FORMULATION AND EVALUATION OF CONTROLLED RELEASE MOSQUITO REPELLENT PATCH

A Thesis Submitted to
Gauhati University

In The Partial Fulfillment of the Requirements for
The Degree of

Master of Pharmacy
In
Pharmaceutics

By
SOMI BORAH
Roll No: MP/11/08
GU Registration No: 075667 of 2011-12

Under the Supervision of:

Mr. BHUPEN KALITA (Internal guide)
Assistant Professor (Pharmaceutics)
Girijananda Chowdhury Institute of Pharmaceutical Science
&
Dr. PRONOBESH CHATTOPADHYAY (External Guide)
Scientist D and Group Head, Defence Research Laboratory,
Defence Research and Development Organisation, Tezpur

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GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE (GIPS)

N.H. 37, Hatkhowapara, Azara, Guwahati-781017

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE (GIPS)
(A unit of Shrimanta Shankar Academy)
Approved by AICTE & PCI, New Delhi, affiliated to Gauhati University
N.H. 37, Hatkhowapara, Azara, Guwahati-781017.
Telephone: (0361) 2843405
Email: gips_guwahati@rediffmail.com

CERTIFICATE

This is to certify that the thesis entitled ***"Formulation and Evaluation of Controlled Release Mosquito Repellent Patch"*** submitted to Gauhati University in partial fulfillment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmaceutics** is a genuine and legitimate research work carried out by **Somi Borah**, Gauhati University ***Registration No***: ***075667 of 2011-12, Roll no- MP/11/08*** at ***Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Azara, Hatkhowapara, Ghy-17,*** during the academic session 2012-2013 under my supervision and guidance.

I wish her all success in life.

Internal Guide
Mr. Bhupen Kalita
Assistant Professor
Department of Pharmaceutics
GIPS

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE (GIPS)
(A unit of Shrimanta Shankar Academy)
Approved by AICTE & PCI, New Delhi, affiliated to Gauhati University
N.H. 37, Hatkhowapara, Azara, Guwahati-781017.
Telephone: (0361) 2843405
Email: gips_guwahati@rediffmail.com

CERTIFICATE FROM THE PRINCIPAL

This is to certify that the thesis entitled ***"Formulation and Evaluation of Controlled Release Mosquito Repellent Patch"*** submitted to Gauhati University in partial fulfillment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmaceutics** was carried out by **Somi Borah**, Gauhati University ***Registration No: 075667 of 2011-12*** at ***Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Hatkhowapara, Ghy-17*** under my supervision.

I wish her all success in life.

Prof (Dr.) Suvakanta Dash
Principal
GIPS

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE (GIPS)
(A unit of Shrimanta Shankar Academy)
Approved by AICTE & PCI, New Delhi, affiliated to Gauhati University
N.H. 37, Hatkhowapara, Azara, Guwahati-781017.
Telephone: (0361) 2843405
Email: gips_guwahati@rediffmail.com

CERTIFICATE

This is to certify that the topic incorporated in this dissertation "***Formulation and Evaluation of Controlled Release Mosquito Repellent Patch***" an original research work being submitted by ***Somi Borah***, Roll no. MP/11/08 Regd no. 075667 of 2011-12, in partial fulfilment of the requirement for the award of Degree of Master in Pharmacy (M. Pharm) of **Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), affiliated to Gauhati University, Guwahati, Assam** is a bonafide assignment which has been carried out under my direct supervision and guidance during the academic session 2012-2013.

External Guide

Dr. Pronobesh Chattopadhyay

Scientist "D"& Group Head, DRL,

DRDO, Tezpur, Assam

Dr. Pronobesh Chattopadhyay,
DRDS

Scientist
and
Group Head

Defence Research Laboratory
Defence Research &
Development Organization (DRDO)
Ministry of Defence,
Govt. of India
Tezpur-784001, Assam
Phone: +913712258498 (Direct)
Fax: +913712-258534
Email:chattoapdhyay.drdo@gmail.com

Date: 28-02-2013

Certificate

This is to certify that the thesis entitled "Formulation and Evaluation of Controlled Release Mosquito Repellent Patch" by **MS. Somi Borah**, done her M.Pharm dissertation research work in Defence Research Laboratory, Tezpur. The entire work embedded in this thesis was carried out by **MS. Somi Borah** under my supervision and guidance.

(Pronobesh Chattopadhyay)

Dr. Pronobesh Chattopadhyay
Scientist D & Group Head
DEFENCE RESEARCH LABORATORY
DRDO, Ministry of Defence
Govt. Of India, Tezpur (Assam)

GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE (GIPS)
(A unit of Shrimanta Shankar Academy)
Approved by AICTE & PCI, New Delhi, affiliated to Gauhati University
N.H. 37, Hatkhowapara, Azara, Guwahati-781017.
Telephone: (0361) 2843405
Email: gips_guwahati@rediffmail.com

DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled **"*Formulation and Evaluation of Controlled Release Mosquito Repellent Patch*"** is a bonafide and genuine research work carried out by me under the joint supervision of **Dr.Pronobesh Chattopadhyay**, Scientist D, ***Defence Research Laboratory, Tezpur*** & **Mr. Bhupen Kalita**, Assistant Professor, Department of Pharmaceutics, ***Girijananda Chowdhury Institute of Pharmaceutical Science, Hatkhowapara, Azara, Guwahati-17.*** The work embodied in this thesis is original and has not been submitted for the award of degree, diploma, associateship or fellowship of any other university or institution.

Date: ***Somi Borah, B. Pharm***

Place: ***GIPS***

Affectionately Dedicated

To

My Beloved Parents

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Thanks to one an all . . . . .

Somi Borah

SERIAL NO.	ABBREVIATIONS	
1	p^H	Hydrogen ion concentration
2	°C	Degree Celsius
3	hrs	Hours
4	min	Minute
5	Hz	Hertz
6	g	Gram
7	kg	Kilogram
8	ml	Mililiter
9	µl	Microliter
10	mm	Milimeter
11	cm^2	Centimeter square
12	cm^{-1}	Per Centimeter/Inverse Centimeter
13	g/kg	Gram per Kilogram
14	g/ml	Gram per Mililiter
15	g/cm^3	Gram per Cubic Centimeter
16	ng/mole	Nanogram per Mole
17	% w/w	Percentage of Weight by Weight
18	% v/v	Percentage of Volume by Volume
19	ID	Internal diameter
20	CO_2	Carbon dioxide
21	Hcl	Hydrochloric acid
22	EC	Ethylcellulose
23	PVP K-30	Polyvinyl pyrrolidone
24	HPMC	Hydroxypropylmethylcellulose
25	PEG	Polyethylene glycol
26	DMSO	Dimethylsulfoxide
27	ANOVA	Analysis of variance
28	DDT	Dichlorodiphenyltrichloroethane
29	DEET	N, N-Diethyl-meta-toluamide

SERIAL NO.	ABBREVIATIONS	
30	DV	Dengue virus
31	DHF	Dengue hemorrhagic fever
32	DF	Dengue fever
33	DSS	Dengue shock syndrome
34	DENV	Dengue virus inhibitors
35	JE	Japanese encephalitis
36	PR	Percent repellency
37	RI	Repellency index
38	DNA	Deoxyribonucleic acid
39	RNA	Ribo Nucleic Acid
40	GO	Glucose oxidase
41	GPCR	G protein coupled receptors
42	GTP	Guanosine triphosphate
43	cAMP	Cyclic monophosphate
44	LD_{50}	Lethal dose 50%
45	OCT	Octopamine
46	ConA	Concanavalin A
47	PIB	Polyisobutylene
48	ACT	Artemisinin combination therapies
49	IT	Inspiratory time
50	ET	Expiratory time
51	TV	Tidal volume
52	PIF	Peak inspiratory flow
53	PEF	Peak expiratory flow
54	RR	Respiratory rate
55	RT	Respiratory time
56	pEnh	Enhanced pause
57	SFE	Supercritical fluid extraction
58	GC	Gas chromatography

SERIAL NO.	ABBREVIATIONS	
59	MS	Mass spectroscopy
60	GC-TOF-MS	Gas chromatography time-of-flight mass spectrometry
61	FID	Flame ionization detector
62	FTIR	Fourier transform infrared spectroscopy
63	SEM	Scanning electron microscope
64	DRL	Defence Research Laboratory
65	DRDO	Defence Research and Development Organisation
66	WHO	World Health Organization
67	EPA	Environment Protection Agency
68	FDA	Food and Drug Administration
69	OECD	Organisation for Economic Co-operation and Development
70	CPCSEA	Committee for the Purpose of Control and Supervision Of Experiments on Animals

TABLE OF CONTENTS

The present study was aimed to design and evaluate matrix type dermal patches containing oil of cinnamon, lemongrass and eucalyptus with different ratios of ethylcellulose (EC) and polyvinyl pyrrolidone (PVP K-30) as rate controlling polymers by solvent evaporation technique. All the prepared formulations were evaluated for physico-chemical properties, entrapment efficiency and oil release study was analyzed by gas chromatography (GC). The variation in the release profile was studied and based on it H_5 was selected as the optimized formulation on which the repellency and inhalation toxicity study was conducted. The mosquito repellent activity of the patch was evaluated on *Aedes albopictus* in laboratory conditions in terms of percent repellency and repellency index and the results of repellency study represented a steady release and longer protection from mosquitoes.

Keywords: Essential oils, Mosquito repellent, Patch, Gas Chromatography.

1.1. INTRODUCTION:

Mosquito borne diseases are major human and animal health problem in all tropical and subtropical countries. The diseases transmitted include malaria, filariasis, yellow fever, Japanese encephalitis and dengue fever. There has been exploration of various methods over the centuries to combat threats from mosquito borne diseases. With the beginning of the 20th century there grew an interest for use of biological control agents but this was declined with the discovery of insecticidal properties of DDT in 1939. However its deleterious impact on nontarget population and the development of resistance prompted for the search of alternative, simple and sustainable methods of mosquito control (Siriyasatien et al, 2006; Kumar et al, 2006; Shaalan et al, 2009).

Considerable research efforts have proved that essential oil compound and their derivatives are an effective and alternative means of controlling nuisance mosquitoes and their property of rapid degradation in the environment has favoured for its increased specificity (Tripathi et al, 2009). The justification of essential oils as green pesticides lies in the fact that the constituents of all essential oils are moderately toxic or mostly found to be nontoxic to mammals, birds and the aquatic ecosystem (Koul et al, 2008).

Although there exist several advancements in the field of synthetic drug chemistry and antibiotics but plants still continue to be one of the major raw materials for drugs treating various ailments of human. In fact Clinical and Pharmaceutical investigations have helped in elevating the status of various medicinal plants by identifying the role of active principles present in them and exploring their mode of action in human and animal systems (Vishwanathan et al, 2010). However essential

oils due to their volatile nature demand for frequent re-application to maintain its potency. They evaporate completely and thereby their effectiveness is short lived and so complete protection cannot be achieved (Patel et al, 2012).

Therefore keeping this in mind it was aimed for bringing up with a promising development of a sustained release dermal patch for controlling and repelling mosquitoes.

1.2. CONTROLLED DRUG DELIVERY SYSTEM:

The basic principle behind controlled drug delivery system is the release of active agent at a constant rate over a prolonged period or it may be triggered by the surrounding environment or by other external events. It has been developed to alleviate the shortcomings of conventional drug delivery systems. This drug delivery system aims at controlling the rate of drug delivery thereby sustaining the duration of therapeutic activity and/or targeting delivery of drug to desired site of action. An ideal drug delivery system should be inert, biocompatible, mechanically strong, comfortable for the patient, capable of achieving high drug loading, safe from accidental release, simple to administer and remove and easy to fabricate. In controlled release system polymers act as rate controlling matrix former and as release retarding agents. Polymers either of natural or synthetic origin are judiciously combined with drug or other active agent in such a way that the release of active agent occurs in a predesigned manner. The release profile from these systems should be predictable and reproducible (Jain et al, 2011; Chowdary et al, 2011). These systems deliver increased patient compliance by reducing frequency of administration and may also add commercial value to marketed drugs by extending patent protection.

The mechanisms used to achieve these goals are complex and diverse and depend on particular application of concern. Elaborate understanding of these mechanisms is important when designing and manufacturing controlled release systems and also in identifying potential failure modes (Siegel et al, 2012).

1.2.1. Mechanisms of Release: The release behaviour from these systems can be divided into three categories: passive pre-programmed, active pre-programmed and active self-programmed. With the first category (passive preprogrammed) the release rate is predetermined and is irresponsive to external biological stimuli, in the second category (active preprogrammed) the release rate can be controlled by a source external to the body. The last category is characterized by delivery systems whose release rate is controlled by biological stimuli. The release mechanism from matrices has been employed as widely used delivery systems. The "matrix" system indicates three dimensional network wherein drug molecules along with solvent and other excipients are homogenously dispersed. Controlled release is achieved by embedding the active constituent in a hydrophobic matrix (such as wax, polyethylene, polypropylene and ethylcellulose) or hydrophilic matrix (such as hydroxypropylcellulose, hydroxypropylmethylcellulose, methylcellulose, sodium carboxymethylcellulose and alginates). Of all hydrophilic polymers, for decades the most widely employed in formulations has and continues to be hydroxypropylmethylcellulose (HPMC). The release kinetics are affected by many factors such as polymer swelling, polymer erosion, drug dissolution/diffusion characteristics, drug distribution inside the matrix, drug/polymer ratio and system geometry (Grassi et al, 2005; Maderuelo et al, 2011).

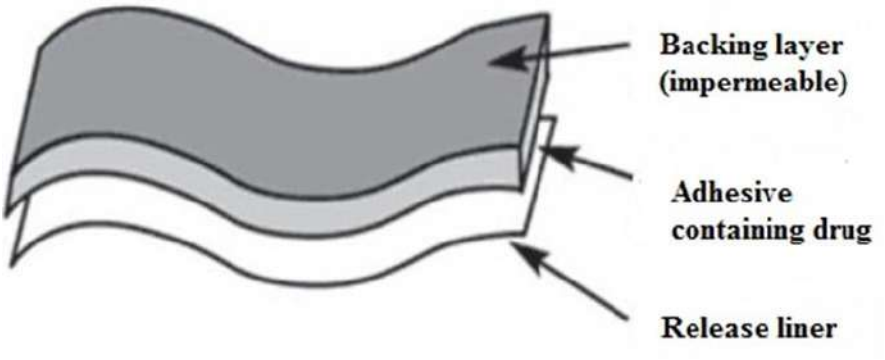


Fig 1.1: Matrix controlled release system

While with a "reservoir system", also called "core-shell system", the drug and the release rate controlling barrier material are completely physically separated. In this system coating or encapsulation is done with slowly dissolving materials like cellulose and polyethylene glycol. Its dissolution depends on the solubility and thickness of the coating. The drug is located at the center of the dosage form, whereas the polymer forms a membrane surrounding this drug depot. This means that the drug is molecularly dispersed within the core of the (wetted) formulation which covers two cases: the drug is molecularly dispersed in the excipients forming the core of the formulation or upon water penetration into the system the drug particles rapidly dissolve (Siepmann et al, 2012; Dusane et al, 2011).

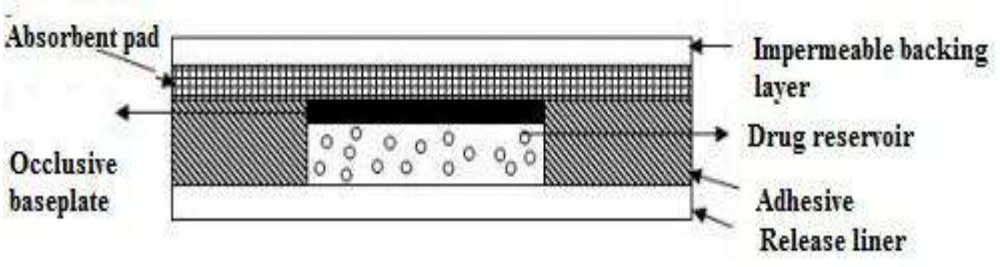


Fig 1.2: Reservoir controlled release system

1.3 POLYMERS IN CONTROLLED DRUG DELIVERY SYSTEM:

Polymers are macromolecules having very large chains, containing a variety of functional groups which can be blended with other low and high molecular weight materials and can be used for various applications. The pharmaceutical applications of polymers range from their use as binders in tablets to viscosity and flow controlling agents in liquids, suspensions and emulsions and used as film coatings to mask the unpleasant taste of a drug, to enhance drug stability and to modify drug release characteristics (Raizada et al, 2010). Polymers forming insoluble or skeleton matrices constitute the first category of retarding materials termed as plastic matrix systems, the second category representing hydrophobic and water insoluble materials and the third group includes polymers forming hydrophilic matrices. The chemical inertness and drug embedding ability of plastic matrix systems have been widely used for sustaining drug release while hydrophobic and waxy materials on other hand are erodable and control release is achieved by pore diffusion and erosion. In the case of polymers belonging to hydrophilic matrix systems, when are exposed to an aqueous medium, they immediately after hydration develops a highly viscous gelatinous surface barrier which controls release of drug and liquid penetration into center of matrix system (Reza et al, 2003).

1.3.1. Characteristic features of Release rate retarding polymers: Release retarding polymers are the sole performers in controlled delivery systems and for which natural, synthetic and semi-synthetic polymers have been employed. Protein, enzymes, muscle fibres, polysaccharides and gummy exudates are the natural polymers used effectively in formulating variety of pharmaceutical products. Some of

the well known natural polymers are chitosan, carrageenan, acacia, agar, gelatin, shellac, guar gum etc. (Pawan et al, 2011). Natural polymers are attractive primarily because they are readily available, capable of enormous number of chemical modifications, potentially degradable and compatible due to their origin (Sharma et al, 2011). Synthetic polymers on the other hand are being used as drug delivery systems as a polymeric drug itself or in combination with small molecule drugs or with biomacromolecules such as proteins and poly (nucleic acids). These polymers often adopt a more active role such as releasing a drug molecule, peptide or oligo/poly (nucleic acid) upon an external stimulus and thereby these polymers are considered as stimuli responsive polymers (Schmaljohann, 2006). These smart polymers or stimuli responsive polymers possess active response to changes in environmental signals and thereby the changes in physico-chemical property are achieved as designed. The various stimulus, physical (temperature, ultrasound, light, electricity, mechanical stress), chemical (p^H, ionic strength) and biological signals (enzymes, biomolecules) have been used as triggering force. The signals can be either artificially supported by 'external' sources or naturally promoted by the 'internal' environment of a certain pathophysiological condition (Kim et al, 2009). With magnetically modulated systems the release rates can be characterized by magnetic field characteristics and mechanical properties of polymer matrix. It was found that the extent of release enhancement occurred as the magnetic field amplitude rises and with ultrasonically controlled systems release rates of substances can be repeatedly modulated at will from a position external to the delivery system. Both bio-erodible and non-erodible polymers were used as drug carrier matrices (Kost et al, 2001). Temperature sensitivity originated from the balance between hydrophilic and hydrophobic segments on

polymer chain and the free energy of mixing, when these polymers undergo abrupt changes in solubility due to increase in environmental temperature (Gariepy et al, 2004). Generally the p^H of body tissue in a healthy human is maintained around 7.4. The variation of p^H along gastrointestinal tract, 1-3 in the stomach and 7 in the intestine has been used as a triggering signal for p^H responsive drug delivery. Basically ionizable moieties such as carboxylic acid, amine, azo, imidazole, pyridine, sulfonamide and thiol groups can afford p^H sensitivity. Lastly polymers responding to biomolecules which have been an interesting subject because they can provide high specificity to polymers responding to physical or chemical stimuli. Famous example of glucose-sensitive polymers utilizing phenyl boronic acid, glucose oxidase (GOx), or concanavalin A (ConA) for insulin delivery to treat diabetes where insulin release could be tightly regulated by a closed-loop feedback system (Kim et al, 2009).

1.4. PATCH AS DRUG DELIVERY SYSTEM:

The discovery of a new medicine is a time consuming as well as expensive procedure so it is quite an innovative approach when improved therapy can be obtained by redesigning the modules. Patches are therapeutic systems defined as self-contained, discrete dosage form which when applied to intact skin delivers the active constituent at a controlled release rate. In USA out of 129 drug delivery candidate under clinical evaluation, 51 are transdermal or dermal systems (Barry, 2001). These systems offer advantages of ease of application, longer duration of action resulting in reduced dosing frequency, non-invasive and above all can be terminated at any time because of self-administration and provide with improved patient compliance. However it also suffers from disadvantages like possibility of local irritation at the

site of application either due to drug, adhesive or by other excipients in the formulation (Arunachalam et al, 2010).

1.4.1. Design and Development of Patch: Generally the formulation of patches are designed by two traditional methods i.e. reservoir and matrix system. With membrane or reservoir system the active constituent is separated by an impervious backing layer and a rate controlling membrane which controls the release at a controlled rate from this membrane in micro-porous or non-porous form. In this type of system the rate of release is zero order and release can be controlled by varying the polymer concentration, permeability co-efficient and thickness of rate controlling membrane whereas in matrix system the active constituent is evenly distributed in a hydrophilic or lipophilic polymer matrix. In fact, a matrix is defined as a well-mixed composite of one or more drugs with gelling agent i.e. hydrophilic polymers. The manufacturing and release rate from the dosage form is controlled mainly by the type and proportion of polymer used in the preparations (Keleb et al, 2010; Vishwakarma et al, 2012). Nowadays vapour patch have come up which are designed to release the active constituents in the form of vapour. In this system the adhesive layer not only serves to adhere the various layers together but also to release vapour. Vapour patches have been used in decongestion which releases essential oils upto 6 hours, are also available as controller vapour patches which improve the quality of sleep and also patches that reduce the quantity of cigarettes that one smokes in a month. The first commercially available vapour patch to reduce smoking was approved in Europe in 2007 (Dhiman et al, 2011).

1.4.2. Marketed Products: The first transdermal patch product was approved by FDA in 1981. It provided the controlled systemic absorption of scopolamine for the prevention of motion sickness (*TransdermScop*, ALZA Corp.) and nitroglycerine for the prevention of angina pectoris associated with coronary artery disease (Transderm-Nitro) (Bhowmik et al, 2010; Saroha et al, 2011). Nilani et al (2011) carried out the formulation and evaluation of wound healing dermal patch from plant *Cocculus hirsutus* on excised wound model. Gupta et al (2009) worked on the formulation of matrix type transdermal patch containing glibenclamide as the active agent and evaluated the various parameters. Barhate et al (2009) prepared ketoprofen patches with polymers HPMC E5, HPMC K_4M, HPMC K100M, Eudrajit RS100 and Eudrajit RL100 with propylene glycol as enhancer. Amongst the various formulations the formulation containing HPMC E5 showed ideal zero order release kinetics. Enayatifard et al (2009) studied the effect of combination of polymers viz. HPMC and ethylcellulose (EC) on release profile and kinetics of Diltiazem hydrochloride from matrices. The results showed a controlled release characteristics from the matrix system which was influenced by combined effect of polymer.

1.4.3. Anatomy of a controlled release patch: The basic components are Release liner, Backing, Overlay, Membrane, Pressure sensitive adhesive and other excipients.

Fig 1.3: Schematic diagram of patch as delivery system

(a) Release liner: During storage the patch is covered by a protective liner,

this is removed and discarded prior to the application of the patch to the skin. Since the liner is in intimate contact with the patch, the liner should be chemically inert. The release liner is composed of a base layer which may be non-occlusive (e.g. paper fabric) or occlusive (e.g. polyethylene, polyvinylchloride) and a release coating layer made up of silicon or teflon. Other materials used for release liner include polyester foil and metalized laminate.

(b) Backing: These are chosen for appearance, flexibility and need for occlusion. It protects the patch from outside environment. The backing layer serves the function of holding the entire system and protects the drug reservoir. Commonly used films are polyester, polyethylene and polyolefin. However an overemphasis on the chemical resistance often may lead to stiffness and high occlusivity to moisture and air which cause the system to lift and possibly irritate the skin during long term wear (Sharma et al, 2011).

(c) Overlay: This overlay secures the patch to the skin of the applicant. Some manufacturers, distributors or medical personnel might suggest placing adhesive tape over the patch if the patch lifts or even falls off.

(d) Membrane: It controls the release of active constituent from the reservoir and multi-layer patches. The elementary way to control the release of a drug is to disperse through an inert polymeric matrix. In this system, the drug is physically blended with polymeric powder (either hydrophilic or lipophilic) and the medicated polymer is then molded into a medicated disc with a defined surface area and controlled thickness. An inverse relationship is thus observed between the release rate and membrane thickness. It may or may not contain rate-controlling membrane but it should be flexible enough not to split or crack on bending or stretching. Some of rate-

controlling membranes are polyethylene sheets, ethylene vinyl acetate copolymer and cellulose acetate.

(e) Pressure sensitive adhesive: The adhesive, a vital component which provide intimate contact between the delivery system and the skin. It serves to adhere to the substrate, in this case skin because of interatomic and intermolecular attractive forces established at interface to provide intimate contact. To obtain this degree of contact, the material must be able to deform under slight pressure, giving rise to the term ‘‘pressure sensitive’’. Generally adhesion involves a liquid like flow resulting in wetting of the skin surface upon the application of pressure and when pressure is removed, the adhesive sets in that state. Widely used pressure sensitive polymers are polyisobutylene (PIB)-based adhesives, acrylics and silicone-based adhesives (Wokovich et al, 2006).

(f) Other excipients: These include penetration enhancer for accelerating the penetration of active ingredients. It should be safe, non-toxic, non-allergenic and pharmacologically inert. Plasticizers are also used to prevent the patches from becoming brittle and should be physically and chemically stable, non-toxic, non-irritant, compatible with other ingredients, colourless and odorless. These have been used in many formulations ranging from 5 to 20% (w/w, dry basis). It is also responsible for adhesiveness of the film with other surfaces or membranes and improvement in the strength of film. Some of its examples are glycerol or sorbitol at 15% w/w, dry basis, phthalate esters, phosphate esters, fatty acid esters and glycol derivatives such as PEG 200 and PEG 400.

(g) Packet: It protects patch against drug loss and contamination during storage and individually packed in heat sealed foil pouches (Alexander et al, 2012).

1.5 INFLUENCE OF MOSQUITO BORNE DISEASES IN PRESENT HEALTH SCENARIO:

1.5.1 Mosquito borne diseases: Since ancient times, mosquitoes have been reported as the source of various ailments affecting human. Comprising approximately 3500 species, mosquitoes are found beyond the tropical and subtropical regions of the world. The chief genera which vector human disease causing pathogens are *Anopheles* (malaria, filariasis), *Aedes* (yellow fever, dengue, chikungunya) and *Culex* (West Nile, Japanese encephalitis, filariasis). Over its life span a female mosquito repeatedly takes a blood meal as protein source to complete egg development. By injecting the saliva which may contain pathogens into the host animal, the pathogens thus complete an obligatory life cycle phase and multiply in the mosquito's salivary glands. This thereby makes female mosquito an ideal transmitter of diverse blood borne pathogens and agents of devastating human diseases (Tolle, 2009; Michel et al, 2005).

1.5.1. (a) Malaria: India being the second populous country in the world, its public health system faces many challenges in terms of implementation of surveillance programs to accurately estimate and control the national malaria burden. It has prevented economic and cultural development in vast regions of the world and continues to be a huge social, economical and health problem today. History reveals that the highest incidence of malaria in India occurred in the 1950s with an estimated 75 million cases and 0.8 million deaths per year (World Health Organization, Country Office for India). Malaria is caused by the protozoal parasites *Plasmodium vivax Grassi* and *Feletti, Plasmodium malariae Feletti* and *Grassi, Plasmodium ovale Stephens*, and *Plasmodium falciparum Welch*, which are transmitted by Anopheles

mosquitoes. Studies suggest that for any form of malaria to be endemic in a certain area a number of requirements must be fulfilled, it needs the presence of a large number of competent anopheline mosquitoes with a sufficient preference for human blood and an exposed human population with enough number of malaria carriers and susceptible individuals for the chain of infection to persist (Adams et al, 2011).

There are six recognised primary vectors of malaria in India viz., *Anopheles culicifacies, An. stephensi, An. dirus, An. fluviatilis, An. minimus* and *An. Sundaicus.* Vectors of secondary importance are *An. annularis, An. varuna, An. jeyporiensis* and *An. philippinensis. An. culicifacies* is the main vector of rural and peri-urban areas and is widespread in peninsular India. It is found in a variety of natural and man-made breeding sites, *An. stephensi* is responsible for malaria in urban and industrial areas, *An. fluviatilis* is the main vector in hilly areas, forests and forest fringes in many states, especially in the east, *An. dirus* and *An. minimus* is the vector in the foothills of North-Eastern states while *An. epiroticus (An. sundaicus),* a brackish-water breeder is restricted to the Andaman and Nicobar Islands. In India National Drug Policy provides guidelines for treatment of malaria. Chloroquine is the first line treatment for vivax malaria and for *P. falciparum* in low risk and chloroquine sensitive areas. In view of reports of treatment failure to chloroquine, artemisinin combination therapies (ACT) have been introduced in the high burden states for the treatment of *P. falciparum* (Dash et al, 2008). On the eve of World Malaria Day, WHO hails global progress in combating malaria, but highlights the need to further reinforce the fight. WHO's new initiative, T3: Test, Treat, Track, urges malaria-endemic countries and donors to move towards universal access to diagnostic testing and antimalarial treatment and to build robust malaria surveillance systems (WHO 2012).

Year	Total malaria cases (million)	Death due to malaria (thousand)
1995	2.93	1151
1996	3.04	1010
1997	2.66	879
1998	2.22	664
1999	2.28	1048
2000	2.03	932
2001	2.09	1005
2002	1.84	973
2003	1.87	1006
2004	1.92	949
2005	1.82	963
2006	1.79	1707
2007	1.51	1310
2008	1.52	924

Table 1.1: Countrywide malaria surveillance data (1995-2008)

Year	State	Remarks
1996	Rajasthan and Haryana	Large number of cases; many deaths in Rajasthan
1997	Gujarat, Goa and West Bengal	4 districts
1998	Goa and Maharashtra	2 districts
1999	Andhra Pradesh, Assam, Bihar and West Bengal	23 districts
2000	Uttar Pradesh, Madhya Pradesh and Karnataka	5 districts
2003	Rajasthan	Large epidemic affecting several districts

2005	Assam, Goa, Haryana, Gujarat, Karnataka and Maharashtra	48 districts
2006	Karnataka and West Bengal	5 districts

Table 1.2: Malaria epidemics in India (1996-2006)

***** Obtained from Strategic Action Plan for Malaria Control in India 2007-2012.

1.5.1. (b) Dengue: Another important mosquito borne disease in the world is dengue viral infections. A wide distribution of dengue virus and their mosquito vectors have been there across many tropical countries in three continents for more than 200 years. The populations which are at risk of acquiring dengue viral infections include those in urban tropical and subtropical areas and it constitutes 40% of the world population. Reports signify that annually 100 million cases of dengue fever and half a million cases of dengue hemorrhagic fever (DHF) occur globally, with an average case of fatality rate around 5%. Potential reasons for the global resurgence and spread of dengue fever and DHF epidemics are assumed due to population growth, uncontrolled urbanization in tropical and subtropical countries, proliferation of breeding sites for *Aedes* mosquitoes and the lack of effective mosquito control. The three major species of mosquitoes belonging to the genus *Aedes,* namely, *A. aegypti, A. albopictus,* and *A. polynesiensis* are known to act as vectors in transmission of dengue infections. *Aedes aegypti*, the most important vector is mainly a container breeder and day biting mosquito and is found in tropical and subtropical areas (Gurugama et al, 2010).

Dengue virus is a positive stranded enveloped RNA virus of the *Flaviviridae* family and there are four serotypes of the virus referred to as DV-1, DV-2, DV-3 and

DV-4. It is composed of three structural protein genes which encode the nucleocapsid or core protein, a membrane associated protein, an enveloped glycoprotein and seven non-structural proteins. The four main characteristic manifestations of dengue illness are continuous high fever lasting 2-7 days, haemorrhagic tendency as shown by a positive tourniquet test, thrombocytopenia and evidence of plasma leakage manifested by haemoconcentration (an increase in haematocrit 20% above average for age, sex and population) and pleural effusion *etc*. (Gupta et al, 2012). Dengue virus causes clinical syndromes in human which ranges from an acute self-limited febrile illness (dengue fever, DF) to a severe and life-threatening vascular leakage and shock (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS). There have been many efforts made for the search of a suitable vaccine but lack of an animal model and the need for a high immunogenicity vaccine against all four serotypes are posing huge challenges in the dengue vaccine development thereby making the search for antiviral products imperative. The antivirals previously designed against flaviviruses mainly focused on inhibition of viral RNA replication. Ribavirin, mycophenolic acid and adenosine analogues are believed to act as inhibitors of the RNA-dependent RNA polymerase. But the low efficacy of these types of compounds, have made it an urgent need for the highly potent DENV (Dengue virus) inhibitors (Alen et al, 2012).

Year	DF/DHF cases	Occurrence of death	Region
1996	10252	423	Delhi
2006	12317	184	21 states/Union Territories
2007	5534	69	18 states

Table 1.3: Severe outbreaks of DF/DHF across India.

*****Obtained from Guidelines for clinical management of DF/DHF/DSS.

1.5.1. (c) Japanese encephalitis: One of the leading causes of acute encephalopathy is encephalitis affecting children and adolescents particularly in the tropics. It is characterized by acute inflammation of brain tissue leading to acute onset of fever, headache, confusion and sometimes seizures. It is transmitted to human beings by insects such as mosquitoes and ticks. Japanese encephalitis (JE) is occasionally transmitted to human being through infected *Culex mosquitoes* after sucking blood from infected amplified host. Mosquitoes proliferate in close association with pigs and other animal reservoirs and are found to spread virus of Japanese encephalitis basically in malnourished children of poor families from rural areas (Kumar et al, 2012). JE is a single stranded positive sense RNA virus. Its structure consists of a genome of 10,976 bases surrounded by a nucleocapsid and lipid membrane. Within the membrane lie the envelope (E), membrane (M) and precursor M (prM) proteins. Other proteins include the NS1-5. The E protein is of particular importance as it binds to cell receptors and initiates infection. The disease tends to occur in rural areas with transmission from April-October in temperate climates or during rainy seasons in tropical/ subtropical areas. Clinical infection varies from fever and aseptic meningitis to seizures (especially in children), paralysis (spastic or flaccid) and coma (Balasegaram et al, 2008). Japanese encephalitis (JE) is a vector-borne viral disease that occurs in South Asia, Southeast Asia, East Asia and the Pacific. It is now an emerging viral disease having international importance because it is invading the previously non-endemic areas. In India it was first recognized in 1955 and since then major outbreaks from different parts of the country have been reported predominantly in rural areas. So far, incidence of JE has been reported from 15 States including Karnataka (Kanojia, 2007).

The main pillar of JE control is the use of a live attenuated vaccine for human, which was developed some 40 years ago. Currently available JE vaccines are relatively safe and effective but drawback is that multiple doses are required. Therefore effective delivery of these vaccines to poor, rural communities remains a formidable challenge and compliance and delivery costs have to be considered. Two vaccine candidates are in late-stage clinical development. The first one is a second-generation, live inactivated, single-dose vaccine grown in Vero cells. It is the yellow fever virus based chimeric vaccine and the second candidate is an attenuated SA 14-14-2 virus strain adjuvanted with aluminum hydroxide and also grown in Vero cells (Erlanger et al, 2009).

1.5.2. Control of Mosquitoes: The efficient way to control these diseases is to control mosquito vector populations and prevent mosquito bites. Studies reflect that insect repellents play an important role in preventing the mosquito vector, deterring an insect from flying to, landing on or biting human and animal skin. Generally the widely used compounds as insect repellents are synthetic chemical repellents but they bear the disadvantage of being not safe for human, especially children, domestic animals because they may cause skin irritation, hot sensation, rashes or allergy (Sritabutra et al, 2011). A mosquito repellent is a substance which when applied to skin, clothing or other surfaces discourages insects (arthropods in general) from landing or climbing on that surface. There are a number of natural and chemical mosquito repellents that work to repel mosquitoes.

Chemical methods	Non-chemical methods	Biological methods
Synthetic repellents: DEET, Permethrin **Natural repellents:** Neem oil, Citronella oil	**Physical method:** Medicated net, Non-medicated net, Mosquito traps **Mechanical methods:** Electric mosquito zapper, Mosquito magnet	By growing some fish species that feeds on mosquito larvae in water bodies

Table 1.4: Mosquito repellent methods

1.5.3. Advanced and Recent Mosquito repellent Approaches: Apart from the methods tabulated above there are some recent approaches in mosquito repellent methods. Fogging is a temporary method of controlling mosquitoes and such other pests but is particularly necessary in the context of health threats from severe bug populations and for an outdoor activity where these pests are unwanted. The method employs a thermal fogger which produces a pesticide fog or smoke by heating the fogging solution with a coil inside the unit. Once this coil warms up, it will produce a nice insect fog that can be directed to areas required. It is ready-to-use fogging solution and each gallon contains 0.5% Pyrethrins and 5.0% Piperonyl Butoxide. Another revolutionary approach is the transdermal technology to deliver a natural mosquito repellent nutrient directly into the blood stream for a complete 24-hour mosquito protection. The active ingredient in the patch is Vitamin B_1 or Thiamine as it is known to be the most effective natural mosquito repellent discovered to date. It was found that female mosquitoes are repulsive to the scent of Thiamine, therefore the

patch works by inducing a controlled amount of Vitamin B_1 into the blood stream (Patel et al, 2012).

Mosquito repellent cream

Mosquito patches

Mosquito repellent spray

Mosquito repellent wipes

Fogging machine

Mosquito repellent liquid

Fig 1.4: List of some of the marketed mosquito repellent products

Recently, the ecological role has been more concentrated on preventing damage from pollution rather than proposing sustainable solutions to different global and local problems faced by human societies. Biological control means the use of different kinds of living things, and/or their derivatives to eliminate pest populations. Various organisms, natural biological control agents can be utilized to control mosquito populations thus avoiding the use of chemicals and harm to the environment in the process. The potential of genetics against mosquito-borne infection has recently been considered in vector control programs. Recently techniques to modify genes of mosquitoes are believed to be an appropriate remedy against malaria and dengue fever. The main purpose is to produce a genetically modified strain of mosquito in the laboratory which does not serve as a carrier of disease and which is competitively superior in the natural habitat such that wild mosquitoes are eventually replaced after the release of genetically altered mosquitoes in nature. However, there are several problems with this approach and it is taking some time to turn the prospects of this technology into practical tools (Kumar et al, 2006). Literature reveals that the predaceous bug *Emesopsi streiti* preys upon adult mosquitoes in bamboo internodes and *Microvelia cavicola* and *Paravelia myersi* fed on adult mosquitoes emerging in tree holes. There are also reports of fly larvae of *Xenoplatyura beaver* preying upon emerging adult mosquitoes in Nepenthes pitcher plants and the dragonflies *Pantala hymenaea* and *Erythemis collocate* attacking swarming of *Anopheles freeborni* after sunset (Shaalan et al, 2009). Also of significance are the most important entomopathogenic fungi for mosquito control. Particular focus is on species belonging to the genera *Lagenidium*, *Coelomomyces*, *Entomophthora*, *Culicinomyces*, *Beauveria* and *Metarhizium*. The use of biological control agents such as predatory fish, bacteria,

protozoa and nematodes have all shown promise as a means to control mosquito populations and progress in these fields has recently been reviewed (Scholte et al, 2004).

1.6. NATURAL PRODUCTS AS POTENTIAL ANTIMOSQUITO AGENTS:

Since the ancient time, plants with secondary metabolites have been used by human to treat infections, health disorders and illness. Mostly higher plants are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavor and aroma industries (Karuppusamy, 2009). Plants produce a vast and diverse assortment of organic compounds, majority of which do not appear to participate directly in growth and development. These substances traditionally referred to as secondary metabolites often are differentially distributed among limited taxonomic groups within the plant kingdom. Their functions many of which remain unknown are being elucidated with increasing frequency. The advancements in plant cell and tissue cultures hold great promise for controlled production of useful secondary metabolites on demand. Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years. In order to obtain high yields suitable for commercial exploitation, efforts have been focused on isolating the biosynthetic activities of cultured cells, achieved by optimizing the cultural conditions, selecting high-producing strains and employing precursor feeding, transformation methods and immobilization techniques (Hussain et al, 2012).

1.6.1. Traditional mosquito repellents and usage custom: The Greek natural philosopher Pliny the Elder (1st century AD) recorded all the known pest control methods in ‘‘Natural History’’. The use of chrysanthemum, pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, d-limonene, camphor and turpentine were among some important phytochemical insecticides widely used in developed countries. The discovery of DDT and subsequent development of organochlorines, organophosphates and pyrethroids suppressed natural product research for insect control. However high cost of synthetic pyrethroids, environment and food safety concerns, the unacceptability and toxicity and increasing insecticide resistance on a global scale argued for stimulated research towards potential botanicals. The plant world comprises a rich reserve of phytochemicals that may be widely used in the place of synthetic insecticides. Various methods were adapted to repel the insects/ mosquitoes (Kishore et al, 2011). This repellency of plant material has been exploited for thousands of years by man, most simply by hanging bruised plants in houses, a practice which is still in wide use throughout the developing countries. Plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later on oil formulations applied to the skin or clothes which was first recorded in writings by ancient Greek, Roman and Indian scholars. The discovery of new plant-based repellents is heavily reliant on ethnobotany. It is the targeted search for medicinal plants through in-depth interviews on folk-lore and traditional medicine. The ethnobotanical surveys is a common practice conducted using structured interviews, combined with the collection of plant voucher specimens to evaluate plant use by indigenous ethnic groups (Maia et al, 2011). Different researchers have observed that

the phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents and ovipositional attractants having deterrent activities. The plant products have been used traditionally to repel or kill the mosquitoes in many parts of the world (Das et al, 2003).

1.6.2. Essential oils as green pesticide: Essential oils are volatile naturally occurring, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are liquid, volatile, rarely coloured, lipid soluble and soluble in organic solvents with a density generally lower than that of water. There are 17,500 aromatic plant species among higher plants and approximately 3,000 esential oils are known out of which 300 are commercially important for pharmaceuticals, cosmetics and perfume industries apart from pesticidal potential. In nature essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reducing their appetite for such plants. They also may attract some insects to favour the dispersion of pollens and seeds or to repel the undesirable. Some of them constitute effective alternatives or complements to synthetic compounds of the chemical industry without showing the same secondary effects (Bakkali et al, 2008).

Literature cited indicates that certain natural products *Zanthoxylum armatum* (Rutaceae); *Azadirachta indica* (Maliaceae) and *Curcuma aromatica* (Zingiberaceae) have been investigated for repellent activity against mosquitoes. *Callistemon rigidus* (bottle brush), *A. indica* (neem) and *Z. armatum* (timur) have been reported to have repellent activity against land leeches also. Repellent action of neem oil in the form of mats and neem cream has been evaluated against mosquitoes. Benzene and methanol

extracts of *Artemisia vulgaris* have been reported to have repellent activity against *Aedes aegypti*. Quelling, an insect repellent produced in China, derived from the extract of the lemon grass and eucalyptus plants were evaluated against mosquitoes. Essential oil obtained from *Vitex negundo* was used as repellent against *Aedes aegypti*. Repellent properties of *Lantana camara* (Vervanaceae) flowers against Aedes mosquitoes have also been reported (Das et al, 2003). Most of the plant based insect repellents currently on the market contain essential oils from one or more of the following plants: citronella (*Cymbopogon nardus*), cedar (*Juniper virginiana*), eucalyptus (*Eucalyptus maculata*), geranium (*Pelargonium reniforme*), lemon-grass (*Cymbopogon excavatus*), peppermint (*Mentha piperita*), neem (*Azadirachta indica*) and soybean (*Neonotonia wightii*) (Choochote et al, 2007).

Typically these oils are liquid at room temperature and get easily transformed from liquid to gaseous state at room or slightly higher temperature without undergoing decomposition. The aromatic characteristics of essential oils provide various functions for the plants including attracting or repelling insects, protecting themselves from heat or cold and utilizing chemical constituents in the oil as defence materials. The concept of "Green Pesticides" refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population. They are safe, ecofriendly and are more compatible with the environmental components than synthetic pesticides. The purified terpenoid constituents of essential oils are moderately toxic to mammals but with few exceptions, the oils themselves or products based on oils are mostly nontoxic to mammals, birds and fish thereby, justifying their placement under "green pesticides" (Koul et al, 2008).

Compound	Animal tested	Route	LD_{50} (mg/kg)
1,8 Cineole	Rat	Oral	2480
Cinnamaldehyde	Guinea pig	Oral	1160
Citral	Rat	Oral	4960
Eugenol	Rat	Oral	2680
d-limonene	Rat	Oral	4600
Linalool	Rat	Oral	>1000
Myrcene	Rat	Oral	5000
Thujone	Mice	Subcutaneous	87.5
Thymol	Rat, Mice	Oral	980,1800
(+) Carvone	Rat	Oral	1640

Table 1.5: Mammalian toxicity of some essential oil compounds (Koul et al, 2008)

The promising herbal medicines should be subjected to phytochemical analysis and identification of active ingredients and clinical trials to confirm their efficacy and safety and also to determine the recommended doses in line with WHO guidelines (Kazembe et al, 2012).

1.6.3. Sources of Essential oils: Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants. These are found in glandular hairs or secretory cavities of plant cell wall and are present as droplets of fluid in the leaves, stems, bark, flowers, roots and/or fruits in different plants. They are constituted by hydrocarbons and oxygenated compounds which are responsible for the distinctive odour of plants (Nerio et al, 2010). Plants produce a high diversity of natural products or secondary metabolites with a prominent function in the protection against predators and microbial pathogens on the basis of their toxic nature and repellence to herbivores and microbes and some of which also involved in defence

against abiotic stress and also important for the communication of the plants with other organisms but these metabolites are insignificant for the growth and developmental processes. There are three major groups of secondary metabolites viz terpenes, phenolics, nitrogen and sulphur containing compound (Mazid et al, 2011).

1.6.4. Extraction and Analysis of Essential oils: The isolation method influences the composition of oil to a large extent . The chemical profile of the essential oil products differs not only in the number of molecules but also in the stereochemical types of molecules extracted. The extracted product can vary in quality, quantity and in composition depending on climate, soil composition, plant organ, age and vegetative cycle stage (Tripathi et al, 2009).

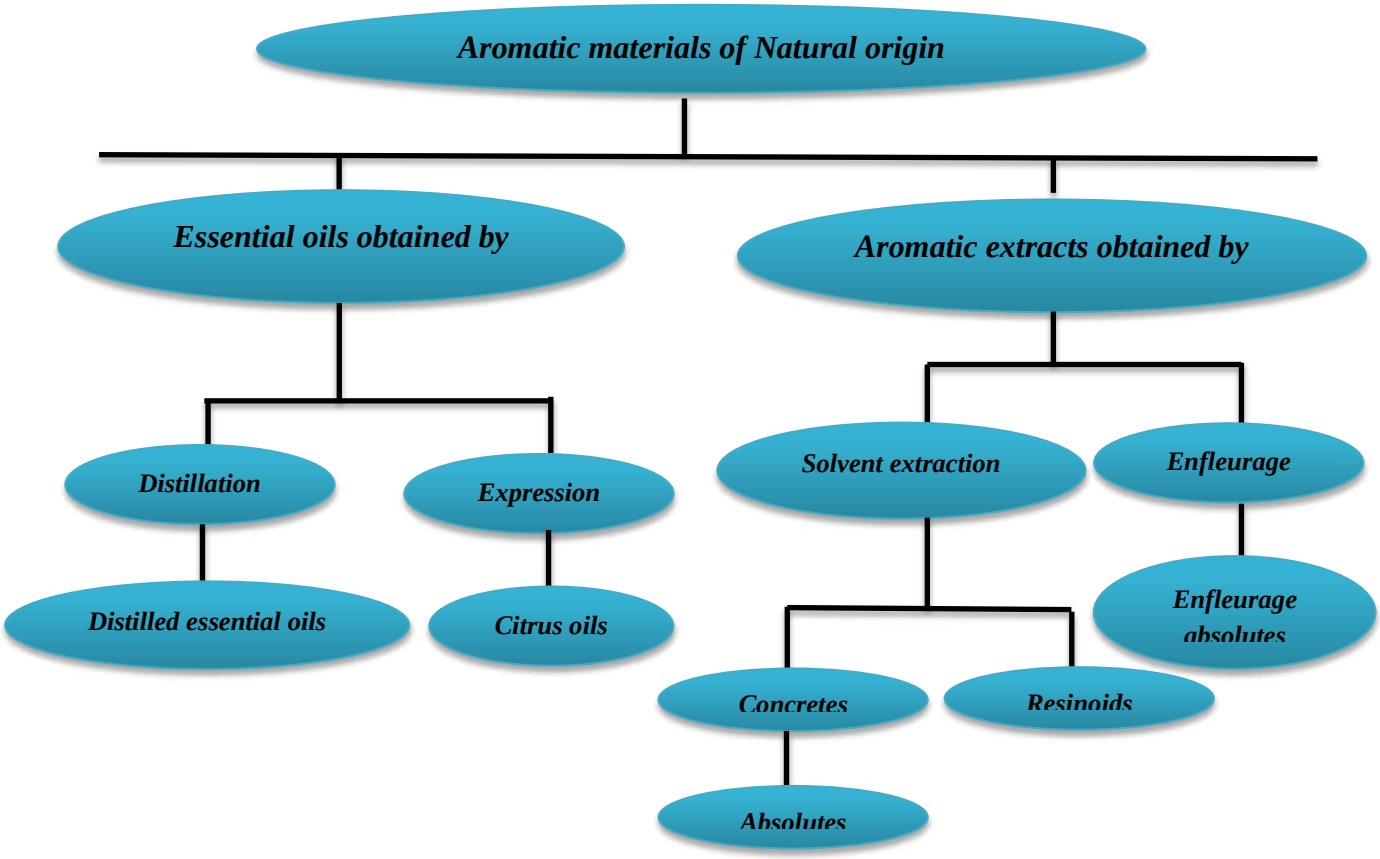


Fig 1.5: Essential oils extraction methods

1.6.4. (a) Extraction techniques of essential oils: Previously essential oils are normally extracted by distillation, steam distillation or organic solvent extraction techniques. In recent time, the supercritical fluid extraction (SFE) has received special attention in the fields of solid material extraction and fractionation of liquid mixtures. Nowadays, the possibility of extracting and fractionating oils (plant and animal) receives widespread interest due to the direct applications in the food and pharmaceutical industries for the generation of high value products. In distillation technique plant materials are dried for 3-4 hours in sunlight followed by introduction of plant material in Clevenger apparatus with solvent while in steam distillation process, steam is generated separately in a boiler and is passed through the distillation tank through a steam coil with the plant material tightly packed above the perforated grid. Steam along with oil vapors is condensed in the condenser and is separated in the oil receiver. Long extraction time, low yield, toxic solvent residue, labor-intensive operation and degradation of thermo-sensitive compounds are the disadvantages involved in using such techniques (Katiyar et al, 2010).

Ideally, extraction procedures should be environment friendly but steam extraction and solvent extraction do not meet these criteria as they generate large volumes of contaminated, hazardous solvents and emit toxic fumes. Recently clean techniques, such as SFE, microwave and ultrasound have been developed for extracting essential oils from complex matrices. Ultrasound assisted extraction has been widely used for the extraction of lipids, proteins, flavoring, essential oils and bioactive compounds carotenoids and polysaccharides. This technique improves extraction efficiency and extraction rate, reduce extraction temperature and increases the selection range of the solvents. The use of microwaves for isolating essential oils

has recently been reported. Microwave technology has allowed the development of rapid, safe and cheap methods for extracting essential oil and does not require samples devoid of water. Supercritical fluid extraction has been used for the extraction of flavors and fragrances from natural materials. This separation technology uses supercritical fluid as the solvent. The main supercritical solvent used is carbondioxide. Carbondioxide is cheap, environment friendly and generally recognized as safe. Another advantage is that it is gaseous at room temperature and ordinary pressure, which makes analyte recovery very simple and provides solvent-free analytes (Xu et al, 2011).

1.6.4. (b) Analytical techniques for essential oil analysis: Chromatography especially gas chromatography (GC) and mass spectroscopy (MS) have been the most applied analytical techniques for essential oil analysis. Due to complexity of essential oil compositions there has been a high demand for sophisticated instruments to analyze them. It is quite expensive in terms of instrumentation, maintenance and even operation costs are also very high. This is a powerful and rapid analytical tool for identification and quantification of almost any compound volatile below 450°C. The principle of operation involves separation of components of sample under test due to partition between gaseous mobile phase and stationary liquid or solid phase. Usually the mobile phase is a carrier gas, generally an inert gas or unreactive gas such as helium, nitrogen, argon, hydrogen and air. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called column. The composition of stationary phase consists of dimethyl polysiloxane phenyl arylene polysiloxane, polyethylene glycol, divinyl benzene etc. (Summerfield, 2010).

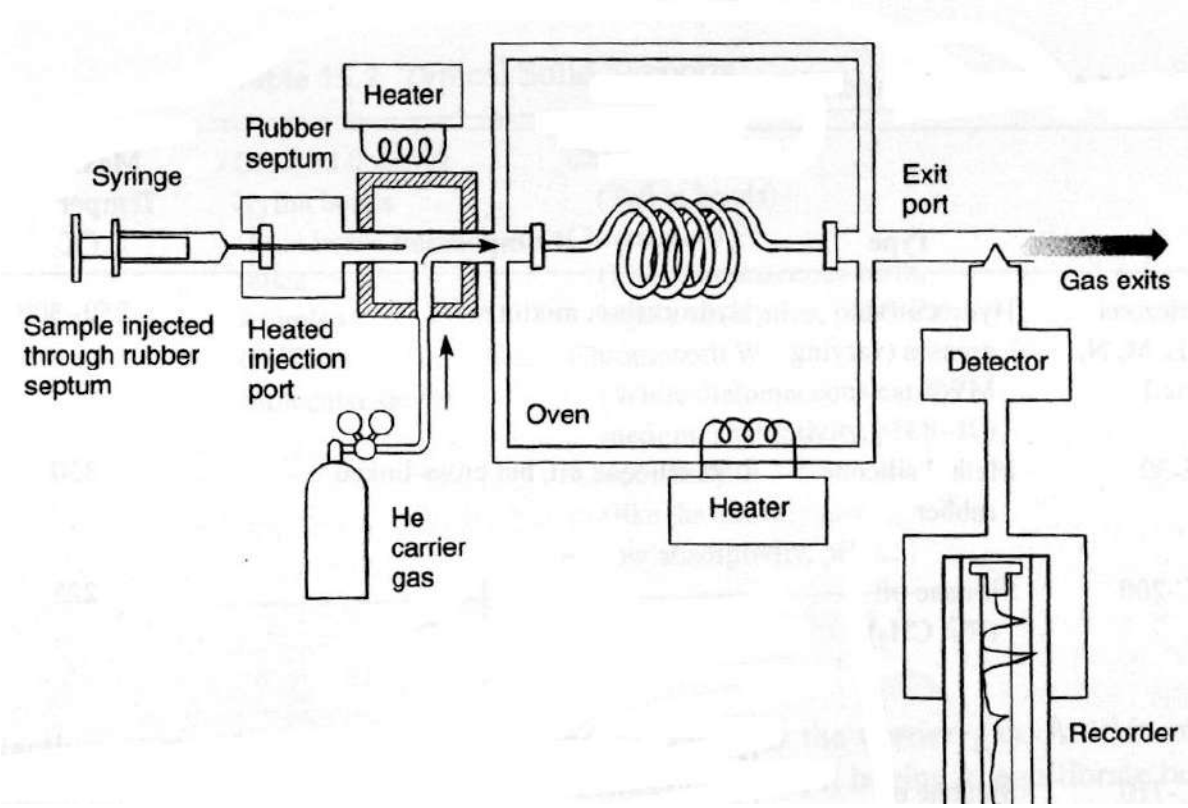


Fig 1.6: Schematic diagram of a gas chromatograph

Stationary phase development seeks to produce more thermally and chemically stable phases, greater selectivity in separation of components by different phase chemistry, better efficiency by preparing more regular surface coating, use different technology to optimize the phase available to the specific regions of the analysis which require better resolution. Once a familiarity with the component elution time is established and thus by combining this with mass spectrometry, the quality of data increases proportionately. Mass spectrometry may give the ability to recognise overlapping peaks and divide relative amount of components where overlaps occur. One of the newer methods proposed to give improved analysis of complex mixtures, especially for deconvolution of overlapping mass spectra, is time-

of-flight mass spectrometry – GC-TOF-MS. Being capable of generating instantaneous spectra, there is no bias arising from the mismatch between scan rate and peak abundance changes in the ion source which may arise with quadrupole mass spectrometers when used for fast GC peaks, so one should expect uniform mass spectra across the whole peak (Marriott et al, 2001). Apart from GC-MS there are also other techniques including GC with flame ionization detection (FID) and determination of retention indices, GC with olfactometric detection and GC with element selective detection (David et al, 2002). The progression from packed to capillary, to multidimensional gas chromatography and then chiral analysis reflects the need or the desire to increase the‘‘separation space’’ of the analysis (Miresmailli et al, 2010).

1.6.5. Chemistry of essential oils: Essential oils are very complex natural mixtures which can contain about 20-60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20-70%) compared to other components present in trace amounts. The volatile components of essential oils can be classified into four main groups: terpenes, benzene derivatives, hydrocarbons and other miscellaneous compounds. Among them monoterpenoids are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures with diverse functions. They are ten carbon hydrocarbon or their related compounds such as acyclic alcohols (linalool, geraniol, citronellol), cyclic alcohols (menthol, isopulegol, terpeniol), bicyclic alcohols (borneol, verbenol), phenols (thymol, carvacrol), ketones (carvone, menthone, thujone), aldehydes (citronellal, citral), acids (chrysanthemic acid) and oxides (cineole). The main group is composed of terpenes and terpenoids

and the other of aromatic and aliphatic constituents, all characterized by low molecular weight (Tripathi et al, 2009).

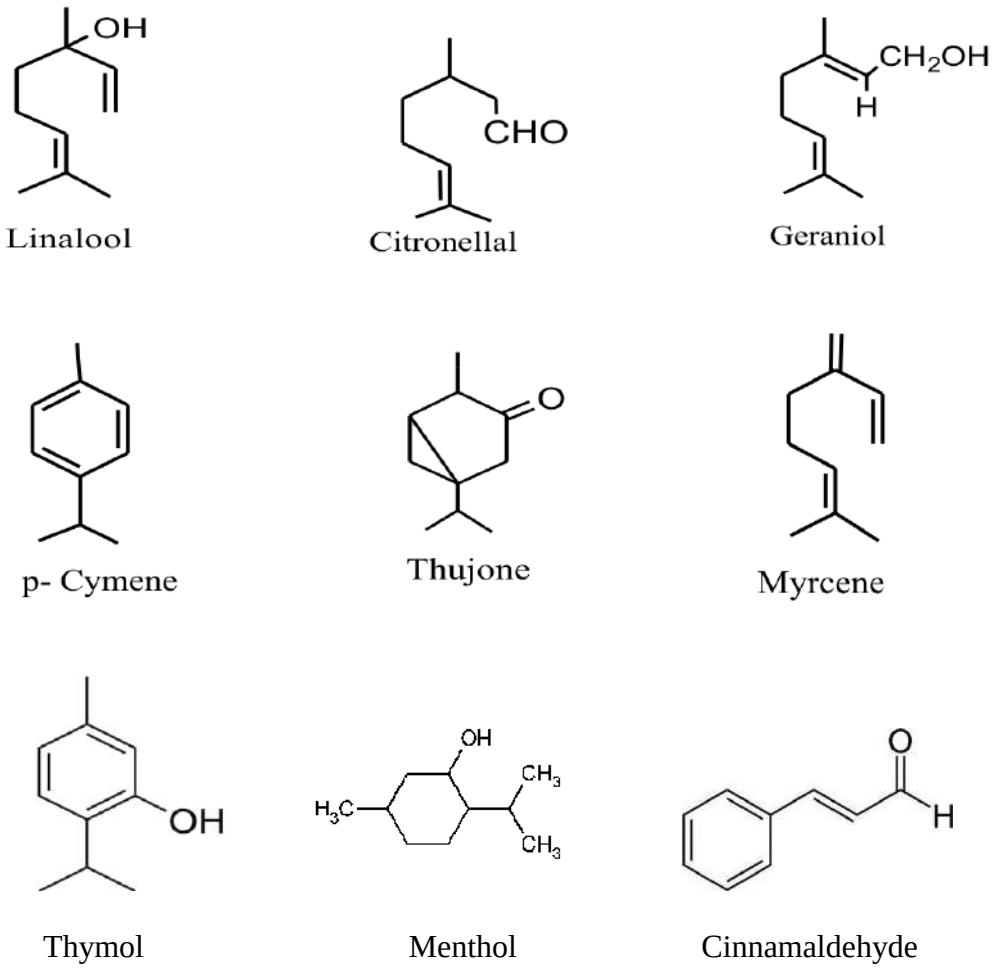


Fig 1.7: Chemical structures of essential oil components

Terpenes form structurally and functionally different classes. They are made from combinations of several 5-carbon base (C5) units called isoprene. The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15) but hemiterpenes (C5), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) also exist. Aromatic compounds are derived from phenylpropane, the aromatic compounds occur less frequently than the terpenes. These compounds comprise: Aldehyde: cinnamaldehyde,

Alcohol: cinnamic alcohol, Phenols: chavicol, eugenol, Methoxy derivatives: anethole, elemicine, estragole, methyleugenols, Methylene dioxy compounds: apiole, myristicine, and safrole (Bakkali et al, 2008).

1.6.6. Olfactory regulation of mosquito-host interactions: The life history strategies of mosquitoes are determined by mating, blood feeding and oviposition. Each of these behaviour is mediated by both internal and external factors. The principal factors affecting mosquito behaviour are temperature, humidity, visual objects and most importantly odor. General mosquito activity and survival is affected by ambient temperature, whereas host temperature may affect host seeking, landing and probing. In the same way humidity influences general activity, survival, host seeking at close range and possibly oviposition. Host seeking and feeding behaviour are much affected by host odors and many mosquito species use olfaction for oviposition. In all likelihood each factor plays an important role in the complex process of identifying and locating appropriate blood-meal hosts. Analysis of blood feeding behaviour in the field as well as in the laboratory is a challenging endeavor not only because blood feeding is a composite behaviour and the sensory input is complex but also because of the variety of feeding strategies exhibited by mosquitoes in their natural habitats and the variations in internal and external stages that affect the expression of the behaviour (Zwiebel et al, 2004).

The human skin is associated with approximately 350 different chemical compounds and it seems unlikely that all these compounds affect host seeking, landing and probing. Studies suggest that lactic acid, unique for human is attractive for *Aedes aegypti*. *Aedes aegypti* does not respond much to other chemical stimuli offered although it is strongly attracted to ethanol extracts of skin residues Studies

with *Anopheles gambiae* have been more successful; this species is attracted to carboxylic acids, amino group containing compounds and to chemical compounds present in incubated human sweat but which are absent from freshly collected sweat. Till now there are no reports on studies relating to the effects of odor blends with these mosquito species although there are reports of one study being cited to show the attraction of *Culex quinquefasciatus* to a blend of CO_2, acetone, 1-octen-3-ol and butyric acid (Zwiebel et al, 2004). The important attractant carbondioxide appears to be of little importance in host discrimination by mosquitoes. Its presence above atmospheric levels does not signify a specific host, although trap capture of some mosquito species that prefer to bite large bovids increases with increased CO_2 release rates. The excretion of sweat glands and sebaceous glands and their microbial breakdown products create the body odour profile of vertebrates. The composition and distribution of sweat glands and skin microflora vary among vertebrate species and are likely to be responsible for the olfactory signature of a species. The density of eccrine sweat gland on human skin is by far the highest among all vertebrates. Human eccrine sweat contains high levels of l-lactic acid (Dekker et al, 2002).

1.6.6. (a) Role of receptors in olfactory regulation: Octopamine (OCT) belonging to the group of biogenic amines plays the major role of neuromodulator in invertebrates. Octopamine is the monohydroxylated analogue of norepinephrine, so it was suggested that the octopaminergic system of invertebrates is the functional correlate of the adrenergic system of vertebrates. Notably only trace amounts of octopamine have been detected in vertebrates and its physiological role is still elusive. Pharmacological studies suggest that octopamine acts through activation of specific GTP-binding protein (G protein)-coupled receptors (GPCRs). According to their

pharmacological properties and intracellular signaling pathways at least two types of octopamine receptors were defined: OCTOPAMINE 1 (OA1) and OCTOPAMINE 2 (OA2) receptors. Activation of OA1 receptors increases the intracellular Ca^{2+} concentration whereas activation of OA2 receptors stimulates adenylate cyclase and thereby increases the concentration of adenosine 3′,5′-cyclic monophosphate (cAMP). The physiological as well as the pharmacological properties of native octopamine receptors were examined in various invertebrate species. However the molecular identification of octopamine receptor genes was lagging behind. But in recent years, molecular cloning efforts of biogenic-amine receptors from insects finally led to the isolation of genes that code for octopamine receptors (Balfanz et al, 2005). Octopamine is released by octopaminergic neurons which are released into the extracellular space through exocytosis to modulate various metabolic activities. Once OCT is released into the extracellular space, it binds to its postsynaptic receptors to elicit a physiological response. However, OCT release in the cytosol and reuptake is regulated by the presence of two types of transporters, i.e., the transporter that carries OCT into secretory vesicles for storage by endocytosis and the transporter that mediates the reuptake of OCT following exocytosis. The binding of octopamine to octopaminergic receptors is coupled with the activation of specific G proteins which leads to transient changes in concentrations of intracellular second messengers. In recent times further advancement in molecular dissection and detailed analysis of octopaminergic signaling in insect nervous systems by using reverse molecular genetic techniques, DNA microarrays and comparison of genome sequencing in more insects may aid in elucidating the molecular mechanism underlying octopamine mediated physiological processes and behavioral changes in insects (Farooqui, 2012).

1.6.7. Mode of action of essential oils: Essential oils being complex mixtures of volatile organic compounds are generally produced as secondary metabolites in plants. They are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones and phenols). Essential oils extracted from different families have shown high repellency against arthropod species. Literature has documented that essential oils and extracts from genus Eucalyptus and Cymbopogan have been traditionally used as effective repellents. The metabolites like the monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are the common constituents in a number of essential oils presenting mosquito repellent activity. Generally the monoterpenoids and sesquiterpenes are associated with repellent properties of several essential oils (Nerio et al, 2010). Amongst the monoterpenes, a majority of them are cytotoxic to plants and animal tissue causing a dramatic reduction in the number of intact mitochondria and golgi bodies thereby impairing respiration, photosynthesis and decreasing the cell membrane permeability. Being volatile in nature so they also act as chemical messengers for insects and other animals, serving as a signal of relatively short duration and alarm the pheromones. Literature cites that hairs on the mosquito antennae are temperature and moisture sensitive. The repellent molecules thus interacts with the female mosquito olfactory receptors thereby blocking the sense of smell which therefore comes as an hurdle in the recognition of host by the mosquitoes (Tripathi et al, 2009).

Studies have indicated that terpenoids containing two functional groups are biologically active as mosquito repellents. The repellent activities of 20 synthesized terpenoids with two functional groups, one being negatively charged end containing

either ester/ether bonds or an ethanol hydroxyl group and the other positively charged end containing alkane groups were assessed. It was revealed that the positive end is more favorable for receptor interactions and its magnitude characterizes the electrophilic nature of the group, consequently the repellent-receptor interactions are most likely to be related to electrophilic interactions. Also it was shown that molecular descriptors such as dipole moment and boiling point are closely related to repellent activity. Generally the dipole moment is related to lipophilicity or specific electrostatic interactions with receptor whereas boiling point/vapor pressure might determine duration of contact with olfactory chemosensilla of mosquitoes (Wang et al, 2008).

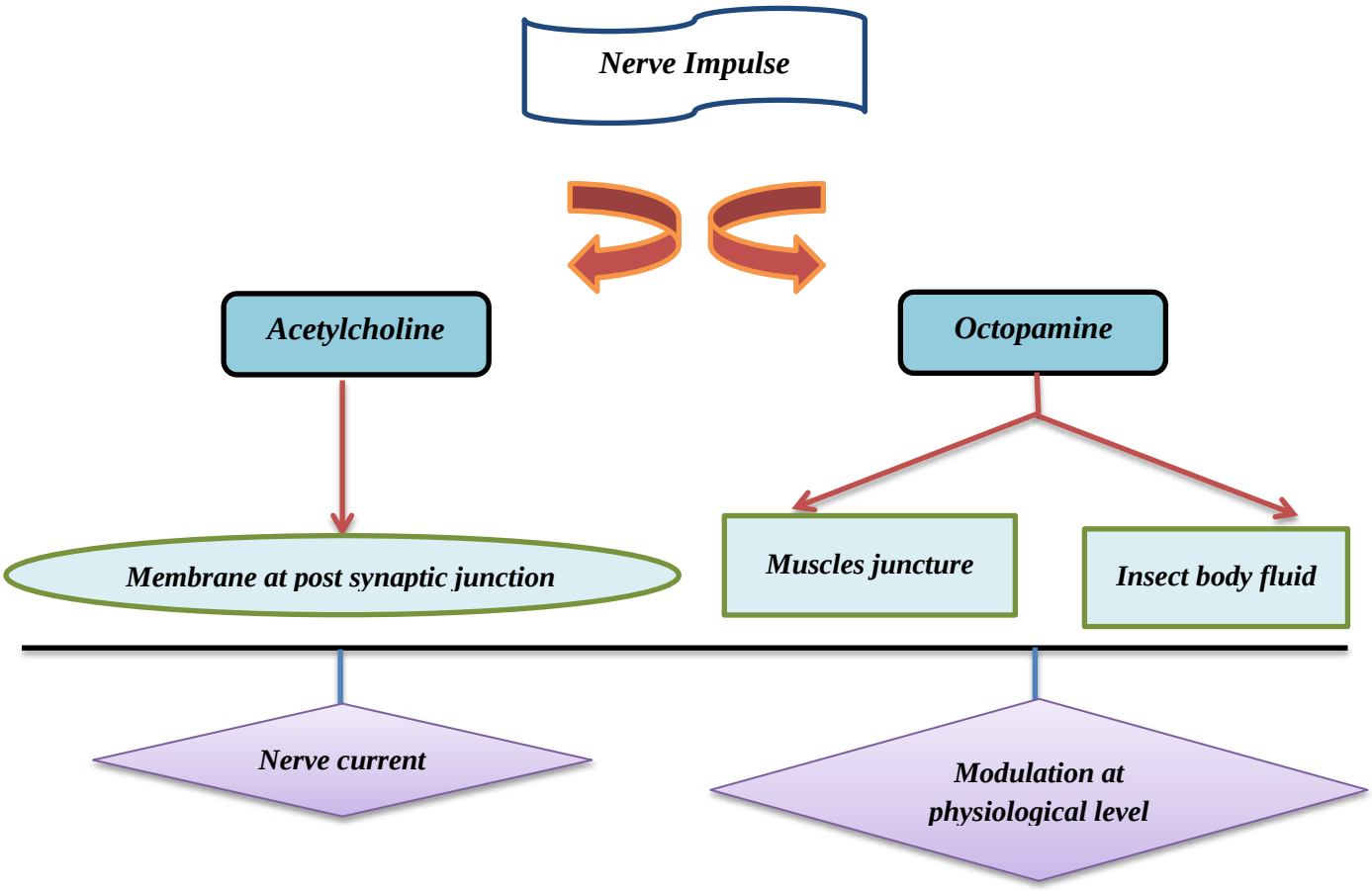


Fig 1.8: Target sites in insects as possible neurotransmitter mediated action of essential oils.

1.6.7. (a) Effect of Synergism on Mode of Action: Although repellent activity of essential oils are generally attributed to some particular compounds but if a synergistic phenomenon is established among these metabolites then it may result in an increased bioactivity compared to isolated components. Also this synergistic effect is showed with mixture of oils. The minor constituents found in low percentages may also act as synergists enhancing the effectiveness of the major constituents through a variety of mechanisms (Omolo et al, 2004). Essential oils act at a vapor phase and are generally effective when freshly applied as they usually dissipate quickly within a short period of time due to their high volatility. However this property can be improved through the development of formulations that would keep the active ingredients onto the skin for a longer period of time. The product composition basically determines the increase in repellency property of essential oils (Trongtokit et al, 2005). Formulation based on creams, polymer mixtures or microcapsules results in increase in repellency duration through controlled release. An example is the successful microencapsulation of *Zanthoxylum limonella* oil in glutaraldehyde crosslinked gelatin (a polymer) in order to improve mosquito repellent properties. Development of new formulations, fixative additives and the production of combined repellents are the possibilities that add up for the effectiveness and greater economic value to repellents obtained from essential oils (Maji et al, 2007).

1.7. PHYTOTOXICITY AND SAFETY OF ESSENTIAL OILS:

Natural based herbicide products have the benefits of absence of “unnatural” ring structures and the presence of few heavy atoms, their short environmental half-lives and their tendency to affect novel target sites. This latter fact is particularly

important since the need for new and improved modes of action is not been required. However agrochemical companies are actively seeking novel mechanisms of action for which they can develop new chemistry (Amri et al, 2012). Perhaps the most attractive aspect of using essential oils and/or their constituents is their favorable mammalian toxicity. Some of the pure essential oil compounds are slightly toxic with rat acute oral LD_{50} values of 2-3g/kg (viz. carvacrol, pulegone) but an essential oil insecticide consisting of a proprietary mixture of essential oil constituents (EcoSMART Technologies Inc.) resulted in no mortality when fed to rats at 2 g/kg, the upper limit required for acute toxicity tests by most pesticide regulatory agencies including the EPA. In that many of the essential oils and their constituents are commonly used as culinary herbs and spices, pesticide products containing certain of these are actually exempt from toxicity data requirements by the EPA (Isman, 2000). Just because a plant has been used for centuries does not mean it is safe or even desirable. Phytotoxicity of essential oils requires serious attention when formulating products for agricultural and landscape use. FDA and EPA have approved many commercial products including essential oils on the GRAS (Generally Recognized As Safe) list. But this does not ensure that the preparation or product will function effectively as formulated or as advertised by a specific producer. There have been several problems reported with the use of tea tree oil. The most common problematic response is contact dermatitis with more than 30 human cases reported in the scientific literature in the 1990s. This can be caused by fresh oil but is enhanced by the formation of degradation products that develop with photodegradation in either open or closed containers. Another bioactive compound d-limonene which is an active constituent of a number of

essential oils like orange, lemon, mandarian and grapefruit. But d-limonene has been reported to cause dermatitis. However these can be controlled if repellent compounds are applied at levels lower than those compounds that are acutely toxic thereby lowering the pesticide load on urban environment (Tripathi et al 2009).

1.8. COMMERCIALIZATION OF ESSENTIAL OIL:

Although considerable research effort in many laboratories throughout the world is being carried out and also an ever-increasing volume of scientific literature on the pesticidal properties of essential oils and their constituents have been evaluated but surprisingly very few pest control products based on plant essential oils have appeared in the market place. Literature cited has identified three barriers to the commercialization of new products of this type: the scarcity of the natural resource, the need for chemical standardization and quality control and difficulties in registration. A number of constituents are available commercially in reasonable purity (95%), and also chemical specifications for even the most complex oils can be provided (Isman, 2000).

1.8.1. Commercialized essential oil products: With over a dozen registered products by the end of 1999, EcoSMART Technologies is aiming to become a world leader in essential oil-based pesticides. It has currently produced aerosol and dust formulations containing proprietary mixtures of essential oil compounds including eugenol and 2-phenethyl propionate aimed at controlling domestic pests (cockroaches, ants, fleas, flies, wasps, etc.). These are marketed to pest control professionals under the brand

name EcoPCO^{R}, with less concentrated formulations for sale to the consumer under the name Bioganic^{TM}. In United States, commercial development of insecticides based on plant essential oils has been greatly facilitated by exemption from registration for certain oils commonly used in processed foods and beverages. This opportunity has led to the development of essential oil-based insecticides, fungicides and herbicides using rosemary oil, clove oil and thyme oil as active ingredients for agricultural and industrial applications and for consumer market. Israel startup Botanocap, founded on oil encapsulation knowledge created at the Ben Gurion University of the Negev, developing a slow release technology for essential etheric oils to prepare environment friendly pesticides. It possesses patents on capturing essential oils in capsules for achieving the delayed release effect. In terms of green pesticide technology the replacement of traditional emulsifiable concentrates (oil) by using oil-in-water micro emulsions as a nano-pesticide delivery system has reduced the use of organic solvent and thereby increase in dispersity, wettability and penetration properties of the droplets has been achieved. The advantages of using pesticide oil-in-water micro emulsions for improving the biological efficacy and reducing the dosage of pesticides would be a useful strategy in green pesticide technology (Isman, 2000; Mohan et al, 2011).

1.8.2. Global market share of essential oil: In recent time essential oil represent a market estimated at US $700.00 million and a total world production of 45,000 tons. Nearly 90% of this production is mainly focused on mint and citrus plants. Thus, presently the widespread activities of essential oils is being considered for both industrial and household uses. Essential oils are now regarded as a new class of ecological products for controlling insect vectors. Therefore the

development of safe and scientifically proven herbal products should be given utmost priority. In the last 40 years the market for pest management products has shown a regular growth of 7-10% per year, a turnover of around 25 billion US dollars. This growth has been accompanied by a deep reorganization of the industrial sector, not only because of many takeovers and amalgamations of companies but also because of modifications in the number and the nature of commercialized insecticidal molecules. Commercial success with these products based on well-known chemistry will likely lead for the development and commercialization of future pesticides based on more exotic essential oils with even greater potency (Tripathi et al, 2009).

2.1. REVIEW OF ARTICLES:

- Siriyasatien et al (2006) has outlined the mosquito-borne diseases still being a major human and animal health problem in many countries. The diseases transmitted by mosquitoes include malaria, filariasis, yellow fever, Japanese encephalitis and dengue fever. It is also discussed that the chemical control used to be the primary strategy for controlling mosquito-borne diseases, but concerns about the impact of the available compounds on human and animal health and the environment, together with the development of insecticide resistance in mosquitoes, has limited the usefulness of this approach.
- Tolle (2009) has discussed about the mosquito borne diseases and their disproportionate impact on poor populations in the developing countries, particularly with respect to children. He has also described that complete and sustained mosquito control is a complicated endeavor; by nature, the chief mosquito vectors, particularly *Aedes aegypti*, exploit the gaps opened by the facts of modern life in many poor, developing settings: weak central authority, favorable ecology and the inescapable effect of poverty and neglect.
- Kumar et al (2006) have discussed on the exploration of more effective and eco-friendly techniques to control mosquitoes. Various organisms, known as natural biological control agents, can be utilized to control mosquito populations, thus avoiding the use of chemicals and harm to the environment in the process. Discussions on the harmful effects of chemicals on non-target populations and the development of resistance to these chemicals in mosquitoes along with the recent resurgence of different mosquito-borne

diseases have prompted to the exploration of alternative, simple and sustainable methods of mosquito control.

- Koul et al (2008) has carried out a study regarding the potential and constraints of essential oils as green pesticides. Recent investigations indicate that some chemical constituents of these oils interfere with the octopaminergic nervous system in insects. As this target site is not shared with mammals, most essential oil chemicals are relatively non-toxic to mammals and fish in toxicological tests and meet the criteria for "reduced risk" pesticides. Some of these oils and their constituent chemicals are widely used as flavoring agents in food and beverages and are even exempt from pesticide registration.
- Katiyar et al (2010) carried out an review on the production of essential oils in health care and its role in the growth and economy of the country with reference to current scenario. It highlights that natural compounds are source of safer or more effective substitutes for artificial pesticides and also provide an alternative way to cure diseases. Large scale productions of aromatic plants are good source of earning of farmers which can play important role for the growth and economy of the country.
- Tripathi et al (2009) focused on the use of essential oils as biopesticide with special emphasis on its chemistry, mode of action, synergism, safety aspects, socioeconomic impacts, sustainability and commercialization aspects. Essential oil compounds and their derivatives are considered to be an alternative means of controlling many harmful insects and their rapid degradation in the environment have increased specificity that favours beneficial insects.

- Bakkali et al (2008) suggested the beneficial effects of essential oils due to prooxidant effects on the cellular level. Their work showed that in eukaryotic cells, essential oils can act as prooxidants affecting inner cell membranes and organelles such as mitochondria. Depending on type and concentration, they exhibit cytotoxic effects on living cells but are usually non-genotoxic. It was reviewed that essential oils are usually devoid of long-term genotoxic risks and moreover some of them show a very clear antimutagenic capacity which could be well linked to an anticarcinogenic activity.
- Zwiebel et al (2004) reviewed significant advances in behavioural, physiological and molecular investigations into mosquito host preference, with a particular emphasis on studies that have emerged in the post-genomic era that seek to combine these approaches. It was concluded that by combining the knowledge that is generated from the behavioral, molecular, neurological and physiological studies as well as additional efforts currently underway, the fundamental elements that underlie critically important vector behaviors such as host selection may be further elucidated.
- Sritabutra et al (2011) investigated the repellent activity of herbal essential oils from garlic (*Allium sativum*), clove (*Syzygium aromaticum*), lemon grass (*Cymbopogon citratus*), citronella grass (*Cymbopogon nardus*), peppermint (*Mentha piperita*), eucalyptus (*Eucalyptus globulus*), orange (*Citrus sinensis*) and sweet basil (*Ocimum basilicum*) and their combinations against *Aedes aegypti* and *Anopheles dirus* under laboratory conditions. Essential oil from lemon grass exhibited protection against both the mosquito species, for Ae. aegypti (98.66±11.56) min protection time and for An. dirus (98.00±15.28)

min protection time. The combinations from eucalyptus oil and sweet basil oil were also effective as repellents and feeding deterrents against *Ae. aegypti* (98.87± 10.28) min protection time.

- Phasomkusolsil et al (2011) studied the repellency to female *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus* of seven essential oils namely *Cymbopogan citratus, Cymbopogan odorata, Cymbopogan nardus, Eucalyptus citriodora, Ocimum basilicum* and *Syzygium aromaticum*. It was found that the percentage repellency increased when the concentration of essential oils increased. In contrast, biting rates decreased when the concentration of essential oils increased.
- Lawal et al (2012) carried out mosquito repellency test of *Eucalyptus globulus*, *Ocimum basilicum* (sweet basil), *Cymbopogon citratus* (lemon grass), *Citrus sinensis* (sweet orange), *Azadirachta indica* (Neem) and *Hyptis suaveolens* by preparing various mosquito repellent preparations. The results showed 8% and 10% formulations to be effective as they showed the ability to repel 100% in first 3 minutes and more than 60% at the end of 1 hour exposure period.
- Adeniran et al (2012) carried out the investigation of a topical formulation from lemongrass oil *(Cymbopogon citratus) Stapf*. for repellent activity against *Acarus sacchari* and mosquitoes respectively. The results exhibited an average of [(194) min (≈3 h) for 0.5 g dose] and [(309) min (≈5 h) for 1.5 g dose] protection time against biting from mosquitoes, (about 50-80%) protection time in comparison to the activity of the best known chemical insect repellent, N, N-diethyl-m-toluamide (DEET).

- Ahad et al (2010) attempted to develop a poly herbal mosquito repellent based on traditional practices and species available in kitchen and garden to provide a reliable, prolonged and complete protection from mosquito bites by killing them. The plant materials included garlic bulb, basil leaves, neem leaves, ajowan seeds, lemon peel, mentha leaves, gold flower petals and cinnamon bark in varying ratios were prepared. The results showed that the formulated repellents were effective, cheaper and non-poisonous than the presently available chemical based marketed mosquito repellents.
- Keleb et al (2010) reviewed on the selection of drug candidates suitable to be formulated as transdermal system and the methods of evaluation were discussed. It was concluded that as successful transdermal drug application requires numerous considerations so the properties of the drug, the characteristics of the transdermal device, selection of in-vivo model and the status of patient's skin are all important for safe and effective drug delivery.
- Wokovich et al (2006) prepared an overview of the types of transdermal, their anatomy, the role of adhesion; the possible adhesion failure modes and how adhesion can be measured were discussed. The adhesive of the transdermal drug delivery system is critical to the safety, efficacy and quality of the product. A decrease in ''adhesion lacking'' by reports, adhesion needs to become an important design parameter and suitable methods required to be made available to assess quality and in vivo performance.
- Saroha et al (2011) have discussed the effectiveness and advantages of patches over other controlled drug delivery system. They have outlined the principles of permeation, various components of transdermal patch, approaches of

transdermal patch, evaluation of transdermal patch, when the patches are to be used and when their use should be avoided are discussed. Also they have discussed regarding the recent development in the field along with the future aspects in this field. The invention of new devices and new drugs which can be incorporated via this system has helped in its increased use in the present time.

- Nilani et al (2011) carried out the wound healing activity of aqueous extract and the mucilage of leaves of plant *Cocculus hirsutus* on excised wound model. Dermal films were prepared using total aqueous extract, mucilage and polyvinyl alcohol as a polymer base. The prepared polysaccharide solutions of the total extract and the isolated mucilage were evaluated for its physiochemical properties and total bacterial count and were also subjected to biodegradation studies. The results indicate statistically significant wound healing response of the dermal patch containing the aqueous extract and the mucilage of leaves of plant *C. hirsutus* when compared with control group and groups subjected to standard marketed medicated dermal patch.
- Uhrich et al (1999) reviewed the chemical issues involved in the design of synthetic polymers used in the controlled release of drugs as before considering the variety and the evolution of these polymeric structures, it is necessary to examine the motivation for achieving controlled release. It was concluded that the ability to impart bioadhesivity, cell specificity, active transport or other specific characteristics into a biocompatible polymer represents an important synthetic challenge.
- Kim et al (2009) reviewed the engineered polymers that have been used in

traditional drug delivery systems and as more recent applications in nanotechnology. The engineered polymers have been utilized for developing advanced drug delivery systems. The development of such polymers has caused advances in polymer chemistry which in turn, has resulted in smart polymers that can respond to changes in environmental condition such as temperature, p^H and biomolecules. However the responses vary widely from swelling to degradation.

- Reza et al (2003) showed that the profile and kinetics of drug release were functions of polymer type, polymer level and physicochemical nature of drug and thereby a controlled plasma level profile of drug can be obtained by judicious combination of polymers and modulation of polymer content in the matrix system. Matrix tablets of theophylline, diclofenac sodium and diltiazem HCl using Kollidon SR, Carnauba wax and Hydroxypropyl methylcellulose were prepared separately by direct compression process. The results showed significant differences among the drug release profile from different classes of polymeric matrices thereby confirming release kinetics to be governed by the type and content of polymer in the matrix system.
- Enayatifard et al (2009) evaluated the effect of hydroxypropyl methylcellulose (HPMC) and ethylcellulose (EC) content on the release profile and kinetics of Diltiazem Hcl from matrices. The results showed that these polymers slowed down the release of Diltiazem HCl from the matrices. It was seen that in the presence of EC when HPMC concentration was increased there was decreased release of diltiazem. Furthermore, incorporation of EC in tablets with HPMC as the matrix was found to control drug release. Kinetic analysis showed that

drug release from the formulations was adequately described by zero order model.

- Arora et al (2002) have prepared matrix-type transdermal patches containing diclofenac diethylamine using different ratios of polyvinylpyrrolidone (PVP) and ethylcellulose (EC) by solvent evaporation technique. The drug matrix film of PVP and EC was casted on a polyvinyl alcohol backing membrane. All the prepared formulations were subjected to physical studies (moisture content, moisture uptake, and flatness), in-vitro release studies and in-vitro skin permeation studies. It concluded that diclofenac diethylamine can be formulated into the transdermal matrix type patches to sustain its release characteristics and the polymeric composition (PVP/EC, 1:2) was found to be the best choice for manufacturing transdermal patches of diclofenac diethylamine among the formulations studied.
- Sahu et al (2012) developed and evaluated transdermal therapeutic system of drug Colchicine with different concentration of drug and hydrophobic (ethylcellulose) polymeric system by the solvent evaporation technique. Formulated transdermal patches were physically evaluated with regard to thickness, weight variation, drug content, flatness, folding endurance, percentage moisture content, percentage moisture loss and water vapour transmission rate. All prepared formulations indicated good physical stability. In vitro permeation studies of formulations were performed by using skin membrane. The release profile of optimized formulation indicated that the permeation of the drug from the patches was governed by a diffusion mechanism.

- Marriott et al (2001) outlines the developmental nature of instrumental approaches to essential oil analysis using gas chromatography. It will help to explain where new technology has been applied to advantage in this field. It demonstrates comprehensive gas chromatography as potentially most powerful separation method for essential oils. This may be for comparative purposes, where one oil is contrasted with other for quality control or investigation of adulteration, to discover new components, or to characterize the chemical classes of compounds present.
- Golmohammad et al (2012) performed gas chromatography to identify and quantify the volatile components composition. The analysis showed that the maximum yield of E-cinnamaldehyde was obtained by methanol: water in (70: 30v/v) ratio by SPE extraction. At optimal condition E-cinnamaldehyde showed highest area percentage of 79.60% in the volatile oils extracted by hydrodistilation and 88.50% by superheated water extraction.
- Tajidin et al (2011) analysed lemongrass oil for its chemical composition and citral contents using gas chromatography-mass spectrometry (GC-MS) analysis at different maturity stages A total of 65 compounds were detected however, only 13 compounds were present at each of the maturity stage. Among 13 compounds, only 7 compounds (β-myrcene, 3-undecyne, neral, geranial, nerol, geranyl acetate and juniper camphor) had a concentration of greater than 1%. The citral content at 6.5 months after planting was higher by 11.4% than at 5.5 months after planting.
- Masetti et al (2006) evaluated the efficacy of commercial insect repellents against *Aedes albopictus* by means of arm in cage assays. Products were

applied to volunteers' forearms and the time elapsed between the application and the first mosquito bite was considered as the time of protection. Each product was tested twice by each subject on two ramdomly selected batches. It was reported that a small number of individuals per cages more accurately represent the conditions in the environment. Off Spray and Autan Active were found to be the most effective repellents and they provided 250 and 220 min protection, respectively.

- Tawatsin et al (2001) have studied the repellency effects of four plant species turmeric, citronella grass, hairy basil and kaffir lime were evaluated in mosquito cages and in a large room for their repellency against three mosquito vectors *Aedes aegypti*, *Anopheles dirus* and *Culex quinquefasciatus*. The oils from turmeric, citronella grass and hairy basil, especially with the addition of 5% vanillin, repelled the three species under cage conditions for up to eight hours. The time between application of the repellents and the second successive bite was recorded as the protection time. It was concluded that the three volatile oils can be formulated with vanillin as mosquito repellents in various forms to replace deet (N,N-diethyl-3-methylbenzamide), the most common chemical repellent currently available.
- Delaunois et al (2009) established a combined model of telemetry and plethysmography in the conscious rat, allowing the reuse of the animals over several successive pharmacodynamic studies. It was discussed that although a shift of some parameters, particularly heart rate and tidal volume was noted with age and body weight but this can easily be managed by appropriate design measures. The effects of the different reference drugs were consistent

with data published in animals and man. Also it was showed that the combined system can detect negative or positive effects on both cardiovascular and respiratory functions with enough sensitivity.

- DeLorme et al (2002) outlined that the evaluation of pulmonary physiological measurements in laboratory animals is an essential tool in many biomedical and toxicological research areas. Recently, an unrestrained single chambered whole-body plethysmograph that utilizes a barometric analysis technique to quantify pulmonary physiological values has gained widespread use. Repeated physiological measurements on the same animals in both the single and double chamber plethysmographs demonstrated that each instrument generated reproducible baseline physiological data. The results indicated that the choice of single or double chamber plethysmograph for physiological measurements should be linked to the study objectives and the type of data required.

2.2. ESSENTIAL OIL REVIEW

2.2.1. Cinnamon Oil:

- Obtained from dried inner bark of the shoots of *Cinnamomum zeylanicum* belonging to family Lauraceae. The bark contains essential oil.

- **Latin Name:** *Cinnamomum verum*
- **Pharmacopeial Name:** Cinnamomi ceylanici cortex
- **Synonym:** Ceylon cinnamon, true cinnamon
- **Physicochemical properties**
  - **Physical state and appearance:** Clear liquid
  - **Colour:** Dark yellow
  - **Odor:** Aromatic
  - **Boiling point:** 249° C
  - **Relative density:** 1.045g/ml
  - **Solubility:** 1 part oil is soluble in 1.5 parts 70% (v/v) ethanol at 30°C yielding a clear solution.
  - **Chemical composition:** Cinnamon contains volatile oils (1-4%) of which

cinnamaldehyde (60-80%), eugenol (10%) and *trans*-cinnamic acid (5-10%); phenolic compounds (4-10%), condensed tannins, catechins and proanthocyanidins, monoterpenes and sesquiterpenes (pinene), calcium-monoterpenes oxalate, gum, mucilage, resin, starch, sugars and traces of coumarin.

- **Storage:** Store in light-resistant container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition. Do not store above 25°C.

❖ **Pharmacology:**

Cinnamon possesses antibacterial and fungistatic properties as well as the promotion of intestinal motility. The *British Herbal Pharmacopoeia* reported its action as bitter. Its essential oil has been reported to demonstrate strong antibacterial antifungal, antiviral, bactericidal and larvicidal actions. Its constituents eugenol, eugenol acetate and methyl eugenol have been reported to enhance trypsin activity *in vitro* and has also shown strong lipolytic action.

- **Toxicity profile:** Acute toxicity of cinnamon in the animals is very low i.e. Benzaldehyde (LD_{50} orally, 1300 mg/kg rat), cinnamaldehyde (LD_{50} orally, 2220 mg/kg in rat), linalool (LD_{50} orally, 2790 mg/kg in rat) and salicylaldehyde (LD_{50} orally, 520 mg/kg in rat).
- **Contraindications:** Allergy to cinnamon and peruvian balsam.
- **Side Effects:** Frequently allergic reactions of skin and mucosa.
- **Use during Pregnancy and Lactation:** Not recommended.
- **Interactions with Other Drugs:** None known.

- **Dosage and Administration:** Internal: Unless otherwise prescribed, 2-4 g per day of cut or ground bark, Infusion or decoction: 0.7-1.3 g in 150 ml water, three times daily, Fluid extract 1:1 (g/ml): 0.7-1.3 ml, three times daily, Tincture 1:5 (g/ml): 3.3-6.7 ml, three times daily, Essential oil: 0.05-0.2 ml.

- **Uses:**

  Cinnamon has been approved for internal use in loss of appetite, dyspeptic complaints such as mild, spastic condition of the gastrointestinal tract, bloating and flatulence. Apart from these it is also effective as antifungal, antiviral, bactericidal and larvicidal activities (Hansel et al, 1992).

2.2.2. Lemongrass Oil:

- Obtained from leaves and aerial parts of plant *Cymbopogan citratus* belonging to family Graminae.

- **Common name:** lemon grass, citronella
- **Synonym:** *Andropogon citratus*

❖ **Physicochemical properties**

- **Physical state and appearance:** Liquid
- **Colour:** Clear yellow
- **Boiling point:** 224 °C
- **Relative density:** 0.887g/ml
- **Chemical composition:** The essential oil of lemon grass (0.2-0.5%) consists mainly of citral. Citral is a mixture of two stereo isomeric monoterpene aldehydes. The trans isomer geranial (40-62%) dominates over the cis isomer neral (25-38%). Further terpenoids in lemon grass oil are nerol, limonene, linalool and beta-caryophyllene. The content of myrcene is low, but still it is enough to make the oil susceptible to oxidative polymerization.
- **Solubility:** Soluble in alcohol and oils and insoluble in water.
- **Storage:** Store in cool place and keep container tightly closed in a dry and well-ventilated place.

❖ **Precautions:**

Lemongrass is not known to be harmful when taken in recommended dosages, though it is important to remember that the long-term effects of taking the herb (in any amount) have not been investigated. The essential oil should not be used internally by children, women who are pregnant or breast-feeding, or people with liver or kidney disease.

❖ **Side effects:**

In rare cases lemongrass essential oil has caused allergic reactions when applied to the skin. To minimize skin irritation the oil should be diluted in carrier oil such as safflower or sunflower seed oil before application. As with

all essential oils, small amounts should be used and only for a limited time. Citral has been reported to irritate the respiratory tract as well as eyes and skin of sensitive people.

- **Interactions:**

  Lemongrass is not known to interact adversely with any drug or dietary supplement.

- **Uses:**

  It clears headaches and helps to combat nervous exhaustion and stress related conditions. It is also useful with respiratory infections such as sore throats, laryngitis and fever and helps prevent spreading of infectious diseases. It is helpful with colitis, indigestion and gastro-enteritis. The oil extracted from lemon grass has also been used as an insect-repellent, as well as to perfume beauty products. It is an important ingredient in several products designed to keep bugs at bay. Beside these properties described above, extracts and the essential oil of lemon grass have also shown antioxidant activity and anti-cancer activity in cases of experimental hepatocarcinogenesis in animals. Cymbopogon citratus extract is reported to have faecal glucuronidase inhibiting activity (Bor, 1953; Clayton et al, 2006).

2.2.3. Eucalyptus Oil:

- Obtained from the fresh leaves of *Eucalyptus globulus* belonging to family Myrtaceae.

- **Common name:** Eucalyptus, fever tree, lemon eucalyptus
- **Physicochemical properties**
- **Physical state and appearance:** Liquid
- **Colour:** Colourless to pale yellow liquid.
- **Boiling point:** 175 °C
- **Relative density:** 0.909g/ml
- **Solubility:** Insoluble in water, Soluble 1 in 5 of alcohol 70%, miscible with alcohol (90%), dehydrated alcohol, oils, fats and paraffins. Miscible with ether, chloroform, glacial acetic acid.
- **Chemical composition:** The essential oil in the leaves is commonly used for medicinal purposes. The quantity of essential oil ranges from less than 1.5 to over 3.5%. On average, between 70-95% of the oil is 1, 8-cineole (eucalyptol). Other major components in the oil are aromadendrene, p-cymene, d-limonene,

alpha-phellandrene, alpha-pinene, beta-pinene, alpha-thujene, d-linalool, cuminaldehyde and terpineol.

- **Storage:** Products containing eucalyptus oil should be stored at a temperature not exceeding 25°C in well filled containers and protect it from light.

❖ **Toxicity profile:**

It contains high levels of phenolics and terpenoids which can be toxic. Some of the specific compounds that can be toxic or cause adverse reactions include: 1,8-cineole, cyanogenic glycosides, rutin and tannins. Based on rodent studies, the oral LD_{50} for eucalyptus oil is very high 4.44g/kg body weight for rats and 3.32g/kg body weight for mice. The LD_{50} is lower when only 1,8-cineole is used 2.48g/kg body weight for rats. The dermal LD_{50} for rabbits is greater than 5g/kg body weight. However, some studies have indicated that citronellal and phellandrene which can be found in some Eucalyptus species are weak mutagenics and carcinogenics respectively.

- **Adverse effects:** Overdoses of the oil in human cause gastro-intestinal burning, abdominal pain, vomiting, convulsions, depress respiration and the central nervous system and may lead to coma and death.

❖ **Uses:**

The cineole based oil is used as component in pharmaceutical preparations to relieve the symptoms of influenza and colds, in products like cough sweets, lozenges, ointments and inhalants. Eucalyptus oil has antibacterial effects on pathogenic bacteria in the respiratory tract. Cineole-based eucalyptus oil is also used as an insect repellent and biopesticide (Adhikari et al, 1992; Alkofahi et al, 1997).

2.3. EXCIPIENTS REVIEW:

2.3.1. Ethylcellulose:

- **Nonproprietary names:** Ethylcellulose, Ethylcellulosum
- **Synonym:** Aquacoat ECD, Aqualon, Ethocel
- **Chemical name:** Cellulose ethyl ether
- **Molecular formula:** $C_{20}H_{38}O_{11}$
- **Chemical structure:** The structure of ethylcellulose is given below:

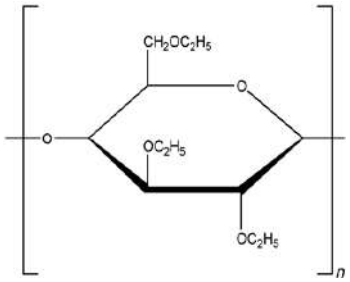


Fig 2.1: Chemical structure of Ethylcellulose

❖ **Physicochemical properties:**

- **Physical state and appearance:** Solid powder
- **Colour:** White to light tan colour
- **Density (bulk):** 0.4g/cm^3
- **Glass transition temperature:** 129-133°C
- **Moisture content:** Ethylcellulose absorbs very little water from humid air or during immersion and that small amount evaporates readily.
- **Specific gravity:** 1.12-1.15g/cm^3
- **Solubility:** Practically insoluble in glycerin, propylene glycol and water. Ethylcellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate and tetrahydrofuran and in mixtures of aromatic

hydrocarbons with ethanol (95%). Ethylcellulose that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol and toluene.

- **Stability:** It is a stable, slightly hygroscopic material. Also it is chemically resistant to alkalis, both dilute and concentrated and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters.
- **Incompatibilities:** Incompatible with paraffin wax and microcrystalline wax.
- **Storage:** Should be stored at a temperature not exceeding 32°C in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

❖ **Pharmacology:**

Ethylcellulose is generally regarded as a nontoxic, non allergenic and nonirritating material. As ethylcellulose is not considered to be a health hazard, the WHO has not specified an acceptable daily intake. Generally the LD_{50} (rabbit, skin): >5g/kg and LD_{50} (rat, oral): >5g/kg according to studies.

❖ **Uses:**

The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethylcellulose coatings are used to modify the release of a drug, to mask an unpleasant taste or to improve the stability of a formulation. High-viscosity grades of ethylcellulose are used in drug microencapsulation. Also in topical formulations, ethylcellulose is used as a thickening agent in creams, lotions or gels, provided an appropriate solvent is used. Ethylcellulose has also been studied as a stabilizer for emulsions and is additionally used in cosmetics and food products.

❖ **Regulatory status:** It is listed in Generally Recognized As Safe category. Accepted for use as a food additive in Europe and also included in the FDA Inactive Ingredients Guide (oral capsules, suspensions and tablets, topical emulsions and vaginal preparations). It has been included in nonparenteral medicines licensed in Europe and included in the Canadian List of Acceptable Non-medicinal Ingredients (Rowe et al, 2006; Wade et al, 1994).

2.3.2. PVP K-30:

- **Nonproprietary names:** Povidone, Povidonum
- **Synonym:** Kollidon, Polyvidone, Polyvinylpyrrolidone
- **Chemical name:** 1-Ethenyl-2-pyrrolidinone homopolymer
- **Molecular formula:** $(C_6H_9NO)_n$
- **Molecular weight:** 111.14ng/mole
- **Chemical structure:** The structure of PVP K-30 is given below:

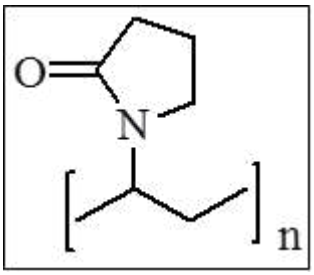


Fig 2.2: Chemical structure of PVP K-30

❖ **Physicochemical properties:**

- **Physical state and appearance:** Powdered solid
- **Colour:** Creamy white
- **Density:** 1.180g/cm^3

- **Melting point:** softens at 150°C
- **Moisture content:** It is very hygroscopic, significant amount of moisture being absorbed at low relative humidities.
- **Solubility:** Freely soluble in acids, chloroform, ethanol (95%), ketones, methanol and water; practically insoluble in ether, hydrocarbons and mineral oil. In water, the concentration of a solution is limited only by the viscosity of the resulting solution, which is a function of the K-value.
- **Stability:** Povidone darkens to some extent on heating at 150°C with a reduction in aqueous solubility. It is stable to a short cycle of heat exposure around 110-130°C; steam sterilization of a povidone aqueous solution does not alter its properties. However aqueous solutions are susceptible to mold growth and consequently require the addition of suitable preservatives.
- **Incompatibilities:** Povidone is compatible in solution with a wide range of inorganic salts, natural and synthetic resins and other chemicals. It forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital, tannin and other compounds. The efficacy of some preservatives may also be adversely affected by the formation of complexes with povidone.
- **Storage:** It may be stored under ordinary conditions without undergoing decomposition or degradation. However, since the powder is hygroscopic, it should be stored in an airtight container in a cool and dry place.

❖ **Pharmacology:**

Povidone is widely used as an excipient, particularly in oral tablets and solutions. When consumed orally, povidone may be regarded as essentially

nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes. It additionally has no irritant effect on the skin and causes no sensitization. Reports of adverse reactions to povidone primarily concern the formation of subcutaneous granulomas at the injection site of intramuscular injections formulated with povidone. Evidence also exists that povidone may accumulate in the organs of the body following intramuscular injection. A temporary acceptable daily intake for povidone has been set by WHO at upto 25mg/kg body-weight. [LD_{50} (mouse, IP): 12g/kg].

- **Uses:**

Although povidone is used in a variety of pharmaceutical formulations, it is primarily used in solid-dosage forms. In tableting, povidone solutions are used as binders in wet-granulation processes. Povidone is used as a solubilizer in oral and parenteral formulations and has been shown to enhance dissolution of poorly soluble drugs from solid-dosage forms. Povidone solutions may also be used as coating agents. It is additionally used as a suspending, stabilizing or viscosity increasing agent in a number of topical and oral suspensions and solutions.

- **Regulatory status:**

It has been accepted for use in Europe as a food additive, included in the FDA Inactive Ingredients Guide (IM and IV injections; ophthalmic preparations; oral capsules, drops, granules, suspensions and tablets; sublingual tablets; topical and vaginal preparations). It is also included in the Canadian List of Acceptable Non-medicinal Ingredients (Rowe et al, 2006; Wade et al, 1994).

2.3.3. Dibutyl Phthalate:

- **Nonproprietary names:** Dibutyl phthalate, Dibutylis phthalas
- **Synonym:** Benzenedicarboxylic acid, di-n-butyl phthalate
- **Chemical name:** Dibutyl benzene-1,2-dicarboxylate
- **Molecular formula:** C_{16} H $_{22}O_4$
- **Molecular weight:** 278.34
- **Chemical structure:** The structure of dibutyl phthalate is given below:

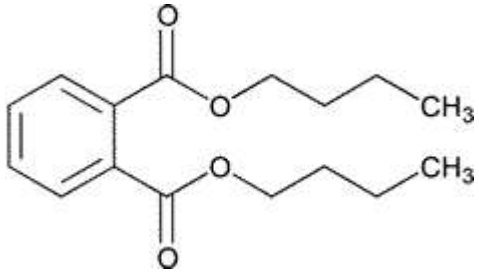


Fig 2.3: Chemical structure of Dibutyl phthalate

❖ **Physicochemical properties:**

- **Physical state and appearance:** Oily viscous liquid
- **Colour:** Colourless or slightly yellow coloured
- **Boiling point:** 340°C
- **Melting point:** approximately 35°C
- **Solubility:** Freely soluble in acetone, benzene, ethanol (95%) and ether; soluble 1 in 2500 of water at 20°C.
- **Incompatibilities:** Dibutyl phthalate reacts violently with chlorine. It also reacts with oxidizing agents, acids, bases and nitrates.
- **Storage:** Dibutyl phthalate should be stored in a well-closed container in a cool, dry, location. Containers may be hazardous when empty since they can contain product residues such as vapors and liquids.

❖ **Pharmacology:**

Dibutyl phthalate is generally regarded as a relatively nontoxic material although it has occasionally been reported to cause hypersensitivity reactions. It is widely used in topical cosmetic and some oral pharmaceutical formulations. LD_{50} (mouse, IV): 0.72g/kg, LD_{50} (mouse, oral): 5.3g/kg, LD_{50} (rat, oral): 8.0g/kg, LD_{50} (rat, IP): 3.05g/kg.

❖ **Uses:**

Dibutyl phthalate is used in pharmaceutical formulations as a plasticizer in film-coatings. It is also used extensively as a solvent particularly in cosmetic formulations such as in anti-perspirants, hair shampoos and hair sprays. In addition to a number of industrial applications, it is also used as an insect repellent, although it is not as effective as dimethyl phthalate.

❖ **Regulatory status:**

It is included in the FDA Inactive Ingredients Guide (oral delayed action, enteric coated, tablets) and also included in nonparenteral medicines licensed in the UK (oral capsules, tablets, granules; topical creams and solutions) (Rowe et al, 2006; Wade et al, 1994)

3.1. AIM OF THE STUDY: The study is aimed to obtain effective and long duration protection against mosquitoes by developing sustained release mosquito repellent patch.

Studies have revealed that mosquito borne diseases have been health hazard and various methods as well as surveillance programmes have been set up for its effective control and prevention. The essential oil have shown effective antimosquito properties both to repel as well as to kill mosquitoes and also as they show safety profile to be used in combatting mosquito borne diseases. Its application in mosquito control was since the 1920s, but the discovery of synthetic insecticides such as DDT in 1939 declined its use in mosquito control programmes. However after facing several problems due to injudicious use and irreversible damage to the ecosystem caused by synthetic insecticides, the focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was again appreciated. The knowledge obtained from previous references suggests that there are different species belonging to various plants which can be effectively used: citronella (*Cymbopogon nardus*), cedar (*Juniper virginiana*), eucalyptus (*Eucalyptus maculata*), geranium (*Pelargonium reniforme*), lemon-grass (*Cymbopogon excavatus*), peppermint (*Mentha piperita*) and neem (*Azadirachta indica*). But these category of oils are volatile in nature and evaporate completely at room temperature and may need more frequent re-application to maintain full protection.

So to overcome this drawback it was aimed at in developing a controlled release patch which will deliver the active constituents i.e. oils at a controlled rate for a prolonged period of time thereby achieving the desired effective protection.

3.2. OBJECTIVES OF THE STUDY:

1. To formulate different formulations of essential oil loaded patch by varying the concentration of polymers, ethylcellulose (EC) and polyvinyl pyrrolidone (PVP K-30) by solvent evaporation method.

2. To characterize all the formulations based on their physico-chemical parameters:

- Physical appearance
- Weight variation
- Surface area measurement
- Measurement of p^H
- Percentage moisture content and uptake
- Measurement of thickness of patch
- Measurement of the extent of flatness

3. To characterize all the formulations based on their experimental parameters:

- Estimation of oil entrapment efficiency in all the formulations by gas chromatography (GC) analysis.
- Determination of in-vitro oil release behaviour of all formulations by gas chromatography (GC) analysis.

4. To characterize the optimized formulation for analytical parameters:

- Determination of surface morphology by scanning electron microscope (SEM) analysis.
- Fourier transform infrared (FTIR) spectral analysis.

- To evaluate mosquito repellency test of the optimized formulation.
- To perform inhalational toxicity of the optimized formulation by Whole Body Plethysmograph.

4. MATERIALS AND METHODS:

4.1. MATERIALS

4.1.1. Chemicals and reagents:

All the materials used in the formulations, evaluations are listed below. The chemicals and solvents used were of analytical reagent grade and were used as such without any further purification. The water used was of high grade after double distillation and deionization.

Sr. No.	Materials	Manufacturer
1	Cinnamon oil	Yarrow chem Products, Mumbai
2	Lemongrass oil	Yarrow chem Products, Mumbai
3	Eucalyptus oil	Himedia Laboratories Pvt. Ltd, Mumbai
4	Ethylcellulose	Himedia Laboratories Pvt. Ltd, Mumbai
5	PVP K30	Himedia Laboratories Pvt. Ltd, Mumbai
6	Chloroform	Spectrochem Pvt. Ltd, Mumbai.
7	Dibutyl phthalate	Himedia Laboratories Pvt. Ltd, Mumbai
8	Dimethyl sulfoxide	Loba Chemie Pvt. Ltd, Mumbai
9	Calcium chloride	Merck Specialities Pvt. Ltd, Mumbai
10	Aluminium chloride	Himedia Laboratories Pvt. Ltd, Mumbai

Table 4.1: List of chemicals and reagents

4.1.2. Equipments and apparatus:

Sr. No.	Equipment	Company
1	Sartorious electronic balance	Model BS 124S, Labtronic
2	Magnetic stirrer	Ikon Instruments, Delhi
3	Hot air oven	Narang Scientific Works, New Delhi
4	Digital micrometer	Mitotyosu, Tokyo, Japan
5	p^H meter	Systronics μ p^H system 361, Ahmedabad
6	Centrifuge	Model CF10, Daihan Scientific Co. Ltd, Korea
7	FTIR spectrophotometer	Model ALPHA-E, Bruker, Germany
8	Scanning electron microscopy (SEM)	Model Jsm-6390LV, Jeol, England
9	Gas chromatography (GC)	Chemito Ceres 800 plus, Thermo Scientific, India
10	Whole Body Plethysmograph	Data sciences International, USA

Table 4.2: List of equipments and apparatus

4.2. METHODS

4.2.1. Fabrication of essential oil loaded patch:

The controlled release dermal patch of the mixture of three essential oils was prepared by the solvent evaporation method.

The solutions of polymer ethylcellulose (EC) and PVP K-30 were prepared in chloroform (3ml) on a magnetic stirrer with magnetic bead at high speed for forming a homogenous mixture. To the above mixture, dibutyl phthalate (71µl) was added as plasticizer and mixed. The oil of cinnamon (150µl), oil of lemongrass (75µl) and oil of eucalyptus (75µl) were added to the above mixture. The mixture was kept under constant stirring to obtain the homogenous and uniform solution of the formulation. As a precautionary measure to prevent the evaporation of the volatile oil components and formation of air bubbles into the mixture, was enclosed by aluminium foil. After continuous stirring for about 2 hrs, when the oil-polymer mixture reaches the semi liquid consistency state then the mixture was casted in mould of 12.56 cm^2 backed by aluminium foil. Inverted funnel was placed over the mould to control the rate of evaporation. The mould was then kept aside for drying at room temperature for 24hrs. After drying the patches were peeled from the mould and preserved in desiccator for further studies.

Fig 4.1: Essential oil embedded dermal patch

In total, five formulations were prepared by varying the ratios of polymer EC and PVP K-30. In all the formulations the total polymer concentration was kept

constant. The essential oils viz. cinnamon oil, lemongrass oil and eucalyptus oil were used in the ratio 2:1:1 respectively. The proposed designs for the preparation of the matrix type patch are given in Table 4.3.

Formulation code	EC:PVPK30 ratio	Chloroform (ml)	Dibutyl-phthalate (%w/w)	Cinnamon, Lemongrass, Eucalyptus (%w/w)
H_1	1:2	3	25	2:1:1
H_2	2:1	3	25	2:1:1
H_3	3:0	3	25	2:1:1
H_4	0:3	3	25	2:1:1
H_5	1.5:1.5	3	25	2:1:1

Table 4.3: Design and development of oil embedded patch

4.2.2. Drug-excipient compatibility study:

Fourier transform infrared (FTIR) technique was used to study the physical and chemical interaction between the mixture of oil and excipients (Model ALPHA-E, Bruker, Germany). The FTIR spectra of the three oils, ethylcellulose, PVP K-30, patches of formulating code (H_1 to H_5) and mixture of oils were carried out by placing the samples on the sampling plate of FTIR spectrophotometer. Then scanning was started and thereby all IR spectra were obtained.

4.2.3. Scanning electron microscopy (SEM):

The main aim behind SEM study is to obtain the topographical characteristics, especially the surface morphology of the prepared patch. The required size was cut

from the patch and mounted onto stubs using double sided adhesive tape and coated with platinum using a sputter. The oil embedded patch as well as blank patch were mounted on the instrument and scanning were performed using (Jeol, Jsm-6390LV, England). The SEM images were taken at the required magnification at room temperature.

4.2.4. Evaluation of oil embedded patch:

- **Physical appearance:**

The patches of all assigned formulation codes were visually inspected for their shape, smoothness, stickiness, clarity, homogeneity, uniformity and flexibility.

- **Weight variation:**

The patches of different formulation codes were used to perform this test. Three patches of each formulation were selected and weighed accurately in a digital balance. The average weight and standard deviation values were calculated from individual weights.

- **Surface area measurement:**

The diameter of each of the formulation of different formulation codes were measured by the millimeter scale and area were calculated.

- **Percentage moisture content:**

Three patches of each formulation code were selected and accurately weighed. The patches were placed in a desiccator containing calcium chloride ($CaCl_2$) and maintained at 40°C for 24 hours. After 24 hours the patches were again weighed to observe the difference in weight from the formula:

% Moisture content (MC) = [Initial weight−Final weight/Final weight] × 100

- **Percentage moisture uptake:**

The test was performed in triplicate and was weighed accurately. The patches were placed in a desiccator containing saturated solution of aluminum chloride ($AlCl_3$) at room temperature for 48 hours with relative humidity maintained at 84%. After 48 hours of exposure the patches were again weighed and the moisture uptake capacity calculated from the formula:

% Moisture uptake (MU) = [Final weight−Initial weight/Initial weight] × 100

- **Thickness of patch:**

The thickness of the patches was measured by using digital micrometer (Mitotyosu, Tokyo, Japan) with an accuracy range of ±0.001 mm and their average and standard deviation values were estimated.

- **Flatness:**

Two longitudinal strips were cut from either side of the patch. The length of each strip was measured and the variation in the length because of uniformity in flatness was measured by determining percent constriction, considering 0 % constriction equivalent to 100% flatness.

% Constriction = [Initial length- Final length/ Final length] × 100

- **p^H measurement:**

The surface p^H of the patch was checked by dissolving the patch in distilled water for 24 hours at room temperature. This was done to allow swelling of the patch. The p^H of the patch was then checked in a p^H meter (Systronics μ p^H system 361, Ahmedabad). Generally p^H range 5.5-6.8 shows skin compatibility.

4.2.5. GC analysis of essential oil:

To identify the main components present in the essential oil of Cinnamon, Lemongrass and Eucalyptus, its GC analysis was carried using (Chemito Ceres 800 plus, Thermo Scientific, India) equipped with an FID detector and Carbowax capillary column (25m × 0.25mm I.D.) Analysis were carried out using nitrogen as carrier gas at a flow rate of 1.5ml/min at a split ratio of 1:50 and the following temperature programmed as initial temperature set at 80° C and a maximum temperature of 280° C with total analysis time of 45 min. Identification of the major components present in the oils were confirmed by comparison with standards.

Fig 4.2: Gas chromatography instrument

4.2.6. Oil entrapment efficiency:

The actual amount of the mixture of oil being entrapped in the different formulations of the matrix type patch was estimated by dissolving the patch in chloroform (10ml) briefly for a period of 2hrs. Then the resulting solution was centrifuged (Model CF10, Daihan Scientific Co. Ltd, Korea) at 3000 rpm for 15 min

to remove the polymeric remnants and thereby the clear supernatant liquid was collected. The determination of entrapment of the mixture of oils was carried out by GC analysis (Chemito Ceres 800 plus, Thermo Scientific, India).

4.2.7. In-vitro oil release study:

The in-vitro release study from the patch was investigated by subjecting patches of all formulation codes under accelerated conditions of high temperature to estimate the sustain release behaviour. Patches of each formulation code were kept in a hot air oven (Narang Scientific Works, New Delhi) and temperature maintained at 40-50°C. From each batch patches were withdrawn after every 2,4,6,8 hrs and extracted by dissolving in chloroform (10ml). These studies were performed in triplicate for each sample to check its reproducibility. The resultant solution was then centrifuged (Model CF10, Daihan Scientific Co. Ltd, Korea) at 3000 rpm for 15 min to remove the polymeric remnants. Then the clear, supernatant liquid was collected. The same procedure was carried out for all the batches. From each sample 0.5µl was injected through the septum for analysis by gas chromatography (Chemito Ceres 800 plus, Thermo Scientific, India).

4.2.8. Repellency study:

The efficacy of a repellent is generally evaluated under laboratory conditions in a mosquito repellent chamber. It is rectangular in shape with wooden frames fitted with glass on both sides. The top of the chamber has standard wire mesh while floor is fitted with wooden sheet. Repellency is determined against any mosquito species by releasing the required number of adult mosquitoes inside and inserting hand of subjects with the repellent applied on it.

Fig 4.3: Mosquito repellent chamber

The repellency test was carried out on the best optimized formulation and triplicate study was done to check for reproducibility. Laboratory reared, three days hungry and 7 to 12 days old adult female *Aedes albopictus* mosquitoes were put into the repellent testing chamber (46× 37× 36 cm) and the study was carried out for a period of 8 hours on three different days by six volunteers. A total of 100 mosquitoes were used in each test, while the testing conditions were (24±2)°C and (65±2)%.

In this study, the right hand (without test patch) was taken as control whereas left hand on to which essential oil loaded path was applied, was used as treated. Readiness of the mosquitoes to bite was checked by exposing right hand for few seconds and the number of mosquito landing or biting (if any) was recorded. The left hand bearing essential oil loaded patch was inserted inside the chamber to check the repellency initially after every half an hour followed by 1 hour interval. Both control and treated hands were exposed for one minute each during the trials. The results were used to calculate the percent repellency and repellency index. Two ways, analysis of variance (ANOVA) followed by Tukey Kramer test of multiple comparison was used to compare the landing and biting repellency.

4.2.9. Inhalational toxicity study:

For assessing the safety or risk associated with any inhalable substance can come from human, animal, and/or in vitro test systems. However studies in animal models provide a distinct view of toxicity as it involves exposure of whole animals in a controlled manner and thereby provide the closest parallel to a human exposure scenario.

Healthy adult Wistar strain albino rats (weighing 210–280 g, 5-8 weeks of age, male) were obtained from Defence Research Laboratory (DRL), Defence Research and Development Organisation (DRDO), Tezpur, Assam, India. The animals were placed in cages, with free access to standard laboratory diet (Pranav Agro Industries Limited, Sangli, Maharastra, India) and provided water *ad libitum*. They were housed in an environmentally-controlled room with temperature of 22±3°C and 40-70% relative humidity with a 12 hr light/dark cycle. All of the animal experimental protocols were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Forest and Environment, Govt. of India.

The inhalational toxicity of the optimized formulation was performed using the Whole Body Plethysmograph. The single chamber plethysmograph utilizes a barometric analysis technique that compares the pressure difference between the animal chamber and a reference chamber to measure airway physiological parameters. The selected animals were randomly placed into plethysmograph and allowed to acclimate. Baseline averages of breathing frequency, tidal volume, inspiratory and expiratory times and airway resistance of all breaths over a 3-min period were recorded initially. The air inlet was positioned on the upper side of the chamber, whereas the air outlet was at lateral end of the chamber. A differential pressure

transducer was connected on one pole to the main chamber and on the second pole to a reference chamber equilibrated with atmospheric pressure by a small channel (1.5 mm).

Fig 4.4: Inhalational chamber

Fig 4.5: Whole Body Plethysmograph

The oil of cinnamon, lemongrass and eucalyptus were made to dissolve in 10% dimethylsulfoxide (DMSO) in the ratio of 1:2. The animals selected for the study are acclimatised to laboratory conditions, 4-5 days prior to the start of experiment. The animals were divided into two groups, control and treated and both groups

consisted of three animals each. The Wistar strain albino rats were introduced sequentially into the inhalation chamber. The homogenous mixture (5ml) was introduced inside the inhalational chamber in the form of mist for a period of 1 hour and exposure was provided for the next 5 hours. During exposure the flow of air through the chamber and the air temperature in the animal's breathing zone was continuously monitored. The entire experiment was carried in accordance to OECD guidelines (Guideline 433). The exposed animals were then checked for any inhalational toxicity by introducing them to the Whole body Plethysmograph (Data sciences International, USA). Pressure signals were amplified, digitized and sampled at 100 Hz by use of the software version iox2, which provided a breath-by-breath analysis of waveforms from which pEnh is derived as a measure of airflow limitation.

5. RESULTS AND DISCUSSION:

5.1. RESULTS

5.1.1. Physical appearance:

Formulation code	Appearance
H_1	Spherical, not smooth, sticky, homogenous
H_2	Spherical, smooth, non-sticky, homogenous
H_3	Spherical, smooth, non-sticky, homogenous
H_4	Spherical, not smooth, sticky, not homogenous
H_5	Spherical, smooth, non-sticky, homogenous

Table 5.1: Characteristic appearances of different formulation

5.1.2. Data obtained from the physico-chemical evaluation of patch:

Formulation code	EC: PVP K30 ratio	Weight variation* (gm)	Surface Area* (cm^2)	Moisture content* (%)	Moisture uptake* (%)	Thickness (mm)*
H_1	1:2	0.39±0.012	13.21±0.040	2.16±1.438	16.56±2.659	0.23±0.014
H_2	2:1	0.39±0.031	13.70±0.122	1.83±0.125	11.11±0.999	0.31±0.021
H_3	3:0	0.36±0.023	13.75±0.104	0.16±0.047	1.73±0.154	0.35±0.007
H_4	0:3	0.39± 0.024	13.72±0.193	4.36±0.634	12.57±1.499	0.35±0.014
H_5	1.5:1.5	0.41±0.011	13.26±0.648	2.13±0.249	8.73±0.739	0.38±0.014

Table 5.2: Parameters obtained from physico-chemical evaluation

*All values are expressed as Mean ± SD (n=3)

5.1.3. Flatness:

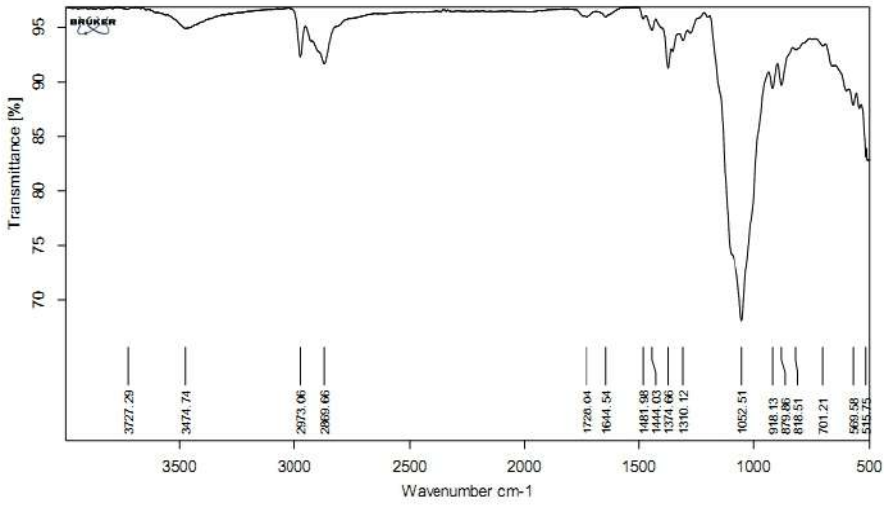
In all the formulations ranging from H_1 to H_5 the calculated flatness was 100%. As 100% flatness refers to 0% constriction so it can be concluded that all the formulations had zero constriction and will not shrink upon application.

5.1.4. p^H measurement:

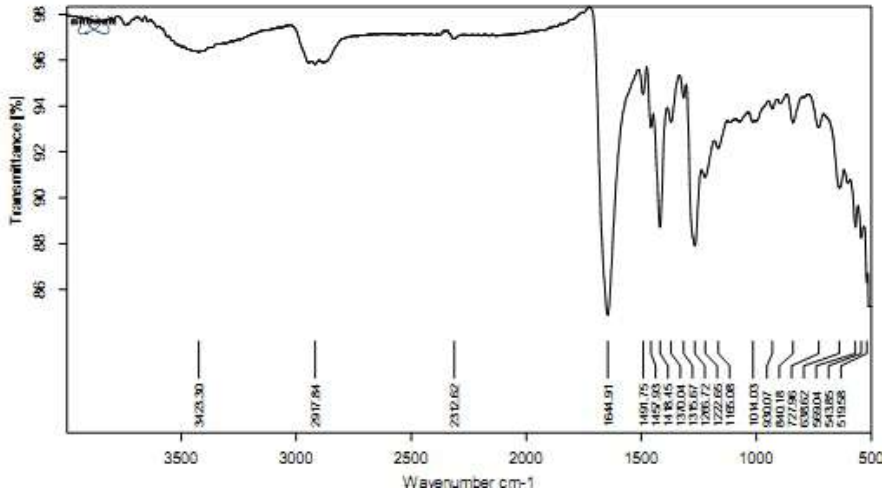
Formulation code	p^H range
H_1, H_2, H_3	6 - 6.8
H_4, H_5	5 – 5.8

Table 5.3: p^H profile of the formulations

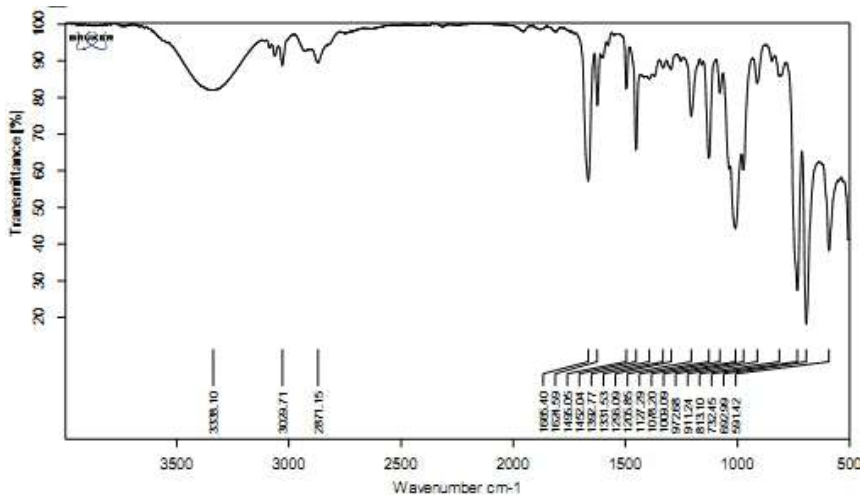
5.1.5. FTIR analysis:



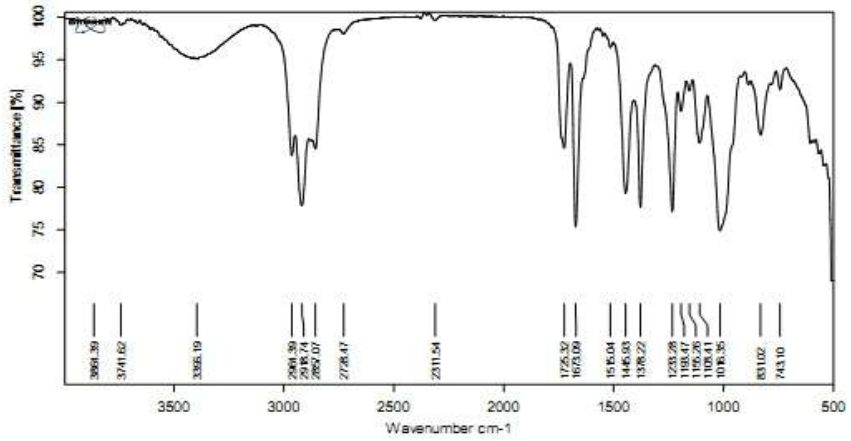
(a)



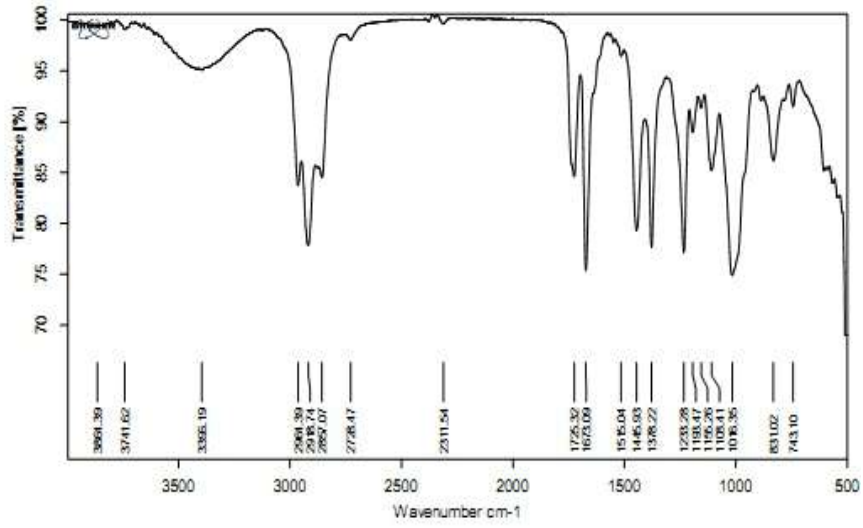
(b)


Transmittance [%]
Wavenumber cm-1
3338.10
3029.71
2871.15

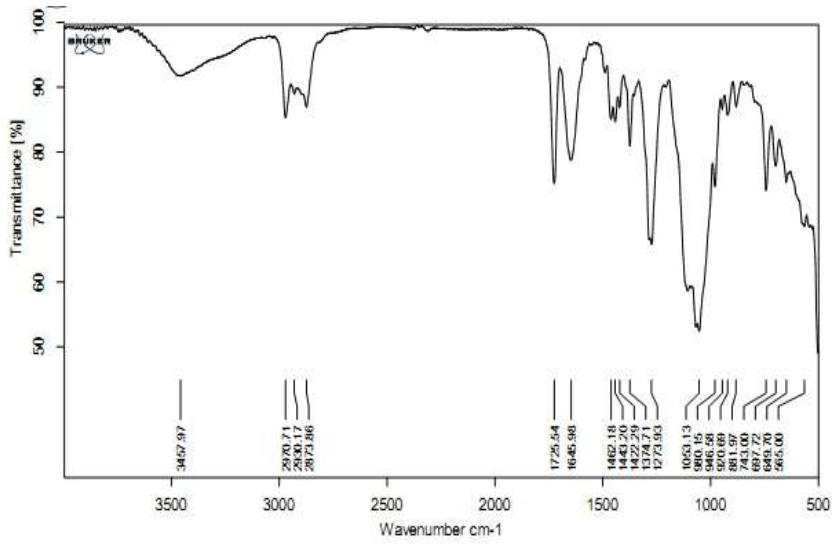
(c)


Transmittance [%]
Wavenumber cm-1
3964.39
3741.62
3366.19
2964.39
2918.74
2852.07
2728.47
2311.54
1725.32
1673.09
1515.04
1445.93
1378.22
1233.28
1193.47
1155.26
1105.41
1076.35
831.02
743.10

(d)


Transmittance [%]
Wavenumber cm-1
3964.39
3741.62
3366.19
2964.39
2918.74
2852.07
2728.47
2311.54
1725.32
1673.09
1515.04
1445.93
1378.22
1233.28
1193.47
1155.26
1105.41
1076.35
831.02
743.10

(e)



(f)

Fig 5.1: FTIR spectra of (a) ethylcellulose, (b) PVP K-30, (c) Cinnamon oil, (d) Lemongrass oil, (e) Eucalyptus oil, (f) H_5 formulation

5.1.6. Scanning electron microscope (SEM) analysis:

(a) **(b)**

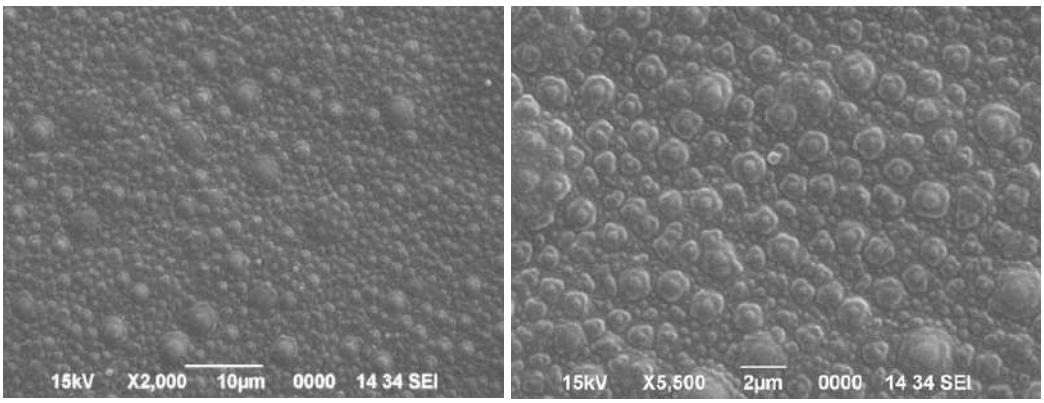


Fig 5.2: SEM images of blank polymeric matrix at 2000 and 5500 resolution respectively

(c) **(d)**

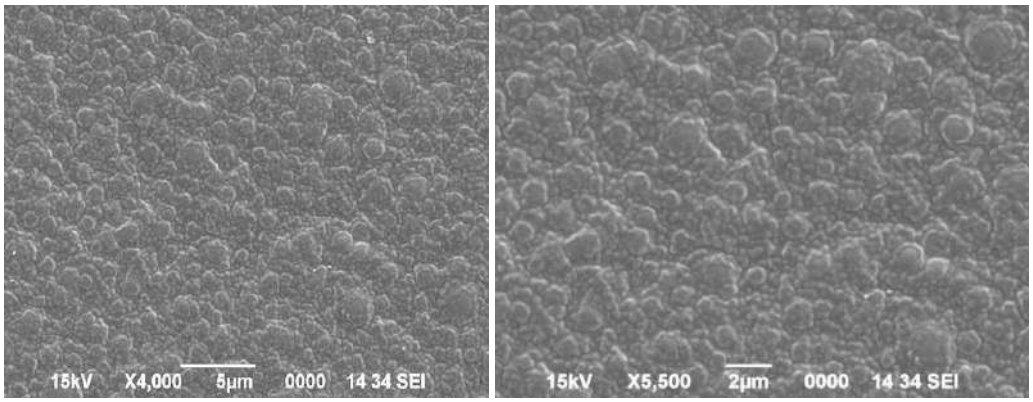


Figure 5.3: SEM images of oil loaded matrix at 4000 and 5500 resolution respectively

5.1.7. GC analysis:

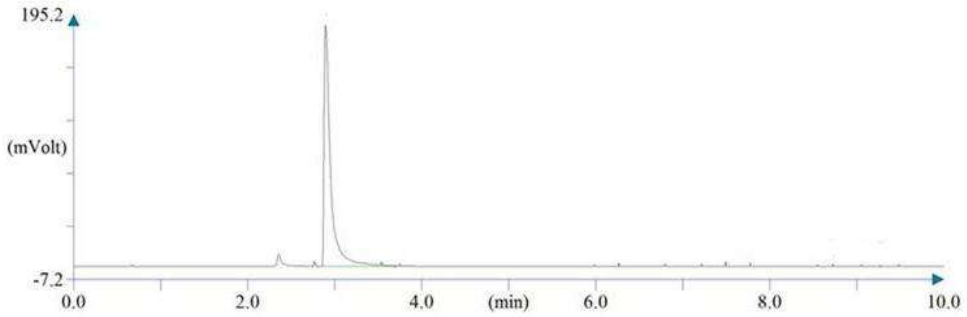


Fig 5.4: Chromatogram of chloroform

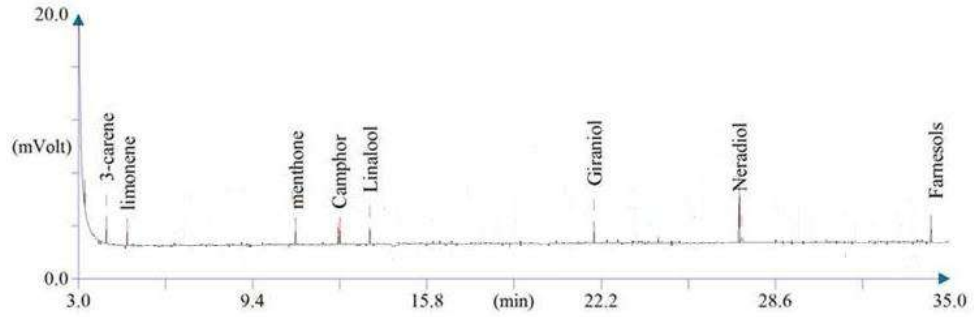


Fig 5.5: Chromatogram of Cinnamon oil

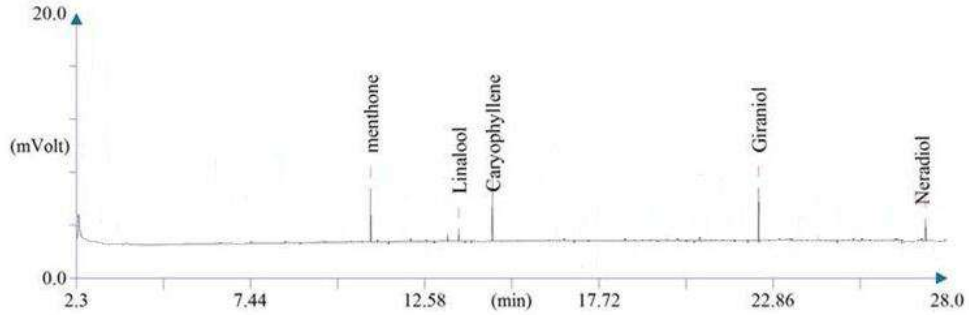


Fig 5.6: Chromatogram of Lemongrass oil

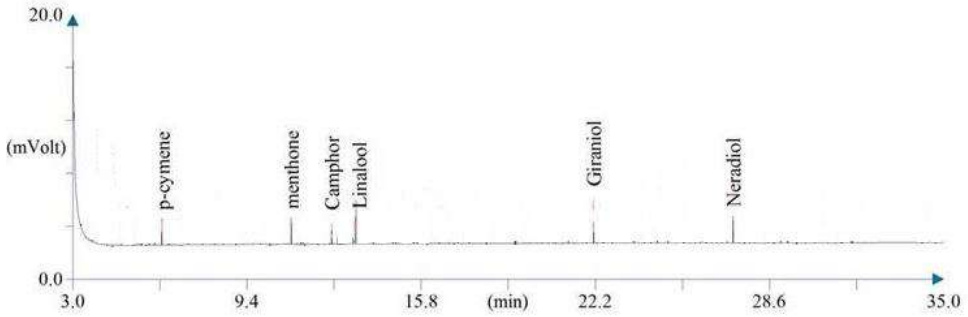


Fig 5.7: Chromatogram of Eucalyptus oil

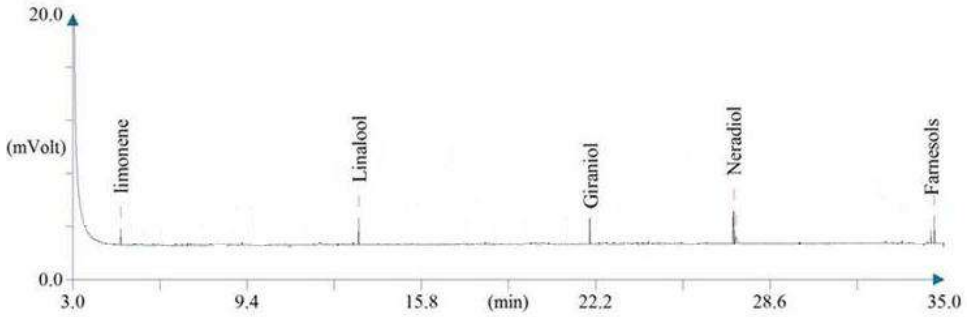


Fig 5.8: Chromatogram of oil mixture

Name of solvent/oil	Component	Retention time (min)
Chloroform		2.90
Cinnamon oil	3-carene	4.03
	limonene	4.80
	menthone	10.98
	camphor	12.55
	linalool	13.70
	giraniol	21.93
	neradiol	27.26
	farnesols	34.46
Lemongrass oil	menthone	10.98
	linalool	13.58
	caryophyllene	14.58
	giraniol	22.43
	neradiol	27.25
Eucalyptus oil	p-cymene	6.28
	menthone	11.03
	camphor	12.52
	linalool	13.51
	giraniol	22.13
	neradiol	27.26
Oil mixture	limonene	4.76
	linalool	13.50
	giraniol	22.00
	neradiol	27.26
	farnesols	34.51

Table 5.4: Detected components and their relative retention time

5.1.8. Calculation of oil entrapment efficiency:

Formulation code	Component	Area entrapped (%)
H_5	Limonene	97.06± 0.92
	Linalool	103.06± 0.98
	Giraniol	97.41± 0.73
	Neradiol	91.40± 0.87
	Farnesols	59.10± 0.52

Table 5.5: Oil entrapment data of the optimized formulation (H_5)

5.1.9. In-vitro oil release study:

Time (hrs)	Linalool (%)	Limonene (%)	Giraniol (%)	Neradiol (%)	Farnesols (%)
0	0	0	0	0	0
2	84.55	80.75	41.31	34.99	42.41
4	97.74	97.5	53.01	37.45	49.21
6	100.4	99.32	53.81	44.73	54.41
8	97.72	98.87	52.95	39.69	47.25

Table 5.6: In-vitro release of the mixture of oils from optimized formulation (H_5)

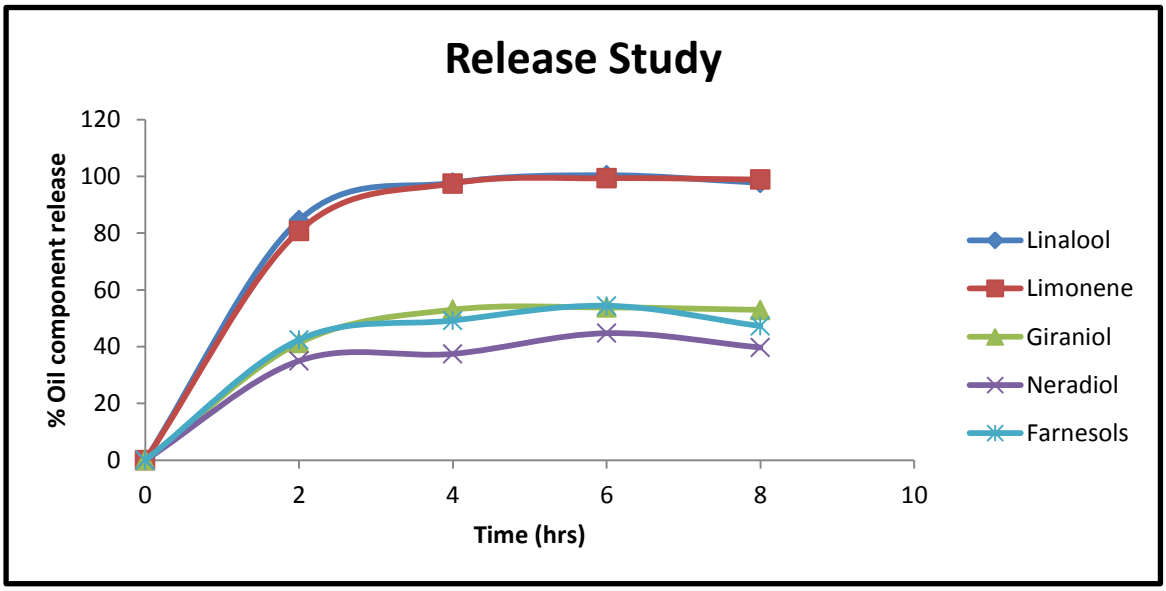


Fig 5.9: % Oil component release from optimized dermal patches (H_5)

5.1.10. Estimation of repellent activity:

Time (min)	R1	R2	R3	Mean	SD
0	5	4	3	4	1
30	3	6	4	4.333333	1.527525
60	4	5	3	4	1
120	4	5	3	4	1
180	3	7	3	4.333333	2.309401
240	3	6	4	4.333333	1.527525

300	4	5	2	3.666667	1.527525
360	4	3	3	3.333333	0.57735
420	5	5	4	4.666667	0.57735
480	5	5	3	4.333333	1.154701

Table 5.7: Mosquito landing data in control

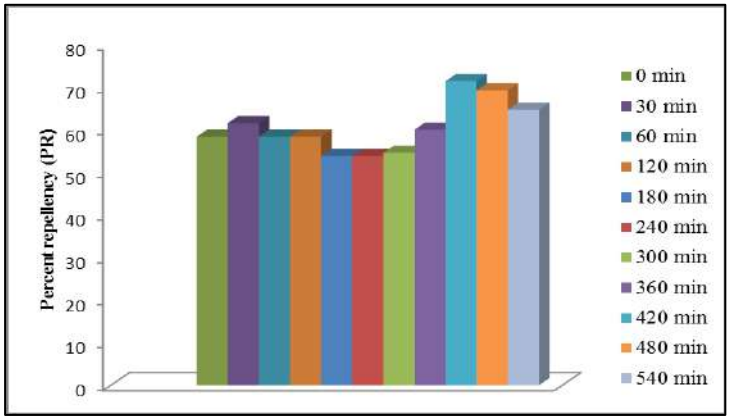
Time (min)	R1	R2	R3	Mean	SD
0	2	3	3	2.66	0.57
30	2	4	2	2.66	1.15
60	1	3	3	2.33	1.15
120	2	3	1	2	1
180	1	4	1	2	1.73
240	1	3	2	2	1
300	2	3	1	2	1
360	1	2	2	1.66	0.57
420	2	1	1	1.33	0.57
480	1	2	1	1.33	0.57

Table 5.8: Mosquito landing data in test

PR	RI
33.5	20.12
38.83	23.89
41.75	26.38
50	33.33
53.81	36.98
50	36.8
45.35	29.32
58.5	41.16
71.45	55.39
69.28	53

Table 5.9: Landing repellency in terms of percent repellency (PR) and repellency index (RI)

(a)



(b)

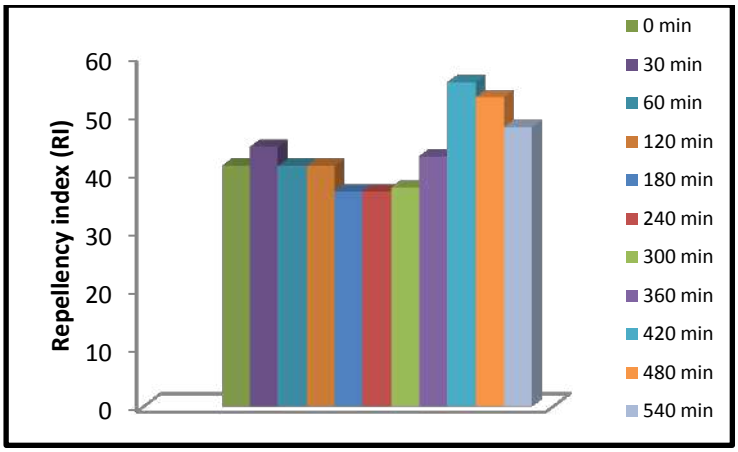


Fig 5.10: Landing repellency of essential oil loaded patch against Aedes albopictus under laboratory conditions (a) Percent repellency (b) Repellency index

Time (min)	R1	R2	R3	Mean	SD
0	1	3	1	1.66	1.15
30	2	1	2	1.666667	0.57735
60	3	3	2	2.666667	0.57735
120	3	2	3	2.666667	0.57735
180	1	6	3	3.333333	2.516611
240	2	2	3	2.333333	0.57735
300	3	2	2	2.333333	0.57735
360	2	5	1	2.666667	2.081666
420	2	3	2	2.333333	0.57735
480	2	4	1	2.333333	1.527525

Table 5.10: Mosquito biting data in control

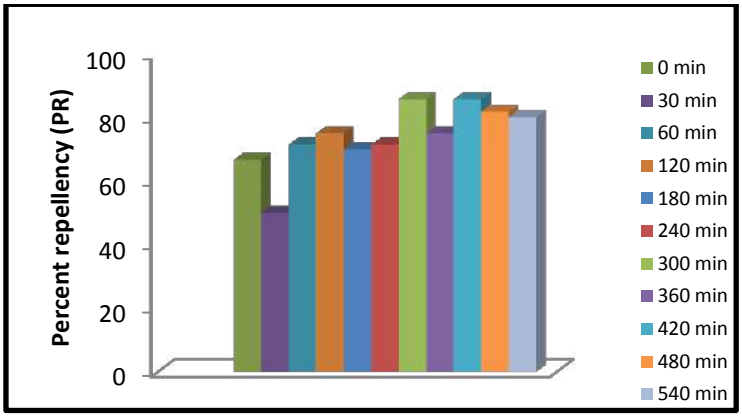
Time (min)	R1	R2	R3	Mean	SD
0	0	1	2	1	1
30	2	0	0	0.666667	1.154701
60	2	1	1	1.333333	0.57735
120	1	0	1	0.666667	0.57735
180	0	2	1	1	1
240	0	1	1	0.666667	0.57735
300	1	0	0	0.333333	0.57735
360	0	2	0	0.666667	1.154701
420	0	0	1	0.333333	0.57735
480	0	1	0	0.333333	0.57735

Table 5.11: Mosquito biting data in test

PR	RI
39.75	24.81
62.5	43.105
50	33.33
75.18	60.24
69.96	53.81
71.67	55.85
85.83	75.18
75.18	60.24
85.83	75.18
85.83	75.18

Table 5.12: Biting repellency in terms of percent repellency (PR) and repellency index (RI)

(a)



(b)

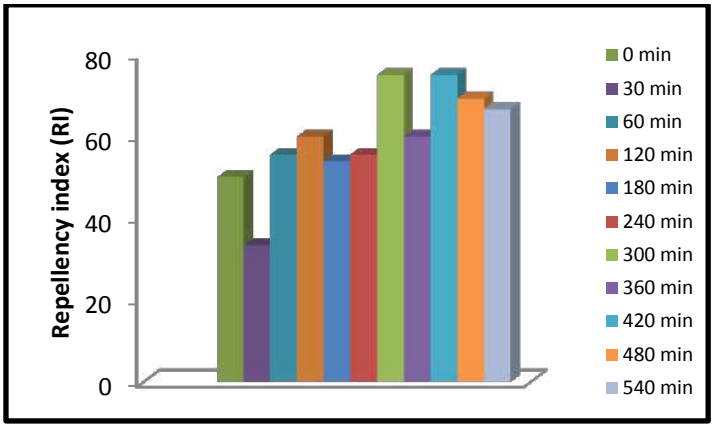


Fig 5.11: Biting repellency of essential oil loaded patch against Aedes albopictus under laboratory conditions (a) Percent repellency (b) Repellency index

5.1.11. Inhalational toxicity study:

Group	Tidal volume (TV)	Breaths per minute (BPM)	pEnh
Control	0.048 ± 0.005	157.80 ± 2.580	0.644 ± 0.028
Test	0.033 ± 0.002	166.67 ± 1.386	0.819 ± 0.097

Table 5.13: Respiratory data expressed as mean ± SD (n=3)

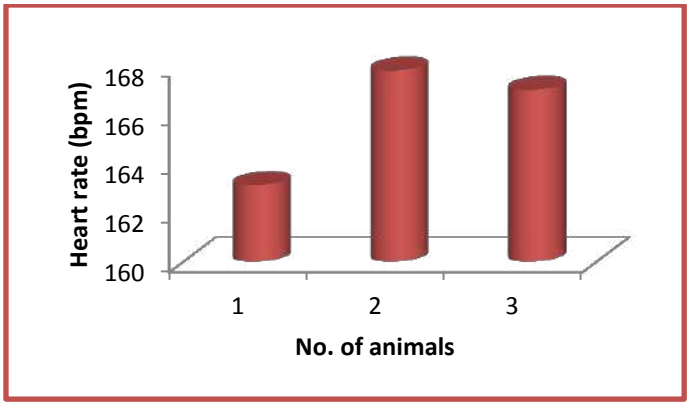


Fig 5.12: Heart rate observed after post exposure of mixture of oils on animals (n=3)

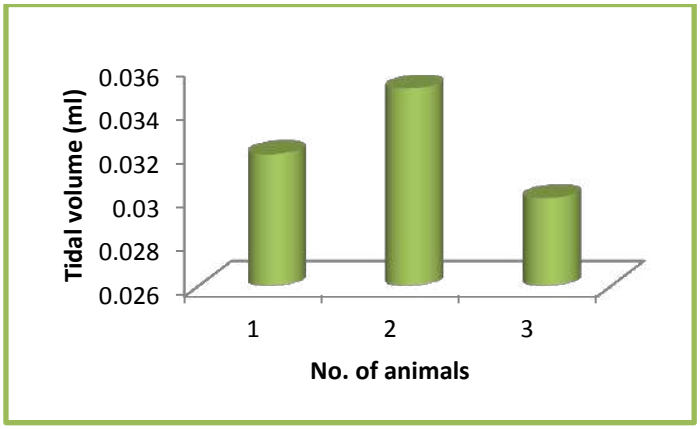


Fig 5.13: Tidal volume observed after post exposure of mixture of oils on animals (n=3)

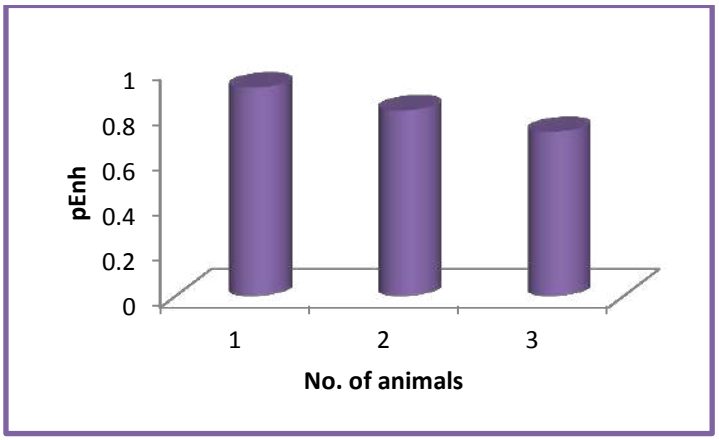


Fig 5.14: pEnh observed after post exposure of mixture of oils on animals (n=3)

5.2. DISCUSSION:

5.2.1. Evaluation of patches:

- **Physical appearance:** The physical appearance of the different formulations is given in Table 5.1. It was seen that formulations containing higher concentration of polymer PVP K-30 showed stickiness and it can be attributed due to the hygroscopic nature of the polymer. But in contrast the formulations containing higher ratio of ethylcellulose irradicates the sticky property and imparts the formulation as non-sticky. It is because of ethylcellulose which absorbs little moisture and is poorly water soluble thus making it a polymer of choice for controlling the release rate.
- **Thickness:** The thickness of patches was measured and their average weight was noted. The thickness results are given in Table 5.2. The result indicates that there was not much difference in thickness within the formulations. The reason being the total polymer concentration in all the formulations was kept constant.
- **Weight variation:** The uniformity in the different formulations was tested and the results of weight variation are given in Table 5.2. The standard deviation values showed negligible differences in all the formulations indicating that there is not much difference in their weight.
- **Surface area:** The surface area of all the formulations is given in Table 5.2 and the values show very negligible difference between them. It is also attributed due to constant polymer concentration among all the formulations.
- **p^H measurement:** The p^H of all the formulations ranging from H_1 to H_5 were checked in a digital p^H meter and the results are given in Table 5.3. Since the

P^H range of normal human skin lies in between 5.5 and 6.8 therefore all the formulations can be referred to be skin compatible and so no skin irritation is expected.

- **Percentage moisture content:** The results of moisture content of all the patches are given in Table 5.2. The results indicated that the moisture content capacity was the highest in case of formulation where PVP K-30 ratio was highest i.e. in H_4 and was the least in case of H_3 where it was negligible. So it can be concluded that PVP K30 due to its hygroscopic nature showed the highest moisture content capacity.
- **Percentage moisture uptake:** The recorded moisture uptake results are given in Table 5.2 and it was seen that the H_3 formulation showed the least moisture uptake as ethylcellulose absorbs little moisture either on exposure to atmosphere or after long immersion in water. It resists oxidation below its softening temperature.

5.2.2. FTIR spectral analysis:

The Fourier transform infrared spectroscopy studies were carried out for the three oils, both the polymers and for the formulation and are shown in figures 5.1 (a) to (f) respectively. The results are summarized as follows:

Sr. No.	IR spectrum	Groups	Peak (cm^{-1})	Stretching/Deformation
1	Ethylcellulose	O–H	3474.74	Stretching
		C–H	2973.06	Aliphatic stretching
		C–H	2869.66	Aliphatic stretching
			1052.51	Cyclohexane ring vibrations
2	PVP K-30	O–H	3423.30	Stretching
		C–H	2917.84	Aliphatic stretching

		C=C	1644.91	Stretching
		C–H	1418.45	Bending
3	Cinnamon oil	O–H	3338.10	Stretching
		C–H	3029.71	Stretching
		C–H	2871.15	Stretching
		C=C	1392.77	Stretching
		C–H	1392.77	Bending
4	Lemongrass oil	O–H	3396.19	Stretching
		C–H	2918.74	Stretching
		C=O	1725.32	Stretching ketone
		N–H	1673.09	Bending
		Aromatic ring	1515.04	Stretching
5	Eucalyptus oil	C–H	2921.60	Stretching
		C–H	1463.83	Bending
		O–H	1359.51	Bending
		C–O	1166.69	Stretching
		C–H	983.41	Bending
6	H_5	O–H	3457.97	Stretching
		C–H	2970.71	Stretching
		C=O	1725.54	Stretching ketone
		C–H	881.97	Bending

Table 5.14: FTIR spectrum of oil, polymer and mixture

The characteristic peaks in figures from 5.1 (a) to (f) clearly show that there is no interaction between the oils and the polymers in the formulation.

5.2.3. SEM analysis:

SEM micrographs of the blank patch and the essential oil embedded patch were carried out at different magnifications as shown in figures 5.2 (a) and (b) and in figures 5.3 (a) and (b) respectively. The blank patch showed globules which are mainly due to polymer crosslinking and shows a porous matrix sysstem. The surface of oil loaded patch on the other hand appears to be not so smooth and shows evenly

distribution of the essential oils in the matrix system without forming any agglomerations.

5.2.4. GC analysis:

The chemical composition of the three essential oils were determined by (Chemito Ceres 800 plus, Thermo Scientific, India) equipped with an FID detector and Carbowax capillary column (25m × 0.25mm I.D.). Also the oil entrapment in the formulated patches and the release behaviour were estimated by GC analysis.

5.2.4.1. Analysis of essential oils:

The chemical composition of cinnamon oil, lemongrass oil, eucalyptus oil and their mixture along with their retention time are shown in Table 5.4. The solvent system chloroform was also individually analysed which showed a retention time of 2.90 min confirming its peak at the same retention time in other analysis as well. In the present study the analysis of cinnamon oil identified 8 compounds comprising 3-carene, limonene, menthone, camphor, linalool, giraniol, neradiol and farnesols. Various literatures also confirmed the presence of these compounds in oil of cinnamon. However variations were seen due to differences in species, geographical location (El-Baroty et al, 2010; Chang et al, 2002). The analysis of lemongrass oil identified 5 compounds comprising menthone, linalool, caryophyllene, giraniol and neradiol. Most of the studies and literature found on lemongrass were focused only on the leaves part. Based on literature data, it appears that giraniol, neral, geraniol, limonene and β-myrcene have been found as major compounds in many other Cymbopogan species (Mirghani et al, 2012; Wei et al, 2010). However, it is noteworthy that the composition of any plant essential oil studies is influenced by

several factors such as local, climatic, seasonal and experimental conditions. Various literatures have also evaluated its repellent activity under laboratory conditions (Sritabutra et al, 2011; Yang et al, 2005). Analysis of eucalyptus oil estimated 6 compounds comprising p-cymene, menthone, camphor, linalool, giraniol and neradiol. Literature cited has confirmed for its chemical composition and has also been evaluated to exhibit anti-mosquito properties (Maciel et al, 2010; Sritabutra et al, 2011; Mandal, 2011). The composite mixture of the three essential oils exhibited the following compounds limonene, linalool, giraniol, neradiol and farnesols when analysed by gas chromatography.

5.2.4.2. Estimation of oil entrapment in formulated patches:

In the present study essential oil loaded dermal patches based on two rate controlling polymers viz; ethylcellulose and PVP K-30 were prepared by solvent evaporation method. By this method the percent entrapment efficiency was found to be the highest in H_5 formulation, given in Table 5.5. The batch H_5 contained an equal concentration of both the polymers which may be attributed due to its increased entrapment. This polymer ratio resulted in the formation of a rigid matrix structure thereby increasing the retention of the essential oil mixture encapsulated.

5.2.4.2. Estimation of in-vitro oil release study:

The in-vitro oil release study was estimated by gas chromatography. The present study investigated the effect of the matrix structure on the release profile of the mixture of oil from the different batches. There was consistent and uniform release of various compounds from H_5 formulation in comparison to other batches. The oil release profile is presented in Table 5.6. The formation of rigid dense

polymeric matrix structure contributed to the uniform release of the various compounds from the formulated patch and thereby was regarded as the best optimized and used for further evaluations.

5.2.5. Estimation of repellent activity:

Landing and biting repellent activity of essential oil loaded mosquito repellent patches against *A. albopictus* performed under laboratory conditions has been presented in figures 5.10 (a) and (b) & 5.11 (a) and (b). There was no difference in both percent repellency (df=32, F=0.5269, p=0.8512) and repellency index (df=32, F=0.5894, p=0.804) for landing of mosquitoes at various time intervals. Similarly, no difference could be observed for percent repellency (df=32, F=0.6206, p=0.7791) and repellency index (df=32, F=0.511, p=0.8604) of biting of *A. albopictus* mosquitoes. Many studies have been carried out to evaluate the repellent activity of herbal based insecticides against vector mosquitoes, which suggest their effectiveness in repelling the mosquitoes and other biting insects (Lawal et al, 2012; Adeniran et al, 2012).The plant based repellent either singly or in combination has been proved to be useful in providing considerable protection against the hematophagous insect in the field (Hazarika et al, 2012). Plant derived repellent has been much accepted because these have no or comparatively lesser harmful effect to the user and environment. Further the chance for developing resistance in mosquitoes is also very limited (Koul et al, 2008; Katiyar et al, 2010).

5.2.6. Estimation of inhalational toxicity study:

The inhalational toxicity of the optimized formulation was performed using the Whole Body Plethysmograph. Respiratory parameters generated from the

plethysmograph system consisted of inspiratory time (IT), expiratory time (ET), peak inspiratory flow (PIF), peak expiratory flow (PEF), tidal volume (TV), respiratory rate (RR), relaxation time (RT) and enhanced pause (pEnh). Air-way resistance in the Whole Body Plethysmograph is determined by calculating the dimensionless parameter enhanced pause (pEnh). Literature cited on inhalational toxicity study revealed the acceptable limit of (pEnh) based on body weight measurement. The value of pEnh depending on body weight varies from 2.2± 0.1 in < 400g body weight, 2.9± 0.1 in 400-500g body weight and 3.0± 0.2 in > 500g body weight (Delaunois et al, 2009; DeLorme et al, 2002). In the present study the value of pEnh was 0.819 ± 0.097 which was within the acceptable limit according to literature cited. The cardiovascular data obtained with the optimized formulation using Whole Body Plethysmograph did not vary much with that obtained with the control group. Therefore it could be concluded that the optimized formulation did not affect any of the respiratory data convincing the formulation to be regarded as environmentally safe.

6. CONCLUSION:

This work demonstrates the successful development of dermal patch through successful incorporation of mixture of essential oils viz; cinnamon oil, lemongrass oil and eucalyptus oil by solvent evaporation method. The development of the patch was designed by varying the ratios of ethylcellulose (EC) and PVP K-30 along with chloroform being the solvent and dibutyl phthalate as the plasticizer.

Essential oils, cinnamon oil, lemongrass oil and eucalyptus oil are reported to have antimosquito properties, thereby the reason behind selecting them in the development of the desired formulation. The patches were subjected to various evaluation parameters such as physical appearance, weight variation, thickness, surface area, flatness, moisture content, moisture uptake, p^H measurement, FTIR study, SEM analysis, entrapment efficiency, in-vitro oil release study, mosquito repellent activity and inhalational toxicity analysis. The thickness, weight variation and surface area were within acceptable limit with low standard deviation values. It also showed 0 % constriction exhibiting good folding endurance. However the moisture content was increased in formulations where PVP K-30 concentration was increased and on the other hand moisture uptake was increased in formulations where ethylcellulose concentration was increased. The FTIR spectral analysis confirmed the integrity and compatibility of the oils with the polymers in the polymer-oil matrix formulations. The SEM analysis reveals uniform distribution of the mixture of oils in the matrix system. The oil entrapment as well as release profile was the highest in case of H_5 formulation with better physico-chemical characteristics and thereby was considered as the best optimized formulation. From the statistical analysis it was seen that the H_5 formulation showed a consistent repellent activity achieved upto 8 hrs of

testing, which suggests that the active components were steadily released from the polymeric matrix. The inhalational toxicity results being within acceptable limits thereby confirmed the feasibility of development of the patch as an effective mosquito repeller as it is environment friendly due to use of herbal components.

It is hoped that the preparation could provide prolonged and controlled release of essential oils from the matrix system and also perform better.

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