5.15 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of *M. arvensis* against *T. rubrum* was recorded as 1.76 mg/ml but it was fungicidal at 2.50 mg/ml. However, the IC_{50} value was recorded as 0.90 mg/ml.

5.4.4 Antidermatophytic activities *E. citriodora* essential oil against *Ef, Mg, Tm* and *Tr*

The efficacy of essential oil of *E. citriodora* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table- 5.16- 5.19 as well as Fig- 5.7 and 5.8.

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.007	0.129	0.303	0.068	0.032	0.032	0.006	0.006	1.023	1.155	1.190	1.138
B	-0.005	0.159	0.396	0.157	0.025	0.022	0.010	0.007	1.097	1.193	1.050	1.139
C	-0.002	0.257	0.407	0.539	0.104	0.159	0.467	1.011	1.091	1.144	1.148	1.396
D	0.001	0.240	0.394	0.513	0.108	0.190	0.826	1.183	1.106	1.129	1.183	1.159
E	-0.003	0.332	0.388	0.573	0.889	1.137	1.053	1.051	1.127	1.145	1.161	1.142
F	0.004	0.865	0.456	0.921	1.107	1.167	1.068	1.055	1.124	1.136	1.139	1.282
G	0.004	0.507	1.006	1.024	0.967	1.019	1.040	1.135	1.134	1.049	1.093	1.158
H	0.010	0.550	1.188	0.998	0.975	1.011	1.026	1.059	1.150	1.165	1.078	1.149

Endpoint
Lm1 530
Automix: Once
Calibrate: Once

Start Read:
2:11 AM 12/29/2011

Wavelength Combination: !Lm1
Mean Temperature: 28.2
Data Type: Absorbance
Plate Blank: Used Lm1 = 0.087
Reader: PLUS384 ROM v1.23 Jun 19 2008

Fig-5.7: O.D. of 96 well plate: *E. citriodora* against *Ef, Mg, Tm* and *Tr;* where column 1 shows Negative Control, Column 2 Media Control, Column 3 Drug Control, Column 4-11 Serial dilution of drug and Column 12 Positive Control.

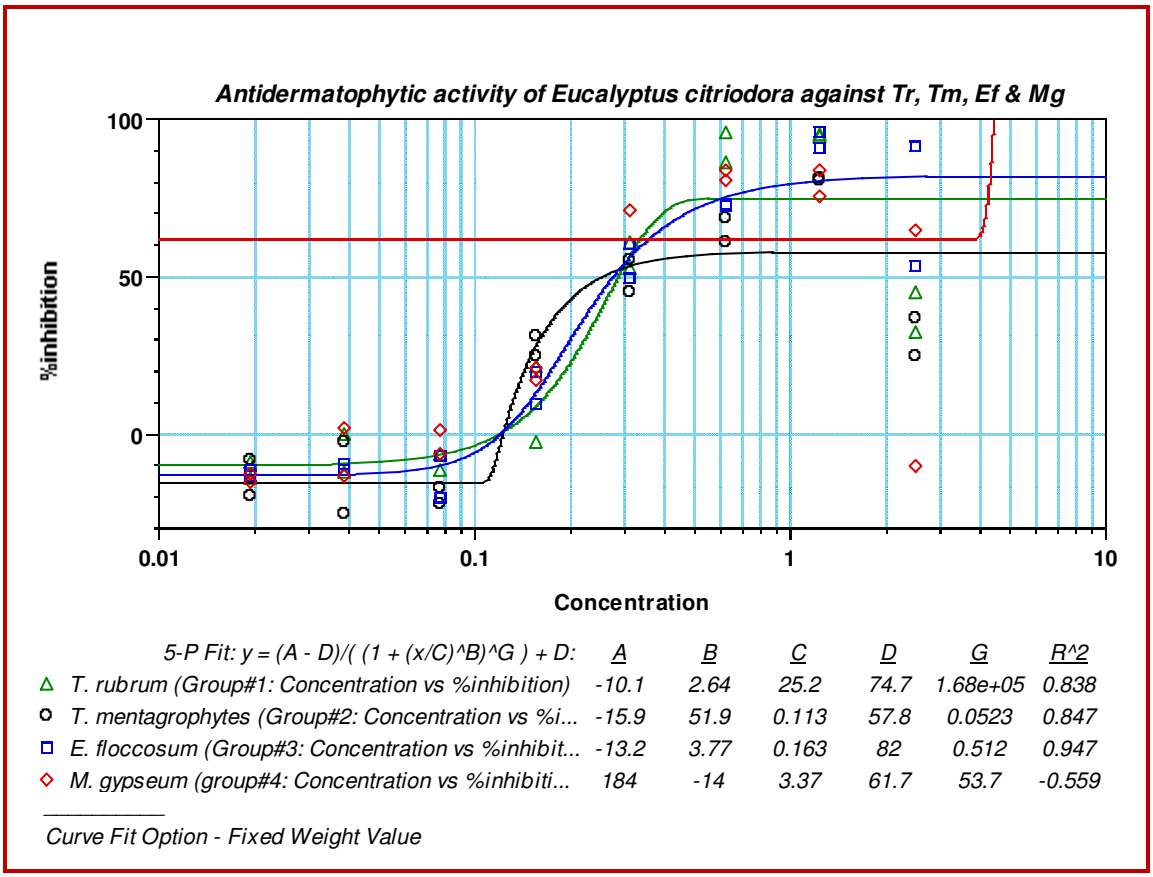


Fig-5.8: Percentage inhibition of dermatophytes against *E. citriodora*

Table-5.16: Antidermatophytic activities *E. citriodora* essential oil against *E. floccosum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	A4	0.854	0.869	0.020	2.3	27.430
		B4	0.883				25.026
BL2	1.250	A5	0.125	0.147	0.031	21.1	89.367
		B5	0.169				85.638
BL3	0.625	A6	1.233	0.662	0.808	122.0	-4.736
		B6	0.091				92.280
BL4	0.313	A7	1.157	1.165	0.011	1.0	1.702
		B7	1.173				0.326
BL5	0.156	A8	1.097	1.111	0.019	1.7	6.815
		B8	1.124				4.530
BL6	0.078	A9	1.135	1.120	0.022	2.0	3.562
		B9	1.104				6.212
BL7	0.039	A10	1.132	1.122	0.014	1.3	3.817
		B10	1.112				5.541
BL8	0.020	A11	1.128	1.121	0.009	0.8	4.225
		B11	1.114				5.346

Based on the observations recorded in table- 5.16 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of *E. citriodora* against *E. floccosum* was recorded as 1.60 mg/ml but it was fungicidal at 2.80 mg/ml. However, the IC_{50} value was recorded as 0.92 mg/ ml.

Table-5.17: Antidermatophytic activities *E. citriodora* essential oil against *M. gypseum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	C4	0.469	0.514	0.064	12.4	36.601
		D4	0.559				24.365
BL2	1.250	C5	0.140	0.141	0.002	1.7	81.099
		D5	0.143				80.640
BL3	0.625	C6	0.236	0.264	0.038	14.5	68.024
		D6	0.291				60.710
BL4	0.313	C7	0.335	0.372	0.052	14.0	54.666
		D7	0.409				44.728
BL5	0.156	C8	0.558	0.534	0.034	6.3	24.500
		D8	0.510				30.977
BL6	0.078	C9	0.903	0.885	0.025	2.8	-22.093
		D9	0.868				-17.320
BL7	0.039	C10	0.757	0.843	0.121	14.4	-2.420
		D10	0.929				-25.555
BL8	0.020	C11	0.885	0.843	0.059	7.0	-19.633
		D11	0.801				-8.329

Based on the observations recorded in table- 5.17 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of *E. citriodora* against *M. gypseum* was recorded as 1.40 mg/ml but it was fungicidal at 2.40 mg/ml. However, the IC_{50} value was recorded as 1.80 mg/ ml.

Table-5.18: Antidermatophytic activities *E. citriodora* essential oil against *T. mentagrophytes*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	A4	0.501	0.454	0.066	14.6	32.315
		B4	0.407				44.985
BL2	1.250	A5	0.038	0.039	0.001	3.6	94.823
		B5	0.040				94.553
BL3	0.625	A6	0.102	0.067	0.049	72.7	86.237
		B6	0.033				95.580
BL4	0.313	A7	0.352	0.322	0.042	13.1	52.367
		B7	0.293				60.439
BL5	0.156	A8	0.761	0.672	0.126	18.8	-2.934
		B8	0.582				21.242
BL6	0.078	A9	0.827	0.807	0.028	3.4	-11.790
		B9	0.787				-6.477
BL7	0.039	A10	0.831	0.786	0.063	8.0	-12.318
		B10	0.742				-0.284
BL8	0.020	A11	0.844	0.825	0.027	3.3	-14.184
		B11	0.806				-8.937

Based on the observations recorded in table- 5.18 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of *E. citriodora* against *T. mentagrophytes* was recorded as 1.08 mg/ml but it was fungicidal at 1.60 mg/ml. However, the IC_{50} value was recorded as 0.64 mg/ ml.

Table-5.19: Antidermatophytic activities *E. citriodora* essential oil against *T. rubrum*

Sample	Concentration	Wells	Values	Mean Values	Std.Dev.	CV%	%inhibition
BL1	2.500	C4	0.539	0.526	0.019	3.5	55.171
		D4	0.513				57.349
BL2	1.250	C5	0.104	0.106	0.003	2.9	91.352
		D5	0.108				90.994
BL3	0.625	C6	0.159	0.175	0.022	12.5	86.762
		D6	0.190				84.184
BL4	0.313	C7	0.467	0.646	0.254	39.3	61.175
		D7	0.826				31.322
BL5	0.156	C8	1.011	1.097	0.122	11.1	15.946
		D8	1.183				1.618
BL6	0.078	C9	1.091	1.099	0.011	1.0	9.260
		D9	1.106				7.996
BL7	0.039	C10	1.144	1.136	0.010	0.9	4.903
		D10	1.129				6.125
BL8	0.020	C11	1.148	1.165	0.025	2.1	4.579
		D11	1.183				1.643

Based on the observations recorded in table- 5.19 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of *E. citriodora* against *T. rubrum* was recorded as 1.20 mg/ml but it was fungicidal at 1.80 mg/ ml. However, the IC_{50} value was recorded as 0.78 mg/ ml.

5.4.5 Antidermatophytic activity of 50% ethanolic leaf extract of *D. indica* against *Ef, Mg, Tm* and *Tr*

The efficacy of 50% ethanolic leaf extract of *D. indica* against the dermatophytes *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table- 5.20 as well as Fig- 5.9 and 5.10.

Plate-4 O.D. of 96 well plate: *Dillenia indica* 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

Fig-5.9: O.D. of 96 well plate: *D. indica* against *Ef, Mg, Tm* and *Tr;* where column 1 shows Negative Control, Column 2 Media Control, Column 3 Drug Control, Column 4-11 Serial dilution of drug and Column 12 Positive Control.

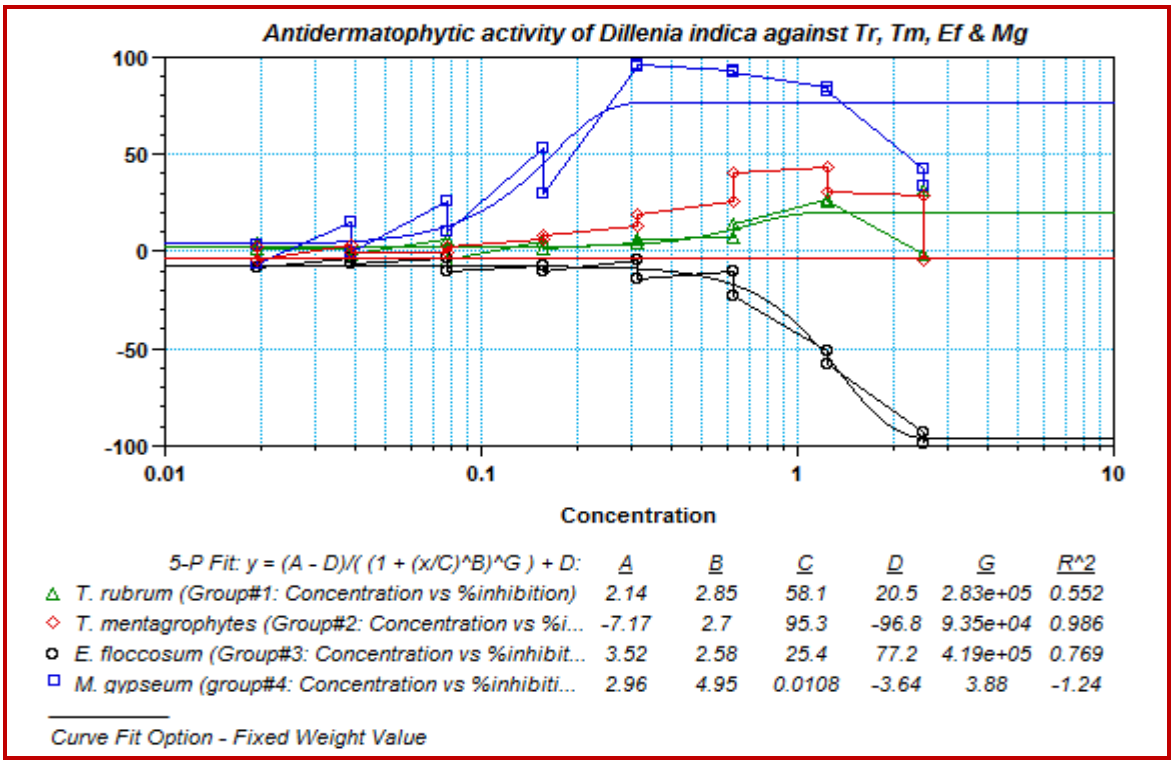


Fig-5.10: Percentage inhibition of dermatophytes against *D. indica*

Table-5.20: Antidermatophytic activities of 50% ethanolic leaf extract of *D. indica*

S. No.	Dermatophytes	Antidermatophytic activities of *D. indica* (mg/ml)		
		MIC	IC_{50}	MFC
1.	***E. floccosum***	NA	NA	NA
2.	***M. gypseum***	2.02	0.83	2.50
3.	***T. mentagrophytes***	2.21	1.14	2.50
4.	***T. rubrum***	2.11	0.89	2.50

NA = Not Applicable

As per the observations recorded in table-5.20 as well as fig-5.9 and 5.10; the ethanolic extract of *D. indica* was not effective against *E. floccosum*; however, it was effective against the rest of the three pathogens. The MIC ranges from 2.02 to 2.21 mg/ ml and the MFC was 2.5 mg/ ml; however, IC_{50} values were recorded in between 0.83 to 1.14 mg/ ml, respectively.

5.4.6 Inoculum Density vis-a-vis Fungicidal Activity

The efficacy of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* as well as 50 % ethanolic extract of *D. indica*, on inoculum density of the test pathogens- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum;* was also determined. The observations recorded are given in table-5.21.

Table-5.21: Efficacy of the oil/ extract on inoculum density of dermatophytes

Essential oils/ active constituents at their MFC	Fungi	Negative Control	Media Control	Drug Control	Mycelial Growth Inhibition (MGI) Inoculum density up to the maximum dose (1 x 10^{18} CFU/ml) taken into consideration								Positive Control
					4	5	6	7	8	9	10	11	12
H. aromatica	*E.f.*	-	-	-	+++	+++	+++	+++	+++	+++	++	+	#
	M.g	-	-	-	+++	+++	+++	+++	+++	+++	++	++	#
	T.m	-	-	-	+++	+++	+++	+++	+++	+++	+++	++	#
	T.r.	-	-	-	+++	+++	+++	+++	+++	+++	+++	++	#
E. citriodora	*E.f.*	-	-	-	+++	+++	+++	+++	+++	+++	++	++	#
	M.g	-	-	-	+++	+++	+++	+++	+++	+++	++	++	#
	T.m	-	-	-	+++	+++	+++	++	+++	+++	++	++	#
	T.r.	-	-	-	+++	+++	+++	+++	+++	++	++	++	#
O. americanum	*E.f.*	-	-	-	+++	+++	+++	++	++	+	+	+	#
	M.g	-	-	-	+++	+++	+++	+++	++	++	+	+	#
	T.m.	-	-	-	+++	+++	+++	+++	+++	++	++	+	#
	T.r.	-	-	-	+++	+++	+++	+++	+++	+	+	+	#
M. arvensis	*E.f.*	-	-	-	+++	+++	+++	+++	+	+	-	-	#
	M.g	-	-	-	+++	+++	+++	+++	++	+	-	-	#
	T.m	-	-	-	+++	+++	+++	+++	+	+	-	-	#
	T.r.	-	-	-	+++	+++	+++	+++	++	+	-	-	#
D. indica	*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-5.21, the minimum fungicidal concentration (MFC) of the oil(s) persisted heavy inoculum density against all; while in case of extract, except *E.f.*; 25% growth inhibition was recorded against *M.g., T.r. & T.m.*

5.4.7 Effect of temperature on the efficacy of oil/ extract

Effect of temperature on the efficacy of oil *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, was also determined. Samples of oil in small vials, each contains 1ml, were exposed at 40, 60 and 80^0 C in hot water bath separately. Further, the oil's efficacy was tested against the test fungi- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum* at their respective minimum fungicidal concentration (MFCs). This was made using the protocols recommended by NCCLS-2002, and the observations were recorded in table-5.22.

Table-5.22: Effect of temperature on the efficacy of oil/ extract

Test Fungi	**Essential oils at their respective MFCs**												Ethanolic extract (50%) at MFC		
	H. aromatica			*E. citriodora*			*O. americanum*			*M. arvensis*			*D. indica*		
	40^0C	**60^0C**	**80^0C**	**40^0C**	**60^0C**	**80^0C**	**40^0C**	**60^0C**	**80^0C**	**40^0C**	**60^0C**	**80^0C**	**40^0C**	**60^0C**	**80^0C**
E. floccosum	+	+	+	+	+	+	+	+	#	+	+	+	+	#	#
M. gypseum	+	+	+	+	+	+	+	+	#	+	+	+	+	#	#
T. mentagro-phytes	+	+	+	+	+	+	+	+	#	+	+	+	+	#	#
T. rubrum	+	+	+	+	+	+	+	+	+	+	+	+	+	#	#

+ = 100% growth inhibition; # = fungal growth observed;

5.4.8 Killing Time of the Essential oil/ Extract, against the Test Pathogens:

The minimum killing time (MKT) of the oil, *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, against the test fungi- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum* was determined and the observations recorded are as follows (table-5.23 to 5.26).

Table-5.23: Killing Time Assay of the oil(s) and extract against *E. floccosum*

Time (hrs)	Conc	Efficacy of the oils and extract against *E. floccosum*				
		Essential oils at different concentrations (mg/ml)				Ethanolic extract (50%)
		H. aromatica	***E. citriodora***	***O. americanum***	***M. arvensis***	***D. indica***
0.1	Pure oil/ext	++	++	++	++	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
1	Pure oil/ext	++	++	++	++	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
6	Pure oil/ext	++	++	++	++	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
12	Pure oil/ext	++	++	++	++	#
	2MIC	++	#	#	#	#
	MIC	+	#	#	#	#
18	Pure oil/ext	++	++	++	++	#
	2MIC	++	#	++	++	#
	MIC	+	#	+	+	#
24	Pure oil/ext	++	++	++	++	#
	2MIC	++	++	++	++	#
	MIC	+	+	+	+	#
30	Pure oil/ext	++	++	++	++	#
	2MIC	++	++	++	++	#
	MIC	+	+	+	+	#

++ = 100% growth inhibition with **cidal** nature; + = 100% growth inhibition with **static** nature;
# = fungal growth observed ext = extract

As per the observation recorded in the table- 5.23; it was observed that hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 12hrs, *O. americanum* and *M. arvensis* took 18 hrs, *E. citriodora* took 24 hrs to kill the test fungi *E. floccosum* while pure oil killed the same just within 0.1 hr (i.e. 6 min).

Moreover, the extract of *D. indica* was not effective against *E.floccosum*.

Table-5.24: Killing Time Assay of the oil(s) and extract against *M. gypseum*

Time (hrs)	Conc	Efficacy of the oils and extract against *M. gypseum*				
		Essential oils at different concentrations (mg/ml)				Ethanolic extract (50%)
		H. aromatica	***E. citriodora***	***O. americanum***	***M. arvensis***	***D. indica***
0.1	Pure oil/ext	++	++	++	++	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
1	Pure oil/ext	++	++	++	++	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
6	Pure oil/ext	++	++	++	++	#
	2MIC	+	#	#	#	#
	MIC	#	#	#	#	#
12	Pure oil/ext	++	++	++	++	#
	2MIC	++	#	+	#	#
	MIC	+	#	#	#	#
18	Pure oil/ext	++	++	++	++	+
	2MIC	++	++	++	#	#
	MIC	+	#	+	#	#
24	Pure oil/ext	++	++	++	++	++
	2MIC	++	++	++	++	#
	MIC	+	+	+	#	#
30	Pure oil/ext	++	++	++	++	++
	2MIC	++	++	++	++	++
	MIC	+	+	+	+	+

++ = 100% growth inhibition with **cidal** nature; **+** = 100% growth inhibition with **static** nature;
# = fungal growth observed ext = extract

As per the observation recorded in the table- 5.24; it was observed that hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 6.0 hrs, *O. americanum* took 12 hrs, *E. citriodora* took 18 hrs and *M. arvensis* took 24 hrs to kill the test fungi *M. gypseum* while, in case of the extract *D. indica* it was recorded 30 hrs. However, pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 18 hrs to kill the same, respectively.

Table-5.25: Killing Time Assay of the oil(s) and extract against *T. mentagrophytes*

Time (hrs)	Conc	Efficacy of the oils and extract against *T. mentagrophytes*				
		Essential oils at different concentrations (mg/ml)				Ethanolic extract (50%)
		H. aromatica	*E. citriodora*	*O. americanum*	*M. arvensis*	*D. indica*
0.1	Pure oil/ext	++	++	++	++	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
1	Pure oil/ext	++	++	++	++	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
6	Pure oil/ext	++	++	++	++	#
	2MIC	+	#	#	#	#
	MIC	#	#	#	#	#
12	Pure oil/ext	++	++	++	++	#
	2MIC	++	+	#	#	#
	MIC	+	#	#	#	#
18	Pure oil/ext	++	++	++	++	#
	2MIC	++	++	++	+	#
	MIC	+	#	+	#	#
24	Pure oil/ext	++	++	++	++	++
	2MIC	++	++	++	++	#
	MIC	+	+	+	#	#
30	Pure oil/ext	++	++	++	++	++
	2MIC	++	++	++	++	+
	MIC	+	+	+	+	#

++ = 100% growth inhibition with **cidal** nature; **+** = 100% growth inhibition with **static** nature;
# = fungal growth observed ext = extract

As per the observation recorded in the table- 5.25; it was observed that hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 6.0 hrs, *E. citriodora* took 12 hrs, *O. americanum* and *M. arvensis* took 18 hrs to kill the test fungi *T. mentagrophytes* while, in case of the extract *D. indica* it was recorded at 24 hrs. Furthermore, pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 24 hrs to kill the test pathogen, respectively.

Table-5.26: Killing Time Assay of the oil(s) and extract against *T. rubrum*

Time (hrs)	Conc	Efficacy of the oils and extract against *T. rubrum*				
		Essential oils at different concentrations (mg/ml)				Ethanolic extract (50%)
		H. aromatica	***E. citriodora***	***O. americanum***	***M. arvensis***	***D. indica***
0.1	Pure oil/ext	**++**	**++**	**++**	**++**	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
1	Pure oil/ext	**++**	**++**	**++**	**++**	#
	2MIC	#	#	#	#	#
	MIC	#	#	#	#	#
6	Pure oil/ext	**++**	**++**	**++**	**++**	#
	2MIC	**+**	**+**	#	#	#
	MIC	#	#	#	#	#
12	Pure oil/ext	**++**	**++**	**++**	**++**	#
	2MIC	**++**	**+**	#	**+**	#
	MIC	**+**	#	#	#	#
18	Pure oil/ext	**++**	**++**	**++**	**++**	#
	2MIC	**++**	**++**	**++**	**+**	#
	MIC	**+**	#	**+**	#	#
24	Pure oil/ext	**++**	**++**	**++**	**++**	**++**
	2MIC	**++**	**++**	**++**	**++**	**+**
	MIC	**+**	**+**	**+**	#	#
30	Pure oil/ext	**++**	**++**	**++**	**++**	**++**
	2MIC	**++**	**++**	**++**	**++**	**+**
	MIC	**+**	**+**	**+**	**+**	#

++ = 100% growth inhibition with **cidal** nature; **+** = 100% growth inhibition with **static** nature;
# = fungal growth observed ext = extract

As per the observation recorded in the table- 5.26; it was observed that hyper lethal conc (i.e. 2MIC) of *H. aromatica* and *E. citriodora* took 6 hrs while *M. arvensis* took 12 hrs and *O. americanum* took 18hrs to kill the test fungi but in case of *D. indica* extract, it was recorded at 24 hrs. Moreover, pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 24 hrs to kill the test pathogen *T. rubrum*.

5.4.9 Fungi-toxic Spectrum of the Essential oil/ Extract:

The range of spectrum of the oil, *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, against some other human pathogenic fungi, available in the lab, viz., *Microsporum auddouinii, M. canis, M. nanum, T. tonsurans* and *T. violaceum* was determined and recorded as follows (table-5.27).

Table-5.27: Fungi-toxic Spectrum of the Essential oil/ Extract against some other pathogenic fungi

Human Pathogenic Fungi Tested	**Essential oils at their respective MFCs (mg/ml)**				**Ethanolic extract (50%)**
	H. aromatica	***E. citriodora***	***O. americanum***	***M. arvensis***	***D. indica***
Microsporum auddouinii	100[c]	100[c]	100[s]	100[s]	100[s]
M. canis	100[c]	100[c]	100[c]	100[c]	100[s]
M. nanum	100[c]	100[c]	100[c]	100[s]	100[c]
T. tonsurans	100[c]	100[c]	100[s]	100[s]	100[s]
T. violaceum	100[c]	100[c]	100[c]	100[c]	100[s]

[s] indicates static; [c] indicates cidal in nature

As per the observation recorded in the table- 5.27; it was observed that *H. aromatica* and *E. citriodora* shows fungicidal efficacy against all the test pathogens, however, *O. americanum* shows fungicidal efficacy against *Microsporum canis*, *M. nanum* and *T. violaceum* and *M. arvensis* shows fungicidal efficacy against *Microsporum canis* and *T. violaceum.* Moreover, the extract of *D. indica* shows fungicidal efficacy against *Microsporum nanum* only.

5.4.10 Comparison with some Synthetic Fungicides/ Antifungal drugs

The efficacy of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica* was also compared with some synthetic antifungal drugs, available in the market viz., Dactrine; Nizaral and Tenderm. The observations were recorded by comparing their minimum inhibitory concentrations (Table 5.28).

Table- 5.28: Comparative MICs of the test samples with some Synthetic Antifungal Drugs

Oil & Trade Name of Antifungal Drugs	**Ingredients**	**Minimum Inhibitory Concentration (mg/ml)**			
		E. floccosum	*M. gypseum*	*T. mentagrophytes*	*T. rubrum*
H. aromatica	E.O.	2.25	0.41	0.20	0.23
E. citriodora	E.O.	1.60	1.40	1.08	1.20
O. americanum	E.O.	2.40	1.24	1.56	1.60
M. arvensis	E.O.	2.48	2.01	1.83	1.76
D. indica	Extract	NA	2.02	2.21	2.11
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

NA = Not Applicable

As per the observation recorded in table 5.28; the MIC of *H. aromatica* have the strongest toxicity against all the test pathogens (ranges from 2.25-0.20 mg/ml) followed by *E. citriodora* (ranges from 1.60-1.08 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.

5.4.11 Fungicidal activity of the selected plants: an over view

The comparative analysis of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* as well as 50 % ethanolic extract of *D. indica*, against the test pathogens viz., *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum* was determined and recorded in table-5.29.

Table: 5.29 Antidermatophytic activities of selected plant secondary metabolites against four dermatophytes reported as MIC, IC_{50} and MFC in (mg/ml)

SN	Samples	Antiderma-tophytic Parameters	Antidermatophytic activity of selected plant secondary metabolites against four dermatophytes				
			Essential oils				50 % ethanolic extract
			H. aromatica	*O. americanum*	*M. arvensis*	*E. citriodora*	*D. indica*
1.	***E. floccosum***	MIC	2.25	2.40	2.48	1.60	NA
		IC_{50}	0.98	1.22	1.26	0.92	NA
		MFC	2.50	3.32	3.82	2.80	NA
2.	***M. gypseum***	MIC	0.41	1.24	2.01	1.40	2.02
		IC_{50}	0.31	0.68	0.98	1.80	0.83
		MFC	0.62	2.50	3.60	2.40	2.50
3.	***T. mentagrophytes***	MIC	0.20	1.56	1.83	1.08	2.21
		IC_{50}	0.12	0.97	0.96	0.64	1.14
		MFC	0.62	2.50	2.50	1.60	2.50
4.	***T. rubrum***	MIC	0.23	1.60	1.76	1.20	2.11
		IC_{50}	0.19	0.88	0.90	0.78	0.89
		MFC	0.62	2.50	2.50	1.80	2.50

5.5 Physico-chemical characterization of the most efficacious plant metabolite(s)

As per the detail *in vitro* investigations, the essential oils of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* were recorded as the most efficacious plant metabolites and subjected for various physio-chemical characterization viz., plant height, appearance, oil yield, odour, boiling point, specific gravity, optical rotation, refractive index and solubility in water. The observations thus recorded are summarized as follows (table-5.30).

Table-5.30: Physio-chemical properties of selected essential oils

Parameter studies	*Homalomena aromatica*	*Ocimum americanum*	*Mentha arvensis*	*Eucalyptus citriodora*
Plant height	0.40-0.80 M	0.5-1.5 M	10-60 Cm	30 - 70 M
Appearance	Light Yellow	Pale yellow liquid	Slightly thick pale yellow liquid	Pale yellow
Oil Yield (%)	0.66 to 1.0	0.6-0.25	0.4-1.2%	1.5-1.8
Odour	refreshing pleasant spicy & typical note of Linalool	Characteristic of basil, spicy	Menthol smell	Characteristic perfume
Boiling Point	75°C	80˚C	68˚C	95˚C
Specific gravity at 25^0C	1.47	0.948 – 0.970	0.894 - 0.901	0.858 to 0.877
Optical rotation at 20^0C	-98	0.459 ± 0.06	-15.50 to -45	-3 to +3
Refractive index at 20^0C	1.468	1.520 – 1.540	1.459 - 1.475	1.450 to 1.468
Solubility in water	Insoluble	Insoluble	Insoluble	Insoluble

5.6 Solubility in various organic solvents

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

sulphoxide (DMSO), ethanol, hexane, methanol, n-butanol, petroleum ether, propanol and solvent ether. (Table- 5.31).

Furthermore, the extract was soluble in water but the oils were insoluble.

Table-5.31: Solubility of selected plant metabolites in organic solvents (1:1) ratio

S. No.	Organic Solvents	Selected Plant Essential oils and 50 % ethanolic extract				
		H. aromatica	***O. americanum***	***M. arvensis***	***E. citriodora***	***D. Indica***
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

5.7 Identification of the active constituents from selected plant essential oils

The essential oils of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* were subjected to identification of their active constituents. The observations were recorded as follows:

5.7.1 Active constituents from *H. aromatica* essential oil using GC MS analysis

The GC-MS analysis of the oil shows Linalool (63.54%), Terpinen-4-o1 (15.97), α-terpineol (2.89), Cryptone (2.45), Spathulenol (1.88), α-cardinol (1.78), and *epi*- α-cardinol (1.60), Humulene epoxide II (1.42) and Oplopanone (1.09) as important constituents (table 5.32).

Table-5.32: Active constituents from *H. aromatica* essential oil

Compound	**RI**	**Area (%)**
α-selinene	949	0.57
Cis- linalool oxide	956	0.91
ρ-cymen-8-ol	979	0.29
Trans-linalool oxide	986	0.73
Linalool	**1002**	**63.54**
Cis-ρ-menth-2-en-1-ol	1024	0.11
Trans- ρ-menth-2-en-1-ol	1057	0.42
Terpinen-4-ol	1069	15.97
Cryptone	1085	2.45
α-terpineol	1119	2.89
Cumin aldehyde	1137	0.79
Car-3en-2-one	1157	0.63
Piperitone	1165	0.29
β-caryophyllene	1197	0.54
α-murolene	1207	0.67
γ-cardinene	1258	0.26
δ-cardinene	1275	0.46
α-calocorene	1289	0.34
Spathulenol	1332	1.88
Ledol	1367	0.37
Humulene epoxide II	1459	1.42
epi- α-cardinol	1487	1.60
α-cardinol	1509	1.78
Oplopanone	1537	1.09

5.7.2 Active constituents from *E. citriodora* essential oil using GC MS analysis

The GC-MS analysis of the essential oil of ***E. citriodora*** shows; Citronellal (83.10%), Citronellol (7.21%), Linalool (2.46%) and Isopulegol (1.92%) as the major constituents (table 5.33).

Table 5.33 Active constituents of *E. citriodora* essential oil

Compounds	**RI**	**Area (%)**
α –thujene	942	0.10
α-pinene	956	0.31
Sabinene	967	0.30
Myrcene	986	0.12
α –terpenene	1015	0.25
ρ-cymene	1019	0.31
Limonene	1032	0.20
1,8-cineole	1039	0.21
Z-β-ocimene	1041	0.20
E-β-ocimene	1049	0.40
Linalool	1085	2.46
Citronellal	**1125**	**83.10**
Isopulegol	1143	1.92
Borneol	1159	0.40
Menthol	1168	0.10
α-terpeneol	1196	0.21
Citronellol	1208	7.21
Nerol	1227	1.01
Geraniol	1248	0.49
Eugenol	1357	0.29
β-caryophyllene	1425	0.20
Caryophyllene oxide	1471	0.21

5.7.3 Active constituents from *O. americanum* essential oil using GC MS analysis

The essential oil of *O. americanum* contains Terpinen-4-ol (45.20%), 1, 8-cineole (18.10%), α-terpineol (4.32%), Trans-Caryophyllene (3.66), Trans-α-Bergamotene (2.79), γ-Terpinene (2.60), cis-Sabinene hydrate (1.95), δ-Guaiene (1.57), trans-Sabinene hydrate (1.49) and l-Linalool (1.40) as the major constituents (table 5.34).

Table-5.34: Active constituents of *O. americanum* essential oil

Compounds	**RI**	**Area (%)**
α-Pinene	935	0.45
β-Pinene	948	0.38
β-Myrcene	974	0.60
α-Thujene	991	0.22
α-Terpinene	1018	0.40
p-Cymene	1029	0.30
l-Limonene	1031	0.70
1,8-Cineole	1034	18.10
γ-Terpinene	1062	2.60
cis-Sabinene hydrate	1068	1.95
α-Terpinolene	1089	0.33
l-Linalool	1097	1.40
trans-Sabinene hydrate	1113	1.49
Oct-1-en-3-ylacetate	1119	0.55
z-β-Terpineol	1127	0.45
Trans-pinene hydrate	1139	0.38
Camphor	1148	0.70
δ-Terpineol	1177	0.38
Terpinen-4-ol	**1197**	**45.20**
α-Terpineol	1234	4.32
Myrtenyl acetate	1257	0.25
Trans-Caryophyllene	1410	3.66
Trans-α-Bergamotene	1434	2.79
Aromadendrene	1478	0.65
Pentadecane	1498	0.30
δ-Guaiene	1506	1.57
Nerolidol	1547	0.39
Heptadecane	1602	0.34
Nonadecane	1669	0.57

5.7.4 Active constituents from *M. arvensis* essential oil using GC MS analysis

The GC-MS analysis of the oil of *M. arvensis* shows 40 constituents, including L-Menthone (31.41), Menthol (23.35%), Iso-menthone (7.98%), Eucalyptol (7.61%), Neo-Menthol (4.70), cis-Piperitone oxide (3.89) and α-Phellandrene (3.29) as the major constituents (table 5.35).

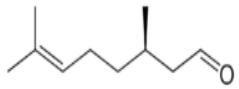
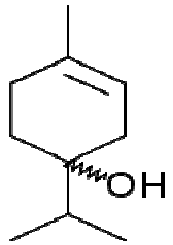
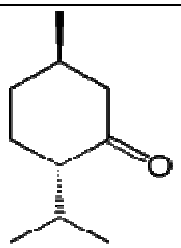
Table-5.35: Active constituents of *M. arvensis* essential oil

Compounds	**RI**	**Area (%)**
Neo-Menthol	1104	4.70
Eucalyptol	1123	7.61
α-Phellandrene	1156	3.29
L-Menthone	**1246**	**31.41**
Linalool	1287	2.21
Menthol	1309	23.35
Iso-menthone	1351	7.98
Trans-Anethole	1396	1.68
cis-Piperitone oxide	1421	3.89
4-Terpineol	1473	0.38
2-Acetylfuran	1521	1.35
α-Terpineol	1537	0.46
α-Pinene	1582	1.17
3-Octanol	1596	1.87
dl-Limonene	1641	1.49
2,5-Dimethyl-3-hexyne-2,5-diol	1678	0.68

5.8 Description of major compounds from bioactive essential oil

The identification of isolated compounds was made by comparing the retention indices of the peaks on the BP-1column with literature values as well as matching with the computer stored *spectra library*. The final confirmation was made by comparing the mass spectra with published data. Further, it was concluded that essential oil of *H. aromatica* contains **Linalool** as the major active compound; *E. citriodora* contains **Citronellal**; *O. americanum* contains **Terpinen-4-ol**; and *M. arvensis* contains **L-Menthone** as the major active compound (Table-5.36).

Table-5.36: General properties of the selected plants

S. No.	Bioactive essential oils	Code	Name of Major Compound	Composition (%)	Molar mass (g/mol)	Compound structure
1.	***H. aromatica***	Eo-1	Linalool	63.54	154.25	
2.	***E. citriodora***	Ca-12	Citronellal	83.10	154.13	
3.	***O. americanum***	Mp-9	Terpinen-4-ol	45.20	154.25	
4.	***M. arvensis***	Ob-10	L-Menthone	31.41	154.24	

6 Discussion

Since the NE States, including Mizoram; are inhabited by tribal populations, beside common health concerns, many people are suffering from skin diseases due to adverse climate, hard work, unhygienic conditions and poor livelihood options. In a study, it has also been estimated that skin diseases account for 34% of all occupational diseases (Spiewak, 2000). Infectious diseases, particularly skin and mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits (Ram *et al.,* (2004). An important group of these skin pathogens are the fungi, among which dermatophytes and *Candida* spp., besides certain pathogenic bacteria, are the most frequently contracted. [Desta B (1993)].

Medical facilities even for primary health care are not easily available especially for the rural and remote populations; several poor people still depend on vaidhayas even for the treatment of serious ailments. Many experiences based on field trips for documentation of traditional knowledge of primary health care system and ethnobotanical heritage of traditional practitioners/ Vaidhayas suggested that most of these people treat common diseases viz., Jwara (fever), Kas (Cough), Pratisyaya (cold) Ajirna (indigestion), Sirsula (headache), Naitra and Danta Rogas etc. using locally available plants/herbs. A few vaidhayas are also known to treat Amavat (Rheumatoid arthritis), Twak Roga (Skin diseases) and burns. Most of these self-styled practitioners did not have any formulations for the treatment of serious ailments. Besides, for skin related problems (especially ring worm infections) there are well known and equally effective herbal remedies which needed to be documented. Furthermore, proper identification, documentation and conservation of such ethno medicinally important plants can be done along with their scientific validation; especially exploration of plant secondary metabolites against the pathogens causing skin diseases in human beings (i.e. dermatophytes), their biochemical analysis is much needed, so that, after detailed investigations some bioactive constituents can be used for drug development, before they are lost forever.

Literatures revealed that the NE region of our country is well known for various forms of non-formal (traditional) medical treatments. The inhabitants of this region have gradually become familiar with the healing properties of the available plants. Several researches on ethno medicinal plants have also been made e.g., in the states of Assam [Hajra (1977); Hajra and Baishya (1981); Kumar (2002); Dutta and Dutta (2005); Sajem and Gosai (2006); Das *et al.,* (2008)]; Meghalaya [Borthakur (1976); Rao (1981); Chhetri *et al.,* (1992); Chetri (1994)]; Manipur [Devi (1990)]; Nagaland [Rao and Jamir (1982); Jamir (1997)]; Arunachal Pradesh [Tiwari (1980); Dam and Hajra (1981); Bhuyan (1989); Rao *et al.,*(2006)] and in Mizoram [Fischer (1938); Mahanti (1994); Singh (1996); Lalramnghinglova and Jha (2000); Sharma *et al.,* (2001); Lalramnghinglova (2003); Rai *et al.,* (2007); Lalfakzuala *et al.,* (2007); Rai and Lalramnghinglova (2010 a); Rai and Lalramnghinglova (2010 b); Kumar (2011)]. However, a consolidated account and analysis of the total use pattern with regard to skin ailment and traditional treatments by different communities is not available and therefore considered in the present study. Moreover, in the present investigation, an attempt has been made to analyze the traditional information available at the grass root level among the tribal communities of Mizoram, and their scientific validation against the pathogens causing ring worm infection/ skin diseases in human beings.

The findings of the present study have been discussed as follows:

6.1 Collection of plants traditionally used against skin diseases/ fungal diseases, and their identification

Literatures reveal that there are various methods for selection of plants for exploring 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, the ethnic groups are very distinct people rich in old- age traditions, cultures and customs, and continue to retain their respective dialects and ethnic identity. However, there are some groups viz., the Mizos, Maras, Pangs and Lais who are gradually losing their valuable indigenous knowledge of traditional medicines, particularly among the younger generations, where as the Chakmas, Brus, Bawms and the Mogs are ethno-bio-culturally rich in their folklore knowledge. In most cases, older people in the community have more knowledge in the field of ethnobotany and they preserve it traditionally. Therefore, in the present study, frequent ethno botanical surveys (Aug 2009- Jan 2011) were made in different localities, so that the informations up to the grass root level can be collected and validated scientifically (table 3.1).

During ethno botanical survey, information on the use of medicinal plants was obtained through structured and semi structured questionnaires, complemented by free interviews and informal conversations. Local practitioners and others with knowledge of plants were also consulted. Inquiries on the prevalence, types, transmission and symptoms of skin diseases along with traditional treatment methods, mode of application, doses etc were recorded properly, and plants were collected for investigations (table 5.1). The plants thus collected were categorized and the most frequently used plants were selected for identification. This was done 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 the School of Earth Sciences & Natural Resources Management, Mizoram University; State Medicinal Plants Board and State Forest Department, Aizawl. Their confirmations were, furthermore, made with the help of experts at the Department of Botany, University of Allahabad as well as 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 the present investigation (table 5.2).

6.2 Screening of Plants

As per the literatures, number of plants belonging to different families, genera and species have been screened against the pathogenic fungi, and 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); Mishra and Dixit (1979); 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).

In the present investigation, based on the literature survey as well as local field visits, twenty traditionally used 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. 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 Rajendra Prasad (2004). Shukla *et al.,* (2011) reported Plants from *Zingiberaceae* to be strong toxicant against *E. floccosum, M. gypseum* and *T. rubrum*.

In the present investigation, out of selected plant families viz., Araceae, Asteraceae, Caesalpiniaceae, Campanulaceae, Convolvulaceae, Dilleniaceae, Dipterocarpaceae, Lamiaceae, Lauraceae, Leguminosae, Loganiaceae, Moraceae, Myrtaceae, Papillionaceae, Saururaceae, Umbelliferae and Verbenaceae; the plants belonging to families Araceae, Lamiaceae and Myrtaceae show strong

antidermatophytic activity against the test pathogens (*E. floccosum, M. gypseum*, *T. mentagrophytes* and *T. rubrum)*.

6.2.2 Various workers observed the variations in the antifungal activity from genus to genus within a family. 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* exhibited 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. However, in another study; the bioactivity of Fabaceae family was reported by Iyenger *et al.,* (1995) and Mukherjee *et al.,* (1996). Antifungal activity of Poaceae family was reported by Pandey *et al.,* (1996); Pandey (1997); Pandey *et al.,* (1997); Shahi (1997); Shahi *et al.,* (1997a, 2002b); and bioactivity of Mrytaceae family was reported by Nenoff *et al.,* (1996); Shahi *et al.,* (1997b); Shahi *et al.,* (1998a); Ali *et al.,* (1999); Shahi *et al.,* (1999a), respectively. In the present investigation; Araceae (*Homalomena aromatica)*, Lamiaceae (*Ocimum americanum, Mentha arvensis*), Myrtaceae (*Eucalyptus citriodora)* and Dilleniaceae (*Dillenia indica*), too shows strong efficacy.

6.2.3 Findings of different studies revealed that all the parts of a particular plant may not always be active. To have a complete picture of distribution of antifungal principle in a plant, different parts such as leaves, stems, flowers and seeds etc. of each plant have been screened. Findings of; 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) shows that antifungal activity was observed using the expressed juices or aqueous extracts of the plants. 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 others 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. Sokovic *et al.,* (2009) used essential oils of *Thymus vulgaris, T. tosevii and Mentha piperita* and *M. spicata* as fungitoxicants and could safely be used as natural preservatives to replace synthetic fungicides in the prevention and cure of some plant, human and animal fungal diseases. In the present investigation, both the ethonolic extract as well as essential oil were investigated against the test pathogens.

6.2.4 Literature reveals that there are a number of methods for antifungal evaluation viz., 'Inverted petri plate method' (Böcher, 1938), 'Slide germination technique' as recommended by American Phytopathological Society (Anonymous, 1943); Modified paper disc technique (Sharvelle and Peletier, 1956); 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); of the plant constituents. In the present investigation, 'disc diffusion method' (a/k/a Kirby-Bauer Technique) as well as 'broth micro dilution method' (NCCLS, 2002) were used for antidermatophytic investigation of *Homalomena*

aromatica Schott., *Eucalyptus citriodora* Hook., *Ocimum americanum* Linn and *Mentha arvensis* Linn as well as 50 % ethanolic extract of *Dillenia indica* Linn.

6.3 *In-vitro* Antidermatophytic Investigations

Literature reveals that 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 both dermatophytic and non-dermatophytic moulds. Furthermore, 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. In this particular investigation, secondary metabolites (essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and ethanolic extract of *D.indica*) were used for detailed *in vitro* investigations against the dermatophytes- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum.* The selected plant secondary metabolites were investigated against the test fungi with special reference to minimum inhibitory concentration, IC_{50} value, nature of toxicity, killing time assay, effect of inoculum density, effect of temperature on toxicity, range of spectrum, comparison with some synthetic antifungals and the most efficacious plants were subjected for physicochemical characterization.

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 shows that very few workers have investigated the MICs of the essential oils, at different ppm, against dermatophytic fungi. 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 *Trichophyton* 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*.

6.3.1.1 In the present investigation, the essential oil of *H. aromatica* shows the minimum inhibitory concentration against *E. floccosum and* was recorded as 2.25 mg/ml and IC_{50} value was 0.98 mg/ml; against *M. gypseum* the MIC was 0.62 mg/ml and IC_{50} value was 0.31 mg/ml; against *T. mentagrophytes* the MIC was 0.20 mg/ml and IC_{50} value was 0.12 mg/ml, while, against *T. rubrum* the MIC was 0.23 mg/ml and the IC_{50} value was recorded as 0.19 mg/ml. (table- 5.4- 5.7; Fig- 5.1 and 5.2.).

6.3.1.2 *O. americanum* shows the minimum inhibitory concentration against *E. floccosum* and was recorded as 2.40 mg/ml and IC_{50} value was 1.22 mg/ml; against *M. gypseum* the MIC was 1.24 mg/ml and IC_{50} value was 0.68 mg/ml; against *T. mentagrophytes* the MIC was 1.56 mg/ml and IC_{50} value was 0.97 mg/ml, while,

against *T. rubrum* the MIC was 1.60 mg/ml and the IC_{50} value was recorded as 0.88 mg/ml (table- 5.8- 5.11; Fig- 5.3 and 5.4).

6.3.1.3 *M. arvensis* shows the minimum inhibitory concentration against *E. floccosum* and was recorded as 2.48 mg/ml and IC_{50} value was 1.26 mg/ml; against *M. gypseum* the MIC was 2.01 mg/ml and IC_{50} value was 0.98 mg/ml; against *T. mentagrophytes* the MIC was 1.83 mg/ml and IC_{50} value was 0.96 mg/ml, while, against *T. rubrum* the MIC was 1.76 mg/ml and the IC_{50} value was recorded as 0.90 mg/ml (table- 5.12- 5.15; Fig- 5.5 and 5.6).

6.3.1.4 *E. citriodora* shows the minimum inhibitory concentration against *E. floccosum* and was recorded as 1.60 mg/ml and IC_{50} value was 0.92 mg/ml; against *M. gypseum* the MIC was 1.40 mg/ml and IC_{50} value was 1.80 mg/ml; against *T. mentagrophytes* the MIC was 1.08 mg/ml and IC_{50} value was 0.64 mg/ml, while, against *T. rubrum* the MIC was 1.20 mg/ml and the IC_{50} value was recorded as 0.78 mg/ml (table- 5.16- 5.19; Fig- 5.7 and 5.8).

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 lowest activity against all tested dermatophytes (8-10 mm).

Antidermatophytic activity of the ethanolic extract of *D. indica* was not effective against *E. floccosum*; but it was effective against the rest of the three pathogens. The MIC ranges from 2.02 to 2.21 mg/ ml and the MFC was 2.5 mg/ ml; as well as IC_{50} values were recorded in between 0.83 to 1.14 mg/ ml, respectively (table- 5.20; Fig- 5.9 and 5.10).

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.,* 1983); *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 0.62 to 2.50 mg/ ml), against all the test pathogens- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum*, respectively (Table-5.4-5.20 & 5.29). Thus, the findings indicate 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 dermatophyte [MIC, IC_{50} and MFC (mg/ml)], an overview

SN	Samples	Antiderma-tophytic Parameters	Antidermatophytic activity of selected plant secondary metabolites against four dermatophytes				
			Essential oils				50 % ethanolic extract
			H. aromatica	*O. americanum*	*M. arvensis*	*E. citriodora*	*D. indica*
1.	*E. floccosum*	MIC	2.25	2.40	2.48	1.60	NA
		IC_{50}	0.98	1.22	1.26	0.92	NA
		MFC	2.50	3.32	3.82	2.80	NA
2.	*M. gypseum*	MIC	0.41	1.24	2.01	1.40	2.02
		IC_{50}	0.31	0.68	0.98	1.80	0.83
		MFC	0.62	2.50	3.60	2.40	2.50
3.	*T. mentagrophytes*	MIC	0.20	1.56	1.83	1.08	2.21
		IC_{50}	0.12	0.97	0.96	0.64	1.14
		MFC	0.62	2.50	2.50	1.60	2.50
4.	*T. rubrum*	MIC	0.23	1.60	1.76	1.20	2.11
		IC_{50}	0.19	0.88	0.90	0.78	0.89
		MFC	0.62	2.50	2.50	1.80	2.50

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 (1956), Inoculum potential is, "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 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.)

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 (culture matched with 0.5 McFarland contained 1x 10^3 CFU/ ml) against the respective MFCs of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* as well as 50 % ethanolic extract of *D. indica* was determined. The observation shows that the minimum fungicidal concentration (MFC) of the tested essential oils still presents with heavy inoculum density, with 100% growth inhibition against all the four pathogens (*E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum*). But in case of extract, the percent growth inhibition was only 25% in case of *M.g., T.r.* and *T.m.* Furthermore, it was not effective against *E. floccosum* (Table- 5.21).

6.3.4 Effect of Thermostability on toxicity of the essential oil

"A fungicide must retain its antifungal activity at the extremes of temperatures" (Wellman, 1967). The disease Tinea corporis occurs worldwide across regions and at various temperature ranges. Any antifungal agent developed against tinea corporis would be expected to have potentiality for temperature tolerance. state Dikshit, 1980; Dubey, 1981; Chandra, 1984; Pandey (1997); Shahi *et al.,* (1997b); Shahi 1997; Shahi *et al.,* (1998a, 1999b, 2002b) reported that the oil of *Cedrus deodara, Ocimum canum, Citrus medica, Cymbopogon pendulus, Eucalyptus* spp, *Trachyspermum ammi, Eucalyptus laveopenia, E. citriodora* and *C. flexuosus* did not lose its activity even upto 70^0C. Shukla *et al.,* (2011) reported the *Curcuma aromatica* oil's efficacy was thermo stable up to 100 ^{0}C for 36 months of storage.

The present investigation shows that oil of *H. aromatica, E. citriodora,* and *M. arvensis* did not lose their efficacy even upto 80^0C, the maximum temperature

taken into consideration. However, the efficacy of *O. americanum* was effective only up to 60^0C. Thus, it can be concluded from the present study that the oil based formulation will be effective with reference to temperature tolerance (Table-5.22).

6.3.5 Killing Time of the Essential oil/ Extract, against the Test Pathogens

Dikshit (1980) reported that the pure oil of *Cedrus deodara* killed *Helminthosporium oryzae* in 2 sec. Renu (1980) reported that the oil of *Cestrum diuranum* killed *Rhizoctonia solani* in 2 hours while that of *Aegle marmelos* in only 1 minute. Chaturvedi & al., (1987) reported the oil of *Andecalymma allicea* killed the fungal pests *Drecshlera oryzae* in 30 minute. Shukla (1998) reported the pure oil of *Cymbopogon flexuosus* killed *Aspergillus flavus* in 30 min and *Penicillium italicum* in 15 sec, while pure oil of *Trachyspermum ammi* killed *A. flavus* in 5 sec and *P. italicum* in 1 sec. Singh (2002) reported the oil of *Mentha spicata* killed *Alternaria alternata, Penicillium variable* and *Aspergillus flavus* in 2 hrs, 3hrs and 5hrs, respectively. Pandey (2008) reported the oil of *Citrus reticulata* killed *Aspergillus flavus*, *A. niger*, *Rhizopus arrhizus* and *Alternaria alternata* in 20 hrs, 20 hrs, 15 hrs and 25 hrs respectively, while, *Cedrus deodara* oil killed the same fungi in 25 hrs, 25 hrs, 10 hrs and 20 hrs respectively; but these investigations were made using the traditional mycelium disc killing methods.

In the present study, the killing time of the oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* as well as 50 % ethanolic extract of *D. indica*, against the test pathogens (dermatophytes) was determined using broth micro dilution method (NCCLS, 2002). The observation shows that, in case of the test fungi *E. floccosum;* the hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 12hrs, *O. americanum* and *M. arvensis* took 18 hrs, *E. citriodora* took 24 hrs to kill the test fungi *E. floccosum* while, pure oil killed the same just within 0.1 hr (i.e. 6 min). The extract of *D. indica,* though, was not effective against *E.floccosum* (table- 5.23)

Similarly, in the case of the test fungi ***M. gypseum****; the* hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 6 hrs, *O. americanum* took 12 hrs, *E. citriodora* took 18 hrs and *M. arvensis* took 24 hrs to kill the test fungi *M. gypseum* while, the extract of *D.*

indica took 30 hrs. On the other hand, pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 18 hrs to kill the same, respectively (table- 5.24).

In the case of ***T. mentagrophytes****;* it was recorded that the hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 6 hrs, *E. citriodora* took 12 hrs, *O. americanum* and *M. arvensis* took 18 hrs to kill the test fungi *T. mentagrophytes* while, the extract of *D. indica* took 30 hrs but pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 24 hrs to kill the same, respectively (table- 5.25).

In ***T. rubrum***; it was observed that the hyper lethal conc (i.e. 2MIC) of *H. aromatica* and *E. citriodora* took 6 hrs while, *O. americanum* and *M. arvensis* took 12 hrs to kill the test fungi but in case of *D. indica* extract, it was recorded 24 hrs. Pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 24 hrs to kill the pathogen *T. rubrum* (table- 5.26).

6.3.6 Antifungal Spectrum

More than one genera of fungi imperfecti group viz; *Epidermophyton*, *Microsporum,* and *Trichophyton* are responsible for Tinea corporis. Thus, for an effective antifungal product development, product needs to be effective against a number of fungal pathogens. Antifungal screening of bioactive chemicals are mainly focused on single or few related group of pathogens. However, substances with broad spectrum antifungal activity show greater promise because of a more widespread adaptation and a potentially larger market value (Kendrick and Zentymer 1957). Some of the oils have shown broad antifungal spectrum, while the others show specific activity. The oils of *Curcuma aromatica* (Rao 1976), *C. angustifolia* (Banerjee and Nigam 1977), *Artemesia absinthium, A. vulgaris, A. vestita* (Kaul *et al.,* 1976), *Ageratum conyzoides* (Sharma *et al.,* 1978), *Cedrus deodara* (Dikshit 1980), *Ocimum canum* and *Citrus medica* (Dubey 1981), *Caesulia axillaris* (Pandey *et al.,* 1982), *Pepromia pellucida* (Singh *et al.,* 1983), *Iberis amara* (Tripathi *et al.,* 1983), *Ocimum adscendens* (Asthana 1984), *Nepeta hindostana* and *Vitex negundo* (Mishra 1991), *Cymbopogon pendulus* (Pandey *et al.,* 1996), *Eucalyptus* spp. (Shahi *et al.,* 1997b), *Cymbopogon flexuosus* and

Trachyspermum ammi (Shahi 1997), Sokovic *et al*.,(2009), exhibited broad antifungal spectrum. However, the essential oils of *Raphanus* sp. (Nahrash 1961), *Juniper* sp. (Salvenas and Razinakaite 1962), *Psoralea corylifolia* (Grover and Rao 1979) and *Feronia elephantum* (Sharma *et al.,* 1978) were found to be specific in nature showing inhibition against one or two fungi only. Shukla *et al.,* (2011) showed oil of *Curcuma aromatica* exhibited broad antifungal spectrum.

In the present study, the oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* exhibited broad fungicidal spectrum inhibiting *Microsporum auddouinii, M. canis, M. nanum* and *T. tonsurans, T. violaceum* completely. This indicates its larger potentiality in the market after successful clinical trials, as therapeutic medication for the control of Dermatophytosis (Table- 5.27).

6.3.7 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 5.28).

Table- 6.2: Comparison of the test samples with some Synthetic Antifungal Drugs

Oil & Trade Name of Antifungal Drugs	**Ingredients**	**Minimum Inhibitory Concentration (mg/ml)**			
		E. floccosum	*M. gypseum*	*T. mentagrophytes*	*T. rubrum*
H. aromatica	E.O.	2.25	0.41	0.20	0.23
E. citriodora	E.O.	1.60	1.40	1.08	1.20
O. americanum	E.O.	2.40	1.24	1.56	1.60
M. arvensis	E.O.	2.48	2.01	1.83	1.76
D. indica	Extract	NA	2.02	2.21	2.11
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 show that the MIC of ***Homalomena aromatica*** have the strongest toxicity against all the test pathogens (ranges from 0.62-2.50 mg/ml) followed by ***Eucalyptus citriodora*** (ranges from 0.64-2.82 mg/ml); which also shows an edge over the synthetic antifungal (Dactrine, Nizaral and Tenaderm), where the efficacy ranges from 0.5- 6.0 mg/ml.

6.3.8 Physico-chemical properties of selected essential oils

The selected essential oils were subjected for various physio-chemical characterization viz., plant height, appearance, oil yield, odour, boiling point, specific gravity, optical rotation, refractive index and solubility in water. The observations thus recorded are given in table-5.30.

6.3.9 Solubility in various organic solvents

The solubility of all selected secondary metabolites in various organic solvents was also investigated and it was found that these were soluble in 13 different organic solvents viz., acetone, alcohols, benzene, chloroform, carbon tetrachloride, dimethyl sulphoxide (DMSO), ethanol, hexane, methanol, n-butanol, petroleum ether, propanol and solvent ether (Table- 5.31).

6.3.10 Description of major compounds from bioactive essential oil

The identification of isolated compounds was made by comparing the retention indices of the peaks on the BP-1column with literature values as well as matching with the computer stored *spectra library*. The final confirmation was made by comparing the mass spectra with published data. Further, it was concluded that essential oil of *H. aromatica* contains **Linalool** as the major active compound; *E. citriodora* contains **Citronellal**; *O. americanum* contains **Terpinen-4-ol**; and *M. arvensis* contains **L-Menthone** as the major active compound (Table-5.32 to 5.36).

Therefore, based on these findings, it was observed that the tested plant metabolite(s) contains not only strong anti-dermatophytic activity, heavy inoculum density, quick killing efficacy, thermo-stability, and broad antimicrobial spectrum but

they (essential oils) have also an edge over the synthetic antifungal drugs, with eco-friendly in nature.

Further, the findings of the present investigation, can also be used as a base line for further *in- vivo* investigations, pre clinical trials, multilocational clinical trials, and the most efficacious bioactive molecule(s) can be made available to the pharmaceutical companies for drug formulations and commercial production as well as for welfare of the society/ tribal communities with proper documentation and conservation of the traditional information, before they are lost forever.

7 Abstract

The present study entitled *'Investigation of Anti-dermatophytic Activity from selected Medicinal Plants of Mizoram'* was carried out during January 2009- December 2012, at the Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl. However, some part of the *in vitro* investigation was carried out at the Biological Product Laboratory, Department of Botany, University of Allahabad, with proper permission.

The findings of the present study are summarizing under the following heads:

- Collection of plants traditionally used against skin diseases/ fungal diseases, and their identification.
- Extraction of the plant secondary metabolites and their antidermatophytic screening.
- Selection of 4-5 most potent plants for detail *in vitro* investigations against the common dermatophytes causing ring worm infection in human beings.
- Physico-chemical characterization of the most efficacious plant metabolite(s).

7.1 The Study area

Mizoram is a beautiful state and is known as the "Scotland of India". It 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.

7.2 Ethno botanical survey

During ethno botanical survey, information on the use of medicinal plants was obtained through structured and semi structured questionnaires, complemented by free interviews and informal conversations. Local practitioners and others with knowledge of plants were also consulted. Inquiries on the prevalence, types, transmission and symptoms of skin diseases along with traditional treatment methods, mode of application, doses etc were recorded properly, and the plants were collected for investigations (table 5.1).

The plants thus collected were categorized and the most frequently used plants were selected for identification. This was done 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 the School of Earth Sciences & Natural Resources Management, Mizoram University; State Medicinal Plants Board and State Forest Department, Aizawl and their confirmations were made with the help of experts at the Department of Botany, University of Allahabad as well as 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 the present investigation (table 5.2).

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

In the present investigation, 20 most frequent ethno-medicinal plants were selected for extraction of the essential oil/ solvent extract, from different parts of the plant, as listed in table 5.2. Clevenger′s apparatus (Clevenger 1928), was used for extraction of the essential oil, however, different solvent (viz., alcohol, acetone, benzene, chloroform, hexane etc), were used for the extract.

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

The antimicrobial screening of these essential oils/ extracts (test sample) was carried out using disc diffusion method. The stock solution of each test samples (at 50mg/ml conc) were prepared separately and subjected against the dermatophytic pathogens *E. floccosum* (MTCC-7880), *M. gypseum* (MTCC-2830), *T. mentagrophytes* (MTCC-8476) and *T. rubrum* (MTCC-3272). The observations were recorded in Table-5.2.

Out of 15 plants; 4 essential oil bearing plants viz., *Homalomena aromatica, Eucalyptus citriodora, Ocimum americanum* and *Mentha arvensis* as well as 50 % ethanolic extract of *Dillenia indica* were recorded more effective against all the test pathogens. The maximum inhibition zone was recorded in *H. aromatica* essential oil (29 mm); followed by *E. citriodora* essential oil (27 mm), *O. americanum* essential oil (23 mm), *M. arvensis* (18 mm) as well as the ethanolic extract of *D.indica* (10 mm).

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

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

7.5.1 Antidermatophytic activity of *H. aromatica* oil against *Ef, Mg, Tm* and *Tr*

The efficacy of the oil of *H. aromatica* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* were recorded in table-5.4- 5.7 as well as Fig- 5.1 and 5.2.

7.5.1.1 Based on the observations recorded in table-5.4 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the oil of *H. aromatica* against *E. floccosum* was recorded as 2.25 mg/ml but it was fungicidal at 2.5 mg/ml; the IC_{50} value was recorded as 0.98 mg/ml.

7.5.1.2 Based on the observations recorded in table-5.5 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the oil of *H. aromatica* against *M. gypseum* was

recorded as 0.41 mg/ml but it was fungicidal at 0.62 mg/ ml; the IC_{50} value was recorded as 0.31 mg/ml.

7.5.1.3 Based on the observations recorded in table-5.6 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the oil of *H. aromatica* against *T. mentagrophytes* was recorded as 0.20 mg/ml but it was fungicidal at 0.62 mg/ml; the IC_{50} value was recorded as 0.12 mg/ml.

7.5.1.4 Based on the observations recorded in table-5.7 as well as fig-5.1 and 5.2 the minimum inhibitory concentration of the essential oil of *H. aromatica* against *T. rubrum* was recorded as 0.23 mg/ml but it was fungicidal at 0.62 mg/ml; the IC_{50} value was recorded as 0.19 mg/ml.

7.5.2 Antidermatophytic activity of *O. americanum* oil against *Ef, Mg, Tm* and *Tr*

The efficacy of the oil of *O. americanum* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table- 5.8- 5.11 as well as Fig- 5.3 and 5.4.

7.5.2.1 Based on the observations recorded in table- 5.8 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the oil of *O. americanum* against *E. floccosum* was recorded as 2.40 mg/ml but it was fungicidal at 3.32 mg/ml; the IC_{50} value was recorded as 1.22 mg/ml.

7.5.2.2 Based on the observations recorded in table- 5.9 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the oil of *O. americanum* against *M. gypseum* was recorded as 1.24 mg/ml but it was fungicidal at 2.50 mg/ ml; the IC_{50} value was recorded as 0.68 mg/ml.

7.5.2.3 Based on the observations recorded in table- 5.10 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the oil of *O. americanum* against *T. mentagrophytes* was recorded as 1.56 mg/ml but it was fungicidal at 2.50 mg/ml; the IC_{50} value was recorded as 0.97.

7.5.2.4 Based on the observations recorded in table- 5.11 as well as Fig- 5.3 and 5.4 the minimum inhibitory concentration of the essential oil of *O. americanum* against *T.*

rubrum was recorded as 1.60 mg/ml but it was fungicidal at 2.50 mg/ml; the IC_{50} value was recorded as 0.88 mg/ml.

7.5.3 Antidermatophytic activity of *M. arvensis* oil against *Ef, Mg, Tm* and *Tr*

The efficacy of essential oil of *M. arvensis* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table- 5.12- 5.15 as well as Fig- 5.5 and 5.6.

7.5.3.1 Based on the observations recorded in table- 5.12 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of ***M. arvensis*** against *E. floccosum* was recorded as 2.48 mg/ml but it was fungicidal at 3.82 mg/ ml; the IC_{50} value was recorded as 1.26 mg/ml.

7.5.3.2 Based on the observations recorded in table- 5.13 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of ***M. arvensis*** against *M. gypseum* was recorded as 2.01 mg/ml but it was fungicidal at 3.60 mg/ ml; the IC_{50} value was recorded as 0.98 mg/ml.

7.5.3.3 Based on the observations recorded in table- 5.14 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of ***M. arvensis*** against *T. mentagrophytes* was recorded as 1.83 mg/ml but it was fungicidal at 2.50 mg/ ml; the IC_{50} value was recorded as 0.96 mg/ml.

7.5.3.4 Based on the observations recorded in table- 5.15 as well as Fig- 5.5 and 5.6 the minimum inhibitory concentration of the oil of ***M. arvensis*** against *T. rubrum* was recorded as 1.76 mg/ml but it was fungicidal at 2.50 mg/ ml; the IC_{50} value was recorded as 0.90 mg/ml.

7.5.4 Antidermatophytic activities *E. citriodora* essential oil against *Ef, Mg, Tm* & *Tr*

The efficacy of essential oil of *E. citriodora* against the test pathogens *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table- 5.16- 5.19 as well as Fig- 5.7 and 5.8.

7.5.4.1 Based on the observations recorded in table- 5.16 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of ***E. citriodora*** against *E. floccosum* was

recorded as 1.60 mg/ml but it was fungicidal at 2.80 mg/ ml; the IC_{50} value was recorded as 0.92 mg/ ml.

7.5.4.1 Based on the observations recorded in table- 5.17 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of ***E. citriodora*** against *M. gypseum* was recorded as 1.40 mg/ml but it was fungicidal at 2.40 mg/ ml; the IC_{50} value was recorded as 1.80 mg/ ml.

7.5.4.2 Based on the observations recorded in table- 5.18 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of ***E. citriodora*** against *T. mentagrophytes* was recorded as 1.08 mg/ml but it was fungicidal at 1.60 mg/ ml; the IC_{50} value was recorded as 0.64 mg/ ml.

7.5.4.2 Based on the observations recorded in table- 5.19 as well as Fig- 5.7 and 5.8 the minimum inhibitory concentration of the oil of ***E. citriodora*** against *T. rubrum* was recorded as 1.20 mg/ml but it was fungicidal at 1.80 mg/ ml; the IC_{50} value was recorded as 0.78 mg/ ml.

7.5.5 Antidermatophytic activity of 50% ethanolic leaf extract of *D. indica* against *Ef, Mg, Tm* and *Tr*

The efficacy of 50% ethanolic leaf extract of *D. indica* against the dermatophytes *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum;* recorded are shown in table- 5.20 as well as Fig- 5.9 and 5.10.

As per the observations recorded in table-5.20 as well as fig-5.9 and 5.10; the ethanolic extract of *D. indica* was not effective against *E. floccosum*; however, it was effective against the rest three pathogens. The MIC was ranges from 2.02 to 2.21 mg/ ml and the MFC was 2.5 mg/ ml and IC_{50} values were recorded in between 0.83 to 1.14 mg/ ml, respectively.

7.5.6 Inoculum Density vis-v-vis Fungicidal Activity

The efficacy of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* as well as 50 % ethanolic extract of *D. indica*, on inoculum density of the test pathogens- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum;* was also

determined. This was made using the protocols recommended by NCCLS-2002. The observation shows that the minimum fungicidal concentration (MFC) of the tested essential oils persisted heavy inoculum density, with 100% growth inhibition against all the four pathogens (*E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum*). But in case of extract, the percent growth inhibition was only 25% in case of *M.g., T.r.* and *T.m.* It was not effective against *E. floccosum* (Table- 5.21).

7.5.7 Effect of temperature on the efficacy of oil/ extract

Effect of temperature on the efficacy of oil *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, was also determined. Samples of oil in small vials containing 1ml each, were exposed at 40, 60 and 80^0C in hot water bath, separately. Further, the oil's efficacy was tested against the test fungi- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum* at their respective minimum fungicidal concentration (MFCs). This was made using the protocols of NCCLS-2002, and the observations were recorded in table-5.22.

7.5.8 Killing Time of the Essential oil/ Extract, against the Test Pathogens

The minimum killing time (MKT) of the pure oil, 2MIC (i.e. hyper lethal concentration) and MICs of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica;* against the dermatophytes- *E. floccosum, M. gypseum, T. mentagrophytes* and *T. rubrum* was also determined. The observations were recorded, pathogenwise (table-5.23 to 5.26), separately.

7.5.8.1 The observations recorded in table- 5.23 shows that in case of test fungi ***E. floccosum****;* the hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 12hrs, *O. americanum* and *M. arvensis* took 18 hrs, *E. citriodora* took 24 hrs to kill the test fungi *E. floccosum* while pure oil killed the same within just 0.1 hr (i.e. 6 min). However, the extract of *D. indica* was not effective against *E.floccosum* (table- 5.23).

7.5.8.2 Similarly, in case of the test fungi ***M. gypseum****; the* hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 6.0 hrs, *O. americanum* took 12 hrs, *E. citriodora* took 18 hrs and

M. arvensis took 24 hrs to kill the test fungi *M. gypseum* while the extract of *D. indica* took 30 hrs. Pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 18 hrs to kill the same, respectively (table- 5.24).

7.5.8.3 Further, in case of ***T. mentagrophytes****;* it was recorded that the hyper lethal conc (i.e. 2MIC) of *H. aromatica* took 6.0 hrs, *E. citriodora* took 12 hrs, *O. americanum* and *M. arvensis* took 18 hrs to kill the test fungi *T. mentagrophytes* while the extract of *D. indica* took 30 hrs. Pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 24 hrs to kill the same, respectively (table- 5.25).

7.5.8.4 Similarly, in case of ***T. rubrum***; it was observed that the hyper lethal conc (i.e. 2MIC) of *H. aromatica* and *E. citriodora* took 06 hrs while, *O. americanum* and *M. arvensis* took 12 hrs to kill the test fungi but in case of *D. indica* extract, it was recorded at 24 hrs. Pure oil took only 0.1 hr (i.e. 6 min), and pure extract took 24 hrs to kill the pathogen *T. rubrum* (table- 5.26).

7.5.9 Fungi-toxic Spectrum of the Essential oil/ Extract

The range of spectrum of the oil, *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica*, against some other human pathogenic fungi, available in the lab, viz., *Microsporum auddouinii, M. canis, M. nanum, T. tonsurans* and *T. violaceum* was also determined. This was done as per the protocols of NCCLS 2002, and the observations were recorded in table-5.27.

7.5.9.1 As per the observation recorded in the table- 5.27; it was observed that *H. aromatica* and *E. citriodora* shows fungicidal efficacy against all the test pathogens, *O. americanum* shows fungicidal efficacy against *Microsporum canis*, *M. nanum* and *T. violaceum* and *M. arvensis* shows fungicidal efficacy against *Microsporum canis* and *T. violaceum.* On the other hand, the extract of *D. indica* shows fungicidal efficacy against *Microsporum nanum* only.

7.5.10 Comparison with some Synthetic Fungicides/ Antifungal drugs

The efficacy of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* and 50 % ethanolic extract of *D. indica* was also compared with some

synthetic antifungal drugs available in the market viz., Dactrine; Nizaral and Tenderm. The observations were recorded by comparing their minimum inhibitory concentrations (Table 5.28).

7.5.10.1 According to the observation recorded in table 5.28; the MIC of *H. aromatica* have the strongest toxicity against all the test pathogens (ranges from 2.25-0.20 mg/ml) followed by *E. citriodora* (ranges from 1.60-1.08 mg/ml); which shows an edge over the synthetic antifungal (Dactrine, Nizaral, Tenaderm) & the efficacy ranges from 0.5- 6.0 mg/ml.

7.5.11 Fungicidal activity of the selected plants: an overview

The comparative analysis of the essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* as well as 50 % ethanolic extract of *D. indica*, against the test pathogerns viz., *E. floccosum; M. gypseum; T. mentagrophytes* and *T. rubrum* was determined and recorded in table-5.29. The same can also be had as follows (table- 7.1):

Table: 7.1 Antidermatophytic activities of selected plant secondary metabolites against four dermatophytes [MIC, IC_{50} and MFC (mg/ml)]

SN	Samples	Antiderma-tophytic Parameters	Antidermatophytic activity of selected plant secondary metabolites against four dermatophytes				
			Essential oils				50 % ethanolic extract
			H. aromatica	***O. americanum***	***M. arvensis***	***E. citriodora***	***D. indica***
1.	***E. floccosum***	MIC	2.25	2.40	2.48	1.60	NA
		IC_{50}	0.98	1.22	1.26	0.92	NA
		MFC	2.50	3.32	3.82	2.80	NA
2.	***M. gypseum***	MIC	0.41	1.24	2.01	1.40	2.02
		IC_{50}	0.31	0.68	0.98	1.80	0.83
		MFC	0.62	2.50	3.60	2.40	2.50
3.	***T. mentagrophytes***	MIC	0.20	1.56	1.83	1.08	2.21
		IC_{50}	0.12	0.97	0.96	0.64	1.14
		MFC	0.62	2.50	2.50	1.60	2.50
4.	***T. rubrum***	MIC	0.23	1.60	1.76	1.20	2.11
		IC_{50}	0.19	0.88	0.90	0.78	0.89
		MFC	0.62	2.50	2.50	1.80	2.50

7.6 Physico-chemical characterization of the most efficacious plant metabolite(s)

In accordance to the detailed *in vitro* investigations, the essential oils of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* were recorded as the most efficacious plant metabolites; and hence, were subjected for various physio-chemical characterization viz., plant height, appearance, oil yield, odor, boiling point, specific gravity, optical rotation, refractive index and solubility in water. The observations thus recorded are compiled in table-5.30.

7.7 Solubility in various organic solvents

The solubility of the selected plant secondary metabolites in various organic solvents was also investigated and it was found that the test samples (essential oil of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* as well as 50 % ethanolic extract of *D. indica* oil) were soluble in 13 different organic solvents viz., acetone, alcohols, benzene, chloroform, carbon tetrachloride, dimethyl sulphoxide (DMSO), ethanol, hexane, methanol, n-butanol, petroleum ether, propanol, solvent (Table- 5.31).

7.8 Identification of the active constituents from selected plant essential oils

The essential oils of *H. aromatica, E. citriodora, O. americanum* and *M. arvensis* were subjected to identification of their active constituents, and their observations were recorded in table 5.32 to 5.36.

7.8.1 Active constituents from *H. aromatica* essential oil using GC MS analysis

The GC-MS analysis of the oil of *H. aromatica* shows Linalool (63.54%), Terpinen-4-o1 (15.97), α-terpineol (2.89), Cryptone (2.45), Spathulenol (1.88), α-cardinol (1.78), and *epi*- α-cardinol (1.60), Humulene epoxide II (1.42) and Oplopanone (1.09) as important constituents (table 5.32).

7.8.2 Active constituents from *E. citriodora* essential oil using GC MS analysis

The GC-MS analysis of the essential oil of ***E. citriodora*** shows; Citronellal (83.10%), Citronellol (7.21%), Linalool (2.46%) and Isopulegol (1.92%) as the major constituents (table 5.33).

7.8.3 Active constituents from *O. americanum* essential oil using GC MS analysis

The essential oil of *O. americanum* contains Terpinen-4-ol (45.20%), 1, 8-cineole (18.10%), α-terpineol (4.32%), Trans-Caryophyllene (3.66), Trans-α-Bergamotene (2.79), γ-Terpinene (2.60), cis-Sabinene hydrate (1.95), δ-Guaiene (1.57), trans-Sabinene hydrate (1.49) and l-Linalool (1.40) as the major constituents (table 5.34).

7.8.4 Active constituents from *M. arvensis* essential oil using GC MS analysis

The GC-MS analysis of the oil of *M. arvensis* shows 40 constituents, including L-Menthone (31.41), Menthol (23.35%), Iso-menthone (7.98%), Eucalyptol (7.61%), Neo-Menthol (4.70), cis-Piperitone oxide (3.89) and α-Phellandrene (3.29) as the major constituents (table 5.35).

Therefore, based on these findings, it can be concluded that the traditionally used plants of the present investigation have been scientifically validated as natural antifungal. The tested plant metabolites contains not only the strong anti-dermatophytic potency, heavy inoculum density, quick killing activity, thermo-stability, and broad antimicrobial spectrum but the essential oils can also be as an alternative to the synthetic antifungal drugs, with eco-friendly in nature.

Further, the findings of the present investigation, can also be used as a base line for further *in- vivo* investigations, pre clinical trials, multilocational clinical trials, and the most efficacious bioactive molecule(s) can be made available to the pharmaceutical companies for drug formulations and commercial production as well as for welfare of the society/ tribal communities with proper documentation and conservation of the traditional information, before they are lost forever.

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