CHAPTER 1

1. INTRODUCTION
INTRODUCTION

The term ‘Pharmacognosy’ was coined by German scientist Seydler in 1815. The pharmacognosy was derived from Greek word Pharmacon (a drug which is meant for dried herb) and gnosis (to acquire knowledge). Hence pharmacognosy, which literally means knowledge of drugs of pharmaceuticals [Kirtikar and Basu, 1918].

The earth is at present covered with 2,50,000 to 5,00,000 species of plants [Borris, 1996]. Out of them a small percentage of medicinal plants are on record [Moerman, 1996]. Hippocrates [in the last 5th century (B.C)] mentioned about 300-400 medicinal plants [Schultes, 1992]. The Indian vedas (Rigveda) are still older (3000 B.C) that reflects medical description. Charaka and Susruta were renowned ancient Indian physicians and Charaka classified about 300 vegetable drugs in to 50 groups [Kirtikar and Basu, 1918]. Dioscorides, in first century
A.D. mentioned about medicinal plants in *De Materia Medica*, which was considered as prototype for modern pharmacopoeias.

The fall of ancient civilization had made destruction or loss of many old documents of medicinal plants and modern pharma had started only when Francois Megendic and Claude Bernard of 19th Century introduced experimental procedures in animals [Stockwell, 1988]. Later Moerman contribution on medicinal plants was very significant who had reported that 1625 species of plants in America were used in food industry and 2564 plants were found as drugs [Klink, 1997].

The use of medicinal plants has gained momentum with the continued search and experience of many generations of physicians and herbal practioners. The plant products are presented in 14 of the 15 therapeutic categories of pharma preparation and they form an important role of health care system in western world [Phillipson and Anderson, 1989]. 25% medicine of all prescription in U.S.A is from natural products and another 25% medicines are the modification of natural products [Franswart, 1990]. According to W.H.O 80% of World inhabitance are relied on tradition medicine for primary health care [Franswart et al., 1985]. However only 25% of modern medicines are derived from plant products [Franswart and Moris, 1976]. The pharmaceutical industries for the preparation of the drugs would depend on minerals, animals, and synthetic, microorganisms, genetic engineering and plant or plants oil. Out of the plants
oil, essential oil is a good source for the drugs, which will have aroma and eco-friendly.

The importance of volatile oils in pharmacy as antimicrobial agent was clearly established by Dorman and Deans 2000. Plants volatile oil are generally isolated from plant material by distillation method and they contain terpenoids, monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpines (C_{20}) and some low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen and sulphur containing compounds, coumarins and homologous of phenyl propionoids [Dorman and Deans, 2000]. The secondary metabolites are potential medical procedure and applications in cosmetic, food [Dorman and Deans, 2000; Ueda et al., 1982; Shelef, 1983; Jay and Rivers, 1984; Gallardo et al., 1987; Baratta et al., 1998; Youdim et al., 1999] and pharmaceutical industries [Janssen et al., 1988; Pelissier et al., 1994; Sapiro et al., 1994; Cai and Wu, 1996]. Therefore these secondary metabolites are proved to have limitless important chemicals that have derived biological properties. Some of the forest indigenous plants have potential antifungal, antibacterial and antiviral properties [Okwue, 1992].

The volatile oils in case of Ocimum canus, Ocimum gratissium, Ocimum trichodon and Ocimum urticifolium are potentially responsible for antimicrobial action and these volatile oils have great effect on microorganisms [Janssen et al., 1989]. Further these plants are used in folk medicines to treat different diseases like upper respiratory tract infection, diarrhea, headache, ophthalmic, skin
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diseases, pneumonia, cough fever and conjunctivitis. Similarly *Xylopia aethiopica* largely found in West South Africa shows antimicrobial activity against the bacteria such as *Staphylococcus aureus*, *E.coli*, *Proteus mirabilis*, *Candida albicans* [Boakyeyiadom et al., 1977]. Though there are plenty of antimicrobial agents available, majority of them have a narrow spectrum of action due to the emergence of new infections which are resistant to conventional drugs that alarms the researchers for the preparation of new drug to be used in combat to microorganisms [Okigbo et al., 2005].

According to W.H.O, infectious diseases are main cause of death especially those involve in skin, mucous membrane and many infections. For instance *Streptococcus pneumonia*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* are gram positive bacteria that causes nosocomial, waterborne, food borne, airborne and skin infections [Delauney and Ermi, 1965]. *Vibrio cholera*, *E.coli*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Neisseria gonorrhoeae* and *Salmonella typhi* are all gram-negative bacteria. *E.coli* causes cholera by the release of enterotoxin. It also causes waterborne infections, dysentery, urinary tract, and food borne infections. The gram-negative bacterium also causes food borne infection. *Proteus vulgaris* causes pneumonia; *Haemophilus influenzae* causes respiratory disease, meningitis, bronchitis, pneumonia and empyemia; *Pseudomonas aeruginosa* causes skin and wound infections, nosocomial infections [Brooks et al., 2001]; *Salmonella typhi* causes typhoid fever and
*Neisseria gonorrhoeae* causes venereal diseases [Brooks et al., 2001; Pelczar et al., 1993; Balows et al., 1992]. *Asperigillus niger, Asperigillus flavus, Tricoderma vibriae, Pencillium rubrum, Chaetomium globosum, Trichophyton mentagrophytes* are the moulds, where as *Candida albicans* is a yeast cell. The mycelium form is mould and yeasts cells do not form mycelium but resemble as fungi [Jagdish chander, 2002]. The fungi cause mycosis infection. Mycosis infections are classified in to different types based on infection. The superficial infection causes tinea nigra, white piedra, black piedra diseases; the cutaneous infection causes dermatophytosis, candidiasis of skin, mucosa and nails; the subcutaneous infection causes sporotrichosis, mycetoma. The endemic infection of fungi causes histoplasosis, blastomycosis; the opportunistic infection causes systemic candidiasis, cryptococcosis, aspringilosis, mucormycosis, pencilliosis [Jagdish chander, 2002].

*Trichophyton mentagrophytes* causes Tinea pedis, which is a chronic infection of the toe webs, hands and nails (onchomycosis) [Chapman and Daniel, 1994]. Initially the symptoms of itching are found between the toes subsequently small vesicles are formed. When the skin is ruptured, a thin fluid tissue are discharged, and finally when cracking upon the wounded region, secondary bacterial infection (*Pseudomonas* and *Staphylococcus*) takes place [Susan and Jimmy, 2006]. When the fungal infection becomes chronic, peeling and cracking of the skin are the principal manifestation accompanied by pain and pruritus [Jagdish chander, 2002; Elewski and Hazen, 1989].
Candida albicans causes candidiasis, which is a cutaneous infection. By the infection of pathogen, oral thrush can occur on the tongue, lips, gums and palate [Chami et al., 2004]. The yeast infection candidiasis also includes invasion of the skin, that is skin become weakened by trauma, burns or maceration [Vijaya Monohar, 2001]. Inter digital involvement between the fingers follows repeated prolonged immersion in water. Candidal invasion of the nails and around nail plate causes onchomycosis a painful erythematous swelling of the nail fold resembling a pyogenic parancychia, which may be eventually destroy the nails [Jagdish chander, 2002]. The secondary infections involves with bacterial infection [Datta et al., 1989; Crislip and Edward, 1989].

Among the infectious microorganisms dermatophyte yeast species and certain bacteria are most frequent [Caceres et al., 1991]. During the last two decades incidence of candidiasis and other fungal infections have increased especially in immuno-compressed host [Diamond, 1991]. It may be due to the non-availability of suitable drugs or toxicity of available drugs like Amphotericin-B [Maddux and Barriere, 1980].

The present thesis work predominantly represents the volatile oils and their activity on microbial and vector control properties. As such a deep study on volatile oils is needed for determining the activities of the volatile oils. The volatile oils are very complex mixture of compounds, which mainly have monoterpenes and sesquiterpenes hydrocarbons with general formula \((C_5H_8)_n\) [Svoboda and Deans, 1995]. Statistical data shows that so far 1000 monoterpenes
and 3000 sesquiterpenes are structured and so many new natural compounds are being identified every year, but their biological activities are known for only some. In some plants like basal, one main constituent (methyl chavicol) predominates [Svoboda et al., 1999]. Generally the action of natural compounds is the result of the combined effect of their active and inactive compounds and most of the components of volatile oils have synergistic effect. The complexity and potency of volatile oils depends on the factors such as time of harvest, genotype, chemo type geographical origin, environment and agronomic condition [Deans and Svoboda, 1988]. Examples for common terpenoids are menthol and camphor (monoterpines) and farnesol and artemisin (sesquiterpenoids).

Volatile oils can monitor the blood circulation, nerve growth, nucleic acid, liver, cholesterol and metabolism in human body. Similarly the volatile oils have a significant effect on influenza virus [Dorman and Deans, 2000]. Further the volatile oils from Nigella sativa, Cuminum cyminum, Papaver somniferum and Osmium sanctum are considered as protective agents against carcinogenesis [Aruna and Sivaramakrishna, 1996]. Volatile oils could act as a chemical defense against plant pathogenic disease [Svoboda and Deans, 1992], as a result many aromatic medicinal plants do not succumb to many diseases of the plant. Further the monoterpenes and sesquiterpenes of volatile oils can acts as strong barrier to fungal infection [Carlton et al., 1992]. The volatile oils exhibit reduction in growth of microorganisms depending on volatile oils concentration and chemical composition [Fyfe and Armstrong, 1998]. Artemisin
(sesquiterpenoids) and derivatives of *Artemisin* are used as antimalarials [Viswakarma, 1990]. Due to that antimalarial property, W.H.O has recommended to develop artemisin as a drug for cerebral malaria.

The present literature till now covers the antimicrobial properties of volatile oils. But the present thesis is further extended to vector control by application of the volatile oils as natural products. As such a brief study on mosquito (*Aedes aegypti*) is necessary.

*Aedes aegypti* is a common mosquito (fig: 1.7.) found all over the world except in some places like Antarctica. This mosquito transmits *Arbo viruses* responsible for yellow fever and dengue hemorrhagic fever (DHF) all over the world [Phillip *et al.*, 2000]. Last few months back, in our country especially the people of Andhra Pradesh state were severely affected by this dengue fever for which no vaccine is so far available. Dengue fever is caused by the *Arbovirus* and spread by genes *Aedes*. Three types of dengue fevers namely 1) Classical dengue fever, 2) Dengue Haemorrhagic fever (DHF), and 3) Dengue Shock Syndrome are commonly found and they are caused by four types of viruses DEN1, DEN2, DEN3 and DEN4 Which are closely related to antigenically [Sharma *et al.*, 2004]. Infection with one sero type provides life long immunity to that virus but not to the others [WHO, 2002]. The first outbreak of dengue fever in India was reported during 1963 in Kolkata. The next major outbreak was reported in Delhi in 1996 with 10,252 cases and 423 deaths [Sharma *et al.*, 2004]. Presently in 2006, 3331 cases and 45 deaths have been reported from
eleven states / Union territories [Kaul et al., 1998]. The classical dengue fever is a self limiting disease and doesn’t kill; where as the other two types (DHF, DSS) are proved to be fatal if prompt treatment was not given [Bir singh and Anil Goswami, 2006]. As such symptoms of the fever are necessary for proper diagnosis. In case of classical dengue fever, the following symptoms are found sudden onset of high fever with feeling of chills, severe headache, pains in muscles and joints, pain behind the eyeballs, extreme weakness, loss of appetite, feeling of nausea, change in taste sensation in mouth, pain in abdomen, mild pain in throat, pinkish red rash appears on the skin. The entire duration of the classical dengue fever lasts for about 5-7 days and the patient recovers [Bir singh and Anil Goswami, 2006]. Symptoms of DHF are as follows, bleeding from nose, gums, blood in stool or in vomiting, bleeding spots on the skin, tourniquet test results positive. In case of DSS, in addition to symptoms of DHF further symptoms are as follows- the person is restless, and the skin feels cold and clammy despite high fever, person may loss consciousness, low pulse rate and low blood pressure. Generally the treatment will be given for reducing the temperature of the fever with paracetamol tablets and hydrotherapy giving plenty of fluid water and providing complete rest to the patient. But in case of DHF and DSS hospitalization, an appropriate investigations are necessary and some times transfusion of fluids or platelets are to be carried out [Bir singh and Anil Goswami, 2006].
Like dengue fever chikungunya is also caused by virus CHIK V. and spread by *Aedes albopictus* mosquito. But in Asia including India *Aedes aegypti* is a main vector for the above disease. The first out break of the disease was recorded in 1963 in Kolkota and was followed by epidemics in Tamilnadu, Andhra Pradesh, and Maharastra during the period between 1964-1965 [Yadav and Shouche, 2003]. The persons affecting with the disease will suffer with moderate to high fever in association with body ache, backache and headache. Joint pains of varying severity occurred with in 2 days of onset of fever and, in decreasing order of affliction, involved knees, ankles, wrist, hands and feet. The joint pains will last for weeks to months [Prasanna *et al.*, 2006]. Inflammation of joints and transient macular rash on ear lobs, neck, trunk and upper extremities are also reported for a few patients but hemorrhage will not occur [Enserink, 2006]. The recent paper published by national institute of virology Pune (fig: 1.15.) [Prasanna *et al.*, 2006] reveals that the people of Andhra Pradesh, Maharastra and Karnataka were severely affected by this disease due to the presence of *Aedes* species in those areas (fig:1.15).

The other diseases spread by other species of mosquitoes are malaria, encephalitis epidemic polyarthritis and filariasis and all these diseases are causing health hazard to human beings [Tyagi and Hiriyan, 2004]. Since mosquito vector is a chief source for transmitting these diseases, it is worthwhile to have a special attention to eradicate the mosquito breeding which automatically have a great impact in controlling the mosquito spread diseases.
Like all diptera, the mosquito passes through four life stages: egg, larvae, pupa and adult. They live in water continuously from the time the egg hatches through the larval and pupa stages until the adult emerge. The egg can remain dormant for several years to wait for favorable conditions. Otherwise it will hatch depending on light and temperature with in a weak and the cycle from egg to adult may takes 1-4 weeks. The adult will survive for 30 days [Subash Chandra, 2002].

Due to its vector in nature, the mosquito has become the major problem to control and considerable number of factors has become advantages to the mosquito for its survival. Mosquito can have the ability to breed even in a teaspoon full of stagnant water. It can hide anywhere in the houses as easily as air enters. The rate of breeding is high and egg can remain live for longer period till the favourable conditions prevail for hatching. Lack of proper drainage system and lack of effective supervision for keeping clean environment has added advantage for providing number of breeding places to the mosquito. Increased use of some insecticides will cause to develop resistance power in mosquito.

Hence proper and systematic care has to be taken by the public, N.G.O and public officers to keep the surroundings clean and hygiene. Further attacking larval breeding places, can reduce the density of the mosquito [Gluber, 1989]. It can be achieved by using insecticide larvicidal organophosphorates or synthetic compounds like N, N-diethyl-3-methyl benzamide [DEET], or some other spray
powder. Though the intensity of these chemicals is excellent, but the toxicity of these synthetic chemicals has some times gives the side effects. More over the constant use of these chemicals will lead to gain the resistance to these mosquitoes [Adebayo, 1999].

Hence the alternative conventional substance is the use of natural product from plants [Consoli and Oliverima, 1994]. In that process several plants namely *Azadirachta indica*, *Osmium basilicum*, *Citronella*, *Galáuiga*, *Thyme*, *Eucalyptus* and *Clove* etc. have been tested and found the repellent activity of volatile oils of these plants against mosquito [Sharma *et al.*, 1993].

These herbal products are ecofriendly, biodegradable, low cost for vector control, which can be used with minimum care [ICMR Bulletin, 2003]. So many plants are very active against mosquitoes and can be used as repellents. They offer a safer alternative to synthetic chemicals and can be obtained by individuals and communities at low cost for protection against mosquitoes.

The quest for the perfect repellent is still a posing problem to the scientist, as it has to fulfill the desired standards. It has to repel against to multiple species of biting mosquitoes, remain effective at least 8hrs, cause no irritation to the skin, causes no synthetic toxicity, be resistant to abrasion, rub-off and greaseless and odourless. But so far no repellent can meat all these criteria [ICMR Bulletine, 2003].
The present doctoral research is an attempt to find a proper herbal product that can act as good repellent against mosquito larvae and good antimicrobial agent. For that purpose, the following six medicinal plants and certain microorganisms of gram-positive bacteria, gram-negative bacteria and fungi including skin diseased pathogens are chosen. Among isolated skin pathogens, *Candida albicans* and *Trichophyton mentagrophytes* belongs to fungi and *Staphylococcus aureus* and *Pseudomonas aeruginosa* belongs to bacteria. The six selected plants are *Allium cepa* L., *Celastrus paniculatus* L., *Curcuma longa* L., *Cuminum cyminum* L., *Leucas aspera* (wild) and *Nigella sativa* L. and description of these selected plants are as follows:

**Allium cepa** L. (Liliaceae):

Vern: *vulli* in Telugu, *Pyas* in Hindi (Fig: 1.6).

*Allium cepa* plants are widely used in all parts of the world as a flavouring vegetable in various types of food and contain many vitamins [Nadkarni, 1954]. They are used in medicine for fever, dropsy, chronic bronchitis, mixed with salt onions are domestic remedy in colic and scurvy. Roasted or otherwise they are applied as a poultice to boils, bruises, wounds etc to relive hot sensation. Raw onion is an antiseptic value through out the alimentary canal. Eaten raw it is a diuretic and emmenagogue mixed with mustard oil it is a good applicant for rheumatic pain and other inflammatory swellings. Mixed with vinegar, onions are useful in sour throat. Cooked with vinegar they are given in jaundice,
spleenic enlargement and dyspepsia. In malarial fevers onions are taken twice a day. Fresh onion juice is moderately bactericidal. The essential oil of onion contains heart stimulant, increases pulse volumes and frequency of systolic pressure, coronary flow and stimulating the intestinal smooth musculature and uterus. It promotes bile production and reduces blood sugar [Chopra et al., 1956]. Extracts of onion acts as insulin to diabetic patients [Collip, 1923].

*Celastrus paniculatus* L. (Celastraceae):


*Celastrus paniculatus* is a large climbing shrub with yellow fruits commonly found in hilly parts of the country. The seeds (Fig: 1.4) are brown and are covered by a scarlet aril. The leaf sap is used as antidote for opium poisoning [Kirtikar and Basu, 1918]. The seeds are bitter, laxative, emetic, and tonic. They are used as stimulant and diaphoretic in rheumatism gout and of various fevers. The volatile oils obtained from seeds are deep reddish yellow and to become thick like honey on keeping. The volatile oils have medicinal value as stimulant [Wealth of India, 1985].

*Curcuma longa* L. (Zingiberaceae):

Vern:- *Pasupu* in Telugu, *Haldi* in Hindi (Fig: 1.3).

*Curcuma longa* is a perennial herb that measures up to 1m high with short stem distributed through out tropical and subtropical regions of the world, widely
cultivated in India and China [Arujo and Leon, 2001]. Its rhizomes are oblong, pyriform, ovate, often short branch. The powder called turmeric, has been in use for its flavouring, as a spice in food preparations and it also has digestive properties [Govindarajan, 1980]. Turmeric powder is further used in medicines such as biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis [Ammon et al., 1992]. It is a old Hindu medicine for treatment of sprains and swelling caused by injury [Ammon and Wahl, 1991].

*Cuminum cyminum* L. (Apiaceae):

Vern: *Jelakara* in Telugu, *Jeera* in Hindi (Fig: 1.2).

*Cuminum cyminum* is a small slender annual herb about 1ft high with much branched, angular or strained with leaves bluish green in colour. The flowers are white or rose coloured. The seeds (Fig: 1.2) are grayish tapering towards both base and apex. The plant is grown extensively in India, China, North Africa and Europe. It is cultivated in all states of India in two seasons either before the Southwest monsoon begins or after the Northeast monsoon ends. Alcoholic extracts of the seeds shows anti fertility effect in rats [Rastogi and Mehrotra, 1993]. The seeds are aromatic odour and hence used as condiment, or an essential ingredient in all mixed spices every powders. The seeds have long been considered stimulant and carminative. They are stomachic, astringent, used in diarrhea and dyspepsia.
**Leucas aspera (wild.) (Labiatae):**

Vern: *Tummi* in Telugu, *Chota halcusa* in Hindi (Fig: 1.5).

*Leucas aspera* (wild.) is a herbaceous much branched, erect or diffuse annual herb with 30-60cm high, more or less found all over India in cultivated fields as a weed. 35 species are found in India and only one species is found in Tropical America. The plant is fragrant and commonly used as antipyretic in villages. The juice of the leaves is used as an external application for psoriasis, chronic skin eruption and painful swellings [Chopra *et al.*, 2002]. The flowers are used for cough and cold in children. An alcoholic extract of leaves shows antibacterial activity against *Micrococcus pyogenes, E.coli* etc. [Wealth of India, 1985].

**Nigella sativa L. (Ranunculaceae):**

Vern: *Nalla Jeeelakaria* in Telugu, *Kaloji* in Hindi (Fig: 1.1).

*Nigella sativa* is a small annual herb 45cms high, distributed and cultivated in Arabia. It is occasionally found as a weed of cultivation in Punjab, Himachal Pradesh and Assam. Flowers are pale or bluish, seeds are trigonous black, rugulose tubercular and the seeds are used as a flowering material and for medicinal purposes. The seeds (Fig: 1.1) are carminative stimulant, diuretic and are used in the treatment of mild cases of puerperal fever. They are externally applied for eruption of skin. Alcoholic extracts of the seed shows antibacterial activity against *Micrococcus pyogenes, E.coli* [Wealth of India, 1985].
For any research work certain objectives are required to reach the target and the present doctoral research work is no exception to this phenomenon and hence placed the following objectives in the present work.

- To determine the efficacy of extracts of selected plants against water borne, food borne and fungal microorganisms.

- To determine the efficacy of plants volatile oil against microorganisms.

- Isolation and identification of fungal skin pathogens from Candidiasis and Tinea pedis diseased patients.

- To determine the efficacy of volatile oils against skin pathogens.

- To determine the inhibitory efficacy of plants volatile oil against bacterial skin pathogens.

- To determine the lethality effect of the volatile oils against *Aedes aegypti*.

- To determine the effective dose of volatile oils along with the protection time for repellency activity.
Fig: 1.1 to 1.6: Photographs of selected plants.
Fig. 1.7: Adult mosquito: *Aedes aegypti*.

Fig. 1.8: Pupa and larvae of *Aedes aegypti*.