CHAPTER-1

Introduction & Review of Literature
INTRODUCTION

The term ‘Pharmacognosy’ was coined by German scientist Seydler in 1815. The pharmacognosy was derived from Greek word Pharmalcon (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 practitioners. “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 an 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}), sesquiterpines (C_{15}), diterpine (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 gratiassium*, *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 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 subtilus*, *Staphylococcus aureus*, *Micrococcus luteus* are gram positive bacteria that causes nosocomial, waterborne, food borne, airborne and skin infections [Delauney and Erni, 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. *Proteus vulgaris* causes pneumonia; *Haemophilus influenzae* causes respiratory disease, meningitis, bronchitis, pneumonia and empyemia; *Pseudomonas aeruginosa* causes skin and wound infections, nosocomial infections...
Asperigillus niger, Asperigillus flavus, Tricoderma vibiae, 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, aspzigilosis, mucormycosis, pencilliosis.

“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 Amphoterine-B [Maddux and Barriere, 1980]”.

The present thesis 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 monoterpiines and sesquiterpiines hydrocarbons with general formula (C$_5$H$_8$)$_n$ [Svoboda and Deans, 1995]”. Statistical data shows that so far 1000 monoterpiines and 3000 sesquiterpiines are structured and
“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 (monoterpenses) and farnesol and artemisin (sesquiterpenoids)”.

The volatile oils have a significant effect on influenza virus [Dorman and Deans, 2000]. Volatile oils could act as a chemical defense against plant pathogenic disease [Svoboda and Deans, 1992]. Further the monoterpenses and sesquiterpenes of volatile oils can acts as strong barrier to fungal infection [Carlton et al., 1992]. 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 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 (DSS) are commonly found and they are caused by four types of viruses DEN1, DEN2, DEN3 and DEN4, which are closely related genetically [Sharma et al., 2004]. "Infection with one serotype provides lifelong 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]. 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; whereas 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 lose 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,
appropriate investigations are necessary and sometimes 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 outbreak of the disease was recorded in 1963 in Kolkota and was followed by epidemics in Tamilnadu, Andhra Pradesh, and Maharasra 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 within 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 lobes, neck, trunk and upper extremities are also reported for a few patients but hemorrhage will not occur [Enserink, 2006]”. The paper published by national institute of virology Pune [Prasanna et al., 2006] reveals that the people of Andhra Pradesh, Maharasra and Karnataka were severely affected by this disease due to the presence of Aedes species in those areas.

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 diphtheria, 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 within a week 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 favorable 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 sometimes 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 *Azadiracha indica, Osmium basilicum, Citronella, Galuiga, 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 meet all these criteria [ICMR Bulletin, 2003]. The following potential medicinal plants used for the present study.

**Leucas aspera (wild.)** (Labiatae):


*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].”

“Achyranthes aspera (Amaranthaceae) a perennial stiff erect herb, is growing up to 1m height. Stems are square, leaves elliptic ovate or broadly rhombate, 5.22 cm long, 2.5 cm broad, and pubescent. The inflorescences are 8-30 cm long, with many single, white or red flowers, 3 -7 mm wide. Flowering time is in summer. The plant is widespread in the world as a weed. In the northern part of India it is known as a medicinal plant in different systems of folk medicine. Plant volatile oils are predominant in many industries, particularly in pharmacy, clinical and food preservative industries. The oil and its constituents are well documented as antimicrobial agents. The volatile oils are complex mixtures of compounds which mainly having monoterpenes, sesquiterpines hydrocarbons with general formula (C$_5$H$_8$)$_n$. Compounds in the seeds of A. aspera are the saponins A and B. They are glycosides of oleanolic acid. The carbohydrate components are the sugars D-glucose, L-rhamnose, D-glucuronicacid (Saponin A). Saponin B is the β-D-galactopyranosyl ester of Saponin A. The content of free oleanolic acid in A. aspera roots is 0.54 %. From the roots ecdysterone and oleanolic acid have been isolated. In the unripe seeds saponines, oleanolic acid, amino acids and hentriacontane, a long chained carbohydrate, have been found. In the shoots an aliphatic dihydroxyketone could be found. Two long chain compounds, isolated from the shoots, have been characterized as 27-cyclohexylheptacosan-7-ol and 16-hydroxy.26-methylheptacosan-2-ol by chemical and spectral investigations. The petrol extract of the shoots produced a yellow semi-solid
mass. From this a pink coloured essential oil with a pleasant odour and an aliphatic alcohol (17-pentatriacontanol) were found.

Objectives of the present work

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

• Identification of plants volatile oil components by GC-MS technique

• 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 protein inhibitory efficacy of plants volatile oil against bacterial skin pathogen.

• 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.
Chapter 1

INTRODUCTION AND REVIEW OF LITERATURE

Fig1.1 *Leucas aspera*

Fig1.2 *Achyranthus aspera*
Chapter 1

INTRODUCTION AND REVIEW OF LITERATURE

REVIEW OF LITERATURE

1.1. HISTORY OF HERBAL PHARMACOLOGY:

“Plants possess active principles employed as medicines. Many indigenous plants have been used, by people on all continents as poultices an infusions dating back to prehistory [Cowan, 1999]. There is an evidence of Neanderthals, living 60000 years ago in Iraq, *Alccarosea* that is still in ethno medicinal use around the world today. The fall of the ancient civilization resulted in the destruction or loss of much of the documentation of plant pharmaceuticals. But many cultures continued in the excavation of the older works as well as building upon them [Cowan, 1999]”.

“Actually there is no authentic record of medicines used by the primitive man. But the *Rig-veda*, which is the oldest book in the library of man supplies, has given valuable information about the medicinal plants [Kirtikar and Basu, 1918]. The knowledge of the medicinal plants must have been accounted in the course of many centuries”. The vedic aryans were acquainted with about 100 medicinal. [Kirtikar and Basu, 1918]. Charaka and Sushruta recorded 300 vegetable medicinal plants in to 50 groups along with their properties. These works were propagated to various Asian countries including China by the Buddhist monks in around 2000 B.C. “According to legend Shen Nong, living about 2000 B.C, was responsible for compiling the first herbal of 365 drugs and their properties. Hence he was called as a father of Chinese medicine [Kao, 1973]. In 1083 the classified pharmaceutical natural history (Sceng Lei Pen Tschao) was published in 1596 with excellent likeness of Ephedra Sinica, [Lewis, 1992] followed by the great pharmacopoeia or the Pandects of Natural History [Li Shih-Chen, 1596]. The last extraordinary compendium Li Shih-Chen, took 50 years to complete and included 1000
of species from China and abroad. After communist revolution in 1949, Mao brought all old and new medical systems under one umbrella for the development of people’s health work. [Kao, 1973]. Traditional Chinese medicine (TCM), which was developed in that way, is still in use at China [Zhou and Baker, 2002]. According to Tang and Leung, the mechanism central approach has dominated research and development of TCM. To many supporters of TCM, the long history of use, tradition, faith and anecdotal reports are considered the best evidence for the efficacy of TCM interventors”.

“The Egyptian medicine is also dates back to 3000 B.C and their pharmaceutical record is the Papyrus Ebers of 1550 B.C” and from them the Greeks have learnt the knowledge of medicine, which paved the way for the origin of western science of medicine in Europe and other countries [Ebbell, 1937]. “The Egyptian-Greek medicine was kept alive by Avicenna, a 10th to 11th century Persian pharmacist and physician who wrote “Canon Medicine” and his work was accepted as authoritative until 17th century [Bender and Thom, 1966]”.

In India, the subsequent development of using herbal medicinal plants was hampered during the period of invasion of Mohammedans. Later it gained momentum only when the British Empire ruled India from 17th century onwards. Sir Late Thomas Lauder Brunton drew an analogy stating that the pharmacy and the use of medicinal plants have been developed in four typical stages as like that of our civilization from Paleolithic age to modern age [Thomas Lauder Brunton, 1886]. “That means, in the olden days crude drugs were employed, prepared in the roughest manner. In the next stage they were be converted in to more active in the form of extracts or solutions, water or alcohol”. “In the third stage the pure active principles separated from crude drugs, were employed e.g.
morphine and quinine”. In the fourth stage instead of getting from natural products, the synthetic substances are prepared for use.

In later decades the use of medicinal plants were neglected all over the world and more so in India. These were contumaciously referred to as “old woman’s” remedies [Jhon footy, 1917]. Roxbergh botanist collected all information about botanical medicinal plants found in India and maintained a book “Flora Indica” [Clarks ed Roxburgh, 1874]. Further Jhon Fleming in 19th century contributed a valuable paper on medicinal plants [Jhon Fleming, 1810]. An International exhibition in 1862 was held in London to exchange views and to develop several indigenous drugs. Thus rapid progress was made for the development of pharmacy and several publications had published the innumerable number of books to meet the challenges for the health of the human beings.

“Natural products have been used to elucidate physiological process. Natural products are the basis of many standard drugs used in modern medicine and even some members of medical profession are not aware of plant origin [Kirtikar and Basu, 1918]. Although the medicinal plants may not always lead to the discovery of novel compounds which may be employed in the treatment of, plants may give valuable insight in to the pathology of diseased conditions or disturbed human minds. For the last 20 years the western societies have realized the significance of hallucinogenic plants in shaping the history of primitive and advanced cultures. Some of these plants contain chemicals capable of inducing visual auditory, tactile, olfactory, gustatory hallucinations or causing artificial psychoses and hence a thorough understanding of a chemical composition of these drugs may lead to discovery of the new drugs for treatment of psychiatric conditions. As a result of the complexity of the human brain and central nervous system,
psychiatry has not been developed as rapidly as other fields of the medicine mainly due to the lack of adequate tools, therefore these drugs may provide the necessary pharmacological tools for the discovery of more appropriate and effective drugs [Cowan, 1999].

“Although the first chemical substance to be isolated from plants was benzoic acid in 1560, the search for useful drugs did not begin until morphine in 1804 was isolated from *Papaver somniferum*. Since then many drugs from higher plants have been discovered and accepted as useful drugs in industrialized countries [Farnsworth, 1985]. In 1875 Williams withering the English physician was succeeded in translating folk knowledge (empirical science) in to new pharmaceuticals by isolating 30 cardiac glycodes from plant *purpurea*. Farnsworth in 1990 estimated that there are atleast 88 ethno botanical derived drugs [Cox, 1997].

Plants seem to have served as models in drug development for three reasons. Firstly atleast 25% of all prescriptions contain active principles extracted from higher plants, which has persisted for the atleast the last 25 years [Farnsworth, 1985] and as many as two-thirds of people in developing countries rely plants as a sources of drugs. Secondly biologically active substances derived from plants may have poor pharmacological or toxicological profiles for use. They can however serve as templates for synthetic modification and structural function studies with anticipation of useful drugs in man will result. “Thirdly many secondary, highly active, plant constituents are found to be useful in studying biological systems and diseased process. W.H.O estimates that 80% of the undeveloped countries rely almost on traditional medicines for their primary health care needs. Since medicinal plants are the backbone for tradition medicine, this means that
more than 3000 million people utilize medicinal plants on regular basis [Farnsworth, 1985]. There is a great demand and potential for medicinal plant research as shown by growing market in medicinal herbs. They are high in value, popular with public interest in natural products and strong competitors for synthetic drugs developed at high cost”.
1.2. REVIEW ON EFFICACY OF VOLATILE OILS AS ANTIMICROBIAL AGENTS:

Nearly 1000 monoterpenes and 3000 sesquiterpenes structures are so far estimated. In other words all the components of the volatile oils are collectively shows their ability. “Essential oils are secondary metabolites of plants. They are volatile, consisting mostly of terpines and oxygenated derivatives and are used for flavour fragrances spices, antiseptic and preservative action. Volatile oils with most effective antimicrobial activity, in descending order, are the phenolic groups, alcohols, aldehydes, ketones, ethers and hydrocarbons [Charai et al., 1996].”

The mode of action of volatile oils is a typical one [Buchbauer and Jirovetz, 1994]. Accordingly the volatile oils, either inhaled or applied to the skin, act by means of their lipophylic fraction reacting with the lipid parts of the cell membranes and as a result, modify the activity of calcium ion channels. At certain levels of dosage the volatile oils saturate the membranes and show the effects similar to the local anesthetics. They can interact with these cell membranes by means of their physiochemical properties and molecular shapes and can influence their enzymes, carriers, ion channels and receptors. The authors Buchbauer and Jirovitz described the physiological effects from human, which include brain stimulation anxiety, reliving sedation and antidepressant activities and increasing the cerebral blood flow. The fragrance compounds are absorbed by inhalation and are able to cross the blood brain barrier and interact with receptors in central nervous system. Bioassay explains the volatile oils action, are usually carried out on mice rats and toads e.g. the influence of peppermint volatile oils on intestinal transport, [Beesley et al., 1996] the effect of volatile oils on skin penetration, [Abdullah
et al., 1996] the effect on skeletal muscle fibers [Fogaca et al., 1997], the screening for analgesic properties [Aydin et al., 1996]. Further the essential oils are used in urology, dermatology, sleep and nervous disorders, laxatives, erosive gastritis, cardiac and vascular system, immunomodulating drugs, cold and cough.

“Plants have a bulk of compounds that act as antimicrobial agents in many cases. These plant compounds serves as plant defense mechanisms against the microorganisms, insects and herbivores. The two phenolic groups namely thymol (5-methyl-2 isopropyl phenol) and carvacrol (5-isopropyl-2-methylphenol) are bactericidal to microorganisms [Kim et al., 1995]. Hence in practice the carvacrol is added to different products as baked goods monoalcoholic beverages and chewing gums [Ultee et al., 1999]. Thymol is another component used in many products including soaps, toothpaste, shampoos, deodorants and mouth washes [sapioro et al., 1994]. Cinamic and caffic acids are common representatives of phenyl propane and derived compounds. The common herbs Tarragon and Thyme contain caffic acid, which is effective against microorganisms [Thomson, 1978; Brantner et al., 1996; Duke, 1985; Wild, 1994]. Eugenol found in Clove volatile oils is considered microbial static against both fungi [Duke, 1985] and bacteria [Thomson, 1978].

Flavones, flavonoids and flavanols of plant extract compounds show effective response to microbial infections. More lipophylic flavonoids disrupt the microbial membranes and thus curb the growth of microorganisms [Tsuchiya et al., 1996]. Catechins, the reduced form of C₃ unit in flavonoids compounds deserved special mention. The teas, which contain catechins, inhibit in in-vitro Vibrio cholera [Borris, 1996], Streptococcus mutants [Batista et al., 1994], Shigella and other microorganisms
Antimicrobial property of tannins is reviewed by Scalbert in 1991. According to his studies tannins can be toxic to phylamentous fungi, yeast and bacteria. Coumarins have antimicrobial property against Candidiasis and Candida albicans and also to gram-positive bacteria. [Fernandez et al., 1996]. Terpines or terpenoids are active against bacteria, [Ahmed et al., 1993.] fungi and viruses. It is reported that 60% of essential oils derivatives are inhibitory to fungi while 30% inhibited bacteria. The triterpenoids butalonic acid that is one of the terpenoids will inhibit to HIV virus.

From this text it is clearly evident that volatile oils and derivatives of volatile oils play a vital role in controlling microorganisms”.

1.3. REVIEW ON EFFICACY OF VOLATILE OILS AS VECTOR CONTROL AGENTS:

Repellency activity and the role of volatile oil of plants are widely studied on several species of the plants. “The ideal repellent would repel multiple species of mosquito, remain effective for atleast eight hours, cause no irritation to the skin or mucous membrane, cause no systemic toxicity, be resistant to abrasion and rub-off and be greaseless and odourless. But no available repellent meets all these criteria [Strauss et al., 1968]. Efforts to find a good repellent have been hampered by several factors that affect the inherent repellency of any chemical [Davis, 1985]. Moreover, different species of mosquitoes may react differently to the same repellent [Rutledge and Collister, 1983]”.

To be effective, a repellent must show an optimal degree of volatility, making it possible for an effective repellent vapour concentration to be maintained at the skin
surface without evaporating so quickly that it loses its effectiveness. Several factors play a role in how effective any repellent is, including the frequency and uniformity of application, the number and species of the organisms attempting to bite, the user’s inherent attractiveness to blood sucking mosquito and the overall activity level of the potential host [Schreck, 1995].

Among all synthetic compounds, DEET is the most effective repellent compound [Davis, 1985]. But the increased concentration of DEET does not proportionately increased the duration of protection against mosquito. Among the herbal repellent, citronella volatile oils is more effective one [Wright, 1975]. But its efficacy is far below to that of DEET.

“After discovery of DDT, the mosquito control has almost based on synthetic organic insecticides [ICMR Bulletin, 2003]. But the extensive use of synthetic insecticides resulted in environmental hazards and also in the development of physiological resistance to major vector species, thus necessitated the need for search and development of environmentally safe, biodegradable, low cost, indigenous methods for vector control. Phytochemicals from plants can be used as an alternative to synthetic insecticides for vector control. A large number of plant extracts have been examined against mosquito vectors [Sukumar et al., 1991]. But a very few plant products have shown practical utility for mosquito control. Studies have revealed that some phytochemicals will act as general toxicant against adult as well as larval stages of mosquitoes while others interfere with growth and development (growth inhibitors) or with reproduction (chemosterilent) or produce olfactory stimuli, thus acting as repellent or attractant”.

Several plants volatile oil show promising response towards larvicidal activity. Essential oils from Citronella plant and Pennyroyal have been employed as insect repellents since ancient times. Insect repellent formulations consisting of volatile oils of Citronella, spirit of Camphor,
volatile oils of Tar, volatile oils of Pennyroyal, and Castor volatile oils have been shown to provide long lasting protection against mosquito [Dover, 1930]. Pine volatile oils were found to be effective as repellent. Volatile oils from Eucalyptus and Caryophyllum species have also been reported to possess satisfactory repellency effects [Mayer, 1952]. The monoterpenes, limonene, terpinolene, citronellon, and camphor, which are common constituents of some volatile oils, have been reported to possess high repellent properties [Ibrahim et al., 1998]. In case of Tagetes species, Tagetes minuta has biocide effect on the larvae and adults of Aedes aegypti [Perich et al., 1994]. Pathak et al., 2000 has found 100 % mortality with steam distilled volatile oils extract from Tagetes erecta against Anopheles stephensi. Similarly volatile oils of Culex Sinensis showed greater insecticidal potency [ICMR Bulletin, 2003].

Alcoholic extracts of leaves and stem of vanilla fragrance with ethyl acetate and aqueous butanol possess larvicidal activity. Azadirachta indica (Neem), Eucalyptus species, Lantana camara, Cymbopogon species, Mentha piperita, Tagetes minuta etc. are extensively studied and found promising results for considering them as good larvicidal and adulticidal agents.

Ignacimuthu et al., (2008) studied the larvicidal activity of Leucas aspera against Culex quinquefasciatus and Aedes aegypti. The hexane extract of L. aspera showed highest activity followed by choloform and ethanol. The LC\textsubscript{50} values of L. aspera against first, second, third and fourth instar larvae of Culex 122.50, 149.97, 193.43 and 230.71 ppm and against Aedes the LC\textsubscript{50} values were 77.40, 144, 199.72 and 257.17 ppm respectively. The results were statistically significant at P<0.05 level.

Ilango et al., (2008) studied the antibacterial activity of Leucas aspera spreng. In this study, the whole plant of Leucas aspera was first defatted with hexane and discarded. Then the remaining marc was successively extracted with ethyl acetate and methanol and both the extract were concentrated under vacuum to yield corresponding ethyl acetate
extract (EAE) and methanolic extract (ME). Both the extracts exhibited a significant antibacterial activity against all the screened microorganisms.

Ramanibai et al., (2012) studied the Larvicidal efficacy of Leucas aspera (willd) extracts against the mosquito larvae of Culex quinquefasciatus. Mosquitoes transmit serious human diseases causing millions of deaths every year. The present study deals with the investigation of larvicidal activity of aqueous and chloroform leaf extract of Leucas aspera (willd.) against mosquito larvae Culex quinquefasciatus. The results were statistically significant at Leucas aspera.

“Mangathayaru et al., (2005) investigated the Antimicrobial activity of Leucas aspera flowers. The methanol extract of Leucas aspera flowers, its fractions, the alkaloidal residue and the expressed flower juice, tested for antimicrobial activity, showed good antibacterial activity for methanol extract and methanol fraction with maximum activity for the alkaloidal residue”.

Mominul Islam et al., (2012) studied the Allelopathic Potentiality of Medicinal Plant Leucas aspera. Excessive use of herbicide in the crop field threatens the environment by destroying flora and fauna. Allelopathic substances could be act as substitutes of chemical herbicides to suppress the target plants. Leucas aspera (Willd.) a herbaceous plant has already provided tangible evidence of its potential with remedies for different sorts of medical problems, but still now, not a single work is reported related to its allelopathic activity. These results indicated that L. aspera may contain growth inhibitory substances and possess allelopathic activity. Therefore, L. aspera could be used as a potential candidate for isolation and identification of allelochemicals, which can lead to
the development of ecologically acceptable bio-herbicides used for sustainable agriculture.

“Chamundeeswari et al., (2011) investigated the anti-inflamatory and antioxidant potential of ethanolic extract of *Leucas aspera* (EELA) in adjuvant arthritis. Complete Freund’s adjuvant served to induce arthritis. This study highlighted the antioxidant and anti-inflammatory potential of *Leucas aspera*. Three major families of compounds present in EELA may explain these activities: catechins (epicatechin, beta epicatechin), flavonoids (procyanidin), phytosterols (beta-sitosterol) apart from glycosides, phenolic compounds and tannins”.

Chamundeeswari et al., (2011) carried out the Acute and Sub-Acute Toxicity Evaluation of Ethanolic Extract of *Leucas aspera* (Lamiaceae) in experimental rats. The present study aimed to evaluate the acute (14 days) and sub-acute (28 days) oral toxicity of ethanolic extract of *Leucas aspera* (EELA) on albino mice and adult Wistar rats. In conclusion, the results of this study suggest the non-toxic nature of the ethanolic extract of *Leucas aspera* at normal therapeutic doses.

“Shirish S. Pingale (2010) investigated the Hatoprotection by fresh juice of *Leucas Aspera* leaves. The aim of the present work is to evaluate the effect of *Leucas aspera* leaves fresh juice against carbon tetrachloride (CCI4) induced liver damage. The observation of markers as well as Light and electron microscope photographs supports the regeneration of liver parenchyma. This proves overall promising effect against liver disorders”.
Rahman et al., (2007) studied Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root. The extract showed significant lethality to brine shrimp with an LC$_{50}$ value.

‘Emi Okuyama et al., (2006) studied about the Diterpenes from *Leucas aspera*, inhibiting Prostaglandin-Induced contractions. Investigation of the inhibitory fraction of *Leucas aspera* on prostaglandin-induced contraction in guinea pig ileum provided four new diterpenes, leucasperones A (1) and B (2) and leucasperols A (3) and B (4), and three new isopimarane glycosides, leucasperosides A, B, and C (5–7), together with the known compounds asperphenamate, maslinic acid, (−)-isololiolide, and linifolioside. The structures of the compounds were determined by detailed spectroscopic analysis. The configurations of 1 and 2 and the acetylated derivatives of 3 and 4 were determined by differential NOE analysis and CD data. Leucasperone A (1), leucasperosides A (5) and B (6), and linifolioside showed inhibition of prostaglandin-induced contractions”.

Mangathayaru et al., (2005) studied the effect of *Leucas aspera* on hepatotoxicity in rats. Swiss albino mice were used for toxicity study, while the hepatoprotective study was carried out in adult male Wistar rats (150-200 g). One-tenth of the maximum tested dose (i.e., 200 mg/kg, p.o.) of the extract was selected for the evaluation of antihepatotoxic activity.

Saundane et al., (2000) investigated the Anti inflammatory and Analgesic activity of various extracts of *Leucas aspera* Spreng. Four different crude extracts- petroleum ether, chloroform, ethanol and water of *Leucas aspera* Spreng were investigated for antiinflammatory and analgesic activities in albino rats and mice, respectively at a dose of 400 mg/kg body weight, orally.
Kannappa Reddy et al., (1992) studied the anti-ulcer activity of *Leucas aspera* spreng. The alcoholic extract of *Leucas aspera* (ALA) was investigated for its antiulcer effect by two experimental models. A significant reduction in acid secretion and ulcer score was observed in rats after ALA treatment. The observed antiulcer effect of ALA may be due to a combination of anti secretary effect and a protective effect on gastric mucosa.

Bagavan et al., (2008) studied the Larvicidal activity of saponin from *Achyranthes aspera*. The acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Acalypha indica, Achyranthes aspera, Leucas aspera, Morinda tinctoria* and *Ocimum sanctum* were studied against the early fourth-instar larvae of *Aedes aegypti* L and *Culex quinquefasciatus*. This study investigates the potential of crude extracts from commonly used medical herbs in India as an environmentally safe measure to control the vector of dengue and lymphatic filariasis.

Ramesh Londonkar et al., (2011) studied the potential Antibacterial and Antifungal Activity of *Achyranthes aspera* L. Petroleum ether, Chloroform and Methanol extract of dried leaves of *Achyranthes aspera*. In conclusion, it appears that *Achyranthes aspera* has non-specific antimicrobial activity.

Elumalai et al., (2009) showed that *Achyranthes aspera* leaf extracts inhibited fungal growth. The aim of the study was to investigate the antifungal activity of various leaves extracts of *Achyranthes aspera* Linn. The results obtained in the present study suggest that the ethanol and methanol extracts of the leaves of *Achyranthes aspera* Linn revealed a significant scope to develop a novel broad spectrum of antifungal herbal formulation.
Triguna N. Misra et al., (1992) extracted an Antifungal essential oil and a long chain alcohol from *Achyranthes aspera*. An essential oil and a new long chain alcohol have been isolated from the shoots of *Achyranthes aspera*. The oil exhibited antifungal activity against *Aspergillus carneus* and the isolated alcohol has been characterized as 17-pentatriacontanol.

Uma A Bhosale et al., (2011) investigated the central nervous system depressant and behavioral activity of an ethanol extract of *Achyranthes aspera* (Agadha) in different animal models. The result of this study reflected that EEAA (400 mg/kg i.p.) decreased locomotor activity, produced muscle relaxation, and showed anxiolytic activity. Conclusions: EEAA exhibit CNS depressant and significant anxiolytic activity comparable to diazepam.

Gajanan Rajpal Deshmukh et al., (2012) evaluated the Eye Irritation Potential of Aqueous Leaf Extract of *Achyranthes aspera* by *In In-vitro* and *In Vivo* Method. The work attempt to investigate the eye irritation potential of aqueous leaf extract of *Achyranthes aspera* by in *in-vitro*, Hen's Egg Chorioallantoic Membrane Test (HET-CAM) and in vivo acute eye irritation test in rabbits. The aqueous extract of *Achyranthes aspera* showed no eye irritation properties both in *in-vitro* and in *vivo* methods when compared with negative control whereas positive controls showed eye irritation potential.

“Anshu Aggarwal et al., (2010) studied the Reduction of oxalate-induced renal tubular epithelial (NRK-52E) cell injury and inhibition of calcium oxalate crystallisation in *in-vitro* by aqueous extract of *Achyranthes aspera*. These studies indicate that *A. aspera* extract besides having a cytoprotective role also has a potential to inhibit both
nucleation and the growth of the CaOx crystals and can prove to be a potent candidate for phytotherapy against urolithiasis”.

“Vasudeva et al., (2007) investigated the Estrogenic and pregnancy interceptory effects of Achyranthes aspera Linn. Root. Achyranthes aspera Linn. (Amaranthaceae) is an abundant indigenous herb in India. Histological studies of the uterus were carried out to confirm this estrogenic activity.

Vasudeva Rao et al., (2006) studied the Effect of Achyranthes aspera on the immunity and survival of Labeo rohita infected with Aeromonas hydrophila. Achyranthes aspera seed was incorporated in the diets (at 0.01%, 0.1% and 0.5%) of Labeo rohita, rohu fingerlings (3.0 ± 0.4 g). These results indicate that Achyranthes aspera stimulates immunity and increases resistance to infection in L. rohita.

Vasudeva et al., (2006) studied the Post-coital antifertility activity of Achyranthes aspera Linn. root. Achyranthes aspera Linn. (Amaranthaceae) is an abundant indigenous herb in India. It is traditionally being used as an abortifacient. The ethanol extract also exhibited estrogenic activity tested in immature ovariectomised female albino rats. Histological studies were carried out to confirm this”.

Vasudeva Rao et al., (2004) observed the enhancement of anti-proteases in Labeo rohita fed with diet containing herbal ingredients. Aqueous root extract of Achyranthes aspera was incorporated in the experimental diet of Labeo rohita (rohu). Control diet was prepared without root extract. Feeding of fishes with experimental diet has significantly (p<0.05) enhanced the serum anti-proteases level than fishes fed with control diet.

Vasudeva Rao et al (2005) studied that dietary incorporation of Achyranthes aspera seed influences the immunity of common carp Cyprinus carpio. Achyranthes aspera seed
(0.5%) was incorporated in the diet for *Cyprinus carpio* (90± 17g): control diet was prepared without the seed of Achyranthes. All these results confirm that *Acharyanthes aspera* enhances the immunity of *Cyprinus carplo*.

**Vetrichelvan et al., (2003)** investigated the Effect of alcohol extract of *Achyranthes aspera* Linn. on acute and subacute inflammation. The antiinflammatory activity of an alcohol extract of *Achyranthes aspera* was tested on carrageenin-induced hind paw oedema and cotton pellet granuloma models in albino male rats.

**Sandhya kumary et al., (2002)** studied the Impact of feeding ethanolic extracts of *Achyranthes aspera* on reproductive functions in male rats. The results suggest that ethanolic extract of *A. aspera* caused reproductive toxicity in male rats and the action may be by suppressing the synthesis of androgen.

**Pankaj Tahiliani et al., (2000)** studied that *Achyranthesaspera* elevates thyroid hormone levels and decreases hepatic lipid peroxidation in male rats. A study was made to evaluate the role of *Achyranthesaspera* on the changes in serum thyroid hormone concentrations and glucose levels in male rats.

**Muhammad Shoaib Akhtar et al., (1991)** investigated the hypoglycaemic effect of *Achyranthes aspera* in normal and alloxan-diabetic rabbits. Blood glucose levels of normal and alloxan diabetic rabbits were determined after oral administration of various doses of *Achyranthes aspera* powdered whole plant and certain aqueous and methanolic extracts.

**Pakrashi et al., (1977)** studied the Abortifacient principle of *Achyranthes aspera* Linn. *Achyranthes apsera* is an abundant indigenous herb in India. Extracts of the whole plant had shown an abortifacient effect in mice.
Kannapa Reddy et al., (1986) studied the Effect of *Leucas aspera* on experimental inflammation and mast cell degranulation. The aqueous and alcoholic extracts of *Leucas aspera* were investigated for their action on experimental inflammation and on mast cell degranulation. Both the extracts exhibited significant anti-inflammatory action of acute and chronic inflammation. The mast cell degranulation induced by propranolol and Carbachol was effectively prevented by pretreatment with *Leucas aspera* extracts.