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# "SCREENING OF PLANT EXTRACTS AND ESSENTIAL OILS FOR LARVICIDAL ACTIVITY IN THE MOSQUITO VECTOR *Culex quinquefaciatus*"

**A PROJECT REPORT**

***Submitted by***


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|---|---|
| **SINDHUJA D.** | **71206214042** |
| **VASUPPRADHA N.** | **71206214051** |
| **VINOTH C.** | **71206214054** |


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**KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE**

**ANNA UNIVERSITY::CHENNAI 600 025**


**APRIL 2010**

# ANNA UNIVERSITY: CHENNAI 600 025

## BONAFIDE CERTIFICATE

Certified that this project report **"Screening Of Plant Extracts And Essential Oils For Larvicidal Activity In The Mosquito Vector *Culex quinquefaciatus*)"** is the bonafide work of **Sindhuja D, Vasuppradha N And Vinoth C.** who carried out the project work under my supervision.

**SIGNATURE**

**Dr. S. SADASIVAM**

**DEAN**

Department of Biotechnology,

Kumaraguru College of Technology,

Coimbatore – 641006.

**SIGNATURE**

**Dr. P.RAMALINGAM**

**SUPERVISOR**

Department of Biotechnology,

Kumaraguru College of Technology,

Coimbatore – 641006.

# CERTIFICATE OF EVALUATION

**COLLEGE** : **Kumaraguru College Of Technology**

**BRANCH** : **Biotechnology**

**SEMESTER** : **Eighth Semester**

| NAME OF THE STUDENT | TITLE OF THE PROJECT | NAME OF THE SUPERVISOR |
|---|---|---|
| **SINDHUJA D.** (71206214042)<br>**VASUPPRADHA N.** (71206214051)<br>**VINOTH C.** (71206214054) | **"Screening Of Plant Extracts And Essential Oils For Larvicidal Activity In The Mosquito Vector *Culex Quinquefaciatus*"** | **Dr. P.RAMALINGAM**<br>**Asst. Professor** |

The Report of the project work submitted by the above students in partial fulfilment for the award of Bachelor of Technology degree in Biotechnology of Anna University was evaluated and confirmed to be their original work. It was submitted for evaluation and viva – voce held on 20th April, 2010.

**(INTERNAL EXAMINER)** **(EXTERNAL EXAMINER)**

DEDICATED TO OUR
BELOVED PARENTS

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[SINDHUJA.D]

[VASUPPRADHA.N]

[VINOTH.C]

# ABSTRACT

## ABSTRACT:

Extracts from the leaves and rhizome of *Curcuma longa, Azadirachta indica, Glycyrrhiza glabra* and the bark extract of *Ervatamia divaricata* using Soxhlet extraction apparatus were evaluated to test the larval toxicity at different concentrations ranging from 1.0 ml/100 ml to 6.0 ml/ 100 ml against the fourth larval instars of *Culex quinquefaciatus* . The mortality rates increased with the concentration of the extract and the exposure time. At higher concentrations of the extract of *Azadirachta indica* (5.0ml/l), 100% mortality was recorded at 150 min. Extracts from the leaves and rhizome of *Curcuma longa* showed 100% mortality at concentrations of 4.0ml/l but at exposure timings of 90 min and 70 min respectively.

Mortality rate of 100 % was observed with *Earvatamia divaricata* only at a concentration of 4.0ml/100ml only at a time of 120 min. A 100% mortality was observed in *Glycyrrhiza glabra* (bark) at the same exposure time but only at a concentration of 6.0ml/100ml. A Combinatorial Study when undertaken showed potent larvicidal activities at lesser concentrations and exposure time for *Azadirachta indica* and *Curcuma longa* (leaves), *Azadirachta indica* and *Ervatamia divaricata* (leaf), *Curcuma longa* and *Ervatamia divaricata* (leaf). A100% mortality was observed at 4.0ml/100ml in 40 min, 5.0ml/100ml in 100 min and 4.0 ml/100ml in 90 min for *Azadirachta indica* and *Curcuma longa* (leaves), *Azadirachta indica* and *Ervatamia divaricata* (leaf) and finally *Curcuma longa* and *Ervatamia divaricata* (leaf) respectively.

The $LC_{50}$ of all the plant extracts when analyzed using SPSS software showed values of 0.571ml/100ml,1.543ml/100ml and 0.159ml/100ml that are lower compared to the $LC_{50}$ values of the individual plant extracts. Essential oil was extracted from *Curcuma longa, Jatropha curcas, Pongamia pinnata, Azadirachta indica, Mangifera indica and Psidium guajava* using steam distillation unit and tested for larvicidal property. Essential oils obtained from *Curcuma longa* was highly effective compared to the other extracts showing 100% larvicidal property at a concentration of 4.0ml/100ml and the mortality. *Mangifera indica* also showed effective mortality at concentrations of 2.0 ml/100ml.

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## LIST OF ABBREVIATIONS:

| ABBREVIATION | EXPLANATION |
|---|---|
| ml | Millilitre |
| µl | Microlitre |
| L | Litre |
| IPCC | Intergovernmental Panel on Climate Change |
| ppm | Parts per million |

# LIST OF FIGURES

# INTRODUCTION

# CHAPTER1

## 1. INTRODUCTION:

### 1.1 Mosquito borne diseases:

Vector-borne diseases including dengue, yellow fever, Japanese encephalitis, malaria, leishmaniasis and filariasis remain severe public heath problems in most of the countries in which they are endemic( Gratz and Jany,1994). Every year, millions of people contract diseases transmitted by insects and these diseases have a significant social and economic impact particularly in tropical countries.

### 1.2 Socio-Economic reasons for the Epidemiology:

Operational, financial and managerial problems together environmental change, vector and pathogen resistance to insecticides and drug, increasing human population, progressive urbanization and population movement, and a shift in emphasis from prevention to emergency response have all contributed to the recent increase in the prevalence of vector-borne diseases (Gubler,1998).

### 1.3 Climate change and malaria in India: Interplay between temperatures and mosquitoes:

Malaria is still a major public health problem in India. The epidemiology of malaria constitutes man as host, four species of protozoan parasite, plasmodia (*Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae* and *Plasmodium ovale*) and anopheline mosquitoes as vectors. Environmental conditions play an important role in the transmission dynamics of malaria, as the parasite has to pass its developmental cycle in the mosquito. The three main climatic factors that affect malaria transmission and distribution are temperature, precipitation and relative humidity. (Pampana E. Textbook of malaria eradication,1969). Climate predicts, to a large degree, the natural distribution of malaria. (Bouma and Vander, 1996).Climate change is a new emerging threat to health, particularly in the context of vector-borne diseases. The Third Assessment Report of the Intergovernmental Panel on Climate

Change (IPCC) (McCarthy et al, 2001) has highlighted that by 2100 the global temperature would increase by 1.8 °C–4 °C. The Fourth Assessment Report of IPCC (2007) has clearly highlighted the possible increase in vector-borne diseases spatially and temporally. The Government of India has also taken initiatives for studying the vulnerability assessment and adaptation measures to address the threat of climate change in the field of vector-borne diseases. The logic behind the possible increase/decrease in malaria transmission in view of climate change is based on the interplay between temperatures and developmental cycles in anopheline mosquitoes.

## 1.4 Statistics on the Epidemiology of Mosquito-borne diseases:

Insect-transmitted disease remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes, 1997). About 40% of the world's population, mostly those living in the poorest countries are at risk of malaria. Of these 2.5 billion people at risk, more than 500 million become severely ill with malaria every year and more than 1 million die from the effects of the disease (WHO, 2007). Although mosquito-borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (Fradin and Day, 2002).

## 1.5 Mosquito Habitation:

Vector mosquitoes carry diseases and lay their eggs in stagnant ditches and sewage treatment ponds or water in tree holes, old tires, clogged gutters, old tin cans, overhead tanks, unused wells and anything else that will hold water. Eggs are laid on or above the water surface, where they usually hatch within two or three days. Herbal products with proven potentials insecticide or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level (Mittal & Subbarao, 2003).

## 1.6 Details of the commonly observed Mosquito colonies in the environment:

- *Anopheles stephensi*
- *Aedes aegypti*
- *Culex quinquefaciatus*
- *Toxorhynchites splendens*

### 1.6.1 *Toxorhynchites splendens:*

This species feed upon plant juice/sap and honey unlike their larval stage which feed upon larvae of other species of mosquitoes. Their preference vanishes out during lack of feed and may also feed upon the larvae of the same species. Thus, this species of mosquitoes are quite useful to human, as they are predatory in nature.

**Table 1.6.1:Details of the commonly found Mosquito Vectors in Southern India**

| Mosquito vector | Disease transmitted | Biting period | Distribution | Control |
|---|---|---|---|---|
| *Aedes aegypti* | Dengue fever, Yellow fever, Chikungunaya | Day time, peak activity in the early morning and late afternoon | Mainly urban in tropical and warm countries. | Source reduction, space sprays, periodical residual sprays and larviciding |
| *Culex quinquefaciatus* | Filariasis | At night, mostly indoors | Tropical countries. | Source reduction, personal protection, space sprays, larvicides. |
| *Anophels stephensi* | Malaria | At night , mostly indoors | Middle eastern and south Asian countries. Especially important in urban areas. | Source reduction, residual sprays, personal protection. |

## 1.7 Life Cycle of a mosquito:

The mosquito goes through four separate and distinct stages of its life cycle: Egg,Larvae, Pupa and Adult.

Eggs are laid one at a time and they float on the surface of the water. In the case of *Culex* species, the eggs are stuck together in rafts of a hundred or more eggs. *Anopheles* and *Aedes* species do not make egg rafts but lay their eggs separately. *Culex* and *Anopheles* lay their eggs on water while *Aedes* lay their eggs on damp soil that will be flooded by water. Most eggs hatch into larvae within 48hrs.

The larva (larvae-plural) lives in the water and come to the surface to breathe. They shed (molt) their skin four times, growing larger after each molting. Most larvae have a siphon and lay parallel to the water surface to get the supply of oxygen through a breathing opening. The larvae feed on the micro organisms and organic matter in the water. On the fourth molt the larvae changes into pupa. The pupal stage is s resting, non-feeding stage. This is the time the mosquito turns into adult. It takes about two days before the adult is fully developed. When development is complete, the pupal skin splits and the mosquito emerges as an adult. *Aedes* mosquitoes are painful and persistent biters, attacking during daylight hours (not at night). *Culex* mosquitoes are also painful and persistent biters, but prefer to attack at dusk and after dark and readily enter dwellings for blood meals.

The mosquito's visual picture is an infrared view produced by its prey's body temperature. The average life span of the female mosquito is 3 to 100 days and for the male it is 10-20 days. The Mosquito adults feed on flower nectar and juices of fruits for flight energy. The female requires the blood meal for egg development. Depending on the species, female mosquitoes may lay 100 to 300 eggs at a time and may average 1000 to 3000 during their lifespan. The mosquito matures from egg to adult in 4 to 7 days. Most mosquitoes remain within 1mile of their breeding site. A few species may range up to 20.00 miles or more.

## 1.8 Mosquito Control:

Several mosquito species belonging to genera *Anopheles, Culex, Aedes* are vectors for the pathogens of various diseases like malaria, Filariasis, Japanese encephalitis (JE), Dengue, Yellow fever, Chikungunya, etc. thus one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes to bite human beings.

### 1.8.1 Physical control method:

The mosquitoes are controlled by various methods. Physical control method used against mosquitoes includes elimination of breeding sites, application of surface films of oil to clog the breathing tubes wrigglers and the use of larvicides (Nathan, 1993). Chemical control use of synthetic insecticides is the most convenient and acceptable method all over the world. However, the extensive application of synthetic pesticides has resulted in environmental contamination and showed negative effect on non target beneficial organisms as well as on human (Harilal and Sahai, 1990 and Deedat 1994). The larvicide used is usually the organophosphate Temephos, although very slightly toxic may cause headaches, loss of memory and irritability (NICC, 2003). Insecticide resistance is becoming a serious problem in pest management. The mosquito *Culex triaeniorhychus* GILES, an important vector of Japanese encephalitis was quite susceptible to organophosphorous insecticides (Ops) during 1960s and 1970s (Buei and Ito, 1974). However, in1982 a high level of resistance to Ops and carbomates was found among mosquitoes collected from Toyama prefecture (Kamimura and Maruyama 1983). During the next few years, the same kind of resistance was detected in the most districts in Japan (Takahashi and Yasutomi, 1987). The mosquito in Toyama appeared to be highly resistance to the most popularly used Ops and carbomates (Watanabe *et al.*, 1991).

### 1.8.2 Biological control method:

Biological control measures include using pathogens (Mulla *et al.*, 1993) as well as aquatic predators affecting different target sites; the chance of development of resistance is remote. Long before the advent of synthetic insecticide era, naturalistic methods of pest control including the use of plants and their derivatives were in vogue in different parts of the world to ward off or kill the annoying insects or pests of agriculture, veterinary and public health importance.

The most effective manner of controlling their transmission is through the control of their vectors. Virtually all of the vector and pest control programs depend on the use of insecticides formulated as larvicides, adulticides, baits or insecticide impregnated bed nets. Due to development of insecticide resistance, toxicological and environmental

considerations and the cost of development and registration, the number of compounds available for use has declined. The recrudescence of vector-borne diseases, the rapid pace of urbanization, lagging development of environmental services in many tropical cities and difficulties encountered in ensuring the community's cooperation in its own protection through environmental measures make imperative the continued availability of pesticides for public health use (Gratz and Jany, 1994).

Different strategies have been devised to reduce the prevalence of malaria and other mosquito-borne diseases in endemic regions of the world. Biological control at the larval stage of development of mosquitoes is one of the techniques which affords a cheap, easy to use, and environment friendly method of malaria control. Natural insecticides are less phytotoxic and do not accumulate chemical residues in flora, fauna and soil.

### 1.8.3 Application of synthetic insecticides:

The discovery of DDT's insecticidal properties in 1939 and the subsequent development of organochlorine and organophosphate insecticides overshadowed the use of herbal products against mosquitoes but also become the major weapon for mosquito control. However, in the field of mosquitocidal insecticides, few new effective and economical insecticides have been developed since the introduction of synthetic pyrethroids. Neem is the one of the few natural products that has gained wide acceptance in some countries as an antifeedant. Recent approval of neem (Isman, 1997) in the USA has stimulated research into other potential botanicals. Other issues prompting researchers to enter the field are the high cost of synthetic pyrethroids, environment and food safety concerns, the unacceptability and toxicity of many organophosphates and organochlorines and increasing insecticides resistance on a global scale (Shalaan, 2005).

Mosquito insecticides used against pest and vectors of human disease (e.g. Fleas, flies and mosquitoes) are spin-offs from agrochemical research and development. The arsenal of safe and cost-effective public health insecticides is being depleted by restrictions for various reasons (e.g. Insecticide resistance unacceptable side effects and non re-registration) and the number of new products launched is dwindling. Mobilizing

public resources and establishment of partnerships to support research and development of public health insecticide is crucial in the post-DDT and post-pyrethroid era (Zaim and Guillet, 2002). In this context, there is a need for exploration of pesticides of alternate source with less environmental hazards; this study intends to identify potential sources of biocide against mosquitoes from plant products. The project aims at the evaluation of the larvicidal potential of the commonly available medicinal plants such as *Glycyrhira glabra* and *Ervatamia divaricata* in combinations with plants that are reported to have potent larvicidal activity.

## 1.9 Phytochemicals as effective Active ingredient for Larvicidal property:

Natural insecticides are less phytotoxic and do not accumulate chemical residues in flora, fauna and soil. Phytochemicals with mosquito larvicidal activity occur in the oils, leaves and roots of plants (Sharma et al., 1998; Ojewole and Shode, 2000; Sosan et al., 2001). An excellent review of the activity of *Azadirachta indica*, and other plants with proven mosquito control potential has been made (Mittal and Subbarao, 2003).

## 1.10 Steam distillation:

Steam distillation (Figure 1.3.1) is a special type of distillation (a separation process) for temperature sensitive materials like natural aromatic compounds. Many organic compounds tend to decompose at high sustained temperatures. If the substances to be distilled are very sensitive to heat, steam distillation can also be combined with vacuum distillation. After distillation the vapors are condensed as usual, usually yielding a two-phase system of water and the organic compounds, allowing for simple separation.

Steam distillation is employed in the manufacture of essential oils, for instance, perfumes. In this method, steam is passed through the plant material containing the desired oils.

## 1.11 Soxhlet extraction:

A Soxhlet extractor (Figure 1.3.2) is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent.

## 1.12 Plants screened for larvicidal activity:

The eight plants chosen to screen for Larvicidal repellent activity are as follows:

1.12.1. *Jatropha curcas*

1.12.2. *Azadirachta indica*

1.12.3. *Pongamia pinnata*

1.12.4. *Mangifera indica*

1.12.5. *Psidium guajava*

1.12.6. *Curcuma longa*

1.12.7. *Glycyrrhiza glabra*

1.12.8. *Ervatamia divaricata*

## 1.12.1. *Jatropha curcas*:

**Scientific classification:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Unranked | : | Angiosperms |
| Unranked | : | Eudicots |
| Unranked | : | Rosids |
| Order | : | Malpighiales |
| Family | : | Euphorbiaceae |
| Genus | : | *Jatropha* |
| Species | : | *J.curcas* |

**Figure 1.12.1** ***Jatropha curcas***

*Jatropha curcas* is a species of flowering plant in the spurge family, Euphorbiaceae, that is native to the American tropics, most likely Mexico and Central America. It is cultivated in tropical and subtropical regions around the world, becoming naturalized in some areas..

## 1.12.2. *Azadirachta indica: indica*

**Figure 1.12.2** ***Azadirachta***

**Scientific classification**:

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Division | : | Magnoliophyta |
| Order | : | Sapindales |
| Family | : | Meliaceae |
| Genus | : | *Azadirachta* |
| Species | : | *A.indica* |

Neem is native to India, Myanmar, Bangladesh, Sri Lanka, Malaysia and Pakistan, growing in tropical and semi-tropical regions . Neem is a fast-growing tree that can reach

a height of 15-20 m (about 50-65 feet), rarely to 35-40 m (115-131 feet). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. All parts of the tree (seeds, leaves, flowers and bark) are reported to have high medicinal poperties.Neem oil has been found to be an effective mosquito repellent.

### 1.12.3. *Pongamia pinnata*:

**Figure 1.12.3 *Pongamia pinnata***

**Scientific classification:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Division | : | Magnoliophyta |
| Class | : | Magnoliopsida |
| Order | : | Fabales |
| Family | : | Fabaceae |
| Genus | : | Pongamia |
| Species | : | P.pinnata |

*Pongamia pinnata* is a deciduous legume tree that grows to about 15–25 meters in height with a large canopy which spreads equally wide.Juices from the plant, as well as the oil, are antiseptic and resistant to pests..

## 1.12.4. *Mangifera indica*:

**Scientific classification:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Unranked | : | Angiosperms |
| Unranked | : | Eudicots |
| Unranked | : | Rosids |
| Order | : | Sapindales |
| Family | : | Anacardiaceae |
| Genus | : | *Mangifera* |
| Species | : | M.indica. |

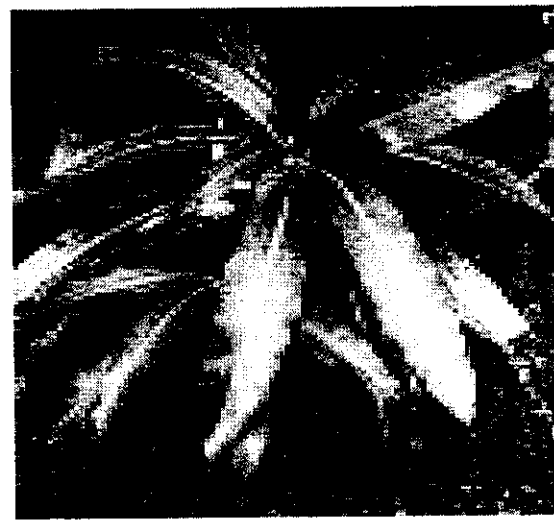


**Figure 1.12.4.** ***Mangifera indica***

## 1.12.5. *Psidium guajava*:

**Figure 1.12.5***Psidiun*

***guajava***

**Scientific classification:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Unranked | : | Angiosperms |
| Unranked | : | Eudicots |
| Unranked | : | Rosids |
| Order | : | Myrtales |
| Family | : | Myrtaceae |
| Genus | : | *Psidium* |
| Species | : | *P. guajava* |

The apple guava or common guava (*Psidium guajava*; known as Goiaba in Brazil and Guayava in parts of The Americas) is an evergreen shrub or small tree native to Mexico, the Caribbean, and Central and South America.

## 1.12.6. *Curcuma longa:*

**Scientific classification:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Class | : | Liliopsids |
| Order | : | Zingiberales |
| Family | : | Zingiberaceae |
| Genus | : | *Curcuma* |
| Species | : | *C. longa* |

**Figure 1.12.6 Leaves of *Cucuma longa***

In Ayurvedic practices, turmeric has many medicinal properties and many in South Asia use it as a readily available antiseptic for cuts, burns and bruises

## 1.12.7. *Glycyrrhiza glabra*: *glabra*

**Figure 1.12.7. *Glycyrrhiza***

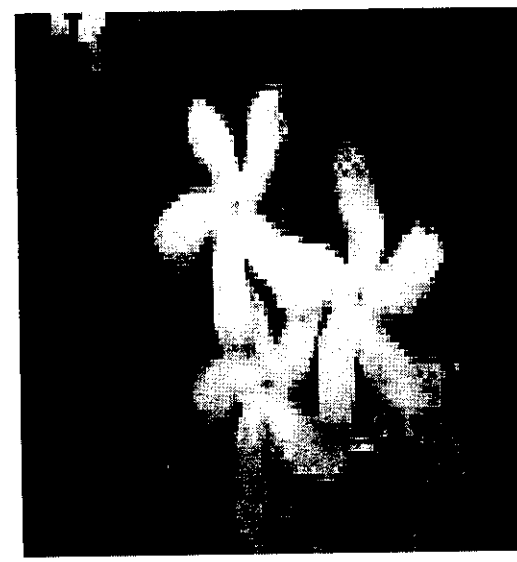
**Scientific classification:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Division | : | Magnoliophyta |
| Class | : | Magnoliopsida |
| Order | : | Fabales |
| Family | : | Fabaceae |
| Genus | : | *Glycyrrhiza* |
| Species | : | *G. glabra* |

Hot water extract of the dried root is taken orally as a gastric ulcers and for amenorrhea. Decoction of the root is taken orally for stomachache. Liquorice may be useful in conventional and naturopathic medicine for both mouth ulcers and peptic ulcers.

## 1.12.8. *Ervatamia divaricata:* *divaricata*

**Figure 1.12.8 *Ervatamia***



**Scientific classification:**

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Subkingdom | : | Tracheobionta |
| Superdivision | : | Spermatophyta |
| Division | : | Magnoliophyta |
| Class | : | Magnoliopsida |
| Order | : | Gentianales |
| Family | : | Apocynaceae |
| Genus | : | *Ervatamia* |
| Species | : | *E.divaricata* |

The root is acrid and bitter; it is employed as a local anodyne and chewed for the relief of toothache. It is rubbed into a thin paste with water and administered as vermicide.The juice of flowers mixed with oil is employed for relieving the burning sensation in sore eyes; it is also applied in skin diseases. The milk juice of the leaves is used as a cooling application for wounds to prevent inflammation.

# REVIEW OF LITERATURE

# CHAPTER2

# 2. REVIEW OF LITERATURE

## 2.1 Development of Synthetic Insecticides:

Conventional insecticides such as malathion, DDT, carbamates and pyrethroids that are generally used for mosquito control are known to cause the problem of environmental pollution, residual effects and resistance by their indiscriminate use. Development of resistance to malathion (Guneady *et al.*, 1989) and to deltamethrin (Chen Wen-Mei, 1990) in adult *C. pipinens* has been reported. Due to the problem of pollution and vector resistance, safe plant products are being tested around the world as pest control agents. Secondary metabolites or the phytochemicals obtained from the indigenous plants with proven mosquito control potential can be used as an alternative to synthetic insecticides under the integrated vector control.

## 2.2 Application of plant products for repellent activity:

Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or repellents for protection against mosquito bites, depending on the type of activity they possess. They can be obtained either from the whole plant or from a specific part by extraction with different types of solvents such as aqueous, methanol, chloroform, acetone, hexane, etc., depending on the polarity of the phytochemicals.

## 2.3 Extraction of plant products:

Of primary consideration is the type of solvent used since polar solvents will extract polar molecules and non-polar solvents will extract non-polar molecules. The purpose of a general screen for bioactivity is to extract as many potentially active constituents as possible. This is achieved by using solvents ranging from water, the most polar with a polarity index (P) of 10.2 to chloroform (relatively non-polar P=4.1) and hexane (non-polar P=0.1) including a number of intermediary solvents such as ethyl alcohols. If incomplete screening of botanical material is attempted, the solvents for phytochemical extractions should be carefully selected because different solvent types can significantly affect the potency of extracted plant compounds (Karmegam *et al.*,

1997). A converse relationship is said to exist between extracts effectiveness and solvent polarity where efficacy increases with decreasing polarity (Mulla and Su, 1999). This not consistent due to difference between the characteristics of active chemicals among plants. Berry and Rodriguez (2003) suggested the use of different solvents based on the type of molecules targeted for extraction. Petroleum ether appears to have been the solvent of choice for some time. Larvicidal activities of the plant extracts vary according to the plant species, the parts of the plant, the geographical location where the plants were grown and the application method.

## 2.3 Phytochemicals for potent larvicidal activity:

If an exceptionally low lethal concentration is detected, the extract may be fractionated in order to locate the particular chemical constituent causing the lethal effect. The purpose of fractionation is thus to produce several simple mixtures of compounds to reduce the number of compounds which may be identified in further analyses. Fractions isolated from the same extract always have different larvicidal activity because they contain different phytochemicals. For instance, Sun *et al.*, (2001) screened ethyl-acetate, n-butyl alcohol and water fractions of alcoholic extract of leaves and stems of Vanilla fragrans against *C. pipiens* larvae. Both n-butyl alcohol and ethyl-acetate fractions were active in bioassays, while the water fraction appeared to contain no substances that inhibited larval growth.

Natural products of plants origin with insecticidal properties have been tried in the recent past for control of variety of insect's pests and vectors. Essential oils of leaf and bark of *Cryptomeria japonica* demonstrated high larvicidal activity against *Ae. Aegypti* (Diptera: Culicidae) larvae (Cheng et al., 2003). Insecticidal activity of plant essential oils has been well-described (Isman, 1999). Azadiractin, the active ingredient of neem has long been recognized for its mosquito larvicidal capability. The extracts of *Murraya koenigii, Coriandrum sativam, Ferula asafezida, Trigonella foenum graceum* were found to be effective and showed encouraging results against *Ae. aegypti* (Harve and Kamath, 2004) mosquito larvae.

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and oviposition attractant and have different activities observed by many researchers (Babu and Murugan, 1998; Venketachalam and Jebasan, 2001 a, b). The larvicidal activities of essential oils of *Ocimum gratissium, Cymbopogon citrus* and *Ageratum conyzoides* were studied against *Ae. Aegpti* and achieved 100% mortality at 120, 200, 300 ppm concentrations respectively (Sosan *et al.*, 2001). Similarly, it was reported that the essential oil of *Ipomoea cairica* Linn.possesses remarkable larvicidal properties as it could produce 100% mortality in the larvae of *C.tritaeniorhynchus, Ae.aegypti, An. Stephensi* and *C.quinquefaciatus* mosquitoes at concentration ranging from 100 to 170 ppm (Thomas *et al.*,2004).

Dwiviedi and Kawasara, (2003) found acetone extract of *Latana camara* to be most effective against *C.quinquefaciatus* larvae. Latha *et al.*, (1999) reported *Piper longum* and *Zingiber wightianum* extracts of 80 mg/l causing complete mortality in *C.quinquefaciatus* and 60 mg/l for *C.sitens*. The $LC_{90}$ values of methanol and ethanol extracts of roots of *Aristolochia saccata*, leaf of *Annona squamosa* and fruits/pericarp of *Gymnopetelum cochinchinensis* against *Ae. Albopictus* and *C.quinquefaciatus* larvae ranged between 31.80 and 155 ppm (Das *et al.*, 2007).

Studies with essential oil of *Ocimum Americans* and *O.grattisium* showed $LC_{50}$ at 67 and 60 ppm respectively against *Ae.aegypti* larvae (Cavalcanti *et al.*, 2004). Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, methanol and acetone extracts of five medicinal plants *Albutilon indicum,Aegle marmelos, Euphorbia thymifolia, Jatropha gossypifolia* and *Solanum torvum* were assayed for their toxicity againat the early fourth-instar larvae of *C.quinquefaciatus* .All extracts showed moderate larvicidal effects; however, the highest larvalmortality was found in petroleum ether extract of *A. indicum* (Rahuman *et al.*,2008).

## 2.4 Effect of plant products against fourth instar larvae:

Larvicidal activity of ethyl acetate, butanol and petroleum ether extracts of five species of Euphorbiaceae plants, *Jatropha curcas, Pedilanthus tithymaloides,*

*Phyllanthus amarus, Euphorbia hirta* and *E.tirucalli* were tested against the early fourth-instar larvae of *Ae,aegypti* and *C.quinquefaciatus*. The LC50 value of petroleum ether extracts of *J.curcas, P. tithymaloides, P.amarus, E.hrita* and *E.tirucalli* were 8.79, 55.26, 90.92, 272.36 and 4.25 ppm respectively against *C.quinquefaciatus* (Rahuman *et al.*, 2007).

A system for biocontrol of malaria and filarial mosquito vectors has been developed using herbal extracts of three *Spilanthes* species, *S.acmella* L.var *oleraceae* Clarke, *S.calva L.* and *S.paniculata* Wall ex DC. Cent percent mortalities was achieved against the late third/early fourth instar larvae of *An. Stephensi* Liston, *A. culicifacies* species and *C.quinquefaciatus* .Say using crude hexane extract obtained from flower heads of *Splianthes* spp. Of the three plant species, *S.acemella* extract proved to be the most effective in inducing complete lethality at minimum doses, the respective $LC_{50}$ and $LC_{90}$ values being 4.57 and 7.83( *A.stephesi)*, 0.87 and 1.92(A. culicifacies) and 3.11 and 8.89 ppm (*C.quinquefaciatus)*. This was followed by *S.calva* and *S.paniculata* extracts respectively (Pandey *et al.*, 2007).

The early fourth instar larvae of *C.quinquefaciatus*, reared in the laboratory were used for larvicidal assay with leaf extracts of *Vitex negundo, V.peduncularis* and *V.altissima*. The methanol extracts of four species possessed varying levels of larvicidal nature. The highest larvicidal activity was found with the extract of *V.trifolia* ($LC_{50}$ = 41.41 ppm) followed by *V.penducularis* and *V.altissima* ($LC_{50}$ = 128.04ppm) and *V.negundo*( $LC_{50}$ =212.57ppm) (Kannathasan *et al.*,2007).

## 2.5 Larvicidal property tested from various fractions of plant extracts extracted using solvents of various polarity index:

The larvicidal activities of various fractions of the hexane extract of the seeds of *Sterculia guttata* against larvae of *Ae.aegypti* and *C.quinquefaciatus* were determined. Bis(2-ethylhexyl) benzene-1,2-dicarboxylate was identified as one of the active principles, displaying chronic toxicity against both types of larvae, with LC50 values of 79 and 64 ppm, respectively (Katade *et al.*,2006a).

Studies on the stem of *Garcinia mangostana* have led to the isolation of one new xanthone mangosharin (2,6-dihydroxy -8- methoxy-5-(-3-methylbut-2-enyl)-xanthone) and six other prenylated xanthones, alpha-mangostin beta-mangostin, garcinone D, 1,6-dihydroxy-3,7-dimethoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2H,6H-pyrano-[3,2-b]-xanthene-6-one All the crude extracts when screened for their larvicidal activities indicated very good larvicidal activity against *Ae.*aegypti(Ee *et al.*,2007).

One hundred and ninety hexane and ethanol extracts from 27 plant species from the Cerrado biome of Brazil were tested for larvicidal activity against third-stage *Ae.*aegypti larvae at 500µg/ml.Fourteen extracts from 7 species showed activity(>65%mortality) against the larvae. Of these *Dugeutia furfuracea, Piptocarpha rotundifolia, Casearia sylvestris* var.*lingua, Serjania lethalis* and *Xtlopia aromatica* were active at 56.6, 162.31, 232.4, 285.76 and 384.37 µg/ml, respectively. *Annona crassiflora* and *Cybistax antisyphilitica* showed activity at 23.06 and 27.61 µg/ml( Rodrigues *et al.*,2006).

The essential oil extract from the forest red gum, *Eucalyptus tereticornis* Sm. (Myrtaceae) was tested against mature and immature mosquito vector *An.stephensi* Liston (Diptera) under laboratory condition. The extract showed the strong larvicidal, pupicidal and adulticidal activity. The leaf oil extracts showed high bioactivity at high doses. Results obtained from the laboratory experiment showed that the leaf extracts suppressed the pupal and adult activity of *An.stephensi* at higher doses. In general, first and second instar larvae were more susceptible to all treatments. Clear dose response relationships were established with the highest dose of 160ppm plant extract evoking almost 100% mortality (Nathan, 2007).

The larvicidal activity of ethanol, hexaneand chloroform soxhlet extracts obtained from *S.guttata* seeds were investigated against the fourth-instar larvae of Dengue fever vector *Ae.*aegypti and the filarial vector *C.quinquefaciatus*. All extracts

including fractions of ethanol extract exhibited 100% larval kill within 24 hr exposure period at 500 ppm concentration ( Katade *et al.*, 2006b).

The larvicidal activity of roots of *Hibiscus abelmoschus* was evaluated against the larvae of mosquitoes in the genera *Anopheles* and *Culex*. Mean median lethal concentration values of the aqueous extract from the roots of *H. abelmoschus* against the larvae of *An.culicifacies, An.stephensi* and *C.quinquefaciatus* were 52.3, 52.6, 43.8 ppm respectively (Due *et al.*, 2006).

Ethanolic and acetone extracts of *Nerium indicum* and *Thuja orientelis* have been studied against third instar larvae of *An.stephensi* and *C.quinquefaciatus*. Ethanolic extract of *N.indicum* was found more effective than its acetone extract against anopheline larvae with $LC_{50}$ values of 185.99 and 148.05 ppm for former and 229.28 and 149.43 ppm for the later after 24 and 48 hrs of exposure. The acetone extract with $LC_{50}$ values of 209.00 and 155.97 ppm was more effective in case of culicine larvae than its ethanolic extract with $LC_{50}$ 494.07 and 194.49 ppm after 24 and 48 hrs of treatments. Ethanolic extract of *T. orientelis* was more effective against both larval species with $LC_{50}$ values of 13.10 and 9.02 ppm after 24 and 48 hrs for anopheline and 22.74 and 16.72 ppm against culicine larvae. The acetone extract showed $LC_{50}$ values of 200.87 and 127.53 ppm against anapheline and 69.03 and 51.14 ppm against *culicine* larvae (Sharma *et al.*, 2005).

Mosquito larvicidal activity of crude carbon-tetra-chloride, methanol and petroleum ether extracts of *Solanum xanthocarpum* fruits was examined against *An.stephensi* and *C.quinquefaciatus*. Among the extracts tested carbon-tetra-chloride extract was the most effective with $LC_{50}$ values of 5.11ppm after 24 hrs and 1.27ppm after 48hrs of treatment against *An.stephens*. In the case of *C.quinquefaciatus* the petroleum ether extract was observed as most toxic with $LC_{50}$ values of 62.62 ppm after 24hrs and 59.45ppm after 48hrs of exposure period respectively (Mohan *et al.*, 2005).

The effects of the neem (*Azadirachta indica* A.Juss)limonoids azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin on *An.stephensi* Liston (Diptera:Culicidae) were investigated. In exploring advantages of pure neem limonoids, the larvicidal, pupicidal, adulticidal and antiovipositional activity of neem limonoids were studied. Azadirachtin, salannin, deacetylgedunin showed high bioactivity at all doses,while the rest of the neem limonoids were less active and were only biologically active at high doses.azadirachtin was the most potent in all experiments and produced almost 100%larval mortality at 1ppm concentration. In general, first to third larval instars were more susceptible to the neem limonoids (Nathan *et al.*, 2005).

Larvicidal activity of *Tagetes patula* essential oil was tested against the fourth instar larvae of *An.stephensi* and *C.quinquefaciatus*. Five different concentrations of essential oil were studied and the results were compared with that of the synthetic insecticide, malathion . *Ae.aegypti* ($LC_{50}$ 13.57ppm) was most susceptible followed by *An.stephensi* ($LC_{50}$ 12.08ppm) and *C.quinquefaciatus* ($LC_{50}$ 22.33ppm) (Dharmagadda *et al.*, 2005).

Oil-resin fractions from *Copilfera reticulate* Ducke (Leguminosae-Caesalpinoideae) were evaluated for larvicidal activity on the third larval instars of *Aedes aegypti* ,in searching foralternative control methods for this mosquito. The most active fractions were $CRM_{1-4}$ (sesquiterpenes) and $CRM_{5-7}$ (labdane diterpenes), which showed LC50 values of 0.2 and 0.8 ppm, respectively (Silva *et al.*, 2007).

Mosquito larvicidal activities of methanolic extracts from different plant parts of red heartwood-type *Cryptomeria japonica* against the fourth instar larvae of *Ae.aegypti* and *Ae.albopictus* were examined. Results of mosquito larvicidal tests demonstrated that the n-hexane fraction of *C.japonica* sapwood methanolic extract had an excellent inhibitory effect against the larvae of *Ae.aegypti* and *Ae.albopictus* and its $LC_{50}$ values were 2.4 and 3.3 μg/ml,respectively, in 24hr(Cheng *et al.*, 2007).

Butterfly pea commonly known as Shankupushpam,is widely used in traditional Indian systems of medicine as a brain tonic and is believed to promote memory and intelligence. A lectin was isolated from the seeds of *Clitoria ternatea* which agglutinated trypsin treated human B erythrocytes (Naeem *et al.*, 2007).

The ethanol extract of the root of *C.ternatea* was evaluated for different neuropharmacological actions in rats and mice. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioral pattern by the head dip and Y-maze test, and reduction in the muscle relaxant activity by the rotarod, 30° inclined screen and fraction tests. Preliminary tests indicate that the ethanol extract of *C.teratea* at doses of 100 and 150 mg/kg showed significant neuropharmacological activity (Boominathan *et al.*, 2003).

The methanol extract of *C.ternatea* L. root blue flowered variety (Family:Fabaceae), was evaluvated for its anti-pyretic potential on normal body temperature and yeast-induced pyrexia in albino rats. Yeast suspension (10ml/kg body wt.) increased rectal temperature after 19 hrs of subcutaneous injection. The extract, at doses of 200,300 and 400 mg/kg body wt., p.o., produced significant reduction in normal body temperature and yeast-provoked elevated temperature in dose-dependent manner. The effect extended up to 5hrs after the drug administration. The anti-pyretic effect of the extract was comparable to that of paracetamol (150mg/kg body wt., p.o.), a standard anti-pyretic agent (Parimaladevi *et al.*, 2004).

The hexane, methanol and water extracts of leaves, stems and roots of white flowered variety of *C.ternatea* (Fabaceae) used by Indian traditional healers for treating ulcer, eye infections, bronchitis, tuberculosis and/or inflammation were screened for invitro antibacterial activities, using disc-diffusion assay, against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pnemoniae*. Minimal inhibitory concentration values were determined with microdilution assay. The methanolic extracts showed the highest activity and no activity was recorded with the water extract.

The hexane and methanolic extracts of roots showed the highest and significant antibacterial activity against both Gram-positive and Gram-negative bacteria. No antibacterial activity was recorded with the stem extracts ( Malabadi *et al.*,2005).

## 2.6 Nematicidal activity of aqueous extracts:

The nematicidal activity of aqueous extracts of leaves and roots of C.ternatea, *Datura stramonium* and *Rauwolfia serpentina*[*Rauvolfia serpentina*] at 6 concentrations (S,S/2,S/4,S/10,S/20 and S/100) was investigated against the second stage juveniles of *M.incognita* by in vitro method. A significant increase in percentage mortality of the larvae was observed with all the leaf extracts compared to the root extracts. All the leaf significantly showed higher nematicidal activity than the root extracts. The leaf extract of *D. stramonium* was the most effective followed by *R. serpentine* and *C.ternatea* (Saxena and Sharma 2005). The aqueous and ethanolic extracts of *C.ternatea* were studied for the antihelminthic property against the soil nematode (Dorylaimus species) (Das *et al.*, 2006).

Plants defend themselves from pathogens and insect pests by a variety of ways, including the production of proteins with antimicrobial and/or insecticidal properties. A highly small protein from seeds of *C.ternatea* was isolated. This protein, designated 'finotin', has broad and potent antifungal, antibacterial and insecticidal properties,raising the possibility that finotin may contribute to the high level of disease and insect resistance observed in *C.teratea* in the field. The effect of direct applications of crude protein preparations on diseases of tomato, beans and the tropical forage Brachiaria under field and greenhouse conditions was studied. The protein showed potent in vitro growth inhibitory effect on the bean angular leaf spot pathogen *Phaeolsariopsis griseola,* the rhizoctonia foliar blight disease pathogen *Rhizoctonia solani* on species of Brachiaria and various important plant pathogenic fungi (Kelemu *et al.*, 2005). The protein 'finotin' , has broad and potent inhibitory effect on the growth of various important fungal pathogens of plants, namely *Fusarium solani, Colletotrichum indemuthianum, Lasiodiplodia thiobromae,*

*Pyricularia gisea,Bipolaris oryzae* and *Colletotrichum gloeosporioides.* It also inhibits the common bean bacterial blight pathogen *Xanthomonas axonopodis* pv. *Phaseoli.* Moreover, finotin has powerful inhibitory properties against the bean bruchids *Zabrotes subfaciatus* and *Acanthoscelides obtectus* (Kelemu *et al.*. 2004).

The literature survey shows that *the Ervatamia divericata and Glycyrrhiza glabra* extracts are extensively studied for its medicinal properties but it has not been studied for its effect of the mosquito larvae. This prompted as to investigate the effects of the extracts of *Ervatamia divericata and Glycyrrhiza glabra against* the larvae of the mosquito vector *C.quinquefaciatus* (filarial vector).

# OBJECTIVES

# CHAPTER3

## 3. OBJECTIVES

**Phase 1**:

Screening of plant extracts and essential oils for larvicidal activity against the fourth instar larvae of the mosquito vector *C.quinquefaciatus* (filarial vector).

**Phase 2:**

Identification of phytochemicals present in the plant extracts that have potent larvicidal activity

**Phase 3:**

To determine the repellent potential of the plant extracts on adult *C.quinquefaciatus* by smoke toxicity test.

# MATERIALS AND METHODS

# CHAPTER 4

# 4.MATERIALS AND METHODS

## 4.1Chemicals required:

### 4.1.1 Extraction:

- Acetone $CH_3COCH_3$ purchased from FISCHER chemicals.

### 4.1.2 Phytochemical Screening:

- Marqui's reagent
- Dragendorff's reagent
- Benedict's reagent
- Neutral 5% ferric chloride solution $FeCl_3$
- Liquid Ammonia $NH_4$
- 0.1% Ferric chloride $FeCl_3$
- 1% aqueous Hydrochloric acid HCl
- Chloroform $CCl_4$
- Conc.Sulphuric acid $H_2SO_4$

### 4.1.3 Antibacterial activity:

- Nutrient agar (NA) purchased from HIMEDIA
- Nutrient broth (NB) purchased from HIMEDIA
- Bacterial cultures, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris and Staphylococcus aureus* obtained from Dept. of Biotechnology, Kumaraguru College of Technology, Coimbatore and maintained in Nutrient Broth (NB) at $37^0$C.

### 4.1.4 Test Coils:

- Coconut shell powder(200 Mesh size)
- 50 % Starch solution
- 10% Gaur Gum solution

## 4.2 Equipments:

### 4.2.1 Essential oil extraction:

- Steam Distillation Apparatus(Fig 2.1)

### 4.2.2 Solvent extraction:

- Soxhlet extraction unit(Fig 2.2)

### 4.2.3 Mosquite rearing:

- Peet Gardy chambers(Fig 2.3)

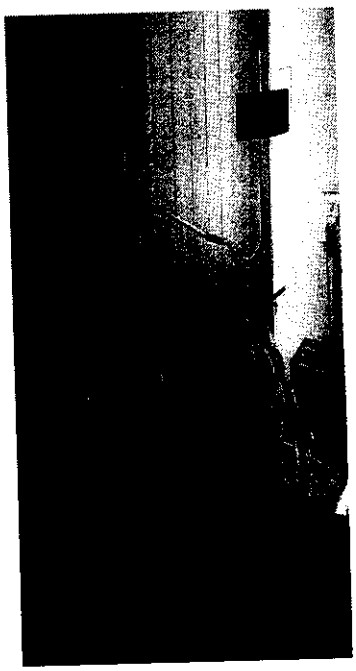


**Fig 2.1 Steam Distillation Apparatus**

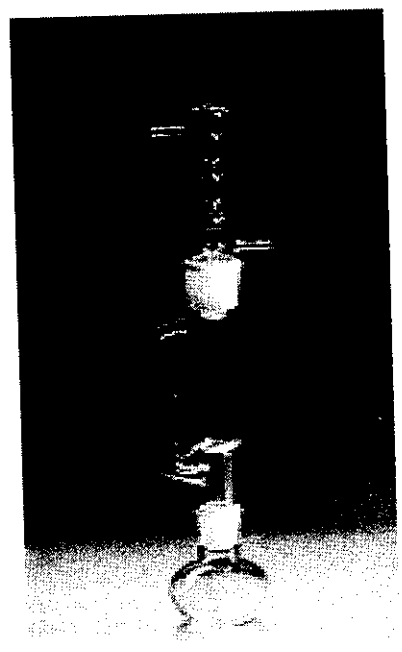


**Fig 2.2 Soxhlet extraction unit**

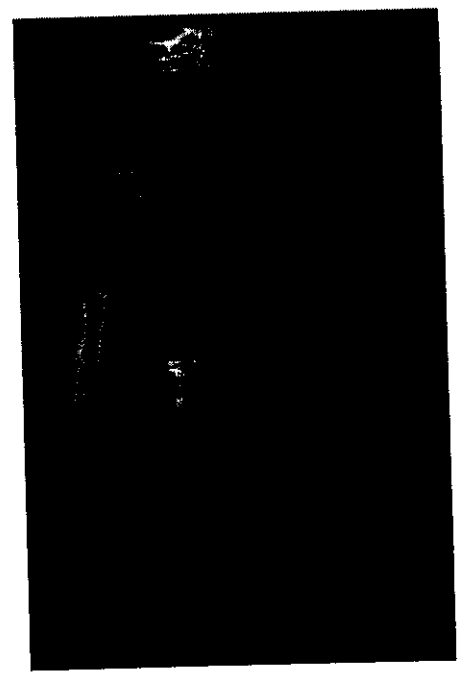


**Fig 2.3 Peet Gardy chambers**

## 4.3 Collection of Plant materials:

Plant materials were collected from the medicinal plant garden in the KCT campus of Azadirachta indica ,*Glycyrhira glabra* ,*Ervatamia divaricata, Pongamia pinnata, Magnifera indica, Curcuma longa and Psidium guajava* were collected from the Medicinal plant garden, KCT while the leaves of *Jatropa curcas* were obtained from Biofarm, Pollachi.

## 4.4 Solvent extraction of plant materials using Soxhlet extraction unit:

The leaves were washed with water and shade dried at room temperature ($26^0C \pm 2^0C$) for 3 days. The dried plant materials were powdered in a laboratory blender and 300 g of the sample was filled in the thimble .The solvent was taken in a round bottomed flask and heated over an electrical mantle. The extraction was done successively with 700 ml of acetone for 8hrs at a temperature of $30^0C$in a Soxhlet apparatus (Vogal 1978). The plant materials were evaporated to dryness to yield powdered samples. All extracts were weighed and stored at $4^0C$ until after use. The volume of solvent added and the yield obtained are tabulated in Table 5.1.

## 4.5 Extraction of essential oils using Steam Distillation:

A closed system of producing steam was also evolved which helped in the collection of oil without losing it into condensed water. The plant materials crushed with distilled water in a mortar and pestle was taken in a 5 $dm^3$ round bottom flask which was to be heated in a mantle. The flask was filled to about half of its capacity with the plants material and then enough water was added into it to completely immerse the material. The flask was heated and steam was produced in the flask, released oil which was carried away by the steam rising out of the flask. The steam carrying oil was led to the condenser on the top and condensed liquid was collected in another flask. The essential oil was extracted by the steam distillation apparatus for 6 hrs .The oil floating on the top of water layer was separated from water by a separatory funnel. The Oils were concentrated by

heating for 20 min over a hot plate. The oils were refrigerated at $4^0$C and used as a test sample. The yield of the essential oil was tabulated (Table 5.2)

## 4.6 Preparation of Test Solution:

### 4.6.1 Solvent extracts:

Two grams of the plant residues were dissolved in 100mL of acetone(Stock solution) considered as 2% stock solution. This stock solution was further diluted in acetone and the following concentrations were prepared(0.2%, 0.3%, 0.4%, 0.5%, 0.6 %).

## 4.7 Rearing of mosquitoes:

A cyclic mosquito colony involves maintenance of four stages:

- Egg
- Larva(4-instars)
- Pupa
- Adult

### 4.7.1. Egg:

The collected eggs were immersed in water to enable hatching. The eggs of the *Culex quinquefaciatus* are in the form of rafts. Each egg raft consists of 150-200 eggs. The time required for egg hatching was 20-24hrs.

### 4.7.2. Larval stage:

- Initially liquid feed containing dog biscuit and yeast ground in the ratio of 60:40 was given to the newly emerged larvae. The usage of yeast is for its fermentation odour.
- Brisk stirring was followed after an interval of 15 min to prevent scum formation. Scum formation may result in oxygen blockade followed by larval mortality.
- Change of tray water was done for every three days.
- Each instar of larvae took around 24-36 hrs.

### 4.7.3. Pupal stage:

- The pupal stage is the non-feeding stage.
- Separation of colonies is made easier using Ice-cold water.
- The mixed stages of larvae and pupa are suspended in ice-cold water which allows separation wherein the pupa floats on the water surface and larvae settles down inactively due to the temperature.
- The entire process was be carried out in a fast pace in about 30-60 secs to prevent mortality.
- The pupal stage was left in the bowl containing water and was kept in cage for emergence.

### 4.7.4. Adult stage:

Feeding was done after three days from the onset of emergence of adults. This starvation facilitated improved fertility of eggs In the laboratory rearing of mosquitoes, artificial membrane feeding method was practiced.

These included materials like,

- Para film as membrane.
- Uninfected blood treated with an anti-coagulant.

EDTA (30mg/100ml of blood sample) was used as the anti-coagulant.

1. Here, Para film fakes the skin membrane
2. The blood was maintained at a temperature of 37°C which mimicked the human body temperature.
3. Stirring of the blood sample was mandatory to obtain uniform conditions.Blood feed was given once in a day. During the rest of the days water pad and soaked raisins were kept in the cage. This intended to feed the male mosquitoes. Water pad and soaked raisins were changed daily. During blood meal the soaked raisins and the water pad were removed from the cage.

## 4.8. Ovi –trap:

- In the laboratory, ovi-traps were kept after two days from the onset of feeding..
- The ovi-trap for the *Culex* consists of only a bowl containing previously reared water to play its habitual environment.
- The ovi-trap is collected after an interval of two days

## 4.9. Collection of eggs:

Long-term preservation of eggs is feasible only for *Ae.aegypti*. The blotting paper is dried in the same bowl for 24hrs. This is necessary for the complete development of the embryo within the eggs. Properly preserved eggs can be maintained for up to 3 yrs. The eggs of Culex and *Anopheles* cannot be preserved. A total of three ovi-traps were kept during its life span in laboratory environments.

## 4.10. Larvicidal activity:

### 4.10.1. Solvent extracts:

The assay was carried out by maintaining a series of such concentrations by dissolving the plant extracts in 40mL distilled water .After about 15 min , 20 early fourth instar larvae of ***Culex quinquefaciatus*** taken on a strainer with fine mesh were transferred to the test medium by tapping. Five replicates were run for each concentration with a final total of 100 larvae tested for each concentration. Simultaneously control tests at the rate of 20 larvae was carried out with the required quantity of acetone. Food containing dog biscuit and yeast ground in the ratio of 60:40 was sprinkled in each treatment.

After the exposure of larvae to corresponding concentrations, their mortality and partial mortality were registered and accordingly the average values were ascertained from the replicates of each concentration.

#### 4.10.1.1. Combinatorial larvicidal activity:

Similar assays were carried out for combinations of *Azadirachta indica* and *Curcuma longa, Azadirachta indica* and *Ervatamia divaricata*(leaf), *Curcuma longa* and

*Ervatamia divaricata* (leaf).Mortality and partial mortality were registered and accordingly the average values were ascertained from the replicates of each concentration.

### 4.10.2. Essential oils:

The concentrated essential oils were dissolved in water to make different concentrations ranging from (1.0%, 2.0%,3.0%, 4.0% & 5.0%). The oil-water solution was stirred for 30 sec with a glass rod. After about 15 min, 20 early fourth instar larvae of ***Culex quinquefaciatus*** taken on a strainer with fine mesh were transferred to the test medium by tapping. Five replicates were run for each concentration with a final total of 100 larvae tested for each concentration. Simultaneously control tests at the rate of 20 larvae were carried out with the required quantity of distilled water. Food containing dog biscuit and yeast ground in the ratio of 60:40 was sprinkled in each treatment.

After the exposure of larvae to corresponding concentrations, their mortality and partial mortality were registered and accordingly the average values were ascertained from the replicates of each concentration.

## 4.11. Antibacterial activity of the plant extracts obtained by Soxhlet extraction:

### 4.11.1 Microbial strains:

Four different strains used for testing the antibacterial activity included *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris and Staphylococcus aureus*. The bacterial strains were cultured on nutrient agar slants. The cultures were maintained by sub culturing periodically and preserved at 4°C prior to use.

### 4.11.2 Screening for antibacterial activity:

### 4.11.2.1 Agar well diffusion method:

The antibacterial activity was tested by agar well diffusion method (Mukherjee et al., 1995).Concentration of 5% of the plant extracts were prepared in acetone The test

organisms were seeded into respective medium by gently mixing 0.1 ml of the 24 h fresh cultures with 35 ml sterile melted agar in sterile Petri-plates. Four 7mm diameter wells were made using sterile borer. The wells were filled with 0.1ml of the sample extract. The antibacterial assay plates were incubated at 37°C for 24 h. The diameter of the zones of inhibition around each of the well was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was recorded.(Table )

### 4.11.2.2 Lawn culture Technique:

The antibacterial activity of the extracts was evaluated by transferring essential oil aseptically to sterile nutrient agar (NA) media at $40^0$C,to obtain concentrations of 1600 ppm and 2400 ppm. Bacteria from NB was swabbed on the surface of plates containing the plant extracts and incubated at $30\pm2^0$C for 1 d. Three replicates of each test was carried out and control tests was run simultaneously without using the plant extracts. The bacterial growth was documented (Table).

## 4.12. Phytochemical screening of the extracts:

Chemical tests were carried out using the extracts from plants, using standard procedures to identify the constituents as described by Harborne(1973).

### 4.12.1. Test for Alkaloids:

### Marqui's test:

About 1ml of leaf extract, 1ml of Marqui's reagent (3ml of concentrated Sulphuric acid + 2 drops of 40% formaldehyde) was added and mixed. A dark orange or purple coloration indicated the presence of alkaloids.

### Dragendorff's test:

About 1ml of leaf extract, 1ml of Dragendorff's reagent was added and mixed. A dark orange or orange red precipitate indicated the presence of alkaloids.

### 4.12.2. Test for Carbohydrates:

**Benedict's test:** To 0.5ml of the filtrate, 0.5ml of the Benedict's reagent was added. The mixture was heated on boiling water bath for 2min. A characteristic red colored precipitate indicates the presence of sugar.

### 4.12.3 .Test for Saponins: (Kokate, 1999)

The extract was diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated cylinder for 15min. 2cm layer of foam indicates the presence of saponins.

### 4.12.4. Test for Phenolic compounds:

**Ferric chloride test**: The extract was diluted to 5ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds

### 4.12.5. Test for Flavonoids:

**Ammonia test:** A small piece of filter paper is dipped to about 1ml of the extract and was exposed to ammonia vapor. Formation of yellow spot on filter paper indicates the presence of flavonoids.

### 4.12.6. Test for Terpenoids:

**Salkowski test:** 5ml of the extract was mixed with the 2ml of chloroform and concentrated sulphuric acid to form a layer. a reddish brown coloration of the interface showed the presence of terpenoids.

### 4.12.7. Test for tannins:

About 0.5 mg of dried powdered samples was boiled in 20ml of water in test tubes then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black coloration.

### 4.12.8. Test for phlobatannins:

Formation of red precipitate when aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid indicates the presence of phlobatannins.The observed results were documented (Table).

## 4.13. Preparation of test coils:

Mosquito coils were prepared following the method of Saini *et al.* (1986), with minor modifications, using 5% of the plant sample containing active ingredient, 10 g sawdust (as binding material) and 10 g coconut shell charcoal powder (as burning material). All three materials were thoroughly mixed with 50% Starch solution and 10 % Gaur gum solution to form a semi-solid paste. Mosquito coils (0.6 cm thickness) were prepared from the semi-solid paste and then dried in the shade (Fig). The control coils were similarly prepared, but without the plant ingredients.

The experiments were conducted in a glass chamber called the Peet Gardy chamber measuring 140 cm x120 cm x 160 cm. A door measuring 60 cm x 30 cm was situated at the front of the chamber. One hundred 3 or 4-day-old blood-starved adult female mosquitoes were released into the chamber and were provided with liquid feed containing dog biscuit and yeast ground in the ratio of 60:40. A negative control group was tested against coils lacking the active ingredient of plant powder, and a positive control group was tested with the experimental group to compare the effectiveness of the plant coils. Mosquitoes were exposed to the vapor of burning coils for 1 h.
The protection provided by the smoke from the plant samples against biting *Culex quinquefaciatus* was calculated. (Table 5.5.1).

## 4.14 Statistical analysis:

To evaluate the 50% lethal concentration of different plants volatile oils against *Aedes aegypti* larvae, standard WHO larval susceptibility test procedure was followed (WHO 1981), and from the results of mortality data, 50% toxicity was assessed by the application of Finney probit statistical method (Finney ,1971).

**Figure 4.14.2: Output generated by the SPSS (16.0) for Probit Analysis indicates Cell counts residues,Confidence limits and Chi-square tests.**

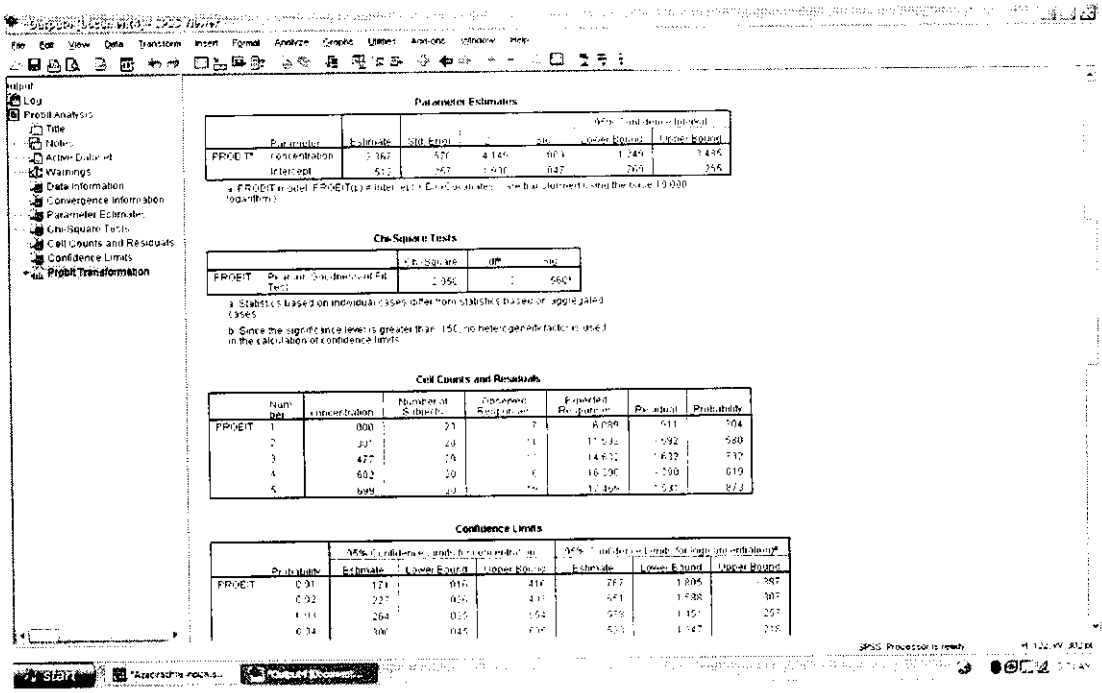
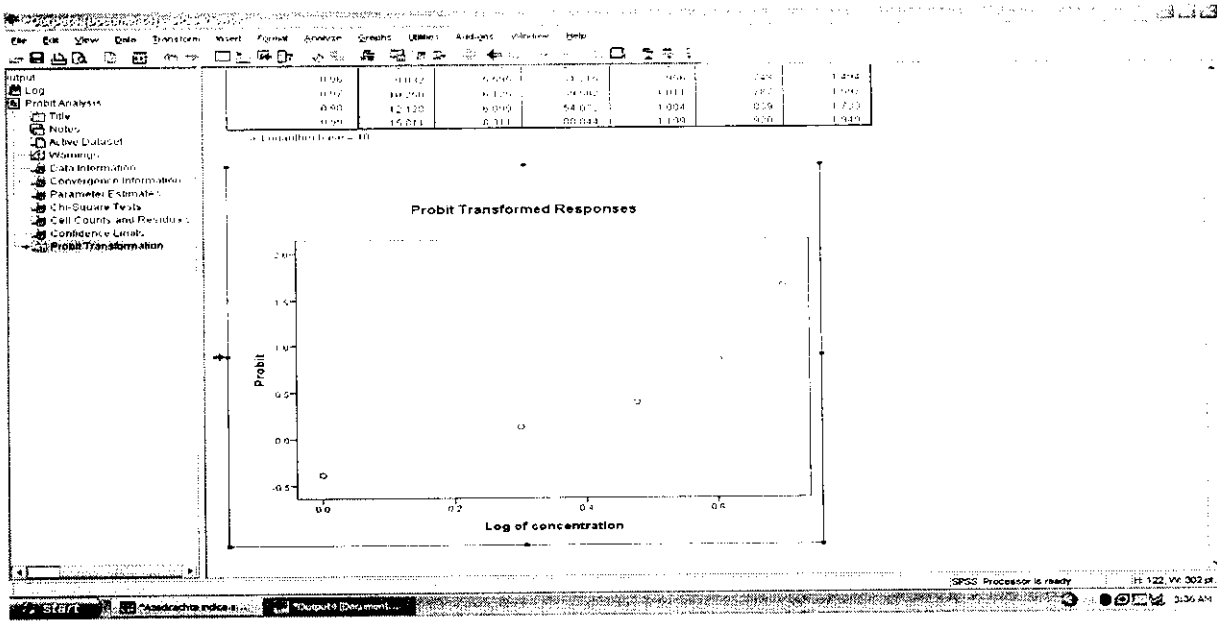



**Figure 4.14.3: Output generated by the SPSS (16.0) for Probit Analysis indicates Probits transformation for $LC_{50}$.**

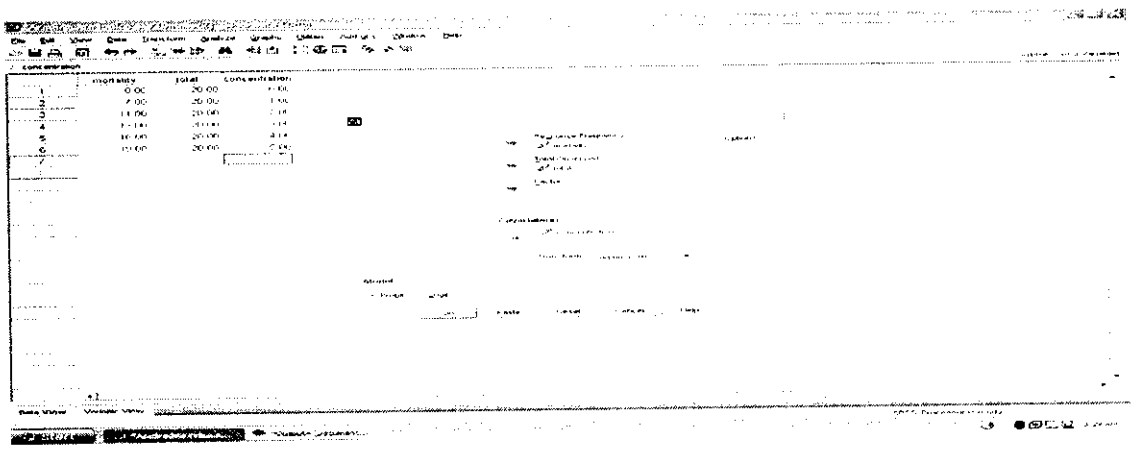


Calculation of Regression: The effect of different plants volatile oils on the mortality of fourth instar *Aedes aegypti* larvae after 24hrs. exposure, were corrected for natural response by the following of Abbott's formulae (Abbott.,1925)

**Abbott's % mortality = ((% Proportion of test mortality - % proportion of control mortality)/ (100 - % proportion of control mortality)) X 100**

$LC_{50}$ confidence intervals were analyzed by means of computerized Probit analysis (SPSS 16.0), yielding a level of effectiveness at 50 and 90% mortality, and 95% confidence interval were used to measure differences between test samples. Based on the log concentration and the mortality percentage values, values of regression equation were obtained. The homogeneity of population could also be tested by Chi-square test (X2). By using median, lethal concentration ($LC_{50}$) values of the different plant volatile oils after 24 hrs. Exposure against fourth instar larvae of *C.quinquefaciatus* and their fiducial limits (95% upper fiducial limit and lower fiducial limit) could be calculated.

**Figure 4.14.1: Mortality data fed into SPSS (16.0) for Probit Analysis**



# RESULTS AND DISCUSSION

# CHAPTER5

## 5. RESULTS AND DISCUSSIONS:

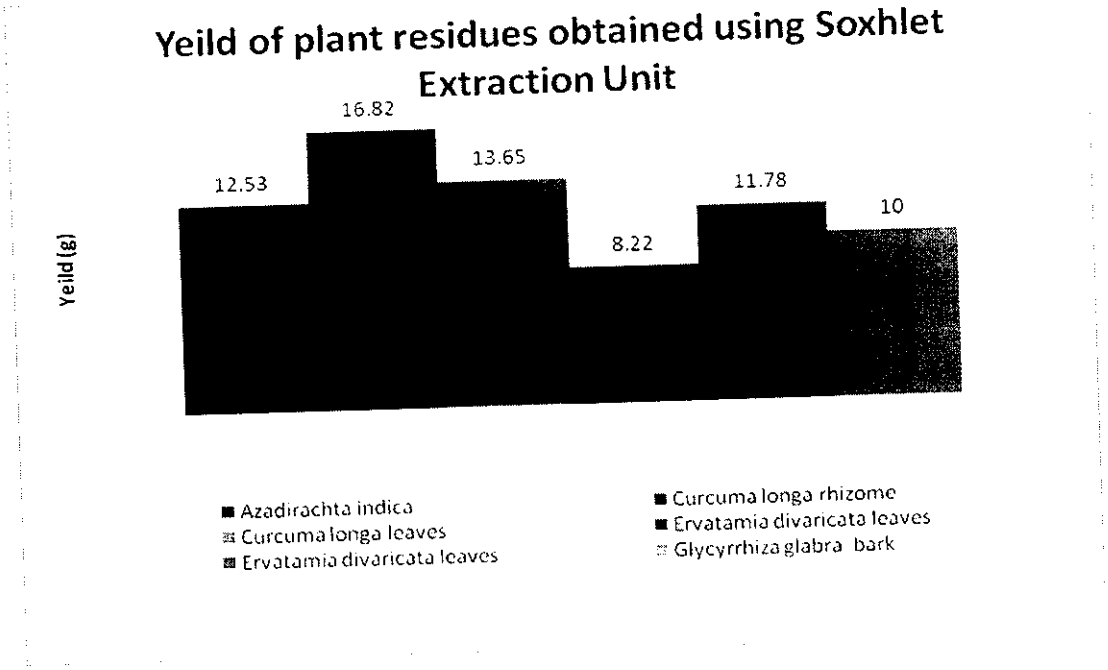
### 5.1 Solvent extraction of plant materials using Soxhlet extraction unit:

The extraction of plant materials by Soxhlet extraction showed a higher yield from the rhizome of *Curcuma longa* compared to the other four plant materials.

**Table 5.1: Volume of Solvent used and the yield of the plant residues obtained**

| S.No | Name of the sample | Part of the plant | Sample weighed(gms) | Volume of solvent(ml) | Yield (gms) |
|---|---|---|---|---|---|
| 1. | *Azadirachta indica* | Leaves | 200 | 400+150+150 | 12.53 |
| 2. | *Curcuma longa* | Rhizome | 200 | 400+150+150 | 16.82 |
| 3. | *Curcuma longa* | Leaves | 200 | 400+150+150 | 13.65 |
| 4. | *Ervatamia divaricata* | Leaves | 200 | 400+150+150 | 08.22 |
| 5. | *Ervatamia divaricata* | Flowers | 200 | 400+150+150 | 11.78 |
| 6. | *Glycyrrhiza glabra* | Bark | 200 | 400+150+150 | 10.00 |

**Figure 5.1: Yield of various plant residues extracted using Soxhlet Extraction Unit**

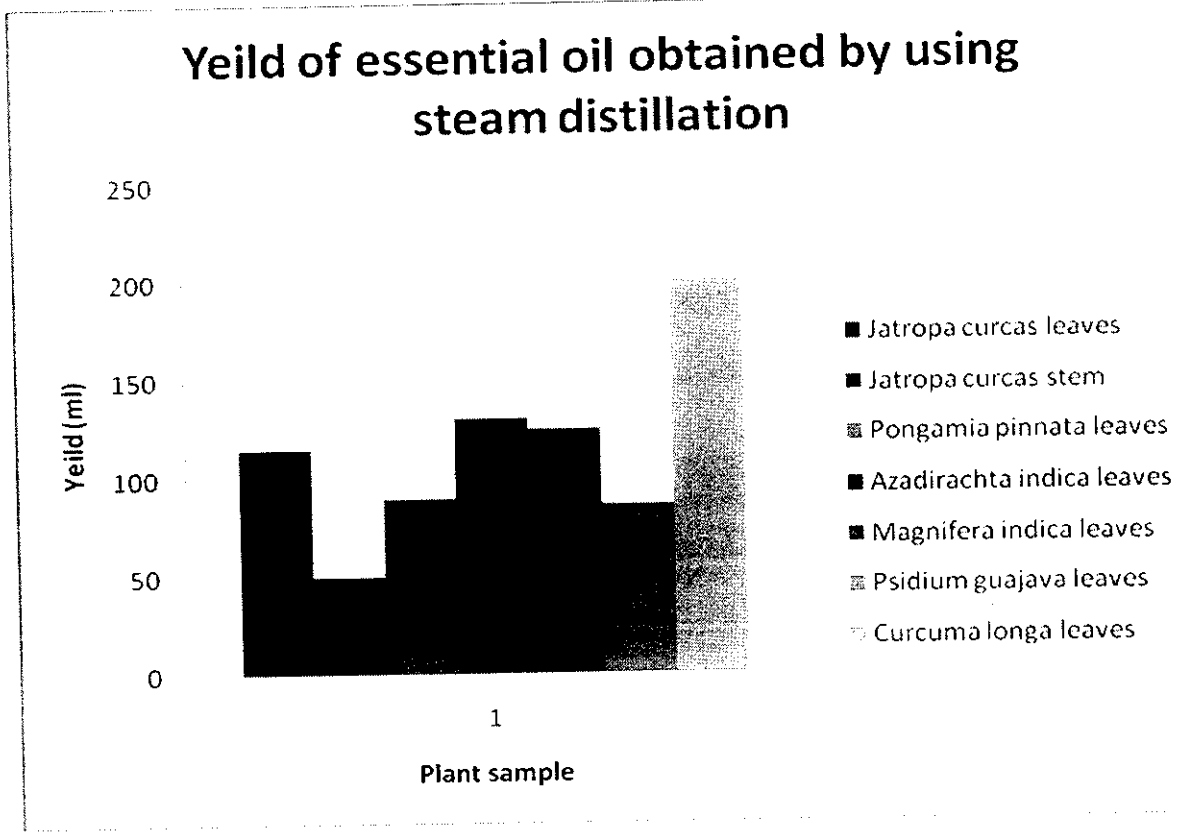



## 5.2 Extraction of Essential oils by Steam distillation:

**Table 5.2: Yield of Essential oils from various plant materials extracted using Steam Distillation**

| S.No | Name of the sample | Part of the plant | Sample weighed(gms) | Volume of water added (lit) | Yield (ml) |
|---|---|---|---|---|---|
| 1. | *Jatropa curcas* | Leaves | 300 | 1.5 | 115 |
| 2. | *Jatropa curcas* | Stem | 300 | 1.5 | 50 |
| 3. | *Pongamia* pinnata | Leaves | 300 | 1.5 | 90 |
| 4. | *Azadirachta indica* | Leaves | 300 | 1.5 | 130 |
| 5. | *Magnifera indica* | Leaves | 300 | 1.5 | 125 |
| 6. | *Psidium guajava* | Leaves | 300 | 1.5 | 86 |
| 7. | *Curcuma longa* | Leaves | 300 | 1.5 | 200 |

**Figure 5.2: Yield of Essential oils from various plant materials**




## 5.2 Larvicidal activity:

### 5.2.1 Plant Extracts by Soxhlet Apparatus:

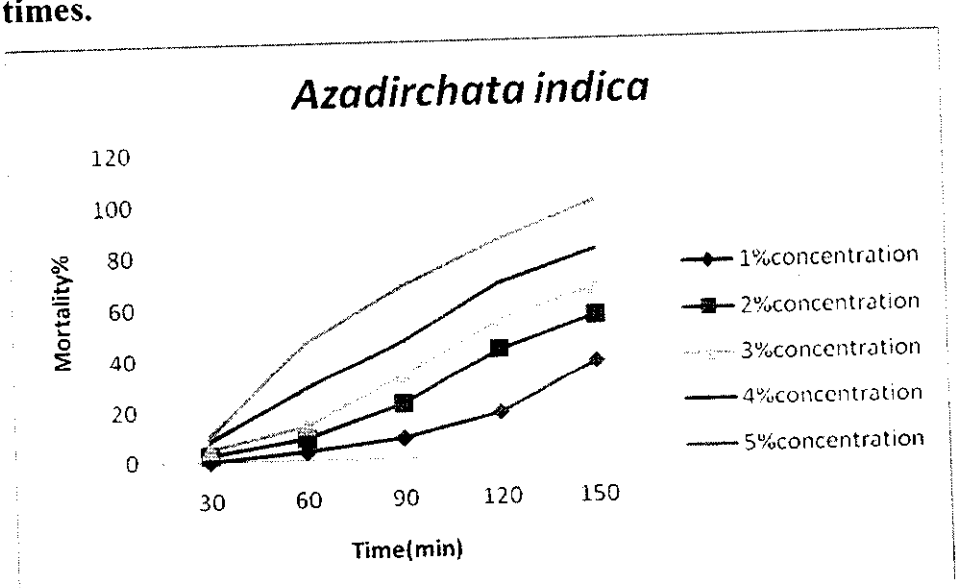
#### *5.2.1.1 Azadirachta indica*:

The larvicidal activity performed for Azadirachta indica was found to be toxic to the 4$^{th}$ Instar larvae of *C.quinquefasciatus*. The mortality rates increased with the concentration of the extract and the exposure time (Table 5.3).At higher concentrations of the extract (5.0ml/l), 100% mortality was recorded.

**Table 5.2.1.1: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of Azadirachta *indica* and various exposure times.**

| Time (min) | Mortality%(Mean ± S.D) | | | | | |
|---|---|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | | | |
| | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 0.0 |
| 30 | 0.0±0.00 | 2.4±0.0 | 4.8±1.92 | 8.0±1.87 | 10±3.08 | 0.0±0.00 |
| 60 | 3.2±0.4 | 8.6±1.05 | 13.6±4.72 | 28±8.86 | 46.2±6.94 | 0.0±0.00 |
| 90 | 8.0±1.91 | 21.6±2.78 | 32.6±7.32 | 46.2±6.94 | 67.8±6.97 | 0.0±0.00 |
| 120 | 17.4±3.28 | 42.6±4.53 | 54±9.02 | 68.2±6.13 | 85.2±3.09 | 1.0±0.00 |
| 150 | 37±4.84 | 55.4±5.98 | 66.4±5.12 | 80.6±8.48 | 99.4±0.02 | 1.0±0.00 |

**Fig5.2.1.1 Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of Azadirachta *indica* and various exposure times.**

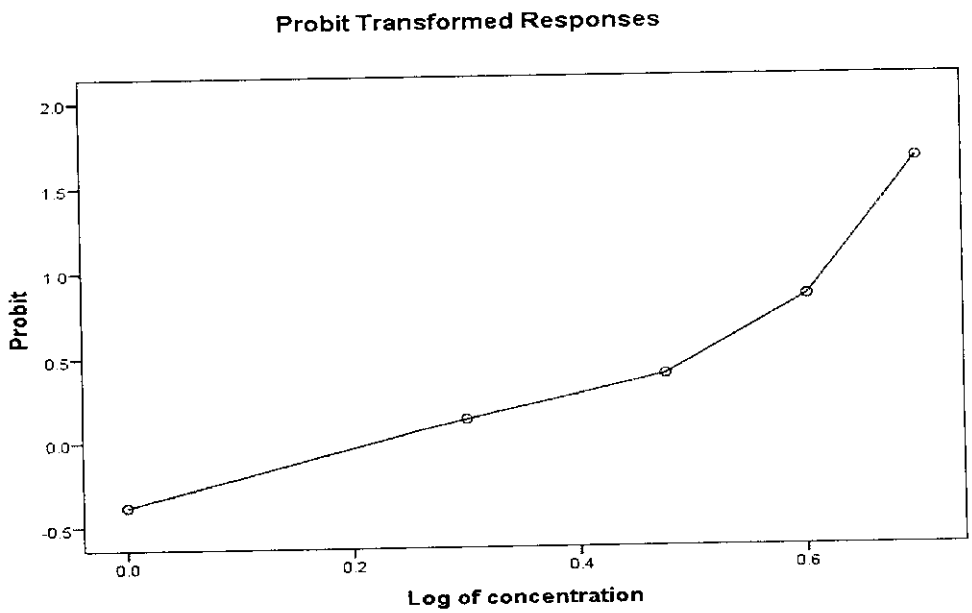


Probit Analysis using SPSS indicated highest probability mortality at higher concentrations (Table 5.2.1.2).

**Table 5.2.1.2: Cell Counts and Residuals**

| Probit Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .000 | 20 | 7 | 6.089 | .911 | .304 |
| 2 | .301 | 20 | 11 | 11.592 | -.592 | .580 |
| 3 | .477 | 20 | 13 | 14.632 | -1.632 | .732 |
| 4 | .602 | 20 | 16 | 16.390 | -.390 | .819 |
| 5 | .699 | 20 | 19 | 17.469 | 1.531 | .873 |

**Figure 5.2.1.2:The Probit transformed Graph generated through SPSSS.**




The $LC_{50}$ was found to be 1.819ml/100ml.

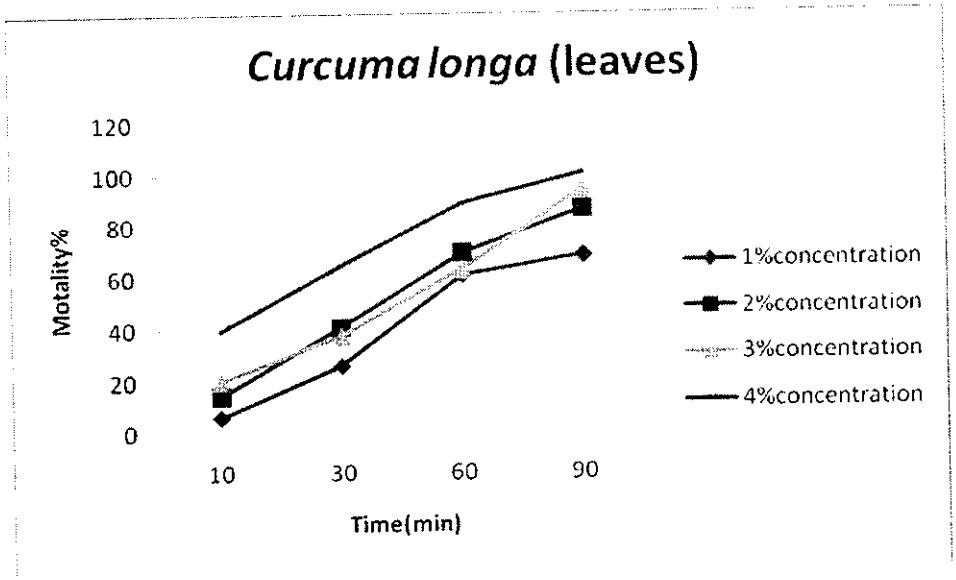
### 5.2.1.2 *Curcuma longa* (leaves):

The larvicidal activity performed for *Curcuma longa* was found to be very toxic to the 4th Instar larvae of *C.quinquefasciatus*. The mortality rates increased with the concentration of the extract and the exposure time (Table 5.5).A 100% mortality was recorded at a concentration of 4.0ml/l. The time required for complete mortality was also only 90 min.

**Table 5.2.1.3: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Curcuma longa* and various exposure times.**

| Time (min) | Mortality%(Mean+S.D) | | | | |
|---|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | | |
| | 1.0 | 2.0 | 3.0 | 4.0 | 0.0 |
| 10 | 6.4±0.10 | 14.4±1.02 | 20.2±1.16 | 39.2±1.02 | 0.0±0.00 |
| 30 | 26.0±1.15 | 40.6±3.12 | 37.2±0.02 | 64.8±0.12 | 0.0±0.00 |
| 60 | 60.8±3.38 | 69.6±0.02 | 63.2±2.00 | 88.4±0.07 | 0.0±0.00 |
| 90 | 68.4±3.54 | 86.4±1.01 | 93.8±0.11 | 100±0.00 | 1.0±0.05 |

**Fig 5.2.1.3 Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Curcuma longa* and various exposure times.**

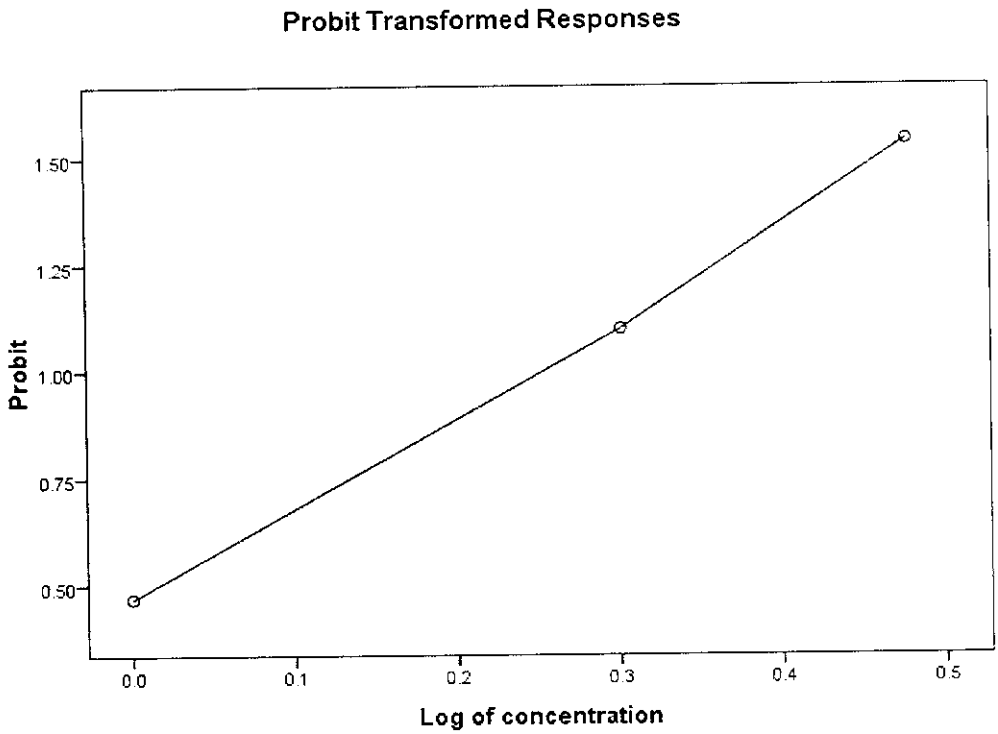



Probit Analysis using SPSS indicated highest probability mortality at higher concentrations (Table 5.2.1.4) and log concentrations of 0.602 showed 0.979%±0.10 mortality

**Table 5.2.1.4:Cell Counts and Residuals**

| Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .000 | 20 | 14 | 13.218 | .382 | .661 |
| 2 | .301 | 20 | 17 | 17.774 | -.494 | .889 |
| 3 | .477 | 20 | 19 | 19.091 | -.331 | .955 |
| 4 | .602 | 20 | 20 | 19.571 | .429 | .979 |

**Figure 5.2.1.2:The Probit transformed Graph generated through SPSS.**




The $LC_{50}$ of the leaves of *Curcuma longa* is 0.879ml/100ml.

### 5.2.1.3 *Curcuma longa (rhizome):*

The mortality rates increased with the concentration of the extract and the exposure time (Table 5.5).A 100% mortality was recorded at a concentration of 4.0ml/l. The time required for complete mortality was also only 70 min.

**Table 5.2.1.5: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Curcuma longa(rhizome)* and various exposure times.**

| Time (min) | Mortality%(Mean±S.D) | | | | |
|---|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | | |
| | 2.0 | 3.0 | 4.0 | 5.0 | 0.0 |
| 10 | 8.2±0.06 | 17.6±2.22 | 22.4±1.83 | 59.2±3.01 | 0.0±0.00 |
| 30 | 26.4±2.31 | 40.8±1.01 | 49.4±1.26 | 80.4±2.10 | 0.0±0.00 |
| 60 | 61.4±0.68 | 69.6±0.33 | 91.2±2.34 | 97.2±0.10 | 0.0±0.00 |
| 70 | 96.4±1.21 | 91.2±1.01 | 100±0.19 | 100±0.00 | 0.0±0.00 |

**Fig 5.2.1.5 Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Curcuma longa(rhizome)* and various exposure times**

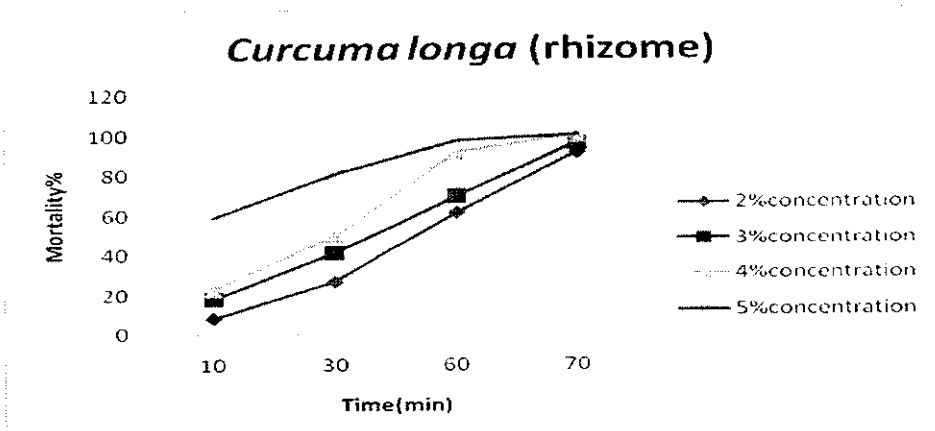
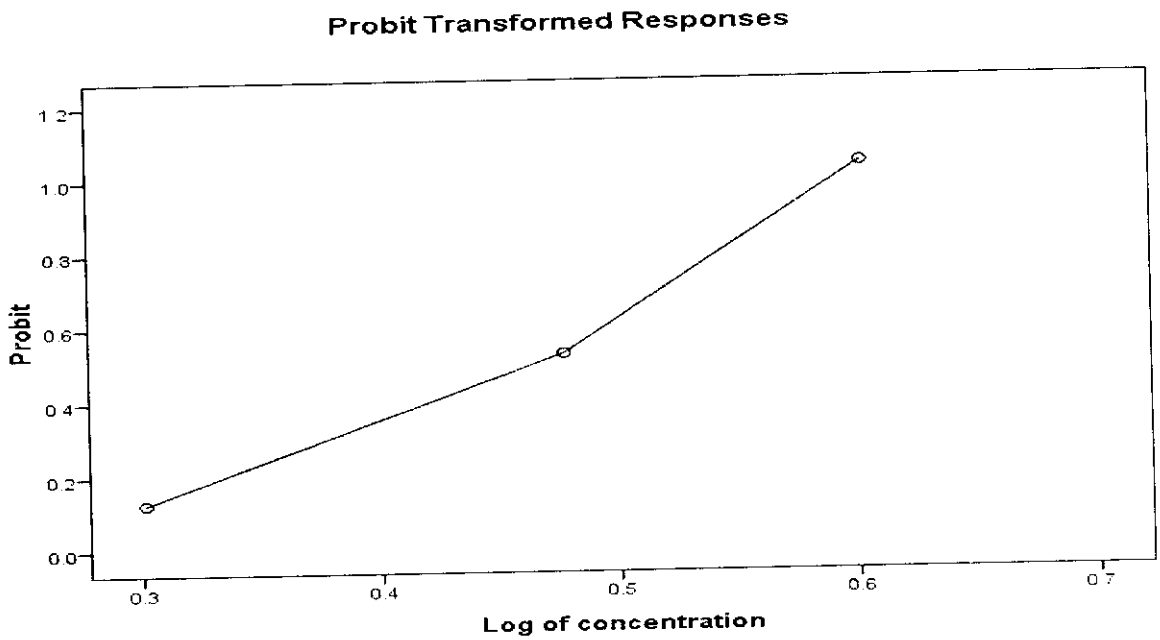


**Table 5.2.1.6:Cell Counts and Residuals**

| Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .301 | 20 | 11 | 9.977 | 1.023 | .499 |
| 2 | .477 | 20 | 14 | 15.299 | -1.299 | .765 |
| 3 | .602 | 20 | 17 | 17.839 | -.839 | .892 |
| 4 | .699 | 20 | 20 | 18.982 | 1.018 | .949 |

**Figure 5.2.1.6: The Probit transformed Graph generated through SPSS**




The $LC_{50}$ of the rhizome of Curcuma *longa* is 0.412ml/100ml.

### 5.2.1.4 *Ervatamia divaricata* (leaves):

**Table 5.2.1.7: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different Concentrations of *Ervatamia divaricata* (leaves) and various exposure times.**

| Time (min) | Mortality%(Mean+S.D) | | | | |
|---|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | | |
| | 1.0 | 2.0 | 3.0 | 4.0 | 0.0 |
| 30 | 0.0±0.10 | 4.8±1.02 | 19.4±0.87 | 44.4±1.02 | 0.0±0.00 |
| 60 | 7.2±1.12 | 10.8±2.11 | 40.6±3.1 | 64.4±0.10 | 0.0±0.00 |
| 90 | 23.2±3.38 | 33.6±0.42 | 64.4±2.20 | 84.2±0.08 | 0.0±0.00 |
| 120 | 37.1±0.53 | 50.6±0.01 | 88.6±1.41 | 100±0.02 | 0.0±0.02 |

**Fig 5.2.1.7: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different Concentrations of *Ervatamia divaricata* (leaves) and various exposure times**

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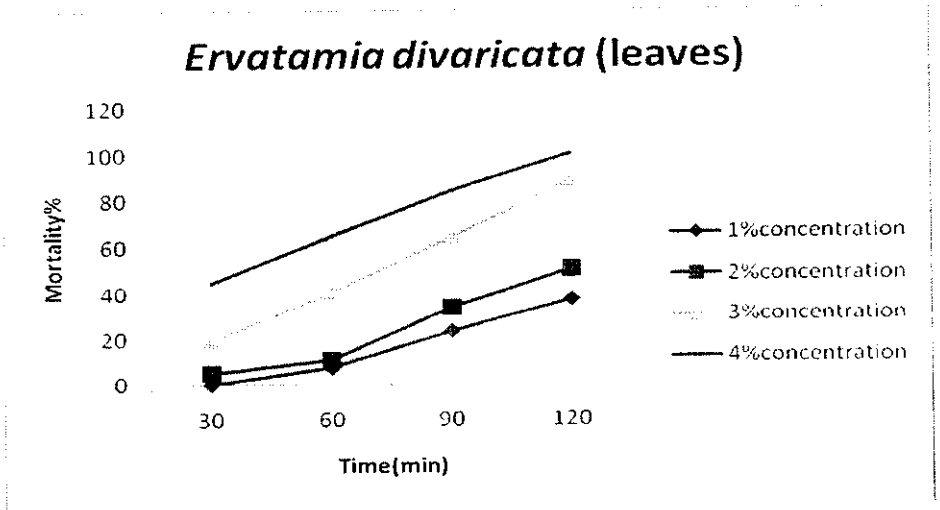
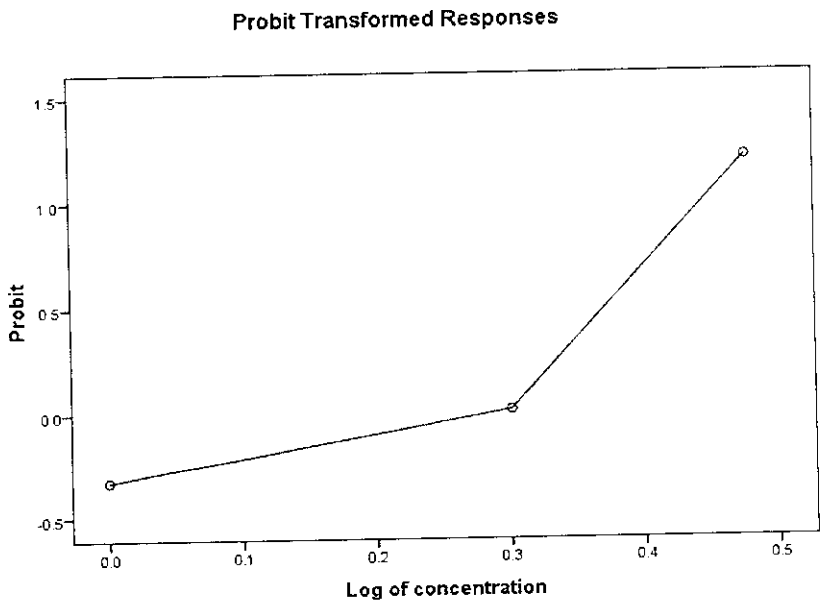


## Table 5.2.1.8:Cell Counts and Residuals

| Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .000 | 20 | 7 | 5.678 | 1.742 | .284 |
| 2 | .301 | 20 | 10 | 13.789 | -3.689 | .689 |
| 3 | .477 | 20 | 18 | 17.363 | .357 | .868 |
| 4 | .602 | 20 | 20 | 18.812 | 1.188 | .941 |

## Figure 5.2.1.8: The Probit transformed Graph generated through SPSS



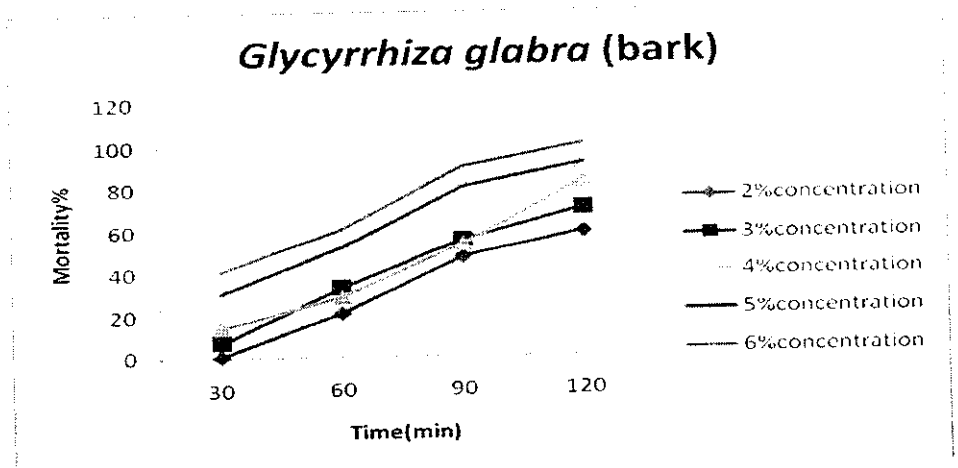

The $LC_{50}$ of the leaves of ***Ervatamia divaricata*** **(leaves)** *is* 1.34ml/100 ml.

*5.2.1.5. Glycyrrhiza glabra*(bark):

**Table 5.2.1.9: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Glycyrrhiza glabra* (bark)**

| Time (min) | Mortality%(Mean+S.D) | | | | | |
|---|---|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | | | |
| | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 0.0 |
| 30 | 0.4±0.01 | 7.0±0.01 | 14.2±1.10 | 30.4±2.07 | 40.8±1.01 | 0.0±0.00 |
| 60 | 20.6±1.91 | 33.0±2.01 | 28.4±1.11 | 51.8±3.21 | 60.6±1.02 | 0.0±0.00 |
| 90 | 47.8±0.58 | 55.0±1.24 | 53.2±3.32 | 80.0±0.11 | 89.6±0.34 | 0.1±0.20 |
| 120 | 58.8±3.21 | 70.0±1.03 | 82.8±0.01 | 91.0±0.54 | 100±0.03 | 0.1±0.20 |

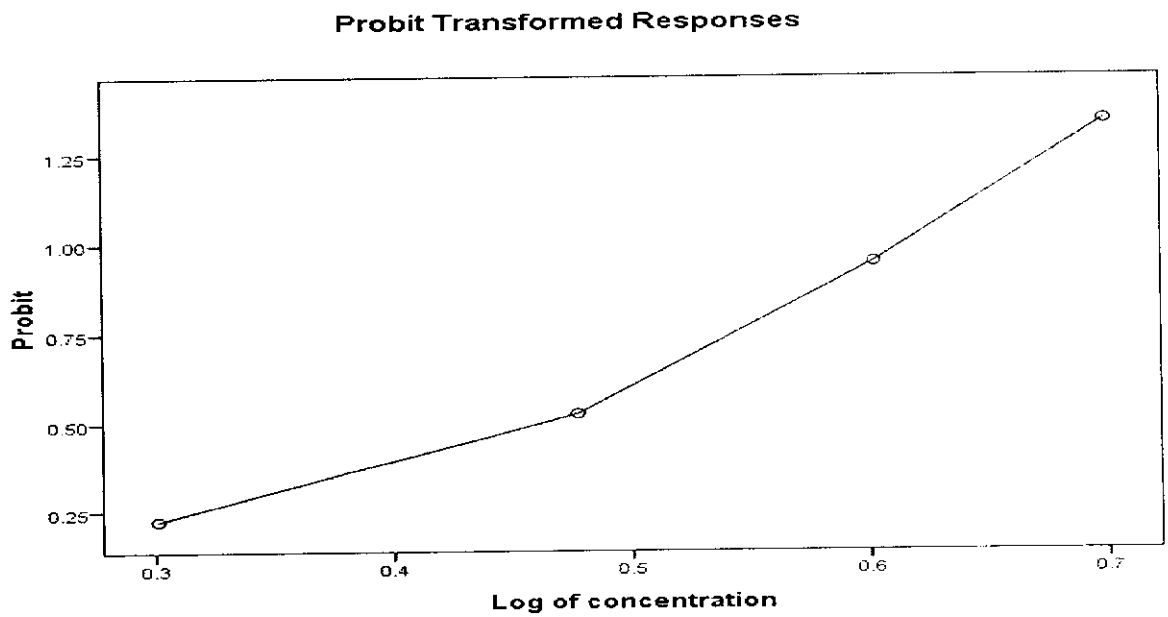
**Fig 5.2.1.9 Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Glycyrrhiza glabra* (bark)**



## Table 5.2.1.10:Cell Counts and Residuals

| Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .301 | 20 | 12 | 10.688 | 1.072 | .534 |
| 2 | .477 | 20 | 14 | 15.040 | -1.040 | .752 |
| 3 | .602 | 20 | 17 | 17.298 | -.738 | .865 |
| 4 | .699 | 20 | 18 | 18.472 | -.272 | .924 |
| 5 | .778 | 20 | 20 | 19.103 | .897 | .955 |

## Figure 5.2.1.10: The Probit transformed Graph generated through SPSS



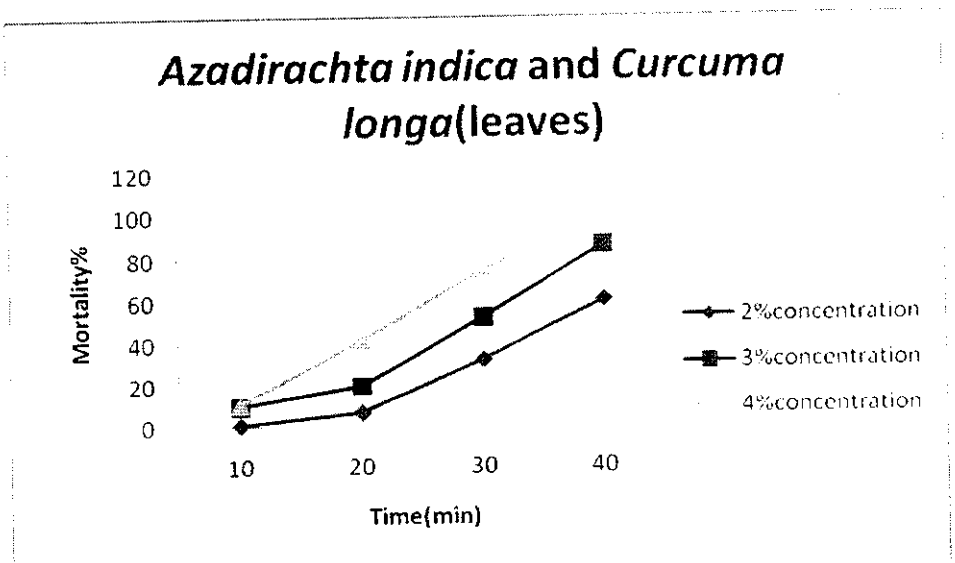

The $LC_{50}$ of the *Glycyrrhiza glabra* (bark) was found to be 1.983ml/100ml.

**5.2.1.6 Combinatorial Study: *Azadirachta indica* and *Curcuma longa* (leaves)**

**Table 5.2.1.11: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Azadirachta indica* and *Curcuma longa*(leaves)**

| Time (min) | Mortality%(Mean+S.D) | | | |
|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | |
| | 2.0 | 3.0 | 4.0 | 0.0 |
| 10 | 1.2±0.02 | 9.8±0.11 | 11.0±0.30 | 0.0±0.00 |
| 20 | 7.0±1.76 | 19.6±2.00 | 41.6±3.12 | 0.0±0.00 |
| 30 | 32.4±0.23 | 52.2±1.22 | 74.2±3.32 | 0.1±0.10 |
| 40 | 60.6±1.21 | 86.4±0.01 | 100±1.02 | 0.2±0.10 |

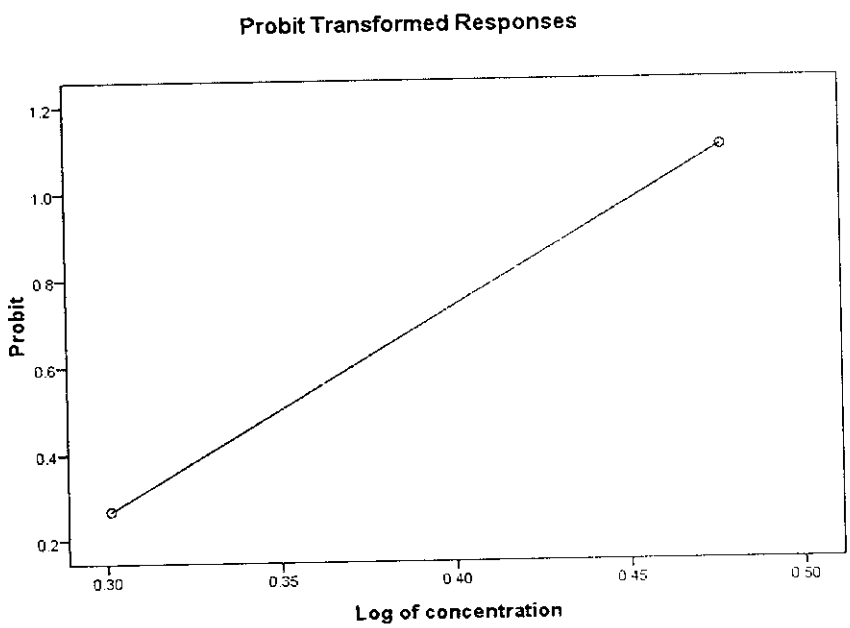
**Fig 5.2.1.11: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Azadirachta indica* and *Curcuma longa*(leaves)**



## Table 5.2.1.12:Cell Counts and Residuals

| Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .301 | 20 | 12 | 11.709 | .411 | .585 |
| 2 | .477 | 20 | 17 | 18.090 | -.810 | .904 |
| 3 | .602 | 20 | 20 | 19.627 | .373 | .981 |

## Figure 5.2.1.12: The Probit transformed Graph generated through spss




The $LC_{50}$ of the leaves of *Azadirachta indica* and *Curcuma longa*(leaves) *is* 0.57ml/100ml.

*5.2.1.7 Azadirachta indica* and *Ervatamia divaricata*(leaf):

**Table 5.2.1.13: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Azadirachta indica* and *Ervatamia divaricata*(leaf)**

| Time (min) | Mortality%(Mean+S.D) | | | |
|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | |
| | 2.0 | 4.0 | 5.0 | 0.0 |
| 40 | 16.6±0.12 | 34.4±0.83 | 45.8±1.20 | 0.0±0.00 |
| 60 | 34.2±0.21 | 67.0±1.76 | 73.8±0.09 | 0.0±0.00 |
| 80 | 58.2±1.13 | 83.6±0.09 | 93.8±2.21 | 0.0±0.10 |
| 100 | 72.8±2.24 | 91.0±0.02 | 100±1.01 | 0.7±0.10 |

**Fig 5.2.1.13 Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Azadirachta indica* and *Ervatamia divaricata*(leaf)**

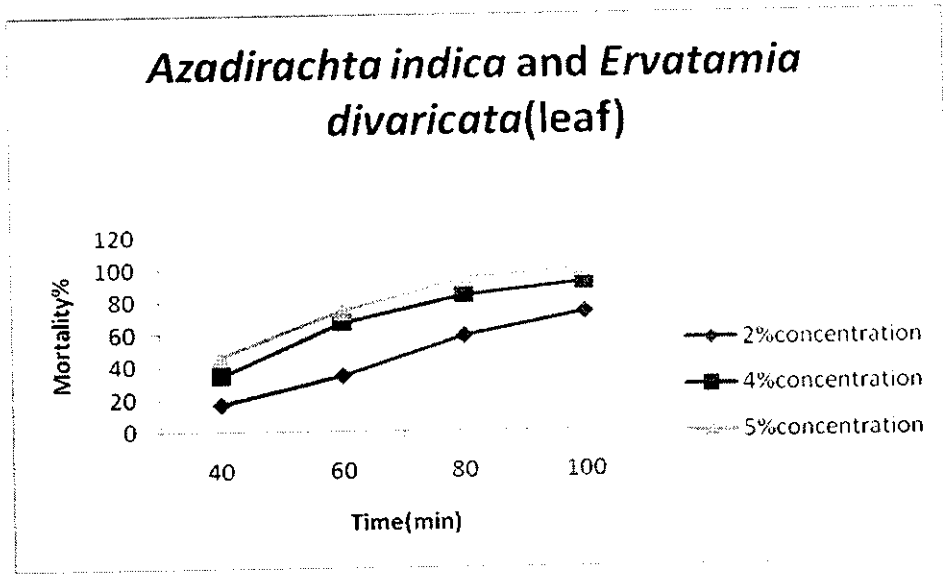
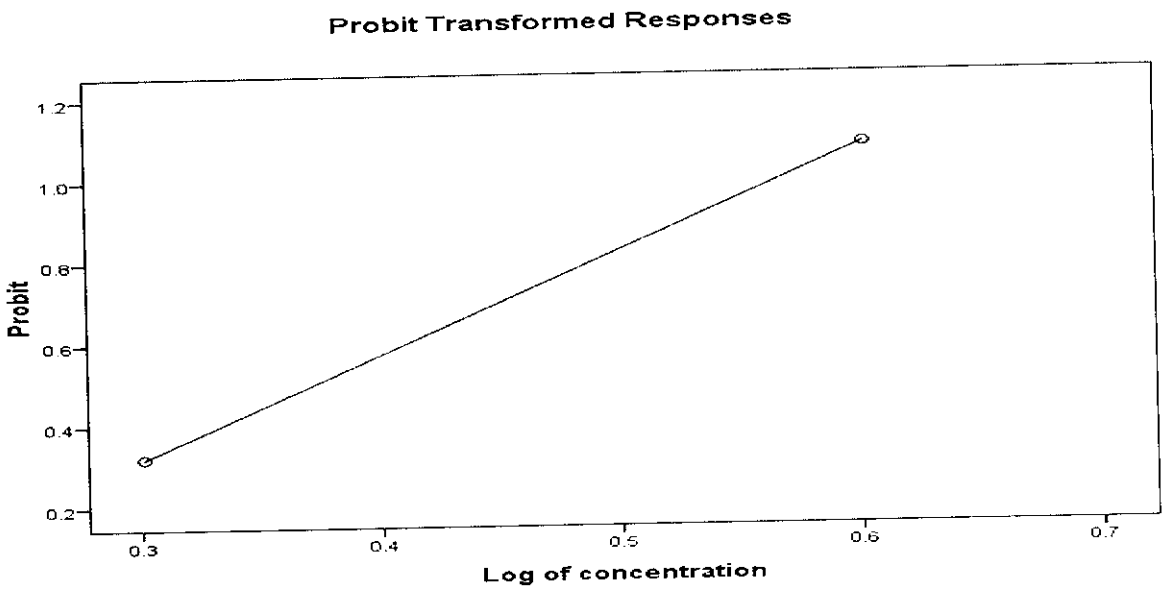


**Table 5.2.1.14:Cell Counts and Residuals**

| Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .301 | 20 | 12 | 12.145 | .355 | .607 |
| 2 | .602 | 20 | 17 | 18.368 | -1.168 | .918 |
| 3 | .699 | 20 | 20 | 19.208 | .792 | .960 |

**Figure 5.2.1.14: The Probit transformed Graph generated through SPSS**



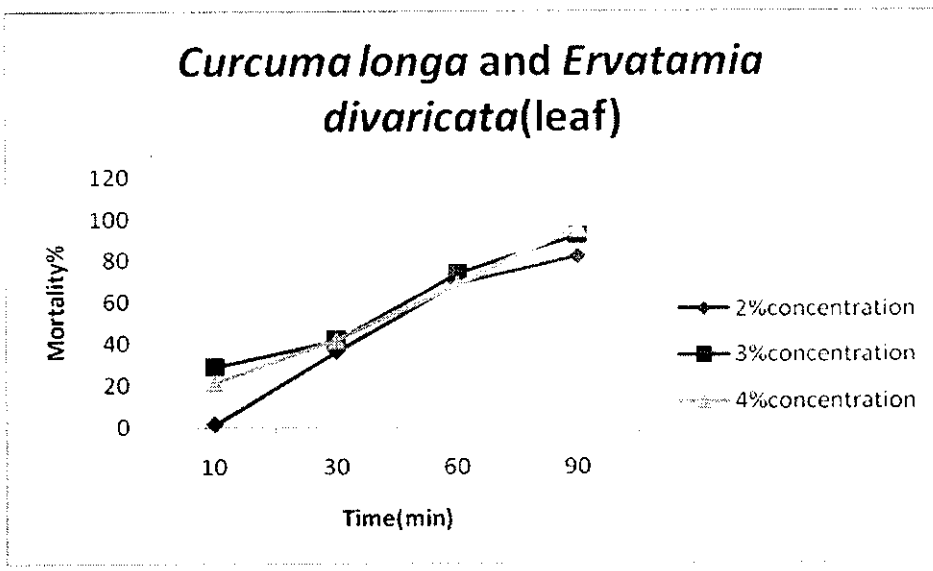

The $LC_{50}$ of *Azadirachta indica* and *Ervatamia divaricata*(leaf) is 0.159ml/100ml

### 5.2.1.8 *Curcuma longa* and *Ervatamia divaricata* (leaf)

**Table 5.2.1.15: Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Curcuma longa* and *Ervatamia divaricata*(leaf)**

| Time (min) | Mortality%(Mean+S.D) | | | |
|---|---|---|---|---|
| | Concentrations(ml/100ml) | | | |
| | 2.0 | 3.0 | 4.0 | 0.0 |
| 10 | 1.50±0.14 | 29.0±0.63 | 21.0±1.30 | 0.0±0.00 |
| 30 | 36.2±1.32 | 41.6±2.10 | 41.8±0.19 | 0.0±0.00 |
| 60 | 69.0±2.12 | 73.8±0.04 | 68.0±0.31 | 0.0±0.10 |
| 90 | 81.6±2.10 | 92.4±0.01 | 96.0±0.01 | 0.7±0.10 |

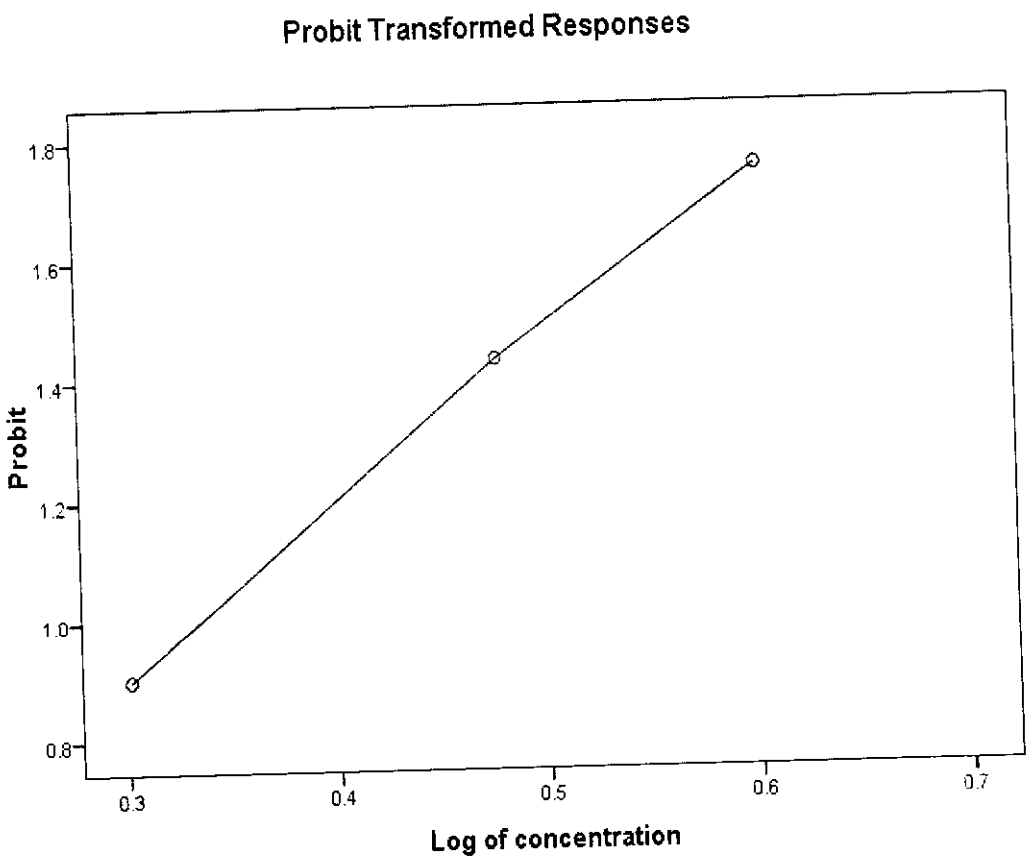
**Fig5.2.1.15 Percent mortality of 4th Instar larvae of *C.quinquefasciatus* at different concentrations of *Curcuma longa* and *Ervatamia divaricata*(leaf)**



## Table 5.2.1.16:Cell Counts and Residuals

| Number | Log Concentration ml/100ml | Number of Subjects | Observed Responses | Expected Responses | Residual | Probability |
|---|---|---|---|---|---|---|
| 1 | .301 | 20 | 16 | 16.349 | -.029 | .817 |
| 2 | .477 | 20 | 18 | 18.418 | .062 | .921 |
| 3 | .602 | 20 | 19 | 19.232 | -.032 | .962 |

## Figure 5.2.1.16 The Probit transformed Graph generated through SPSS




The $LC_{50}$ of ***Curcuma longa*(leaf) and *Ervatamia divaricata*(leaf) is** 0.543ml/100ml.

**Table 5.2.17: $LC_{50}$ of the plant extracts**

| S.No | Name of the sample | $LC_{50}$ ml/100ml |
|---|---|---|
| 1. | *Azadirachta indica*(Leaves) | 1.819 |
| 2. | *Curcuma longa*(Rhizome) | 0.412 |
| 3. | *Curcuma longa*(Leaves) | 0.879 |
| 4. | *Ervatamia divaricata*(Leaves) | 1.34 |
| 5. | *Glycyrrhiza glabra(bark)* | 1.983 |
| 6. | *Azadirachta indica*(Leaves) and *Curcuma longa*(Leaves) | 0.571 |
| 7. | *Curcuma longa*(Leaves) and *Ervatamia divaricat*(Leaves) | 0.543 |
| 8. | *Ervatamia divaricata*(Leaves) and *Azadirachta indica* (Leaves) | 0.159 |

The mortality rates increased with the concentration of the extract and the exposure time. At higher concentrations of the extract of *Azadirachta indica* (5.0ml/l), 100% mortality was recorded at 150 min. Extracts from the leaves and rhizome of *Curcuma*

*longa* showed 100% mortality at concentrations of 4.0ml/l but at exposure timings of 90 min and 70 min respectively.

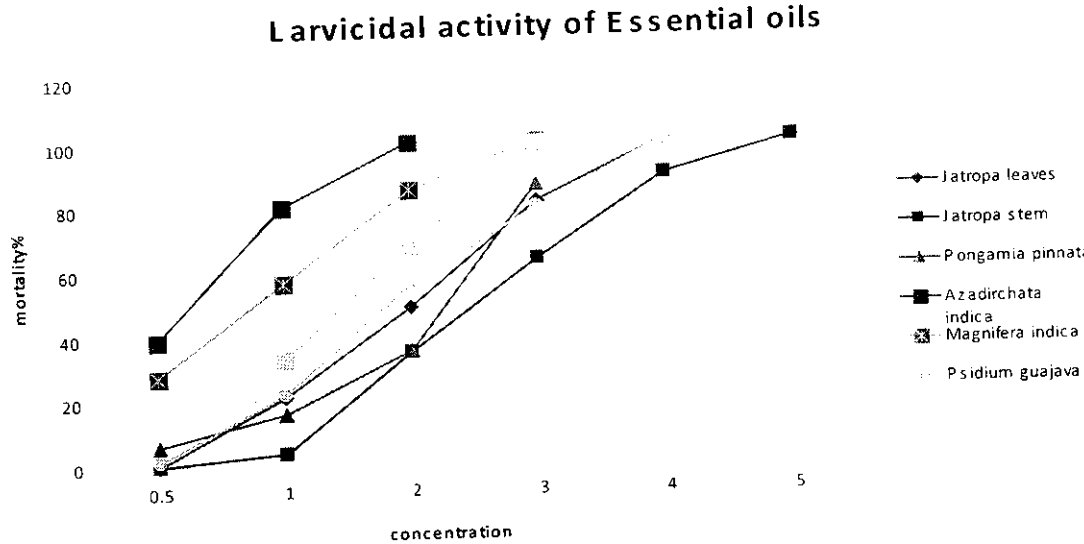
Mortality rate of 100 % was observed with *Earvatamia divaricata* only at a concentration of 4.0ml/100ml only at a time of 120 min. A 100% mortality was observed in *Glycyrrhiza glabra*(bark) at the same exposure time but only at a concentration of 6.0ml/100ml.A Combinatorial Study when undertaken showed potent larvicidal activities at lesser concentrations and exposure time for *Azadirachta indica* and *Curcuma longa*(leaves), *Azadirachta indica* and *Ervatamia divaricata*(leaf), *Curcuma longa* and *Ervatamia divaricata*(leaf).A 100% mortality was observed at 4.0ml/100ml in 40 min,5.0ml/100ml in 100 min and 4.0 ml/100ml in 90 min for *Azadirachta indica* and *Curcuma longa*(leaves) , *Azadirachta indica* and *Ervatamia divaricata*(leaf) and finally *Curcuma longa* and *Ervatamia divaricata*(leaf) respectively.

The $LC_{50}$ of all the plant extracts when analyzed using SPSS software showed values of 0.571ml/100ml,1.543ml/100ml and 0.159ml/100ml that are lower compared to the $LC_{50}$ values of the individual plant extracts

## 5.2.2 Larvicidal activity of Essential oils:

Essential oil was extracted from *Curcuma longa, Jatropa curcas, Pongamia pinnata, Azadirachta indica ,Magnifera indica and Psidium guajava* using steam distillation unit and tested for larvicidal property. Essential oils obtained from *Curcuma longa* was highly effective compared to the other extracts showing 100% larvicidal property at a concentration of 4.0ml/100ml and the mortality. *Magnifera indica* also showed effective mortality at concentrations of 2.0 ml/100ml.(Figure 5.2.2.1)

**Figure: 5.2.2.1 Larvicidal activity of Essential oils**




Essential oil was extracted from *Curcuma longa*, *Jatropha curcas*, *Pongamia pinnata*, *Azadirachta indica*, *Mangifera indica and Psidium guajava* using steam distillation unit and tested for larvicidal property. Essential oils obtained from *Curcuma longa* was highly effective compared to the other extracts showing 100% larvicidal property at a concentration of 4.0ml/100ml and the mortality. *Mangifera indica* also showed effective mortality at concentrations of 2.0 ml/100ml.

## 5.3 Antibacterial activity of plant extracts:

### 5.3.1 Lawn culture technique

The plant extracts obtained by the Soxhlet extraction were effective against all the Bacterial strains when grown in nutrient agar medium. (Table 5.3.1.1 and Table 5.3.1.1).This preliminary qualitative tests were carried out to test the antibacterial properties of the extracts. The comparative analysis done using two different concentrations of 1600ppm and 2400 ppm showed that the higher concentrations effectively inhibited bacterial growth in all the plant extracts except for the plant extract of *Ervatamia divaricata*(flowers).(Table 5.3.1.1)

**Table 5.3.1.1: Antibacterial property of *Azadirachta indica* leaves, *Curcuma longa* Leaves, *Curcuma longa* rhizome at two different concentrations**

| Name of the Bacterium | Presence/absence of growth | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | *Azadirachta indica* Leaves | | *Curcuma longa* Leaves | | *Curcuma longa* rhizome | |
| | | Concentrations(ppm) | | | | | |
| | | 1600 | 2400 | 1600 | 2400 | 1600 | 2400 |
| *Bacillus subtilis* | Lawn* | No Growth | No Growth | No Growth | No Growth | No Growth | No Growth |
| *Escherichia coli* | Lawn* | No Growth | No Growth | No Growth | No Growth | No Growth | No Growth |
| *Proteus vulgaris* | Lawn* | Growth present | No Growth | Growth present | No Growth | No Growth | No Growth |
| *Staphylococcus aureus* | Lawn* | Growth present | No Growth | No Growth | No Growth | No Growth | No Growth |

**Table 5.3.1.2 : Antibacterial property of *Ervatamia divaricata* leaves, *Ervatamia divaricata* Leaves, *Glycyrrhiza glabra* leaves at two different concentrations**

| Name of the Bacterium | Presence/absence of growth | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | *Ervatamia divaricata* leaves | | *Ervatamia divaricata* flowers | | *Glycyrrhiza glabra* bark | |
| | | Concentrations(ppm) | | | | | |
| | | 1600 | 2400 | 1600 | 2400 | 1600 | 2400 |
| *Bacillus subtilis* | Lawn* | Growth present | No Growth | No Growth | No Growth | Growth present | Growth present |
| *Escherichia coli* | Lawn* | No Growth | No Growth | No Growth | No Growth | No Growth | No Growth |
| *Proteus vulgaris* | Lawn* | Growth present | No Growth | Growth present | No Growth | No Growth | No Growth |
| *Staphylococcus aureus* | Lawn* | Growth present | No Growth | Growth present | No Growth | No Growth | No Growth |

## 5.3.2 Well Diffusion Technique:

Antibacterial tests when carried out for the plant extracts with bacterial strains such as *Bacillus subtilis, Escherichia coli, Proteus vulgaris* and *Staphylococcus aureus.* The Zone of Inhibitions when measured showed that the Plant extracts were least effective against *Proteus vulgaris.* The extracts of *Glycyrrhiza glabra* was seen to possess antibacterial activity next to Curcuma longa (Chainani-Wu, 2003).

**Table 5.3.2.1: Antibacterial property of *Azadirachta indica* leaves, *Curcuma longa* Leaves, *Curcuma longa* rhizome at two different concentrations.**

| Name of the Bacterium | Zone of inhibitions(mm) | | | |
|---|---|---|---|---|
| | Concentrations of 5% | | | |
| | Control | *Azadirachta indica* leaves | *Curcuma longa* leaves | *Curcuma longa* Rhizome |
| *Bacillus subtilis* | Lawn* | 1.2 | 0.1 | 0.45 |
| *Escherichia coli* | Lawn* | 1.0 | 0.4 | 0.5 |
| *Proteus vulgaris* | Lawn* | 0.2 | 0.1 | 0.2 |
| *Staphylococcus aureus* | Lawn* | 0.5 | 0.4 | 0.45 |

**Table 5.3.2.2: Antibacterial property of *Ervatamia divaricata* leaves, *Ervatamia divaricata* leaves, *Glycyrrhiza glabra* bark at two different concentration.**

| Name of the Bacterium | Zone of inhibitions(mm) | | | |
|---|---|---|---|---|
| | Concentrations of 5% | | | |
| | Control | *Ervatamia divaricata leaves* | *Ervatamia divaricata Flowers* | *Glycyrrhiza glabra Bark* |
| *Bacillus subtilis* | Lawn* | 0.0 | 0.0 | 0.3 |
| *Escherichia coli* | Lawn* | 0.8 | 0.1 | 1.0 |
| *Proteus vulgaris* | Lawn* | 0.1 | 0.0 | 0.2 |
| *Staphylococcus aureus* | Lawn* | 0.55 | 0.2 | 0.3 |

| | |
|---|---|
| *1.C.l rhizome-Curcuma longa rhizome* | *2.A.i- Azadirachta indica* leaves |
| *3.E.d flowers-Ervatamia divaricata flowers* | *4.G.g-Glycyrrhiza glabra bark* |
| *5.C.l leaf-Curcuma longa* leaves | *E.d leaves-Ervatamia divaricata leaves* |

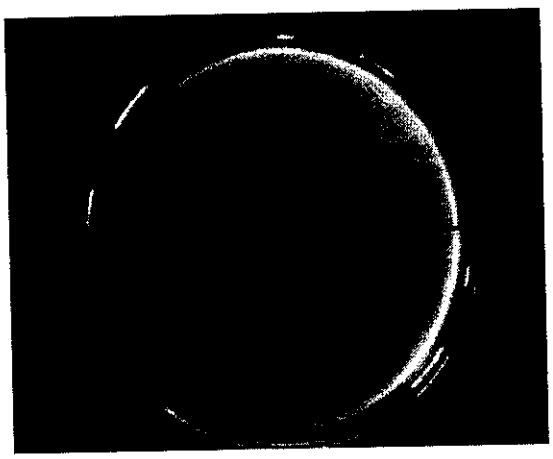


**Figure 5.3.2.1Antibacterial activity of *Curcuma longa* (rhizome) and *Azadirachta indica* on *Proteus vulgaris*.**

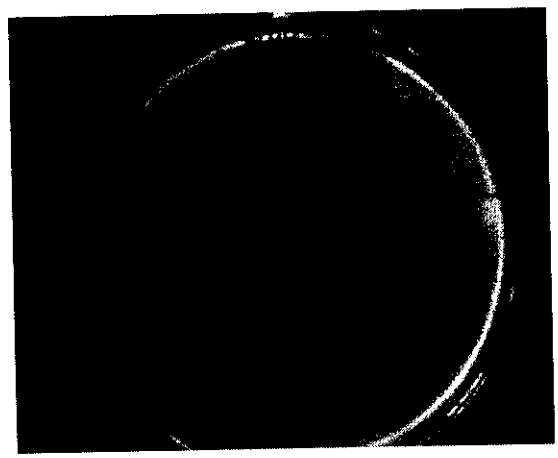


**Figure 5.3.2.2 Antibacterial activity of *Curcuma longa* (rhizome) and *Azadirachta indica* on *Staphylococcus aureus***

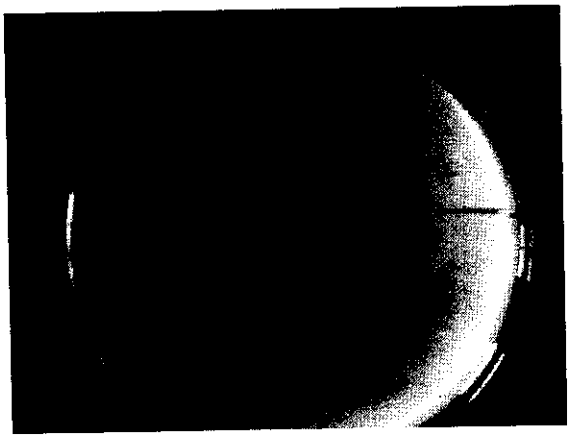


**Figure 5.3.2.3 Antibacterial activity of *Curcuma longa* (rhizome) and *Azadirachta indica* on *Bacillus subtilis***

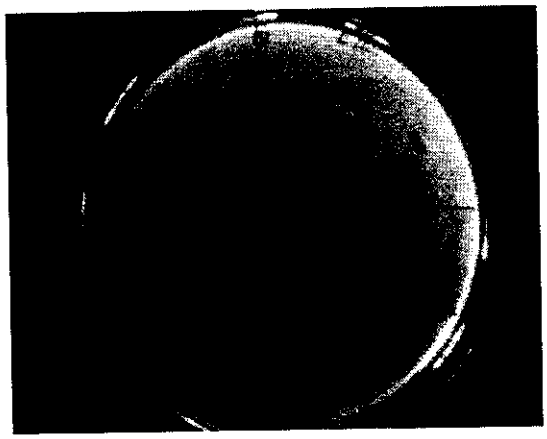


**Figure 5.3.2.4 Antibacterial activity of *Curcuma longa* (rhizome) and *Azadirachta indica* on *Escherichia coli***

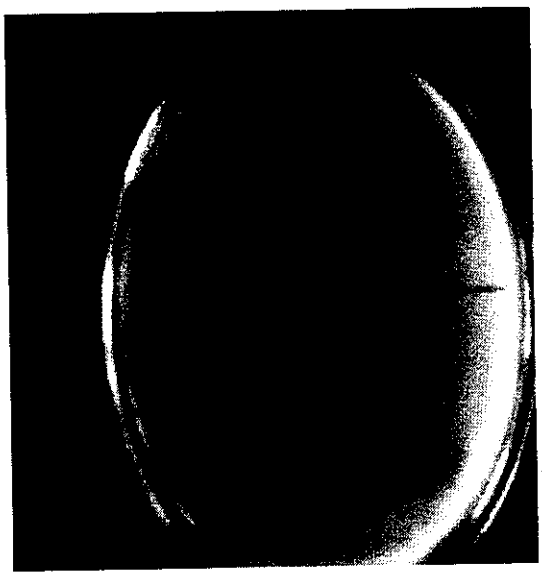


**Figure 5.3.2.5 Antibacterial activity of *Ervatamia divaricata* flowers and *Glycyrrhiza glabra* bark on *Proteus vulgaris***

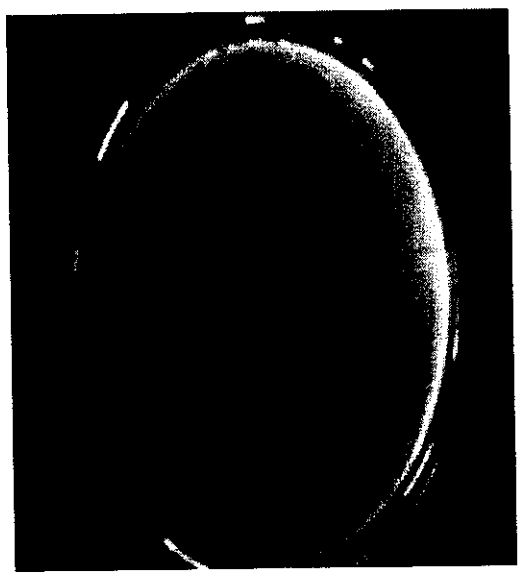


**Figure 5.3.2.6 Antibacterial activity of *Ervatamia divaricata* flowers and *Glycyrrhiza glabra* bark on *Bacillus subtilis***

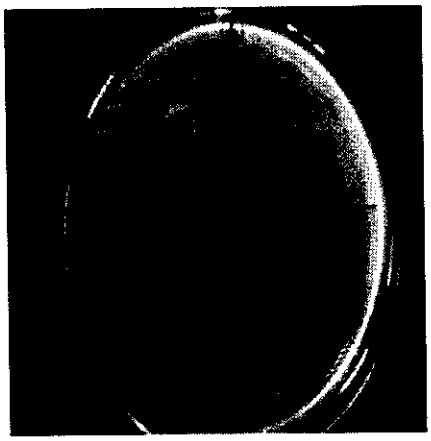


**Figure 5.3.2.7 Antibacterial activity of *Ervatamia divaricata* flowers and *Glycyrrhiza glabra* bark on *Staphylococcus aureus.***

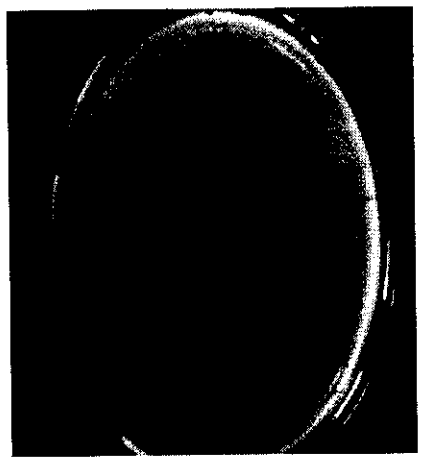


**Figure 5.3.2.8 Antibacterial activity of *Ervatamia divaricata* flowers and *Glycyrrhiza glabra* bark on *Escherichia coli***

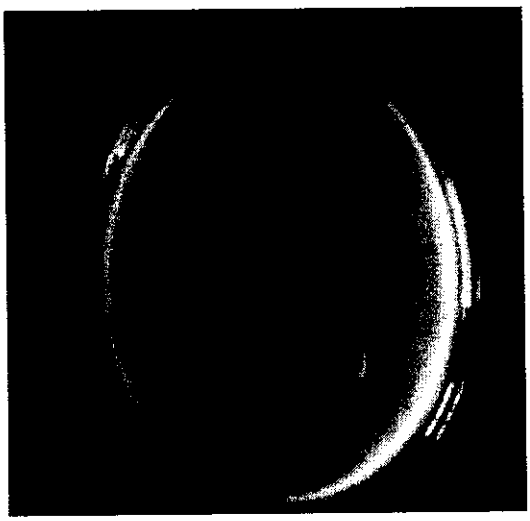


**Figure 5.3.2.9 Antibacterial activity of *Curcuma longa* leaves and *Ervatamia divaricata leaves* on *Proteus vulgaris***

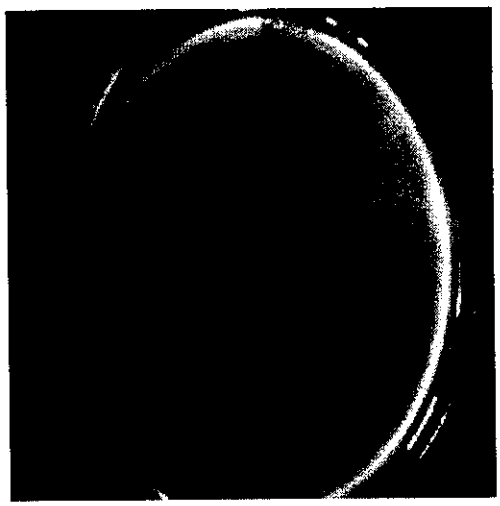


**Figure 5.3.2.10 Antibacterial activity of *Curcuma longa* leaves and *Ervatamia divaricata leaves* on *Bacillus subtilis***

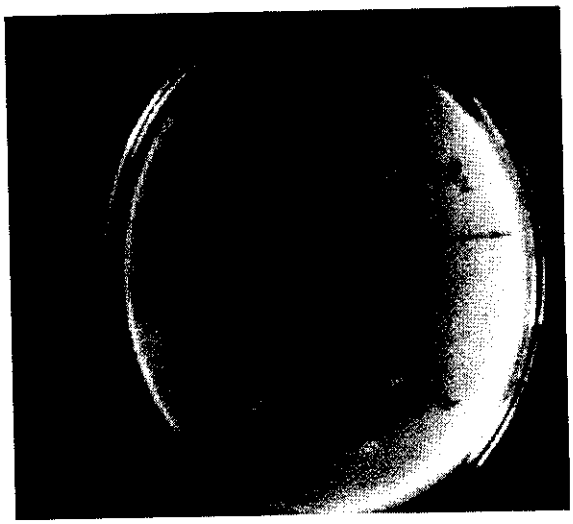


**Figure 5.3.2.11 Antibacterial activity of *Curcuma longa* leaves and *Ervatamia divaricata leaves* on *Staphylococcus aureus.***

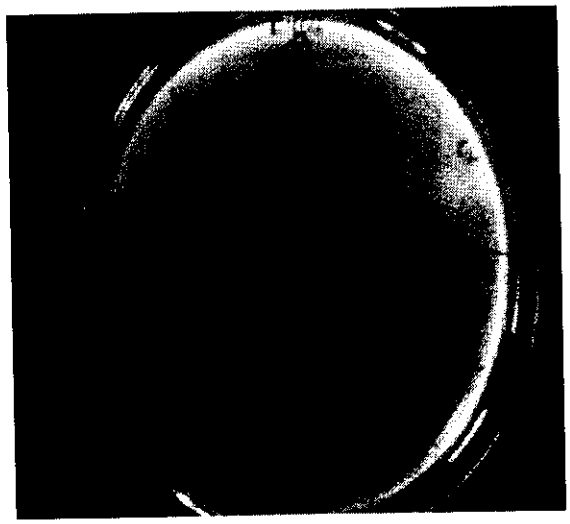


**Figure 5.3.2.12 Antibacterial activity of *Curcuma longa* leaves and *Ervatamia divaricata leaves* on *Escherichia coli***

The antibacterial tests carried out showed that the rhizome of *Curcuma longa and* the bark extract of *Glycyrrhiza glabra* bark at a concentration of 5% was very effective on *Bacillus subtilis* and *Escherichia coli* (Figure 5.3.2.6 and Figure 5.3.2.7).

## 5.4 Phytochemical Screening:

Phytochemical screening was done for the Plant extracts extracted using solvent.The results are tabulated as in Table 5.4.1.

## Table 5.4.1.:Results of Phytochemical Screening for the plant extracts

| Tests | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| Alkaloids | Marqui's test | - | - | + | - | - | + | - |
| | Dragendorff's test | + | + | + | + | + | + | + |
| Carbohydrates | | + | - | + | + | - | + | - |
| Saponins | | + | - | - | + | - | + | + |
| Phenols | | + | - | - | + | - | - | + |
| Flavonoids | | + | - | + | - | - | + | + |
| Terpenoids | | + | - | - | - | + | + | + |
| Tannins | | + | + | + | + | + | + | + |
| phlobatannins | | - | - | - | - | - | - | - |

1. *E.d flowers-Ervatamia divaricata flowers(hydrophobic)*
2. *C.l leaf-Curcuma longa* leaves
3. *C.l rhizome-Curcuma longa rhizome*
4. *A.i- Azadirachta indica* leaves

5. *E.d leaves-Ervatamia divaricata leaves*
6. *G.g-Glycyrrhiza glabra bark*
7. *E.d flowers-Ervatamia divaricata flowers(hydrophilic)*

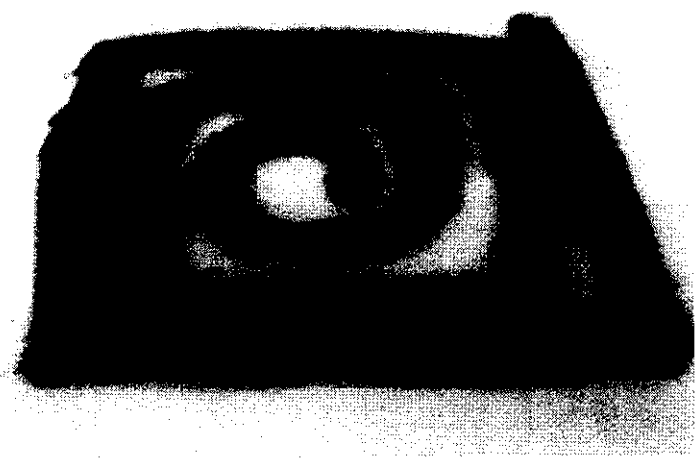
## 5.5 Smoke toxicity tests:

Smoke toxicity tests were carried out for the *Ervatamia divaricata* plant extract which showed a mortality percentage in the unfed mosquitoes.(Table 5.5.1).

**Table 5.5.1 Smoke toxicity effects of *Ervatamia divaricata on C.quinquefaciatus***

| Plant samples used | No of mosquitoes tested | No.Fed mosquitoes | No. un fed mosquitoes | | | % unfed over negative control |
|---|---|---|---|---|---|---|
| | | | Alive | Dead | Total | |
| *Ervatamia divaricata* | 50±5 | 11 | 24 | 16 | 40 | 25 |
| Negative control | 50±5 | 35 | 14 | 0 | 14 | 0 |
| Positive control | 50±5 | 07 | 21 | 22 | 43 | - |

**Figure 5.5.1: Test coils containing 10 % *Ervatamia divaricata* plant extract as the active ingredient**



# SUMMARY AND CONCLUSION

# CHAPTER 6

## SUMMARY AND CONCLUSION

The project carried out to study the larvicidal property of extracts of the leaves of *Azadirachta indica*, *Curcuma* longa and *Ervatamia divaricata*, the rhizome extract of *Curcuma* longa, the bark extract of *Glycyrrhiza glabra* has showed efffective larvicidal properties. The $LC_{50}$ of the individual extracts were higher comparable to the $LC_{50}$ values of the combined extracts. The results obtained were found to be more potent than the acetone extract of *Lantana camara* which was reported to be the most effective against *C.quinquefaciatus* larvae. The combined larvicidal activity of *Ervatamia divaricata* (leaves) and *Azadirachta indica* (leaves) which is 0.159 ml/100ml will be more effective on the mosquito vector *C.quinquefaciatus* than previous reported data from *Aristolochia saccata and Annona squamosa* that ranged between 31.80 and 155 ppm. . The highest larvicidal activity was found with the extract of *Curcuma longa* (rhizome) ($LC_{50}$ = 142 ppm)

The results of the larvicidal activity of the essential oils of *Pongamia pinnata*, *Mangifera indica, Psidium guajava* and *Jatropha curcas* were also highly effective with complete mortality achieved at relatively higher concentrations as in *Azadirachta indica* and *Curcuma* longa. The essential oils of *Pongamia pinnata* exhibited a 100% mortality at 3.0ml/100ml .

This study has paved the way to screen more plant extracts for mosquito repellent activity which can be made commercially viable.

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# REFERENCE


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