EFFICACY OF SELECTED HERBS AGAINST DANDRUFF

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CERTIFICATE

This is to certify that the dissertation entitled **"EFFICACY OF SELECTED HERBS AGAINST DANDRUFF"** submitted to the Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore, in partial fulfilment of the requirements for the award of the Degree of **MASTER OF PHILOSOPHY IN LIFE SCIENCES** is a record of original research work done by **D. MALARVIZHI** during the period of her study in the Department of Life Sciences, Avinashilingam Institute for Home Science and Higher Education for Women Deemed University, Coimbatore, under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree / Diploma / Associateship / Fellowship or other similar title to any candidate of any University and it represents entirely an independent work on the part of the candidate.

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19.8.04

Signature of the Guide

Sarojini Sukumar
19.8.04.

Forwarded

Signature of the Head of the Department

DECLARATION

I hereby declare that the dissertation entitled **"EFFICACY OF SELECTED HERBS AGAINST DANDRUFF"** submitted to the Avinashilingam Institute for Home Science and Higher Education for Women, Deemed University, Coimbatore, in partial fulfilment of the requirements for the award of the degree of **MASTER OF PHILOSOPHY IN LIFE SCIENCES** is a record of original supervision and guidance of Dr. (Tmt.) **S. PARVATHI, M.Sc., Dip.Ed., (Madras), M.Phil, (Bharathiar), Ph.D., (Avinashilingam Deemed University)**, Department of Life Sciences and that it has not formed the basis for the Award of any Degree / Diploma / Associateship / Fellowship or other similar title of any candidate of any University.

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Signature of the Candidate

S. Parvathi 19.8.04

Signature of the Guide

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ACKNOWLEDGEMENT

The Lord is my shepherd : I have everything I need
He lets me rest in fields of green grass and
Leeds me to quiet pools of fresh water"

- Holy Bible

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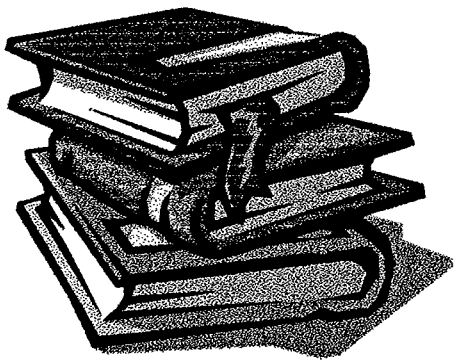

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INTRODUCTION

I INTRODUCTION

India is blessed with incredible biological diversity. It is not only counted among the 12 mega gene centres of the world, but the Eastern Himalayas and the Western Ghats which are 2 of the 25 global 'Hot spots' of biodiversity are located here. India is also placed 10th among the plant rich nations of the world and 4th among the Asian countries.

The World Health Organisation (WHO) has estimated that 90 per cent of the people in the world rely on traditional medicine for primary health care needs (Sinha, 1996). Many of the medicines in the present generation are based on herbal remedies used by natives. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developing world. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as plant metabolites in one or more parts of these plants.

Plant kingdom is an everlasting reservoir of wonderful molecules with varying biological activities. All traditional systems of medicine show that plants have provided mankind of large variety of patent drugs to alleviate suffering from diseases.

To protect and sustain the existing treasures of herbs, Herbal Bio-Med Foundation (HBMF) has been formed by the intellectual society of India, registered under the societies Registration Act of 1860. About 30 per cent of ingredients of all allopathic medicines and 100 percent of Ayurvedic, Unani and homeopathy medicine come from the plants (Aziz and Shivchandran, 2000).

Use of herbal medicines can be traced back as far as 2100 B.C. in ancient China and India. Since the Vedic period, the first written reports date back to 600 B.C. and therefore, it is necessary to ensure that Ayurveda plays a major role in our health administration.

Around one million traditional medicinal professionals are serving the community with the herbal medicine (Swaminathan, 2000). The herbal drugs are popular for their safety, efficacy, cultural acceptability and less side effects. These drugs also cure age related diseases.

About two hundred and ninety three plant species were screened on a variety of biological activities including anticancer, chemotherapeutic and pharmacological activities (Turner, 1965). Chemical investigation and biological screening of about 300 wild tribal medicinal plants have been carried out by many researchers (AICRPE, 1993).

It is interesting and relevant to note that 80% of Chinese populations get remedy for diseases from plants. Ayurvedic literature describes about 2000 medicinal plants. Siddha literature describes about 300 medicinal plants of which, few are used for the treatment of superficial mycoses (Rajarajan, 2003). The future of natural product's drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner.

Recently, many international authorities and agencies, including the World Health Organisation, European Agency for the Evaluation of Medicinal products and European Scientific Cooperation of phytomedicine, US Agency for Health Care Policy and Research, European pharmacopoeia commission

and Department of Indian system of Medicine have started creating new mechanisms to induce and regulate quality control and standardization of herbal medicine.

It is estimated that world market for plant derived drug may account for about Rs.2,00,000 crores. Presently, Indian contribution is around Rs.2300 crores. Indian export of raw drugs has steadily grown at 26% to Rs.165 crores in 1991-92. The trade of medicinal plants in India is estimated to be about Rs.550 crores/annum. It is predicted that India is growing to be one of the world's largest economy in the next millennium.

Identification of action of a particular compound against a specific disease is a challenging and long process with generally two approaches, first, selecting a disease and testing several possible plant extracts and compounds that could inhibit enzymes associated with that disease. Second, by selecting an ethnobotanically identified plant and screen extracts (or) compounds from these plants in a range of assays.

There are two types of plant chemicals, one is the primary constituents such as common sugars, carbohydrates, proteins, amino acids and chlorophylls which are universally present in all kinds of plants, whether medicinal (or) non-medicinal. Second is the other type of chemicals which make up all the remaining plant constituents such as alkaloids, terpenoids, phenolics, agetogenin etc, and which do not have essential role in plant metabolism and vary in their distribution from plant to plant.

Many medicinal plants are used in Dandruff such as, D*atura stramonium* L., *Cynodon dactylon* pers., *Phyllanthus emblica* Linn., *Indigofera tinctoria* Linn., *Aristolochia bracteata* Retz. *Citrus medica* Linn. (Rutaceae),

Aloe vera Bur F. *Hibiscus Rosa-sinensis* Linn., *Andrographis paniculata* L., *Eclipta alba* Hassle., *Santalum album* Linn *Urtica dioica* L., and *Wrightia tinctoria*. R.Br.

In the present investigation, an effort was made to find out the effect of the selected two herbal plants namely *Lippia nodiflora* Rich. (Verbenaceae) and *Thea sinensis* Linn. (Theaceae) in curing the Dandruff. (Plate 1, 2,3 and 4)

LIPPIA NODIFLORA

Lippia nodiflora belongs to the family verbenaceae. Juice of the leaves, fruits mixed with pepper and oil are dried in the sun till the water evaporates. Thus, oil if rubbed, on the head before having a bath, it removes Dandruff. It possess a very strong antibacterial and antifungal capacity (Nadkarni, 1954).

THEA SINENIS Linn.

Thea sinensis belongs to the family Theaceae. Tea leaves oil has antifungal activity and, thus may be useful in the treatment of Dandruff (David, 2003).

PLATE -1

Lippia nodiflora **Rich.**

PLATE -2

Thea Sinensis **Linn.**

PLATE -3

Habit of Thea Sinensis **Linn.**

PLATE -4

Inflorescence of Thea Sinensis **Linn.**

PLANTS MORPHOLOGY

S.No.	CHARACTERS	*LIPPIA NODIFLORA*	*THEA SINENSIS*
1.	Total genera and species	75 genera and 3000 species.	18 genera and 550 species.
2.	Distribution	Throughout India, Ceylon, Africa, tropics and subtropics.	Tropics and subtropics.
3.	Leaves	Simple, opposite, decussate, subsessile, obovate, spathulate, serrate towards the apex.	Simple, alternate, leathery and evergreen, exstipulate.
4.	Inflorescence	A condensed terminal spike.	Solitary.
5.	Flower	Sessile, bracteate, ebracteolate, cyclic, complete, pentamerous, hetero chlamydeous, zygomorphic, bisexual and hypogynous.	Bracteate (few small bracts). complete, regular, bisexual and hypogynous.
6.	Calyx and Corolla	Sepal 5, small, fused, membranous, deeply bipartite Valvate aestivation. petals 5 Gamopetalous, bilabiate upper lip 2 lobed rounded, lower lip 3 lobed with the middle lobe being large, Imbricate aestivation.	Sepals 5-7, free persistent sepal, imbricate aestivation. Petals (4-) 5 (-9 (or) more), free (or) basically connate, Imbricate aestivation.
7.	Androecium	Stamens 4, free, altemipetalous and epipetalous, didynamous, filaments short, anthers dithecous and introrse.	Stamens numerous, rarely 5-10-15, free (or) in bundles (or) united into a tube.
8.	Gynoecium	Ovary superior, bicarpellary, syncarpous, bilocular with one ovule in each locule on axile placentation, ovules anatropous to orthotropous.	Ovary superior, carpels 2-10, syncarpous, axile placentation, anatropous ovule.
9.	Fruit	Drupe	Capsule (or) dry drupe
10.	Seed	Without endosperm	Without endosperm
11.	Floral formula	$\uparrow\!\varphi$,$K_{(5)}$, $C_{(5)}$, A_4, $\underline{G}_{(2)}$	$\uparrow\!\varphi$,$K_{(5)}$, $C_{(5)}$, $A\infty$, $\underline{G}_{(3)}$

SKIN DISEASES:

The skin is an extremely complicated organism, consisting of epidermis with the hair and nails, the papillary and reticular layers of the cutis, the subcutaneous connective tissue and the sebaceous and sudoriferous glands. It has three important functions to perform-1. sensation,2. secretion and 3. Excretion. It also protects internal organs from the effects of various morbid processes to which they are liable. The important skin diseases are Psoriasis, Dandruff, Tubercles, Tinea, Eczema, Pigmentary diseases, Purpura, Scabies, Scleroderma, Boils and Carbuncles, Psoriatic arthritis etc (Rustonjee, 2004).

The investigator has carried out a study on treatment of Dandruff through herbal medicines.

DANDRUFF:

It is a kind of Dermatitis, mostly found on the scalp. A typical thin mica like, scaling on scratching with acute burning and itching sensation will be noticed. Scurf (or) Dandruff is a desquamation from the surface of the skin, where plates of detached epidermis come out. The affected part is called squamae. Scurf consists of a mixture of epidermis and sebaceous matter. Dandruff is not contagious and is rarely serious, it can be embarrassing and surprisingly persistent.

CAUSES:

1. Dandruff has been formed on dry skin, oily skin, Shampooing too often (or) not often enough, a poor diet, stress and the use of the many fancy styling products.

2. Dandruff causing fungus lives on the scalps of most healthy adults without causing problems.

3. All skin cells die and are replaced by new cells. Because the cells renew themselves slowly, this process usually is not noticeable.

4. An overgrowth of these organisms, increased oil production, hormonal fluctuations, stress, illness, neurologic disorders such as parkinson's disease, a suppressed immune system, infrequent shampooing and even heredity may contribute to the development of Dandruff.

RISK FACTORS:

1. Dandruff usually begins at puberty.
2. Because far more men than women have Dandruff, some researchers think male harmones may play a role in Dandruff.
3. The fungus feeds on oils in the scalp.
4. Neurologic diseases such as Parkinson's disease are more likely to develop seborrheic dermatitis and Dandruff.

Self care steps such as learning to manage stress, not to shampoo often, cut back on styling products, eating a healthy diet and getting a little sun can be taken to reduce Dandruff.

THE NEED FOR THE STUDY:

1. The plant kingdom is an invaluable source of chemical products and has significant biological properties. So it is pertinent to exploit it for medicinal properties as they cause minimal or no side effects.

2. Of the 6,00,000 plants present on the earth, only 6 percent have been tested pharmacologically. About 50 percent of the medicines used today are based on biogenetic materials and only 10 percent are pure preparations from healing plants.

3. These plants are easily available and cheap.

4. The tribal people are using the medicinal plants commonly without any scientific basis. It is essential to conduct scientific experiments for evaluation on human beings and to develop certain drugs for curative purpose.

OBJECTIVES:

The objectives of the present study are:

1. To make a pharmacognostic study of the two plants namely,
   (i) *Lippia nodiflora* Rich. (ii) *Thea sinensis* Linn. for preventing Dandruff.
2. To analyse the biochemical constituents of the entire plants of *Lippia nodiflora* and leaves of *Thea sinensis.*
3. To prepare the extract of the leaves in oils.
4. To identify the causal organism of Dandruff.
5. To culture the fungus using Sabouraud's Dextrose Agar medium.
6. To find out the sensitivity of the prepared extracts and newly formulated herbal oils upon the fungus in culture media.
7. To made a clinical study on patients who are suffering from Dandruff.

II REVIEW OF LITERATURE

The herbal medicines are being used by about 80 per cent of the world population for primary health care in the developing countries. India is blessed with incredible biological diversity. It is tribal and folklore traditions, particularly the traditional medicinal systems, are rich and unique. These drugs are popular for its safety, efficacy, cultural acceptability and less side effects. These drugs cure age related diseases for which modern medicines have not been completely successful (Ghosh, 2000). The literature being owned to the study of "Efficacy of selected herbs against Dandruff" has been reviewed under the following headings.

1. Ethnobotanical studies
2. Pharmacognostic studies
3. Phytochemical studies
4. Clinical studies
5. Microbial studies

1. ETHNOBOTANICAL STUDIES:

This includes identification studies that have sceptical and mainly descriptive (either ethnobotanical (or) ethnomedical) leading to a chance of the medicinal property of some plants.

According to Nakamura (1984), *Primula obconica* plants used in dermatitis, patch test reactions to flower, leaf and stem were positive in both cases.

According to Kritikar and Basu (1987) the bark and seeds of *Wrightia tinctoria* have the same therapeutic properties as those of *H.antidysentrica*. The leaves are used for Dandruff.

Badruzzaman *et al.* (1988) examined that *Datura stramonium* juice, extracted from fruits is applied to the scalp for Dandruff.

Inge (1990) in his book "Reader's Digest Magic and Medicine of plants" reported the use of *Mullein's golden* flowers to cure certain skin diseases. Tradition also asserts that it partially thickens hair and enriches its colour, burdock root controls Dandruff and stinging nettle conditions hair as well as helps to cure Dandruff.

Vander Naf *et al.* (1991) made ethnopharmacognostical survey of *Azadirachta indica*. Antifungal effects were reported of gedunin against *polyporus wood rot* and a leaf extract against *Alternaria altenata*.

Walter and Memory (1990) in their study had mentioned that an infusion of Rosemary mixed with a little borax, used daily and massaged into the root hairs, is considered to be an efficacious home remedy for Dandruff.

The bark of *Wrightia tinctoria* is very good for piles and skin disease, reported in "Reader's Digest Magic and Medicine of Plants" (1990).

Kurian (1994) reported in his book on "Plants that heal" has mentioned that *Persea americana* is used as an antidandruff medicine. It has the property of preventing hair fall and promote the hair growth.

Sivarajan and Indira (1994) in their study had mentioned that *Hibiscus rosa-sinensis* is extensively used for blackening of hair. Leaves and flowers are reported in treating Dandruff and promoting the growth of hair.

Warrier *et al.* (1996) in their book on "Indian medicinal plants" has mentioned that the leaves and seeds of *Wrightia tinctoria* are used for Psoriasis, Dandruff and Leprosy.

Sairam (1998) reported the use of *Azadirachta indica, Glycyrrhiza glabra, Murraya koenigii, Phyllanthus emblica, Papaver somnifera* and *Sesamum indicum* for Dandruff in his book on "Home remedies-volume I".

Kurian (1999) reported that the plant *Glycine max* is one of the best food for malnutrition as it contains very high percentage of proteins. It is responsible for the curing properties of Dandruff, Dermatitis, eye diseases and skin diseases. It is also used for promoting growth of hair. Application of soya bean over the head, removes Dandruff.

According to Bhupinder *et al.* (2000) *Malus sylvestris* the mini doctor (apple), controls the wear and tear of nerve cells. Apple is generally recommended by the doctors for its rich medicinal values for common ailments such as Acidity, Acne, Anaemia, Dandruff, Epilepsy etc.

George and Roger (2000), in his book "Encyclopedia of medicinial plants" pointed out that the leaves and flowers of Balm tree having the properties of antifungal and antiseptic, are used in treating Dandruff, Psoriasis, Eczema etc.

George and Roger (2000) has reported, *Persa gratissima* and *Citrus lemon* eliminates Dandruff, prevents hairloss and add strength and smoothness to the hair.

Ghose (2000) in his report on "Plants for Hair care" has mentioned that *Allium sativum, Cinchona spp.* etc were used for Dandruff and Baldness.

Syed and Sharma (2000) reported that Burdock roots control Dandruff. The stinging nettle conditions hair as well as helps to cure Dandruff.

Anandakumar *et al.* (2003) reported that Petroleum ether, Chloroform, Acetone and Methanol extracts of the leaves of *Wrightia tinctoria* is having antibacterial and antifungal effects against *Pseudomonas aeruginosa, Staphylococcus aureus, E.coli, Aspergillus niger, Candida sps* and *Malassezia furfur.*

Gemmer and De Angelis (2002) carried out research on herbal plant extract of *Saw palmetto* for curing the Dandruff.

Rajarajan (2003) reported that *Lawsonia inermis, Terminalia catapa, Terminalia chebula and Ocimum basilicum* were used against the fungus causing Dandruff like *Malassezia furfur* and *Candida albicans.*

Sankar *et al.* (2003) observed that Dandruff is caused by the fungus *Pityrosporum ovale* and *Pityrosporum orbiculare.* Medicinal plants such as *Aloe vera* leaves, *Citrus aurentifolia* (fruits) and *Raphanus sativus* (root) are capable of curing the Dandruff.

According to Vijayakumar *et al.* (2003), the aqueous extracts of *Aloe vera, Eucalyptus globulus, Wrightia tinctoria* and *Phyllanthus emblica* were tested at different concentrations (25, 50, 75 and 100%) against the fungus *Pityriasis versicolor* causing Dermatitis, Dandruff etc.

2. PHARMACOGNOSTIC STUDIES

From time immemorial human beings depend on medicinal plants for their survival against diseases. It has been in existence by "Trial and Error" method. But in this sophisticated, computerized and highly advanced world, people are efficient to find out the chemical compounds responsible for curing a particular disease.

Agarwal (1982) carried out pharmacognostic studies on the leaf of *Clerodendron infortunatum* for proper identification. Later he also discussed the fluorescence characteristics of the above leaf.

Datta *et al.* (1982) made a pharmacognostic evaluation of the root bark of *Emblica officinalis*. Macro, micro, morphological characteristics, physical constants, histo chemistry, UV fluorescence of the root bark of *Emblica officinalis* were examined to ascertain the identity of the drug.

Subramanian *et al.* (1986) made a pharmacognostical and pharmacological studies on fresh and dried leaves of *Albizzia lebbeck*.

Pataskar *et al.* (1989) reported the macro and microscopic structure of *Lavendula bipinnata* to bring out its pharmacognostic characters.

Jan (1989) applied the drugs with diversified chemical structure in the treatment of *Pityriasis versicolor*. Local applications of solutions containing Selenium sulphide, Sodium hyposulphite, Jolnattate, Alcoholic solution of iodine, Propylene glycol, Zinc pyrithione and whitefields ointment are practised for various lengths of time for the treatment of *Pityriasis versicolor*.

Gopalakrishnan and Solomon (1991) examined the macroscopic and microscopic characters of leaf and stem of *Ocimum basilicum*.

Sivarajan and Indira (1994) carried out research on pharmacognostic characters of root, leaves and flowers of *Datura metal* which is a source of drug throughout the country.

Tripathi (1994) in his book "Essentials of medicinal pharmacology" made pharmacognostic studies on *cyclopirox olamine.* The newer drug was effective in *Pityriasis versicolor, Dermal candidiasis* and *Tinea* infection.

Rashid and Ahmad (1995) made pharmacognostic and pharmacological studies on the leaves of *Jasminum grandiflorum* used as crude drugs.

Sasikala *et al.* (1995) carried out research on the pharmacognosy of leaves of *Clerodendron inerme* in curing certain skin diseases.

Dash *et al.* (2000) conducted a study on some pharmacognostical characteristics of *Tragia involucrata* roots. It is used for various medicinal uses in the indigenous system of medicine especially for the infection of skin.

Gopalakrishnan and Venkataraman (2000) made a pharmacognostic study on three commerical samples of the roots of *Trianthema decandra*.

Santhi and Jagadeesan (2000) reported that the pharmacognostical studies on aerial parts of *Toddalia asiatica var gracilis* revealed the medicinal properties, used by the Indian system of medicine.

Tamilarasi *et al.* (2000) studied the phytochemical and pharmacological evaluation of *Ampelocissus latifolia*. They exhibit significant antiinflammatory activity that may be due to its inhibitory effects of histamin, kinin and prostaglandins release.

Rajarajan (2003) reported that *Pongamia pinnata, Cassia auriculata, Ocimum basilicum, Lawsonia inermis, Terminalia catapa, Artimesia nilagrica* and *Hemidesmus indicus* were potential sources of drugs for superficial mycoses.

Damodaran and Venkataraman (2003) conducted a study on the pharmacognostical and morphological characters of *Cassia alata.*

Dhandapani and Balu (2003) observed the pharmacognostical characters of *Moringa pterygosperma.*

Indra (2003) carried out preliminary pharmacological studies on five plants namely *Centella asiatica, Cynodon dactylon, Leucas aspera, Ocimum sanctum and Solanum trilobatum.*

Pharmacognostical and Preliminary Phytochemical studies on *Nicandra physaloides* were undertaken by Mahadevan *et al.* (2003).

Santhi and Balu (2004) studied the Pharmacognostical characters of the leaf of *Aegle marmelos.*

The microscopic characters of aerial part, physical constant values, extractive values, behaviour of treatment with different reagents of powdered leaves of *Plantago erosawall* were studied to fix some pharmacognostical parameters. The preliminary phytochemical studies also were carried out by Sathyavathi *et al.* (2003).

Sheela and Anamika (2003) examined the psychopharmacological effects of the leaves of the plants *Polyscias paniculata Var. variegata.*

Shrishailappa *et al.* (2003) analysed the pharmacological evaluation, which include ash and extractive values, HPTLC and fluorescence analysis and also phytochemical tests of *Caesalpinia sappan* Heartwood.

PHYTOCHEMICAL STUDIES

Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one (or) more parts of these plants. These plant metabolites, according to their composition are grouped as alkaloids, glycosoides, carticosteroids, essential oils etc.

Penfold and Grant (1925) made the first scientific study of Tea tree oil in the 1920's in Australia. Tea tree has a long history of detailed research with a special focus on antibacterial and antifungal effect. It contains a terpinene and y-terpinene, the main ingredient is terpinent-4-ol known for the antimicrobial effect.

Nadkarni (1954) reported that the plant *Psoralea corylifolia,* has an alkaloid vernonin responsible for the curing the skin diseases.

Roy and Mitra (1958) invented that the *Thea sinensis* yield caffeine and tannin from the tea infusion. Dust tea undoubtedly produces excess tannin in the infusion.

Rastogi and Mehrotra (1991) studied that the *Delonix elata* bark yielded L(-) paragine, aspartic acid, beta amyrin and beta sisosterol and beta-D-glucopyranoside.

Ganamanickam (1979) demonstrated the production of isoflavonoid & phytoalexins by bean seeds, having antimicrobial activity.

Rastogi and Mehrotra (1993) reported that *Cardiospermum helicabum* plant extract stabilised lysosomal membrane and prevented enzyme leakage. The drug may prevent cellular and extracellular injuries by stabilising lysosomal membrane.

Narayanaswamy and Mahadevan (1983) reported the presence of phytoalexins from germinating seeds of ground nut (*Arachis hypogea*).

Shuster (1984) stated that individual sensitivity is important in Dandruff and seborrheic dermatitis, with sensitive individuals experiencing intense symptoms to minimal numbers of *Malassezia.*

Gueho and Boekhout (1998) reported that *Malassezia restricta* and *M. globosa* were the predominant species present in the scalp, with high enough frequency relevant etiologic agents for Dandruff.

Pant (1985) carried out research on *Azadirachta indica*, a mixture of sulphurous compounds from the steam distillate of fresh mature leaves (against *Trichophyton mentagraphytes* in a concentration of 25 μg/ml.

Ahmed *et al.* (1993) studied the morphological, anatomical and preliminary phytochemical characters of bark and leaf samples of plants viz, *Jatropha curcas,* and *J. gossipifolia.*

A phytochemical study of the forest plants of South Gujarat with special reference to the medicinal plants were discussed by Joshi and Submis (1989). The paper deals with phytochemical screening for alkaloids, saponins and tannins of 182 samples consisting of 147 species which are

used as medicine either in indigenous system (or) by aboriginal tribes of South Gujarat forests.

Rastogi and Mehrotra (1991) isolated Gallic, P-coumaric and caffeic acids from leaves of *Thea sinensis*. TLC identification of seed oil showed the presence of Palmitic, stearic, oleic acid and linoleic acids as major components and lauric, myristic and orucic acids as minor components. Campesterol, brassicasterol, stigmasterol and β-sitosterol were identified in sterol fraction.

The Preliminary phytochemical investigation of the plant *Cardiospermum helicabum* showed the presence of saponin in root and fruit (Arasu, 1995).

One cup extract of *Trichodesma indicum* is taken internally to cure skin diseases. It is applied for skin infection. Incanine, Incanine N-Oxide, Nikanine, Nikanine-N-Oxide, supinine, Trichodesmine and Trichodesmine-N-Oxide alkaloids were present in this plant. (Rajak and Rai, 1996).

Determination of aristolochic acid in traditional chinese medicinal prescriptions containing *Radix aristolochiae* by HPTLC was evaluated by Chen *et al.* (1997).

Kamil *et al.* (2000) studied the phytochemistry and phytotherapeutic uses of members of the genus *Portulaca.*

Ku (2000) observed that matrine and Oxymatrine from the dried roots of *Sophora subprostata* is commonly used in chinese herbal drug in Taiwan.

Joshi (2000) reported that *Wrightia tinctoria* seeds yield 30-49 per cent fixed B-sits-sterol, B-amyrin and bark accetate benzoate.

Pierard and Hermanns, (2000) pointed out in his book "From Axioms to New Insights into Dandruff", that P&G beauty scientistis developed a triaminoacid system, specifically designed to replace these last building blocks. Scanning focussed Ion Beam Analysis techniques have been used to prove the penetration of these aminoacids into the very cortex of the hair. On drying these amino acids from bonds within the cortex, which can dramatically improve the physical properties of the hair fibre itself.

The phytochemical and antimicrobial studies of extracts of *Solanum xanthocarpum* was carried out by Udayakumar *et al.* (2000).

Kim and Parkinseon (2001) reported that the saponin compounds (Saikosaponin c, a and d) in *Buypleurum falcattum* were partially purified by solvent partitioning of the herbal extract using diethyl ether, distilled water, n-butanol and acetone.

Jirovetz *et al.* (2002) investigated the aroma compounds of the essential oils from fruit (fresh pulp and peels) and leaves (Fresh and dried) of the African custard apple *Annona sensgalensis* from cameroun.

Li (2002) extracted imperialine and imperialine-3 β-glucoside from *Fritilaria pallidiflora* by HPLC method.

Jeychandran *et al.* (2003) in a study found that the leaves of *Phyla nodiflora* potential source of salicylic acid finds use in many of the chemotherapeutics.

Manisha and Modhimitr (2003) screened the young leaves of the six medicinal plants namely *Rauwolfia tetraphylla*, *Andrographis paniculata*, *Piper longum*, *Terminalia arjuna* and *Plumbago zeylanica.*

The preliminary phytochemical investigation of the petroleum ether and ethanol extracts of the stem of *Glycosmis pentaphylla* showed the presence alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins and tannins (Raju *et al.* 2003).

A report of Mayo Foundation for Medical Education and Research (MFMER) (1998-2004) states that Zinc pyrithione shampoos contain the antibacterial and antifungal agent Zinc pyrithione which cures Dandruff and Seborrbeic dermatitis.

CLINICAL STUDIES

Clinical studies were undertaken to find out the mode of infection, symptoms and their exact remedies. It is interesting to note 80 per cent of chinese population get remedy for diseases from plants. Ayurvedic literature describes about 2000 medicinal plants. Siddha literature describes about 3000 medicinal plants of which few are used for the treatment of superficial mycoses. However, these observations were based on uncontrolled clinical trials by application of extracts on affected parts of skin.

Penfold and Grant (1925) reported the use of tea tree oil in cosmetics and toiletries and was found to be the best medicine to cure the Dandruff.

Walker (1972) made a clinical investigation of tea tree for a variety of foot problem.

Kishore *et al.* (1982) examined the fungitoxic activity of leaves of some higher plants. Ethanol extracts of 31 plant species were screened for their fungitoxicity against *Colletotrichum falcatum*, *Rhizoctonia solani* and *Malassezia furfur*. The leaves of *Allamanda cathartica* and *Artabotrys hexapetalus* showed 100 per cent toxicity against both the fungi. Leaves of *Aegle marmelos*, *Clerodendron viscosum*, *Cosmos spp*, *Kergandia reticulata* and *Sapindus emarginatus* exhibited significant inhibition of *Candida falcatum*, Yeasts and Moulds.

Shuster (1984) reported that Anti-androgens are the major drugs used for hair growth. It is essential to take these drugs with a form of contraception. Cyproterone is a commonly used anti-androgen. Spironolactone works as an anti-androgen as well as diuretic.

Sing et al. (1984) observed the fungitoxic properties of essential oil of *Mentha arvensis var piperascens*. This essential oil exhibited strong fungitoxicity against *Pityriasis versicolor*, mould, yeasts etc.

Inge (1986) in his book "Magic and Medicine of plants", mentioned that the plant *Urtica dioica* is rich in Protein, Iron and Vitamins A and C and make a healthful tea and soup. A tea made from the seeds is used in modern herbal medicine as a hair tonic, growth stimulant and antidandruff shampoo.

Walsh and Longstaff (1987) reported that Tea tree oil was used in anti inflammatory, antifungal and athletes foot products.

Williams *et al.* (1990) reported that the tea tree's properties aid in the repair of skin damage caused by sun, acne, dry skin, fungal diseases

and various other problems. The U.S. Government has approved the use of tea tree oil in cosmetic formulations. The oil is non-irritating to almost all areas of the body, allowing healthy skin.

Altmen (1991) undertook a clinical study of Tea tree focussing on skin sensitivity and irritation potential.

Raychaudhuri (1991) in his book "Recent advances in medicinal, aromatic and spice crop" (Vol I) mentioned that the seeds of *Anamirta cocculus* made into paste and applied on the scalp mixed with hair oil, removes the Dandruff.

De and Weyland (1991) investigated contact dermatitis, which is associated with tea tree in extreme cases.

Tong *et al.* (1992) made a clinical investigation of Tea tree, which is used in the treatment of *Tinea pedis*.

A clinical study on the *Malassezia furfur* was undertaken by Bhutani (1993).

Sivarajan and Indira (1994) reported that *Solanum capsicoides,* is an effective remedy for all skin diseases like Scabies, Eczema, Dandruff, Leprosy and Ulcers. Leaves, fruit and oil from the seeds are also medicinal. Oil from the seeds applied to the head, removes the Dandruff.

Southwell (1996) investigated the antibacterial, antifungal and germicidal effect of Tea tree oil.

Apted (1991) and her colleagues tested over 200 healthy adult volunteers for allergy to tea tree oil. Two tests were performed using ten

different samples of 100 per cent tea tree oil. Various common allergens were also tested to determine the relative reactivity of the population study.

Southwell (1997) made a clinical study of tea tree oil on skin irritancy and cineole as a potential irritant.

RIRDC (1999) reported that irritant reactions reflect damage to the surface of the skin, and can be controlled by decreasing the exposure time and more importantly the concentration of the substance.

In the view of Aziz and Shivchandran (2000), most people would regard Psoriasis and Dandruff as a fairly serious skin diseases may be no more than a rather usual response to some form of irritation. Plants also play an important role in combating skin diseases like Psoriasis, Eczema, Dandruff, Ringworm etc.

George and Roger (2000) in his book "Encyclopedia of medicinal plants" noted that the lemon tree, used to prevent the Dandruff, can be used in lotion with dissolved juice.

Vegetal tar of Pinetree is used to regenerate the skin, as an emollient, to eliminate fungi and skin parasites. (George and Roger, 2000)

Pierard and Hermanns (2000), in randomised clinical trials found that hair loss due to Dandruff in most women did not respond either to the 5 reductase inhibitor finasteride or to androgen receptor blockade with cyproterone acetate.

Satchell (2000) made a clinical study on the tea tree oil shampoo which was effective in the treatment of Dandruff. Tea tree oil has antifungul

activity and thus, may be useful in the treatment of Dandruff. Patients washed their hair daily, leaving the shampoo for three minutes before rinsing. After using the tea tree oil shampoo, patient in each group was completely free of Dandruff at the end of the four weeks.

Levin and Maibach (2002) reported that the natural remedies, (eg. Tea extracts, other herbs, hydroxy acids, essential fatty acids, essential oils, ascorbic acid and vitamin E) seem promising in treating a wide variety of dermatological disorders, including inflammation, phytotoxicity, Dandruff, Atopic dermatitis, and poison oak.

Tanaka (2003) examined the clinical studies of superficial mycoses from the nail, hair and skin scraping.

Udayakumar *et al.* (2000) observed the medicinal plants used in the treatment of skin diseases in sebarkantha district, Gujarat, in the survey was conducted during 1997-2000. Along with the plants used are *Ficus hispida*, *Acacia chundra, Tamarindus indica* etc.

MICROBIAL STUDIES

Microbes cause several diseases. In order to prevent these diseases the particular causative microorganism should be investigated and then implement correct and effective remedy.

Penfold and Grant (1925) reported that Tea leaves is effective, when used pure or with various emollients, humectants, surfactants, and ingredients in products of all kinds. As a valuable ingredient, tea leaves has first and foremost a pronounced antifungal and antibacterial effect, which is beneficial both in application and for the shelf life and integrity of the product itself.

Purushothaman *et al.* (1979) found that the B-solamarine the major glycoalkaloid present in *Solanum trilobatum* has antibacterial and antifungal activity. It shows promising result in two cancer test.

Goushterov *et al.* (1983) observed the antimicrobial and antimycotic activity of the extracts of the leaves, stems and flowers of *Chrysanthemum indicum.*

Jain and Saxena (1983) observed *in vitro* antimicrobial efficacy of the essential oil from the flowers of *Heterophragma quadriloculare*. It was very active against *Microsporum gypseum, Malassezia furfur, E.coli* and *Aspergillus niger*.

Ju Rc and Chou cc (1983) reported that ethanolic extract of *Piper betle* leaf exhibited strongest antimicrobial activity against various bacteria, moulds and yeast.

Walsh and Longstaff (1987) observed the antimicrobial effect of tea tree on oral pathogens.

Badruzzaman *et al.* (1988) reported that *Acacia arabica* leaves have the antimicrobial properties. Decoction of leaves mixed with coconut oil, and ointment made, is then applied for different skin disease (Scabies, Ringworm, Dandruff, Eczema, etc.).

The phytochemical analysis and antimicrobial effects of *Aloe* species were undertaken by Ahmad *et al.* (1993).

Hernandez *et al.* (1993) studied about the antimicrobial activity of *Visnea mocanera* leaf extracts using *Candida albicans, Candida tropicans, Candida guillermondii, Saccharomyces cerevisae and Cryptococcus albidus*.

Geetha *et al.* (2001) studied the activity of *Ocimum sanctum* against the enteric pathogens. The studies analyse the effect of alcoholic extracts of *O.sanctum* against the enteric pathogens and amp.

Study of radioproductivity, anticarcinogenic and antioxidant properties of the Indian holy basil *Ocimum sanctum* was undertaken by Umadevi (2001).

Verma *et al.* (2001) studied the phytochemical characters and antimicrobial activity of *Vitex negundu* leaves.

Weseler *et al.* (2002) studied the antifungal effect of Australian tea tree oil on *Malassezia pachydermatis* isolated from canines suffering from cutaneous skin diseases.

Arul *et al.* (2003) tested the antifungal activity of *Tridax procumbens* extracts.

Maheswaran and Balaji (2003) analysed the dermatophytic fungi *Trichophyton rubrum, Epidermophyton floclosum, Microsporum canis* and *M.audouinii* and used various plant extracts against these fungi.

Margarita and Hunter (2003) observed the superficial mycoses as a source of great physical and in addition psychological discomfort, which can be severe for esthetic reasons.

The preliminary screening of *Aristolochia bracteata, Lippia nodiflora, Mukia maderaspatana* and *Wedelia calendulacea* for antifungal activity against *Candida albicans* and dermatophytes (Murugan et al. 2003).

Philip *et al.* (2003) described the antifungal activity of petroleum ether, acetone, alcoholic and aqueous extracts of aerial parts of *Calotropis procera* against *Rhizopus japonicum*, *Rhodotarula glutinis* and *Candida albicans*.

Sangameshwaran *et al.* (2003) described the antifungal activity of aerial parts of *Euphorbia hirta* against *Aspergillus niger*, *Candida albicans* and *Candida tropicals*.

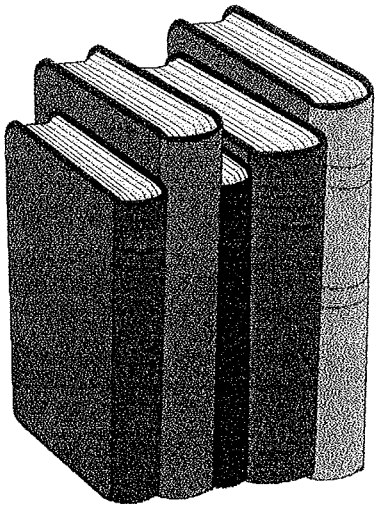
Sangameswaran *et al.* (2003) studied about the antifungal activity of extracts leaves of *Croton tiglium.* The test extracts were evaluated for antifungal activity against *C. serratus* and *C. albicans*.

According to Sankar *et al.* (2003) Dandruff is caused by the fungus *Malassezia furfur*. Several types of medicines are prescribed to the patients but the herbal medicines are the best for Dandruff, as they possess effective phytochemicals.

Senthil Kumar *et al.* (2003) reported that the extracts of *Croton tiglium* have been effective against *Microsporum gypseum*, *Actinomyces serratus* and *Candida albicans* using filter paper disc agar diffusion technique.

Vadivelan *et al.* (2003) documented that *Cassia alata* showed high antifungal activity aganist *A.niger*, *C.albicans* and *C.tropcals*.

Herbal medicines are regarded as remedies for diseases by the vast majority of the world's public. The present study deals with the isolation and screening of microbial contaminants of *Karunai* and *Sowbhagya Sunti Lehiyam* which was reported by Dharani *et al.* (2004).



MATERIALS AND METHODS

III MATERIALS AND METHODS

India has one of the world's most sophisticated indigenous medical cultures, with an unbroken tradition coming down across more than four millennia. Though this medical heritage is many centuries old, even today, millions of people are using herbal medicine because it is very cheap and safe. Government and non-Government organizations are carrying out many researches in the area of alternative medicine.

The plants *Lippia nodiflora* and *Thea sinensis* possessing medicinal properties for Dandruff were listed from Siddha, Ayurveda and Tamil medicinal literatures. About 15 kg of the above plants were collected at their flowering stage during September - November (2003). The plant material was identified and authenticated at its fresh condition from the Botanical survey of India, Southern Circle, Coimbatore and was made into Herbaria and kept for future reference.

The collected leaf materials were washed with tap water to remove the adhering dust, followed by rinsing with distilled water. They were shade dried for one month, powdered by using Wiley Mill (0.5mm) at the Sugarcane Breeding Institute, Coimbatore.

3.1 PHARMACOGNOSTIC STUDY

The various methods used in the study include,

1. Powder study
2. Organoleptic study
3. Fluorescence analysis
4. Preparation of the plant extract
5. Preliminary phytochemical studies

6. Biochemical analysis
7. Study of microbial factor in Dandruff to analyse the sensitivity
8. Oil preparation
9. Sabouraud's Dextrose Agar (SDA) medium
10. Clinical study on human beings.

3.1.1 POWDER STUDY

About 5 kg. of entire plants of *Lippia nodiflora* and only the leaves of *Thea sinensis* were collected and dried in shade, for three months. After the drying process it was powdered in Sugarcane, Breeding Institute using the Wiley mill (0.5mm) for pharmacognostic studies (Wallis, 1950) (Plate 5 & 6).

3.1.2 ORGANOLEPTIC EVALUATION

The colour variation and taste are the basis for this test as given by Jackson and Snowdown (1968).

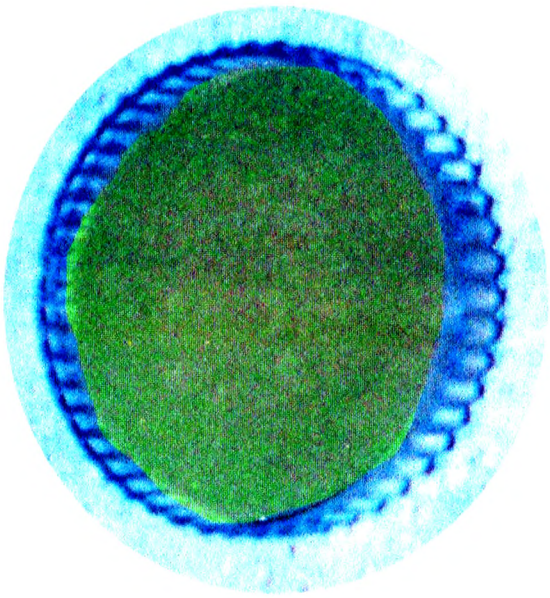
3.1.3 FLUORESCENCE EVALUATION

The fluorescence properties were studied under UV light adopting the method described by Kokoski *et al.* (1958) and chase and Pratt (1949). The colour of the fluorescence emitted by the powder was studied. Employing the colour chart of Ridgeway (1886), identified the properties. The selected herbal powders of *Lippia nodiflora, Thea sinensis* were treated with certain chemicals under long UV and short UV in the UV chambers.

3.1.4 PREPARATION OF THE PLANT EXTRACT

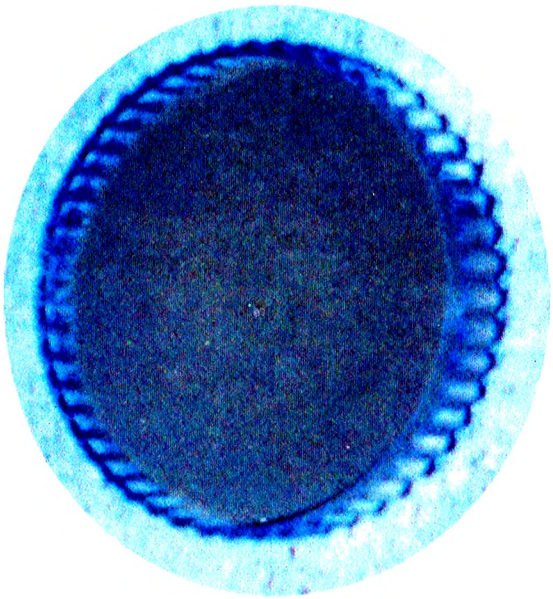
Extracts were obtained by means of dissolvent applied to the active parts of the plant. The solvent was finally evaporated and only the

PLATE -5



Powder Of *Lippia nodiflora* Rich.

PLATE -6



Powder Of *Thea sinensis* Linn.

active components remained. The most usual dissolvents are Ethylalcohol, Petroleum Ether, Chloroform and Hot water.

By Soxhlet Apparatus

The extraction of solids is generally applied to the removal of natural products from dried tissue originating from plants by soxhlet extraction apparatus. 10g of leaf powder was weighed using an electronic balance (Denver, 210) and made into 8 packets using filter paper (10'A grade SD's Xerohaze'). The leaf powder in the individual packet was subjected to extraction with 250 ml petroleum Ether, Chloroform and Ethanol (60^0 - 80^oC) according to the increasing order of polarity using soxhlet apparatus for 8 hours. After extraction was over the solution was distilled for solvent removal using distillation apparatus. The extract was kept in water bath for further evaporation of solvent and the residue obtained was stored in the refrigerator for further use.

TYPES OF EXTRACTS

1. Hot water extract
2. Petroleum Ether extract
3. Chloroform extract
4. Ethanol extract

1. Hot Water Extraction Method

20 gm of plant powder was weighed accurately in the electronic balance (Denver, 210) and poured in 250 ml hot water. This was kept for two hours and filtered with the help of a filter paper. The supernatant solution was used for further study. Water extraction is useful for the amateur herbalist and in certain clinical situation.

Petroleum Ether, Chloroform and Ethanol extracts:

The soxhelt methods were used for preparation of other extracts.

The extract was then filtered and concentrated by vacuum evaporation. The phytochemical studies and alkaloid tests were done by using the extract.

DISTILLATION

It was done by means of a device called a still. The water inside the still was heated to boiling point. The volatile active ingredients of the plants which lie over the water were carried by the water vapor. That vapor, which contained the active principles of the plants, passed through a refrigerating circuit where it is cooled and condensed forming a liquid.

3.1.5 PRELIMINARY PHYTOCHEMICAL STUDIES

Preliminary phytochemical test of the extract was performed by specific reagents. The extract was subjected to qualitative chemical tests for detection of Cellulose, Protein, Fixed oil and Fat, Flavonoid, Quinone, Saponin, Suberin, Sugar, Steroid, Alkaloid and Phenol.

3.1.6 BIOCHEMICAL STUDIES

ESTIMATION OF TOTAL PROTEIN

The protein was estimated by Lowry *et al.* (1951).

PRINCIPLE

The blue colour developed by the reduction of the phosphomolybdic phosphotungstic components in the Folin-Ciocalteau reagent

by the amino acids tyrosine and tryptophan present in the protein and the colour developed by the biuret reaction of the protein with alkaline cupric tartrate were measured in Lowry's method.

MATERIALS REQUIRED

1. 2% Sodium carbonate in O.I N Sodium Hydroxide (Reagent A).
2. 0.5% Copper sulphate in 1% Potassium Sodium tartarate (Reagent B)
3. Alkaline copper solution (Reagent C)
4. Folin - Ciocalteau Reagent (Reagent D)
5. Protein solution (Stock standard)
6. Working standard

10ml of stock was diluted to 50 ml with distilled water.

PROCEDURE

1. 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard were pipetted out into a series of test tubes.
2. 0.1 ml and 0.2 ml of the sample extract were pipetted out in two other test tubes.
3. The volume was made to 1 ml in all the test tubes. A tube with 1 ml of water serves as the blank.
4. 5ml of reagent C was added to each tube including the blank. The mixture was mixed well and allowed to stand for 10 minutes.
5. Then 0.5 ml of reagent D was added, mixed well and incubated at room temperature in the dark for 30 minutes and blue colour developed.
6. The reading was taken at 660nm.

CALCULATION

$$\text{Protein in mg / gm} = \frac{\text{mg of protein}}{\text{Volume of the test standard}} \times \text{Concentration of the standard}$$

ESTIMATION OF TOTAL CARBOHYDRATE

The total carbohydrate was estimated by the method of Hedge and Hofreiter (1962).

PRINCIPLE

Concentrated Sulphuric acid Hydrolyses the glycosidic bond of carbohydrate to the given Mono saccharides which were then dehydrated to furfural. The furfural reacted with Anthrone (10-keto 9, 10 dihydro anthracene) to give the blue green coloured complex which was measured colorimetrically at 640 nm.

MATERIALS REQUIRED

1. 30% KOH Solution:

   30g of KOH was dissolved in 100 ml of distilled water.

2. 0.2% Anthrone in Sulphuric acid:

   200 mg of Anthrone in 100 ml of Con.sulphuric acid.

3. Stock standard solution:

   100 mg of glucose dissolved in 100 ml of distilled water.

4. Working standard solution:

   5 ml of stock standard solution was diluted to 100 ml with distilled water (50mg/ml).

PROCEDURE

1. Various concentrations of the working standard solution (0.2, 0.4, 0.6, 0.8 and 1 ml) were taken into a series of test tubes.
2. Made up the volume in each tube to 1 ml with distilled water.
3. Kept the tubes in an ice bath and added slowly 0.2 ml of Anthrone reagent with constant stirring.
4. A blank was set up using 1 ml of water and 4 ml of Anthrone reagent. Heated all the tubes in a boiling water bath for 5 minutes.
5. A blue green colour developed was read at 640 nm.
6. A standard graph was plotted with concentration on X-axis and optical density on Y-axis.
7. From the graph the amount of total carbohydrate present in the given sample was calculated.

CALCULATION

$$\text{Amount of Carbohydrate present in 100 mg of the sample} = \frac{\text{mg of Glucose}}{\text{Volume of test sample}} \times 100$$

ESTIMATION OF TOTAL PHENOL

The total Phenols were estimated by the method of Malick and Singh (1980).

PRINCIPLE

Phenols react with phosphomolybdic acid in Folin - Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue) that can be estimated colorimetrically at 650 nm.

MATERIALS REQUIRED

1. 80% ethanol
2. Folin - Ciocalteau reagent
3. $Na_2 Co_3$ - 20%
4. Stock standard solution:

   100mg catechol in 100 ml water.
5. Working standard solution:

10 ml of stock standard solution was diluted to 100 ml with distilled water.

PROCEDURE

1. 0.5 to 1.0 g of the sample was weighed and ground in a morter and pestle in 10 times volume at 80% Ethanol.
2. The homogenate was centrifuged at 10,000 rpm for 20 minutes, the supernatant was collected, the residue was re-extracted with 5 times the volume of 80% ethanol, centrifuged and supernatant was collected.
3. The supernatant was evaporated to dryness.
4. The residue was then dissolved in known volume of distilled water (5 ml).
5. Different aliquots (0.2 to 2 ml) were pipetted out into test tubes.

6. The volume in each tube was made to 3 ml with water.
7. Then 0.5 ml of Folin-Ciocalteau reagent was added.
8. After 3 minutes, 2 ml of 20% $Na_2 Co_3$ solution was added to each test tube.
9. The mixture was mixed thoroughly, the tubes were placed in a boiling water bath for exactly one minute, cooled and the absorbance as read at 650 nm.

CALCULATION

$$\text{Amount of Phenol present in 100 mg of sample} = \frac{\text{mg of Phenol}}{\text{Volume of test standard}} \times \text{Concentration of the standard}$$

ESTIMATION OF TOTAL SOLUBLE SUGAR

The amount of total soluble sugars present in the extracts can be estimated by Anthrone and Phenol Sulphuric acid reagents.

ANTHRONE METHOD

The concentration of pentoses, hexoses, disaccharides including sucrose, lactose, maltose and hexuronic acids present either freely (or) along with polysaccharides can be estimated by using this method. Anthrone, 10 -keto - 9, 10-dihydroanthracene, a reduction product of anthroquinone, reacts by green colour in a diluted solution and a blue colour in a concentration solution.

REAGENTS

Anthrone Reagent:

Dissolved 2g of Anthrone in 1 litre of Concentrated H_2So_4 prepared freshly.

PROCEDURE

1. Pipetted out the aliquots of 1 ml of the extract into test tubes.
2. To each tube, added 4 ml of the Anthrone reagent allowing the reagent to run down the sides of the test tube.
3. Placed a glass marble on top of each tube to prevent loss of water by evaporation. Placed the tubes in a boiling water bath for 10 min.
4. Removed and cooled them to room temperature in a water bath.
5. Treated a reagent blank similarly, measured the absorbance of the blue green solution at 625 nm, calculated the amount of sugars present in the extract, using a standard curve prepared from glucose.

3.1.7 STUDY OF MICROBIAL FACTOR IN DANDRUFF TO ANALYSE THE SENSITIVITY

The Dandruff strain was collected from the following area patients at Coimbatore, Pollachi and Palani. The macroscopic examination was carried out to analyse the colour, odour, quantity, consistency of strain and observations were recorded.

Microscopic examination was carried out with Dandruff affected patients to find out the causal organisms of the above.

3.1.8 OIL PREPARATION

(MEDICATED OIL (OR) TAILAS)

The whole plant of *Lippia nodiflora* and leaves of *Thea sinensis* were used as the sample. They were powdered after drying. It was discharged into a glass container.

The oil was prepared from the extract of the selected medicinal plants.

- *Lippia nodiflora* oil (Plate 7)
- *Thea sinensis* oil and (Plate 8)
- Mixture of two extracts (Plate 9)

PREPARATION METHOD

250 gms of the two selected herbal plant powder was added to 1000 ml of boiled water. This decoction were kept till over night at room temperature. After filtration of the decoction, it was boiled till the whole of the watery portion was evaporated. After that 750 ml of oil was added and then boiled for 30 minutes.

Storing

The prepared herbal oil applied externally after the preparation, does not require special precaution other than being kept in a tightly closed bottle. The bottles were stored in dry and dark place.

NEWLY PREPARED ANTIDANDRUFF HERBAL OIL

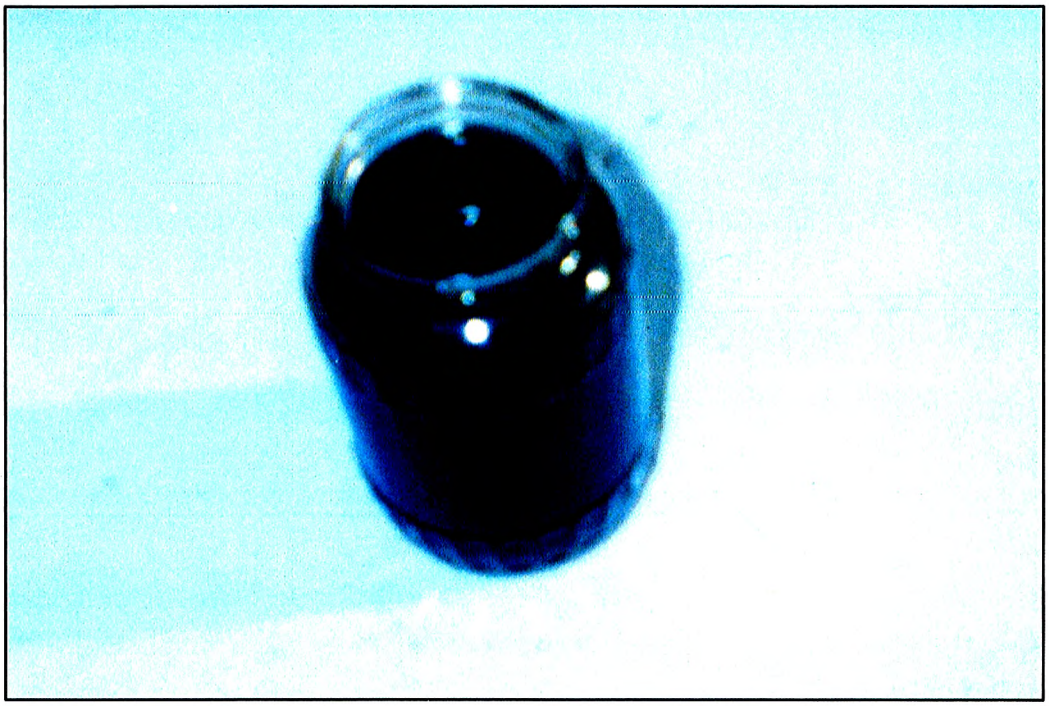
PLATE - 7

Lippia nodiflora Oil

PLATE - 8

Thea sinensis Oil

PLATE - 9



Mixture of Oil
(Lippia nodiflora and Thea sinensis)

IN VITRO AND CLINICAL TRIAL TO ANALYSE THE EFFECT OF NEWLY PREPARED HERBAL OIL AND PLANT EXTRACTS

Dandruff:

Dandruff is a kind of Dermatitis, mostly found on the scalp. It was characterized by the production of scales all over the surface of the scalp. This is a mild form of **seborrheic Dermatitis**. A typical thin mica like, scaling on scratching with acute burning and itching sensation will be noticed. Rough papules are seen with itching, without any discharge.

Symptoms of Dandruff:

The symptoms of Dandruff are, white, oily - looking flakes of dead skin that dot your hair and shoulders and an itchy, scaling scalp, seborrheic dermatitis, scalp ringworm and contact dermatitis.

Common reasons that trigger Dandruff:

* *Pityrosporum ovale* is the main reason
* Sebaceous glands secretion
* Heavy dosage shampoos with not perfect conditioners
* Hormone changes
* Vitamin B - complex deficiency
* Fat deficiency
* Heavy sweat and high temperature
* Tight caps and scarfs
* Ultra violet rays
* Use of hair sprays
* Severe mental stress

Collection of Dandruff:

Dandruff strains were collected from the skin lesions by scrubbing with a blunt scalpel. Scrubbing the skin first with 70% alcohol may help to reduce bacterial contamination. Then the strain should be placed in a folded piece of paper (preferably black) for transfer to the laboratory.

Laboratory investigation:

The specimens are placed on slides in drops of 20-30% KOH and add one drop of Methylene blue, cover with coverslips and warm gently. The treatment dissolves the host cell and the highly refractile fungal elements can then be seen, by using the microscope with a dry objective.

Method of Culture:

The strain was collected from the highly infected patients. The Dandruff was collected in sterile paper from the patients, namely Ranganayaki and Suguna from Pollachi, Poornima and Krithika from Coimbatore, Raja and Ramprasath from Palani for *in vitro* culture to know the sensitivity.

3.1.9 PREPARATION OF SABOURAUD DEXTROSE AGAR MEDIUM

Standard Formula:

Ingredients	Gms / Litre
Mycological peptone	10.00 gms
Dextrose	40.00 gms
Agar	15.00 gms
Final P^H (at 25°C)	5.6 ± 0.2

Direction:

Suspended 65 gms in 1000 ml distilled water. It was heated to boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes (Plate 10).

It was used for culturing yeast, Moulds and Aciduric microorganism.

PROCEDURE

1. Collected strain was inoculated in Sabouraud Dextrose Agar medium aseptically along with Chloramphenicol to prevent bacterial contamination.
2. A thin layer of olive oil was spread on the medium.
3. The petriplates were placed in the inoculation chamber at 37°C for 5-14 days to observe the growth of microorganism.
4. Creamy colonies was observed in the mother culture.
5. Smear from culture showed predominantly budding yeast cells and short irregular mycelium microscopically.
6. Then the mother culture was placed in another petriplate for pure culture.
7. The sensitivity disc was placed in the pure culture to know the sensitivity of the plant extracts and prepared herbal oil.

PREPARATION OF THE SENSITIVITY DISC:

Disc diffusion technique was used for the yeast like fungus. The *in vitro* antifungal assay was employed (Rajarajan, 2003).

The Whatsman filter paper was used as a sensitivity disc. Nearly 0.5 cm of disc was used to know the sensitivity.

The newly prepared herbal oils and extracts were used to know the sensitivity. The micropipette was used to pour the herbal extracts and oils (250 km) inside the disc by overlapping method and allowed it to dry. After the drying process, it was kept inside the inoculation chamber for 5-14 days to analyse the sensitivity.

3.1.10 EXTERNAL CLINICAL STUDY ON HUMAN BEINGS

Human Data Collection:

A clinical study was launched upon 60 patients selected from Palani, Coimbatore and Pollachi. The patients who were having chronic Dandruff were thoroughly investigated.

Dosage of the patients:

The newly prepared herbal oil was given to the patients. The oil was applied and massaged on the scalp daily and after 1 or 2 hours hair wash was done with shikakai herbal powder.

Patients with Dandruff were treated for 15 days by dividing into three groups viz., A, B and C.

Group A:

The prepared herbal oil of *Lippia nodiflora* was given to 20 patients in Kanakkanpatti and Palani.

Group B:

The prepared herbal oil of *Thea sinensis* was given to 20 patients in Pollachi.

Group C:

The prepared herbal oil of mixture of both extracts *(Lippia nodiflora* and *Thea sinensis)* was given to 20 patients in Coimbatore.

After 15 days, the response to treatment was assessed based on the symptomatic improvement. The efficacy of the drug over the diseases was evaluated after the treatment. Based on the clinical data, the total duration of the treatment can be extended further.

RESULTS AND DISCUSSION

IV RESULTS AND DISCUSSION

Numerous drugs have entered the international pharmacopeia through the study of ethnopharmacology and traditional medicine.

There is not only an increasing international awareness of the value of natural plant products in the development of new drugs but also an urgency to examine the plant kingdom for new pharmaceuticals due to continual loss of plant lore in traditional culture. Presently, more than three fourth of the drugs manufactured in Unani and Ayurvedic systems are derived from hardly hundred plants.

Drugs used in traditional system of medicine are all crude drugs in their natural state. During 20th century the progress in chemical techniques and with the growth of pharmaceutical industry, crude drugs were replaced gradually by chemical (synthetic) drugs. Synthetic drugs can produce remarkable life saving results in acute diseases but cannot be used often with treatments of chronic diseases.

A herb (or) crude drug as used in a traditional system of medicine is a complex potpouri of compound, some beneficial, some harmful, some vitamins and some even toxic but all integrated under a certain rule (natural) to make a crude drug function in the same way as a single chemical agent. The crude drug, thus acts as a single chemical agent without any side effects.

Thus a crude drug have substances amalgamated in a fashion where some chemicals counter balance the undersirable effects of the other, ultimately to give only beneficial effect, it is the reason why generally natural medicines induce fewer side effects than conventional western drugs.

The observation and the results indicate the effect of *Lippia nodiflora* and *Thea sinensis* powders on Dandruff in human beings. The powder study, phytochemical and the microbial study of the Dandruff strain of the patients were studied and observed in the laboratory. The results are narrated in the following parameters. They are as follows.

1. Organoleptic study
2. Fluorescence analysis
3. Phytochemical screening
4. Biochemical screening
5. Microbial study of the Dandruff
6. Microbial sensitivity study
7. Human data (Clinical study) collection. clinical trials.

1. ORGANOLEPTIC STUDY

The powder study of two plants namely *Lippia nodiflora* and *Thea sinensis* indicated the organoleptic characters like colour, odour and taste. The colour of the plant powders and the taste were observed and results were recorded. (Table I and Plate 5 and 6).

TABLE I. ORGANOLEPTIC STUDY OF THE POWDER

S.No.	Organoleptic Characters	Name of the Plants	
		Lippia nodiflora	*Thea sinensis*
1.	Part used	Whole plant	Leaves
2.	Colour	Green	Dark green
3.	Texture	Smooth	Leathery
4.	Taste	Acrid, Bitter, Astringent	Astringent

The above results showed similarity with the findings of Kritikar and Basu (1987) who reported the green colour, bitter and astringent powder in Wrightia leaves. This coincides with the microscopic, organoleptic and fluorescence studies conducted in *Albizzia lebbeck*, *Cassia auriculata, Clitoria ternatea* and *Delonix elata* (Indra, 2003).

Fluorescence Analysis

The fluorescence properties are found to be a valuable aid in the identification of the powdered drug. Many substances, both of plant and animal origin, exhibit flourescence characteristics, both qualitatively and quantitatively when exposed to UV radiation. Since the solvent and pH are capable of modifying the fluorescence of many substances, the powder is treated with different chemicals and then observed under UV light (Kokoshi *et al.,* 1958 and Chase and Partt, 1949).

The fluorescence properties were studied under UV and visible light and the behaviour of the plant powder with different reagents were studied and the fluorescence emitted by the powder were observed under ordinary and long ultra violet at 254 nm. (Table II and III)

TABLE II. FLUORESCENCE ANALYSIS OF *Lippia nodiflora*

S.No.	Treatments with Chemical Reagents	Observations	
		Visible Light	UV Light
1.	Powder	Pale green colour	Dark green
2.	Powder + 1N Hcl	Pale pink colour	Dark pink colour
3.	Powder + 1N NaOH	Yellowish brown	Dark brown
4.	Powder + 1N NaOH in Methanol	Dark green	Blackish brown
5.	Powder + 50% H_2So_4	Brown	Black

TABLE III. FLUORESCENCE ANALYSIS OF *Thea sinensis*

S.No.	Treatments with Chemical Reagents	Observations	
		Visible Light	UV Light
1.	Powder	Dark green	Blackish green
2.	Powder + 1N Hcl	Dark yellow	Brownish yellow
3.	Powder + 1N NaOH	Brown	Blackish brown
4.	Powder + 1N NaOH in Methanol	Dark brown	Black
5.	Powder + 50% H_2So_4	Dark shiny brown	Blackish shiny brown

Mary *et al.* (1980) in their study differentiated two species of *Valerina L.* on the basis of morphology, fluorescent analysis and microscopic characters.

In the present study, the same test has been conducted under the long UV and short UV rays and green, pale green and brown, dark brown colour has been noticed on treatment with water extract, Petroleum Ether extract, Chloroform extract, Alcoholic extract and Acetone extract. The two plant powders manifest fluorescence where in various shades of greens are found to be predominant, proving their purity (Table IV and Table V).

TABLE IV. FLUORESCENCE ANALYSIS OF *Lippia nodiflora*

S.No.	Treatments with Extracts	Long UV	Short UV
1.	Water	Green	Pale green
2.	Petroleum ether	Dark green	Green
3.	Chloroform	Dark green	Pale green
4.	Alcohol	Green	Pale green
5.	Acetone	Dark green	Green

TABLE V. FLUORESCENCE ANALYSIS OF *Thea sinensis*

S.No.	Treatments with Extracts	Long UV	Short UV
1.	Water	Dark brown	Brown
2.	Petroleum ether	Dark green	Green
3.	Chloroform	Green	Pale green
4.	Alcohol	Dark green	Green
5.	Acetone	Dark green	Pale green

The same test has been conducted, under long and short UV rays with the extract of ***Thea sinensis.***

The behaviour of the plant powder was treated with certain chemicals and the colour changes were observed and the results were recorded and placed in Table VI.

TABLE VI. FLUORESCENCE ANALYSIS OF PLANT POWDER

Lippia nodiflora* and *Thea sinensis

S.No.	Treatments	Colour Under Visible Light	
		Lippia nodiflora	***Thea sinensis***
1.	Powder	Green	Dark green
2.	Powder + NaOH	Yellowish brown	Blackish brown with broth
3.	Powder + 1N NaOH in Methanol	Dark green	Dark brown
4.	Powder + Hcl	Dark pink colour	Dark yellow colour
5.	Powder + NaOH + H_2O	Dark yellowish green	Dark brownish black with broth
6.	Powder + Ethanol	Pale yellowish green	Bright lemon yellowish colour
7.	Powder + 50% HNO_3	Orange colour with broth	Dark reddish orange colour
8.	Powder + 50% H_2So_4	Blackish brown	Dark shiny brown
9.	Powder + Ferric - Chloride	Green with mild broth	Dark green with broth
10.	Powder + Glacial acetic acid	Yellowish green	Dark yellow

Pandey *et al.* (1984) and Gupta *et al.* (1977) treated plant powders with different chemical reagents and observed their behaviour. In the present research similar pharmacognostic study has been done.

So the above mentioned fluorescent behaviour helps to identify and discriminate different species of *Lippia* and *Thea.*

Phytochemical Screening

The active principles in a drug are most important in curing the diseases and the supreme task is to link the drugs according to the constituents, in order to construct a pharmaco - chemical method that leads to pharmacognostical studies (Pasquale, 1984).

Phytochemical analysis intend to serve as a major resource for information on analytical and instrumental methodology in plant science as was reported in "Phytochemical analysis" (Ling, 2004).

In the present study, it has been found that Cellulose, Protein, Starch, Quinone, Flavonoid, Suberin, Sugar, Steroid, Phenol, Fat, Saponin were present. All the phytochemical tests showed the positive result except starch. The reports were narrated in Table VII.

Level of vitamin A is increased after the consumption of green tea. Vitamin A is reported to play a vital role in suppressing carcinogenesis by increasing immunity to tumours through several mechanisms. Vitamin A deficiency has been associated with a higher incidence of cancer and increased carcinogenesis (Taehibana *et al.*, 1984).

Rastogi and Mehrotra (1991) in their phytochemical analysis of *Thea sinensis L.* observed the presence Gallic acid, P-coumari and caffeic acids isolated from leaves and palmitic, stearic, oleic and linoleic acids as major components and lauric, myristic, gaeloleic and erucic acids as minor components identified by TLC in seed oil; campesterol, brassicasterol, stigmasterol and β-sitosterol identified in sterol fraction.

A complex mixture of hydrocarbons like calamene and β-caryophyllene, phenethyl alcohol, linalool, P-cymen-3ol and oxygenated compounds like Methyl salicylate were noticed in their pharmacognostical study on the plant *Lippia nodiflora* by Rastogi and Mehrotra (1993).

Verma *et al.,* (1993) have detected the presence of Starch, Cellulose, Tannin, Sugar, Protein, Fat and Latex in the aerial roots and *Ficus bengalensis,* during their phytochemical study.

Thamaraikani and Poornima (2003) reported in their phytochemical analysis of *Artemesia parviflora* Roxb. presence of Carbohydrates, Proteins, Aminoacids, Tannins, Phenols, Reducing sugars and Chlorophyll in the alcoholic extract of fresh leaves.

Joshi and Submis (1989) have discussed in their paper about the phytochemical screening for Alkaloid, Saponin and Tannins of 182 samples consisting of 142 species which are used as a medicine either in indigenous system (or) by original tribes of South Gujarath forests.

TABLE VII. PHYTOCHEMICAL TEST FOR THE PLANT EXTRACT

S.No.	Test for	Nature of Colour change		Phytochemical change
		Lippia nodiflora	*Thea sinensis*	
1.	**CELLULOSE TEST** Extract + Iodine followed by H_2SO_4	Black colour	Black colour	Cellulose present
2.	**STARCH TEST** Extract + Iodine solution	No colour change	No colour change	Starch absent
3.	**PROTEIN TEST** Extract + Millions reagent	Yellow colour	Bright yellow colour	Protein present
4.	**PROTEIN TEST** Picric acid solution was added to the substance	Yellow colour	Yellow colour	Protein present
5.	**FIXED OIL AND FAT TEST** The powder was added with Sudar III	Shiny orange colour	Shiny orange colour	Fat and oil present
6.	**FLAVONOID TEST** The substance was added with 10% NaOH	Yellow colour	Dark yellow colour	Presence of Flavonoid
7.	**QUINONE TEST** The substance was added with conc. Hcl.	Pink colour	Lemon yellow colour	Quinone present
8.	**SAPONIN TEST** substance shaken in water	Brothing absent	Brothing absent	Saponin present
9.	**SAPONIN TEST** Substance was added with Lead acetate	White precipitate	White precipitate	Saponin present
10.	**SUBERIN TEST** The powder was heated with conc. H_2SO_4	Black colour	Black colour	Suberin present
11.	**SUGAR TEST** The powder was added with Anthrone reagent followed by conc. H_2SO_4 and heated.	Blue colour	Blue colour	Sugar present

S.No.	Test for	Nature of Colour change		Phytochemical change
		Lippia nodiflora	*Thea sinensis*	
11.	**SUGAR TEST** The powder was added with anthrone reagent followed by conc. H_2SO_4 and heated.	Blue colour	Blue colour	Sugar present
12.	**STEROID TEST** Substance was added with chloroform and a drop of Acetic acid and heated a drop of conc H_2SO_4 was added along the side of the tube.	Red colour	Red colour	Steroid present
13.	**STEROID TEST** Chloroform and conc. H_2SO_4 is added to alcoholic plant extract.	Red colour	Pale red colour	Steroid present
14.	**PHENOL TEST** Plant extract + Ferric Chloride solution.	Blackish green in colour	Blackish green in colour	Phenol present

Lippia nodiflora and *Thea sinensis* contains Saponin and Alkaloids. So, these chemicals may constitute the mechanism of curing of different diseases.

James and James (2002) have conducted phytochemical investigation of plants used in African traditional medicine which will help the future generation to trace out new medicines for different types of diseases (with these phytochemicals). Preliminary phytochemical analysis for Alkaloids, Carbohydrates, Tannins, Phenols, Flavonoids and Saponin were carried out in *Maliqueca langifolia* as was reported by Kannan and Jagadeesan (2001).

Green tea is a rich source of Flavonoids which are 10 to 20 times more powerful than vitamin C and has the ability to retard the progression of eye lens cataract.

Flavonoids are antioxidants molecules found in plant sources such as fruits, flowers, roots, stems, tea, wines, grains and vegetables. They are responsible for the beautiful colouring of plant structures. Flavonoids have antiviral, antifungal, anti-flammatory and carcinogenic effects *in vitro.*

Green tea is consumed daily between the meals (or) after meals in Japan and other Asian countries. In recent years green tea and its major polyphenolics have been demonstrated to prevent chemically induced tumours in a variety of experimental animal model system.

However, green tea catechins exhibited excellent ability in scavenging H_2O_2 and O_2, the precursors and hydroxide. The individual catechins and theoflavins which were isolated from green tea and black tea irrespectively and effectively inhibited the production of superoxide generated by phenazrine methosulphate and NADH, the excellent superoxide scavenging ability of catechins and theoflavins may be related to their potent antimutagenic and anticarcinogenic activities (Chen *et al.*, 1997).

Green tea is the rich source of vitamin - C (370 ± 2.5mg/100g). The catalase activity is high in green tea (i.e.,) 2.4±0.6. It is found to possess highest peroxidase activity (i.e) 217.3±0.85 (Revathi, 2003).

In the present investigation, the combined extract exhibited the black colour, which showed the presence of alkaloids (Table VIII). This coincides with the results of Sankar and Datta (1989) who had conducted phytochemical analysis of leaf of *Melia azadirach* Linn. and have found that leaves of *M.azadirach* contained alkaloids. *Lippia nodiflora* and *Thea sinensis* also showed positive results during the alkaloid test.

TABLE VIII. TEST FOR ALKALOIDS

S.No.	Test for	Reagents	Nature of Colour changing	Phytochemical change
1.	Alkaloid	Extract + Mayer's Reagent	Black colour	Alkaloid present
2.	Alkaloid	Extract + Wagner's Reagent	Black colour	Alkaloid present
3.	Alkaloid	Extract + Suberin	Black colour	Alkaloid present
4.	Alkaloid	Extract + Chloroform + Con.H_2SO_4	Black colour	Alkaloid present
5.	Alkaloid	Dragendraff's Reagent	Black colour	Alkaloid present

Biochemical Analysis

The biological activities of the medicinal plants are mainly due to their biochemical constituents. Most of the biochemicals found in an organism are the result of the metabolic pathways. These biochemicals are formed as intermediary products.

However the early 90's have seen a huge surge of scientific research on green tea. Consequently, exciting claims started to spread that

green tea reduced the risk of cancer and protect body against heart attacks (Gitanjali, 1992).

With green tea the leaves did not oxidize but are steamed and packed to better presence of the natural active substance of the leaf. Green tea protects the body against cardiovascular disease, stroke cataract, aging DNA damage (Srinivasan, 2002).

Qualitative phytochemical analysis

The extracts were subjected to qualitative chemical tests for detection of various plant constitutents. These tests indicate the presence of protein, carbohydrate, phenolic compounds and reducing sugar etc.

Estimation of protein

Biochemical studies on the leaf powder of the plants *Lippia nodiflora* and *Thea sinensis* in powdered and extract condition reveal that the protein content in 100g leaves of *Lippia nodiflora* in powdered form it has been 5.72 g, 6.02 g, 6.47 g, 7.01 g and 7.52 g per 100 g powder. In case of water extract of the plant, the protein content has been 6.03 g, 6.72 g, 7.11g, 7.93 g and 8.42 g (Table IX and Fig 1).

In the powdered form of *Thea sinensis* it has been 6.42 g, 6.89g, 7.29 g 7.73 g and 8.17 g per 100 g powder. In case of water extract of the plant, the protein content has been 7.13 g, 7.92 g, 8.43 g, 8.94 g and 9.19 g (Table X and Fig. 2).

TABLE IX. ESTIMATION OF TOTAL PROTEIN IN *Lippia nodiflora*

S.No.	Volume of Test sample used for the test	Protein Value (g/100g)	
		In powdered form	In water extract
1.	0.2	5.72	6.03
2.	0.4	6.02	6.72
3.	0.6	6.47	7.11
4.	0.8	7.01	7.93
5.	1.0	7.52	8.42

TABLE X. ESTIMATION OF TOTAL PROTEIN IN T*hea sinensis*

S.No.	Volume of Test sample used for the test	Protein Value (g/100g)	
		In powdered form	In water extract
1.	0.2	6.42	7.13
2.	0.4	6.89	7.92
3.	0.6	7.29	8.43
4.	0.8	7.73	8.94
5.	1.0	8.17	9.19

Only a light variation was observed in the values of protein present in dried powder and water extracts of *Lippia nodiflora* and *Thea sinensis.*

The protein content was estimated in the above two plants. When we compare the protein content in these two plants, the amount of protein in *Thea sinensis* was found to be greater than in *Lippia nodiflora.*

When we compare the two samples taken from the same plants, in which one is in powder form, other is water extract, the later is found to contain more amount of protein.

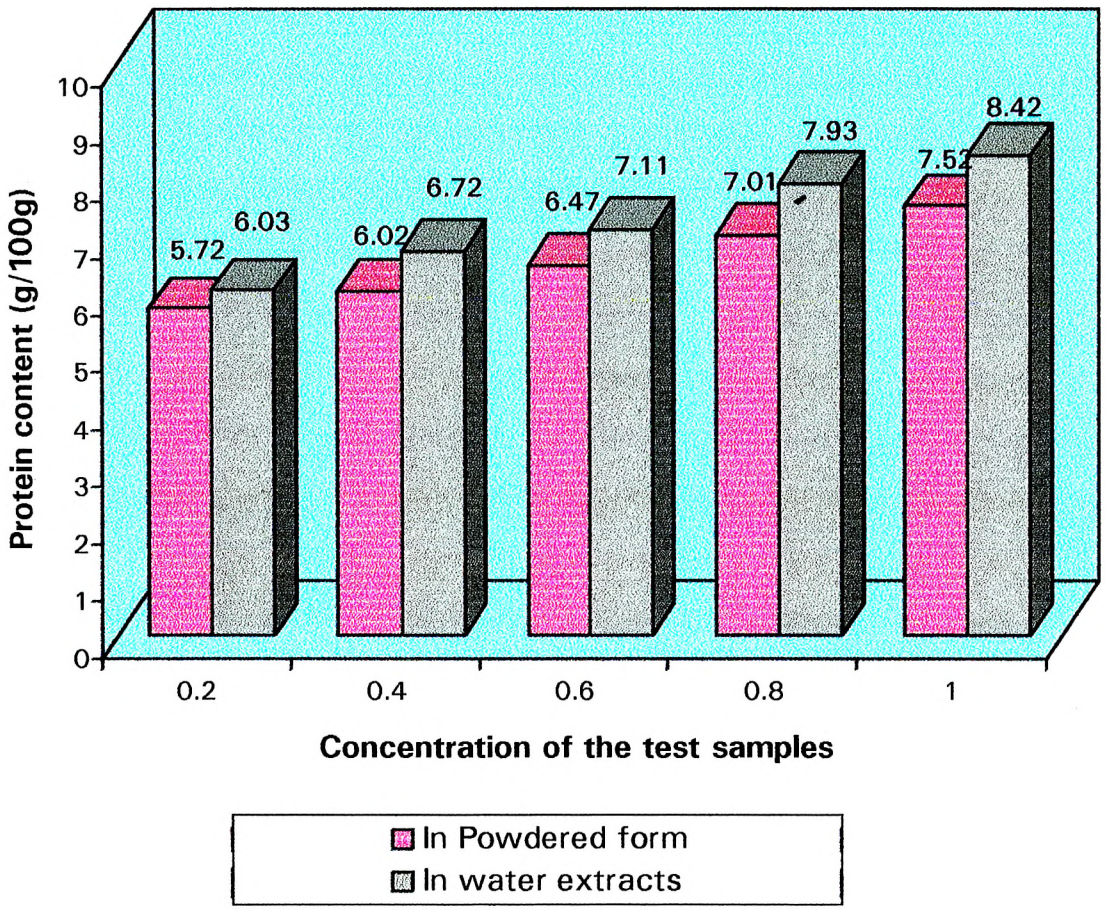


FIGURE 1. ESTIMATION OF TOTAL PROTEIN IN *Lippia nodiflora*.

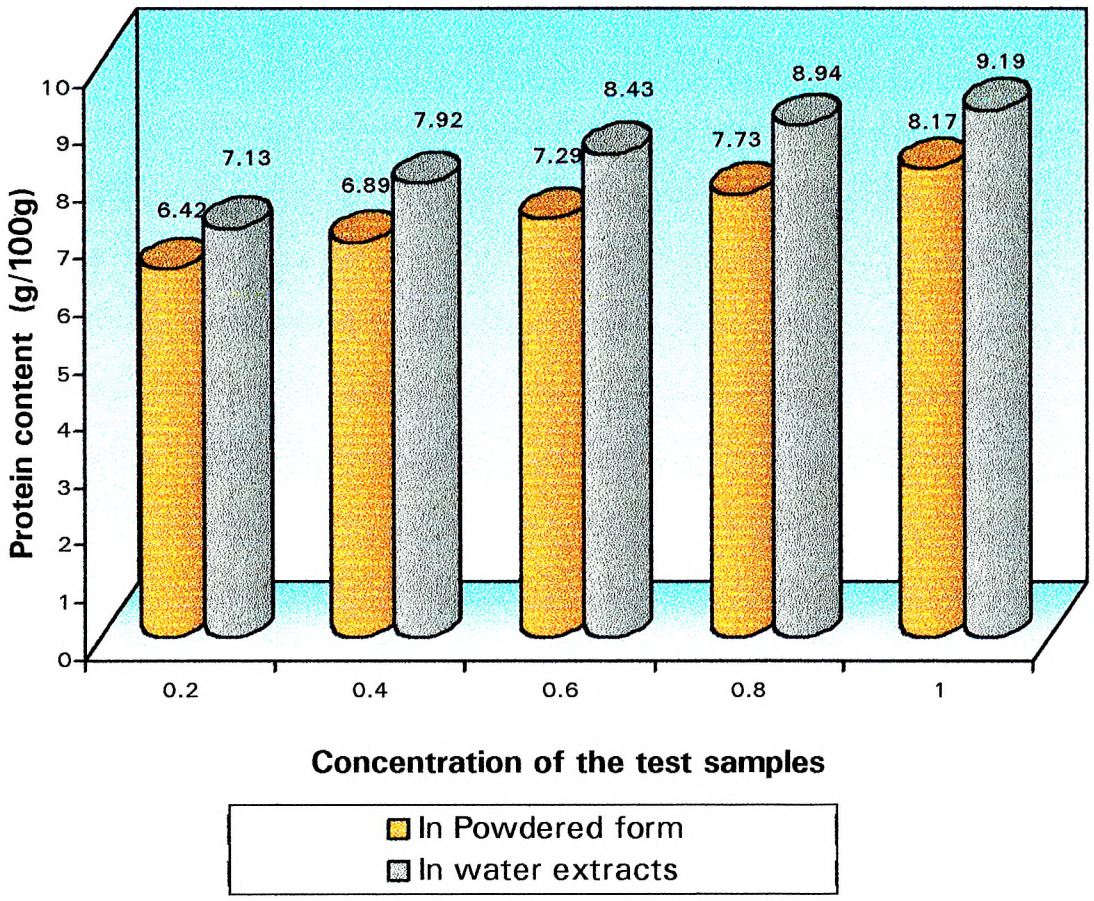


FIGURE 2. ESTIMATION OF TOTAL PROTEIN IN *Thea sinensis*.

The protein content of arugampul (*Cynodon dactylon*), karpuravalli (*Coleus aromaticus)* and thulasi *(Ocimum sanctum)* were found to be more in 40% saturation of ammounium sulphate fractionation and for thulasi 60% saturation of ammonium sulphate fractionation. (Annapurani and Bhagavathi, 2001).

Some lichens have high protein content. For example *Pelifigeria canina* have 21% *and Lobaria isidiosa* have 22% Protein (Ramakrishnan and Venkat Rao, 1995).

The protein content of six green leaves were Green tea (6.0 g), *Amaranthus* (3.2g), Agathi (8.5g), Mint (5.0g), Coriander (3.5g) and *Fenugreek* (4.4g). Among the six leaves the protein content is found to be maximum in *Agathi* (i.e.,) 8.5 g/100g when compared to the other. The protein content of *Amaranthus* is less (i.e.,) 3.2 g/100g. The protein content of green tea, mint, coriander and *Fenugreek* was less than Agathi. (Revathi, 2003)

Udayakumar *et al.* (2003) also noticed and discussed about the amount of protein present in the plant *Solanum xanthocarpum*. He estimated the protein from the leaves of *S.xanthocarpum* by Lowry method.

Estimation of total carbohydrate

Carbohydrates are a class of energy yielding substances. 100 g leaves of *Lippia nodiflora* in powdered form contains 10.02 g, 10.71g, 11.22g, 11.57g and 12.32 g of carbohydrate and the amount of carbohydrate present in water extract was 11.12, 11.72g, 12g, 12.22g, 12.83 g and 13.17 g (Table XI and Fig 3).

TABLE XI. ESTIMATION OF CARBOHYDRATE IN *Lippia nodiflora*

S.No.	Volume of Test sample used for the test	Carbohydrate Value (g/100g)	
		In powdered form	In water extract
1.	0.2	10.02	11.12
2.	0.4	10.71	11.72
3.	0.6	11.22	12.22
4.	0.8	11.57	12.83
5.	1.0	12.32	13.17

TABLE XII. ESTIMATION OF CARBOHYDRATE IN *Thea sinensis*

S.No.	Volume of Test sample used for the test	Carbohydrate Value (g/100g)	
		In powdered form	In water extract
1.	0.2	11.03	13.22
2.	0.4	11.58	13.97
3.	0.6	12.02	14.62
4.	0.8	12.84	15.19
5.	1.0	13.01	15.78

In *Thea sinensis*, the powdered form of leaves contained 11.03g, 11.58g, 12.02g, 12.84 g and 13.01g. In case of water extract, the carbohydrate content has been 13.22 g, 13.97g, 14.62 g, 15.19g and 15.78 g (Table XII and Fig. 4).

The carbohydrate content was estimated in the above two plants. When we compare the carbohydrate content in these two plants, the amount of carbohydrate in *Thea sinensis* was found to be greater than *Lippia nodiflora.*

When we compare the two sample (i.e. powder and water extracts) taken from the same plants, the water extracts have more amount of carbohydrate compared with powder forms as shown in Table XI and XII.

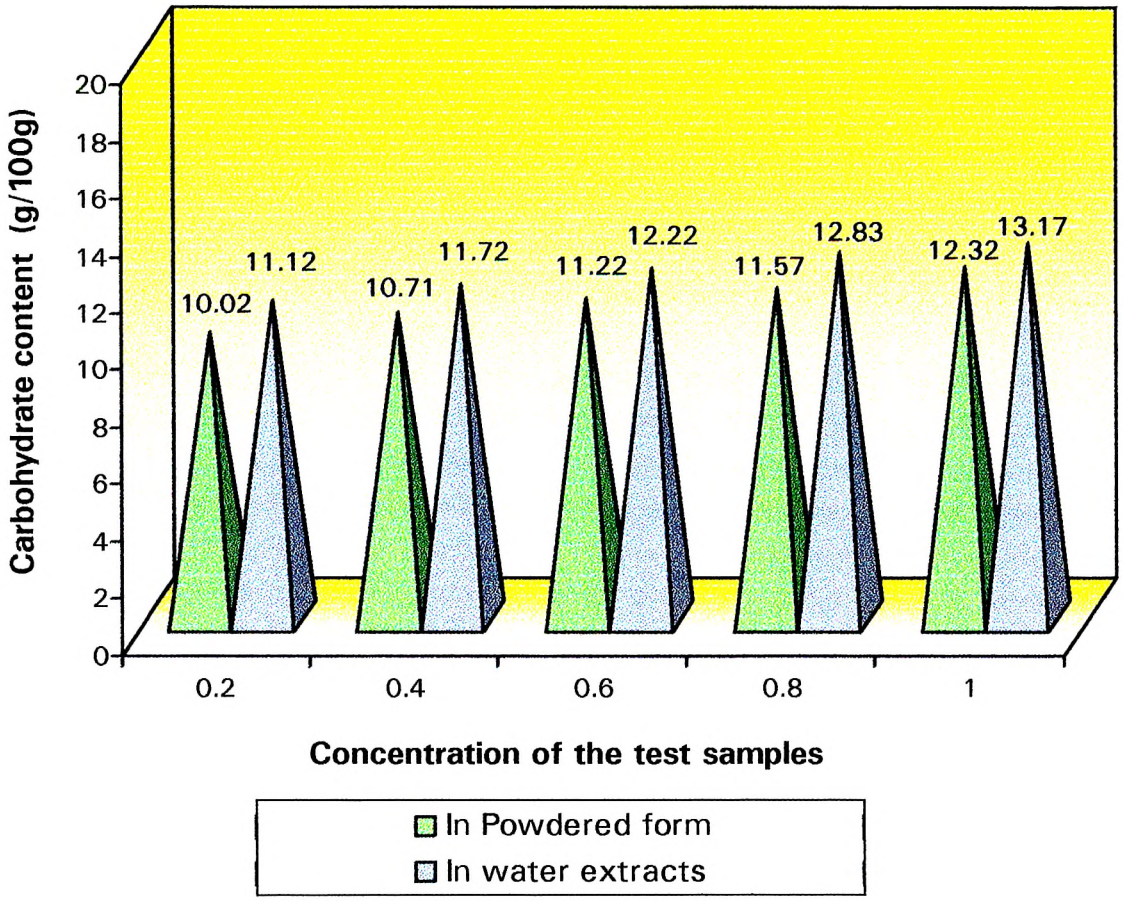


FIGURE 3. ESTIMATION OF TOTAL CARBOHYDRATE IN *Lippia nodiflora*.

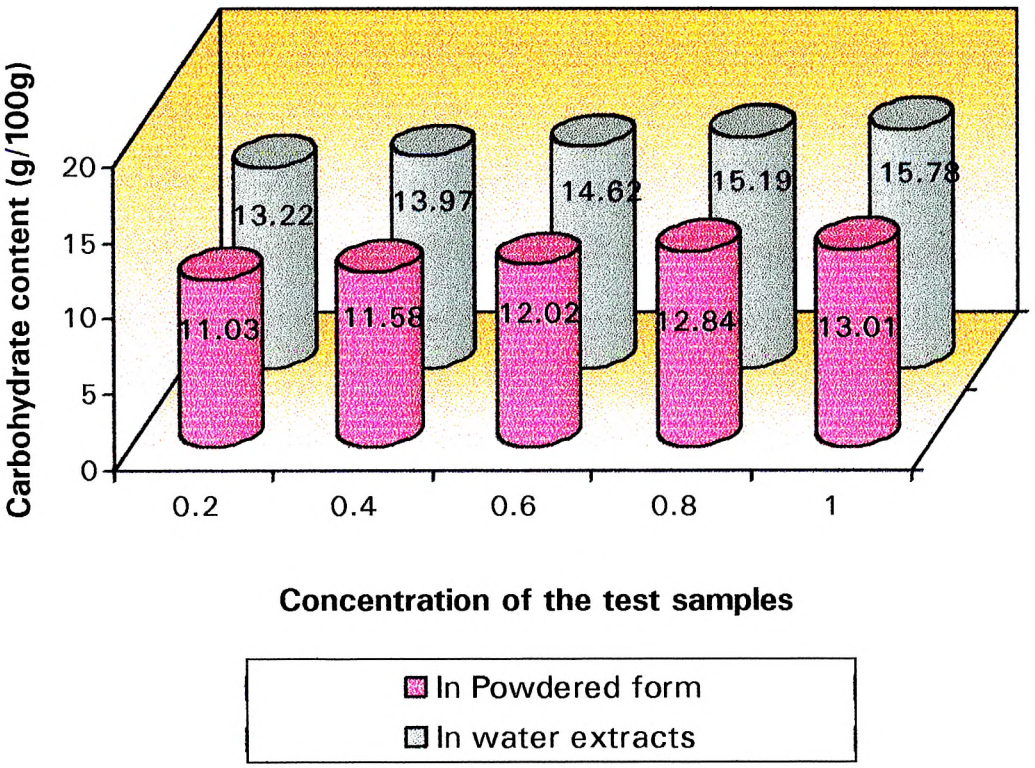


FIGURE 4. ESTIMATION OF TOTAL CARBOHYDRATE IN *Thea sinensis*.

Naseer *et al.* (2003) estimated carbohydrate by Anthrone reagent method in *Amaranthus viridis* and *Spinaccae olcracca* in which *A. viridis* have higher carbohydrate content (3.562 mg/g) than *Spinanceae oleracea. Lippia nodiflora* and *Thea sinensis* have higher percentage (13.17g and 15.78g) of carbohydrate when compared with *A. viridis.*

The green tea is the richest in carbohydrate content (i.e.,) 12 ± 0.5 g/100g. Agathi has the least content of carbohydrate (.e,) 0.04 ± 100g. The carbohydrate content of the coriander is surprisingly comparable to that of *Fenugreek* (i.e) 6.3 ± 0.2g/100g, 6.0 ± 0.13 g/100g respectively (Revathi, 2003).

Drumstick leaves and curry leaves contain high amount of carbohydrate when compared to other green leaves. The amount of carbohydrate present in these greens are 12.5g/100g, 18.7 g/100 mg respectively (Ramakrishnan and Venkat Rao, 1995).

Estimation of total phenol

The amount of phenol present in 100g of leaves of *Lippia nodiflora* in powdered form has been 10.32g, 10.79g, 11.05g, 11.47g and 12.02g. The content of phenol in water extract of the plant has been 11.25g, 11.97g, 12.52g, 13.73g and 14.22g.

In the leaf powder of *Thea sinensis* the phenol content has been 17.02 g, 17.49 g, 18.03 g, 18.77 g and 19.03 g. The content of phenol in water extract has been 19.21 g, 20.28 g, 20.97 g, 21.63g and 22.45 g.

The data on phenolic content recorded in all the two forms of *Lippia nodiflora* and *Thea sinensis* is presented in the Table XIII, XIV and Fig. 5, 6).

TABLE XIII. ESTIMATION OF PHENOL IN *Lippia nodiflora*

S.No.	Volume of Test sample used for the test	Phenol Value (g/100g)	
		In powdered form	In water extract
1.	0.2	10.32	11.25
2.	0.4	10.79	11.97
3.	0.6	11.05	12.52
4.	0.8	11.47	13.73
5.	1.0	12.02	14.22

TABLE XIV. ESTIMATION OF TOTAL PHENOL IN *Thea sinensis*

S.No.	Volume of Test sample used for the test	Phenol Value (g/100g)	
		In powdered form	In water extract
1.	0.2	17.02	19.21
2.	0.4	17.49	20.28
3.	0.6	18.03	20.97
4.	0.8	18.77	21.63
5.	1.0	19.03	22.45

The phenol content was estimated in the above plants. When we compare the phenol content in these two plants, the amount of phenol in *Thea sinesis* was found to be greater than *Lippia nodiflora*.

When we compare the two samples taken from the same plants, in which one is in powder form and the other is water extract, the later is found to contain more amount of phenol.

The amount of phenol present in *Lippia nodiflora* and *Thea sinensis* was assessed and it was compared with the findings of Amudhan *et al.* (1999). They explained the total phenol profile in some rice varieties in relation to infestation by Asian rice gall midge (or) *Seolia Oryzae.*

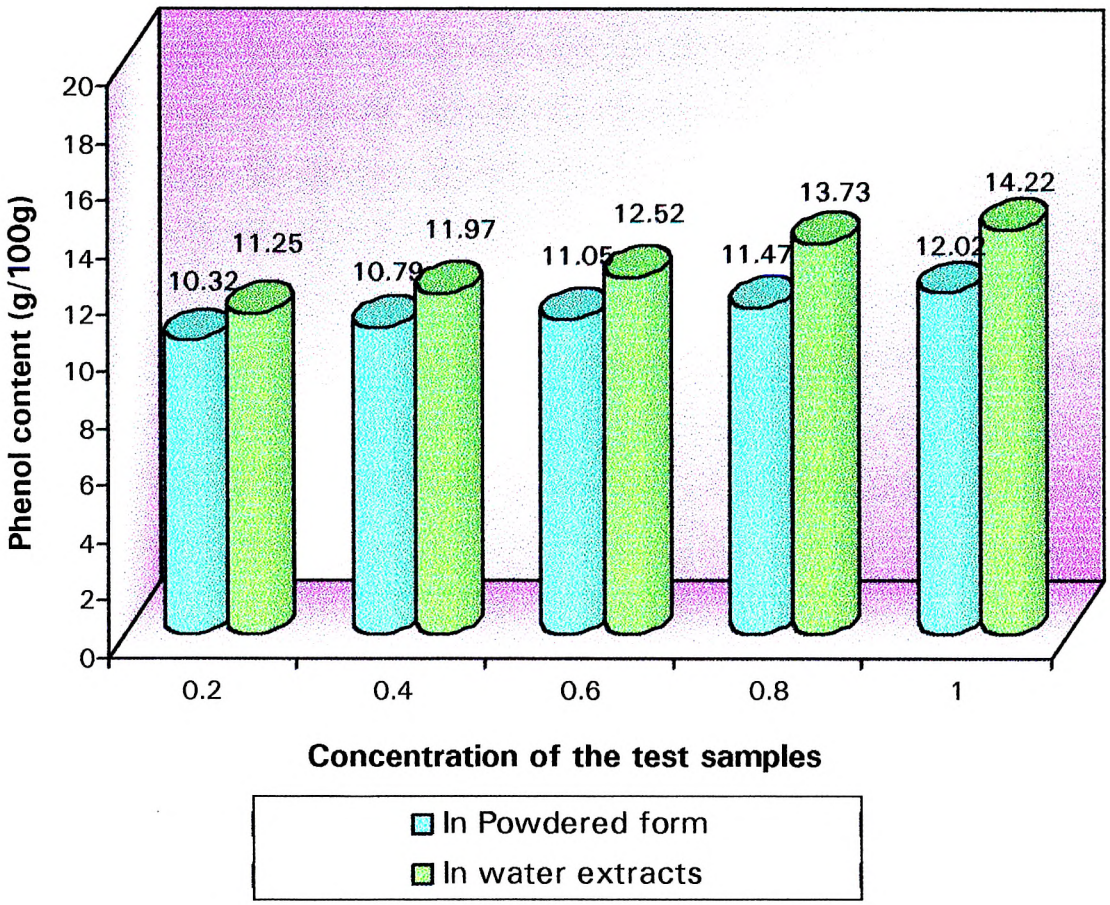


FIGURE 5. ESTIMATION OF TOTAL PHENOL IN *Lippia nodiflora.*

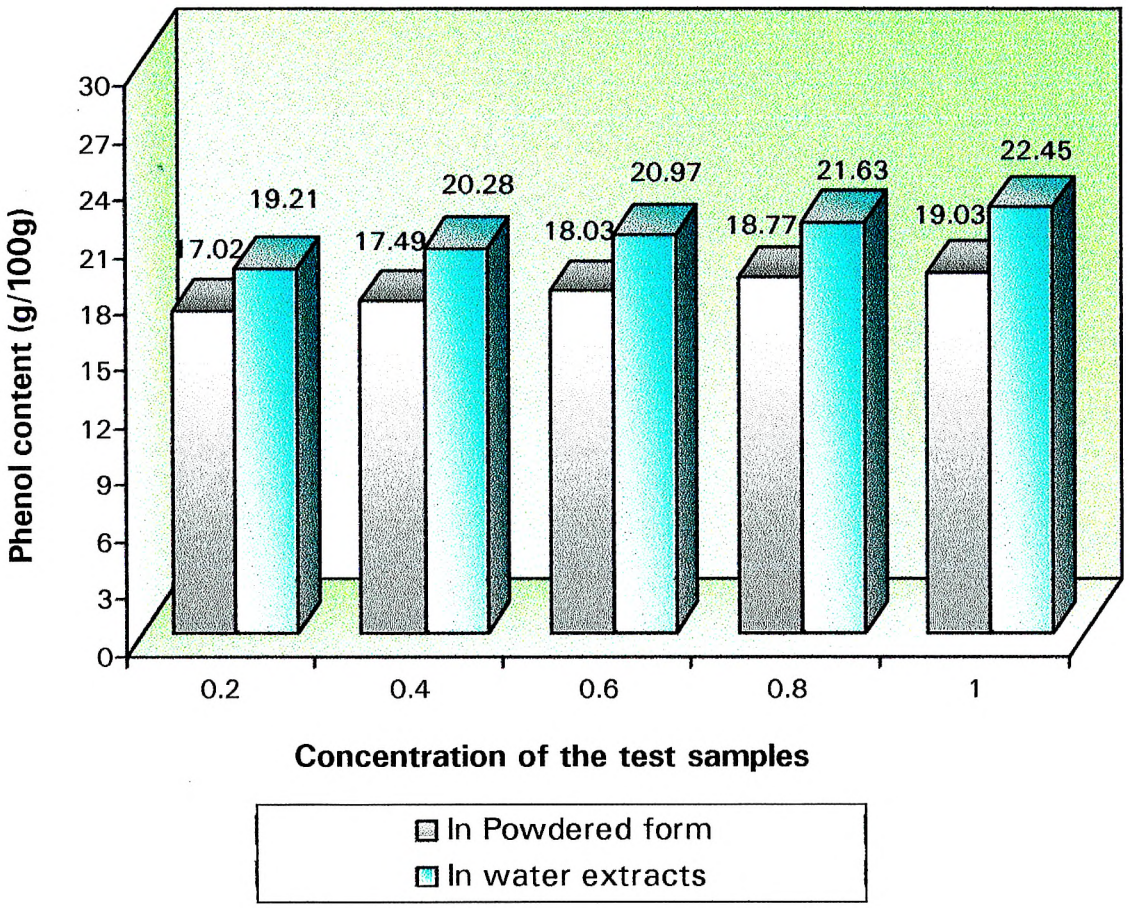


FIGURE 6. ESTIMATION OF TOTAL PHENOL IN *Thea sinensis*.

Apple showed 12.1 mg/100g of total phenols. In herbs like Rose mary and willow herbs high phenolic activities were found. A high phenolic content was found in berries (Kahkonen *et al.,* 1999).

The antioxidants principles, phenolics and phospholipid classes from mango seed, kernals were extracted using the organic solvents. The extract contains 69.5 mg/100g of phospholipids. The antioxidant and anticancer potential of phenolic compounds isolated from olive oils shows, simple and complete phenolic compounds which posses antioxidant properities which may have a protective action in human health (Dinesh *et al.,* 2000).

Estimation of Total soluble sugar

Estimation of total soluble sugar in the leaf of *Lippia nodiflora* showed 9.19g, 9.82 g, 10.37g, 10.97g and 11.43 g in 100g of the powdered form. The water extract of this plant has 10.25g, 10.77g, 11.22g, 11.67g and 12.11g of soluble sugar. So the water extract of the *Lippia nodiflora* possesses more total soluble sugar than in the powdered form which is depicted in Table XV and Fig.7.

In *Thea sinensis* the 100 g of the powdered form of the leaf contain 10.03 g, 10.55 g, 11.01 g, 11.67 g and 12.07 g of the total soluble sugar. The water extract of this plant has 11.15 g, 11.65 g, 12.01 g, 12.77 g and 13.25 g of total soluble sugar. So the water extract of *Thea sinensis* possesses more total soluble sugar than in the powdered form, which is depicted in Table XVI and fig. 8.

TABLE XV. ESTIMATION OF TOTAL SOLUBLE SUGAR IN *Lippia nodiflora*

Sl.No.	Volume of the test sample used for the test	Total Soluble Sugar (g/100g)	
		powdered form	In water extract
1.	0.2	9.19	10.25
2.	0.4	9.82	10.77
3.	0.6	10.37	11.22
4.	0.8	10.97	11.67
5.	1.0	11.43	12.11

TABLE XVI. ESTIMATION OF TOTAL SOLUBLE SUGAR IN *Thea sinensis*

Sl.No.	Volume of the test sample used for the test	Total Soluble Sugar (g/100g)	
		powdered form	In water extract
1.	0.2	10.03	11.15
2.	0.4	10.55	11.65
3.	0.6	11.01	12.01
4.	0.8	11.67	12.77
5.	1.0	12.07	13.25

When we compare the total soluble sugar in these two plants, the amount of soluble sugar in *Thea sinensis* was found to be greater than in *Lippia nodiflora.*

Amount of total soluble sugar present in leaves of extract and dried powder of *Lippia nodiflora* and *Thea sinensis* was observed and then it was compared and discussed with other findings such as total soluble sugar in the leaves of steroid and non-steroid plants (Habib and Seema, 2003).

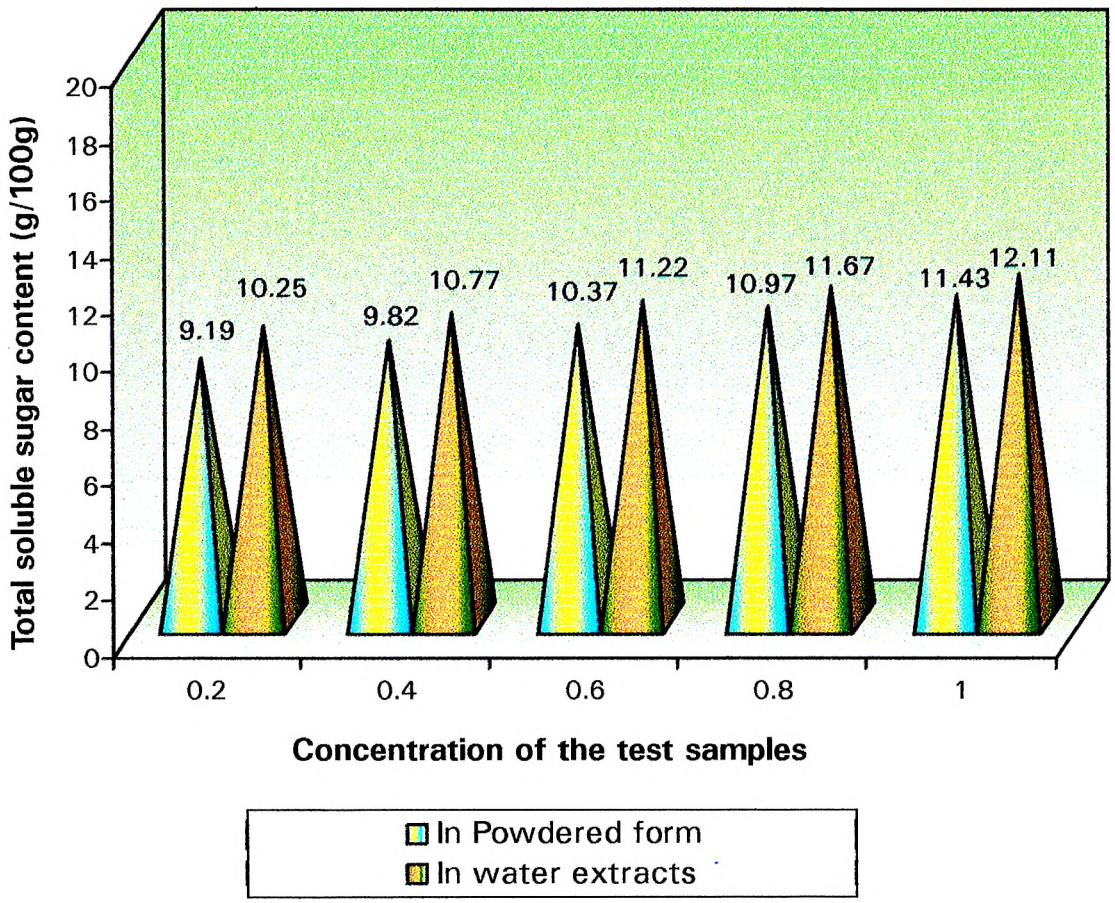


FIGURE 7. ESTIMATION OF TOTAL SOLUBLE SUGAR IN *Lippia nodiflora*.

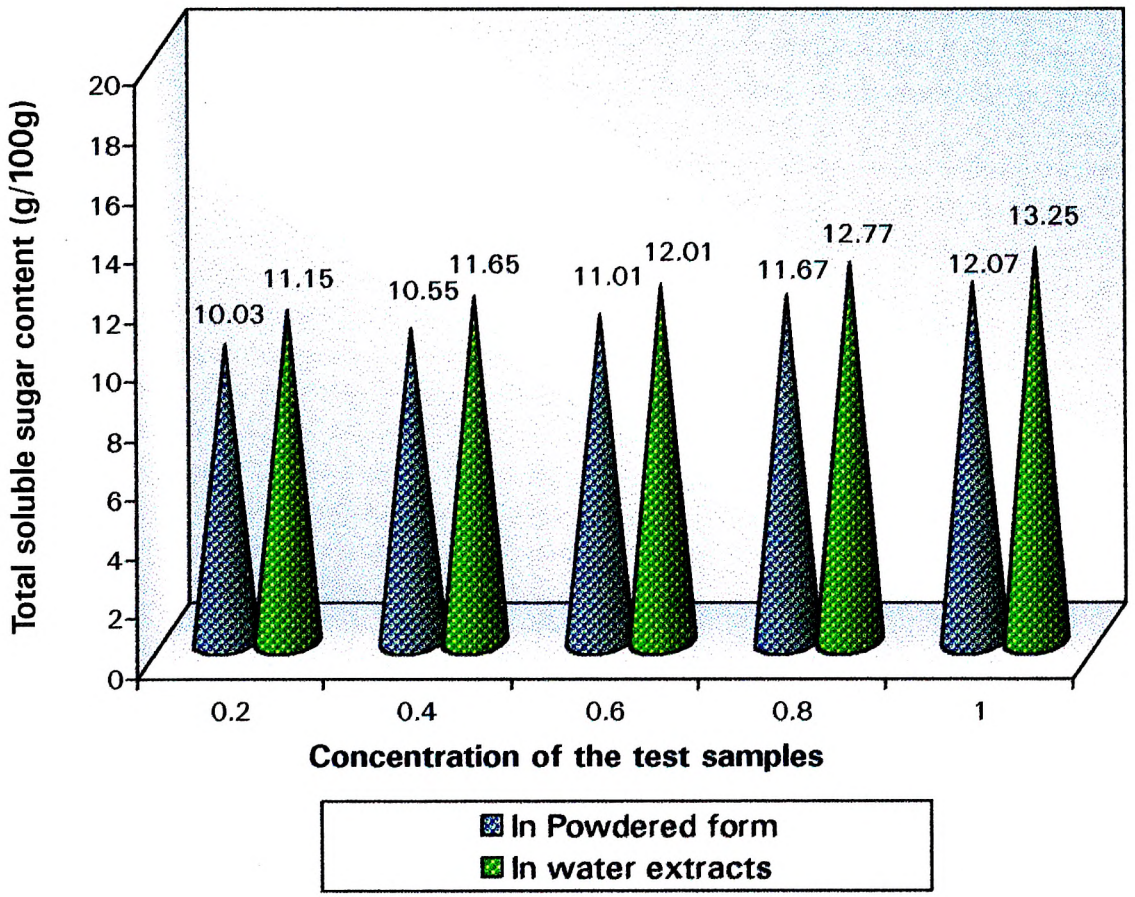


FIGURE 8. ESTIMATION OF TOTAL SOLUBLE SUGAR IN *Thea sinensis.*

DANDRUFF STUDY

In the macroscopic study of Dandruff patient, the scalp was yellowish white scaly in appearance with bad odour (Table XVII). Under microscopic examinations, it showed cluster of small, round budding yeast cells which were interspersed among septate branching hyphae (Table XVIII).

TABLE XVII. MACROSCOPIC EXAMINATION OF DANDRUFF

NAME OF THE PATIENTS : Mrs. Ranganayaki and Suguna from Pollachi.
Miss: Poornima and Krithika from Coimbatore.
Mr. Raja and Ramprasath from Palani.

Sl.No.	Macroscopic Examination of Dandruff Strain	Results
1.	Quantity of Dandruff	5 gm
2.	Consistency	Powdery scales
3.	Odour	Bad odour
4.	Colony appearance	Creamy colonies
5.	Colour	Yellowish white

TABLE XVIII. MICROSCOPIC EXAMINATION OF DANDRUFF STRAIN

Sl.No.	Microscopic Examination of Dandruff Strain	Results
1.	Fungi	Present
2.	Bacteria	Absent
3.	Nature of the cell	Budding yeast cell present
4.	Parasite	Absent
5.	Pigmented cells	Absent
6.	Branched filaments	Present
7.	Mycelium	Present
8.	Elastic fibres	Absent
9.	Curschmann's spirals	Absent
10.	Charcot - Leyden crystals	Absent
11.	Nature of the fungi	Lipophilic fungi present

4.1.5 STUDY OF MICROBIAL FACTOR IN DANDRUFF

In vitro study of Dandruff was conducted in the Jebi lab at Pollachi.

The scales were removed from the scalp of the affected person who had Dandruff problem by blunt scalpel. It was mounted on a slide in 10% KOH solution and stained with Methylene blue 1% for 1 min. and was observed under microscope. The round, budding cell and short hyphae spaghetti and meat balls are characteristic of the fungus *Malassezia furfur* (Jan, 1989). The identified fungus was cultured in the sabouraud's Dextrose Agar medium and it was used for further study (Plate 10, 11,12, 13, 14 and 15).

4.1.7 MICROBIAL SENSITIVITY STUDY

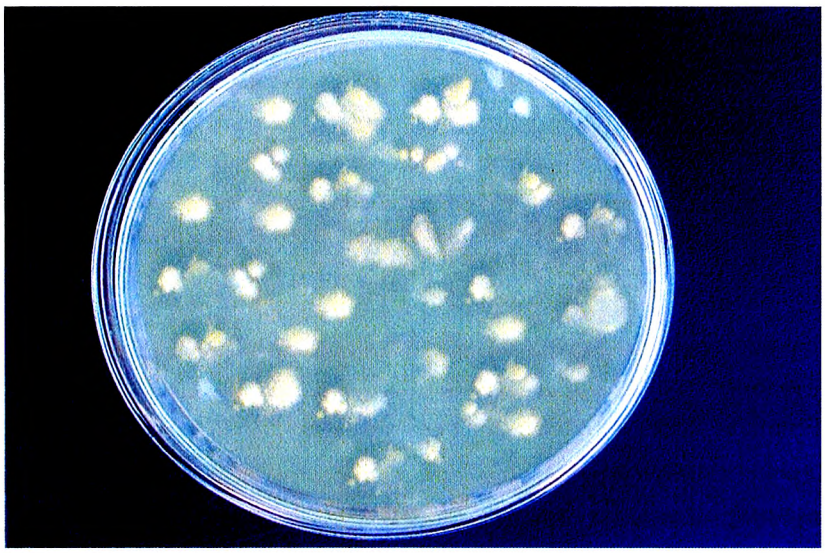
Leaf extracts and prepared herbal oils of *Lippia nodiflora* and *Thea sinensis* were investigated for antifungal activities against *Malassezia furfur* at 0.5 cm disc using disc diffusion and overlapping method. After 3-4 days, a well noticed inhibition zone was found. So the extracts and oils showed high degree of inhibitory activity against *Malassezia furfur.*

The microbial sensitivity test was conducted and the inhibition zone was measured horizontally. Leaf extracts of *Lippia nodiflora* showed a notable inhibition zone (Plate 16 to 19). The petroleum Ether extract showed an inhibition zone nearly 2.5 cm, Chloroform extract showed nearly 3 cm and Ethanol extract showed nearly 4cm inhibition zone. The newly prepared herbal oil showed nearly 4.5 cm inhibition zone.

PLATE - 10

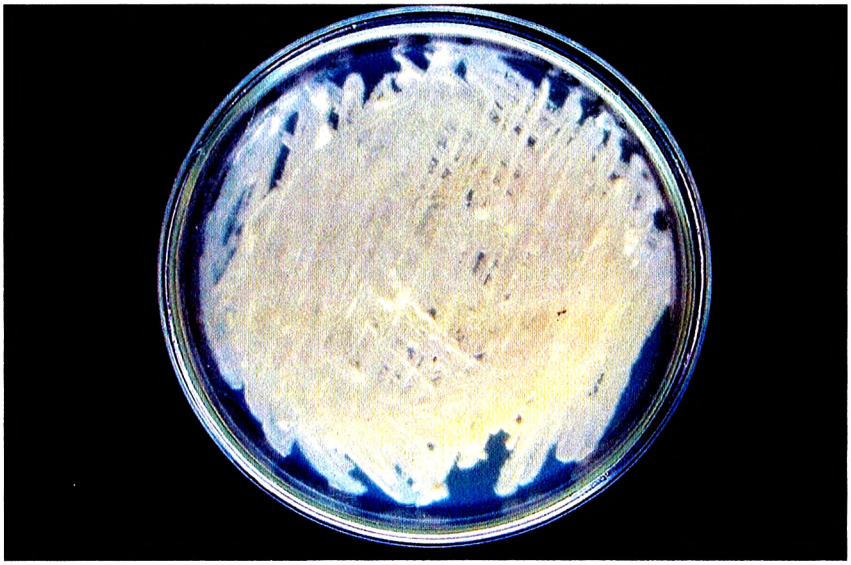
Culture Media for *Malassezia furfur*

PLATE - 11



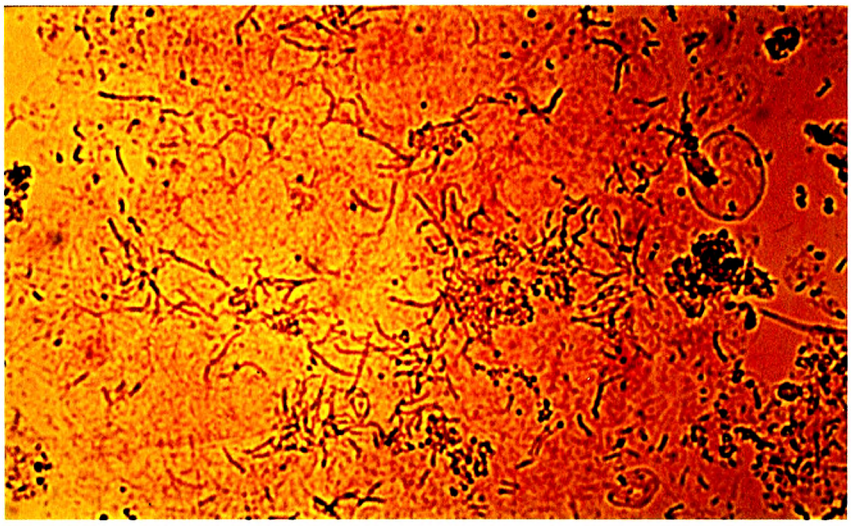
Master Plate of *Malassezia furfur*

PLATE - 12



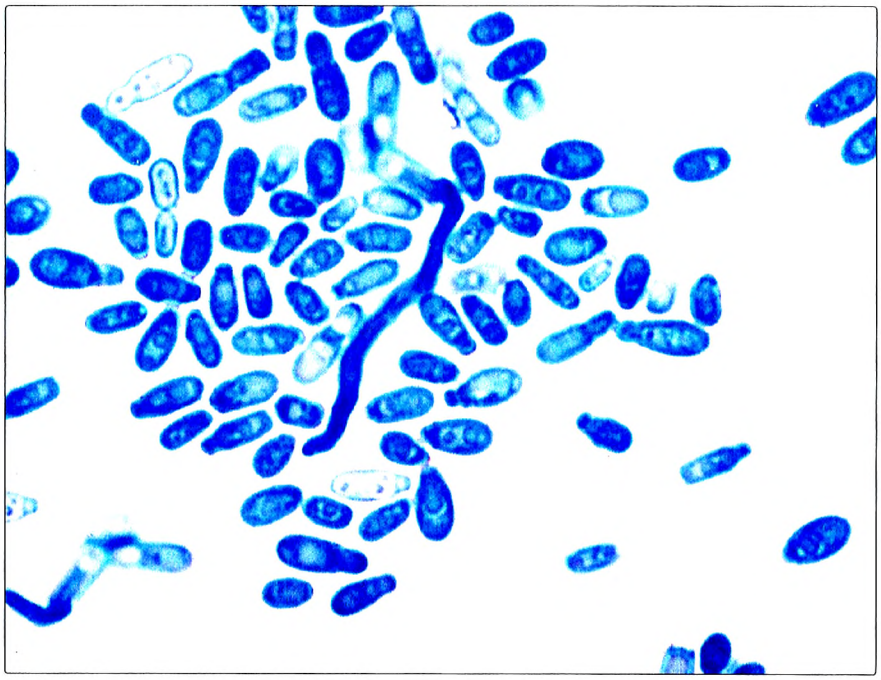
Pure Culture of *Malassezia furfur*

PLATE - 13



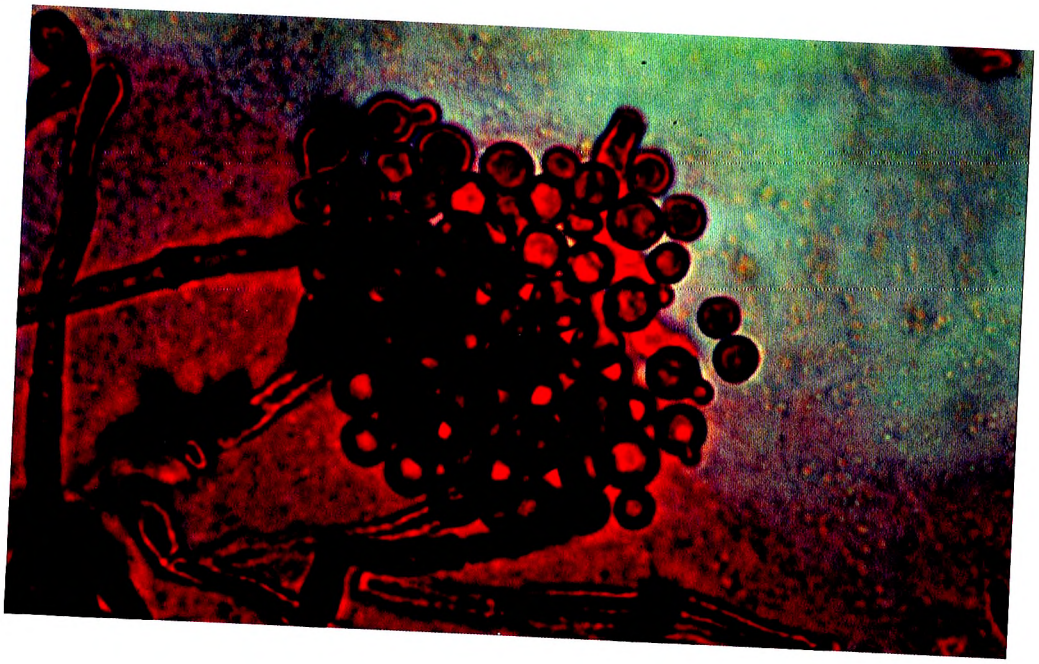
Morphology of *Malassezia furfur*

PLATE - 14



Microscopic view of *Malassezia furfur*

PLATE - 15



Cluster of Yeast Cells of *Malassezia furfur*

Leaf extracts of *Thea sinensis* showed a well notable inhibition zone (Plate 20-23). The Petroleum Ether extract showed an inhibition zone nearly 2.5 cm, Chloroform extract showed nearly 2.5 cm and Ethanol extract showed nearly 4.5 cm inhibition zone. The newly prepared herbal oil showed nearly 5 cm inhibition zone.

The mixture of extracts of two plants (*Lippia nodiflora* and *Thea sinensis)* used for preparing herbal oil showed nearly 6.5 cm inhibition zone (Plate 24).

When we compare the both *Lippia nodiflora* and *Thea sinensis* equal inhibitory zone of 2.5 cm in petroleum ether extract was noticed. Among the two plants, Chloroform extract of *Lippia nodiflora* showed higher inhibitory zone (3 cm) than *Thea sinensis* (2.5 cm). Similarly the ethanol extracts and prepared oil of Thea sinensis showed more inhibition zone, than *Lippia nodiflora.*

The combination of *Lippia nodiflora* and *Thea sinensis* oil showed remarkably higher inhibitory zone of 6.5 cm than the other.

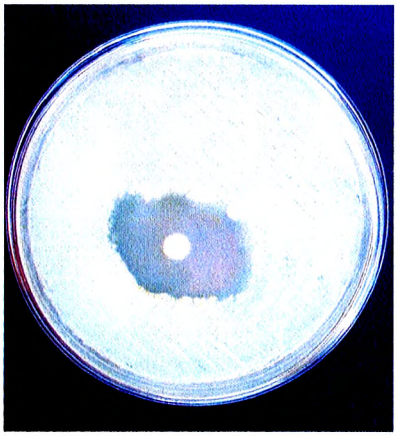
The magnitude of the antiyeast activity demonstrated by the hexane extract of stems from *Plectranthus* suggest that these plant tissues could contain chemical compounds useful in treating infections which was reported by Alabshi *et al.* (1999).

Sudhir (2002) reported that the plant *Boenninghausenia albiflora* oil possesses antifungal activity against *Candida spp* and *Malassezia furfur.*

Anandakumar *et al.* (2003) experimented the extracts of *Wrightia tinctoria* against *Candida albicans* which is an yeast like fungus, *Malassezia*

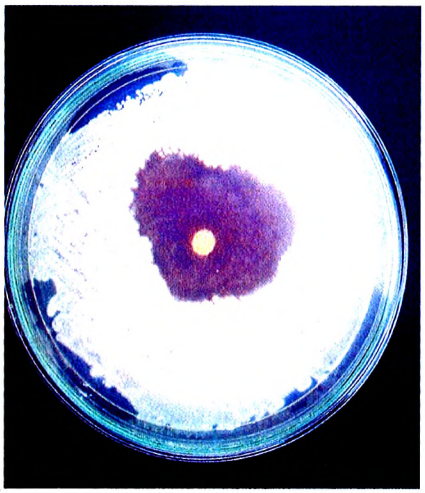
ANTIFUNGAL SENSITIVITY TEST

PLATE - 16



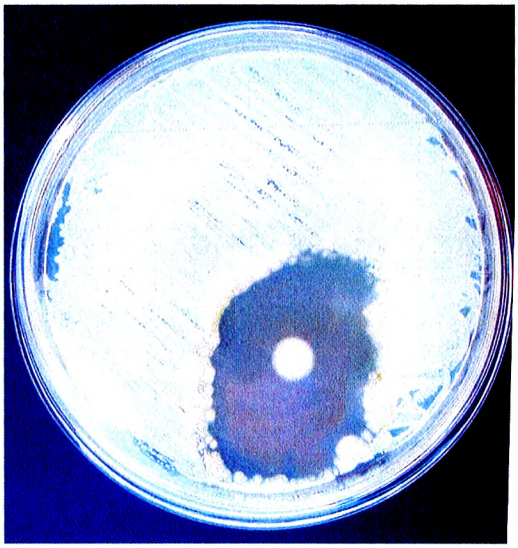
Antifungal extracts of petroleum Ether (*Lippia nodiflora* Rich) Showing the sensitive Zone.

PLATE - 17



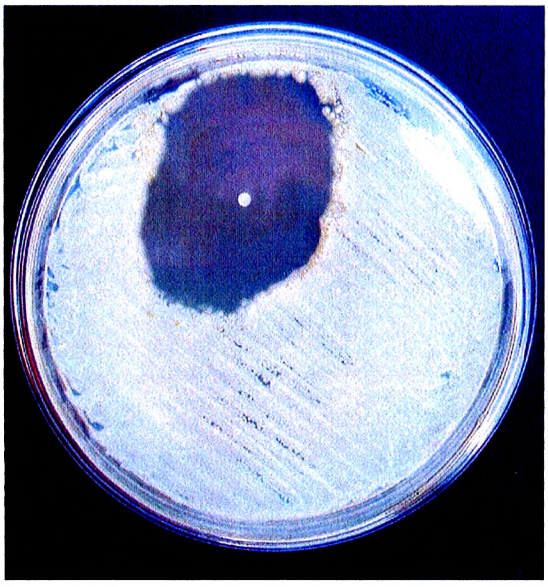
Antifungal extracts of Chloroform (*Lippia nodiflora* Rich) Showing the sensitive Zone.

PLATE - 18



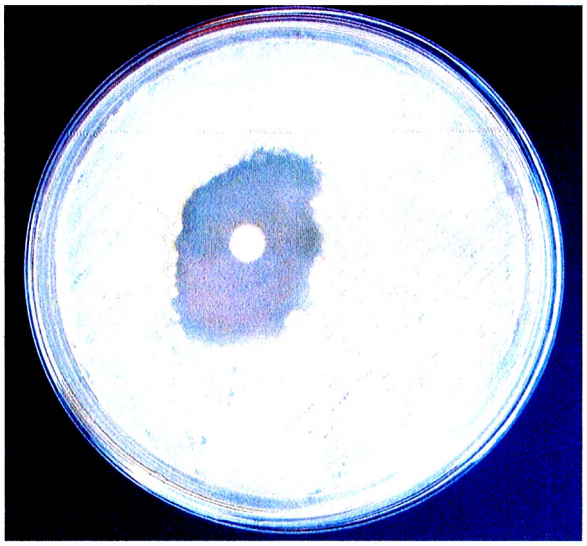
Antifungal extracts of Ethanol (*Lippia nodiflora* Rich) Showing the sensitive Zone.

PLATE - 19



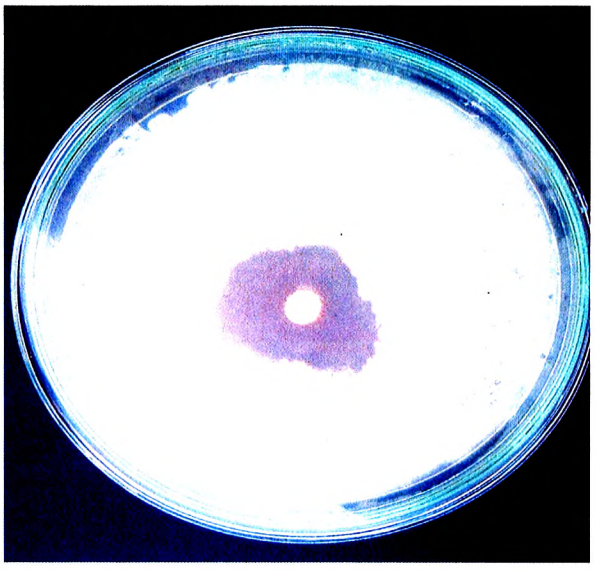
Antifungal Oil (*Lippia nodiflora* Rich) Showing the sensitive Zone.

PLATE - 20



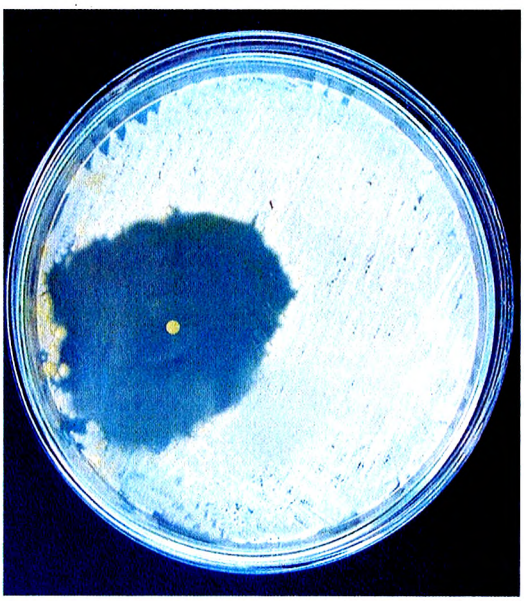
Antifungal extracts of Petroleum Ether (*Thea sinensis* Linn.) Showing the sensitive Zone.

PLATE - 21



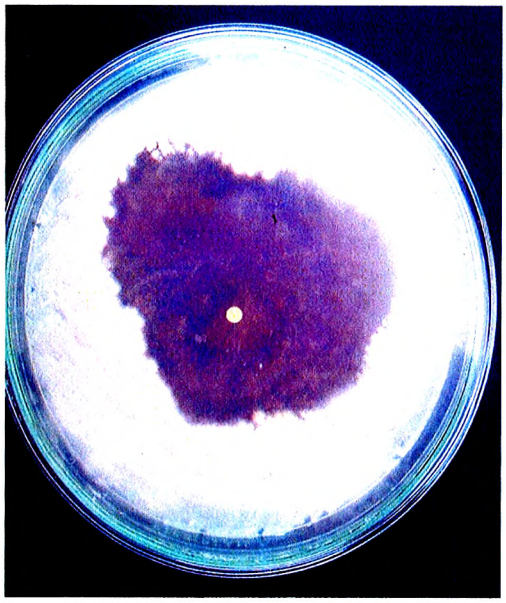
Antifungal Extracts of Chloroform (*Thea sinensis* Linn.) Showing the sensitive Zone.

PLATE - 22



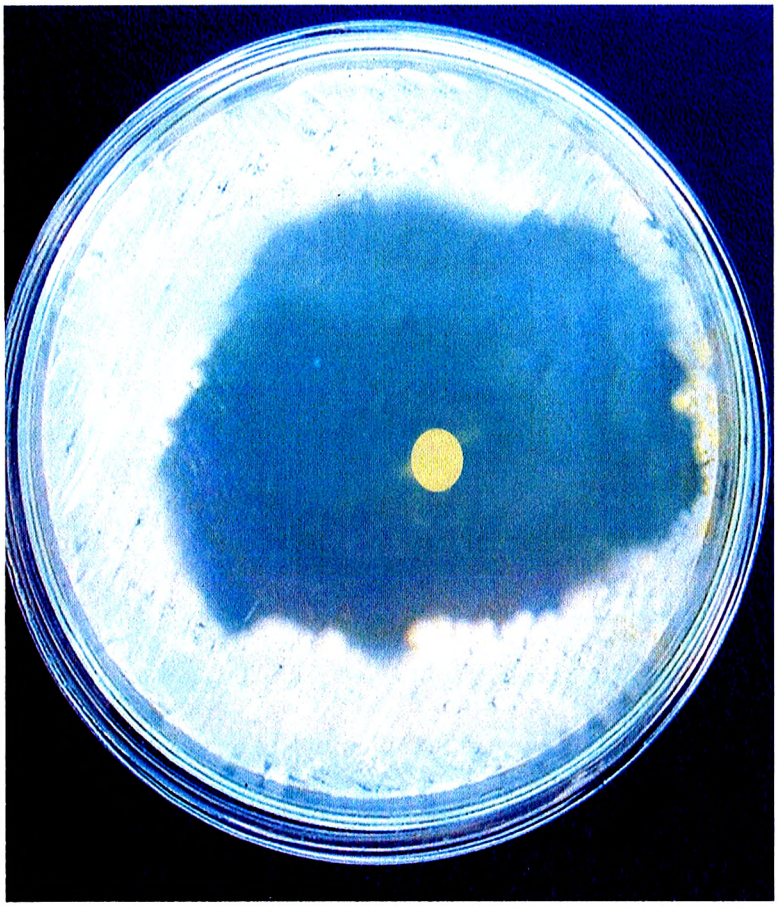
Antifungal extracts of Ethanol(*Thea sinensis* Linn.) Showing the sensitive Zone.

PLATE - 23



Antifungal Oil (*Thea sinensis*, Linn.) Showing the sensitive Zone.

PLATE - 24



Mixture of Oil (*Lippia nodiflora and Thea sinensis*) Showing the sensitive Zone.

furfur by disc diffusion method. The extracts showed high sensitivity against these fungi which proves the antifungal activity of the plant *Wrightia tinctoria.*

Superficial Mycotic infections affecting skin, hair and nails are common among the people living in hot and humid Climates (Bhutani, 1993). Since nail, hair and skin scraping are readily obtained for direct microscopic examination and culture studies superficial mycoses can easily be identified (Tanaka *et al.,* 2003).

Non Keratinolytic fungi cannot penetrate the keratin; feed on the surface lipid film, hence live on and not in the skin, as *Malassezia furfur* (Bhutani, 1993).

4.1.8 HUMAN DATA (CLINICAL STUDY) COLLECTION

The present study was launched with a view to assess the effect of selected herbal plant extracts (*Lippia nodiflora* and *Thea sinensis*) in oil form on Dandruff. From the above results, it is evident that the treatment of the *Lippia nodiflora* and *Thea sinensis* on Dandruff showed excellent effects after completing the course of treatment. The investigation was carried out *in vitro* and external application.

Totally 60 Dandruff patients under 3 groups namely Group A, Group B and Group C were treated with the oil I, oil II and oil III (Table (XIX, XX, XXI). The prepared herbal oils have different ingradients (Table XXII) Maximum number of patients belonged to the Age group of 20-45.

TABLE XIX. CLINICAL STUDY - GROUP A

S.No.	Name of the Patients	Address	Age	Sex	Identified Diseases	Duration of the Course 15 Days
1.	C. Kuppusamy	North Street, Kanakkanpatti and Pottampatti (PO), Palani (TK).	19	Male	Dandruff	15 Days
2.	D. Thamilarasi	North Street, Kanakkanpatti (PO), Palani (TK).	25	Female	Dandruff	15 Days
3.	M. Vani	Bazaar Street, Kanakkanpatti (PO), Palani (TK).	29	Female	Dandruff	15 Days
4.	A. Raja	East Street, Kanakkanpatti (PO),Palani (TK).	31	Male	Dandruff	15 Days
5.	N. Sheela	East Street, Kanakkanpatti (PO), Palani (TK).	19	Female	Dandruff	15 Days
6.	Selva Bharathi	T. Nagar (P.O), Palani (TK).	22	Female	Dandruff	15 Days
7.	A. Umamaheswari	Housing Unit, Palani.	23	Female	Dandruff	15 Days
8.	A. Ram Prasath	Cheran Jeva Nagar, Palani.	22	Male	Dandruff	15 Days
9.	S. Kanagaraj	Sathya Nagar, Palani.	29	Male	Dandruff	15 Days
10.	N. Senthil	Sathya Nagar, Palani.	31	Male	Dandruff	15 Days
11.	A. Senthil Rani	North Street, Eramanaikanpatti	22	Female	Dandruff	15 Days
12.	A. Uma	North Steet, Eramanaikanpatti	31	Female	Dandruff	15 Days
13.	N. Rajammal	Middle Street, Eramanaikanpatti	39	Female	Dandruff	15 Days
14.	S. Ramasamy	P. N. Pudhur,Kanakkanpatti (PO), Palani (TK).	42	Male	Dandruff	15 Days
15.	N. Karuppusamy	P. N. Pudhur,Kanakkanpatti (PO), Palani (TK).	45	Male	Dandruff	15 Days
16.	N. Lakshmi	West Street,Kanakkanpatti (PO), Palani (TK).	39	Female	Dandruff	15 Days
17.	P. Arumugam	Rajapuram Pudhoor, Kanakkanpatti (PO), Palani (TK).	43	Male	Dandruff	15 Days
18.	T. Kaliyappan	Rajapuram Pudhoor, Kanakkanpatti (PO), Palani (TK).	40	Male	Dandruff	15 Days
19.	Kurshith Bhegam	New Ayakudi, Obulapuram (PO),Palani. (TK).	23	Female	Dandruff	15 Days
20.	S. Mohamed Abdulla	Hatjee Street, Palani.	44	Male	Dandruff	15 Days

TABLE XX. CLINICAL STUDY - GROUP B

S.No.	Name of the Patients	Address	Age	Sex	Identified Diseases	Duration of the Course 15 Days
1.	P. Ranganayaki	Sanganpalayam (PO), Pollachi.	27	Female	Dandruff	15 Days
2.	A. Suguna	Sanganpalayam (PO), Pollachi.	25	Female	Dandruff	15 Days
3.	S. Geetha	Sanganpalayam (PO), Pollachi.	17	Female	Dandruff	15 Days
4.	S. Narmatha	Sanganpalayam (PO), Pollachi.	16	Female	Dandruff	15 Days
5.	A. Saroja	Kottoor (PO), Pollachi.	40	Female	Dandruff	15 Days
6.	B. Ranganathan	Jakkara Palayam(PO), Pollachi.	45	Male	Dandruff	15 Days
7.	A. Perumalsami	Jakkara Palayam(PO), Pollachi.	42	Male	Dandruff	15 Days
8.	N. Shek Abdhulla	Pallivasal, Anaimalai.	39	Male	Dandruff	15 Days
9.	A. Parveen Banu	Pallivasal, Anaimalai.	29	Female	Dandruff	15 Days
10.	S. Devi	Siddhoor (PO), Pollachi.	17	Female	Dandruff	15 Days
11.	S. Karuppusamy	Siddhoor (PO), Pollachi.	39	Male	Dandruff	15 Days
12.	T. Velathal	Kottoor (PO), Pollachi.	41	Female	Dandruff	15 Days
13.	K. Kuppusamy	Indra Nagar. Anaimalai.	24	Male	Dandruff	14 Days
14.	S. Sekar	Indra Nagar, Anaimalai.	25	Female	Dandruff	14 Days
15.	A. Kalaivani	Near Jhangam Theatre, Pollachi	22	Female	Dandruff	14 Days
16.	N. Senthil Kumar	Pollachi	27	Male	Dandruff	14 Days
17.	S. Jeevanantham	Pollachi	29	Male	Dandruff	14 Days
18.	A. Saraswathy	Kinathukadavu, Pollachi.	40	Female	Dandruff	14 Days
19.	S. Rani Marie	Kinathukadavu, Pollachi.	41	Female	Dandruff	14 Days
20.	A.Manikkavasagam	Sethumadi, Pollachi.	42	Male	Dandruf	14 Days

TABLE XXI. CLINICAL STUDY - GROUP C

S.No.	Name of the Patients	Address	Age	Sex	Identified Diseases	Duration of the Course 15 Days
1.	S. Poornima	Saibaba Colony, Coimbatore.	19	Female	Dandruff	15 Days
2.	R. Krithika	Saibaba Colony, Coimbatore.	19	Female	Dandruff	15 Days
3.	R. Rathika	T.V.S. Coimbatore	26	Female	Dandruff	15 Days
4.	R. Vasantha Sekar	P. N. Pudhoor, Coimbatore.	44	Male	Dandruff	15 Days
5.	A. Pooja	P. N. Pudhoor, Coimbatore.	12	Female	Dandruff	15 Days
6.	S. Manjula	Saibaba Koil, Coimbatore.	32	Male	Dandruff	15 Days
7.	A. Krishnasamy	Saibaba Koil, Coimbatore	39	Male	Dandruff	15 Days
8.	S. Selvaraj	Sanganoor, Coimbatore	32	Female	Dandruff	15 Days
9.	T. Surya Murthi	Sanganoor, Coimbatore	29	Female	Dandruff	15 Days
10.	A. Lakshmi	Ukkadam, Coimbatore	27	Female	Dandruff	15 Days
11.	S.Sri Mahalakshmi	Kasthuribai Illam, Coimbatore	19	Female	Dandruff	15 Days
12.	T. Indhumathi	Kasthuribai Illam, Coimbatore	32	Male	Dandruff	15 Days
13.	V. Karthik	Masakkali Palayam, Coimbatore.	29	Male	Dandruff	15 Days
14.	S. Siva Kumar	Sanganoor, Coimbatore	34	Female	Dandruff	15 Days
15.	T. Kruthika	Kasthuribai Illam, Coimbatore	19	Male	Dandruff	15 Days
16.	S. Hari	T.V.S. Coimbatore	25	Male	Dandruff	15 Days
17.	D. Siva Samy	Masakkali Palayam, Coimbatore.	43	Male	Dandruff	15 Days
18.	T. Mathan Kumar	Peelamedu, Coimbatore.	32	Female	Dandruff	15 Days
19.	S. Swetha	Peelamedu, Coimbatore.	13	Female	Dandruff	15 Days
20.	S. Praneetha	Saibaba Colony, Coimbatore.	29	Female	Dandruff	15 Days

In group A, out of 20 patients, eight have recovered from Dandruff completely, with in two weeks and four patients showed marked relief. Moderate relief was noticed in 3 patients and 3 got mild relief. Two patients have shown less improvement (Fig.9).

In group B, out of 20 patients, twelve showed complete relief within two weeks, 3 patients marked relief, two patients moderate relief and two patients mild relief. Only one patient has shown less improvement (Fig.10).

In group C, out of 20 patients, sixteen have recovered from Dandruff completely within two weeks, two patients marked relief, one patient showed moderate relief and one patients mild relief as reported in Table XXIII and Fig. 11.

CRITERIA OF ASSESSING / TO TREATMENT

The response to treatment was assessed based on symptomatic improvement.

Some symptomatic improvements are

*Hair loss was completely stopped

* Itching was completely stopped

* White, oily looking flakes of dead skin was shed down

The treatment of single drug of *Lippia nodiflora,* (oil I) and *Thea sinensis* specially (oil II) showed moderate relief compared to that of the combined effect of the mixed oil of *Lippia nodiflora* and *Thea sinensis* (oil III) (Table XXII and Fig.13).

However, in Siddha Clinic the plants *Lippia nodiflora* and *Thea sinensis* along with other plants are used to prepare the medicated herbal oils. The newly prepared herbal oils were safe to the patients who were suffering from Dandruff when applied on the scalp externally.

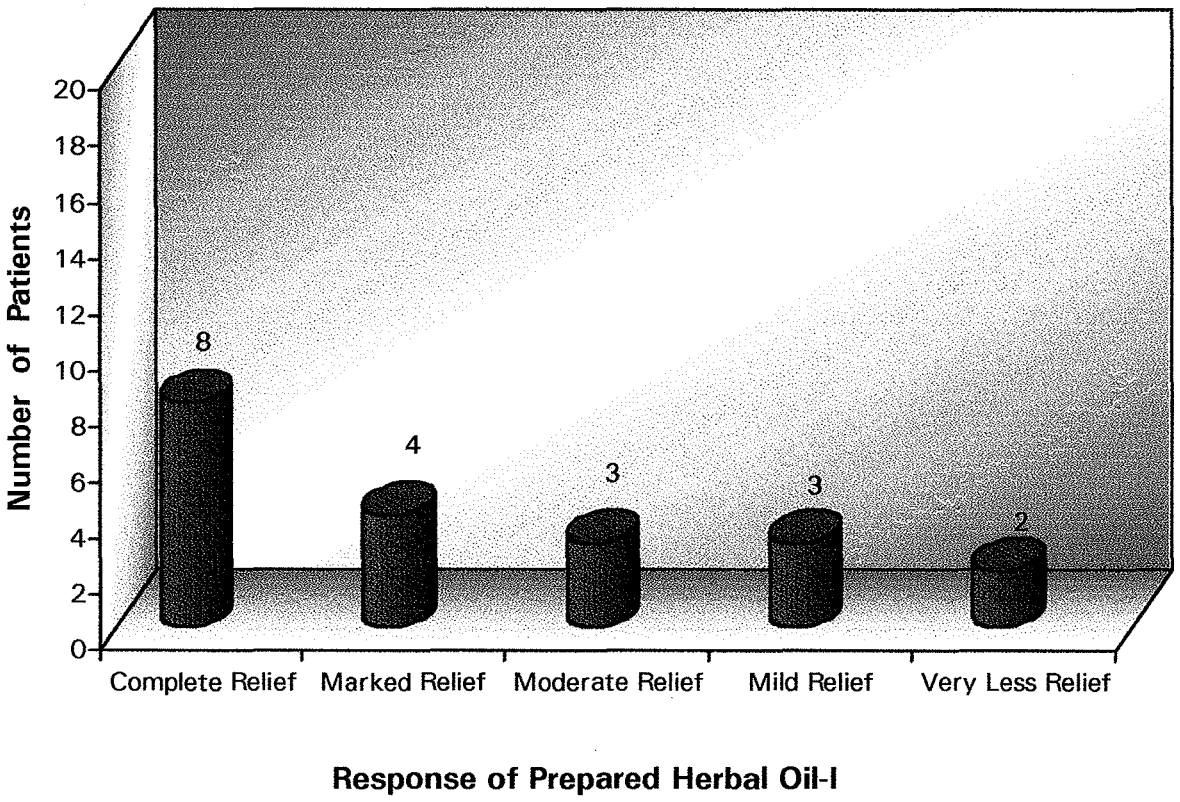


FIGURE 9. RESPONSE OF PREPARED HERBAL OIL-I IN GROUP A.

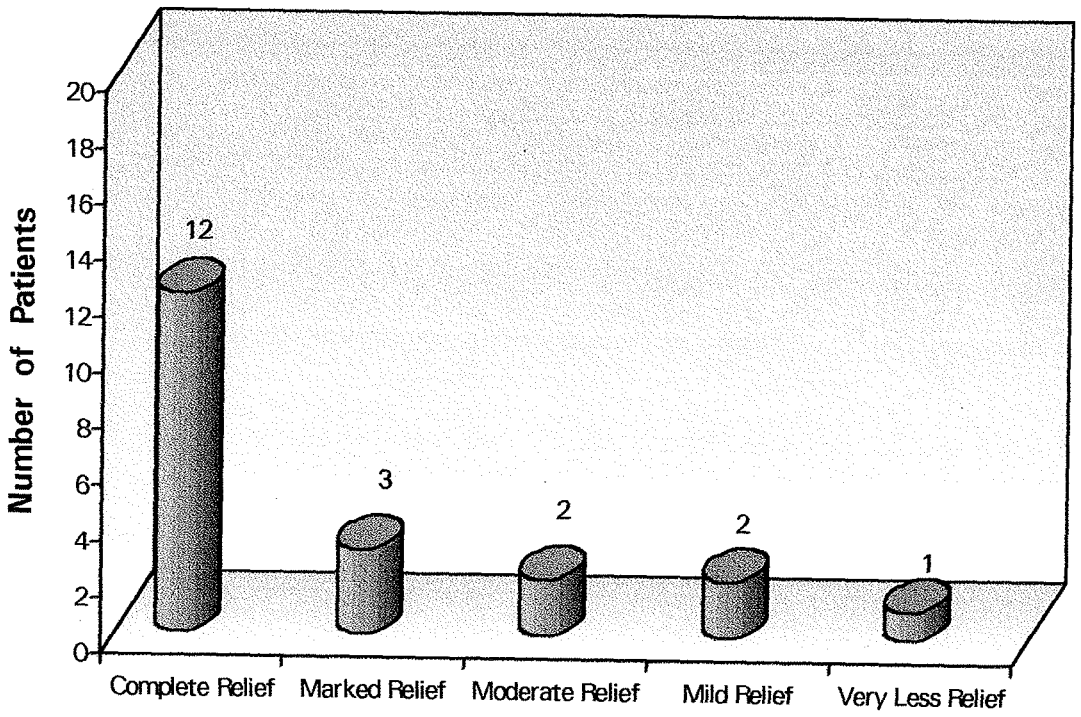


FIGURE 10. RESPONSE OF PREPARED HERBAL OIL-II IN GROUP B.

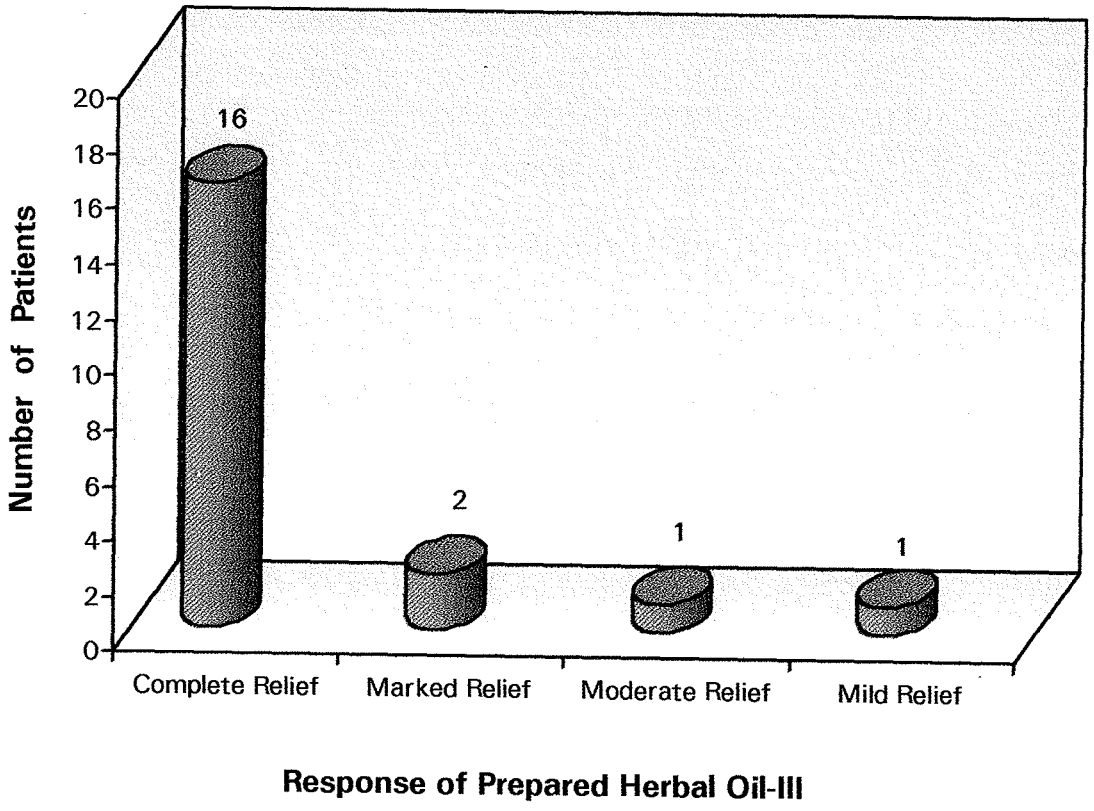


FIGURE 11. RESPONSE OF PREPARED HERBAL OIL-III IN GROUP C.

In the present investigation, newly prepared herbal oils (Plate 7, 8 & 9) are found to nullify the effect of the unknown toxins of these plants. So the oils showed no side effect against the patients. The total clinical improvement of patients in the treatment of Dandruff was depicted in Fig.12.

The present clinical study indicated that the combined effect of these two plants showing excellent result to cure Dandruff. In Group A, 75 per cent of them were recovered and Group B 85 per cent showed the same. In group C, 95 per cent of them were recovered from the Dandruff (Fig.13). Totally more than 90 per cent of the patients were cured without any side effect. (Fig.14).

TABLE XXII. STUDY OF MICROBIAL FACTOR IN DANDRUFF *IN VITRO* CLINICAL TRIAL TO ANALYSE THE SENSITIVITY OF THE PREPARED OILS

S.No.	Name of the Patients	Prepared Oils	Ingredients	Results
1.	Ranganayaki Poornima	Oil I	Gingelly oil + Lippia nodiflora	Moderate Sensitive
2.	Raja Ranganayaki	Oil II	Gingelly oil + Thea sinensis	Moderate Sensitive
3.	Raja Poornima	Oil III	Gingelly oil + Lippia nodiflora + Thea sinensis	High Sensitive

TABLE XXIII. RESULTS OF THE TREATED PATIENTS OF DANDRUFF

Relief	Number of Patients		
	Group : A	Group : B	Group : C
Complete Relief	8	12	16
Marked Relief	4	3	2
Moderate Relief	3	2	1
Mild Relief	3	2	1
Less Relief	2	1	–

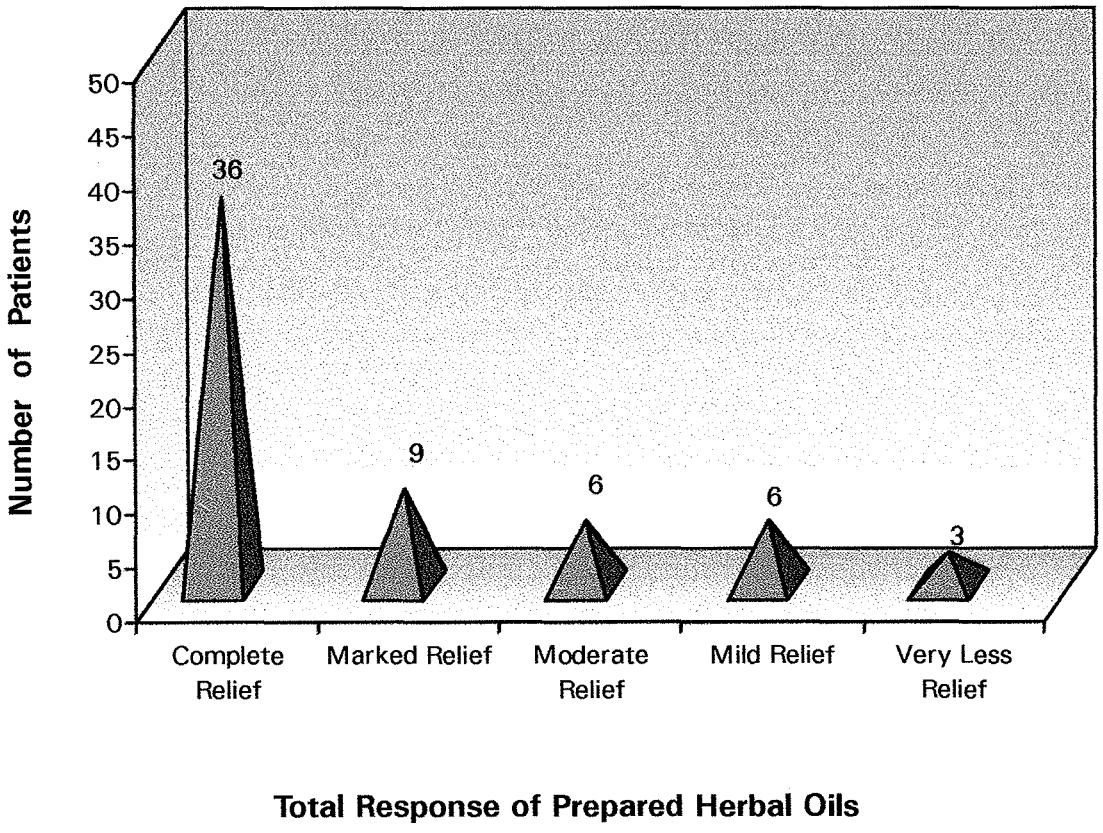


FIGURE 12. CLINICAL IMPROVEMENT OF PATIENTS IN THE TREATMENT OF EXTERNAL APPLICATION FOR DANDRUFF.

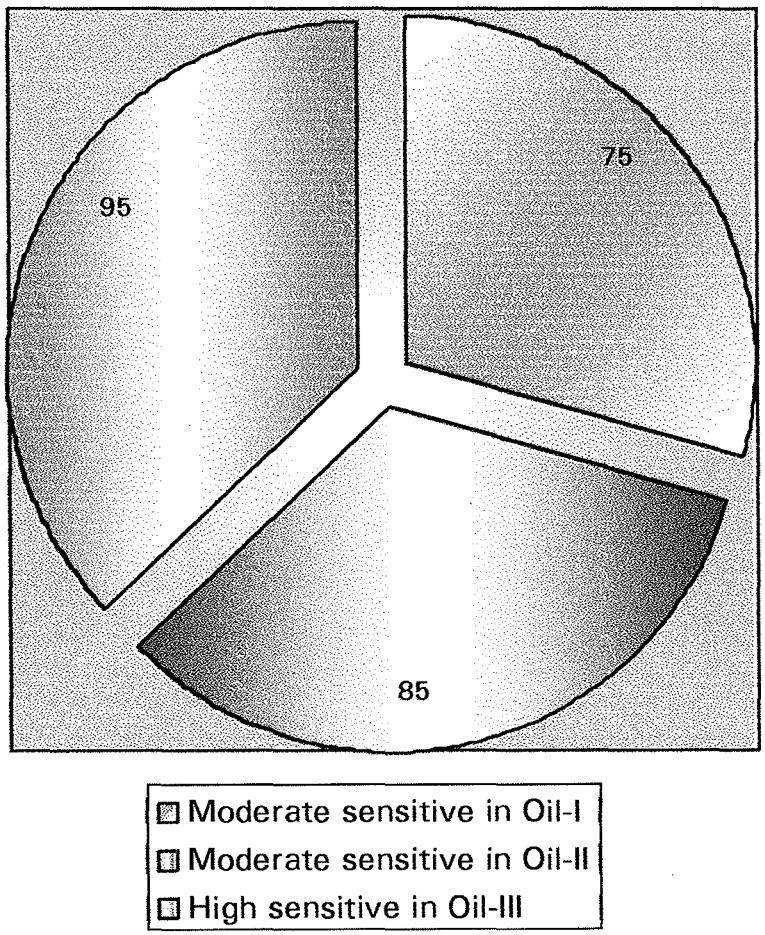


FIGURE 13. SENSITIVITY AGAINST THE DANDRUFF FOR USING PREPARED OILS.

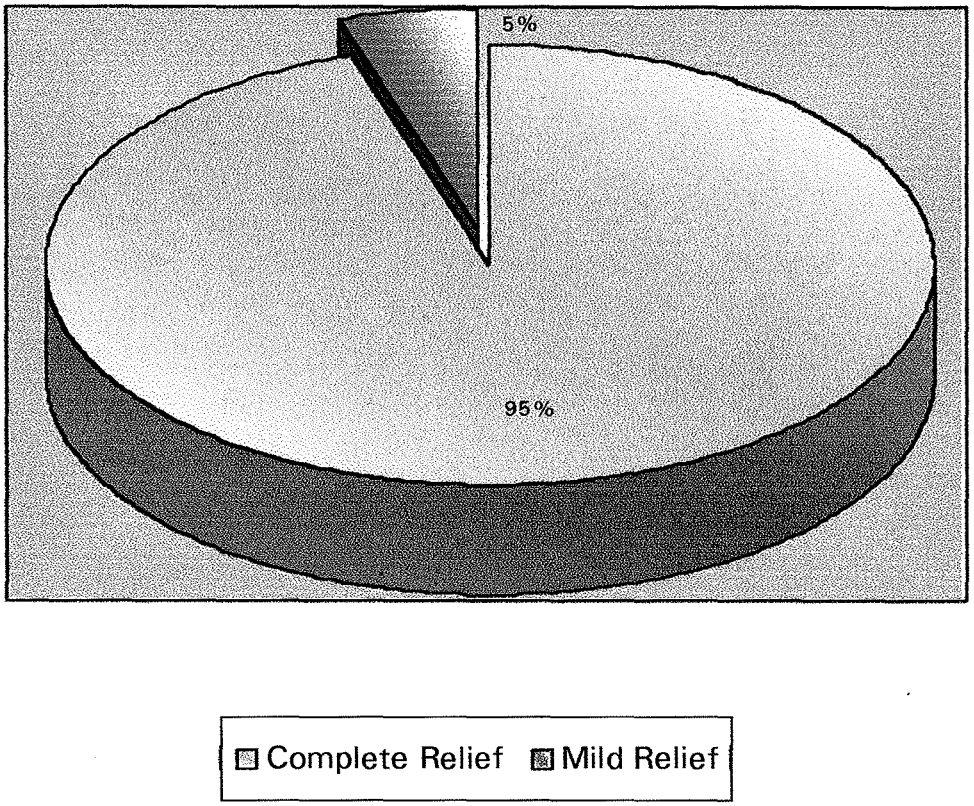


FIGURE 14. TOTAL PERCENTAGE OF RELIEF IN GROUP A, GROUP B, GROUP C.

Thirughnanam (1997) reported that the oil prepared from the juice of *Lippia nodiflora* was used for Dandruff weekly twice.

He also reported in his book on "Herbal medicine", the Juice of *Cynodon dactylon* mixed with coconut oil was used to cure the Dandruff.

Infusion and decoctions of *Phyllanthus emblica* mixed with cow milk (½ l), coconut water (½ l), and gingelly oil (*Phyllanthus* thaila) is applied externally to cure the Dandruff (Thirughnanam, 1997).

Hari (1993) reported in his book "Home remedies", the flowers of *Azadirachta indica (Meliaceae)* mixed with gingelly oil was used to cure the Dandruff.

The juice of *Indigofera tinctoria* and *Aristolochia* mixed with gingelly oil and coconut oil is boiled till the whole of the watery portion is evoporated and nothing but oil remains. The prepared oil is used to cure the Dandruff and other skin diseases. (Hari, 1993).

The curing responses of Dandruff patients were depicted in Table XXIII. The clinical improvement and the days taken for the recovery were shown in Fig.12.

The results of both subjective and objective parameters of the study reveals that the plants *Lippia nodiflora* and *Thea sinensis* in the form of oil have a significant action in the reduction of clinical symptom of "Dandruff scales in the scalp". The above plants possess saponin. So the antifungal activity of these plants may be due to the presence of saponin. This result is being supported by the reports of Weseler et al., (2002) in which antifungal

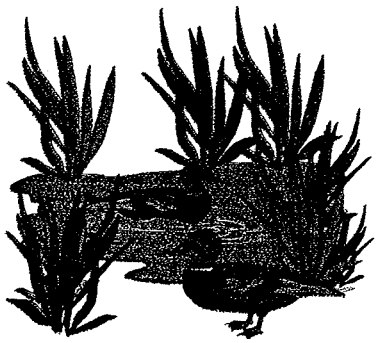
activity has been demonstrated in the crude saponin fraction of *Asparagus officinalis*. The activity was specific to *Microsporum, candida* etc.

The presence of alkaloid also reported in *Lippia nodiflora* and *Thea sinensis* may be responsible for the curence of these diseases. This finding coincides with the findings of Nadkarni (1954) who observed vernonin (an alkaloid) in *Psoralea corylifolia* which is responsible for the curing of Dandruff.

In Ayurveda and Unani the descriptions regarding the properties and actions of the plants *Lippia nodiflora* and *Thea sinensis* is generally depicted.

It has been realised now that the medicinal herbs are going to play an important role in the future Materia medica of the world. Most of the herbal researchers have started doing research from the traditional folklore information. This work has been going on effectively even though more than half portions of the Medicinal wealth have not yet been explored.

Majority of herbal medicines appeared to be safe if used correctly, but they can do harm and it is time to make it mandatory to conduct trials to evaluate the safety and efficacy of herbal medicines.



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