STUDIES ON THE EFFECT OF PLANT SECONDARY METABOLITES IN THE CONTROL OF MOSQUITO VECTORS

Thesis submitted to the
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IN

ENVIRONMENTAL SCIENCE

by

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on the effect of plant secondary metabolites in the control of mosquito vectors**", submitted to the University of Calicut by Mrs. Rathy M.C., in partial fulfillment of the award of the degree of Doctor of Philosophy in Environmental Science is a *bona fide* record of the research work carried out by her under my supervision and guidance. No part of the present work has formed the basis for the award of any other degree or diploma previously.

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This is to certify that the corrections /modifications suggested by both the adjudicators have been incorporated in the thesis entitled **"Studies on the effect of plant secondary metabolites in the control of mosquito vectors"**,submittedby **Mrs. Rathy, M.C., Research Scholar in Environmental Sciences, Department of Botany, University of Calicut.**

The corrected thesis is submitted to the University of Calicut,with reference to the letter No144833/RESEARCH-C-ASST-1/2017/Admn dated 23/03/2018.

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DECLARATION

The thesis entitled **Studies on the effect of plant secondary metabolites in the control of mosquito vectors,** submitted by me in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Environmental Science of the University of Calicut is an original research work carried out by me under the guidance and supervision of Dr. C.C. Harilal, Assistant Professor, Division of Environmental Science of the Department of Botany, University of Calicut. No part of the work formed the basis for the award of any other degree or diploma of any University.

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18th July 2017

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Rathy.M.C

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ABBREVIATIONS

Ae.		*Aedes*
An.		*Anopheles*
Ar.		*Armigeres*
Cx.		*Culex*
HAT		Hours After Treatment
HPTLC		High Performance Thin Layer Chromatography
JE		Japanese encephalitis
LC/Q-TOF/MS		Liquid Chromatography / Quadruple – Time of Flight / Mass Spectrometry
LC_{50}		Lethal concentration
LF	:	Lymphatic filariasis
mg/l		Milligram/Litre
mg/ml		Milligram/Milli Litre
^{o}C		Degree Celsius

GENERAL INTRODUCTION

Insects are an ancient group of organisms, which are persisting for millions of years. They refer to a diverse group of organisms, serving beneficial as well as detrimental effects on all other life forms, including human beings (Little 1957). When insects are considered on a worldwide basis, the most injurious ones in terms of epidemiology are mosquitoes, houseflies, sandflies, black flies, tse-tse flies, soft ticks, fleas, lice and mites. Among these, mosquitoes play a significant role in the spread of epidemics (Gubler 1998; Jomon et al. 2009; Amala et al. 2011) . Throughout history, mosquitoes had been a constant impediment and threat to human health and development, owing to their ability to support pathogens and facilitate diseases spread (Service 1996; Rozendaal 1997).

Mosquitoes are cosmopolitan in distribution and are prevalent throughout the tropical and temperate regions, excluding Antarctica and a few islands (Rajendran 2000). These insects are very successful in adjusting to different climatic conditions, occupy special niches in the environment and breed within a short period of time (Dhanalakshmi 2013).

Mosquitoes not only cause nuisance by their bites, but also transmit deadly diseases like Malaria, Yellow fever, Dengue fever, Chikungunya, Japanese Encephalitis, Filariasis, Eastern Equine Encephalitis, St Louis Encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis, West Nile Virus fever and many others. Among approximately 3500 known mosquito species, more or less 10% are regarded as efficient vectors of pathogenic agents of infectious diseases, having high impact on human health and welfare (Lalrotluanga et al. 2012). The data on the prevalence of mosquito-borne diseases is inadequate, but it is roughly estimated that more than 700

million people world wide are infected with mosquito-borne diseases, annually (Mwine et al. 2005; Maurya et al. 2009).

Ever in the epidemiological history of our country, mosquito borne diseases have prime importance. Malaria is probably the most rampant, devastating vector-borne disease in the tropics and subtropics (Mwine et al. 2010), causing high extent of morbidity and mortality in the world (Tikar et al. 2011; Chalannavar et al. 2013). Malaria is endemic in about 90 countries, especially in Africa, Asia, South America and in Caribbean islands. There are about 400 species of Anopheles mosquitoes, but only 10 species are responsible for transmitting Malaria (Park 1994). About 2- 4 billion world's population lives in areas where there is definite risk (Rosenthal 2004). Dengue is yet another serious and fast emerging tropical mosquito–borne disease. More than 2.5 billion people are at risk of Dengue fever and have become endemic in more than 100 countries (Nkya et al. 2014; Yadav et al. 2014). This disease is spread by the species *Aedes aegypti* (Ahmed et al. 2011; Rajasekaran and Duraikannan 2012). Dengue fever has been receiving manifold attention from health authorities and researchers all over India, from the time of an outbreak of Dengue fever and Dengue haemorrhagic fever in Delhi during 1996 (Sumodhan 2003). Presently the disease is of frequent occurrence in the state of Kerala (DHS 2014).

Japanese Encephalitis (JE) is a common mosquito borne Viral Encephalitis, rampant in eastern and southern Asia, causing significant morbidity and mortality in the World (Thenmozhi et al. 2006; Erlanger et al. 2009; Tiwari et al. 2012). Culex mosquitoes, especially *Culex vishnui* and *Culex tritaeniorhynchus* are considered to be the principal vectors of Japanese Encephalitis (Singh et al. 2012). Lymphatic Filariasis (LF) is a parasitic and infectious tropical disease caused by filarial nematode worms; *Wuchereria bancrofti* and *Brugia malayi*, transmitted by the mosquito *Culex quinquefasciatus* (Sabesan et al. 2010; Kannathasan et al. 2011). Chikungunya is

a relatively rare and benign form of viral fever caused by an alpha virus that is spread through infected *Aedes aegypti* (Swaroop et al. 2007). In addition to *Aedes aegypti, Aedes albopictus* is is also reported to cause Chikungunya in Asian region. Yellow fever, the original viral haemorrhagic disease, is transmitted by infected mosquitoes like *Culex fatigans* and *Aedes aegypti*. Upto 50% of severely affected persons will die from yellow fever. Every year there are around 200, 000 cases of illness and 30, 000 deaths from yellow fever through out the world. Over the past two decades, the number of yellow fever cases has increased due to declining immunity to infection and other changes in environmental conditions (Verma et al 2014; WHO 2014). West Nile Fever (WNF) and Rift Valley Fever (RVF) are emerging diseases, causing epidemics outside their natural range of distribution by mosquitoes of the *Culex* group, including *Culex pipiens* and *Culex quinquefasciatus*, which are ubiquitous mosquitoes in temperate and tropical regions (Ozer 2007; Amraoui et al. 2012).

Thus mosquito vectors have a profound role in the establishment and spread of many dreadful diseases. The outbreak of diseases is naturally associated with water sources, as mosquitoes are inevitably linked with aquatic breeding sites for the development of their juveniles (Vyas 2008). During their life cycle, mosquitoes pass through stages like egg, larva, pupa and adult, of which the first three stages need stagnant water. It is reported that the larvae and pupae of all species live in water (Ogbeibu 2001; Jaime 2016). Inadequate water management has resulted in man-made mosquitogenic conditions, facilitating their proliferation and thereby disease outbreaks. Rapid urbanization and man-made changes of the environment continues to expand mosquito-breeding habits (Norris 2004; Afolabi 2013). Apart from this, most species of mosquito vectors possess certain peculiar characters, which make them successful and so difficult to control. Some of them are short gestation period, high fecundity rate, high dispersal potential, high resistance to insecticides etc. (Kalyanasundaram and Das 1985; Sreelatha and Pillai 1996; Narayanan and Pillai 1996; Sukumaran

1997; Mehra and Hirdhar 2002), which facilitate them to survive over a wide range of environmental conditions.

In many areas, the incidence of mosquito-borne diseases has increased largely as a result of decreased efficacy of vector-control programmes and subsequent increase in vector mosquito populations. To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential.Their control has become increasingly difficult in recent times due to the indiscriminate use of synthetic insecticides, which has disrupted natural biological control systems and thereby the resurgences in mosquito populations. It has also resulted in the development of resistance (Brown 1986) and undesirable effects on non-target organisms (Alfonso-parra 2016). This has initiated a search for alternative methods of mosquito control.

Plants are considered as a rich source of bioactive compounds (Wink 1993) and hence can be an alternative source of mosquito control agents. Natural products of plant origin with insecticidal properties have been tried in the recent past for the control of a variety of insect pests and vectors (Das et al. 2007). Diverse plant species are the major sources of safe and biodegradable chemicals, which can be screened for mosquito repellent and insecticidal activities (Mittal and Rao 2003; Govindarajan et al. 2011; Raveen et al. 2012; Annapoorani 2014; Yadav et al. 2015; Jayapriya and Shoba 2015; Mohankumar et al. 2016). Phytochemicals have more effects that are specific and can be usefully integrated with other control measures. It can have comprehensive design, appropriate and effective management protocols with less impairment to the environment and non-target organisms, when compared to synthetic ones (Pitasawat et al. 2007; Aktar 2009; Silva et al. 2009; Kweka et al. 2008; Azokou et al. 2013; Jayabalan et al. 2013). The absence of residual effect has made it an effective substitute for chemical pesticides (Jolivett 1998).

The plant kingdom, which is a rich source of active components for mosquito control, still lies fully unexplored. As Kerala is gifted with its rich plant diversity, such a search is worthwhile. The focus is now on environment friendly pesticides and biological control methods based on plants. Environment friendly pesticides are those chemicals, which suppress the insect populations below the economic threshold, which is being accepted by the ecosystem without creating an imbalance in it (Ansari 1993). In this search for environment friendly pesticides the 'Botanical' or natural organic pesticides have emerged as the best candidate specifically due to their cost effectiveness. The widely known Botanical pesticides are azadirachtin, nicotine, pyrethrum, rotenone, quassins etc. These are secondary metabolites synthesized by plants during the co-evolution of plants and insects, to serve as defense chemicals against insect attack (Nagasampagi 1993).

As stated, the plant diversity comprises a reservoir of diverse group of chemical compounds such as carbohydrates, proteins, alkaloids, phenols, lipids, tannins, glycosides, resins and volatile oils (Dubey et al. 2008; Dan 2002), which cause physiological effects and behavioral changes on pests that could be tapped for use as pesticides. These secondary compounds have an ecological role as pollinator, attractants, chemical adaptations to environmental stresses, chemical defenses against micro organisms, insects, higher predators and even other plants but no apparent function in plant's primary metabolism (Balandrin et al. 1985). Thus, the use of biologically active plant materials with these ecological properties has attracted considerable interest (Ananthakrishnan 1990).

Various environment friendly insecticides derived from plants are currently in use. Botanical compounds like pyrethrum, rotenone, nicotine, quassins, neem oil/ cake etc. are used for developing ecofriendly pesticides (Nair et al. 1976). Among the Botanicals, neem ranks first as the king of bio pesticides in view of its excellent insecticidal, insect antifeedant, growth initiatory and

repellent activities. Neem is very close to an ideal biopesticide as it exhibits excellent efficiency for insect control, show relatively low mammalian toxicity, does not affect the non-target and beneficial organisms, biodegradable and is available in abundance in various countries (Collins and Blackwell 2000). In addition to this, there are other plants like *Ocimum sanctum, Leucas aspera, Allium sativum, Cannabis sativum, Tridax procumbens, Calophyllum inophyllum* etc. which are widely applicable against insects (Sreenivasan and Perumalsamy 1993).

As stated earlier, secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of an organism. Secondary metabolites aid a plant in important functions such as protection, competition and species interactions, but are not necessary for survival. One important defining quality of secondary metabolites is their specificity. Usually, secondary metabolites are specific to an individual species (Oomah 2003; Sasson 2005). The major secondary metabolites in plants belong to the category of Terpenoids, Alkaloids and Phenolics (Cannon et al. 2001).

Terpenoids are one of the largest groups of secondary metabolites in the form of lipids. These organic volatile substances occur widely in fruits, flowers, stems, bark and roots of almost all plant parts having aromatic properties (Krishnaiah et al. 2009). Alkaloids are nitrogen-containing organic constituents that occur mainly in plants having a basic character and containing at least one nitrogen in a heterocyclic ring (Verma 2014). The wide spread distribution of alkaloids in all parts of plants has stimulated the search for the function of these compounds in the general metabolism of plants. Phenolics are plant metabolites essential for the growth and reproduction of plants, and are produced as a response for defending injured parts against pathogens (Waghorn 2003; Chandra et al. 2017). They are categorized as secondary metabolites derived from carbohydrates. There is little evidence that phenolics play a key role in plant

growth and development. However, the flavonoid pigments in flowers and fruits serve as insect attractants. Allelopathic and antimicrobial properties have been ascribed to certain phenolics (Fabricant and Farnsworth 2001). Thus economically feasible plant secondary metabolites are considered as a potential alternative approach to various stages and species of mosquito control owing to their excellent properties like non toxicity on non target organism, environmental safety, cheap availability, occurrence of rich source of bioactive compounds etc. (Rajkumar and Jebanesan 2005; Pavela et al. 2005; Kostic et al. 2008; Elango et al. 2009; Uma Devi et al. 2010; Borah et al. 2010; Govindarajulu et al. 2015; Santos et al. 2012; Syed ali et al. 2013; Pratheeba et al. 2015; Hemalatha et al. 2015; Vignesh et al. 2016). Plant based insecticides for the management of mosquitoes necessitates the preliminary screening of plants to assess their efficacy in mosquito control and selecting the plants with high potency for further study (Koushik and Saini 2008). For the development of ecofriendly insecticides, selection of plants for the extraction and isolation of active components are important.

In light of the above, the present investigation is carried out to evolve a biological or eco-friendly control over the mosquitoes using plants of the locality by evaluating the effects of secondary metabolites contained within them on the survival of mosquito larvae. The objectives of the present study are consolidated as:

1. Screening of plants and preparation of extracts.
2. Assessment of the lethal effect of plant extracts on the survival of mosquito larvae. Identification of the optimum conditions offering maximum insecticidal efficiency and confirmation of lethality.
3. Isolation / characterization of compounds having insecticidal properties from plants using separation techniques.

The elucidation of the findings of the present study has also been carried out in three chapters as follows:

- Chapter I. Laboratory trials on the rearing of mosquitoes and standardization of growth conditions.
- Chapter II. Screening for larvicidal activity of plant extracts on mosquito vectors
- Chapter III. Isolation and characterization of phytochemicals having insecticidal properties

CHAPTER I

LABORATORY TRIALS ON THE REARING OF MOSQUITOES AND STANDARDIZATION OF GROWTH CONDITIONS

Introduction

Vector-borne diseases have emerged as a major public health issue in various countries (Kumar 2011; Shidaraddi et al. 2015), including India (Varshini and Kanagappan 2015). Rapid urbanization, industrialization, excessive population growth coupled with rural to urban migration, land use alteration and issues related to water management have facilitated increase in mosquito-breeding habits and there by proliferation of vectors (Thomas 2013). About 3500 species of mosquitoes have been detected globally, out of these 16 genera are identified from India (Barraud 1934; Hazra and Dash 2008; Suhasini and Sammaiah 2014). Kerala has a significantly higher diversity of mosquitoes including vector species. As many as 118 species of mosquitoes under 15 genera have been recorded from Kerala (Lucy and Subramanian 2007). The most important among them, which are of nuisance to human beings belongs to the genera Anopheles, Culex, Aedes, Mansonia, Haemagogus, Armigeres, Culiseta, Sabethes and Psorophora (VCRC 1989; Sumodan 2014). Among different mosquito species, a few are vectors of dreadful diseases such as Malaria, Filariasis, Dengue fever, Japanese encephalitis etc. Their population has gone considerably high in recent times. Bringing interruption in the transmission of vector parasites through vector control is clearly the most effective disease control strategy. Knowledge on the variety of environmental preferences and life histories is a pre requisite for

deriving strategies for their effective control (Service 1996; Annapoorani 2013).

The life cycle of mosquitoes has four-stages, namely egg, larva, pupa and adult. The eggs are usually laid in water or in places likely to be submerged later. The eggs may be laid singly as in Anopheles, Armigeres or Aedes, or collectively in egg rafts as in Culex. When the eggs are laid in water they may hatch within few days, but those laid out of water remain unhatched until submergence. The larvae are always aquatic. They are active and voracious creatures feeding on algae and other organic matter. The larval period may be as short as 7 to 10 days, but may extend to several months depending on the temperature and other environmental conditions. On the fourth moult, the mosquito larvae transform into the pupae. The pupae are unusual in that they remain mobile and responsive to external stimuli. The pupal period lasts for 1 to 5 days under favorable conditions. The pupal skin then splits along the back and the adult mosquito extricates itself. After the emergence of adults, they rest on the water or the edges of the breeding habits during which their wings unfold and the body wall gets hardened. The life span of the adults varies with the species, but often extends from several weeks to months. The biting and feeding habits of mosquitoes vary from species to species. Some species bite human beings (anthropophilic) as the females require blood meal for oviposition. Certain other species are animal biting (zoophilic). The male mosquitoes feed on nectar. Odour, temperature and carbon dioxide emanating from the body are the attraction of the females towards the host (VCRC 1989). After obtaining the blood meal, the mosquitoes rest. By the time the blood is fully digested and the ovaries and the eggs are fully developed. The gravid mosquitoes choose appropriate habitat to lay the eggs. The eggs undergo further development in the aquatic habitat and thus the life cycle continues (Latha 1998).

Mosquito colonies are needed in bulk in laboratories to carry out studies on vector biology, vector-parasite interactions, insecticide susceptibility, vaccine studies etc. It is important to maintain the original gene pool, physiological and behavioral characteristics of the insects under study, as much as possible. The quantity of experimental insects can be influenced by food availability, climatic conditions, etc. As these factors can strongly influence the outcome of experiments and thereby results, precise culture conditions need to be maintained (Lazzari et al. 2004; Takken 2005).

As the present study is intended to assess the larvicidal properties of phytochemicals in vector control, the availability of mosquitoes for experimentation in required quantities with respect to species specificity, genetic purity, age structure etc. are of prime importance. The present chapter discusses different aspects of the rearing process of mosquitoes under laboratory condition and standardization of conditions offering better efficiency.

Review of literature

Several factors can influence the colonization of mosquito species in the rearing process under laboratory conditions. A variety of nutritive and climatic factors are indispensable for larval growth, pupation and adult emergence under aseptic conditions in the laboratory (Singh and Brown 1957; Akov 1962).

Most adult females need only one blood meal to complete an oviposition cycle, but some individuals, especially the smaller ones, may require more than one blood meal before they complete their first gonotrophic cycle (Briegel 1990).

Clements (1992) studied the survivorship in mosquitoes related to temperature and nutritional availability under laboratory conditions. Lyimo

and Takken (1993) and Briegel and Horler (1993) reported that blood source influences feeding success, female fecundity, egg hatching and survival rates in mosquito species under laboratory conditions.

Wallace and Merritt (1999) carried out laboratory experiments with *Anopheles quadrimaculatus* and reported that larval survival was greater with enhanced food resources. It is reported that sugar availability can influence the nutrition-seeking behavior of both male and female mosquitoes and also reduce the the blood-feeding frequency of female mosquitoes (Chen et al. 2007). Spitzen and Takken (2005) revealed that the quality of experimental insects can be influenced by population density, food availability and climatic conditions.

Murthy and Rani (2009) demonstrated that the light intensity affect the development of the various stages in the life cycle of the mosquitoes. Costa (2010) revealed that overcrowding will result in slow development of larvae under laboratory conditions. It is also discussed that the problem can be overtaken by dilution of population, which would enhance the gain of adequate nutrition to the larval population.

Dodson et al. (2012) and Araujo et al. (2012) reported that temperature, humidity, light intensity and adequate food accelerates larval development and improves adult fitness in successful mosquito rearing.

Panigrahi et al. (2014) stated that the duration of light acts as the most important factor in oviposition behavior of Aedes mosquitoes under laboratory conditions. The experiment proved that the Aedes species laid the maximum number of eggs with normal 12 h light and dark phases (LD 12: 12).

The main focuses of the entire study is to assess the larvicidal properties of selected plants on the genus Aedes, Culex and Armigeres.The literature available on the rearing of above mosquito species under laboratory conditions is less and fragmentary in nature. An attempt has been carried out in the present study to assess the impact of temperature and humidity on the growth, reproduction and subsequent development of mosquito larvae belong to the above species under laboratory conditions.The present study, apart from giving insights on the standardization of growth conditions has also contributed to the production of specific mosquito species in bulk quantities for further experimentation.

Materials and Methods

Considering the significance of certain vectors in transmitting diseases in a public health perspective, three important mosquito species which are widely distributed in and around human habitats of Kerala were selected for the present study. The selected organisms include *Aedes albopictus* Skuse, *Culex sitiens* Wiedemann and *Armigeres subalbatus* Coquillett.

For the present study, larvae of three species were collected from different breeding habitats such as coconut shells, discarded cans (*Aedes albopictus* Skuse), mangrove habitats, coirpits (*Culex sitiens* Wiedemann) and from polluted water sources (*Armigeres subalbatus* Coquillett). The systematic position together with a description of individual species is given below:

***Aedes albopictus* Skuse**

Kingdom : Animalia

Phylum : Arthropoda

Class : Insecta

Order : Diptera

Family : Culicidae

Genus : Aedes

Species : *Aedes albopictus*

Aedes albopictus Skuse is an aggressive mosquito, which is ranked as one of the most invasive species worldwide (Plate1a). It is recognized as adangerous vector (Puggioli et al. 2016) involved in the transmission of several viral pathogens, including the West Nile virus, Yellow fever virus, St. Louis encephalitis, Dengue fever and Chikungunya. Their feeding activity is mostly during day time. Adults are known as tiger mosquitoes due to their conspicuous patterns of white stripe on black bodies, with a distinguishing white stripe down the center, beginning at the dorsal surface of the head and continuing along the thorax. The body size of individuals depends on larval density and nutritional availability. It is native to Eastern Asia, stretching into India, Japan, and several islands in the Pacific (Ayers et al. 2002). *Aedes albopictus* has become a significant pest in many communities and proven to be very difficult to suppress or to control due to their remarkable ability to adapt to various environments, their close contact with humans and their reproductive biology (Polaszek 2006; Diallo 2005).

The eggs of Aedes mosquitoes do not have a frill or floats but are elongate-oval in shape. Eggs of *Aedes albopictus* are laid along the sides of artificial or man-made containers and will hatch when water levels rise above the location of the egg, submerging it. It can remain dry for months but still remain viable and hatch when they become flooded with water (Goma 1966; Harwood and James 1979).

***Culex sitiens* Wiedemann**

Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Diptera
Family	:	Culicidae
Genus	:	Culex
Species	:	*Culex sitiens*

Culex sitiens Wiedemann is a medium sized species, with dark scaledproboscis, clearly marked median pale ring, which occupies about 1/5 of their total length (Plate1b). *Culex sitiens* breeds primarily in brackish water habitats, in tidal marshes and mangrove swamps. Their larvae and pupae are highly salinity tolerant, occurring in fresh, brackish and even pure seawater (Prummongkol 2009). They feed indoors and outdoors and rest outside during the day. Adult females are nocturnal biters and feed on humans as well as other mammals and birds. The developmental cycle of *Culex sitiens* are reported to take a total of 16 days at 24^{o}C, with eggs hatching in two days after being laid on the water surface. *Culex sitiens* is an insect native to coastal areas of the Oriental Region, Eastern Africa, South western Asia, Madagascar, Ryukyu Archipelago, Korea, northern Australia and many islands in the South Pacific (Becker 2010). *Culex sitiens* is a potential vector for Japanese encephalitis, Ross River Fever and Filariasis (Vythilingam et al. 2002).

***Armigeres subalbatus* Coquillett**

Kingdom	:	Animalia
Phylum	:	Arthropoda
Class	:	Insecta
Order	:	Diptera
Family	:	Culicidae
Genus	:	*Armigeres*
Species	:	*Armigeres subalbatus*

Armigeres subalbatus Coquillett is widely distributed throughout Southeast and East Asia (Kirti and Kaur 2015). They are commonly found close to human dwellings, especially in sub-urban areas with poor sanitation (Muslim et al. 2013). This mosquito also colonizes bamboo stumps, artificial containers, tree holes, hollow logs, banana stumps, fruit shells and husks, fallen leaves and artificial containers having organic matter and small collections of ground water. The females of a number of species readily attack and viciously bite humans.

The phenology of adults also seems to be strongly seasonal, with latitudinal variation. Rainfall has been suggested as an important factor for *Armigeres subalbatus* population changes, with significant adult abundance during and after high rainfall (Chaves et al. 2015). *Armigeres subalbatus* has been incriminated as a competent vector of Japanese encephalitis virus (Chen et al. 2000). It has also been reported to be a vector of filarial worm *Wuchereriabancrofti* in India and the dog heartworm *Dirofilariaimmitis* in Peninsular Malaysia (Muslim et al. 2013; Chaves et al. 2015) (Plate 1c).

Rearing studies on mosquito species

The rearing studies on mosquitoes were carried out following Clemons (2010) and Gerberc (1970) with minor modifications. For this, mosquito larvae collected from natural breeding sites (coconut shells, mangrove habitats, drainage water sources from lavatories etc.) were used. Samples from similar habitats were pooled in the laboratory and were subjected to species level identification using standard manual (Baraud 1934). In the laboratory, the larvae were transferred to separate trays and fed with appropriate amount of food (oats and yeast powder in the ratio 3:1) until the larvae were transformed into the pupae stage. The pupae were collected from the trays and transferred to glass containers containing 500ml of natural growth medium with the help of a dipper and kept in separate mosquito cages for adult emergence. The entire procedure is given in Plate 2. After complete emergence, the mosquitoes were again identified and species confirmed before rearing. After identification, the adults of *Aedes albopictus, Culex sitiens* and *Armigeres subalbatus* were transferred to colony cages of size 1 x 1 x 1 ft. maintained separately.

The laboratory level colonization and subsequent experimentation on the three species was achieved in cages separately. For each species, 10 cages were maintained, with sufficient number of adults. Each cage was maintained at a specific temperature and humidity using lighted bulbs and damp clothes. Both the temperature (degree celcius) and humidity (%) were measured using a thermo hygrometer (HTC-1). In accordance with earlier reports possible on temperature and humidity (Chaves et al. 2015) favoring oviposition rate and hatchout percentage, temperature ranging from 27-28°C and humidity range nearing 70% was maintained in rearing cages. Each rearing cage was provided with a 200 ml bottle with a cotton wick that is soaked with 5-10% sucrose/ glucose solution. The adult female mosquitoes were fed on blood, procured from slaughter houses and added with anti coagulant to facilitate egg laying by artificial membrane methods; Mishra feeder and latex condoms

(Mishra et al. 2005). The mosquitoes were allowed to feed in accordance with their requirements. A control set was also maintained.

A small filter paper, wrapped to an oval shape, was kept in a china dish containing ~100 ml tap water (pH 6.4) for (*Aedes albopictus*) ; saline water (*Culex sitiens*) and foul smelling water (*Armigeres subalbatus*) inside the respective cages for oviposition. The number of eggs laid by respective species of mosquitoes was estimated within a period of two days and the resultant temperature and humidity was recorded.

Collection and care of eggs, larvae and pupae

Egg rafts of *Culex sitiens,* single eggs of *Aedes albopictus* and *Armigeres subalbatus* received under culture conditions were transferred to bowls containing respective growth medium (tap water, filtered saline water and seasoned water containing dog biscuit and yeast). A pinch of food (oats and yeast powder in the ratio 3:1) was added to it (except for Armigeres) and eggs were allowed to hatch to larvae during the forthcoming days. The larvae were made to undergo four moults. The pupae were allowed to emerge into adults in the forthcoming days. The adults (both males and females) were then kept in the rearing cages, fed on 5-10% sucrose before they were again blood-fed to begin the next cycle.

Results and Discussion

The laboratory level rearing studies on the three species was achieved in cages separately. For each species, 10 cages including control were maintained, containing sufficient number of adults. Each cage was maintained at a specific temperature and humidity for finding out the optimum condition which favoured higher extent of egg laying by mosquitoes and the conditions of hatch out from egg to larvae. The conditions provided for the rate of oviposition and percentage of hatch out of vectors belonging to the species *Aedes albopictus, Culex sitiens and Armigeres subalbatus* are given in tables 1.1, 1.2 and 1.3 respectively.

Table 1.1: Mean±S.D of oviposition rate and hatch out percentage of *Aedes albopictus* under different temperature and humidity

Aedes albopictus							
Cage No.	**Mean**		**Mean±S.D**	**S.E**	**Mean±S.D**	**S.E**	**Hatch%**
	Temperature°C	**Humidity%**	**Egg**		**Larvae**		
1	27.03	72.74	220.14±28.90	66.38	208.4±27.00	63.27	94.67
2	27.05	71.65	207.14±37.36	62.46	194.29±44.19	58.58	93.80
3	27.07	71.87	212.29±41.17	64.01	204.43±45.62	61.81	96.30
4	27.19	75.21	241.14±28.21	72.71	232.29±30.66	70.25	96.33
5	27.27	72.49	199.86±52.76	60.26	189.00±49.89	57.63	94.57
6	27.42	73.87	219.57±49.21	66.20	207.71±49.90	62.63	94.60
7	27.54	74.10	246±35.30	74.17	229.0±34.05	67.37	93.09
8	27.59	73.81	222.86±43.87	67.19	211.14±46.21	64.57	94.74
9	28.15	74.51	205.29±39.60	61.90	189.14±34.81	57.59	92.13
10	34.23(Control)	60.65	147.86±10.54	44.58	131.14±9.77	39.54	88.69

In the case of *Aedes albopictus*; an increase inthe number of eggs (246±35.30) was observed at a temperature of 27.54 ^{o}C and humidity of 74.10% respectively, whereas a decrease in number of eggs (147.86±10.54) was observed in control at a temperature of 34.23^{O}C and humidity of 60.65%. In the case of larvae, highest number (232.29±30.66) was observed at a temperature of 27.19^{O}C and humidity of 75.21%. Larval number was decreased to 131.14±9.77 at a temperature of 34.23^{O}C and humidity of 60.65% in control (Table 1.1). Hence a temperature ranging from 27.19 - 27.54 and humidity ranging from 74.10 to 75.21 was noted to be ideal for egg laying and larval emergence in *Aedes albopictus*.

Table 1.2: Mean±S.D of oviposition rate and hatch out percentage of *Culex sitiens* under different temperature and humidity

Culex sitiens							
CageNo.	**Mean**		**Mean±S.D**	**S.E**	**Mean±S.D**	**S.E**	
	Temperature°C	**Humidity%**	**Egg raft**		**Larvae**		**Hatch%**
1	27.03	72.74	3.92±1.73	0.50	1120.25±513.50	148.24	93.33
2	27.05	71.65	3.92±1.73	0.50	1217.33±531.48	153.43	96.59
3	27.07	71.87	3.33±1.72	0.50	904.17±498.12	143.80	84.09
4	27.19	75.21	3.83±1.27	0.37	1103.08±266.31	76.88	91.92
5	27.27	72.49	3.42±1.31	0.38	1039.08±397.89	114.86	85.44
6	27.42	73.87	3.33±1.72	0.50	882.92±482.51	139.29	78.75
7	27.54	74.10	3.75±1.42	0.41	1142.42±444.16	128.22	91.36
8	27.59	73.81	3.83±1.47	0.42	1122.58±462.67	133.56	89.61
9	28.15	74.51	3.83±1.27	0.37	1181.17±343.15	99.06	89.47
10	34.23 (Control)	60.65	2.58±1.08	0.31	821±371.89	107.36	80.49

In the case of *Culex sitiens* the number of egg raft and thereby eggs (Christophers 1945) and larvae were found to be reduced with increase in temperature and humidity. Highest number (3.92±1.73) of egg raft was observed at a temperature of 27.03^{O}C and humidity of 72.74%. Lowest number of egg raft was observed with control at 34.23^{O}C and humidity of 60.65%. In the case of larvae, highest number (1217.33±531.48) was observed at a temperature of 27.05 ^{O}C and humidity of 71.65%. Larval number (821±371.89) was found to be decreased with an increased temperature (34.23^{O}C), and decreased humidity (60.65%). Hence a temperature ranging from 27.03 - 27.05 ^{O}C and humidity ranging from 71.65 - 72.74% was found to be ideal for oviposition and hatch out of mosquitoes.

Table 1.3: Mean±S.D of oviposition rate and hatch out percentage of *Armigeres subalbatus* under different temperature and humidity

Armigeres subalbatus							
Cage No.	**Mean**		**Mean±S.D**	**S.E**	**Mean±S.D**	**S.E**	**Hatch%**
	Temperature°C	**Humidity%**	**Egg**		**Larvae**		
1	27.03	72.74	172.29±58.91	22.27	159.43±62.35	23.57	92.54
2	27.05	71.65	213.14±34.89	13.19	206.14±34.67	13.10	96.72
3	27.07	71.87	248.29±38.74	14.64	241.00±34.79	13.15	97.06
4	27.19	75.21	239.14±29.11	11.00	228.71±28.23	10.67	95.64
5	27.27	72.49	281.71±33.7	12.65	273.71±33.60	12.70	97.16
6	27.42	73.87	225.71±65.76	24.85	216.57±66.76	25.23	95.95
7	27.54	74.10	264.71±62.81	23.74	257.86±53.43	20.19	97.41
8	27.59	73.81	289.29±28.77	10.88	273.86±20.87	7.89	94.67
9	28.15	74.51	212.00±41.26	15.59	207.71±39.52	14.94	97.98
10	34.23 (Control)	60.65	163.86±49.00	18.52	147.43±46.89	17.72	89.97

An increase in egg and larvae of *Armigeres subalbatus* was found at a temperature of 27.59^{O}C. Similarly an increase in egg and larvae was observed at a humidity of 74.51% (egg) and 73.81% (larvae) respectively. A reduction in egg and larvae of *Armigeres subalbatus* was found in control at a temperature and humidity of 34.23^{O}C and 60.65% respectively. The correlation between temperature and humidity with the hatch out of mosquitoes belonging to the species *Aedes albopictus*, *Culex sitiens* and *Armigeres subalbatus* are given in Table 1.4

Table 1.4.Correlation of various factors with the hatch out of larvae

Hatch out	Temperature	Humidity
Aedes albopictus	-0.87075	0.764647
Culex sitiens	-0.09983	0.550037
Armigeres subalbatus	-0.6806	0.752858

Throughout experimentation, the temperature and humidity were found to influence the rearing of mosquito species to a greater extent. In the case of *Aedes albopictus*, *Culex sitiens*and *Armigeres subalbatus*, reduction in the number of egg was observed with the increase in temperature, evidenced by lesser number of oviposition and larval emergence in control. Thus a temperature range of 27.19 to 27.54 ^{O}C and a humidity range of 74.10-75.21% were noted to be ideal for culture conditions of *Aedes albopictus*; a temperature range of 27.03 to 27.05^{O}C and humidity range of 71.65-72.74% for *Culex sitiens* and a temperature of 27.59 ^{O}C and humidity of 74.51% was noted to be ideal for set for *Armigeres subalbatus*.

Also in the present experiment, the number of hatch out of larvae was correlated positively with humidity and negatively with temperature. Ramsey (1988) described the correlation of oviposition rate to temperature and humidity. High temperatures associated with low humidity resulted in a

decrease in the hatching rate of mosquito larvae. Alto and Juliano (2001) also revealed that the hatch out rate of *Aedes albopictus* increased with increased humidity and decreased temperature.

Subsequent rearing of mosquitoes for the production of larvae for larvicidal studies were carried out in accordance with the present conditions standardized in the laboratory.

Summary and Conclusion

Mosquito larvae, collected from natural habitats were pooled in the laboratory and subjected to species level identification using standard manual. The screened larvae were reared to adults in the laboratory under controlled conditions. From these adults, the first generation larvae were produced at varying temperature and humidity. The larvae in the growth medium were fed with oats and yeast in the ratio 3:1. The number of eggs, larvae and hatchout percentages were estimated for each species of mosquito reared at a varying condition of temperature and humidity.

The result of the present study revealed that a temperature ranging from 27.19 to 27.54^{O}C and humidity ranging from 74.10 to 75.21% was ideal for oviposition and larval emergence in *Aedes albopictus*. Likewise a range of temperature from 27.03 to 27.05^{O}C and humidity from 71.65-72.74% was ideal for the growth of *Culex sitiens*. In case of *Armigeres subalbatus* a temperature of 27.59^{O}C and humidity of 74.51% was noted to be ideal for egg and larval production. Also the number of hatch out of larvae was correlated positively with humidity and negatively with temperature.

The conditions standardized for higher production of egg and larvae were maintained throughout the rearing process for the production of larvae for larvicidal bioassay.

CHAPTER II

SCREENING FOR LARVICIDAL ACTIVITY OF PLANT EXTRACTS ON MOSQUITO VECTORS

Introduction

Mosquitoes are pestiferous vectors which are responsible for the transmission of various dreadful diseases, causing millions of deaths every year (Arivoli et al. 2011). Their eradication is becoming a pre requisite for establishing proper health and hygiene. Environmental improvement is one option, which relates to those measures, which help in preventing the occurrence of breeding and resting places of mosquitoes (Arunachalam et al.1992). Another approach for the control of mosquito borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings (Sharma 1996). Most mosquito control programmes target on the larval stage at their breeding sites with larvicides since adulticides may reduce the adult population only temporarily. Thus it has been confirmed that the most efficient approach to check the population of mosquitoes is to target the larvae (Chung et al. 2009; Conti et al. 2013). Over these years, indiscriminate use of chemical insecticides has resulted in the development of resistance by these organisms, resulting in rebounding vectorial capacity. Moreover, such chemicals have given rise to serious environmental issues. This has led to the search for phytochemicals, which are having several advantages over the chemical insecticides in the control of vectors. The present endeavor is to assess the larvicidal potential of the aqueous extracts of selected plants against mosquito larvae.

Review of literature

Mosquitoes are pests and vectors of dreadful diseases. Several mosquito species belonging to the genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like Malaria, Filariasis, Japanese encephalitis, Dengue fever, Yellow fever etc. One of the approaches for the control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings (Sharma et al. 2001). Herbal products with proven potential as 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 (Benjamin and Pandian 1997). Several studies have emphasized the importance of research and development of herbal substances for controlling mosquitoes (Shaalan et al. 2005). Botanical insecticides may serve as suitable alternatives to synthetic ones in future, as they are environmentally safe, effective and inexpensive (Chaithong et al. 2006).

Attempts have already been reported to assess the larvicidal properties of plants. Jaswanth et al. (2002) evaluated the larvicidal activity of *Annona squamosa* leaves against *Culex quinquefasciatus*. Omena et al. (2007) screened out the larvicidal activities of some Brazilian medicinal plants against *Aedes aegypti*. Kaushik and Saini (2008) highlighted the larvicidal activity of the leaf extract of *Millingtonia hortensis* against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. Larvicidal effect of *Lantana camara* against *Aedes aegypti* and *Culex quinquefasciatus* was reported by Sathish kumar and Manimegalai (2008). Larvicidal activity of *Leucus aspera* (Wild.) against the larvae of *Culex quinquefasciatus* and *Aedes aegypti* was recorded by Maheswaran et al. (2008). Nikkon et al. (2009) studied the larvicidal activity of crude extracts from the stem and fruits of *Duranta repens* against the larvae of *Culex quinquefasciatus.* Vasudevan et al.

(2009) evaluated the efficiency of extracts of dried fruits of *Piper nigrum* against the larvae of *Culex quinquefasciatus.* Rahuman et al. (2009b) evaluated the efficacy of extracts against *Culex quinquefasciatus*. Abubakar et al. (2009) focused on the combined effect of *Aloe barbadensis* and *Bryophyllum pinnatum* on mosquito population as an effective anti-mosquito agent. Maniafu et al. (2009) analysed the larvicidal activity of extracts from three species of *Plumbago* against *Anopheles gambiae.* The LC_{50} values of *Plumbago zeylanica* (hexane extract), *Plumbago stenophylla* (chloroform extract) and *Plumbago dawei* (ethyl acetate extract) were 6.4 µg/mL, 6.7 µg/mL and 4.1 µg/mL respectively. Larvicidal efficacy of leaf extracts of *Catharanthus roseus* and *Lantana camara* against the life stages of mosquito vector *Aedes aegypti* was carried out by Remia and Logaswamy (2010). The leaf extracts of *Catharanthus roseus* was more potent than *Lantana camara*. The LC_{50} of *Catharanthus roseus* was 75.31 ppm for the second instar larvae, whereas it was 156.85 ppm for fourth instar and 207.83 ppm for the pupae. Kalu et al. (2010) reported the larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. Larvicidal activity of *Trigonella foenum* and *Nerium oleander* leaves against mosquito larvae was observed by Lokesh et al. (2010). Screening of the weed plant species *Croton bonplandianum* for larvicidal activity on *Aedes aegypti* was attempted by Jeeshna et al. (2010). Larvicidal efficacy of latex and extract of *Calotropis procera* against *Culex quinquefasciatus* and *Anopheles stephensi* was analysed by Shahi et al. (2010). Okigbo et al (2010) investigated the potency of petroleum ether leaf extracts of *Azadirachta indica*, *Ocimum gratissimum and Hyptis suaveolens* as larvicides against Culex mosquito. Mwine et al. (2011) reported the evaluation of larvicidal properties of the latex of *Euphorbia tirucalli* against the larvae of *Anopheles* mosquitoes. Singha et al. (2011) investigated the synergistic effect of crude and methanol extract of *Croton caudatus* and *Tiliacora acuminate* (flowers)

against the larval form (*Culex quinquefasciatus*). Kundu et al. (2013) evaluated the larvicidal activity of crude and ethyl acetate extract of matured seed coat of *Cassia sophera* against *Culex quinquefasciatus.*

Gomathi et al. (2014) studied the larvicidal efficacy of medicinal plant extracts for the control of mosquito vectors. Durga et al. (2014) tested the larvicidal activity of methanol, ethyl acetate, hexane and acetone extracts of *Wedelia chinensis* against the early fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Nkya et al. (2014) screened petroleum ether, ethyl acetate and methanolic extracts of *Moringa oleifera* and revealed possible larvicidal activity against mosquito. The larvicidal efficiency of leaf extracts (hexane, diethyl ether, dichloromethane and methanol) of *Eucalyptus globulus* and *Centella asiatica* against two different strains of *Aedes aegypti* and *Anopheles stephensi* was investigated by Nair et al. (2014). Dass and Mariappan (2014) studied the larvicidal and pupicidal efficacy of *Lawsonia inermis* and *Murraya exotica* leaves against *Culex quinquefasciatus*. The LC_{50} values of *Murraya exotica* for larvae and pupae were 135.539 ppm and 178.571 ppm respectively. Likewise for *Lawsonia inermis* it was 139.057 and 188.151 for the pupa. Baranitharan and Dhanasekaran (2014) assessed the larvicidal potential of the leaf extract from the medicinal plant *Croton sparciflorus* against three important mosquito vectors - *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. Nayak and Rajani (2014) attempted the properties of leaf extract of *Vitex negundo* against *Culex quinquefasciatus.* Yogalakshmi et al. (2014) carried out studies to evaluate the larvicidal activity of potential essential oil from *Cestrum nocturnum* (Solanaceae) against *Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti.* Yadav et al. (2014) screened some weeds (*Vernonia cinerea, Prosopis juliflora, Hyptis suaveolens and Malvastrum coromandelianum*) against *Aedes albopictus*.

Dohutia et al. (2015) carried out mosquito larvicidal propertiesof indigenous plants of North East India against *Anopheles stephensi, Stegomyia aegypti* and *Culex quinquefasciatus* mosquitoes. Ajaegbu et al. (2016) evaluated the larvicidal activity of crude extracts (methanol, hexane, dichloromethane, acetone, ethyl acetate and methanol) of *Spondias mombin* leaf against larvae of *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus*. Zareen et al. (2016) investigated the larvicidal activity of Eucalyptus leaf extract against larvae of Anopheles mosquitoes. Setiawan et al. (2017) evaluated the potential of *Pinus merkusii* tree bark extract against the larvae of *Aedes aegypti* through larvicidal bioassays. Most of the studies revealed the efficacy of plant extracts in varying degrees.

Materials and methods

The present chapter envisages assessing the larvicidal activity of aqueous extracts from plants belonging to varied taxonomic groups in the control of mosquito vectors collected from breeding sites and reared from eggs in the laboratory. The experiment has been undertaken through the following steps:

Plant collection and Processing

The selection of plants was carried out based on their reported medicinal / aromatic properties andlocal availability. Standard keys for taxonomic identification (Gamble 1935) and services of experts in taxonomy were sought for the identification of plant specimens. Species descriptions were supplemented with genus name, species name and author citations. Herbaria of plants were prepared and preserved. Photographs were taken and depicted as plates.

Preparation of extracts

In the present study 120 plant species were screened for their anti-larval activity. The list of plants attempted is given in Table 2.1 and their photographs in plates 3- 8. The materials were taken from healthy plants free from dust, dirt and other impurities and were brought to the laboratory for subsequent processing. The washed plant materials were chopped properly and kept in clean trays. For preparation of extracts, approximately twenty grams (20gms) of respective plant material (wholeplant, shoot, leaves, flower, fruit, Seed, dry bulb, rhizome, scape or peduncle and phylloclade) was taken and ground in a homogenizer using distilled water. The extract was filtered and the filtrate was made upto 1000 ml with distilled water and retained as stock solution for further experimentation. Serial dilution of the stock solutions was prepared for assessing treatment efficiencies.

General Screening for larvicidal activity

Mosquito larvae, collected from heterogeneous breeding sites were used in the present study. The larvae were pooled in the laboratory and subjected to screening for larvicidal activity. For screening, twenty larvae (third instar), each were introduced into treatment trays containing 250 ml of their natural growth medium prepared in ordinary water added with oats and yeast in the ratio 3:1. To the treatment set, respective concentrations of the plant extract (0.5, 1.0, 2.0, 4.0 and 8.0 ml) were added from the stock solution. A control was maintained, containing only larvae and natural growth medium. Mortality counts of larvae were monitored at regular intervals i.e. 6, 12, 24, 48, 72 and 96 Hours after Treatment (HAT). Larvae were considered dead if they settle and remain motionless in the bottom of the test beaker with no response to light or mechanical stimulus or not recovering life functions even after being transferred to their growth medium (Murugan et al. 2007; Arivoli et al. 2011). The bioassay for each plant was repeated three times with

three different sets of stock solutions and with three different batches of mosquito larvae.

Species specific larvicidal bioassay

Bioassay for species specific larvicidal activity was carried out using WHO procedure (WHO 1981), with minor modifications. For this, the plants which were found to be effective in general screening were used to assess their larvicidal activities on specific mosquito vectors like *Aedes albopictus* Skuse, *Culex sitiens* Wiedemann and *Armigeres subalbatus* Coquillett. The experirmental layout was similar to that of general screening, except for mosquito larvae, which were brought from species specific rearing facilities maintained under laboratory conditions (Chapter I).

Statistical analysis

The concentration at which mortality observed (mg/ml) was corrected using Abbott's formula (Abbott 1925).

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

All data were subjected to Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) for windows version 16.0. This is being carried out to determine the significant differences, if any, in the effects of different plant extracts at the same concentration. Duncan's new Multiple Range Post Hoc test was used to separate the means at $p < 0.01$ for significant data after ANOVA analysis. $LC_{50}$was calculated using Probit-Regression method (Finney 1971).

Results and Discussion

The toxicity of aqueous extracts of 120 species of plants was experimented against third instar larvae of mosquitoes. Details of plants used for the present study and conditions at which highest mortality has been noticed are depicted in Table 2.1.

Table 2.1 List of plant species used for the preparation of aqueous extracts and their impact on Mosquito larvae

SI No	Plants	Family	Plant parts used	Condition at which larval mortality noticed	Mortality%
1	*Acalypha hispida* Burm.f.	Euphorbiaceae	Leaf	8ml96 hours	0
2	*Acalypha indica* L.	Euphorbiaceae	Leaf	8ml 96 hours	0
3	*Adenanthera pavonina* L.	Fabaceae	Leaf	8ml 96 hours	0
4	*Adenocalymma alliaceum* (Lam.) Miers	Bignoniaceae	Leaf	0.5ml 96 hours	100
5	*Adhatoda vasica* Nees	Acanthaceae	Leaf	8ml 96 hours	0
6	*Aegle marmelous* (L.) Correa	Rutaceae	Leaf	8ml 96 hours	0
7	*Aerva lanata* (L.) Juss.	Amaranthaceae	Shoot	8ml 96 hours	0
8	*Allamanda cathartica* L.	Apocynaceae	Leaf	8ml 96 hours	0
9	*Allium sativum* L.	Amaryllidaceae	Dry Bulb	0.5ml 48 hours	100
10	*Aloe vera* (L.) Burm.f.	Asphodelaceae	Leaf	8ml 96 hours	85
11	*Alpinia purpurata* (Vieill.) K. Schum.	Zingiberaceae	Leaf	0.5ml 72 hours	100
12	*Anisomelos malabarica* (L.) R.Br.ex Sims	Lamiaceae	Leaf	8ml 96 hours	60
13	*Apium graveolens* L.	Apiaceae	Leaf	8ml 12 hours	100

14	*Aristolochia indica* L.	Aristolochiaceae	Leaf	8ml 96 hours	0
15	*Artocarpus heterophyllus* Lam.	Moraceae	Leaf	8ml 96 hours	100
16	*Averrhoa bilimbi* L	Oxalidaceae	Leaf	8ml 96 hours	0
17	*Azadiracta indica* (A.Juss.)	Meliaceae	Leaf	8ml 96 hours	95
18	*Bacopa monnieri* (L.) Edwall	Plantaginaceae	Leaf	8ml 96 hours	95
19	*Bauhinia purpurea* L.	Fabaceae	Leaf	8ml 96 hours	0
20	*Blumea oxyodonta* DC.	Asteraceae	Shoot	4ml 24 hours	100
21	*Boerhaavia diffusa* Var.hirsuta kuntze	Nyctaginaceae	Leaf	8ml 96 hours	0
22	*Calotropis gigantea* (L.) R. Br.	Apocynaceae	Leaf	1ml 96hours	100
23	*Calycopteris floribunda* (Roxb.) Poir	Combretaceae	Leaf	8 ml 96 hours	0
24	*Canna indica* L.	Cannaceae	Leaf	8 ml 96 hours	0
25	*Capsicum annuum* L.	Solanaceae	Leaf	8 ml 96 hours	95
26	*Careya arborea* Roxb.	Lecythidaceae	Fruit	8 ml 96 hours	0
27	*Carica papaya* L.	Caricaceae	Leaf	4 ml 96 hours	100
28	*Cassia fistula* L.	Fabaceae	Leaf	8 ml 96 hours	0
29	*Chromolaena odorata* (L.) R.M. King & H. Rob.	Asteraceae	Leaf	4 ml 72 hours	100
30	*Chrysanthemum morifolium* Ramat.	asteraceae	Leaf	8 ml 96 hours	80
31	*Citharexylum spinosum* Kunth.	Verbenanaceae	Leaf	8 ml 96 hours	0
32	*Citrus medica* L.	Rutaceae	Leaf	0.5ml 24 hours	100
33	*Citrus reticulata* Blanco	Rutaceae	Peel	0.5ml12 hours	100
34	*Cleome viscose* L.	Cleomaceae	Shoot	8 ml96 hours	80
35	*Clerodendron viscosum* Vent.	Lamiaceae	Leaf	8 ml96 hours	95

36	*Clitoria ternatea* L.	Fabaceae	Leaf	8 ml 96 hours	0
37	*Codiaeum variegatum* (L.) Rumph. ex A. Juss.	Euphorbiaceae	Leaf	8 ml 96 hours	35
38	*Coleus aromaticus* (Roxb.) Benth.	Lamiaceae	Leaf	8 ml 96 hours	55
39	*Coleus blumei* Benth.	Lamiaceae	Leaf	8 ml 96 hours	0
40	*Commelina diffusa* Burm.f.	Commelinaceae	Leaf	8 ml 96 hours	0
41	*Coriandrum sativum* L.	Apiaceae	Leaf	8 ml 96 hours	90
42	*Cosmos sulphureus* Cav.	Asteraceae	Shoot	8 ml 96 hours	85
43	*Couroupita guianensis* Aubl.	Lecythidaceae	Flower	8 ml 96 hours	75
44	*Crassocephalum crepidioides* (Benth.) S. Moore	Asteraceae	Leaf	8 ml 96 hours	80
45	*Cryptostegia grandiflora* Roxb. ex R. Br.	Apocynaceae	Leaf	8 ml 96 hours	60
46	*Cucurbita pepo* L.	Cucurbitaceae	Leaf	8ml 96 hours	0
47	*Cuphea hyssopifolia* Kunth	Lythraceae	Leaf	8 ml 96 hours	0
48	*Curcuma longa* L.	Zingiberaceae	Rhizome	1 ml 96 hours	100
49	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Leaf	8ml 96 hours	95
50	*Cyperus rotundus* fo.latifolius kuk.	Cyperaceae	Leaf	8ml 96 hours	0
51	*Datura metel* L.	Solanaceae	Leaf	8 ml 96 hours	65
52	*Desmodium gangeticum* Blanco	Fabaceae	Leaf	8 ml 96 hours	0
53	*Dieffenbachia seguine* (Jacq.) Schott	Araceae	Leaf	8 ml 96 hours	50
54	*Eclipta alba* (L.) Hassk.	Asteraceae	Leaf	8 ml 96 hours	0
55	*Eichhornia crassipes* (Mart.) Solms	Pontederiaceae	Leaf	8ml 96 hours	20
56	*Elephantopus scaber* L.	Asteraceae	Wholeplant	8ml96 hours	65

57	*Emilia sonchifolia* (L.) DC.	Asteraceae	Leaf	8 ml 96 hours	95
58	*Epipremnum pinnatum* cvAureum Nicolson.	Araceae	Leaf	8 ml 96 hours	0
59	*Eucalyptus tereticornis* Sm.	Myrtaceae	Leaf	4 ml 12 hours	100
60	*Euphorbia antiquorum* L.	Euphorbiaceae		8 ml 96 hours	0
61	*Gliricidia sepium* (Jacq.) kunth ex Walp	Fabaceae	Leaf	8ml 96 hours	90
62	*Glycosmis pentaphylla* (Retz.) DC.	Rutaceae	Leaf	8ml 96 hours	95
63	*Hemidesmus indicus* (L.) R. Br. ex *Schult.*	Apocynaceae	Wholeplant	8 ml 96 hours	50
64	*Hibiscus rosa-sinensis* L.	Malvaceae	Leaf	8 ml 96 hours	0
65	*Hyptis suaveolens* (L.) Poit	Lamiaceae	Shoot	4 ml 48 hours	100
66	*Ixora coccinea* Comm. ex Lam.	Rubiaceae	Leaf	8 ml 96 hours	0
67	*Lantana camara* L.	Verbenaceae	Leaf	8 ml 72 hours	100
68	*Lawsonia inermis* L.	Lythraceae	Leaf	8 ml 96 hours	0
69	*Leucas aspera* (wild.)	Lamiaceae	Shoot	8 ml 96 hours	90
70	*Macaranga peltata* Boivin ex Baill.	Euphorbiaceae	Leaf	8 ml 96 hours	0
71	*Mangifera indica* L.	Anacardiaceae	Leaf	8 ml 72 hours	100
72	*Mentha arvensis* L.	Lamiaceae	Leaf	8 ml 72 hours	100
73	*Mesua ferrea* L.	Calophyllaceae	Leaf	2 ml 96 hours	100
74	*Millettia pinnata* (L.) Panigrahi	Fabaceae	Leaf	8 ml 96 hours	65
75	*Mimosa pudica* L.	Fabaceae	Whole Plant	2 ml 72 hours	100
76	*Mitracarpus hirtus*(L.) DC.	Rubiaceae	Leaf	8 ml 96 hours	95
77	*Momordica charantia* L.	Cucurbitaceae	Fruit	0.5 ml 24 hours	100
78	*Morinda pubescens* Sm.	Rubiaceae	Leaf	8 ml 96 hours	0

79	*Moringa oleifera* Lam.	Moringaceae	Leaf	8 ml 96 hours	0
80	*Murraya koenigii* (L.) Spreng.	Rutaceae	Leaf	8 ml 24 hours	100
81	*Musa X paradisiaca* L	Musaceae	Scape (Peduncle)	0.5ml 24 hours	100
82	*Nerium oleander* L.	Apocynaceae	Leaf	8 ml 96 hours	80
83	*Ochroma grandiflorum* Rowlee	Malvaceae	Fruit	8 ml 96 hours	95
84	*Ocimum gratissimum* L.	Lamiaceae	Leaf	8 ml 48 hours	100
85	*Opuntia dillenii* (Ker Gawl.) Haw.	Cactaceae	Phylloclade	8 ml 96 hours	85
86	*Phyllanthus amarus* Schumach. & Thonn.	Phyllanthaceae	Leaf	8 ml 96 hours	0
87	*Phyllanthus emblica* L.	Phyllanthaceae	Dry Seed	8 ml 96 hours	95
88	*Physalis minima* L.	Solanaceae	Leaf	8 ml 96 hours	95
89	*Pimenta dioica* (L.) Merr.	Myrtaceae	Leaf	0.5ml 96 hours	100
90	*Piper betle* L.	Piperaceae	Leaf	8 ml 96 hours	40
91	*Polyalthia longifolia* (Sonn.) Thwaites	Annonaceae	Leaf	0.5ml 48 hours	100
92	*Pouteria campechiana* (Kunth) Baehni	Sapotaceae	Leaf	8 ml 96 hours	0
93	*Praecitrullus fistulosus* (stocks) Pangalo	Cucurbitaceae	Leaf	8 ml 96 hours	0
94	*Psidium guajava* L.	Myrtaceae	Leaf	8 ml 96 hours	0
95	*Ricinus communis* L.	Euphorbiaceae	Seed	0.5ml 6 hours	100
96	*Saraca asoca* (Roxb.) De Wilde	Fabaceae		8 ml 96 hours	0
97	*Saritaea magnifica* (W. Bull) Dugand	Bignoniaceae	Leaf	0.5ml 24 hours	100
98	*Scoparia ducis* L.	Plantaginaceae	Shoot	8 ml 96 hours	0
99	*Senna alata* (L.) Roxb.	fabaceae	Leaf	8 ml 96 hours	0
100	*Sida rhombifolia* L.	Malvaceae	Leaf	8 ml 72 hours	100

101	*Solanum nigrum* L.	Solanaceae	Leaf	8 ml 96 hours	80
102	*Spilanthes calva* DC.	Asteraceae	Flower	0.5ml 24 hours	100
103	*Synedrella nodiflora* (L.) Gaertn.	Asteraceae	Whole	8 ml 96 hours	55
104	*Syzygium cumini* (L.) Skeels	Myrtaceae	Seed	8 ml 96 hours	80
105	*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.	Apocynaceae	Leaf	8 ml 96 hours	0
106	*Tagetes erecta* L.	Asteraceae	Flower	8 ml 96 hours	60
107	*Tamarindus indica* L.	Fabaceae	Leaf	8 ml 96 hours	0
108	*Tephrosia purpurea* (L.) Pers.	Fabaceae	Leaf	8 ml 96 hours	0
109	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Dry Fruit	4 ml 96 hours	100
110	*Terminalia chebula* Ret.	Combretaceae	Dryfruit	8 ml 96 hours	75
111	*Thevetia neriifolia* Juss.ex Steud.	Apocynaceae	Leaf	8 ml 96 hours	0
112	*Tridax procumbens* L.	Asteraceae	Flower	8 ml 72 hours	100
113	*Trigonella foenum graecum* L.	Fabaceae	Seed	8 ml 96 hours	90
114	*Vernonia cinerea* (L.) Less.	Asteraceae	Whole Plant	8 ml 96 hours	85
115	*Vetiveria zizanioides* (L.) Nash	Poaceae	Root	8 ml 96 hours	40
116	*Vinca rosea* L.	Apocynaceae	Leaf	8 ml 24 hours	100
117	*Vitex negundo* L.	Lamiaceae	Leaf	4 ml 72 hours	100
118	*Wedelia trilobata* (L.) Hitchc	Asteraceae	Leaf	8 ml 96 hours	25
119	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome	0.5 ml 6 hours	100
120	*Ziziphus jujuba* (L.) Lam.	Rhamnaceae	Dry Seed	8 ml 96 hours	100

Out of 120 plants attempted, 77 plants were found to be effective in the control of mosquito larvae at varying concentrations and retention times

(Table 2.1). Aqueous extracts of 31 plants showed 100% and 46 plants upto 95% larvicidal activity against the third instar larvae of mosquitoes (Table 2.2, figures 2.1- 2.5). Of the 31 plants, extracts from thirteen species, ie. *Adenocalymma alliaceum* (Lam.) Miers, *Allium sativum* L., *Alpinia purpurata* (Vieill.) K. Schum., *Citrus reticulata* Blanco, *Citrus medica* L. *Momordica charantia* L., *Pimenta dioica* (L.) Merr., *Polyalthia longifolia* (Sonn.) Thwaites, *Ricinus communis* L., *Saritaea magnifica* (W. Bull) Dugand, *Spilanthes calva* DC., *Zingiber officinale* Roscoe and *Musa X paradisiaca* L. at 0.5 ml (Fig. 2.1) ; *Calotropis gigantea* (L.) R.Br.and *Curcuma longa* L. at 1ml (Fig 2.2) ; *Mesua ferrea* L. and *Mimosa pudica* L. at 2ml (Fig. 2.3) ; *Carica papaya* L., *Chromolaena odorata* (L.) R.M. King & H. Rob., *Vitex negundo* L., *Hyptis suaveolens* (L.) Poit, *Terminalia bellirica* (Gaertn.) Roxb., *Eucalyptus tereticornis* Sm.at 4ml (Fig.2.4) and *Apium graveolens* L., *Lantana camara* L., *Ocimum gratissimum* L., *Sida rhombifolia* L., *Tridax procumbens* L., *Vinca rosea* L., *Ziziphus jujuba* (L.) Lam., *Murraya koenigii* (L.) Spreng.at 8ml (Fig. 2.5) concentrations showed 100% larvicidal activity at varied retention time. The LC_{50} were calculated (Table 2.3 and Figure 2.6 to 2.8). Mortalities were recorded in respective control sets. Remaining 46 plant species only showed moderate (20-95%) larvicidal activity compared to others and thus these species were redundant from further bioassays.

Table 2.2: Larvicidal activity of plant extracts showing 100% mortality with various concentration and retention time

SI No	Name of plant	Plant parts used	Concentration of the extract (ml in 250 ml of growth medium)	Hours After Treatment (HAT)					
				6	12	24	48	72	96
1	*Adenocalymma alliaceum* (Lam.) Miers	Leaf	control	0	0	0	0	0	0
			0.5	0	0	5	15	65	15
			1	0	5	30	45	20	
			2	0	0	100			
			4	0	0	100			
			8	0	15	85			
2	*Allium sativum* L	Dry bulb	control	0	0	0	0	0	0
			0.5	5	20	25	25	20	5
			1	10	90				
			2	20	80				
			4	50	50				
			8	70	30				
3	*Alpinia purpurata* (Vieill.) K. Schum.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	40	20	20	20
			1	0	0	90	0	10	
			2	10	0	90			
			4	5	0	95			
			8	10	0	90			
4	*Citrus reticulata* Blanco	Peel	control	0	0	0	0	0	0
			0.5	95	5				
			1	95	5				
			2	100					
			4	100					
			8	100					
5	*Citrus medica* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	15	25	50	10	
			1	0	0	70	30		
			2	0	0	100			
			4	0	0	100			
			8	0	0	100			
6	*Momordica charantia* L.	Fruit	control	0	0	0	0	0	0
			0.5	0	5	95			
			1	0	15	85			
			2	0	20	80			
			4	10	30	60			
			8	15	40	45			
7	*Pimenta dioica* (L.) Merr.	Leaf	control	0	0	0	0	0	0
			0.5	5	5	10	20	30	30
			1	0	5	35	20	30	10
			2	0	35	40	25		

			4	25	40	35			
			8	25	50	25			
8	*Polyalthia longifolia* (Sonn.) Thwaites	Leaf	control	0	0	0	0	0	0
			0.5	0	0	30	40	30	
			1	0	0	30	35	35	
			2	0	0	10	40	50	
			4	0	0	40	60		
			8	0	0	50	50		
9	*Ricinus communis* L.	Seed	control	0	0	0	0	0	0
			0.5	100					
			1	100					
			2	100					
			4	100					
			8	100					
10	*Saritaea magnifica* (W. Bull) Dugand	Leaf	control	0	0	0	0	0	0
			0.5	15	25	25	35		
			1	0	15	65	20		
			2	0	50	50			
			4	0	0	100			
			8	0	0	100			
11	*Spilanthes calva* DC.	Flower	control	0	0	0	0	0	0
			0.5	30	15	55			
			1	40	30	15	15		
			2	90	10				
			4	100					
			8	100					
12	*Zingiber officinale* Roscoe	Rhizome	control	0	0	0	0	0	0
			0.5	100					
			1	100					
			2	100					
			4	100					
			8	100					
13	*Musa X paradisiaca* L.	Scape (Peduncle)	control	0	0	0	0	0	0
			0.5	20	25	55			
			1	35	30	35			
			2	30	70				
			4	20	80				
			8	20	80				
14	*Calotropis gigantea* (L.) R. Br.	Leaf	control	0	0	0	0	0	0
			0.5	0	10	0	0	10	75
			1	0	10	10	0	30	50
			2	0	30	0	0	20	50
			4	10	10	10	20	20	30
			8	10	20	30	20	20	
15	*Curcuma longa* L.	Rhizome	control	0	0	0	0	0	0
			0.5	0	0	0	0	10	5
			1	0	10	10	15	15	10
			2	20	30	40	10		
			4	50	50				

			8	60	40				
16	*Mesua ferrea* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	5	5	10
			1	0	5	10	15	15	25
			2	0	10	25	25	15	25
			4	0	20	40	20	20	
			8	0	20	70	10		
17	*Mimosa pudica* L.	Whole plant	control	0	0	0	0	0	0
			0.5	0	0	0	25	30	0
			1	0	5	20	25	30	0
			2	0	10	35	30	25	
			4	0	10	35	55		
			8	0	10	45	45		
18	*Carica papaya* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	5	5	5
			1	0	0	5	5	5	10
			2	0	0	5	5	10	15
			4	0	0	20	25	25	30
			8	0	10	50	40		
19	*Chromolaena odorata* (L.) R.M. King & H. Rob.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	5
			1	0	0	5	5	5	5
			2	0	0	15	25	15	25
			4	0	0	40	20	20	20
			8	0	0	10	40	30	20
20	*Eucalyptus tereticornis* Sm.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	0
			1	0	0	10	5	15	5
			2	0	40	20	5	5	5
			4	60	40				
			8	75	25				
21	*Hyptis suaveolens* (L.) Poit	Leaf	control	0	0	0	0	0	
			0.5	5	5	30	40	20	0
			1	10	5	65	10	10	
			2	15	5	60	10	10	
			4	50	10	20	20		
			8	45	10	45			
22	*Terminalia bellirica* (Gaertn.) Roxb.	Dry fruit	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	0
			1	0	0	0	0	0	0
			2	0	0	0	5	5	5
			4	0	0	0	20	40	40
			8	0	0	70	10	20	
23	*Vitex negundo* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	10	20	40	0
			1	0	0	10	30	30	0
			2	0	0	0	45	10	5
			4	0	0	30	55	15	
			8	10	20	10	60		

24	*Apium graveolens* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	0
			1	0	0	0	10	10	5
			2	0	5	5	20	20	5
			4	0	20	40	20	5	5
			8	0	20	40	30	10	
25	*Lantana camara* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	0
			1	0	0	0	0	0	0
			2	0	0	0	5	5	0
			4	0	5	5	10	20	0
			8	10	10	15	25	40	
26	*Ocimum gratissimum* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	30	0
			1	0	0	0	15	35	0
			2	0	0	0	10	35	20
			4	0	10	20	50	15	0
			8	50	30	10	10		
27	*Sida rhombifolia* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	20	0
			1	0	0	20	0	20	0
			2	0	0	40	0	40	0
			4	0	0	40	0	60	
			8	0	0	60	0	40	
28	*Tridax procumbens* L.	Flower	control	0	0	0	0	0	0
			0.5	10	90				
			1	40	60				
			2	45	55				
			4	55	45				
			8	80	20				
29	*Vinca rosea* L.	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	0
			1	0	0	0	0	0	0
			2	0	0	0	0	0	0
			4	5	10	15	5	5	5
			8	15	30	55			
30	*Ziziphus jujuba* (L.) Lam.	Dry fruit	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	0
			1	0	0	0	0	0	0
			2	0	0	0	0	5	5
			4	0	0	20	20	5	5
			8	0	0	30	40	10	20
31	Murraya koenigii (L.) Spreng	Leaf	control	0	0	0	0	0	0
			0.5	0	0	0	0	0	0
			1	0	0	0	0	0	0
			2	0	0	0	0	0	0
			4	0	0	5	15	25	5
			8	20	40	40			

Figure 2.1: Percentage mortality (100%) of mosquito larvae exposed to aqueous crude plant extracts at 0.5ml concentration with various retention time

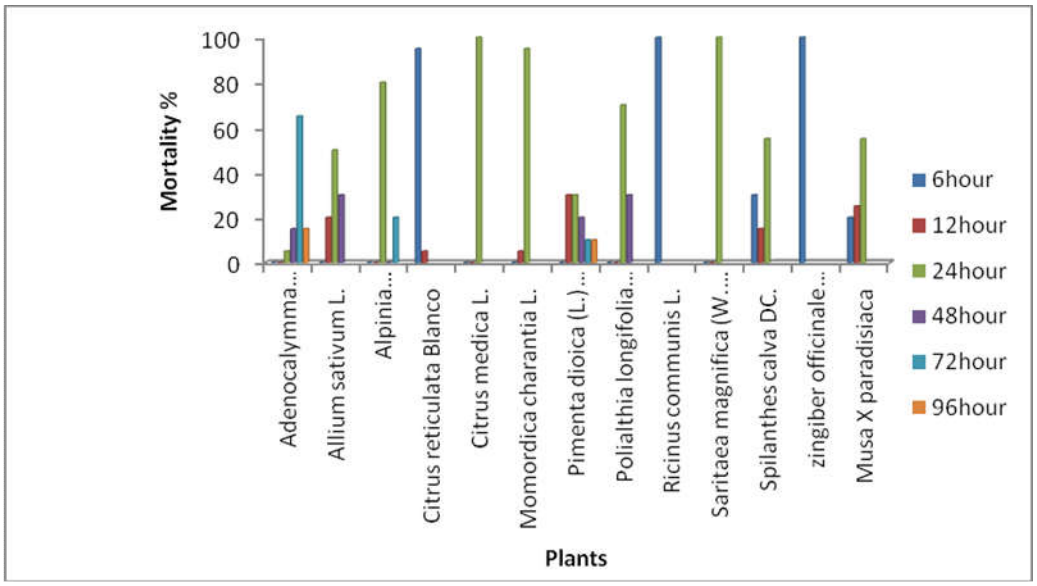


Figure 2.2: Percentage mortality (100%) of mosquito larvae exposed to aqueous crude plant extracts at 1ml concentration with various retention time

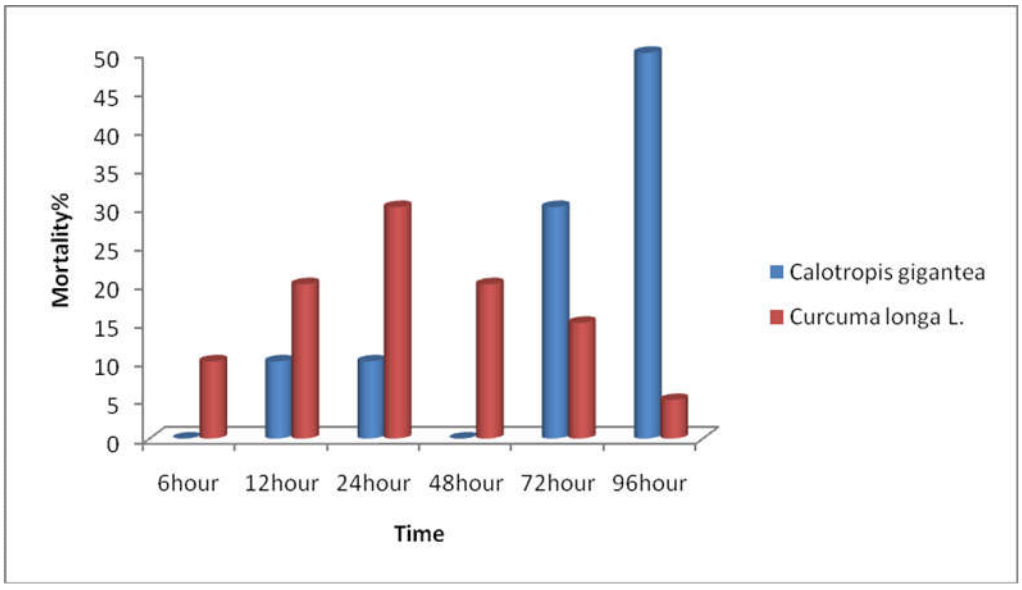


Figure 2.3: Percentage mortality (100%) of mosquito larvae exposed to aqueous crude plant extracts at 2ml concentration with various retention time

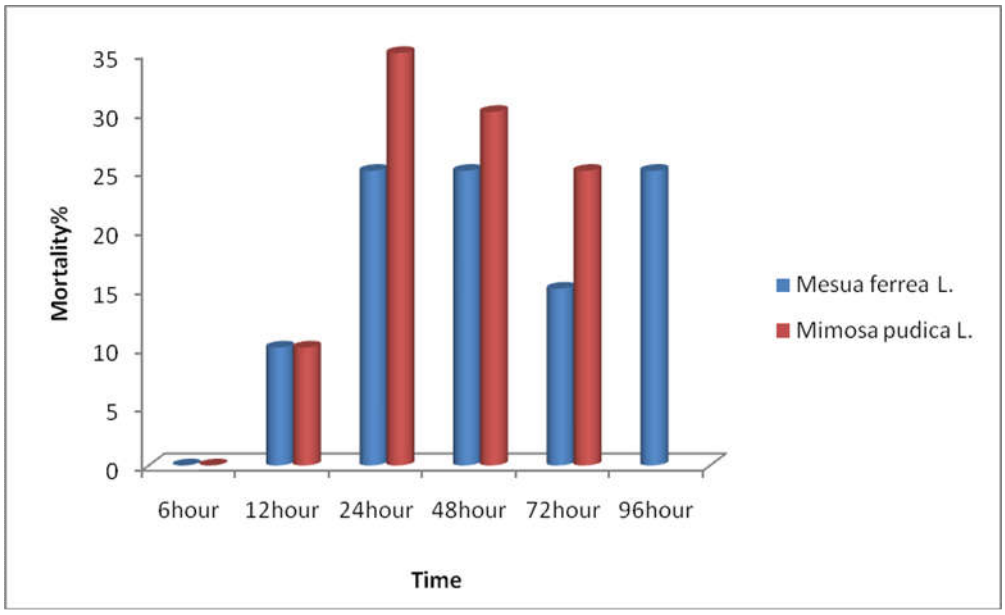


Figure 2.4: Percentage mortality (100%) of mosquito larvae exposed to aqueous crude plant extracts at 4ml concentration with various retention time

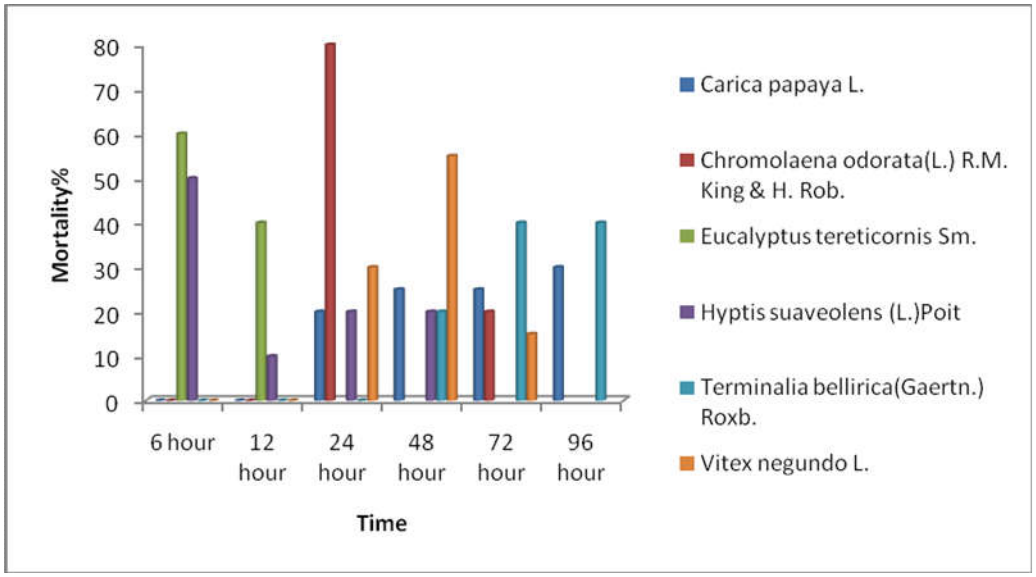


Figure 2.5: Percentage mortality (100%) of mosquito larvae exposed to aqueous crude plant extracts at 0.5ml concentration with various retention time

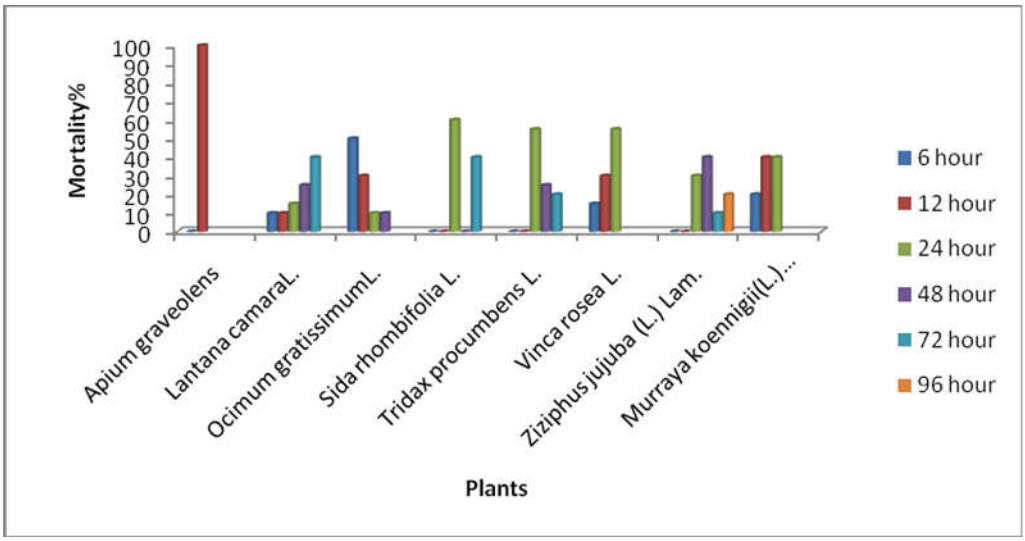


Table2.3: The LC_{50}values of promising plant extracts against mosquito larvae

SI No	Plants	LC_{50}(mg/ml)
1	*Adenocalymma alliaceum* (Lam.) Miers	1.764
2	*Allium sativum* L.	0.0002
3	*Alpinia purpurata* (Vieill.) K. Schum.	0.012
4	*Citrus reticulata* Blanco	0.552
5	*Citrus medica* L.	5.768
6	*Momordica charantia* L.	0.002
7	*Pimenta dioica* (L.) Merr.	2.732
8	*Polyalthia longifolia* (Sonn.) Thwaites	71.24
9	*Ricinus communis* L.	1.333
10	*Saritaea magnifica* (W. Bull) Dugand	1.696
11	*Spilanthes calva* DC.	3.939
12	*Zingiber officinale* Roscoe	1.316
13	*Musa X paradisiaca* L	1.327
14	*Mesua ferrea* L.	1.927
15	*Mimosa pudica* L.	8.651
16	*Calotropis gigantea* (L.) R.Br.	0.681
17	*Curcuma longa* L.	0.0024

18	*Carica papaya* L.	492.388
19	*Chromolaena odorata* (L.) R.M. King & H. Rob.	46.416
20	*Eucalyptus tereticornis* Sm.	0.258
21	*Hyptis suaveolens* (L.) Poit	3.348
22	*Terminalia bellirica* (Gaertn.) Roxb.	316.228
23	*Vitex negundo* L.	100.277
24	*Apium graveolens* L.	3.364
25	*Lantana camara* L.	316.228
26	*Ocimum gratissimum* L.	15.067
27	*Sida rhombifolia* L.	2.613
28	*Tridax procumbens* L.	2.1544
29	*Vinca rosea* L.	316.228
30	*Ziziphus jujuba* (L.) Lam.	316.228
31	*Murraya koenigii* (L.) Spreng.	316.228

Figure 2.6. LC_{50}value of plants against mosquitoes at 96 hours retention time

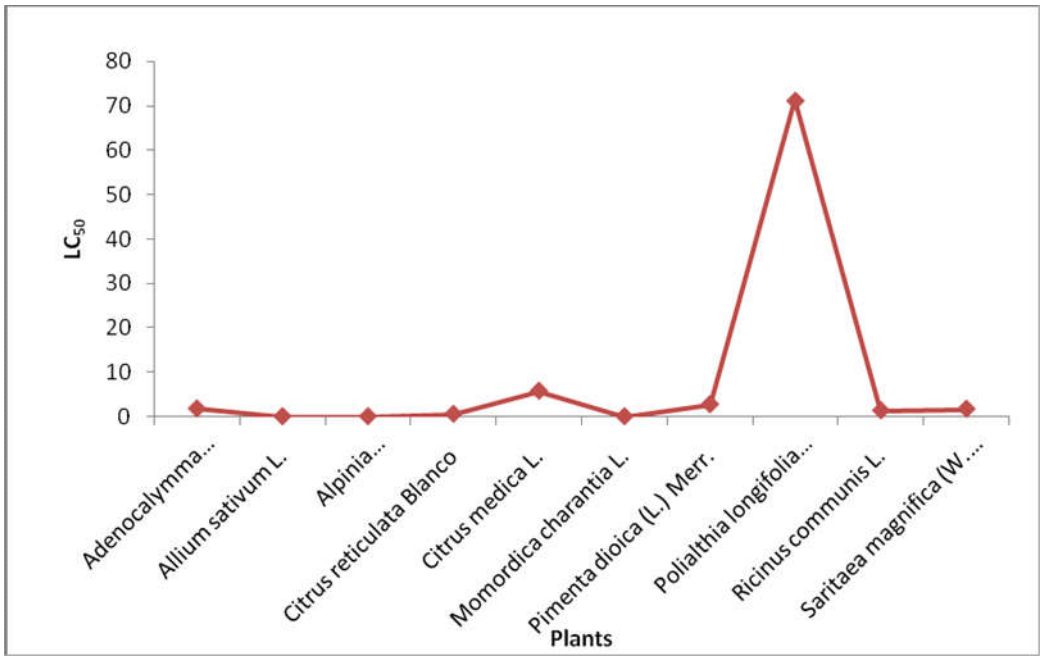


Figure 2.7 LC_{50}value of plants against mosquitoes at 96 hours retention time

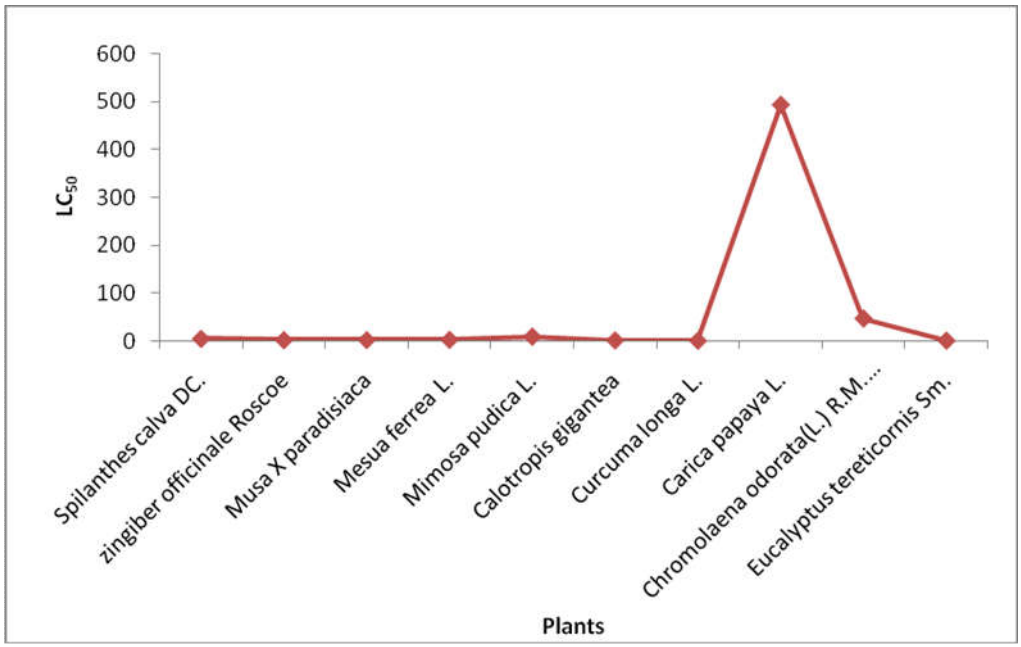
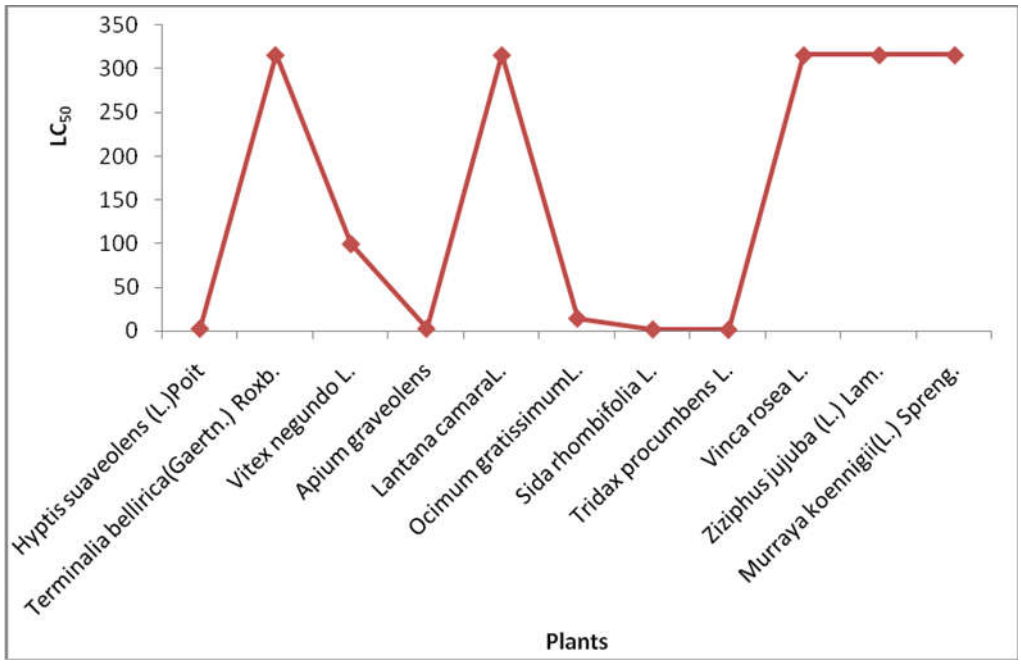


Figure 2.8 LC_{50}value of plants against mosquitoes at 96 hours retention time



Thus the present study confirms the larvicidal efficacy (100%) of 31 plants from an array of 120 plants screened. The lethality of these plants on species specific mosquito vectors were carried out and the results are represented.

Screening studies on species specific larvae reared under laboratory conditions

Based on mortality percentages, thirty one plant species were screened out for their larvicidal efficiencies. These plants were further checked for their species specific larvicidal properties on *Aedes albopictus*, *Culex sitiens* and *Armigeres subalbatus.* The larvae required for such studies were obtained from the rearing protocol mentioned in Chapter I. The bioassay was carried in the same way as that of general screening, except for the utilization of species specific vectors which are genetically pure.

The data so obtained were subjected to Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) for windows, version 16.0. This was carried out to determine significant differences in the effects of different plant extracts at the same concentration. Duncan's New Multiple Range Post Hoc test was used to separate the means at $P<0.01$ for significant data after ANOVA analysis.$LC_{50}$was calculated using Probit- Regression method (Finney 1971).

The efficacy of plant extracts on the third instar larvae of *Aedes albopictus* at varying concentration and retention time are depicted in Table 2.4 to 2.8. The efficacy of plant extracts on the third instar larvae of *Culex sitiens* at varying concentration and retention time are depicted in Table 2.10 to 2.14. Similarly the efficacy of plant extracts on the third instar larvae of *Armigeres subalbatus* at varying concentration and retention time are depicted in Table 2.16 to 2.20.

Table 2.4. The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Aedes albopictus* at 0.5ml concentration and varying retention time

Plants	0.5ml											
	Mean 6HAT	SD 6HAT	Mean 12HAT	SD 12HAT	Mean 24HAT	SD 24HAT	Mean 48HAT	SD 48HAT	Mean 72HAT	SD 72HAT	Mean 96HAT	SD 96HAT
Spilanthes calva DC.	12.33	2.52	7.67	2.52								
Hyptis suaveolens (L.) Poit	3.33	3.21	2.67	1.15	6.67	2.52	7.33	2.52				
Vinca rosea L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.5	0.71	0	0
Allium sativum L	19.33	1.15	2.00	.								
Vitex negundo L.	2.33	0.58	3.67	0.58	5.67	1.15	1.67	0.58	2.67	0.58	0.67	1.15
Ocimum gratissimum L.	0.00	0.00	5.00	0.00	3.00	0.00	4.00	0.00				
Alpinia purpurata (Vieill.) K. Schum.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Mimosa pudica L.	3.67	0.58	6.33	1.53	10.00	1.00						
Terminalia bellirica (Gaertn.) Roxb.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Saritaea magnifica (W. Bull) Dugand	0.00	0.00	15.00	1.00	3.67	1.53	2.00	0.00				

Ricinus communis L.	20.00	0.00	0.00	0.00								
Zingiber officinale Roscoe	20.00	0.00	0.00	0.00								
Tridax procumbens L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Citrus reticulata Blanco	20.00	0.00	0.00	0.00								
Pimenta dioica (L.) Merr.	7.33	1.15	12.67	1.15								
Sida rhombifolia L.	0.00	0.00	0.00	0.00	2.67	0.58	2.67	0.58	1.00	0.00	0.50	0.71
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carica papaya L.	2.33	0.58	3.33	1.15	4.67	2.08	4.00	1.73	2.00	1.00	1.00	0.00
Chromolaena odorata (L.) R.M. King & H. Rob.	0.00	0.00	0.00	0.00	1.00	1.00	4.00	1.00	3.33	1.53	0.00	0.00
Polyalthia longifolia (Sonn.) Thwaites	0.00	0.00	0.00	0.00	4.67	0.58	6.00	2.00	6.33	1.53	2.00	0.00
Mesua ferrea L.	5.00	1.00	7.00	1.00	7.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calotropis gigantea (L.) R. Br.	4.33	1.53	8.67	1.15	7.00	1.73					0.00	0.00
Momordica charantia L.	2.67	2.08	6.33	1.53	11.00	1.73						

Musa X paradisiaca L.	0.00	0.00	0.00	0.00	0.00	0.00			0.00	0.00		
Adenocalymma alliaceum (Lam.) Miers	5.33	0.58	14.67	0.58								
Apium graveolens L.	1.33	0.58	2.33	0.58	4.33	1.53	3.67	0.58			1.00	1.00
Curcuma longa L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Citrus medica L.	4.67	1.53	7.33	1.15	2.67	2.52	2.00	1.73	0.67	1.15	0.00	0.00
Lantana camara L.	0.67	1.15	2.33	3.21	4.00	2.65	5.00	2.65	0.67	0.58	0.67	0.58
Murraya koenigii (L.) Spreng	0.67	1.15	2.33	3.21	4.00	2.65	5.00	2.65	0.67	0.58	0.67	0.58
Eucalyptus tereticornis Sm.	0.00	0.00	0.00	0.00	0.33	0.58	0.67	0.58	2.00	0.00	0.50	0.71
Grand Total	4.37	0.62	3.90	0.80	3.43	0.99	2.29	0.79	1.40	0.51	0.412	0.278

Table 2.4 (a). Anova table showing activity against 0.5ml concentration and retention time

Concentration 0.5ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	187.994	39	4.820	81.862	.000
	Within Groups	4.711	80	.059		
	Total	192.705	119			
12 hour	Between Groups	154.060	39	3.950	49.761	.000
	Within Groups	6.351	80	.079		
	Total	160.411	119			
24 hour	Between Groups	85.899	39	2.203	30.222	.000
	Within Groups	5.830	80	.073		
	Total	91.729	119			
48 hour	Between Groups	51.927	39	1.331	16.627	.000
	Within Groups	6.406	80	.080		
	Total	58.334	119			
72 hour	Between Groups	24.696	39	.633	20.413	.000
	Within Groups	2.482	80	.031		
	Total	27.178	119			
96 hour	Between Groups	5.684	39	.146	4.440	.000
	Within Groups	2.626	80	.033		
	Total	8.309	119			

Five plants, namely *Spilanthes calva* DC.(12.33 ±2.52), *Allium sativum* L (19.33±1.15), *Ricinus communis* L.(20±0), *Zingiber officinale* Roscoe (20±0), and *Citrus reticulata* Blanco (20±0) were highly significant at 0.5ml concentration at 6 hours retention time (F value= 81.862; p value < 0.01). Three plants namely *Pimenta dioica* (L.) Merr. (12.67±1.15), *Adenocalymma alliaceum* (Lam.) Miers (14.67±0.58) *Saritaea magnifica* (W. Bull) Dugand (15.00±1.00) were highly significant at 0.5ml concentration at 12 hours retention time (F value= 49.761; p value < 0.01). Two plants namely *Mimosa pudica* L.(10.00±1.00) and *Momordica charantia* L. (11±1.73) were highly

significant at 0.5ml concentration and 24 hours retention time (F value= 30.222 p value < 0.01). *Lantana camara* L. (5.00±2.65), *Murraya koenigii* (L.) Spreng (5.00±2.65), *Polyalthia longifolia* (Sonn.) Thwaites (6.00±2.00), Hyptis *suaveolens* (L.) Poit (7.33±2.52) were highly significant at 0.5ml concentration and 48 hours retention time (F value= 16.627 p value < 0.01). *Polyalthia longifolia* (Sonn.) Thwaites (6.33±1.53; 2.00±0.00) was highly significant at 0.5ml concentration at 72 and 96hours retention time (F value= 20.413 p value < 0.01; F value= 4.440 p value < 0.01) (Table 2.4 and 2.4(a)).

Table 2.5.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Aedes albopictus* at 1ml concentration and varying retention time

Plants						1ml						
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	6HAT	6HAT	12HAT	12HAT	24HAT	24HAT	48HAT	48HAT	72HAT	72HAT	96HAT	96HAT
Spilanthes calva DC.	12	2	8	2								
Hyptis suaveolens (L.) Poit	3.67	2.08	1.67	0.58	10.67	2.08	4	1				
Vinca rosea L.	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.58	0.5	0.71	0	0
Allium sativum L.	20.00	0.00										
Vitex negundo L.	2.33	0.58	4.33	0.58	8.00	3.61	2.33	1.53	1.33	1.53	0.67	1.15
Ocimum gratissimum L.	0.00	0.00	5.00	0.00	3.00	0.00	4.00	0.00				
Alpinia purpurata (Vieill.) K. Schum.	1.33	2.31	4.67	4.62	9.33	4.16	7.00	4.24				

Mimosa pudica L.	2.67	0.58	7.67	0.58	9.67	0.58						
Terminalia bellirica (Gaertn.) Roxb.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			0.00	0.00
Saritaea magnifica (W. Bull) Dugand	17.33	0.58	2.00	0.00	1.00	0.00						
Ricinus communis L.	20.00	0.00										
Zingiber officinale Roscoe	20.00	0.00										
Tridax procumbens L.	0.00	0.00	0.67	1.15	0.33	0.58	1.00	1.00	1.33	1.53	5.33	7.57
Citrus reticulata Blanco	20.00	0.00										
Pimenta dioica (L.) Merr.	9.67	3.21	10.33	3.21								
Sida rhombifolia L.	2.00	0.00	2.67	0.58	3.00	1.00	1.33	0.58	1.33	0.58	1.00	0.00

Ziziphus jujuba (L.) Lam.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carica papaya L.	5.67	1.53	7.33	1.15	7.00	2.65						
Chromolaena odorata (L.) R.M. King & H.Rob.	0.00	0.00	3.00	1.00	3.33	2.08	5.00	1.00	5.33	1.53	0.00	0.00
Polyalthia longifolia (Sonn.) Thwaites	0.00	0.00	0.00	0.00	8.67	1.15	5.00	1.00	4.00	1.73	1.33	0.58
Mesua ferrea L.	7.00	1.73	10.67	2.89	2.33	1.15						
Calotropis gigantea (L.) R. Br.	6.67	1.53	13.33	1.53								
Momordica charantia L.	2.00	1.00	9.33	1.15	8.67	2.08						
Musa X paradisiaca L.	3.33	0.58	5.67	0.58	5.33	2.08	1.67	0.58	1.33	1.53	0.00	.
Adenocalymma alliaceum (Lam.) Miers	6.67	2.08	13.33	2.08								

Apium graveolens L.	3.00	1.00	3.67	1.15	4.67	0.58	6.67	2.31	2.00	0.00	0.00	.
Curcuma longa L.	0.00	0.00	0.00	0.00	4.67	0.58	3.00	1.00	0.67	0.58	0.00	0.00
Citrus medica L	8.67	3.21	9.67	1.15	2.50	2.12						
Lantana camara L.	2.33	1.53	5.00	1.00	12.00	2.00						
Murraya koenigii (L.) Spreng	2.33	1.53	5.00	1.00	12.00	2.00						
Eucalyptus tereticornis Sm.	6.00	2.00	6.67	1.15	1.67	0.58	1.00	0.00	1.50	0.71	0.00	0.00
Grand Total	5.96	0.94	5.17	1.08	5.12	1.35	2.82	0.99	1.61	0.87	0.69	0.93

Table 2.5 (a) Anova table showing activity against 1ml concentration and retention time

Concentration1mll		Sum of Squares	df	Mean Square	F	Sig.
6hour	Between Groups	209.534	39	5.373	57.539	.000
	Within Groups	7.470	80	.093		
	Total	217.004	119			
12 hour	Between Groups	126.088	39	3.233	44.965	.000
	Within Groups	5.752	80	.072		
	Total	131.840	119			
24 hour	Between Groups	115.029	39	2.949	36.481	.000
	Within Groups	6.468	80	.081		
	Total	121.496	119			
48 hour	Between Groups	47.157	39	1.209	13.382	.000
	Within Groups	7.228	80	.090		
	Total	54.385	119			
72 hour	Between Groups	30.511	39	.782	16.794	.000
	Within Groups	3.727	80	.047		
	Total	34.237	119			
96 hour	Between Groups	24.390	39	.625	6.469	.000
	Within Groups	7.734	80	.097		
	Total	32.125	119			

Five plants namely *Saritaea magnifica* (W. Bull) Dugand (17.33±0.58), *Allium sativum* L (20±0), *Ricinus communis* L.(20±0), *Zingiber officinale* Roscoe (20±0), *Citrus reticulata* Blanco (20±0) were highly significant at 1ml concentration at 6 hours retention time (F value= 57.539; p value < 0.01).

Four plants namely *Pimenta dioica* (L.) Merr. (10.33±3.21), *Mesua ferrea* L. (10.67±2.89) *Adenocalymma alliaceum* (Lam.) Miers (13.33±2.08) and *Calotropis gigantea* (L.) R. Br. (13.33±1.53) were highly significant at 1ml concentration at 12 hours retention time (F value= 44.965; p value < 0.01). Seven plants namely *Momordica charantia* L. (8.67±2.08), *Polyalthia longifolia* (Sonn.) Thwaites (8.67±1.15), *Alpinia purpurata* (Vieill.) K. Schum. (9.33±4.16), *Mimosa pudica* L. (9.67±0.58), *Hyptis suaveolens* (L.) Poit (10.67±2.08), *Lantana camara* L. (12.00±2.00) and *Murraya koenigii* (L.) Spreng (12.00±2.00) were highly significant at 1ml concentration and 24 hours retention time (F value= 36.481; p value < 0.01). Four plants such as *Hyptis suaveolens* (L.) Poit (4±1), *Polyalthia longifolia* (Sonn.) Thwaites (5.00±1.00), *Chromolaena odorata* (L.) R.M. King & H. Rob. (5.00±1.00) and *Apium graveolens* L. (6.67±2.31) were highly significant at 0.5ml concentration and 48 hours retention time (F value=13.382; p value < 0.01).*Chromolaena odorata* (L.) R.M. King & H. Rob. (5.33±1.53) is highly significant at 1ml concentration and 72 hours retention time (F value= 16.794; p value < 0.01). *Tridax procumbens* L. (5.33±7.57) was highly significant at 1ml concentration and 96 hours retention time (F value= 6.469; p value< 0.01) (Table 2.5 and 2.5(a)).

Table 2.6.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Aedes albopictus* at 2ml concentration and varying retention time

2ml												
Plants	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
	6HAT	**6HAT**	**12HAT**	**12HAT**	**24HAT**	**24HAT**	**48HAT**	**48HAT**	**72HAT**	**72HAT**	**96HAT**	**96HAT**
Spilanthes calva DC.	9.67	5.51	10.33	5.51								
Hyptis suaveolens (L.) Poit	4.33	1.53	2.00	1.73	9	1.73	4.67	1.53				
Vinca rosea L.	0.00	0.00	1.67	2.89	5.33	4.51	5.67	3.06	3.5	4.95	4.5	6.36
Allium sativum L	20.00	0.00										
Vitex negundo L.	4.67	0.58	7.33	2.31	2.67	0.58	2.33	0.58	2.5	0.71	1	1.41
Ocimum gratissimum L.	0.00	0.00	16.67	1.15	3.00	1.00	1.00	.				
Alpinia purpurata (Vieill.) K. Schum.	0.67	1.15	5.00	4.36	8.33	1.53	9.00	1.41				
Mimosa pudica L.	7.67	7.64	7.67	5.51	14.00	.						
Terminalia bellirica (Gaertn.) Roxb.	0.00	0.00	0.00	0.00	2.67	0.58	5.33	0.58	4	1.00	5	4.36
Saritaea	20.00	0.00										

magnifica (W. Bull) Dugand												
Ricinus communis L.	20.00	0.00										
Zingiber officinale Roscoe	20.00	0.00										
Tridax procumbens L.	0.00	0.00	0.67	1.15	1.00	1.00	3.67	3.79	7.67	5.13	7.5	7.78
Citrus reticulata Blanco	20.00	0.00										
Pimenta dioica (L.) Merr.	12.00	0.00	8.00	0.00								
Sida rhombifolia L.	2.00	0.00	3.33	0.58	5.33	2.52	6.33	1.53	3.50	2.12	2	.
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.00	0.00	1.67	0.58	2.00	1.00	2.00	1.00	1.33	0.58
Carica papaya L.	7.00	1.00	8.33	3.51	4.67	2.52						
*Chromolaena odorata (*L.) R.M. King & H. Rob.	0.00	0.00	1.67	0.58	6.33	1.53			4.33	1.15	0	0.00
Polyalthia longifolia (Sonn.) Thwaites	5.00	1.00	6.33	2.08	8.67	1.15						
Mesua ferrea L.	9.67	2.08	6.67	0.58	3.67	2.31						

Calotropis gigantea (L.) R. Br.	7.67	0.58	12.33	0.58								
Momordica charantia L.	6.33	1.53	9.67	1.53	4.00	1.00						
Musa X paradisiaca L.	4.00	1.00	10.67	1.15	4.67	1.53						
Adenocalymma alliaceum (Lam.) Miers	9.67	2.08	10.33	2.08								
Apium graveolens L.	6.33	1.53	5.67	0.58	6.00	2.65	1.67	1.15	1.00	.		
Curcuma longa L.	0.00	0.00	4.33	0.58	6.00	1.73	7.00	2.65	2.67	0.58		
Citrus medica L	11.33	1.53	8.67	1.53								
Lantana camara L.	3.00	1.73	6.33	1.53	10.00	3.00						
Murraya koenigii (L.) Spreng	3.00	1.73	6.33	1.53	10.00	3.00						
Eucalyptus tereticornis Sm.	15.00	1.73	3.67	1.53	1.33	0.58						
Grand Total	7.39	1.09	6.29	1.71	5.63	1.75	4.69	1.62	3.46	2.08	3.05	3.42

Table 2.6 (a) Anova table showing activity against 2ml concentration and retention time

Concentration 2ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	223.147	39	5.722	38.891	.000
	Within Groups	11.770	80	.147		
	Total	234.917	119			
12 hour	Between Groups	130.307	39	3.341	20.341	.000
	Within Groups	13.141	80	.164		
	Total	143.448	119			
24 hour	Between Groups	85.119	39	2.183	11.895	.000
	Within Groups	14.679	80	.183		
	Total	99.798	119			
48 hour	Between Groups	67.124	39	1.721	16.047	.000
	Within Groups	8.580	80	.107		
	Total	75.704	119			
72 hour	Between Groups	42.906	39	1.100	8.779	.000
	Within Groups	10.025	80	.125		
	Total	52.932	119			
96 hour	Between Groups	22.919	39	.588	3.554	.000
	Within Groups	13.228	80	.165		
	Total	36.147	119			

Six plants such as *Eucalyptus tereticornis* Sm. (15.00±1.73), *Allium sativum* L. (20±0), *Saritaea magnifica* (W. Bull) Dugand. (20±0), *Ricinus communis* L.(20±0), *Zingiber officinale* Roscoe (20±0) and *Citrus reticulata* Blanco (20±0) were highly significant at 2ml concentration at 6 hours

retention time (F value= 38.891; p value < 0.01). Two plants such as *Calotropis gigantea* (L.) R. Br. (12.33±0.58) and *Ocimum gratissimum* L. (16.67±1.15) were highly significant at 2ml concentration at 12 hours retention time (F value= 20.341; p value < 0.01). Eight plants such as *Apium graveolens* L. (6.00±2.65), *Curcuma longa* L. (6.00±1.73), *Chromolaena odorata* (L.) R.M. King & H. Rob. (6.33±1.53), *Alpinia purpurata* (Vieill.) K. Schum. (8.33±1.53) *Polyalthia longifolia* (Sonn.) Thwaites (8.67±1.15), *Hyptis suaveolens* (L.) Poit (9±1.73), *Lantana camara* L. (10.00±3.00) and *Murraya koenigii* (L.) Spreng (10.00±3.00) were highly significant at 2ml concentration and 24 hours retention time (F value= 11.895; p value < 0.01). Seven plants such as *Hyptis suaveolens* (L.) Poit (4.67±1.53), *Alpinia purpurata* (Vieill.) K. Schum. (9.00±1.41), *Terminalia bellirica* (Gaertn.) Roxb. (5.33±0.58), *Vinca rosea* L. (5.67±3.06), *Sida rhombifolia* L. (6.33±1.53), *Curcuma longa* L. (7.00±2.65) and *Chromolaena odorata* (L.) R.M. King & H. Rob. (6.33±1.53) were highly significant at 2ml concentration and 48 hours retention time (F value=16.047; p value < 0.01). *Polyalthia longifolia* (Sonn.) Thwaites (6.33±1.53) is significant at 2ml concentration and 72 hours retention time (F value= 8.779; p value < 0.01). Two plants such as *Tridax procumbens* L. (7.5±7.78) and *Terminalia bellirica* (Gaertn.) Roxb. (5±4.36) were highly significant at 2ml concentration and 96 hours retention time (F value= 3.554; p value < 0.01) (Table 2.6 and 2.6(a)).

Table 2.7.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Aedes albopictus* at 4ml concentration and varying retention time

Plants	4ml											
	Mean 6HAT	SD 6HAT	Mean 12HAT	SD 12HAT	Mean 24HAT	SD 24HAT	Mean 48HAT	SD 48HAT	Mean 72HAT	SD 72HAT	Mean 96HAT	SD 96HAT
Spilanthes calva DC.	19.33	1.15	2	.								
Hyptis suaveolens (L.) Poit	9.00	1.00	3.33	0.58	6	1.73	1.67	1.15				
Vinca rosea L.	0.67	1.15	4.33	3.51	4.33	3.51	5.67	2.52	5	.	10	.
Allium sativum L	20.00	0.00										
Vitex negundo L.	5.00	3.00	9.67	5.51	3.50	2.12	2.50	0.71	2	.	2	.
Ocimum gratissimum L.	0.00	0.00	18.33	0.58	1.67	0.58						
Alpinia purpurata (Vieill.) K. Schum.	1.00	1.73	6.67	8.08	12.33	9.81						
Mimosa pudica L.	7.00	4.24	10.00	6.00	16.00	.						
Terminalia bellirica (Gaertn.) Roxb.	0.00	0.00	0.00	0.00	2.67	0.58	5.67	0.58	4.33	0.58	5	4.36

Saritaea magnifica (W. Bull) Dugand	20.00	0.00										
Ricinus communis L.	20.00	0.00										
Zingiber officinale Roscoe	20.00	0.00										
Tridax procumbens L.	0.00	0.00	0.67	1.15	1.67	0.58	5.33	5.86	9.67	5.13	4	4.24
Citrus reticulata Blanco	20.00	0.00										
Pimenta dioica (L.) Merr.	17.67	0.58	2.33	0.58								
Sida rhombifolia L.	6.00	1.00	14.00	1.00								
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.00	0.00	4.00	1.00	5.33	0.58	4.67	0.58	6	0
Carica papaya L.	6.67	3.06	13.33	3.06								
Chromolaena odorata (L.) R.M. King & H. Rob.	0.00	0.00	3.00	1.00	6.33	0.58	10.67	0.58				
Polyalthia longifolia (Sonn.) Thwaites	5.00	1.00	6.33	2.08	8.67	1.15						

Mesua ferrea L.	12.00	1.00	8.00	1.00								
Calotropis gigantea (L.) R. Br.	10.67	1.53	9.00	1.73								
Momordica charantia L.	10.67	3.51	8.67	2.52	2.00	.						
Musa X paradisiaca L	6.00	0.00	9.00	2.65	5.00	2.65						
Adenocalymma alliaceum (Lam.) Miers	20.00	0.00										
Apium graveolens L.	8.33	2.52	11.67	2.52								
Curcuma longa L.	6.33	1.15	13.67	1.15								
Citrus medica L.	13.67	4.16	6.33	4.16								
Lantana camara L.	4.67	1.53	7.00	3.00	7.67	2.52						
Murraya koenigii (L.) Spreng	4.67	1.53	7.00	3.00	7.67	2.52						
Eucalyptus tereticornis Sm.	16.33	2.08	3.33	2.52	1.00	.						
Grand Total	9.38	1.19	7.11	2.39	5.66	2.26	5.26	1.71	5.13	2.10	5.4	2.87

Table 2.7(a) Anova table showing activity against 4ml concentration and retention time

Concentration 4ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	257.567	39	6.604	54.394	.000
	Within Groups	9.713	80	.121		
	Total	267.281	119			
12 hour	Between Groups	141.756	39	3.635	16.035	.000
	Within Groups	18.135	80	.227		
	Total	159.891	119			
24 hour	Between Groups	86.024	39	2.206	8.730	.000
	Within Groups	20.213	80	.253		
	Total	106.237	119			
48 hour	Between Groups	66.894	39	1.715	26.506	.000
	Within Groups	5.177	80	.065		
	Total	72.071	119			
72 hour	Between Groups	48.594	39	1.246	22.163	.000
	Within Groups	4.498	80	.056		
	Total	53.092	119			
96 hour	Between Groups	35.482	39	.910	5.664	.000
	Within Groups	12.851	80	.161		
	Total	48.333	119			

Nine plants such as *Eucalyptus tereticornis* Sm. (16.33±3.33), *Pimenta dioica* (L.) Merr. (17.67±2.33), *Spilanthes calva* DC. (19.33±2), *Allium sativum* L (20±0), *Saritaea magnifica* (W. Bull) Dugand (20±0), *Ricinus*

communis L.(20±0), *Zingiber officinale* Roscoe (20±0), *Citrus reticulata* Blanco (20±0) and *Adenocalymma alliaceum* (Lam.) Miers (20±0) were highly significant at 4ml concentration at 6 hours retention time (F value= 54.394; p value < 0.01). Four plants such as *Carica papaya* L. (13.33±3.06), *Curcuma longa* L. (13.67±1.15), *Sida rhombifolia* L. (14.00±1.00) and *Ocimum gratissimum* L. (18.33±0.58) were highly significant at 4ml concentration at 12 hours retention time (F value= 16.035; p value < 0.01).Six plants such as *Hyptis suaveolens* (L.) Poit (6±1.73), *Chromolaena odorata* (L.) R.M. King & H. Rob. (6.33±0.58), *Lantana camara* L. (7.67±2.52), *Murraya koenigii* (L.) Spreng (7.67±2.52), *Polyalthia longifolia* (Sonn.) Thwaites (8.67±1.15) and *Alpinia purpurata* (Vieill.) K. Schum. (12.33±9.81) were highly significant at 4ml concentration and 24 hours retention time (F value= 8.730; p value < 0.01). Plant *Chromolaena odorata* (L.) R.M. King & H. Rob. (10.67±0.58) is highly significant at 4ml concentration and 48 hours retention time (F value= 26.506; p value < 0.01). Plant *Tridax procumbens* L. (5.13±4) was highly significant at 4ml concentration and 72 hours retention time (F value= 22.163; p value < 0.01). Two plants such as *Terminalia bellirica* (Gaertn.) Roxb. (5±4.36) and *Ziziphus jujuba* (L.) Lam. (6±0) were highly significant at 4ml concentration and 96 hours retention time (F value= 5.664; p value< 0.01) (Table 2.7 and 2.7(a)).

Table 2.8.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Aedes albopictus* at 8ml concentration and varying retention time

Plants	8ml											
	Mean 6HAT	SD 6HAT	Mean 12HAT	SD 12HAT	Mean 24HAT	SD 24HAT	Mean 48HAT	SD 48HAT	Mean 72HAT	SD 72HAT	Mean 96HAT	SD 96HAT
Spilanthes calva DC.	19.67	0.58	1	.								
Hyptis suaveolens (L.) Poit	12.33	1.53	3	1	4.67	2.31						
Vinca rosea L.	4.00	5.66	3.67	3.06	6.00	4.58	2.67	0.58	9	.	6	.
Allium sativum L	20.00	0.00										
Vitex negundo L.	6.00	2.65	8.33	4.16	3.50	0.71	5.00	1.41				
Ocimum gratissimum L.	0.00	0.00	20.00	0.00								
Alpinia purpurata (Vieill.) K. Schum.	1.00	1.73	6.33	7.51	12.67	9.24						
Mimosa pudica L.	5.00	0.00	12.00	5.20	14.00	.						
Terminalia bellirica (Gaertn.) Roxb.	0.00	0.00	0.00	0.00	3.00	1.00	6.33	0.58	4.67	0.58	4	3.46
Saritaea magnifica (W. Bull) Dugand	20.00	0.00										

Ricinus communis L.	20.00	0.00										
Zingiber officinale Roscoe	20.00	0.00										
Tridax procumbens L.	0.00	0.00	0.67	1.15	5.67	8.08	5.00	5.29	8.50	0.71	9	.
Citrus reticulata Blanco	20.00	0.00										
Pimenta dioica (L.) Merr.	18.00	0.00	2.00	0.00								
Sida rhombifolia L.	8.67	1.15	11.33	1.15								
Ziziphus jujuba (L.) Lam.	5.67	0.58	12.67	0.58	1.67	0.58						
Carica papaya L.	8.00	1.00	12.00	1.00								
Chromolaena odorata (L.) R.M. King & H. Rob.	0.00	0.00	3.67	1.53	6.67	2.08	9.67	0.58				
Polialthia longifolia (Sonn.) Thwaites	20.00	0.00										
Mesua ferrea L.	12.67	1.53	7.33	1.53								
Calotropis gigantea (L.) R. Br.	9.67	1.53	10.33	1.53								
Momordica charantia L.	10.33	3.51	9.67	3.51								

Musa X paradisiaca L.	7.00	1.00	8.33	2.52	4.67	2.31						
Adenocalymma alliaceum (Lam.) Miers	20.00	0.00										
Apium graveolens L.	10.00	2.65	10.00	2.65								
Curcuma longa L.	9.00	1.73	11.00	1.73								
Citrus medica L	17.67	0.58	2.33	0.58								
Lantana camara L.	5.33	1.53	9.33	2.31	5.00	3.61						
Murraya koenigii (L.) Spreng	5.33	1.53	9.33	2.31	5.00	3.61						
Eucalyptus tereticornis Sm.	17.67	0.58	2.33	0.58								
Grand Total	10.74	1.00	7.36	1.98	6.04	3.46	5.73	1.69	7.39	0.64	6.33	3.46

Table 2.8 (a) Anova table showing activity against 8ml concentration and retention time

Concentration 8ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	251.535	39	6.450	42.081	.000
	Within Groups	12.261	80	.153		
	Total	263.797	119			
12 hour	Between Groups	138.728	39	3.557	20.361	.000
	Within Groups	13.976	80	.175		
	Total	152.704	119			
24 hour	Between Groups	85.108	39	2.182	6.764	.000
	Within Groups	25.809	80	.323		
	Total	110.916	119			
48 hour	Between Groups	59.798	39	1.533	23.555	.000
	Within Groups	5.207	80	.065		
	Total	65.005	119			
72 hour	Between Groups	41.676	39	1.069	9.920	.000
	Within Groups	8.618	80	.108		
	Total	50.293	119			
96 hour	Between Groups	25.159	39	.645	4.567	.000
	Within Groups	11.301	80	.141		
	Total	36.460	119			

Eleven plants such as *Citrus medica L* (17.67±0.58), *Eucalyptus tereticornis* Sm. (17.67±0.58), *Pimenta dioica* (L.) Merr. (18.00±0), *Spilanthes calva* DC. (19.67±0.58), Allium *sativum* L (20±0), *Saritaea magnifica* (W. Bull) Dugand (20±0), *Ricinus communis* L. (20±0), *Zingiber officinale* Roscoe (20±0), *Citrus reticulata* Blanco (20±0), Polyalthia *longifolia* (Sonn.) Thwaites (20±0) and *Adenocalymma alliaceum* (Lam.)

Miers (20±0) were highly significant at 8ml concentration at 6 hours retention time (F value= 42.081; p value < 0.01). Plant *Ocimum gratissimum* L. (20±0) was highly significant at 8ml concentration at 12 hours retention time (F value = 20.361; p value < 0.01). Three plants such as *Vinca rosea* L. (6±4.58), *Chromolaena odorata* (L.) R.M. King & H. Rob. (6.67±2.08) and *Alpinia purpurata* (Vieill.) K. Schum. (12.67±9.24) were highly significant at 8ml concentration and 24 hours retention time (F value= 6.764; p value < 0.01). Plant *Chromolaena odorata* (L.) R.M. King & H. Rob. (9.67±0.58) was highly significant at 8ml concentration and 48 hours retention time (F value= 23.555; p value < 0.01). Plant *Terminalia bellirica* (Gaertn.) Roxb. (4.67±0.58) is highly significant at 8ml concentration and 72 hours retention time (F value= 4.567; p value < 0.01). Plant *Terminalia bellirica* (Gaertn.) Roxb. (4±3.46) is highly significant at 4ml concentration and 96 hours retention time (F value = 5.664; p value < 0.01) (Table 2.8 and 2.8(a)).

Altogether twelve plants among thirty one are showing strongest larvicidal effects in all concentration and retention time against *Aedes albopictus* larvae. Their LC_{50}values are calculated (Table 2.9).

Table 2.9.LC_{50}values of *Aedes albopictus*

SI. No.	**Plants**	LC_{50}mg/ml
1	*Allium sativum* L	0*
2	*Saritaea magnifica*(W. Bull) Dugand	0.0010
3	*Ricinus communis* L.	0*
4	*Zingiber officinale* Roscoe	0*
5	*Citrus reticulata* Blanco	0*
6	*Polialthia longifolia* (Sonn.) Thwaites	13.15
7	*Hyptis suaveolens* (L.) Poit	11.94
8	*Alpinia purpurata* (Vieill.) K. Schum.	0.060
9	*Pimenta dioica* (L.) Merr.	0.0001
10	*Chromolaena odorata* (L.) R.M. King &H. Rob.	1.98
11	*Adenocalymma alliaceum* (Lam.) Miers	0.002
12	*Lantana camara* L.	7244.36

0* represents plants having 100 % mortality at lowest concentration

Table 2.10.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Culex sitiens* at 0.5ml concentration and varying retention time

0.5ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	7.33	1.15	11.00	1	1.67	0.58						
Calotropis gigantea (L.) R. Br.	9.33	2.31	9.67	1.53	1.50	0.71						
Citrus medica L	9.33	2.31	10.00	1.73	1.00	0.00						
Hyptis suaveolens (L.) Poit	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0	0.00	0
Musa Xparadisiaca L	6.33	0.00	6.67	2.89	7.00	3.00		2.65				
Saritaea magnifica (W. Bull) Dugand	1.00	1.00	8.67	1.15	9.67	1.53						
Eucalyptus tereticornis Sm.	1.00	1.00	2.67	0.58	4.33	0.58	7.00	2.65	5.00	3		
Allium sativum L	20.00	0.00										
Citrus reticulata Blanco	20.00	0.00										
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0
Vitex negundo L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0
Ricinus communis L.	20.00	0.00										
Mimosa pudica L.	0.00	0.00	3.67	3.79	5.00	1.00	6.00	2.00	0.67	1.15	0.00	0
Murraya koenigii (L.) Spreng	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Terminalia bellirica (Gaertn.) Roxb.	0.00	0.00	0.00	0.00	1.67	1.53	3.67	1.53	2.00	1.73	0.67	1.15

Tridax procumbens L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polyalthia longifolia (Sonn.) Thwaites	0.00	0.00	0.00	0.00	1.67	0.58	3.00	0.00	3.33	0.58	7.33	4.62
Curcuma longa L.	0.00	1.15	2.00	0.00	3.33	0.58	4.67	0.58	4.67	0.58	4.00	1.00
Lantana camara L.	12.33	10.69	7.67	10.69								
Carica papaya L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Momordica charantia L.	4.33	0.58	11.67	2.89	6.00	1.41						
Vinca rosea L.	1.00	2.31	5.67	9.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alpinia purpurata (Vieill.) K. Schum.	5.00	0.58	7.00	1.00	7.00	2.65	1.50	0.71				
Ocimum gratissimum L.	1.33	0.58	2.33	0.58	4.33	1.53	3.67	0.58	3.67	1.53	0.33	0.58
Sida rhombifolia L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Adenocalymma alliaceum(Lam.) Miers	0.00	0.00	3.33	2.87	3	1.73	1.33	0.58	1.33	0.58	11	1
*Chromolaena odorata (*L.) R.M. King & H. Rob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zingiber officinale Roscoe	20.00	0.00										
Pimenta dioica (L.) Merr.	0.00	0.00	9.00	1.00	7.00	2.00	4.00	1.73				
Mesua ferrea L.	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.58	1.67	0.58	0.33	0.58
Apium graveolens L.	0.00	0.00	1.33	0.58	4.00	1.00	5.67	0.58	1.67	0.58	2.33	2.31
Grand Total	4.61	6.71	3.81	4.89	2.63	3.08	2.15	2.58	1.36	1.95	0.98	2.32

Table 2.10 (a) Anova table showing activity against 0.5ml concentration and retention time

Concentration 0.5 ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	172.404	30	5.747	30.239	.000
	Within Groups	11.783	62	.190		
	Total	184.187	92			
12 hour	Between Groups	96.531	30	3.218	11.224	.000
	Within Groups	17.775	62	.287		
	Total	114.305	92			
24 hour	Between Groups	57.986	30	1.933	20.482	.000
	Within Groups	5.851	62	.094		
	Total	63.837	92			
48 hour	Between Groups	43.237	30	1.441	40.450	.000
	Within Groups	2.209	62	.036		
	Total	45.446	92			
72 hour	Between Groups	23.558	30	.785	19.515	.000
	Within Groups	2.495	62	.040		
	Total	26.053	92			
96 hour	Between Groups	18.168	30	.606	10.283	.000
	Within Groups	3.652	62	.059		
	Total	21.820	92			

Four plants such as *Zingiber officinale* Roscoe (20 ±0), *Allium sativum* L (20±0), *Citrus reticulata* Blanco (20±0) and *Ricinus communis* L. (20±0) were highly significant at 0.5ml concentration at 6 hours retention time (F value= 30.239; p value < 0.01). Eight plants such as *Musa X paradisiaca* L (6.67±2.89), *Alpinia purpurata* (Vieill.) K. Schum (7.00±1), *Saritaea*

magnifica (W. Bull) Dugand (8.67±1.15), *Pimenta dioica* (L.) Merr. (9.00±1), *Calotropis gigantea* (L.) R. Br. (9.67±1.53), *Citrus medica* L (10.00±1.73), *Spilanthes calva* DC. (11±1) and *Momordica charantia* L. (11.67±2.89) were highly significant at 0.5ml concentration at 12 hours retention time (F value= 11.224; p value < 0.01). Four plants such as *Musa X paradisiaca* L. (7.00±3.00), *Alpinia purpurata* (Vieill.) K. Schum (7.00±2.65), *Pimenta dioica* (L.) Merr. (7.00±2) and *Saritaea magnifica* (W. Bull) Dugand (9.67±1.53) were highly significant at 0.5ml concentration and 24 hours retention time (F value= 20.482 p value < 0.01). Three plants such as *Apium graveolens* L. (5.67±0.58), *Mimosa pudica* L. (6.00±2.00) and *Eucalyptus tereticornis* Sm. (7.00±2.65) are highly significant at 0.5ml concentration and 48 hours retention time (F value = 40.450; p value < 0.01).Four plants such as *Polyalthia longifolia* (Sonn.) Thwaites (3.33±0.58), *Ocimum gratissimum* L. (3.67±1.53), *Curcuma longa* L.(4.67±0.58) and *Eucalyptus tereticornis* Sm. (5.00±3) were highly significant at 0.5ml concentration and 72 hours retention time (F value= 19.515; p value < 0.01). Two plants such as *Adenocalymma alliaceum* (Lam.) Miers (11±1) and *Polyalthia longifolia* (Sonn.) Thwaites (7.33±4.62) were highly significant at 0.5ml concentration and 96 hours retention time (F value = 10.283; p value < 0.01) (Table 2.10 and 2.10(a)).

Table 2.11.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Culex sitiens* at 1ml concentration and varying retention time

1ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	8.00	0	10.00	0	2.00	0.00						
Calotropis gigantea (L.) R. Br.	8.67	1.15	10.00	0.00	2.00	0.00						
Citrus medica L	8.67	1.15	10.00	0.00	2.00	0.00						
Hyptis suaveolens (L.) Poit	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0	0.00	0
Musa X paradisiaca L	6.33	2.08	5.33	0.58	8.33	1.53		.				
Saritaea magnifica (W. Bull) Dugand	16.33	1.53	3.33	1.15	1.00	.						
Eucalyptus tereticornis Sm.	3.67	0.58	4.33	0.58	12.00	1.00						
Allium sativum L	20.00	0.00										
Citrus reticulata Blanco	20.00	0.00										
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.67	1.15	0.67	1.15	1.00	1.00	1.33	1.53	5.33	7.57
Vitex negundo L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ricinus communis L.	20.00	0.00										
Mimosa pudica L.	1.00	0.00	8.33	3.51	10.67	3.06						
Murraya koenigii (L.) Spreng	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Terminalia bellirica (Gaertn.) Roxb.	2.33	0.58	2.33	0.58	2.67	0.58	1.67	0.58	1.33	0.58	0.67	0.58
Tridax procumbens L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polyalthia longifolia (Sonn.) Thwaites	0.00	0.00	0.33	0.58	3.00	0.00	5.33	1.53	11.00	2.65	1.00	.

Curcuma longa L.	2.00	0.00	7.33	0.58	2.33	0.58	2.00	0.00	2.67	1.15	3.00	1.73
Lantana camara L.	13.33	11.55	20.00	.								
Carica papaya L.	0.00	0.00	14.00	3.61	2.00	0.00	1.33	1.53	1.00	1.41		
Momordica charantia L.	8.00	5.29	9.67	0.58	3.50	2.12						
Vinca rosea L.	3.33	4.62	3.33	5.77	6.00	5.66	6.00	0.00	1.00			
Alpinia purpurata (Vieill.) K. Schum.	8.33	1.00	5.67	1.53	5.00	1.00						
Ocimum gratissimum L.	3.00	1.00	3.67	1.15	4.33	1.15	6.33	2.89	1.67	0.58	0.00	0.00
Sida rhombifolia L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Adenocalymma alliaceum (Lam.) Miers	7.00	2.08	9.00	0.58	4.00							
Chromolaena odorata (L.) R.M. King & H. Rob.	0.00	0.00	1.00	0.00	4.00	0.00	5.33	0.58	6.00	1.00	2.67	1.53
Zingiber officinale Roscoe	20.00	0.00										
Pimenta dioica (L.) Merr.	0.00	0.00	11.67	2.89	5.33	0.58	4.50	0.71				
Mesua ferrea L.	5.67	2.65	6.33	1.53	1.67	0.58	2.33	2.31	1.33	1.15	0.00	.
Apium graveolens L.	0.00	0.00	1.00	0.00	4.67	0.58	4.00	1.00	2.67	1.15	4.67	3.21
Grand Total	5.99	7.07	5.09	4.79	3.40	3.41	2.37	2.50	2.07	3.10	1.43	2.85

Table 2.11(a) Anova table showing activity against 1ml concentration and retention time

Concentration 1ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	169.170	30	5.639	20.977	.000
	Within Groups	16.667	62	.269		
	Total	185.837	92			
12 hour	Between Groups	99.832	30	3.328	12.046	.000
	Within Groups	17.127	62	.276		
	Total	116.960	92			
24 hour	Between Groups	60.197	30	2.007	14.182	.000
	Within Groups	8.772	62	.141		
	Total	68.969	92			
48 hour	Between Groups	34.242	30	1.141	10.268	.000
	Within Groups	6.892	62	.111		
	Total	41.134	92			
72 hour	Between Groups	34.438	30	1.148	25.066	.000
	Within Groups	2.839	62	.046		
	Total	37.278	92			
96 hour	Between Groups	16.189	30	.540	4.293	.000
	Within Groups	7.794	62	.126		
	Total	23.984	92			

Five plants such as *Saritaea magnifica* (W. Bull) Dugand (16.33±1.53), *Allium sativum* L(20±0), *Citrus reticulata* Blanco (20±0), *Ricinus communis* L.(20±0) and *Zingiber officinale* Roscoe(20±0) were highly significant at 1ml

concentration and 6 hours retention time (F value= 20.977; p value < 0.01). Nine plants such as *Curcuma longa* L. (7.33±0.58), *Mimosa pudica* L. (8.33±3.51), *Adenocalymma alliaceum* (Lam.) Miers (9.00±0.58), *Momordica charantia* L. (9.67±0.58), *Spilanthes calva* DC.(10±0), *Calotropis gigantea* (L.) R. Br. (10±0), *Citrus medica* L (10±0), *Pimenta dioica* (L.) Merr. (11.67 ±2.89) and *Carica papaya* L. (14.00±3.61) were highly significant at 1ml concentration and 12 hours retention time (F value= 12.046; p value < 0.01). Five plants such as *Alpinia purpurata* (Vieill.) K. Schum. (5.00±1.00), *Pimenta dioica* (L.) Merr. (5.33±0.58), *Musa X paradisiaca* L (8.33±1.53), *Mimosa pudica* L. (10.67±3.06) and *Eucalyptus tereticornis* Sm. (12.00±1.00) were highly significant at 1ml concentration and 24 hours retention time (F value= 14.182 value < 0.01). Four plants such as *Apium graveolens* L. (4.00±1.00), *Polyalthia longifolia* (Sonn.) Thwaites (5.33±1.53), *Chromolaena odorata* (L.) R.M. King & H. Rob. (5.33±0.58) and *Ocimum gratissimum* L. (6.33±2.89) are highly significant at 1ml concentration and 48 hours retention time (F value = 10.268; p value < 0.01). Plant *Polyalthia longifolia* (Sonn.) Thwaites (11.00±2.65) was highly significant at 1ml concentration and 72 hours retention time (F value = 25.066; p value < 0.01). Four plants such as *Chromolaena odorata* (L.) R.M. King & H. Rob. (2.67±1.53), *Curcuma longa* L. (3.00±1.73), *Ziziphus jujuba*(L.) Lam. (5.33±7.57) and *Apium graveolens* L. (4.67±3.21) were highly significant at 1ml concentration and 96 hours retention time (F value = 4.293; p value < 0.01) (Table 2.11 and 2.11(a)).

Table 2.12The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Culex sitiens* at 2ml concentration and varying retention time

2ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	9.00	0	8.00	0	3.00	0.00						
Calotropis gigantea (L.) R. Br.	9.33	0.58	8.67	1.15	3.00	0.00						
Citrus medica L	9.33	0.58	8.67	1.15	3.00	0.00						
Hyptis suaveolens (L.) Poit	0.00	0.00	0.00	0.00	0.67	1.15	0.00	0	0.00	0	0.00	0
Musa X paradisiaca L	6.33	0.00	6.67	1.53	7.00	2.65						
Saritaea magnifica (W. Bull) Dugand	17.33	1.15	2.67	1.15								
Eucalyptus tereticornis Sm.	4.67	0.58	15.33	0.58								
Allium sativum L	20.00	0.00										
Citrus reticulata Blanco	20.00	0.00										
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.67	1.15	1.33	1.15	3.67	3.79	7.33	5.03	7.50	7.78
Vitex negundo L.	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	1.33	0.58	2.33	0.58
Ricinus communis L.	20.00	0.00										
Mimosa pudica L.	6.00	5.29	12.00	5.29	2.00	0.00						
Murraya koenigii (L.) Spreng	0.00	0.00	0.00	0.00	0.00	0.00	1.33	1.15	2.00	1.00	1.00	0.00
Terminalia bellirica (Gaertn.) Roxb.	2.00	0.00	3.00	0.00	5.00	2.00	7.00	1.73	3.50	2.12	2.00	.
Tridax procumbens L.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Polyalthia longifolia (Sonn.) Thwaites	0.00	0.00	0.67	0.58	4.67	0.58	6.33	1.15	8.33	1.53		
Curcuma longa L.	2.00	0.00	8.33	1.53	1.67	1.53	1.33		4.00	3.46	0.00	0.00

Lantana camara L.	13.33	11.55	20.00	.								
Carica papaya L.	0.00	0.00	20.00	0.00								
Momordica charantia L.	10.33	1.15	8.67	1.53	3.00	.						
Vinca rosea L.	10.33	3.79	9.67	4.93								
Alpinia purpurata (Vieill.) K. Schum.	13.00	1.53	7.00	1.00								
Ocimum gratissimum L.	6.33	1.53	5.67	0.58	6.00	2.65	1.67	1.15	0.50	0.71	0.00	.
Sida rhombifolia L.	0.00	0.00	0.00	0.00	2.33	0.58	7.00	2.00	0.00		0.00	
Adenocalymma alliaceum (Lam.) Miers	12.33	1.00	7.67	1.15								
Chromolaena odorata(L.) R.M. King & H. Rob.	0.00	0.00	2.33	0.58	3.33	1.53	5.00	0.00	7.67	1.53	1.67	0.58
Zingiber officinale Roscoe	20.00	0										
Pimenta dioica (L.) Merr.	0.00	0.00	16.67	0.58	3.33	0.58						
Mesua ferrea L.	15.67	1.53	3.67	1.15	1.00	0.00						
Apium graveolens L.	0.00	0.00	2.00	1.00	4.67	0.58	5.67	0.58	2.67	0.58	0.00	.
Grand Total	7.33	7.26	6.25	5.83	2.76	2.27	3.33	2.94	3.38	3.53	1.39	2.71

Table 2.12 (a) Anova table showing activity against 2ml concentration and retention time

Concentration 2ml		Sum of Squares	df	Mean Square	F	Sig.
tt6	Between Groups	191.344	30	6.378	26.228	.000
	Within Groups	15.077	62	.243		
	Total	206.421	92			
tt12	Between Groups	127.053	30	4.235	18.408	.000
	Within Groups	14.265	62	.230		
	Total	141.318	92			
tt24	Between Groups	38.792	30	1.293	12.249	.000
	Within Groups	6.545	62	.106		
	Total	45.337	92			
tt48	Between Groups	45.242	30	1.508	26.674	.000
	Within Groups	3.505	62	.057		
	Total	48.748	92			
tt72	Between Groups	42.168	30	1.406	13.599	.000
	Within Groups	6.408	62	.103		
	Total	48.577	92			
tt96	Between Groups	9.105	30	.304	3.551	.000
	Within Groups	5.300	62	.085		
	Total	14.405	92			

Eight plants such as *Adenocalymma alliaceum* (Lam.) Miers (12.33±1.00), *Alpinia purpurata* (Vieill.) K. Schum. (13.00±1.53), *Mesua ferrea L.* (15.67±1.53), *Saritaea magnifica* (W. Bull) Dugand (17.33±1.15),

Allium sativum L (20±0), *Citrus reticulata* Blanco (20±0), *Ricinus communis* L. (20±0) and *Zingiber officinale* Roscoe (20±0) were highly significant at 2ml concentration and 6 hours retention time (F value= 26.228; p value < 0.01).Three plants such as *Eucalyptus tereticornis* Sm. (15.33±0.58), *Pimenta dioica* (L.) Merr. (16.67±0.58) and *Carica papaya* L. (20±0) were highly significant at 2ml concentration and 12 hours retention time (F value= 18.408; p value < 0.01). Five plants such as *Polyalthia longifolia* (Sonn.) Thwaites (6.33±1.15), *Apium graveolens* L. (4.67±0.58), *Terminalia bellirica* (Gaertn.) Roxb. (5.00±2.00), *Ocimum gratissimum* L. (6.00±2.65) and *Musa X paradisiaca* L (7.00±2.65) are highly significant at 2ml concentration and 24 hours retention time (F value = 12.249; p value < 0.01). Five plants such as *Chromolaena odorata* (L.) R.M. King & H. Rob. (5.00±0.00), *Apium graveolens* L. (5.67±0.58), *Polyalthia longifolia*(Sonn.) Thwaites (6.33±1.15), *Sida rhombifolia* L. (7.00±2.00) and *Terminalia bellirica* (Gaertn.) Roxb. (7.00±1.73) were highly significant at 2ml concentration and 48 hours retention time (F value= 26.674 p value < 0.01). Three plants such as *Ziziphus jujuba*(L.) Lam.(7.33±5.03), *Chromolaena odorata* (L.) R.M. King & H. Rob. (7.67±1.53) and *Polyalthia longifolia* (Sonn.) Thwaites (8.33±1.53) were highly significant at 2ml concentration and 72 hours retention time (F value= 13.599 p value < 0.01).Two plants such as *Vitex negundo* L. (2.33±0.58) and *Ziziphus jujuba* (L.) Lam. (7.50±7.78) were highly significant at 2ml concentration and 96 hours retention time (F value= 3.551 p value < 0.01) (Table 2.12 and 2.12(a)).

Table 2.13.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Culex sitiens* at 4ml concentration and varying retention time

Plants	4ml											
	Mean 6HAT	SD 6HAT	Mean 12HAT	SD 12HAT	Mean 24HAT	SD 24HAT	Mean 48HAT	SD 48HAT	Mean 72HAT	SD 72HAT	Mean 96HAT	SD 96HAT
Spilanthes calva DC.	10.00	0	10.00	0								
Calotropis gigantea (L.) R. Br.	13.33	5.77	10.00	0.00								
Citrus medica L	13.33	5.77	10.00	0.00								
Hyptis suaveolens (L.) Poit	0.00	0.00	1.67	2.89	5.00	0.00	6.00	1.73	0.33	0.58	0.00	0
Musa X paradisiaca L	0.00	2.65	6.33	3.21	8.67	1.15	5.00					
Saritaea magnifica (W. Bull) Dugand	18.67	1.15	2.00	0.00								
Eucalyptus tereticornis Sm.	20.00	0.00										
Allium sativum L	20.00	0.00										
Citrus reticulata Blanco	20.00	0.00										
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.67	1.15	1.33	1.15	5.00	5.29	9.67	4.93	3.33	3.21

Vitex negundo L.	0.00	0.00	0.00	0.00	1.33	0.58	3.67	1.15	4.00	1.00	4.00	1.73
Ricinus communis L.	20.00	0.00										
Mimosa pudica L.	6.00	5.29	11.67	6.35	2.33	1.53						
Murraya koenigii (L.) Spreng	0.00	0.00	2.00	0.00	4.33	1.15	5.00	0.00	6.00	1.00	2.67	2.08
Terminalia bellirica (Gaertn.) Roxb.	6.33	1.53	13.67	1.53								
Tridax procumbens L.	0.00	0.00	3.67	0.58	5.67	0.58	8.67	0.58	0.33	0.58	0.00	0.00
Polyalthia longifolia (Sonn.) Thwaites	0.00	0.00	2.67	0.58	6.67	1.53	7.00	0.00	3.67	2.08		
Curcuma longa L.	2.67	1.15	17.33	1.15								
Lantana camara L.	13.33	11.55	20.00	.								
Carica papaya L.	0.00	0.00	20.00	0.00								
Momordica charantia L.	9.67	0.58	6.33	1.53	10.00	.						
Vinca rosea L.	11.00	3.46	9.00	4.36								
Alpinia purpurata	20.00	0.00										

(Vieill.) K. Schum.												
Ocimum gratissimum L.	8.00	2.00	12.00	2.00								
Sida rhombifolia L.	3.33	4.16	12.33	5.51	13.00	.						
Adenocalymma alliaceum (Lam.) Miers	14.67	1.73	5.33	0.58								
Chromolaena odorata (L.) R.M. King & H. Rob.	0.00	0.00	2.33	0.58	3.00	1.73	4.33	0.58	10.00	3.46		
Zingiber officinale Roscoe	20.00	0.00										
Pimenta dioica (L.) Merr.	0.00	0.00	18.33	0.58	1.67	0.58						
Mesua ferrea L.	16.33	1.53	3.67	2.08								
Apium graveolens L.	0.00	0.00	3.33	0.58	7.00	1.00	5.67	2.08	4.00	1.00		.
Grand Total	8.70	7.89	7.87	6.29	4.69	3.08	5.59	2.34	4.75	4.02	2.00	2.36

Table 2.13(a) Anova table showing activity against 4ml concentration and retention time

Concentration 4 ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	222.713	30	7.424	20.929	.000
	Within Groups	21.992	62	.355		
	Total	244.705	92			
12 hour	Between Groups	130.326	30	4.344	9.975	.000
	Within Groups	27.000	62	.435		
	Total	157.327	92			
24 hour	Between Groups	51.289	30	1.710	8.754	.000
	Within Groups	12.109	62	.195		
	Total	63.397	92			
48 hour	Between Groups	58.573	30	1.952	30.268	.000
	Within Groups	3.999	62	.065		
	Total	62.572	92			
72 hour	Between Groups	51.131	30	1.704	32.225	.000
	Within Groups	3.279	62	.053		
	Total	54.410	92			
96 hour	Between Groups	11.498	30	.383	10.343	.000
	Within Groups	2.297	62	.037		
	Total	13.796	92			

Eleven plants such as *Calotropis gigantea* (L.) R. Br. (13.33±5.77), *Citrus medica* L (13.33±5.77), *Adenocalymma alliaceum* (Lam.) Miers (14.67±1.73), *Mesua ferrea* L. (16.33±1.53), *Saritaea magnifica* (W. Bull)

Dugand (18.67±1.15), *Eucalyptus tereticornis* Sm. (20±0), *Allium sativum* L (20±0), *Citrus reticulata* Blanco (20±0), *Ricinus communis* L. (20±0), *Alpinia purpurata* (Vieill.) K. Schum. (20±0) and *Zingiber officinale* Roscoe (20±0) were highly significant at 4ml concentration and 6 hours retention time (F value= 20.929; p value < 0.01). Nine plants such as *Vinca rosea* L.(9.00±4.36), *Spilanthes calva* DC.(10±0), *Mimosa pudica* L.(11.67±6.35), *Sida rhombifolia* L.(12.33±5.51), *Ocimum gratissimum* L.(12.00±2.00), *Terminalia bellirica* (Gaertn.) Roxb. (13.67±1.53), *Curcuma longa* L. (17.33±1.15), *Pimenta dioica* (L.) Merr. (18.33±0.58) and *Carica papaya* L. (20±0) were highly significant at 4ml concentration and 12 hours retention time (F value= 9.975; p value < 0.01). Five plants such as *Hyptis suaveolens* (L.) Poit (5.00±0.00), *Tridax procumbens* L. (5.67±0.58), *Polyalthia longifolia* (Sonn.) Thwaites (6.67±1.53), *Apium graveolens* L. (7.00±1.00) and *Musa X paradisiaca* L (8.67±1.15) were highly significant at 4ml concentration and 24 hours retention time (F value = 8.754; p value < 0.01). Two plants such as *Polialthia longifolia* (Sonn.) Thwaites (7.00±0.00) and *Tridax procumbens* L. (8.67±0.58) were highly significant at 4ml concentration and 48 hours retention time (F value= 30.268 p value < 0.01).Two plants such as *Ziziphus jujuba* (L.) Lam. (9.67±4.93) and *Chromolaena odorata* (L.) R.M. King & H. Rob. (10.00±3.46) were highly significant at 4ml concentration and 72 hours retention time (F value= 32.225 p value < 0.01). Plants such as *Ziziphus jujuba* (L.) Lam. (3.33±3.21) and *Vitex negundo* L. (4.00±1.73) were highly significant at 4ml concentration and 96 hours retention time (F value= 10.343 p value < 0.01) (Table 2.13 and 2.13(a).

Table 2.14.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Culex sitiens* at 8ml concentration and varying retention time

8ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	12.00	0	8.00	0								
Calotropis gigantea (L.) R. Br.	14.67	4.62	8.00	0.00								
Citrus medica L	14.67	4.62	8.00	0.00								
Hyptis suaveolens (L.) Poit	0.00	0.00	3.00	2.65	8.33	3.51	4.00	1.73	2.00	2.83	2.50	3.54
Musa X paradisiaca L	1.00	3.21	9.67	2.08	9.33	1.15	1.00					
Saritaea magnifica (W. Bull) Dugand	20.00	0.00										
Eucalyptus tereticornis Sm.	20.00	0.00										
Allium sativum L	20.00	0.00										
Citrus reticulata Blanco	20.00	0.00										
Ziziphus jujuba (L.) Lam.	0.00	0.00	0.67	1.15	6.33	7.51	4.33	4.93	5.67	4.04	9.00	.
Vitex negundo L.	0.00	0.00	2.33	0.58	3.00	1.00	4.67	1.15	5.67	0.58	4.33	0.58
Ricinus communis L.	20.00	0.00										
Mimosa pudica L.	10.00	2.00	9.00	2.00	1.00	0.00						
Murraya koenigii(L.) Spreng	0.00	0.00	2.33	0.58	5.33	0.58	7.00	1.73	4.67	3.06	2.00	.
Terminalia bellirica (Gaertn.) Roxb.	8.33	1.53	11.67	1.53								
Tridax procumbens L.	0.00	0.00	6.67	1.53	7.00	0.00	6.33	1.53				
Polyalthia longifolia (Sonn.) Thwaites	0.00	0.00	3.67	1.53	7.33	1.15	7.67	0.58	4.00	.		
Curcuma longa L.	5.33	1.53	14.67	1.53								
Lantana camara L.	13.33	11.55	20.00	.								
Carica papaya L.	0.00	0.00	20.00	0.00								
Momordica charantia L.	17.00	1.53	3.00	2.65								
Vinca rosea L.	12.33	4.00	7.67	4.93								

Alpinia purpurata (Vieill.) K. Schum.	20.00	0										
Ocimum gratissimum L.	9.67	2.08	10.33	2.08								
Sida rhombifolia L.	3.67	3.51	13.00	3.00	10.00	.						
Adenocalymma alliaceum (Lam.) Miers	17.33	1.15	2.67									
Chromolaena odorata (L.) R.M. King & H. Rob.	0.00	0.00	2.00	0.00	4.33	1.53	5.67	0.58	8.00	2.00		
Zingiber officinale Roscoe	20.00	0										
Pimenta dioica (L.) Merr.	0.00	0.00	19.67	0.58	1.00	.						
Mesua ferrea L.	17.67	0.58	2.33	0.58								
Apium graveolens L.	0.67	1.15	4.67	0.58	11.00	3.00	3.67	2.31	0.00	.	0.00	
Grand Total	9.70	8.12	7.69	5.84	6.25	3.82	5.24	2.45	5.00	3.08	3.63	2.97

Table 2.14(a) Anova table showing activity against 8ml concentration and retention time

Concentration 8ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	232.951	30	7.765	29.976	.000
	Within Groups	16.061	62	.259		
	Total	249.012	92			
12 hour	Between Groups	119.876	30	3.996	10.997	.000
	Within Groups	22.529	62	.363		
	Total	142.404	92			
24 hour	Between Groups	74.965	30	2.499	15.755	.000
	Within Groups	9.834	62	.159		
	Total	84.799	92			
48 hour	Between Groups	51.648	30	1.722	24.976	.000
	Within Groups	4.274	62	.069		
	Total	55.922	92			
72 hour	Between Groups	33.804	30	1.127	12.171	.000
	Within Groups	5.740	62	.093		
	Total	39.544	92			
96 hour	Between Groups	8.739	30	.291	2.964	.000
	Within Groups	6.092	62	.098		
	Total	14.831	92			

Thirteen plants such as *Spilanthes calva* DC (12.00±0), *Calotropis gigantea* (L.) R. Br. (14.67±4.62), *Citrus medica* L (14.67±4.62), *Adenocalymma alliaceum* (Lam.) Miers (17.33.±1.15), *Mesua ferrea* L. (17.67±0.58), *Momordica charantia* L. (17.±1.53), *Saritaea magnifica* (W.

Bull) Dugand (20±0), *Eucalyptus tereticornis* Sm. (20±0), *Allium sativum* L (20±0), *Citrus reticulata* Blanco (20±0), *Ricinus communis* L. (20±0), *Alpinia purpurata* (Vieill.) K. Schum. (20±0) and *Zingiber officinale* Roscoe (20±0) were highly significant at 8ml concentration at 6 hours retention time (F value= 29.976; p value < 0.01). Five plants such as *Lantana camara* L. (20±0), *Curcuma longa* L. (14.67±1.53), *Terminalia bellirica* (Gaertn.) Roxb. (11.67±1.53), *Mimosa pudica* L. (9±2) and *Musa X paradisiaca* L (9.67±2.8) were highly significant at 8ml concentration and 12 hours retention time (F value= 10.997; p value < 0.01). Two plants such as *Hyptis suaveolens* (L.) Poit (8.33± 3.51) and *Tridax procumbens* L. (7.00±0) were significant at 8ml concentration and 24 hours retention time (F value= 15.755; p value < 0.01). Plants such as *Murraya koenigii* (L.) Spreng (7.00±1.73) *Polyalthia longifolia* (Sonn.) Thwaites (7.67± 0.58) at 48 hours; *Chromolaena odorata* (L.) R.M. King & H. Rob. (8.00 ± 2.00) at 72 hours and *Ziziphus jujuba* (L.) Lam. (9.00±0) at 96 hours were significant at 8ml concentration (F value = 1.722; p value < 0.01; F value = 1.127; p value < 0.01; F value = 0.291; p value < 0.01) respectively (Table 2.14 and 2.14(a)).

Altogether thirteen plants among thirty one were showing strongest larvicidal effects in all concentration and retention time against *Culex sitiens* larvae. Their LC_{50}values were calculated (Table 2.15).

Table 2.15.LC_{50} values of plants against *Culex sitiens*

SI. No.	Plants	LC_{50}mg/ml
1	*Musa Xparadisiaca* L.	0.012
2	*Saritaea magnifica* (W. Bull) Dugand	0.0010
3	*Eucalyptus tereticornis* Sm.	19.92
4	*Allium sativum* L	0*
5	*Citrus reticulata* Blanco	0*
6	*Ricinus communis* L.	0*
7	*Polyalthia longifolia* (Sonn.) Thwaites	0*
8	*Carica papaya* L.	0*
9	*Alpinia purpurata* (Vieill.) K. Schum.	0.060
10	*Adenocalymma alliaceum* (Lam.) Miers	1.98
11	*Chromolaena odorata* (L.) R.M. King & H. Rob.	18.93
12	*Zingiber officinale* Roscoe	0*
13	*Pimenta dioica* (L.) Merr.	0*

0* represents 100 % mortality occurred in lower concentration in short time.

Table 2.16.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Armigeres subalbatus* at 0.5ml concentration and varying retention time

0.5ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	7.33	1.154	11	1	1.67	0.58						
Calotropis gigantea (L.) R. Br.	9.33	2.3	9.67	1.53	1.5	0.71						
Citrus medica L	9.33	2.31	10	1.73	1	0						
*Hyptis suaveolens (*L.) Poit	0	0										
Musa X paradisiaca L	3.67	2.08	4.67	2.52	11.67	0.58						
Saritaea magnifica (W. Bull) Dugand	1	1	8.67	1.15	9.67	1.53						
Eucalyptus tereticornis Sm.	1	1	2.33	0.58	4.33	0.58	6.67	2.31	5.67	3.79		
Allium sativum L	20	0										
Citrus reticulata Blanco	20	0										
Ziziphus jujuba (L.) Lam.	0	0	0	0	0	0	0	0	0	0	0	0
Vitex negundo L.	0	0	0	0	0	0	0	0	0	0	0	0
Ricinus communis L.	20	0										

Mimosa pudica L.	6	5.29	3.67	3.79	5	1	6	2	0.67	1.15	0	0
Murraya koenigii (L.) Spreng	0	0	0	0	0	0	0	0	0	0	0	0
Terminalia bellirica (Gaertn.) Roxb.	2	0	0	0	2	1	3.33	1.15	2	1.73	0	0
Tridax procumbens L.	0	0	0	0	0	0	0	0	0	0	0	0
Polyalthia longifolia (Sonn.) Thwaites	0	0	0	0	1.33	0.58	3	0	3.33	0.58	10.33	0.58
Curcuma longa L.	0.67	1.15	2	0	3.33	0.58	4.67	0.58	4.67	0.58	4	1
Lantana camara L.	12.33	10.69	5.67	7.23	6	.						
Carica papaya L.	0	0	0	0	0	0	0	0	0	0	0	0
Momordica charantia L.	3.67	0.58	13.33	2.31	6	2.83						
Vinca rosea L.	1.33	2.31	5.33	9.24	0	0	0	0	0	0	0	0
Alpinia purpurata (Vieill.) K. Schum.	1.33	0.58	2.33	0.58	4.33	1.53	3.67	0.58	3.33	1.15	1	.
Ocimum gratissimum L.	1.33	0.58	2.33	0.58	4.33	1.53	3.67	0.58	3.67	1.53	0.33	0.58
Sida rhombifolia L.	0	0	0	0	0	0	0	0	0	0	0	0

Adenocalymma alliaceum (Lam.) Miers	5.67	1.15	8.67	1.15	3	1						
Chromolaena odorata (L.) R.M. King & H. Rob.	0	0	0	0	0	0	0	0	0	0	0	0
Zingiber officinale Roscoe	14.33	0.58	5.67	0.58								
Pimenta dioica (L.) Merr.	0	0	9	1	7.33	1.53	3.33	2.08				
Mesua ferrea L.	0	0	0	0	0.33	0.58	2	2	5.5	4.95	0.5	0.71
Apium graveolens L.	0	0	1.33	0.58	4	1	5.33	1.15	1.67	0.58	2	1.73
Grand Total	4.53	1.06	3.91	1.32	2.95	0.69	2.32	0.69	1.79	0.94	1.14	0.31

Table 2.16(a) Anova table showing activity against 0.5ml concentration and retention time

Concentration 0.5ml		Sum of Squares	df	Mean Square	F	Sig.
6hour	Between Groups	156.330	30	5.211	24.983	.000
	Within Groups	12.932	62	.209		
	Total	169.262	92			
12 hour	Between Groups	95.737	30	3.191	13.482	.000
	Within Groups	14.676	62	.237		
	Total	110.413	92			
24 hour	Between Groups	60.580	30	2.019	17.727	.000
	Within Groups	7.063	62	.114		
	Total	67.643	92			
48	Between Groups	43.462	30	1.449	31.058	.000
	Within Groups	2.892	62	.047		
	Total	46.354	92			
72	Between Groups	28.217	30	.941	10.307	.000
	Within Groups	5.658	62	.091		
	Total	33.875	92			
96	Between Groups	25.475	30	.849	43.888	.000
	Within Groups	1.200	62	.019		
	Total	26.674	92			

Four plants such as *Zingiber officinale* Roscoe (14.33±0.58), *Allium sativum* L (20±0), *Citrus reticulata* Blanco (20±0) and *Ricinus communis* L. (20±0) were highly significant at 0.5ml concentration at 6 hours retention

time (F value= 24.983; p value < 0.01). Seven plants such as *Saritaea magnifica* (W. Bull) Dugand (8.67±1.15), *Adenocalymma alliaceum* (Lam.) Miers (8.67±1.15), *Pimenta dioica* (L.) Merr. (9.00±1), *Calotropis gigantea* (L.) R. Br. (9.67±1.53), *Citrus medica* L (10.00±1.73), *Spilanthes calva* DC. (11±1) and *Momordica charantia* L. (13.33±2.31) were highly significant at 0.5ml concentration at 12 hours retention time (F value= 13.482; p value < 0.01). Two plants such as *Saritaea magnifica* (W. Bull) Dugand (9.67±1.53) and *Musa X paradisiaca* L(11.67±0.58) were highly significant at 0.5ml concentration and 24 hours retention time (F value= 17.727 p value < 0.01). Four plants such as *Curcuma longa* L.(4.67±0.58), *Apium graveolens* L.(5.33±1.15), *Mimosa pudica* L.(6.00±2.00) and *Eucalyptus tereticornis* Sm.(6.67±2.31) were highly significant at 0.5ml concentration and 48 hours retention time (F value= 31.058; p value < 0.01). Five plants such as *Alpinia purpurata* (Vieill.) K. Schum. (3.33±1.15), *Polyalthia longifolia (Sonn.) Thwaites* (3.33±0.58), *Ocimum gratissimum* L. (3.67±1.53), *Curcuma longa* L. (4.67±0.58) and *Eucalyptus tereticornis* Sm.(5.67±3.79) were highly significant at 0.5ml concentration and 72 hours retention time (F value= 10.307; p value < 0.01). *Polyalthia longifolia* (Sonn.) Thwaites (10.33±0.58) was highly significant at 0.5ml concentration and 96 hours retention time (F value= 43.888; p value < 0.01) (Table 2.16 and 2.16(a)).

Table 2.17.The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Armigeres subalbatus* at 1ml concentration and varying retention time

1ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	8	0	10	0	2	0						
*Calotropis gigantea (*L.) R. Br.	8.67	1.155	10	0	2	0						
Citrus medica L	8.67	1.16	10	0	2	0						
Hyptis suaveolens (L.) Poit	0	0	0	0	0	0	0	0	0	0	0	0
Musa X paradisiaca L	5	0	9.33	1.15	5.67	1.15						
Saritaea magnifica (W. Bull) Dugand	16.33	1.53	3.33	1.15	1	.						
Eucalyptus tereticornis Sm.	3.67	0.58	4	0	12.33	0.58						
Allium sativum L	20	0										
Citrus reticulata Blanco	20	0										
Ziziphus jujuba (L.) Lam.	0	0	0	0	0	0	0	0	0	0	0	0
Vitex negundo L.	0	0	0	0	0	0	0	0	0	0	0	0
Ricinus communis L.	20	0										
Mimosa pudica L.	6	5.29	8.33	3.51	11	3.61	2	.				
Murraya koenigii (L.) Spreng	0	0	0	0	0	0	0	0	0	0	0	0
Terminalia bellirica (Gaertn.) Roxb.	6.33	1.53	2.33	0.58	2.67	0.58	2.67	2.08	2.67	2.08	0.67	0.58
Tridax procumbens L.	0	0	0	0	0	0	0	0	0	0	0	0
Polyalthia longifolia (Sonn.) Thwaites	0	0	0.33	0.58	3	0	5.33	1.53	11.33	2.08		
Curcuma longa L.	2	0	5.67	2.08	2.33	0.58	2.33	0.58	2.67	1.15	5	1
Lantana camara L.	13.33	11.55	20	.								
Carica papaya L.	0	0	0	0	17	1	1.67	1.15	1.33	0.58		

Momordica charantia L.	6	5.29	9.67	0.58	4	1.41						
Vinca rosea L.	2.67	4.62	4	6.93	12	0	7	1.41	1	.		
Alpinia purpurata (Vieill.) K. Schum.	3	1	3.67	1.15	3.67	2.31	6.33	2.89	1.67	0.58		
Ocimum gratissimum L.	3	1	3.67	1.15	4.33	1.15	6.33	2.89	1.67	0.58	0	0
Sida rhombifolia L.	0	0	0	0	0	0	0	0	0	0	0	0
Adenocalymma alliaceum (Lam.) Miers	10.33	2.08	9	2.65								
Chromolaena odorata (L.) R.M. King & H. Rob.	0	0	1	0	4	0	5.33	0.58	6	1	2.67	1.53
Zingiber officinale Roscoe	16.33	0.58	3.67	0.58								
Pimenta dioica (L.) Merr.	0	0	11.67	2.89	5.33	0.58	4.5	0.71				
Mesua ferrea L.	3	2.65	5.67	2.08	3	1.73	4	1.41	5	4.24	0	.
Apium graveolens L.	0	0	1	0	4	1	4	1	2.67	1.15	4	2.65
Grand Total	5.88	1.29	4.87	1.00	4.05	0.65	2.86	0.95	2.25	0.90	1.03	0.52

Table 2.17(a) Anova table showing activity against 1ml concentration and retention time

Concentration 1ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	165.883	30	5.529	17.847	.000
	Within Groups	19.209	62	.310		
	Total	185.092	92			
12 hour	Between Groups	90.815	30	3.027	10.441	.000
	Within Groups	17.976	62	.290		
	Total	108.791	92			
24 hour	Between Groups	82.750	30	2.758	16.653	.000
	Within Groups	10.269	62	.166		
	Total	93.019	92			
48 hour	Between Groups	41.687	30	1.390	9.478	.000
	Within Groups	9.090	62	.147		
	Total	50.777	92			
72 hour	Between Groups	38.236	30	1.275	18.725	.000
	Within Groups	4.220	62	.068		
	Total	42.456	92			
96 hour	Between Groups	15.042	30	.501	18.118	.000
	Within Groups	1.716	62	.028		
	Total	16.758	92			

Five plants such as *Saritaea magnifica* (W. Bull) Dugand (16.33±1.53), *Zingiber officinale* Roscoe (16.33±0.58), *Allium sativum* L (20±0), *Citrus reticulata* Blanco (20±0) and *Ricinus communis* L.(20±0) were highly

significant at 1ml concentration and 6 hours retention time (F value= 17.847; p value < 0.01).Ten plants such as *Curcuma longa* L.(5.67±2.08), *Mesua ferrea* L.(5.67±2.08), *Mimosa pudica* L. (8.33±3.51), *Adenocalymma alliaceum* (Lam.) Miers (9.00±2.65), *Musa X paradisiaca* L. (9.33±1.15), *Momordica charantia* L. (9.67±0.58), *Spilanthes calva* DC.(10±0), *Calotropis gigantea* (L.) R. Br. (10±0), *Citrus medica* L (10±0) and *Pimenta dioica*(L.) Merr. (11.67 ±2.89) were highly significant at 1ml concentration and 12 hours retention time (F value= 10.441; p value < 0.01). Two plants such as *Eucalyptus tereticornis* Sm.(12.33±0.58) and *Carica papaya* L.(17.00±1.00) were highly significant at 1ml concentration and 24 hours retention time (F value= 16.653; p value < 0.01). Six plants such as *Vinca rosea* L. (7.00±1.41), *Apium graveolens* L. (4.00±1.00), *Polyalthia longifolia* (Sonn.) Thwaites (5.33±1.53), *Chromolaena odorata* (L.) R.M. King & H. Rob. (5.33±0.58) *Alpinia purpurata* (Vieill.) K. Schum. (6.33±2.89) and *Ocimum gratissimum* L. (6.33±2.89) were highly significant at 1ml concentration and 48 hours retention time (F value = 9.478; p value < 0.01). Plants such as *Polyalthia longifolia* (Sonn.) Thwaites (11.33±2.08) at 72 hrs and *Curcuma longa* L. (5.00±1.00) at 96 hrs were highly significant at 0.5ml concentration (F value= 18.725; p value < 0.01; 18.118; p value < 0.01) respectively (Table 2.17 and 2.17(a)).

Table 2.18 The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Armigeres subalbatus* at 2ml concentration and varying retention time

2ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	9	0	8	0	3	0						
Calotropis gigantea (L.) R. Br.	9.33	0.577	8.67	1.15	3	0						
Citrus medica L	9.33	2.31	10	1.73	1	0						
Hyptis suaveolens (L.) Poit	0	0	0	0	0	0	0	0	0	0	0	0
Musa X paradisiaca L	7	2.65	6.67	1.53	6.33	1.53						
Saritaea magnifica (W. Bull) Dugand	17.33	1.15	2.67	1.15								
Eucalyptus tereticornis Sm.	4.67	0.58	15.33	0.58								
Allium sativum L	20	0										
Citrus reticulata Blanco	20	0										
Ziziphus jujuba (L.) Lam.	0	0	0.67	1.15	1.33	1.15	4.67	5.51	6	5.29	5.33	6.66
Vitex negundo L.	0	0	0	0	0	0	0	0	1.33	0.58	2.33	0.58
Ricinus communis L.	20	0										
Mimosa pudica L.	10	2	12	5.29	2	0						
Murraya koenigii (L.) Spreng	0	0	1.67	1.53	3.67	1.53	5.33	0.58	4	2	2	1.73
Terminalia bellirica (Gaertn.) Roxb.	8.33	1.53	3	0	5	2	7	1.73	3.5	2.12	2	.
Tridax procumbens L.	0	0	3.67	0.58	5.67	0.58	8.67	0.58	1	1	0	0
Polyalthia longifolia (Sonn.) Thwaites	0	0	0.33	0.58	4.33	0.58	6.33	2.08	9	2.65		

Curcuma longa L.	2	0	4.33	3.51	2.67	0.58	2.67	1.15	8.33	2.08	0	.
Lantana camara L.	13.33	11.55	20	.								
Carica papaya L.	0	0	20	0								
Momordica charantia L.	11.33	1.15	7	1.73	2.5	0.71						
Vinca rosea L.	9.67	3.79	10.33	3.79	0	0						
Alpinia purpurata (Vieill.) K. Schum.	6.33	1.53	5.67	0.58	6	2.65	1.67	1.15	1	.		
Ocimum gratissimum L.	6.33	1.53	5.67	0.58	6	2.65	1.67	1.15	0.5	0.71	0	.
Sidarhombifolia L.	0	0	0	0	3.33	1.53	9	3.61	0	.	0	.
Adenocalymma alliaceum (Lam.) Miers	12	1	7.33	1.53								
Chromolaena odorata (L.) R.M. King & H. Rob.	0	0	2.33	0.58	3.33	1.53	5	0	7.67	1.53	1.67	0.58
Zingiber officinale Roscoe	16.67	1.15	3.33	1.15								
Pimenta dioica (L.) Merr.	0	0	16.67	0.58	3.33	0.58						
Mesua ferrea L.	15.67	1.53	3.33	0.58	1.5	0.71						
Apium graveolens L.	0	0	2	1	4	1	5	1	2.33	0.58	0	.
Grand Total	7.37	1.10	6.45	1.14	3.09	0.88	4.39	1.43	3.44	1.69	1.21	1.59

Table 2.18 (a) Anova table showing activity against 2ml concentration and retention time

Concentration 2ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	181.981	30	6.066	24.619	.000
	Within Groups	15.277	62	.246		
	Total	197.258	92			
12 hour	Between Groups	118.712	30	3.957	16.249	.000
	Within Groups	15.099	62	.244		
	Total	133.811	92			
24 hour	Between Groups	41.685	30	1.389	14.194	.000
	Within Groups	6.069	62	.098		
	Total	47.754	92			
48 hour	Between Groups	50.003	30	1.667	21.199	.000
	Within Groups	4.875	62	.079		
	Total	54.877	92			
72 hour	Between Groups	48.425	30	1.614	21.571	.000
	Within Groups	4.640	62	.075		
	Total	53.065	92			
96 hour	Between Groups	9.960	30	.332	4.957	.000
	Within Groups	4.153	62	.067		
	Total	14.112	92			

Six plants such as *Mesua ferrea* L. (15.67±1.53), *Zingiber officinale Roscoe* (16.67±1.15), *Saritaea magnifica* (W. Bull) Dugand (17.33±1.15), *Allium sativum* L.(20±0), *Citrus reticulata* Blanco (20±0) and *Ricinus*

communis L. (20±0) were highly significant at 2ml concentration and 6 hours retention time (F value= 24.619; p value <0.01). Four plants such as *Adenocalymma alliaceum* (Lam.) Miers (7.33±1.53) *Eucalyptus tereticornis* Sm. (15.33±0.58), *Pimenta dioica* (L.) Merr. (16.67±0.58) and *Carica papaya* L. (20±0) were highly significant at 2ml concentration and 12 hours retention time (F value= 16.249; p value < 0.01). Six plants such as *Apium graveolens* L. (4.00±1.00), *Polyalthia longifolia* (Sonn.) Thwaites (4.33±0.58), *Terminalia bellirica* (Gaertn.) Roxb. (5.00±2.00) *Alpinia purpurata* (Vieill.) K. Schum. (6.00±2.65), *Ocimum gratissimum* L. (6.00±2.65) and *Musa X paradisiaca* L (6.33±1.53) were highly significant at 1ml concentration and 24 hours retention time (F value = 14.194; p value < 0.01). Three plants such as *Polyalthia longifolia* (Sonn.) Thwaites (6.33±2.08), *Terminalia bellirica* (Gaertn.) Roxb. (7.00±1.73) and *Sida rhombifolia* L. (9.00±3.61) were highly significant at 1ml concentration and 48 hours retention time (F value= 21.199 value < 0.01). Three plants such as *Chromolaena odorata* (L.) R.M. King & H. Rob. (7.67±1.53), *Curcuma longa* L. (8.33±2.08) and *Polyalthia longifolia* (Sonn.) Thwaites (9.00±2.65) were highly significant at 2ml concentration and 72 hours retention time (F value= 21.571 p value < 0.01). Plant *Ziziphus jujuba* (L.) Lam. (5.33±6.66) was highly significant at 2ml concentration and 96 hours retention time (F value= 4.957 p value < 0.01) (Table 2.18 and 2.18(a)).

Table 2.19 The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Armigeres subalbatus* at 4ml concentration and varying retention time

4ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	10	0	10	0								
Calotropis gigantea (L.) R. Br.	13.33	5.77	10	0								
Citrus medica L	13.33	5.77	10	0								
Hyptis suaveolens (L.) Poit	0	0	1.67	2.89	5	0	6	1.73	1.67	2.08	0	0
Musa X paradisiaca L	6.33	3.21	8.67	1.15	5	2.65						
Saritaea magnifica (W. Bull) Dugand	18.67	1.15	2	0								
Eucalyptus tereticornis Sm.	20	0	0	0								
Allium sativum L	20	0										
Citrus reticulata Blanco	20	0										
Ziziphus jujuba (L.) Lam.	0	0	0.67	1.15	5.67	8.14	2	1	8.67	6.66	4.5	3.54
Vitex negundo L.	0	0	0	0	1	1	3.67	1.15	4	1	4	1.73
Ricinus communis L.	20	0										
Mimosa pudica L.	0	0	11.67	6.35	2.33	1.53						
Murraya koenigii (L.) Spreng	0	0	1.67	1.53	3.67	1.53	5.33	0.58	4	2	2	1.73
Terminalia bellirica (Gaertn.) Roxb.	20	0	13.67	1.53								
Tridax procumbens L.	0	0	3.67	0.58	5.67	0.58	8.67	0.58	1	1	0	0
Polialthia longifolia (Sonn.) Thwaites	0	0	2.67	0.58	6.67	1.53	7	0	3.67	2.08		
Curcuma longa L.	2.67	1.155	17.33	1.15								
Lantana camara L.	13.33	11.55	20	.								
Carica papaya L.	0	0	20	0								

Momordica charantia L.	13.67	0.58	6	0	1	.						
Vinca rosea L.	10	3.46	10	3.46								
Alpinia purpurata (Vieill.) K. Schum.	8	2	12	2								
Ocimum gratissimum L.	8	2	12	2								
Sida rhombifolia L.	3.33	4.16	12.33	5.51	13							
Adenocalymma alliaceum (Lam.) Miers	14	1.73	6	1.73								
Chromolaena odorata (L.) R.M. King & H. Rob.	0	0	2.33	0.58	3	1.73	4.33	0.58	10.33	2.89		
Zingiber officinale Roscoe	18	0	2	0								
Pimenta dioica (L.) Merr.	0	0	18.33	0.58	1.67	0.58						
Mesua ferrea L.	16.67	1.53	3.33	1.53								
Apium graveolens L.	0	0	3.33	0.58	6	1	5	1.73	3.67	0.58	0	.
Grand Total	8.69	1.42	7.91	1.29	4.59	1.84	5.25	0.92	4.63	2.29	1.75	1.40

Table 2.19 (a) Anova table showing activity against 4ml concentration and retention time

Concentration 4ml		Sum of Squares	df	Mean Square	F	Sig.
6hour	Between Groups	201.315	30	6.710	20.287	.000
	Within Groups	20.509	62	.331		
	Total	221.824	92			
12 hour	Between Groups	126.024	30	4.201	9.853	.000
	Within Groups	26.433	62	.426		
	Total	152.457	92			
24 hour	Between Groups	44.727	30	1.491	6.485	.000
	Within Groups	14.253	62	.230		
	Total	58.980	92			
48 hour	Between Groups	52.443	30	1.748	119.090	.000
	Within Groups	.910	62	.015		
	Total	53.353	92			
72 hour	Between Groups	44.723	30	1.491	13.714	.000
	Within Groups	6.739	62	.109		
	Total	51.463	92			
96 hour	Between Groups	9.584	30	.319	6.598	.000
	Within Groups	3.002	62	.048		
	Total	12.586	92			

Eleven plants such as *Calotropis gigantea* (L.) R. Br. (13.33±5.77), *Citrus medica L* (13.33±5.77), *Momordica charantia* L. (13.67±0.58), *Adenocalymma alliaceum* (Lam.) Miers (14.00±1.73), *Mesua ferrea* L. (16.67±1.53), *Zingiber officinale* Roscoe (18.00±0.00), *Saritaea magnifica* (W. Bull) Dugand (18.67±1.15), *Eucalyptus tereticornis* Sm. (20±0), *Allium*

sativum L. (20±0), *Citrus reticulata* Blanco (20±0) and *Ricinus communis* L. (20±0) were highly significant at 4ml concentration and 6 hours retention time (F value= 20.287; p value < 0.01). Eight plants such as *Mimosa pudica* L. (11.67±6.35), *Sida rhombifolia* L. (12.33±5.51), *Alpinia purpurata* (Vieill.) K.Schum. (12.00±2.00), *Ocimum gratissimum* L. (12.00±2.00), *Terminalia bellirica* (Gaertn.) Roxb. (13.67±1.53), *Curcuma longa* L. (17.33±1.15), *Pimenta dioica* (L.) Merr. (18.33±0.58) and *Carica papaya* L. (20±0) were highly significant at 4ml concentration and 12 hours retention time (F value = 9.853; p value < 0.01). Eight plants such as *Chromolaena odorata* (L.) R.M. King & H. Rob. (3.00±1.73), *Murraya koenigii* (L.) Spreng (3.67±1.53), *Ziziphus jujuba* (L.) Lam. (5.67±8.14), *Musa X paradisiaca* L (5.00±2.65), *Hyptis suaveolens* (L.) Poit (5.00±0), *Tridax procumbens* L. (5.67±0.58), *Apium graveolens* L. (6.00±1.00) and *Polyalthia longifolia* (Sonn.) Thwaites (6.67±1.53) were highly significant at 4ml concentration and 24 hours retention time (F value = 6.485; p value < 0.01). *Tridax procumbens* L. (8.67±0.58) was highly significant at 4ml concentration and 48 hours retention time (F value= 21.199 p value < 0.01).Two plants such as *Ziziphus jujuba* (L.) Lam. (8.67±6.66) and *Chromolaena odorata* (L.) R.M. King & H. Rob.(10.33±2.89) were highly significant at 4ml concentration and 72 hours retention time (F value = 13.714 p value < 0.01). Plant *Vitex negundo* L. (4.00±1.73) was highly significant at 4ml concentration and 96 hours retention time (F value = 6.598 p value < 0.01) (Table 2.19 and 2.19(a)).

Table 2.20The efficacy (Mean± S.D) of plant extracts on the third instar larvae of *Armigeres subalbatus* at 8ml concentration and varying retention time

8ml												
Plants	**Mean 6HAT**	**SD 6HAT**	**Mean 12HAT**	**SD 12HAT**	**Mean 24HAT**	**SD 24HAT**	**Mean 48HAT**	**SD 48HAT**	**Mean 72HAT**	**SD 72HAT**	**Mean 96HAT**	**SD 96HAT**
Spilanthes calva DC.	12	0	8	0								
Calotropis gigantea (L.) R. Br.	14.67	4.62	8	0								
Citrus medica L	14.67	4.62	8	0								
Hyptis suaveolens (L.) Poit	0	0	2.67	3.06	8.33	3.51	2.67	2.08	1.33	2.31	2	3.46
Musa X paradisiaca L.	7.67	5.13	8.67	1.15	5.5	6.36						
Saritaea magnifica (W. Bull) Dugand	20	0	0	0								
Eucalyptus tereticornis Sm.	20	0	0	0								
Allium sativum L	20	0										
Citrus reticulata Blanco	20	0										
Ziziphus jujuba (L.) Lam.	0	0	0.67	1.15	1.67	0.58	6.33	4.73	8.33	0.58	9	.
Vitex negundo L.	0	0	3	1	3	1	4.67	1.15	5.33	1.15	4	0
Ricinus communis L.	20	0										
Mimosa pudica L.	0	0	9	2	1	0						
Murraya koenigii (L.) Spreng	0	0	1	1	4.67	2.52	5.33	0.58	6.67	1.15	3.5	3.54
Terminalia bellirica (Gaertn.) Roxb.	20	0	11.67	1.53								
Tridax procumbens L.	0	0	6.67	1.53	7	0	6.33	1.53				
Polyalthia longifolia (Sonn.) Thwaites	0	0	3.67	1.53	7.33	1.15	7.67	0.58	4	.		
Curcuma longa L.	5.33	1.53	14.67	1.53								

Lantana camara L.	13.33	11.55	20									
Carica papaya L.	0	0	20	0								
Momordica charantia L.	17.67	1.53	2.33	1.53								
Vinca rosea L.	12	4	8	4								
Alpinia purpurata (Vieill.) K. Schum.	9.67	2.08	10.33	2.08								
Ocimum gratissimum L.	9.67	2.08	10.33	2.08								
Sida rhombifolia L.	3.67	3.51	13	3	10	.						
Adenocalymma alliaceum (Lam.) Miers	17.67	1.15	2.33	1.15								
Chromolaena odorata (L.) R.M. King & H. Rob.	0	0	2	0	4	1	4.67	0.58	9.33	1.15		
Zingiber officinale Roscoe	18.67	1.15	2	0								
Pimenta dioica (L.) Merr.	0	0	19.67	0.58	1	.						
Mesua ferrea L.	17.67	0.58	2.33	0.58								
Apium graveolens L.	0.67	1.15	4.33	0.58	10	3	3.33	2.08	0	.	0	0
Grand Total	9.52	1.44	7.23	1.15	5.29	1.91	5.13	1.66	5.00	1.27	3.70	1.75

Table 2.20 (a) Anova table showing activity against 8ml concentration and retention time

Concentration8ml		Sum of Squares	df	Mean Square	F	Sig.
6 hour	Between Groups	214.233	30	7.141	25.231	.000
	Within Groups	17.548	62	.283		
	Total	231.781	92			
12 hour	Between Groups	115.524	30	3.851	10.394	.000
	Within Groups	22.970	62	.370		
	Total	138.494	92			
24 hour	Between Groups	58.332	30	1.944	11.690	.000
	Within Groups	10.312	62	.166		
	Total	68.644	92			
48 hour	Between Groups	48.596	30	1.620	24.663	.000
	Within Groups	4.072	62	.066		
	Total	52.668	92			
72 hour	Between Groups	46.232	30	1.541	32.202	.000
	Within Groups	2.967	62	.048		
	Total	49.199	92			
96 hour	Between Groups	9.610	30	.320	2.537	.001
	Within Groups	7.829	62	.126		
	Total	17.439	92			

Thirteen plants such as *Vinca rosea* L.(12±4), *Spilanthes calva* DC.(12±0), *Calotropis gigantea* (L.) R. Br.(17.33±1.15), *Citrus medica* L. (14.67±4.62), *Momordica charantia* L.(17.67±1.53), *Adenocalymma*

alliaceum (Lam.) Miers (17.67±1.15), *Mesua ferrea* L. (17.67±0.58), *Zingiber officinale* Roscoe (18.67±1.15), *Saritaea magnifica* (W. Bull) Dugand (20±1.15), *Eucalyptus tereticornis* Sm. (20±0), *Allium sativum L.* (20±0), *Citrus reticulata* Blanco(20±0) and *Ricinus communis* L. (20±0) were highly significant at 8ml concentration and 6 hours retention time (F value= 25.231; p value < 0.01). Five plants such as *Terminalia bellirica* (Gaertn.) Roxb. (11.67±1.53), *Sida rhombifolia* L. (13.00±3.00), *Curcuma longa* L. (14.67±1.53), *Pimenta dioica* (L.) Merr. (19.67±0.58) and *Carica papaya* L. (20±0) were highly significant at 8ml concentration and 12 hours retention time (F value= 10.394; p value < 0.01). Four plants such as *Tridaxprocumbens* L. (7±0), *Polialthia longifolia* (Sonn.) Thwaites (7.33±1.15), *Hyptis suaveolens* (L.) Poit (8.33±3.51) and *Apium graveolens* L. (10.00±3.00) were highly significant at 8ml concentration and 24 hours retention time (F value = 11.690; p value < 0.01). Four plants such as *Murraya koennigii* (L.) Spreng (5.33±0.58), *Ziziphus jujuba* (L.) Lam. (6.33±4.73), *Tridax procumbens* L. (6.33±1.53) and *Polyalthia longifolia* (Sonn.) Thwaites (7.67±0.58) were highly significant at 8ml concentration and 48 hours retention time (F value= 24.663; p value < 0.01). Two plants such as *Ziziphus jujuba* (L.) Lam. (8.33±0.58) and *Chromolaena odorata* (L.) R.M. King & H. Rob. (9.33±1.15) were highly significant at 8ml concentration and 72 hours retention time (F value= 32.202; p value < 0.01). Plant *Vitex negundo* L.(4.00±0) was highly significant at 2ml concentration and 96 hours retention time (F value= 2.537 p value > 0.01). Among the significant plants, ten plants were showing strongest larvicidal effects in all concentrations. Their LC_{50} values were noted (Table 2.21 and 2.20(a)).

Table 2.21.LC_{50}values of plants against *Armigeres subalbatus*

SI. No.	Plants	LC_{50}mg/ml
1	*Calotropis gigantea* (L.) R. Br.	7.15
2	*Citrusmedica* L	7.15
3	*Saritaea magnifica* (W.Bull) Dugand	0.009
4	*Allium sativum* L	0*
5	*Citrus reticulata* Blanco	0*
6	*Ricinus communis* L.	0*
7	*Momordica charantia* L.	0.86
8	*Adenocalymma alliaceum* (Lam.) Miers	0.008
9	*Zingiber officinale* Roscoe	2.70
10	*Pimenta dioica* (L.) Merr.	0

0* represents 100 % mortality occuring in lowest concentration

Biopesticides may serve as suitable alternatives to chemical insecticides in future as they are relatively safe, inexpensive and widely distributed. The present exercises reveal that the aqueous extracts from 31 plants / plant parts have effective larvicidal properties against third instar larvae of mosquitoes. Also all the 31 plants expressed toxicity towards three mosquito vectors such as *Aedes albopictus, Culex sitiens* and *Armigeres subalbatus.*

As stated in the review, previous studies are also available on the insecticidal properties of plant extracts (Arnason et al. 1981). Chapagain and Wiesman (2005) showed that aqueous extracts of the Balanites plant can be used as environment-friendly and sustainable insecticide to control mosquitoes. Vasanthraj et al (2009) made an attempt to screen *Vitex negundo* Linn. for its larvicidal activity against three different mosquito species, *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti.* The aqueous extract of this plant was found to be effective against *Culex quinquefasciatus* with IC_{50} values of 167.88 ppm, *Anopheles stephensi* with the IC_{50} values of 167.88 ppm and *Aedes aegypti* with IC_{50} values of 231.17 ppm. Overall

observation revealed that the plant *Vitex negundo* Linn. has significant larvicidal activity against *Culex quinquefasciatus*, *Anopheles stephensi* and moderately effective against *Aedes aegypti*. Tandon and Sirohi (2010) assessed the larvicidal properties of four plants against *Culex quinquefasciatus* larvae. Govindarajan (2010) revealed that the crude extract of *sida acuta* has an excellent potential for controlling *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* mosquitoes with LC_{50}values ranging between 38 to 48mg/L. Kamaraj et al (2011) studied larvicidal activity of medicinal plant extracts of *Annona squamosa, Chrysanthemum indicum* and *Tridax procumbens* against *Anopheles subpictus* and *Culex tritaeniorhynchus*. The results showed moderate effects after 24 hours of exposure with LC_{50} values ranging between 39.98 to104.94 mg/l. Alvarez et al (2016) screened 28 plant extracts against *Aedes aegypti* and *Aedes albopictus*. In the study, leaf of *Capsicum frutescens* exhibited 100% mortality after 24 hours against both mosquito species. Varghese et al (2016) in invitro studies of aqueous leaf extract of *Garcinia gummi* gutta Linn. against mosquito larvae revealed larvicidal activity against late III or early IV instars of mosquito larvae. A 50% larvicidal activity was observed for all the test concentrations, except at the lowest concentration of 45 mg/ml. A 100% mortality rate was observed at concentrations of 180 and 225 mg/ml of the extract.

Studies by Kalyanasundaram and Das (1985), Narayan and Narayanapillai (1996), Kamali (2001), Kumar et al. (2012) and Samuel Tennyson (2015) reported the larvicidal action of terrestrial plant extracts with varying concentrations for achieving significant mortality of mosquito larvae. Plants such as *Momordica charantia* (Singh et al. 2006; Prabakar and Jebanesan 2004), *Ocimum gratissimum* (Kamaraj and Rahuman 2010; Mgbemena (2010), *Vitex negundo* (Krishnan et al. 2007), *Apium graveolens* (Choochate et al 2005), *Carica papaya* (Rawani et al. 2009), *Citrus medica*

(Sujatha et al.1988), *Spilanthes calva* (Pandey et al. 2007), *Mimosa pudica* (Kamaraj 2010), *Mesua ferrea* (Singha et al. 2011), *Calotropis gigantea* (Kumar et al. 2012), *Musa X paradisiaca* (Bagavan and Rahuman 2011), *Alpinia purpurata* (Santos et al. 2012), *Terminalia bellirica* (Ilango and Malarvizhi 2016), *Carica papaya* (Malathi and Vasugi 2015; Rawani 2012) ; *Eucalyptus tereticornis* (Nathan 2007) ; *Vinca rosea* (Sharma et al. 2016) have already been reported. The present study also confirms the efficiency of these plants with respect to their larvicidal properties.

The present study gains significance as it revealed / confirmed that the aqueous extracts of 31 plants have 100% larvicidal activity with very low concentration and retention time against the third instar larvae of mosquitoes with LC_{50} values ranging between 0.002 to 316.228 mg/ml.

Plant species such as *Adenocalymma alliaceum* (Lam.) Miers (LC_{50}1.76 mg/ml), *Allium sativum* L. (LC_{50} 0.002mg/ml), *Alpinia purpurata* (Vieill.) K. Schum. (LC_{50} 0.012mg/ml), *Citrus reticulata* Blanco (LC_{50} 0.552mg/ml), *Citrus medica* L. (LC_{50} 5.768mg/ml), *Momordica charantia* L. (LC_{50} 0.002mg/ml), *Pimenta dioica* (L.) Merr.(LC_{50} 2.732mg/ml), *Ricinus communis* L. (LC_{50} 1.33mg/ml), *Saritaea magnifica* (W. Bull) Dugand(LC_{50} 1.696mg/ml), *Spilanthes calva* DC. (LC_{50} 3.939mg/ml), *Zingiber officinale* Roscoe (LC_{50} 1.316mg/ml) *Musa X paradisiaca* L. (LC_{50} 1.327mg/ml) and *Polyalthia longifolia* (Sonn.) Thwaites. (LC_{50} 71.24mg/ml) at 0.5 ml concentration; *Calotropis gigantea* (L.) R. Br. (LC_{50} 0.681mg/ml) and *Curcuma longa* L. (LC_{50} 0.02mg/ml) at 1ml; *Mesua ferrea* L. (LC_{50} 1.927mg/ml) and *Mimosa pudica* L. (LC_{50} 8.651mg/ml) at 2ml; *Hyptis suaveolens* (L.) Poit (LC_{50} 3.348 mg/ml), *Carica papaya* L. (LC_{50} 492.388mg//ml) *Chromolaena odorata* (L.) R.M. King & H. Rob. (LC_{50} 46.416 mg/ml), *Vitex negundo* L. (LC_{50} 100.277mg/ml), *Terminalia bellirica* (Gaertn.) (LC_{50} 316.228mg/ml) and *Eucalyptus tereticornis* Sm. (LC_{50} 0.258mg/ml) at 4ml and *Apium graveolens* L (LC_{50} 3.364mg/ml), *Ocimum*

gratissimum L. (LC_{50} 15.067mg/ml), *Sida rhombifolia* L. (LC_{50} 2.613 mg/ml), *Lantana camara* L (LC_{50} 316.228mg/ml) *Vinca rosea* L. (LC50 316.228mg/ml), *Ziziphus jujuba* (L.) Lam. (LC_{50} 316.228mg/ml), *Murraya koenigii* (L.) (LC_{50} 316.228mg/ml) and *Tridax procumbens* L. (LC_{50} 2.154mg/ml) at 8ml concentrations showed 100% larvicidal activity at varying retention time.

From among the 31 plants, 7 plants (*Allium sativum* L.*, Ricinus communis* L., *Zingiber officinale* Roscoe., *Citrus reticulata* Blanco., *Pimenta dioica* (L.) Merr., *Adenocalymma alliaceum* (Lam.) Miers.and *Saritaea magnifica* (W. Bull) Dugand.) were statistically significant in all concentrations and retention time with significant LC_{50} values against all the three mosquito species. Of these seven plants *Allium sativum* L.; *Zingiber officinale* Roscoe (Singha and Chandra, 2011) ; *Citrus reticulata* Blanco (Akram et al. 2010; Akono et al. 2015) and *Ricinus communis* L. (Nazar et al. 2009) were also reported to be efficient in exhibiting larvicidal properties. The present study thus brings for the first time the efficacy of three plants (*Adenocalymma alliaceum* (Lam.) Miers*., Pimenta dioica* (L.) Merr. and *Saritaea magnifica* (W. Bull) Dugand.) in the control of mosquito vectors belonging to the species *Aedes albopictus, Culex sitiens* and *Armigeres subalbatus*. These promising plants have the potential to be included in the formulations of new and safe control products against mosquito vectors. As these plant species are distributed throughout the country, it can help minimize the dependence on expensive synthetic pesticides, generate local employment and also to stimulate local efforts to enhance public health. Further studies on the larvicidal mode of action, their effects on non-target organisms and formulations for improving their insecticidal potency are to be carried out for further standardization.

Summary and Conclusion

The primary aim of this work was to formulate ways and means of controlling mosquito larvae using aqueous extracts of plant origin. One hundred and twenty plant species belonging to 42 varied families were screened for this purpose. Aqueous extracts of selected plants / plant parts were prepared (0.5, 1.0, 2.0, 4.0 and 8.0 ml) and tested against mosquito larvae for a period of 96hours. Mortality percentages and LC_{50} were calculated as per WHO protocols and standards. Attempts were also carried out to assess the lethality of plant materials on specific mosquito species like *Aedes albopictus, Culex sitiens* and *Armigeres subalbatus*.

The present study confirms the larvicidal efficacy of altogether 77 plants from an array of 120 plants screened. Of seventy seven, thirty one plants were proved to be 100% efficient in exhibiting larvicidal properties. The present investigation brings out the efficiency of three plants (*Adenocalymma alliaceum* (Lam.) Miers., *Pimenta dioica* (L.) Merr. and *Saritaea magnifica* (W. Bull) Dugand.), which are not reported in any literature and are having larvicidal efficiency. These three promising plants have the potential, to be included in the formulations of new and safe control products, especially in the control of mosquito species with medical importance (Aedes, Culex and Armigeres). As these plant species have a cosmopolitan distribution, they can help minimize the dependence on expensive synthetic pesticides, generate local employment and stimulate local efforts to enhance public health.

Thus in addition to the confirmation of plant species which are reported to be effective in larvicidal property, the present study brings out three novel plant species (*Adenocalymma alliaceum* (Lam.) Miers*., Pimenta dioica* (L.) Merr.and *Saritaea magnifica* (W. Bull) Dugand.), which can be treated as an effective source of phytochemicals for the control of mosquito larvae belonging to three taxonomic groups like Aedes, Culex and Armigeres.

CHAPTER III

ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS HAVING INSECTICIDAL PROPERTIES.

Introduction

Phytochemicals are botanicals which are naturally occurring in plant resources (Shahi et al. 2010). They are stored by plants mainly as secondary metabolites, which serve as a means of defense mechanism. Secondary metabolites such as alkaloids, steroids, terpenoids, tannins and flavonoids from different plants have been reported earlier for their insecticidal properties (Hartzell and Wilcoxon 1941; Shaalan et al. 2005).The plant products or plant-derived compounds are promising alternatives to synthetic insecticides in controlling insect pests of medical importance as these are environmentally safe, biodegradable, low cost and can be used with minimum care by individuals and communities (Haldar et al. 2012; Bhattacharya and Chandra 2014). Some herbal products such as nicotine obtained from tobacco leaves; anabasine and lupinine, the alkaloids extracted from Russian weed, *Anabasis aphylla* (Campbell et al. 1993), rotenone from *Derris elliptica* (Zubairi and Jaeis 2004) and pyrethrums from *Chrysanthemum cinererifolium* flowers (Ghosh et al. 2012) have been used as natural insecticides earlier.

The present chapter is an extension of the previous chapter (chapter 2) which elucidated the efficacy of *Adenocalymma alliaceum, Pimenta dioica* and *saritaea magnifica* against mosquito vectors *Aedes albopictus, Culex sitiens* and *Armigeres subalbatus.* The present study aims at screening / characterization of phyto-constituents present in the extracts which are responsible for larval toxicity, based on phytochemical screening techniques

like High Performance Thin Layer Chromatography (HPTLC) and Liquid Chromatography – Quadrupole Time - of - Flight Mass Spectrometry (LC-Q-ToF-MS).

Review of literature

Several studies reported the bio control potentialof active ingredients isolated from plants in the control of mosquito vectors. Das et al. (1996) carried out studies on the toxic effects of alkaloids on the larvae of *Culex quinquefasciatus*. In this study on the toxicity of certain alkaloids and their derived products isolated from *Glycosmis pentaphylla, Murraya koenigii* and *Piper nigrum*, they revealed that the alkaloid piperine obtained from *piper nigrum* has the highest toxicity on mosquito larvae. Limoneim, nomilein and the limonoides from *Citrus reticulata* is reported to be a strong inhibitor on *Culex quinquefasciatus* (Jayaprakasha et al.1997).

Nivasarkar et al. (2001) revealed that alpha-terthienyl, a naturally occurring secondary plant metabolite found in abundance in the roots of Tagetes species (Asteraceae) can act as a potential larvicide. Ramsewak et al. (2001) isolated five compounds from the seeds of *Dirca palustris* using a hexane extract and suggested that some of these new compounds with their novel mode of bioactivity may prove useful in the development of safe insecticides for the future. Zhang et al. (2002) studied antimalarial bioassay of isolated indole alkaloid, decursivine from the leaves and stems of *Rhaphidophora decursiva* by direct fractionation. Saraf and Dixit (2002) elucidated a major constituent (Spilanthol) from flower heads of *Spilanthes acmella* Murr.having effective ovicidal, larvicidal and pupicidal activity against Anopheles, Culex and Aedes mosquitoes.

Carvalho (2003) studied the larvicidal activity of the essential oil from *Lippia sidoides* against *Aedes aegypti* owing to the presence of alkyl phenol

derivatives (thymol) isolated by GC method. Saxena et al. (1992) reported that plants may well prove to be the source of new antimalarial drug in view of the success with the two important chemotherapeutic agents, quinine and artemisinin, both of which are derived from plants. In this study they revealed that the recently developed isolation and characterization techniques together with the development of new pharmacological testing have led to interest in plant derived products as a source of new drugs. Yang et al. (2003) suggested that Emodin, isolated from *Cassia obtusifolia* seed showed larvicidal activity against three mosquito species.

The larvicidal effect of aqueous extracts of *Hemidesmus indicus* (roots), *Gymnema sylvestre* and *Ecliptaprostrata* (leaves) were tested against *Culex quinquefasciatus* larvae by Khanna and Kannabiran (2007). This study highlighted crude saponin and tannin as major constituents responsible for larvicidal properties, which could be developed and used as natural insecticides for mosquito control. Akinmoladun et al. (2007) screened the aqueous and methanolic extracts of *Chromolaena odorata* for phytochemical constituents and detected alkaloids as the major one. Idu et al. (2007) screened preliminary phytochemicals on extracts of *Senna alata* using water, methanol, chloroform and petroleum ether as solvents and showed the presence of phenols, tannins, anthraquinoes, saponin and flavonoids.

Kumar and Manimegalai (2008) carried out larvicidal and preliminary analysis of both ethanol and methanol extracts of leaves and flowers of *Lantana camara*on *Aedes aegypti* and *Culex quinquefasciatus*. Krishan et al (2008) reported the isolation of mosquito larvicidal bioactive saponin from an indigenous plant (*Tridax procumbens*) found in Indian subcontinent as a weed. Senthilkumar et al. (2009) studied the leaf extract of *Blumea mollis* against *Culex quinquefasciatus.* They analysed the chemical constituents by GC and GC–MS and revealed that extracts contained 39 compounds. This

could be useful as a safe, natural larvicidal agent against mosquito. Cheng et al. (2009) isolated tectoquinone from red heartwood-type *Cryptomeria japonica* which exhibited the strongest larvicidal activity against two mosquito species. Rahuman et al. (2008) identified the mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn).

Chemical composition and larvicidal activity against *Aedes aegypti* larvae by essential oils from four Guarea species was analysed by Magalhaes et al. (2010). Sutthanont et al. (2010) also investigated the chemical composition and larvicidal potential of essential oils from edible plants against mosquito vectors. Umadevi et al. (2010) assessed the toxic effects of methanolic extracts of leaves of *Artemisia parviflora* against malarial vector *Anopheles stephensi*. The biological activity of the plant extract might be due to the presence of toxic compounds such as 1-8 Cineole, Germacrene D, Artemisia Ketone, Sabinyl acetae etc. Mathivanan et al. (2010) determined the larvicidal and phytochemical properties of *Ervatamia coronaria and Callistemon rigidus* leaf extracts against *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus.* Mgbemena (2010) assessed the comparative efficacy of *Azadirachta indica*, *Ocimum gratissimum* and *Cymbopogon citratus*extracts on *Aedes aegypti* mosquitoes and found out varying phytochemicalsresponsible for treatment. Serena et al. (2010) attempted to evaluate the insecticidal property of the methanol and water extracts of *Duranta erecta* and showed the presence of sugars, tannins, saponin, steroids, alkaloids, phenols, flavonoids, glycosides, triterepens and carboxylic acid through phytochemical analysis.

Savithramma et al. (2011) reported the qualitative phytochemical analysis of 18 different plant species of medicinal value and confirmed the presence of various phytochemicals like saponin, terpenoids, steroids, anthocyanins, coumarins, fatty acids, tannins, leucoanthocyanins and

emodins. The results suggested that the phytochemical properties may cure various ailments due to potential antioxidants. Talontsi et al. (2011) evaluated mosquito larvicidal activity of *Zanthoxylum lemairei* (Rutaceae) against the malarial vector *Anopheles gambiae*. Four alkaloids (6 - acetonyl - N - methyl - dihydrodecarine, 10 - O - demethyl - 17 - O - methyl isoarnottian amide, nitidine and chelerythrine) were isolated from the plant and achieved 100% mortality at 1000 mg/L. These findings could be useful in their search for newer, more selective, biodegradable and natural larvicidal compounds or can be used as lead compounds for the development of larvicides. Zhu and Tian (2011) studied *Blumea martiniana* and their isolated compounds as potential larvicides against *Anopheles anthropophagus,* which could be useful in the search for safer natural larvicides. Kannathasan et al. (2011) reported methyl-p-hydroxybenzoate from the leaves of *Vitex trifolia,* explored for mosquito control. Maragathavalli et al. (2012) evaluated mosquito larvicidal activity and phytochemical screening of methanol extract of leaves of *Azadirachta indica*. Presence of caproic acid, 4-butoxy butanol, oleic acid, decanoic acid, N-methyl N-N-di (2-(4-pyridyl) ethyl) - (2-pyridyl) ethylamine were found in GC MS analysis of leaf extract. Edriss et al. (2012) evaluated extracts prepared from two asclepiadaceous plants, viz., *Solenostemma argel* (seeds and leaves) and *Calotropis procera* as natural larvicides against *Anopheles arabiensis*. Singha et al. (2012) studied mosquito larvicidal potentiality of *Holoptelea integrifolia* crude leaf extract against *Culex vishnui* and reported that the toxicity may be due to the presence of secondary metabolites of saponin, steroid, tannin and phenol.

Essential oils of spices/aromatic medicinal plants, particularly *Piper capense* (Matasyoh et al.2011), *Foeniculum vulgare* and *Tagetes patula* (Rana and Rana 2012) carry large quantities of mosquito larvicidal constituents, which could be exploited for the development of safer and effective management of mosquitoes. Park and Park (2012) tested larvicidal activities

of *Pogostemon cablin*, *Amyris balsamifera*, and *Daucus carota* essential oils against *Culex pipiens pallens*. Four active compounds such as β-eudesmol, elemol, patchoulol, and carotol were isolated from the three oils and showed significant results against mosquito vectors. Vatandoost (2012) studied the larvicidal activity of essential oil extracted from an indigenous plant *Kelussia odoratissima* mozaffarian and larvicidal activity of the essential oil of *Kelussia odoratissima* against two mosquito species, *Anopheles stephensi* and *Culex pipiens* and evaluated the main constituents as Z-ligustilide, 2-octen-1-ol acetate, E-ligustilide and Butylidene phthalide.

Imam et al. (2014) carried out solvent extraction of the bark of *Cassia arereh* and assessed for *in vitro* larvicidal, antiplasmodial and cytotoxicity properties due to the presence of anthraquinones, flavanoids, terpenes, steroles and tannins. The larvicidal activity of crude extracts of *Larrea cuneifolia* and its metabolite Nordihydroguaiaretic acid against *Culex quinquefasciatus* was observed by Batallanet al. (2013). Kim et al.(2013) examined the mosquito larvicidal activities of active constituent isolated from *Tabebuia avellanedae* bark and its structurally related derivatives were examined against the fourth instar larvae of *Aedes aegypti, Culex pipiens pallens* and *Ochlerotatus togoi*. The most toxic compound 1, 4-naphthalenedione (1.26 mg/L) was found to be very much effective against *Culex pipiens pallens* larvae and less effective to non-target species.

Gutierrez et al. (2014) evaluated the larvicidal effects of leaf and stem/bark extracts of *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii* on the larvae of *Aedes aegypti*. Phytochemical screening of the extracts was carried out to determine the active toxic compounds. Akhila and Vijayalakshmi (2015) had undertaken studies on the phytochemical profiling of papaya leaf extract using Liquid Chromatography-Mass Spectroscopy (LCMS).Babatunde et al. (2016) elucidated the phytochemical components of

extracts of *Ocimum canum* and investigated the larvicidal activity of extracted essential oil against *Anopheles gambiae*larvae.

Materials and Methods

Plant selection andcollection

Plants like *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* were selected for the present study for the characterization of phytochemicals, as they are proved to have larvicidal properties on mosquito vectors like *Aedes albopictus, Culex sitiens* and *Armigeres subalbatus.*For phytochemical studies, plant materials were collected from pollution free environments. Their identity is confirmed with standard manuals and consultation with experts. The Botanical descriptions of plants selected for the present study are as follows:

***Adenocalymma alliaceum* (Lam.) Miers.**

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Equisetopsida
Order	:	Lamiales
Family	:	Bignoniaceae
Genus	:	Adenocalymma
Species	:	*Adenocalymma alliaceum* (Lam.) Miers.

Adenocalymma alliaceum (Garlic vine), a dazzling ornamental vine (Plate 10 a) with garlic-like smell, is a native of Amazon rainforest. The plant is a decorative evergreen vine, 6-8 feet tall. The leaves are arranged opposite to each other and are having garlic-like odour. *Adenocalymma alliaceum*, like the well-known Bignonias to which it is closely related, is a climber of surpassing charm which finds a ready welcome everywhere because of its

refreshing evergreen foliage and enchanting masses of exquisitely coloured flowers. The garlic vine bear clusters of funnel or cone shaped purple to pale or white flowers, which become lighter on ageing. Full bloom with large number of floral bunches is seen during November-December. The plant thrives on a rich loamy soil in hot and moist conditions. Commonly propagated by layering, the cuttings also produce root when planted in a sandy soil. *Adenocalymma alliaceum* has a long history associated with the herbal medical systems in Peru and Brazil as an analgesic, anti-inflammatory and anti-rheumatic. Indian tribes in Amazon basin use the poultice of its barks on bumps, swellings and inflammatory conditions of the skin. An infusion or leaves in decoction is used for rheumatism, arthritis, uterine disorders and epilepsy. Leaves are also used as a common remedy for cold, flu, pneumonia, coughs, fever and headache. It is also effective as a mosquito repellent and snake repellent (Shrankhla et al. 2012).

***Pimenta dioica* (L.) Merr.**

Kingdom : Plantae

Phylum : Spermatophyta

Class : Equisetopsida

Order : Myrtales

Family : Myrtaceae

Genus : Pimenta

Species : *Pimenta dioica*(L.) Merr.

Pimenta dioica (L.) Merr.is commonly known as allspice, which belongs to Myrtaceae family. It possesses an aromatic taste and flavour resembling a mixture of cinnamon, cloves and nutmeg, hence the name

allspice. *Pimenta dioica* (Plate 10 b) is native to the Caribbean region, especially Jamaica and Cuba. The trees grow naturally at a mean average temperature of 18°C - 24°C. Allspice is a small evergreen tree up to 15 meter tall with a pale brown bark. Leaves are simple, opposite, entire, oblong - elliptical, 6-20 cm long, punctuate with pellucid glands which give off the odour of all-spice when crushed. The flowers are small and whitish with a peculiar aroma. They are present in groups of cymes. They are structurally hermaphrodite, but functionally dioecious. Those trees which bear no fruit are male trees, wherein the flowers will have above 100 stamens and the flowers in bearing female trees have around 50 stamens. The receptacle has four cream-coloured calyx lobes, spreading at anthesis and persistent in the fruit. Petals are four, whitish and quickly deciduous. The style is white with a yellow stigma. In females, the style is slightly shorter and the stigma longer than in the barren trees. The ovary is inferior and 2-celled, usually with one ovule in each cell. Plants flower during March-June and the fruit, which is a berry, matures 3-4 months later. For spice purpose, it is picked when it is fully developed. The fruits have two kidney-shaped seeds (Parthasarathy 2007). Like clove, pimento requires quite specific environmental conditions to flourish. *Pimenta dioica* is widely planted in warm regions of the world as an ornamental plant, valued for its fragrance and attractive habit. This plant has various uses. The dried, green-mature fruit is the commercial flavourant and curing agent. Young woody shoots of pimento are popularly made into walking sticks and umbrella handles. It is used as an aromatic stimulant in digestive troubles, as an adjuvant to tonics and purgatives, as an anodyne against rheumatism and neuralgia. The essential oils of *Pimenta dioica* leaves and fruits are utilized in food industry, tanning industries as well as in perfumery compositions and cosmetic products. The therapeutic properties of the essential allspice oils are anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acaricidal carminative, muscle relaxant, rubefacient, stimulant and

tonic. Pimento oil can be helpful for the digestive system, for cramp, flatulence, indigestion and nausea. Further, the essential oils can help in cases of depression, nervous exhaustion, tension and stress (Weiss 2002).

***Saritaea magnifica* (W. Bull) Dugand**

Kingdom	:	Plantae
Phylum	:	Spermatophyta
Class	:	Equisetopsida
Order	:	Lamiales
Family	:	Bignoniaceae
Genus	:	Saritaea
Species	:	*Saritaea magnifica* (W. Bull) Dugand

Saritaea magnifica, commonly known as glow vine is a native to Colombia and Ecuador. The plant is an evergreen tropical climber with very spectacular flowering (Plate 10c). It climbs by tendrils, 3-7 m in length. Stems cylindrical, lepidote, compressed at the nodes, interpetiolar zone not glandular; cross section of the mature stem normal. Leaves opposite, 2-foliolate, sometimes with a simple tendril of short duration; leaflets 4.2-11.5 × 3.1-6.4 cm, obovate, chartaceous, with the venation slightly prominent on both surfaces, the apex obtuse, the base cuneate or decurrent, the margins entire; upper surface dull, minutely lepidote; lower surface dull, sparsely lepidote with domatia in the axils of the basal secondary veins; petioles and petiolules lepidote, the petioles 1.7-2.8 cm long, the petiolules 0.3-1.6 cm long; pseudostipules foliaceous, 0.6-4.2 cm long. Flowers few, in corymbiform panicles, usually terminal; pedicels 4-6 mm long. Calyx yellowish green, simple, crateriform, 7-8 mm long, truncate, lepidote; corolla purple-pink, tubularcampanulate, 8-9 cm long, glabrous or minutely glandular, the tube pubescent inside, the throat white inside with purple-pink

lines, the lobes unequal, 2.2-3.1 cm long; stamens 4, didynamous, inserted; ovary linear, 4 mm long, glandularlepidote, with two locules, the ovules in 2 series per locule; disc hypocrateriform-pulviniform, 1 mm high. Capsule linear, compressed, coriaceous, brown, 20-25 × 1-1.2 cm; seeds numerous, oblong, 2-winged, the hyaline wings membranaceous. The large heads of showy rosy mauve to purple coloured, bell-shaped flowers with hairy yellow throat, borne at the end of the branches often display all year-round. This plant is regarded as one of the outstanding climbers of the world. The plant needs a warm-subtropical or tropical climate to be seen at its best, as well as well-drained moisture-retaining soil with lots of humus. The plant is propagated from seeds and stem cuttings (Bor and Raizada 1990).

Preparation of plant materials

The plant materials (leaves) were washed with tap water, blotted, shade dried at room temperature and subjected to pulverization. The Pulverized plant parts were subjected to solvent extraction (Yadav and Agarwala 2011; Bargah 2015) in a Soxhlet apparatus using various solvents according to their polarity.

Soxhlet extraction

Soxhlet extraction is carried out when the desired compound has solubility in a solvent and the impurity is insoluble in that solvent. From each sample, 20gm of powdered plant material was uniformly packed into a thimble and extracted with three different solvents (250ml) namely Petroleum ether, Chloroform and Ethanol, individually for 6 to 8 hours or till the solvent in siphon tube of an extractor become colorless (Vogel 1978).The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The extracts were concentrated and evaporated to dryness in a water bath for

complete removal of solvents and stored at 4^{o}C in a refrigerator for further analysis.

Isolation of compounds

Isolation of phyto-constituents from the plant extract was carried out by analytical techniques like High Performance Thin layer Chromatography (HPTLC). Prior to that, the extracts were subjected to a preliminary screening for the identification of specific categories of compounds present in the plant material.

Preliminary phytochemical screening of leaf extracts

The Petroleum ether, Chloroform and Ethanol extracts from leaves were separately subjected to preliminary phytochemical tests using standard methods by following the procedure of Sofowora (1982) ; Harborne (1983) ; Trease and Evans (1989) ; Adetuyi and Popoola et al. (2001) and Tiwari et al.(2011).

Detection of Alkaloids (Hager's Test)

Extract (0.5gm) was treated with dilute Hydrochloric acid and filtered. Filtrate (2ml) was treated with Hager's reagent (saturated aqueous solution of picric acid) and observed. Formation of a prominent yellow precipitate indicated the presence of alkaloids.

Detection of Carbohydrates (Fehling's Test)

Extracts (0.5gm) were dissolved individually in 5 ml distilled water and filtered. The filtrates were boiled with 3-4 drops of Fehling's solution A and B for 2 minutes. The resultant solution was observed for orange red precipitate, which indicated the presence of carbohydrates.

Detection of Glycosides (Keller Killiani Test)

Extracts (0.5gm) were treated with 2ml of glacial acetic acid and a drop of 5 % (w/v) $FeCl_3$ added to it. Formation of a brown ring indicated the presence of glycosides.

Detection of Saponins (Froth Test)

Extracts (0.5gm) were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15minutes.Formation of 1cm layer of foam indicated the presence of saponins.

Detection of Terpenoids (Salkowski's Test)

Extracts (0.5gm) were treated with 2ml of chloroform and filtered. Filtrates were treated with few drops of concentrated H_2SO_4. A reddish brown precipitate produced immediately indicated the presence of Terpenoids.

Detection of Steroids (Libermann Burchard's Test)

Extracts (0.5gm) were treated with Chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicated the presence of steroids.

Detection of Phenols (Ferric Chloride Test)

Extracts (0.5gm) were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Detection of Tannins (Gelatin Test)

To the extract (0.5gm), 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

Detection of Flavonoids (Alkaline Reagent Test)

Extracts (0.5gm) were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids.

Detection of proteins (Xanthoproteic Test)

The extracts (0.5gm) were treated with few drops of concentrated nitric acid. Formation of yellow colour indicated the presence of proteins.

The results of these tests were used in the presumptive identification of various metabolities contained within the plants.

High Performance Thin Layer Chromatography (HPTLC) Analysics

HPTLC fingerprint pattern of the petroleum ether, chloroform and ethanol extract were developed by using Camag HPTLC system (Switzerland) equipped with Linomat -V applicator fitted with a 100 µL syringe, Camag TLC scanner-III with CATS 4 software for interpretation of data. The HPTLC analysis was facilitated by the Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Malappuram District, Kerala. Chromatographic conditions used are stationary phase aluminium backed pre-coated silica gel plates Merck 60 F_{254} (0.2 mm thickness). Samples were applied to the plate as bands at 10 mm from the bottom of the plate by using CAMAG ATS 4. The plate was developed up to 80 mm in ascending mode with solvent system, Toluene: Ethyl acetate (8:2 v/v), at room temperature (28 ± 2°C) in a Twin Trough Chamber (Camag, Switzerland) which was previously saturated with mobile phase. After development, the air dried plate was scanned at 254 nm and in 366 nm, in CAMAG TLC SCANNER-III using Deuterium lamp with win CATS software. The Rf values of the resolved spots were noted and the resulting fingerprint was observed (Sulaiman et al. 2014).

Identification and characterization of bioactive compound by LC-Q-ToF-MS.

The isolated compounds were identified using an analytical techniqueLiquid Chromatography - Quadrupole Time - of - Flight Mass Spectrometry(LC - Q - ToF - MS). LC-Q-ToF-MS is a significant analytical tool for identification of the known compounds and elucidation of unknown compounds in natural products. The ethanolic extract was used for LC/Q-TOF/MS analysis using the facility available at the Inter University Instrumentation Center, Mahatma Gandhi University, Kottayam, Kerala. The Instrument used was Acquity H class (Waters) Ultra Performance Liquid Chromatography and Xevo G2 (Waters) Quadrapole-Time-of-Flight (Q-TOF). BEH C18 column (50 mm × 2.1 mm × 1.7 μm) was used at a flow rate of 0.3 ml/min. The total run time was 8 min. The source type was ElectroSpray Ionization (ESI) with the capillary temperature of 135°C. Capillary voltage of positive mode of ESI was 3.50 KV and for negative mode, it was 2.50 KV. The mobilization gas flow was nitrogen at 0.3 ml/min, approximately. A full scan analysis ranging from 50 to 1000 m/z was performed. A lock spray ionization source is present along with the waters Q-TOF equipment that performs on line calibration using leucine - enkephalin ([M + H] + m/z 556.2771) for providing accurate and reproducible molecular masses of parent and product ions. MSE centroid technology generating stick graphs were employed for data acquisition in which two separate scan functions were performed which generates parent ions in first scan at low collision energy of 6 eV and the second at high collision energy between 20 to 30 eV providing fragment ions. The elemental composition tool Mass Lynx V4.1 software was installed in the instrument for calculating the accurate mass and predicting elemental composition. The accurate mass obtained from the LC-Q-ToF-MS was compared with the available literature for the confirmation of the compound and structure (Ravunni and Benjamin 2016).

Results and Discussion

For quantification / characterization, Soxhlet extraction of 20 gm of the leaves of *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* were carried out with (1) non-polar solvent petroleum ether (60-80^{o}c; PI-0.1), which yielded 1.014, 1.089 and 1.024 gms respectively (2) moderately non-polar solvent chloroform (55-60^{o}c; PI-4.1), which yielded 1.161, 1.251 and 1.052 gms respectively and (3) polar solvent ethanol(75-80^{o}c;PI-5.4), which yielded1.254, 1.319 and 1.251gms respectively (Table 3.1).

Table 3.1.Details of extraction of plants using different solvents

	Solvents	***Adenocalymma alliaceum***	***Pimenta dioica***	***Saritaea magnifica***
Colour	Petroleum ether	Dark Yellow	Dark Greenish Yellow	Dark Yellow
	Chloroform	Dark Greenish Yellow	Dark Greenish Yellow	Dark Greenish Yellow
	Ethanol	Dark Brownish Green	Dark Brownish Green	Dark Brownish Green
% of yield(gm)	Petroleum ether	1.014	1.089	1.024
	Chloroform	1.161	1.251	1.052
	Ethanol	1.254	1.319	1.251
% of Extraction		37.49	39.76	34.76

The preliminary phytochemical screening is a means of evaluating the potential phyto compounds present in the extract. Phytochemical screening of petroleum ether, chloroform and ethanol extracts of *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* are depicted in Table 3.1

Table 3.2. Results on the preliminary phytochemical screening of plants using varied solvents

Plants / Constituents	Petroleum ether			Chloroform			Ethanol		
	Adenocalymma alliaceum	***Pimenta dioica***	***Saritaea magnifica***	***Adenocalymma alliaceum***	***Pimenta dioica***	***Saritaea magnifica***	***Adenocalymma alliaceum***	***Pimenta dioica***	***Saritaea magnifica***
Alkaloids	+	+	+	+	+	+	+	+	+
Carbohydrates	+	-	-	+	-	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	-	+	+	+	+
Terpenoids	+	+	+	+	-	+	+	+	+
Steroids	+	+	+	+	-	+	+	+	+
Phenols	+	-	-	-	-	-	+	+	+
Tannin	-	+	+	+	-	-	+	+	+
Flavonoids	+	-	+	+	+	+	+	+	+
Protein	-	-	-	-	-	-	+	+	+

+ indicates presence; - indicates absence.

The Petroleum ether, chloroform and ethanol extract of the leaves of *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* were subjected to preliminary phytochemical tests to get an assumption of the active ingredients responsible for larval mortality. The results of *Adenocalymma alliaceum* showed the presence of alkaloids, flavonoids, glycosides, saponins, terpenoids, steroids, phenols and carbohydrates but tannin and protein were not represented in petroleum ether extract. Similarly in chloroform extract, presence of alkaloids, flavonoids, glycosides, saponins, terpenoids, steroids, carbohydrates and tannin but phenol and protein were not represented. However, all the components were represented in ethanol extract. The petroleum ether extracts of *Pimenta dioica* showed the presence of alkaloids, glycosides, saponin, terpenoids, steroids and tannin while carbohydrates, phenols, flavonoids and proteins were not represented. In chloroform extract, alkaloids, glycosides and flavonoids where represented whereas other components were not represented. However, all the components were represented in ethanol extract.The petroleum ether extracts of *Saritaea magnifica* showed presence of alkaloids, glycosides, saponins, terpenoids, steroids, flavonoids and tannins but carbohydrates, phenols and protein were not represented. Chloroform extracts showed presence of alkaloids, glycosides, saponin, terpenoids, steroids, flavonoids and carbohydrates while phenol, tannin and proteins were not represented. The ethanolic extracts of *Saritaea magnifica* showed the presence of all components. Thus the phytochemical screening revealed the presence of various metabolites in one or the other solvent. Its further characterization is elucidated in HPTLC analysis.

HPTLC Analysis

In HPTLC analysis, each plant extract showed distinct bands under UV visualization and the compounds detected and confirmed accordingly with their Rf values (Tables 3.3 to 3.29 and Figures 3.1 to 3.15).

The HPTLC fingerprinting patterns of the petroleum ether extract of *Adenocalymma alliaceum* was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract.

Figure 3.1 HPTLC three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Adenocalymma alliaceum* at 254 nm

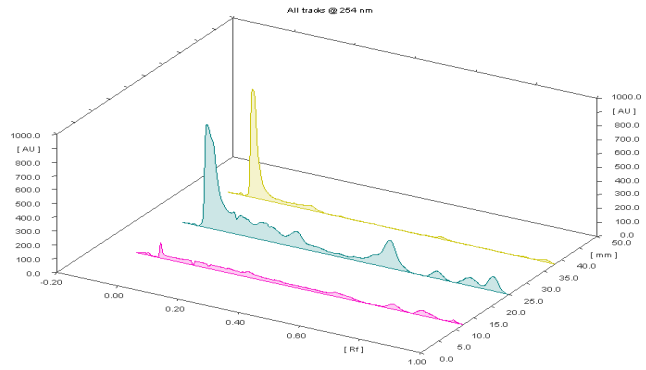


Figure-3.2: HPTLC image after derivatization observed at 254 nm.

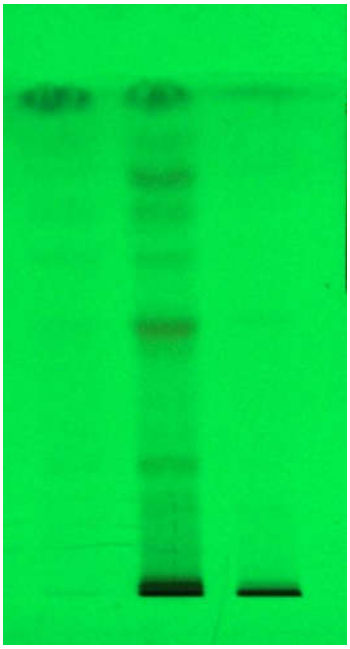


Table 3.3.Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	1.2 AU	-0.04 Rf	17.6 AU	4.52 %	-0.01 Rf	0.3 AU	344.0 AU	3.76 %	unknown *
2	-0.01 Rf	1.5 AU	0.00 Rf	109.5 AU	28.10 %	0.03 Rf	24.7 AU	1099.0 AU	12.01 %	unknown *
3	0.08 Rf	25.2 AU	0.09 Rf	29.5 AU	7.56 %	0.11 Rf	0.0 AU	560.5 AU	6.13 %	unknown *
4	0.11 Rf	4.4 AU	0.14 Rf	34.4 AU	8.82 %	0.16 Rf	18.3 AU	948.6 AU	10.37 %	unknown *
5	0.16 Rf	19.4 AU	0.17 Rf	31.4 AU	8.07 %	0.20 Rf	29.7 AU	727.6 AU	7.95 %	unknown *
6	0.28 Rf	29.1 AU	0.29 Rf	39.5 AU	10.13 %	0.36 Rf	19.1 AU	1508.5 AU	16.49 %	unknown *
7	0.55 Rf	13.8 AU	0.58 Rf	30.8 AU	7.92 %	0.66 Rf	4.2 AU	1460.4 AU	15.96 %	unknown *
8	0.72 Rf	3.1 AU	0.77 Rf	38.5 AU	9.87 %	0.82 Rf	2.6 AU	1067.1 AU	11.66 %	unknown *
9	0.83 Rf	0.3 AU	0.87 Rf	37.9 AU	9.73 %	0.93 Rf	0.1 AU	1215.1 AU	13.28 %	unknown *
10	0.95 Rf	2.2 AU	0.97 Rf	20.6 AU	5.28 %	0.99 Rf	0.6 AU	218.9 AU	2.39 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Phenol	6	Alkaloid
2	Alkaloid	7	Terpenoid
3	Flavonoid	8	Glycosides
4	Flavonoid	9	Terpenoid
5	Terpenoid	10	Steroids

In the extract using petroleum ether, a total of ten peaks (Rf -0.01, 0.03, 0.11, 0.16, 0.20, 0.36, 0.66, 0.82, 0.93, 0.99) were observed in the chromatogram and the components at Rf 0.36, 0.66, 0.93, 0.03 were present in significant level (Table 3.3; Figure 3.1).

Table 3. 4 Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	0.6 AU	0.00 Rf	749.6 AU	40.39 %	0.08 Rf	45.2 AU	23981.9 AU	41.69 %	unknown *
2	0.08 Rf	146.7 AU	0.09 Rf	156.4 AU	8.43 %	0.10 Rf	39.4 AU	1842.9 AU	3.20 %	unknown *
3	0.10 Rf	111.6 AU	0.12 Rf	144.8 AU	7.80 %	0.16 Rf	30.0 AU	4486.8 AU	7.80 %	unknown *
4	0.16 Rf	103.0 AU	0.20 Rf	127.6 AU	6.87 %	0.21 Rf	36.2 AU	3691.2 AU	6.42 %	unknown *
5	0.21 Rf	106.9 AU	0.22 Rf	111.3 AU	5.99 %	0.26 Rf	33.4 AU	2752.5 AU	4.78 %	unknown *
6	0.26 Rf	63.5 AU	0.29 Rf	115.8 AU	6.24 %	0.36 Rf	38.1 AU	4856.4 AU	8.44 %	unknown *
7	0.47 Rf	17.9 AU	0.49 Rf	28.8 AU	1.55 %	0.50 Rf	26.5 AU	579.0 AU	1.01 %	unknown *
8	0.52 Rf	28.7 AU	0.61 Rf	201.2 AU	10.84 %	0.68 Rf	2.9 AU	9119.2 AU	15.85 %	unknown *
9	0.73 Rf	2.3 AU	0.76 Rf	53.9 AU	2.91 %	0.81 Rf	0.6 AU	1417.4 AU	2.46 %	unknown *
10	0.83 Rf	1.3 AU	0.87 Rf	61.4 AU	3.31 %	0.91 Rf	19.7 AU	1981.7 AU	3.44 %	unknown *
11	0.91 Rf	19.7 AU	0.95 Rf	105.3 AU	5.67 %	0.99 Rf	0.2 AU	2817.9 AU	4.90 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	7	Saponin
2	Saponin	8	Terpenoid
3	Flavonoid	9	Terpenoid
4	Alkaloid	10	Saponin
5	Terpenoid	11	Steroids
6	Alkaloid	-	-

The HPTLC fingerprinting patterns of the chloroform extract of *Adenocalymma alliaceum* was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total eleven peaks (Rf 0.08, 0.10, 0.16, 0.21, 0.26, 0.36, 0.50, 0.68, 0.81, 0.91, 0.99) were observed in the chromatogram and the components at Rf 0.08, 0.68 were present in significant level (Table 3.4; Figure 3.1).

Table 3.5. Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	1.2 AU	-0.04 Rf	16.2 AU	1.85 %	-0.03 Rf	2.5 AU	137.8 AU	0.79 %	unknown *
2	-0.02 Rf	2.5 AU	0.00 Rf	785.1 AU	89.83 %	0.08 Rf	28.5 AU	15259.7 AU	87.86 %	unknown *
3	0.17 Rf	26.0 AU	0.20 Rf	36.1 AU	4.13 %	0.24 Rf	4.2 AU	1117.3 AU	6.43 %	unknown *
4	0.60 Rf	4.2 AU	0.62 Rf	18.5 AU	2.12 %	0.66 Rf	3.8 AU	426.4 AU	2.45 %	unknown *
5	0.94 Rf	3.9 AU	0.97 Rf	18.1 AU	2.07 %	0.99 Rf	5.1 AU	428.1 AU	2.46 %	unknown *

Peak	Assigned Compounds
1	Alkaloid
2	Saponin
3	Steroid
4	Terpenoid
5	Steroid

The HPTLC fingerprinting patterns of the ethanol extract of *Adenocalymma alliaceum* was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total five peaks (Rf -0.03, 0.08, 0.24, 0.66, 0.99) were observed in the chromatogram and the components at Rf 0.08 was present in significant level (Table 3.5; Figure 3.1).

Figure 3.3 HPTLC Three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Adenocalymma alliaceum* at 366 nm

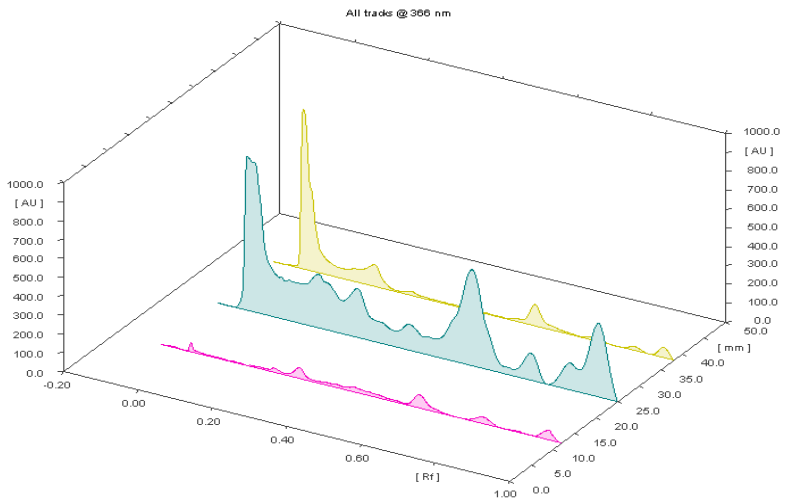


Figure 3.4 HPTLC fluorescence after derivatization observed at 366 nm.

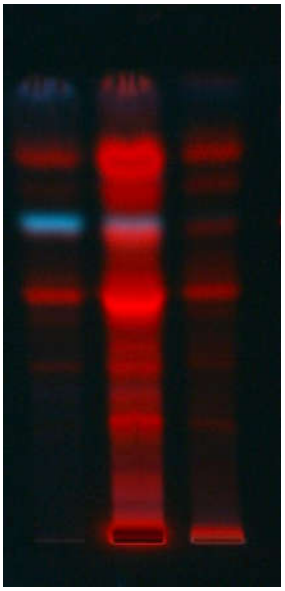


Table 3.6 Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.01 Rf	0.6 AU	0.00 Rf	48.0 AU	15.57 %	0.03 Rf	6.8 AU	449.3 AU	6.45 %	unknown *
2	0.21 Rf	9.0 AU	0.23 Rf	21.4 AU	6.93 %	0.25 Rf	7.5 AU	351.6 AU	5.04 %	unknown *
3	0.26 Rf	10.1 AU	0.29 Rf	57.8 AU	18.74 %	0.33 Rf	9.8 AU	1260.4 AU	18.08 %	unknown *
4	0.41 Rf	12.0 AU	0.44 Rf	25.4 AU	8.24 %	0.46 Rf	19.0 AU	677.5 AU	9.72 %	unknown *
5	0.58 Rf	5.2 AU	0.62 Rf	69.3 AU	22.48 %	0.69 Rf	4.2 AU	2093.2 AU	30.03 %	unknown *
6	0.74 Rf	0.0 AU	0.80 Rf	35.0 AU	11.34 %	0.83 Rf	0.3 AU	1046.5 AU	15.01 %	unknown *
7	0.93 Rf	3.1 AU	0.97 Rf	51.5 AU	16.70 %	0.99 Rf	3.3 AU	1091.5 AU	15.66 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Alkaloid	5	Saponin
2	Saponin	6	Terpenoid
3	Terpenoid	7	Steroids
4	Steroids	-	-

The HPTLC fingerprinting patterns of the Petroleum ether extract of *Adenocalymma alliaceum* was developed at 366nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total seven peaks (Rf 0.03, 0.25, 0.33, 0.46, 0.69, 0.83, 0.99) were observed in the chromatogram and the components at Rf 0.69 was present in significant level (Table 3.6; Figure 3.3).

Table 3.7 Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	1.4 AU	0.00 Rf	819.4 AU	32.71 %	0.09 Rf	15.8 AU	32633.9 AU	29.53 %	unknown *
2	0.10 Rf	203.1 AU	0.12 Rf	215.2 AU	8.59 %	0.13 Rf	38.9 AU	4409.8 AU	3.99 %	unknown *
3	0.15 Rf	208.3 AU	0.20 Rf	282.5 AU	11.28 %	0.26 Rf	74.5 AU	17287.9 AU	15.64 %	unknown *
4	0.26 Rf	174.8 AU	0.30 Rf	257.5 AU	10.28 %	0.35 Rf	12.3 AU	11392.8 AU	10.31 %	unknown *
5	0.40 Rf	83.8 AU	0.44 Rf	138.3 AU	5.52 %	0.48 Rf	39.4 AU	5880.7 AU	5.32 %	unknown *
6	0.50 Rf	94.4 AU	0.61 Rf	507.1 AU	20.24 %	0.70 Rf	37.6 AU	29854.3 AU	27.01 %	unknown *
7	0.72 Rf	33.3 AU	0.77 Rf	142.1 AU	5.67 %	0.81 Rf	0.1 AU	4565.0 AU	4.13 %	unknown *
8	0.82 Rf	0.4 AU	0.87 Rf	142.9 AU	5.71 %	0.90 Rf	17.0 AU	4500.9 AU	4.07 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Terpenoid	5	Phenols
2	Terpenoid	6	Flavonoid
3	Terpenoid	7	Terpenoid
4	Terpenoid	8	Saponin

The HPTLC fingerprinting patterns of the chloroform leaf extract of *Adenocalymma alliaceum* was developed at 366nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total eight peaks (Rf 0.09, 0.13, 0.26, 0.35, 0.48, 0.70, 0.81, 0.90) were observed in the chromatogram and the components at Rf 0.09, 0.70 were present in significant level (Table 3.7; Figure 3.3).

Table 3.8 Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	4.2 AU	0.00 Rf	848.5 AU	71.32 %	0.10 Rf	54.2 AU	19645.9 AU	65.07 %	unknown *
2	0.15 Rf	69.1 AU	0.19 Rf	115.7 AU	9.73 %	0.25 Rf	9.2 AU	4445.5 AU	14.72 %	unknown *
3	0.26 Rf	8.9 AU	0.30 Rf	24.9 AU	2.09 %	0.33 Rf	12.2 AU	695.5 AU	2.30 %	unknown *
4	0.43 Rf	9.8 AU	0.45 Rf	21.5 AU	1.81 %	0.49 Rf	7.1 AU	569.6 AU	1.89 %	unknown *
5	0.55 Rf	6.1 AU	0.58 Rf	19.0 AU	1.60 %	0.59 Rf	16.9 AU	344.8 AU	1.14 %	unknown *
6	0.59 Rf	16.5 AU	0.63 Rf	114.8 AU	9.65 %	0.70 Rf	13.3 AU	3400.5 AU	11.26 %	unknown *
7	0.76 Rf	4.7 AU	0.79 Rf	22.4 AU	1.88 %	0.82 Rf	3.0 AU	574.1 AU	1.90 %	unknown *
8	0.86 Rf	2.5 AU	0.90 Rf	22.9 AU	1.92 %	0.93 Rf	0.1 AU	514.4 AU	1.70 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	5	Saponin
2	Saponin	6	Flavonoid
3	Terpenoid	7	Glycosides
4	Alkaloid	8	Terpenoid

The HPTLC fingerprinting patterns of the ethanol leaf extract of *Adenocalymma alliaceum* was developed at 366nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total, eight peaks (Rf 0.10, 0.25, 0.33, 0.49, 0.59, 0.70, 0.82, 0.93) were observed in the chromatogram and the components at Rf 0.10 was present in significant level (Table 3.8; Figure 3.3).

Figure 3.5. HPTLC three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Adenocalymma alliaceum* at 550 nm

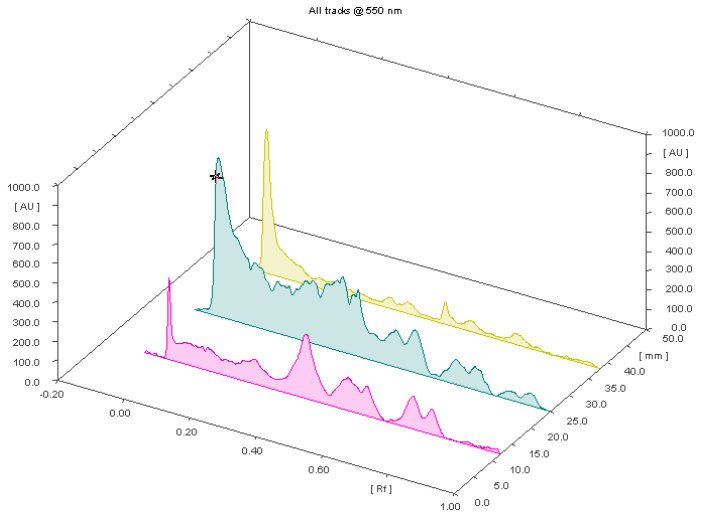


Figure 3.6 HPTLC fluorescence after derivatization observed at 550 nm**.**

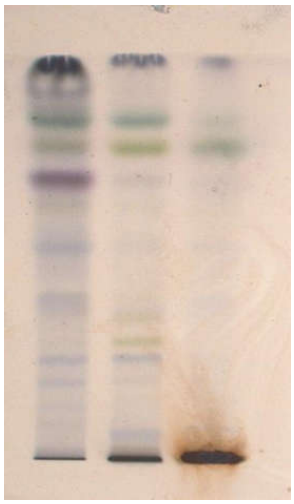


Table 3.9 Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	1.6 AU	-0.00 Rf	415.6 AU	22.27 %	0.02 Rf	32.9 AU	3871.3 AU	6.98 %	unknown *
2	0.02 Rf	93.2 AU	0.05 Rf	111.8 AU	5.99 %	0.07 Rf	33.6 AU	3572.6 AU	6.44 %	unknown *
3	0.08 Rf	101.8 AU	0.10 Rf	114.4 AU	6.13 %	0.10 Rf	33.2 AU	1581.9 AU	2.85 %	unknown *
4	0.11 Rf	97.0 AU	0.11 Rf	118.6 AU	6.36 %	0.16 Rf	72.3 AU	3335.5 AU	6.01 %	unknown *
5	0.21 Rf	81.7 AU	0.25 Rf	126.5 AU	6.78 %	0.32 Rf	52.8 AU	6494.8 AU	11.70 %	unknown *
6	0.33 Rf	57.5 AU	0.41 Rf	326.2 AU	17.48 %	0.48 Rf	49.2 AU	15626.4 AU	28.16 %	unknown *
7	0.48 Rf	49.3 AU	0.54 Rf	168.7 AU	9.04 %	0.58 Rf	11.7 AU	8086.5 AU	14.57 %	unknown *
8	0.58 Rf	113.1 AU	0.60 Rf	152.7 AU	8.18 %	0.65 Rf	1.3 AU	3808.5 AU	6.86 %	unknown *
9	0.68 Rf	13.6 AU	0.74 Rf	168.0 AU	9.00 %	0.77 Rf	39.5 AU	5372.8 AU	9.68 %	unknown *
10	0.77 Rf	69.8 AU	0.79 Rf	132.0 AU	7.07 %	0.84 Rf	0.7 AU	3216.8 AU	5.80 %	unknown *
11	0.87 Rf	0.1 AU	0.90 Rf	14.4 AU	0.77 %	0.93 Rf	3.9 AU	286.3 AU	0.52 %	unknown *
12	0.94 Rf	6.2 AU	0.96 Rf	17.5 AU	0.94 %	0.97 Rf	14.7 AU	240.4 AU	0.43 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	7	Glycoside
2	Terpenoid	8	Glycoside
3	Saponin	9	Steroid
4	Flavonoid	10	Flavonoid
5	Flavonoid	11	Terpenoid
6	Phenols	12	Flavonoid

The HPTLC fingerprinting patterns of the petroleum ether extract of *Adenocalymma alliaceum* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total, twelve peaks (Rf 0.02, 0.07, 0.10, 0.16, 0.32, 0.48, 0.58, 0.65, 0.77, 0.84, 0.93, 0.97) were observed in the chromatogram and the components at Rf 0.48, 0.58 were present in significant level (Table 3.9; Figure 3.5).

Table 3.10 Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	9.4 AU	-0.01 Rf	815.6 AU	19.01 %	0.09 Rf	37.5 AU	39216.6 AU	30.00 %	unknown *
2	0.09 Rf	291.6 AU	0.10 Rf	327.1 AU	7.62 %	0.16 Rf	10.0 AU	12031.0 AU	9.20 %	unknown *
3	0.16 Rf	210.4 AU	0.18 Rf	265.7 AU	6.19 %	0.19 Rf	48.9 AU	4681.3 AU	3.58 %	unknown *
4	0.20 Rf	245.4 AU	0.21 Rf	258.0 AU	6.01 %	0.22 Rf	19.4 AU	3155.4 AU	2.41 %	unknown *
5	0.24 Rf	252.4 AU	0.26 Rf	309.4 AU	7.21 %	0.27 Rf	31.3 AU	6952.5 AU	5.32 %	unknown *
6	0.27 Rf	303.4 AU	0.28 Rf	321.1 AU	7.48 %	0.30 Rf	47.0 AU	6236.4 AU	4.77 %	unknown *
7	0.30 Rf	251.0 AU	0.36 Rf	359.2 AU	8.37 %	0.36 Rf	47.4 AU	12305.8 AU	9.41 %	unknown *
8	0.36 Rf	351.7 AU	0.37 Rf	384.4 AU	8.96 %	0.39 Rf	54.3 AU	6755.5 AU	5.17 %	unknown *
9	0.39 Rf	261.2 AU	0.42 Rf	340.3 AU	7.93 %	0.46 Rf	35.1 AU	10205.1 AU	7.81 %	unknown *
10	0.46 Rf	135.8 AU	0.46 Rf	141.0 AU	3.29 %	0.49 Rf	16.3 AU	3131.7 AU	2.40 %	unknown *
11	0.49 Rf	116.4 AU	0.54 Rf	192.4 AU	4.48 %	0.56 Rf	31.3 AU	7082.3 AU	5.42 %	unknown *
12	0.56 Rf	132.5 AU	0.59 Rf	218.7 AU	5.10 %	0.64 Rf	16.1 AU	7182.1 AU	5.49 %	unknown *
13	0.66 Rf	17.1 AU	0.72 Rf	127.6 AU	2.97 %	0.75 Rf	76.4 AU	4964.3 AU	3.80 %	unknown *
14	0.76 Rf	77.6 AU	0.77 Rf	118.8 AU	2.77 %	0.83 Rf	0.1 AU	3608.7 AU	2.76 %	unknown *
15	0.84 Rf	0.3 AU	0.89 Rf	46.8 AU	1.09 %	0.91 Rf	27.4 AU	1446.2 AU	1.11 %	unknown *
16	0.91 Rf	26.2 AU	0.94 Rf	64.2 AU	1.50 %	0.98 Rf	3.1 AU	1787.7 AU	1.37 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Alkaloid	9	Steroid
2	Flavonoid	10	Alkaloid
3	Tannin	11	Flavonoid
4	Alkaloid	12	Flavonoid
5	Terpenoid	13	Saponin
6	Flavonoid	14	Terpenoid
7	Alkaloid	15	Saponin
8	Terpenoid	16	Flavonoid

The HPTLC fingerprinting patterns of the chloroform leaf extract of *Adenocalymma alliaceum* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total sixteen peaks (Rf 0.09, 0.16, 0.19, 0.22, 0.27, 0.30, 0.36, 0.39, 0.46, 0.49, 0.56, 0.64, 0.75, 0.83, 0.91, 0.98) were observed in the chromatogram and the components at Rf 0.09 was present in significant level (Table 3.10; Figure 3.5).

Table 3.11 Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	5.1 AU	-0.05 Rf	23.6 AU	1.78 %	-0.04 Rf	13.8 AU	285.6 AU	0.81 %	unknown *
2	-0.04 Rf	15.2 AU	-0.01 Rf	742.4 AU	56.11 %	0.13 Rf	39.0 AU	21001.9 AU	59.27 %	unknown *
3	0.13 Rf	39.1 AU	0.15 Rf	56.3 AU	4.25 %	0.17 Rf	46.2 AU	1263.3 AU	3.56 %	unknown *
4	0.17 Rf	47.8 AU	0.19 Rf	53.8 AU	4.07 %	0.22 Rf	29.4 AU	1489.4 AU	4.20 %	unknown *
5	0.26 Rf	29.5 AU	0.28 Rf	34.7 AU	2.62 %	0.31 Rf	23.3 AU	1004.4 AU	2.83 %	unknown *
6	0.33 Rf	23.3 AU	0.37 Rf	60.4 AU	4.56 %	0.39 Rf	33.1 AU	1601.0 AU	4.52 %	unknown *
7	0.40 Rf	38.1 AU	0.42 Rf	62.8 AU	4.75 %	0.46 Rf	16.8 AU	1744.3 AU	4.92 %	unknown *
8	0.51 Rf	17.4 AU	0.53 Rf	117.3 AU	8.86 %	0.57 Rf	24.9 AU	2173.6 AU	6.13 %	unknown *
9	0.58 Rf	25.4 AU	0.61 Rf	59.0 AU	4.46 %	0.66 Rf	14.5 AU	1966.3 AU	5.55 %	unknown *
10	0.71 Rf	16.5 AU	0.74 Rf	57.4 AU	4.34 %	0.80 Rf	12.5 AU	2040.9 AU	5.76 %	unknown *
11	0.80 Rf	13.1 AU	0.81 Rf	18.7 AU	1.41 %	0.83 Rf	5.1 AU	302.2 AU	0.85 %	unknown *
12	0.89 Rf	5.2 AU	0.90 Rf	17.8 AU	1.35 %	0.91 Rf	8.6 AU	157.8 AU	0.45 %	unknown *
13	0.95 Rf	13.6 AU	0.97 Rf	18.9 AU	1.43 %	0.99 Rf	2.4 AU	406.4 AU	1.15 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Steroid	8	Terpenoid
2	Terpenoid	9	Terpenoid
3	Saponin	10	Flavonoid
4	Alkaloid	11	Terpenoid
5	Terpenoid	12	Saponin
6	Terpenoid	13	Steroid
7	Steroid	-	-

The HPTLC fingerprinting patterns of the ethanol extract of *Adenocalymma alliaceum* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total thirteen peaks (Rf -0.04, 0.13, 0.17, 0.22, 0.31, 0.39, 0.46, 0.57, 0.66, 0.80, 0.83, 0.91, 0.99) were observed in the chromatogram and the components at Rf 0.13 was present in significant level (Table 3.11; Figure 5).

In the study on *Adenocalymma alliaceum* predominant compounds such as alkaloids, terpenoids, saponins, phenols and glycosides were noticed in the petroleum ether extract. In chloroform extract, saponins, terpenoids,

flavonoids and alkaloids and in ethanol extract, saponins and terpenoids were tentatively identified by comparison of Rf values with authentic database published (Yamunadevi et al. 2011;Gomathi et al. 2012).

Figure 3.7 HPTLC three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Pimenta dioica* at 254 nm

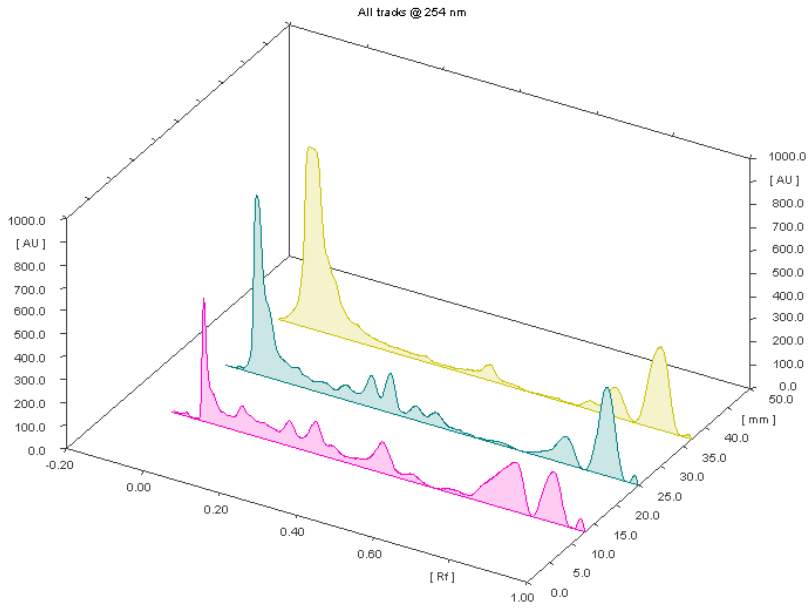


Figure-3.8: HPTLC image after derivatization observed at 254 nm.

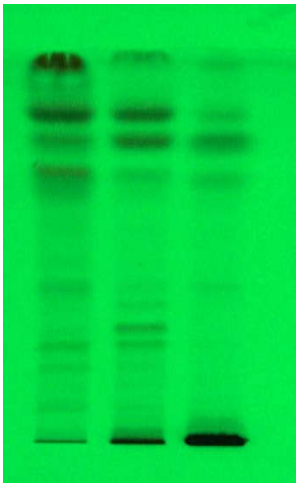


Table 3.12. Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	4.0 AU	-0.04 Rf	15.1 AU	0.92 %	-0.03 Rf	0.8 AU	179.3 AU	0.38 %	unknown *
2	-0.01 Rf	3.0 AU	0.01 Rf	535.5 AU	32.39 %	0.05 Rf	31.2 AU	7258.4 AU	15.45 %	unknown *
3	0.08 Rf	54.7 AU	0.11 Rf	114.1 AU	6.90 %	0.14 Rf	34.1 AU	3417.9 AU	7.28 %	unknown *
4	0.15 Rf	64.5 AU	0.16 Rf	69.5 AU	4.21 %	0.20 Rf	48.2 AU	2095.6 AU	4.46 %	unknown *
5	0.20 Rf	48.3 AU	0.23 Rf	109.4 AU	6.62 %	0.26 Rf	48.6 AU	2856.6 AU	6.08 %	unknown *
6	0.26 Rf	49.0 AU	0.30 Rf	137.9 AU	8.34 %	0.33 Rf	47.7 AU	3916.6 AU	8.34 %	unknown *
7	0.33 Rf	48.1 AU	0.34 Rf	53.5 AU	3.23 %	0.37 Rf	24.3 AU	1212.8 AU	2.58 %	unknown *
8	0.40 Rf	27.0 AU	0.47 Rf	132.7 AU	8.03 %	0.52 Rf	17.9 AU	4975.6 AU	10.59 %	unknown *
9	0.52 Rf	18.0 AU	0.55 Rf	33.0 AU	2.00 %	0.60 Rf	1.2 AU	1075.1 AU	2.29 %	unknown *
10	0.62 Rf	1.9 AU	0.66 Rf	17.7 AU	1.07 %	0.68 Rf	10.9 AU	504.5 AU	1.07 %	unknown *
11	0.70 Rf	10.4 AU	0.82 Rf	214.3 AU	12.96 %	0.86 Rf	0.5 AU	12227.7 AU	26.03 %	unknown *
12	0.86 Rf	0.7 AU	0.92 Rf	220.5 AU	13.34 %	0.96 Rf	1.4 AU	7250.5 AU	15.44 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Alkaloid	7	Terpenoid
2	Saponin	8	Terpenoid
3	Alkaloid	9	Saponin
4	Terpenoid	10	Terpenoid
5	Terpenoid	11	Terpenoid
6	Terpenoid	12	Flavonoid

The HPTLC fingerprinting patterns of the petroleum ether extract of *Pimenta dioica* was developed at 254 nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total twelve peaks (Rf -0.03, 0.05, 0.14, 0.20, 0.26, 0.33, 0.37, 0.52, 0.60, 0.68, 0.86, 0.96) were observed in the chromatogram and the components at Rf 0.86 was present in significant level (Table 3.12; Figure 3.7).

Table 3.13. Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	1.1 AU	-0.04 Rf	15.9 AU	0.81 %	-0.03 Rf	10.3 AU	144.3 AU	0.26 %	unknown *
2	-0.03 Rf	10.5 AU	0.00 Rf	781.8 AU	39.64 %	0.09 Rf	75.4 AU	21191.6 AU	38.46 %	unknown *
3	0.10 Rf	76.1 AU	0.11 Rf	82.2 AU	4.17 %	0.16 Rf	41.1 AU	2157.4 AU	3.92 %	unknown *
4	0.16 Rf	41.4 AU	0.18 Rf	50.5 AU	2.56 %	0.20 Rf	33.1 AU	1433.6 AU	2.60 %	unknown *
5	0.21 Rf	33.5 AU	0.24 Rf	67.6 AU	3.43 %	0.26 Rf	44.2 AU	1817.1 AU	3.30 %	unknown *
6	0.26 Rf	44.2 AU	0.30 Rf	139.2 AU	7.06 %	0.33 Rf	55.7 AU	3557.0 AU	6.46 %	unknown *
7	0.33 Rf	56.0 AU	0.35 Rf	173.4 AU	8.79 %	0.38 Rf	18.7 AU	3694.1 AU	6.70 %	unknown *
8	0.39 Rf	18.8 AU	0.42 Rf	64.7 AU	3.28 %	0.45 Rf	31.6 AU	1749.9 AU	3.18 %	unknown *
9	0.45 Rf	32.1 AU	0.48 Rf	60.7 AU	3.08 %	0.51 Rf	13.5 AU	1626.8 AU	2.95 %	unknown *
10	0.52 Rf	13.5 AU	0.54 Rf	15.2 AU	0.77 %	0.58 Rf	2.3 AU	339.7 AU	0.62 %	unknown *
11	0.62 Rf	8.4 AU	0.64 Rf	14.8 AU	0.75 %	0.68 Rf	3.1 AU	422.7 AU	0.77 %	unknown *
12	0.73 Rf	12.6 AU	0.81 Rf	119.8 AU	6.07 %	0.85 Rf	0.6 AU	4765.2 AU	8.65 %	unknown *
13	0.86 Rf	1.3 AU	0.92 Rf	386.1 AU	19.58 %	0.96 Rf	0.3 AU	12199.6 AU	22.14 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Alkaloid	8	Saponin
2	Alkaloid	9	Saponin
3	Flavonoid	10	Eugenol
4	Terpenoid	11	Terpenoid
5	Terpenoid	12	Terpenoid
6	Terpenoid	13	Flavonoid
7	Tannin	-	-

The HPTLC fingerprinting patterns of the chloroform extract of *Pimenta dioica* was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total thirteen peaks (Rf -0.03, 0.09, 0.16, 0.20, 0.26, 0.33, 0.38, 0.45, 0.51, 0.58, 0.68, 0.85, 0.96) were observed in the chromatogram and the components at Rf 0.09, 0.96 were present insignificant level (Table 3.13; Figure 3.7).

Table 3.14. Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	8.2 AU	0.01 Rf	788.5 AU	55.59 %	0.12 Rf	75.3 AU	36301.9 AU	62.34 %	unknown *
2	0.29 Rf	19.5 AU	0.31 Rf	26.2 AU	1.84 %	0.33 Rf	8.4 AU	548.4 AU	0.94 %	unknown *
3	0.42 Rf	19.3 AU	0.48 Rf	68.7 AU	4.84 %	0.51 Rf	11.2 AU	2170.7 AU	3.73 %	unknown *
4	0.53 Rf	9.9 AU	0.54 Rf	12.6 AU	0.89 %	0.56 Rf	2.0 AU	185.0 AU	0.32 %	unknown *
5	0.70 Rf	1.1 AU	0.74 Rf	38.1 AU	2.68 %	0.76 Rf	29.0 AU	885.2 AU	1.52 %	unknown *
6	0.76 Rf	29.1 AU	0.80 Rf	125.8 AU	8.87 %	0.85 Rf	0.3 AU	4313.1 AU	7.41 %	unknown *
7	0.85 Rf	0.3 AU	0.92 Rf	358.7 AU	25.29 %	0.97 Rf	0.3 AU	13829.1 AU	23.75 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	5	Terpenoid
2	Terpenoid	6	Flavonoid
3	Flavonoid	7	Flavonoid
4	Saponin	-	-

The HPTLC fingerprinting patterns of the ethanol extract of *Pimenta dioica* was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total seven peaks (Rf -0.12, 0.33, 0.51, 0.56, 0.76, 0.85, 0.97) were observed in the chromatogram and the components at Rf 0.12, 0.97 were present in significant level (Table 3.14; Figure 3.7).

Figure 3.9. HPTLC three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Pimenta dioica* at 366 nm

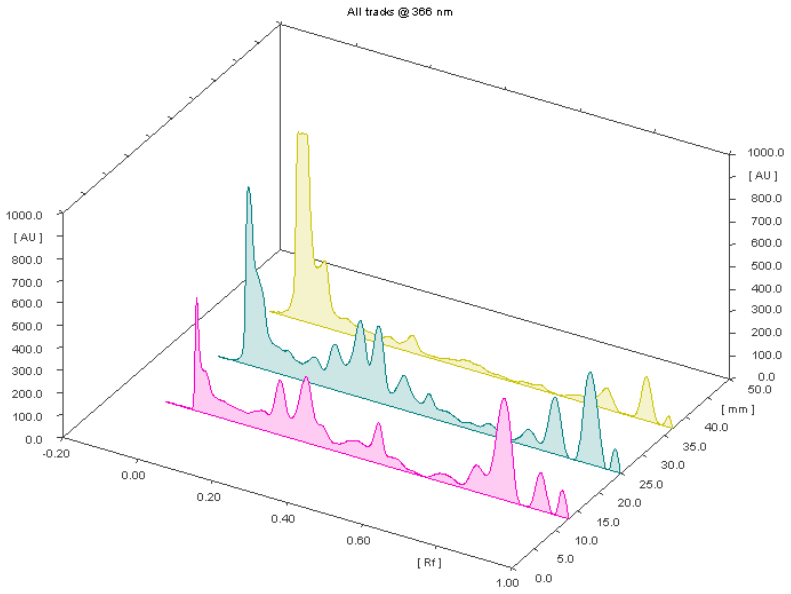


Figure- 3.10: HPTLC image after derivatization observed at 366nm.

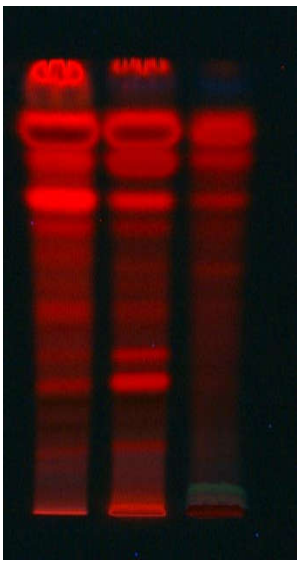


Table 3.15. Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.01 Rf	1.9 AU	0.01 Rf	502.0 AU	22.10 %	0.06 Rf	32.6 AU	7951.0 AU	13.08 %	unknown *
2	0.06 Rf	75.4 AU	0.08 Rf	77.5 AU	3.41 %	0.12 Rf	58.2 AU	2666.9 AU	4.39 %	unknown *
3	0.14 Rf	53.9 AU	0.19 Rf	85.8 AU	3.78 %	0.20 Rf	74.2 AU	2864.9 AU	4.71 %	unknown *
4	0.20 Rf	75.5 AU	0.23 Rf	242.8 AU	10.69 %	0.26 Rf	37.7 AU	5942.3 AU	9.78 %	unknown *
5	0.26 Rf	87.8 AU	0.30 Rf	291.3 AU	12.82 %	0.37 Rf	32.3 AU	10329.6 AU	16.99 %	unknown *
6	0.37 Rf	32.3 AU	0.44 Rf	71.8 AU	3.16 %	0.46 Rf	58.1 AU	3224.2 AU	5.30 %	unknown *
7	0.46 Rf	58.4 AU	0.49 Rf	179.6 AU	7.91 %	0.52 Rf	40.1 AU	3905.2 AU	6.42 %	unknown *
8	0.52 Rf	40.8 AU	0.54 Rf	45.1 AU	1.99 %	0.58 Rf	10.6 AU	1283.4 AU	2.11 %	unknown *
9	0.62 Rf	0.1 AU	0.67 Rf	37.4 AU	1.65 %	0.71 Rf	13.2 AU	1510.4 AU	2.48 %	unknown *
10	0.72 Rf	13.4 AU	0.76 Rf	118.9 AU	5.23 %	0.78 Rf	35.8 AU	3304.2 AU	5.44 %	unknown *
11	0.78 Rf	86.8 AU	0.83 Rf	450.8 AU	19.85 %	0.88 Rf	0.2 AU	14373.6 AU	23.65 %	unknown *
12	0.89 Rf	0.6 AU	0.93 Rf	168.4 AU	7.41 %	0.96 Rf	0.6 AU	3427.1 AU	5.64 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Tannin	7	Terpenoid
2	Saponin	8	Steroid
3	Terpenoid	9	Glycosides
4	Terpenoid	10	Terpenoid
5	Terpenoid	11	Terpenoid
6	Steroid	12	Flavonoids

The HPTLC fingerprinting patterns of the petroleum ether leaf extract of *Pimenta dioica* was developed at 336nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total twelve peaks (Rf 0.06, 0.12, 0.20, 0.26, 0.37, 0.46, 0.52, 0.58, 0.71, 0.78, 0.88, 0.96) were observed in the chromatogram and the components at Rf 0.88, 0.37 were present in significant level (Table 3.15; Figure 3.9).

Table 3.16. Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	6.3 AU	0.00 Rf	795.7 AU	26.28 %	0.08 Rf	20.4 AU	21848.3 AU	26.41 %	unknown *
2	0.10 Rf	109.7 AU	0.11 Rf	115.7 AU	3.82 %	0.15 Rf	75.8 AU	3045.7 AU	3.68 %	unknown *
3	0.15 Rf	76.2 AU	0.18 Rf	121.1 AU	4.00 %	0.20 Rf	78.0 AU	3689.0 AU	4.46 %	unknown *
4	0.21 Rf	78.3 AU	0.24 Rf	204.2 AU	6.74 %	0.26 Rf	24.7 AU	5491.0 AU	6.64 %	unknown *
5	0.26 Rf	125.0 AU	0.30 Rf	342.0 AU	11.30 %	0.33 Rf	57.0 AU	9810.2 AU	11.86 %	unknown *
6	0.33 Rf	160.4 AU	0.35 Rf	342.4 AU	11.31 %	0.38 Rf	31.2 AU	8101.3 AU	9.79 %	unknown *
7	0.39 Rf	61.5 AU	0.42 Rf	156.6 AU	5.17 %	0.47 Rf	53.7 AU	5207.9 AU	6.30 %	unknown *
8	0.47 Rf	53.9 AU	0.49 Rf	107.3 AU	3.54 %	0.52 Rf	49.3 AU	2475.6 AU	2.99 %	unknown *
9	0.53 Rf	49.5 AU	0.54 Rf	50.6 AU	1.67 %	0.58 Rf	22.1 AU	1343.7 AU	1.62 %	unknown *
10	0.62 Rf	26.0 AU	0.65 Rf	50.5 AU	1.67 %	0.70 Rf	9.1 AU	1643.4 AU	1.99 %	unknown *
11	0.71 Rf	10.2 AU	0.75 Rf	76.9 AU	2.54 %	0.78 Rf	30.7 AU	2167.6 AU	2.62 %	unknown *
12	0.79 Rf	31.3 AU	0.83 Rf	254.5 AU	8.41 %	0.86 Rf	0.6 AU	6450.6 AU	7.80 %	unknown *
13	0.88 Rf	0.0 AU	0.92 Rf	409.9 AU	13.54 %	0.96 Rf	3.9 AU	11451.1 AU	13.84 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Terpenoid	8	Terpenoid
2	Flavonoid	9	Steroid
3	Terpenoid	10	Flavonoids
4	Terpenoid	11	Terpenoid
5	Terpenoid	12	Terpenoid
6	Glycosides	13	Flavonoid
7	Steroid	-	-

The HPTLC fingerprinting patterns of the chloroform extract of *Pimenta dioica* was developed at 336nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total thirteen peaks (Rf 0.08, 0.15, 0.20, 0.26, 0.33, 0.38, 0.47, 0.52, 0.58, 0.70, 0.78, 0.86, 0.96) were observed in the chromatogram and the components at Rf 0.08, 0.96 were present in significant level (Table 3.16; Figure 3.9).

Table 3.17. Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	2.4 AU	0.02 Rf	838.8 AU	49.07 %	0.05 Rf	58.4 AU	25040.8 AU	51.38 %	unknown *
2	0.05 Rf	259.4 AU	0.07 Rf	298.6 AU	17.47 %	0.11 Rf	59.4 AU	7366.1 AU	15.11 %	unknown *
3	0.11 Rf	59.5 AU	0.13 Rf	65.0 AU	3.80 %	0.21 Rf	23.1 AU	2855.7 AU	5.86 %	unknown *
4	0.22 Rf	24.9 AU	0.24 Rf	40.2 AU	2.35 %	0.27 Rf	25.3 AU	1030.6 AU	2.11 %	unknown *
5	0.27 Rf	25.4 AU	0.31 Rf	78.0 AU	4.56 %	0.35 Rf	9.2 AU	2052.4 AU	4.21 %	unknown *
6	0.37 Rf	9.7 AU	0.46 Rf	35.6 AU	2.08 %	0.47 Rf	28.8 AU	1540.6 AU	3.16 %	unknown *
7	0.48 Rf	29.3 AU	0.48 Rf	30.3 AU	1.78 %	0.52 Rf	9.9 AU	625.9 AU	1.28 %	unknown *
8	0.59 Rf	4.6 AU	0.61 Rf	14.1 AU	0.82 %	0.62 Rf	12.9 AU	242.0 AU	0.50 %	unknown *
9	0.62 Rf	12.7 AU	0.64 Rf	21.4 AU	1.25 %	0.68 Rf	0.3 AU	491.3 AU	1.01 %	unknown *
10	0.70 Rf	0.9 AU	0.82 Rf	92.6 AU	5.42 %	0.86 Rf	0.3 AU	3530.3 AU	7.24 %	unknown *
11	0.89 Rf	0.0 AU	0.93 Rf	194.7 AU	11.39 %	0.96 Rf	1.2 AU	3964.2 AU	8.13 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	7	Terpenoid
2	Flavonoid	8	Terpenoid
3	Alkaloid	9	Terpenoid
4	Terpenoid	10	Terpenoid
5	Terpenoid	11	Flavonoid
6	Steroid	-	-

The HPTLC fingerprinting patterns of the ethanol extract of *Pimenta dioica* was developed at 336nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total eleven peaks (Rf 0.05, 0.11, 0.21, 0.27, 0.35, 0.47, 0.52, 0.62, 0.68, 0.86, 0.96) were observed in the chromatogram and the components at Rf 0.05 was present in significant level (Table 3.17; Figure 3.9).

Figure 3.10. HPTLC three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Pimenta dioica* at 550 nm

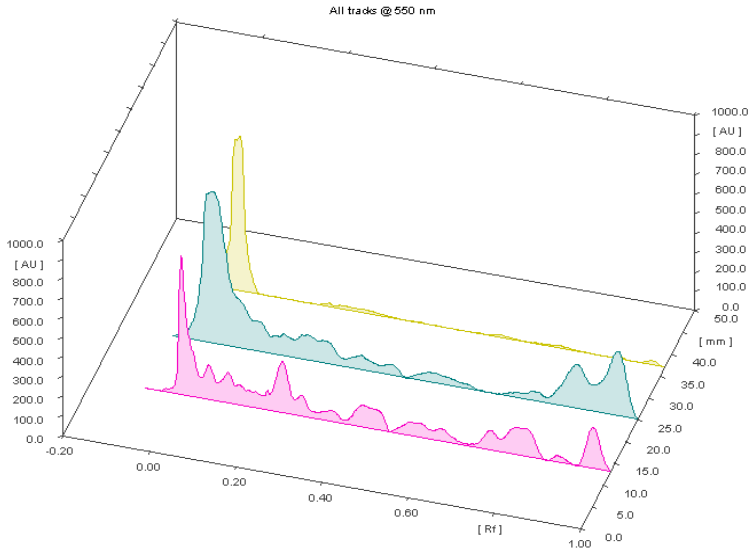


Figure-3.11: HPTLC image after derivatization observed at 550nm.

Table 3.18. Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	8.2 AU	-0.05 Rf	14.2 AU	0.71 %	-0.04 Rf	0.7 AU	111.9 AU	0.21 %	unknown *
2	-0.02 Rf	5.1 AU	0.01 Rf	650.8 AU	32.57 %	0.06 Rf	55.7 AU	11252.5 AU	21.06 %	unknown *
3	0.10 Rf	90.2 AU	0.12 Rf	125.3 AU	6.27 %	0.14 Rf	17.8 AU	2852.1 AU	5.34 %	unknown *
4	0.14 Rf	119.5 AU	0.15 Rf	124.1 AU	6.21 %	0.20 Rf	34.4 AU	4351.5 AU	8.15 %	unknown *
5	0.20 Rf	94.9 AU	0.23 Rf	153.7 AU	7.69 %	0.26 Rf	31.9 AU	4859.9 AU	9.10 %	unknown *
6	0.26 Rf	92.2 AU	0.30 Rf	175.7 AU	8.79 %	0.33 Rf	74.3 AU	5475.1 AU	10.25 %	unknown *
7	0.34 Rf	73.2 AU	0.35 Rf	75.5 AU	3.78 %	0.37 Rf	53.9 AU	1477.0 AU	2.76 %	unknown *
8	0.37 Rf	54.0 AU	0.38 Rf	71.9 AU	3.60 %	0.40 Rf	55.4 AU	1109.4 AU	2.08 %	unknown *
9	0.41 Rf	58.3 AU	0.45 Rf	83.8 AU	4.19 %	0.52 Rf	29.5 AU	4670.0 AU	8.74 %	unknown *
10	0.53 Rf	30.4 AU	0.56 Rf	50.7 AU	2.54 %	0.59 Rf	37.2 AU	1801.0 AU	3.37 %	unknown *
11	0.59 Rf	37.7 AU	0.63 Rf	97.5 AU	4.88 %	0.71 Rf	2.9 AU	3777.5 AU	7.07 %	unknown *
12	0.72 Rf	3.6 AU	0.75 Rf	48.9 AU	2.45 %	0.78 Rf	38.6 AU	1254.7 AU	2.35 %	unknown *
13	0.78 Rf	39.1 AU	0.83 Rf	241.9 AU	12.11 %	0.88 Rf	1.0 AU	8483.4 AU	15.88 %	unknown *
14	0.90 Rf	5.3 AU	0.93 Rf	84.2 AU	4.21 %	0.96 Rf	0.7 AU	1942.1 AU	3.64 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Steroid	8	Steroid
2	Tannin	9	Terpenoid
3	Alkaloid	10	Glycoside
4	Terpenoid	11	Terpenoid
5	Terpenoid	12	Terpenoid
6	Terpenoid	13	Terpenoid
7	Terpenoid	14	Flavonoid

The HPTLC fingerprinting patterns of the petroleum ether extract of *Pimenta dioica*was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total fourteen peaks (Rf -0.04, 0.06, 0.14, 0.20, 0.26, 0.33, 0.37, 0.40, 0.52, 0.59, 0.71, 0.78, 0.88, 0.96) were observed in the chromatogram and the components at Rf 0.06 was present in significant level (Table 3.18; Figure 3.10).

Table 3.19. Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	0.5 AU	0.00 Rf	775.0 AU	41.03 %	0.11 Rf	54.8 AU	23747.9 AU	43.53 %	unknown *
2	0.11 Rf	64.8 AU	0.13 Rf	87.6 AU	4.64 %	0.14 Rf	35.4 AU	1626.8 AU	2.98 %	unknown *
3	0.15 Rf	85.4 AU	0.16 Rf	90.3 AU	4.78 %	0.20 Rf	72.1 AU	3182.8 AU	5.83 %	unknown *
4	0.21 Rf	72.1 AU	0.23 Rf	112.4 AU	5.95 %	0.26 Rf	30.8 AU	3418.4 AU	6.27 %	unknown *
5	0.26 Rf	81.3 AU	0.30 Rf	196.7 AU	10.41 %	0.33 Rf	36.0 AU	6047.5 AU	11.08 %	unknown *
6	0.33 Rf	107.4 AU	0.35 Rf	173.2 AU	9.17 %	0.38 Rf	72.9 AU	4415.3 AU	8.09 %	unknown *
7	0.40 Rf	72.2 AU	0.42 Rf	107.3 AU	5.68 %	0.47 Rf	30.3 AU	3412.1 AU	6.25 %	unknown *
8	0.47 Rf	31.9 AU	0.49 Rf	48.8 AU	2.59 %	0.50 Rf	43.7 AU	923.0 AU	1.69 %	unknown *
9	0.55 Rf	31.1 AU	0.56 Rf	33.3 AU	1.76 %	0.58 Rf	25.1 AU	543.6 AU	1.00 %	unknown *
10	0.60 Rf	24.4 AU	0.63 Rf	39.4 AU	2.09 %	0.70 Rf	0.4 AU	1600.4 AU	2.93 %	unknown *
11	0.71 Rf	2.3 AU	0.74 Rf	19.1 AU	1.01 %	0.78 Rf	0.1 AU	506.8 AU	0.93 %	unknown *
12	0.80 Rf	2.8 AU	0.84 Rf	47.8 AU	2.53 %	0.87 Rf	0.3 AU	977.7 AU	1.79 %	unknown *
13	0.87 Rf	0.8 AU	0.92 Rf	157.7 AU	8.35 %	0.97 Rf	0.5 AU	4155.8 AU	7.62 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Flavonoid	8	Terpenoid
2	Alkaloid	9	Glycoside
3	Terpenoid	10	Flavonoid
4	Terpenoid	11	Terpenoid
5	Terpenoid	12	Terpenoid
6	Glycoside	13	Flavonoid
7	Steroid	-	-

The HPTLC fingerprinting patterns of the chloroform extract of *Pimenta dioica* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total thirteen peaks (Rf 0.11, 0.14, 0.20, 0.26, 0.33, 0.38, 0.47, 0.50, 0.58, 0.70, 0.78, 0.87, 0.97) were observed in the chromatogram and the components at Rf 0.11 was present in significant level (Table 3.19; Figure 3.10).

Table 3.20. Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	5.6 AU	0.01 Rf	768.1 AU	69.03 %	0.13 Rf	99.9 AU	38762.7 AU	82.47 %	unknown *
2	0.27 Rf	3.1 AU	0.30 Rf	16.0 AU	1.44 %	0.35 Rf	0.0 AU	410.1 AU	0.87 %	unknown *
3	0.40 Rf	1.6 AU	0.43 Rf	21.7 AU	1.95 %	0.44 Rf	19.9 AU	330.7 AU	0.70 %	unknown *
4	0.45 Rf	20.6 AU	0.47 Rf	26.4 AU	2.37 %	0.49 Rf	15.2 AU	594.1 AU	1.26 %	unknown *
5	0.49 Rf	16.1 AU	0.50 Rf	19.4 AU	1.74 %	0.51 Rf	0.6 AU	159.9 AU	0.34 %	unknown *
6	0.51 Rf	0.0 AU	0.54 Rf	20.8 AU	1.87 %	0.55 Rf	18.4 AU	241.8 AU	0.51 %	unknown *
7	0.56 Rf	21.2 AU	0.58 Rf	22.8 AU	2.05 %	0.60 Rf	3.1 AU	362.2 AU	0.77 %	unknown *
8	0.60 Rf	3.2 AU	0.63 Rf	26.7 AU	2.40 %	0.68 Rf	8.5 AU	912.5 AU	1.94 %	unknown *
9	0.76 Rf	2.6 AU	0.80 Rf	28.0 AU	2.52 %	0.81 Rf	6.6 AU	396.3 AU	0.84 %	unknown *
10	0.81 Rf	7.2 AU	0.83 Rf	23.7 AU	2.13 %	0.85 Rf	3.0 AU	393.1 AU	0.84 %	unknown *
11	0.87 Rf	13.2 AU	0.92 Rf	139.1 AU	12.50 %	0.97 Rf	7.8 AU	4439.8 AU	9.45 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Flavonoid	7	Saponin
2	Saponin	8	Terpenoid
3	Saponin	9	Terpenoid
4	Alkaloid	10	Flavonoid
5	Flavonoid	11	Flavonoid
6	Flavonoid	-	-

The HPTLC fingerprinting patterns of the ethanol extract of *Pimenta dioica* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total eleven peaks (Rf 0.13, 0.35, 0.44, 0.49, 0.51, 0.55, 0.60, 0.68, 0.81, 0.85, 0.97) were observed in the chromatogram and the components at Rf 0.13 was present in significant level (Table 3.20; Figure 3.10).

In the case of *Pimenta dioica* the predominant compounds such as terpenoids and tannins were in the petroleum ether extract. In chloroform extract, alkaloids, flavonoids and terpenoids were noticed, where as in ethanolic extract, saponins and flavonoid were tentatively identified in comparison of Rf values with authentic data published (Yamunadevi et al.2011; Gomathi et al.2012).

Figure 3.11. HPTLC Three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Saritaea magnifica* at 254 nm

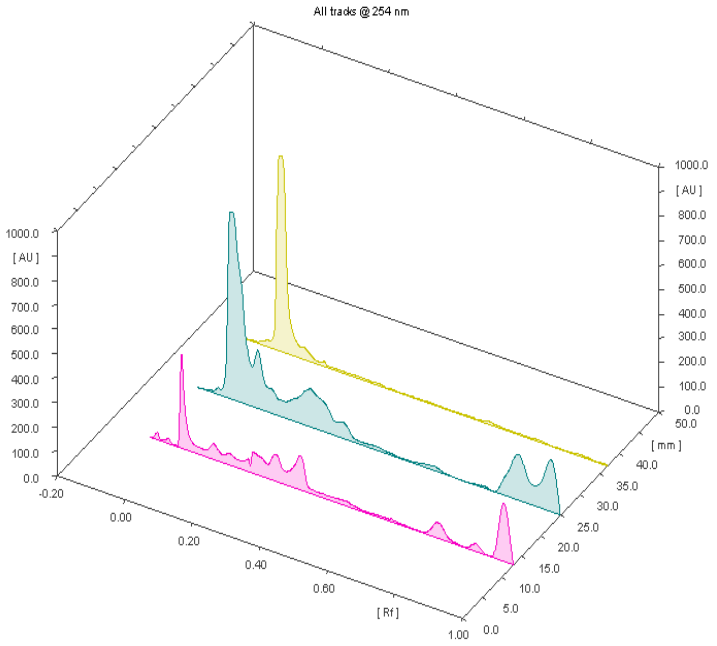


Figure-3.12: HPTLC image after derivatization observed at 254nm

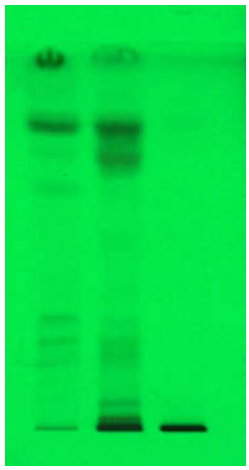


Table 3.21. Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.6 AU	-0.05 Rf	28.6 AU	2.77 %	-0.04 Rf	3.8 AU	250.1 AU	1.22 %	unknown *
2	-0.04 Rf	3.9 AU	-0.02 Rf	20.5 AU	1.99 %	-0.01 Rf	1.1 AU	217.4 AU	1.06 %	unknown *
3	0.00 Rf	2.4 AU	0.02 Rf	380.2 AU	36.85 %	0.06 Rf	26.8 AU	4573.4 AU	22.36 %	unknown *
4	0.08 Rf	25.5 AU	0.11 Rf	63.0 AU	6.11 %	0.14 Rf	29.6 AU	1573.3 AU	7.69 %	unknown *
5	0.14 Rf	30.2 AU	0.16 Rf	45.6 AU	4.42 %	0.18 Rf	32.7 AU	1030.0 AU	5.04 %	unknown *
6	0.19 Rf	31.7 AU	0.21 Rf	50.9 AU	4.93 %	0.22 Rf	25.8 AU	860.7 AU	4.21 %	unknown *
7	0.22 Rf	38.3 AU	0.23 Rf	86.7 AU	8.41 %	0.27 Rf	56.6 AU	2376.0 AU	11.62 %	unknown *
8	0.27 Rf	56.7 AU	0.29 Rf	107.1 AU	10.38 %	0.32 Rf	52.1 AU	2738.2 AU	13.39 %	unknown *
9	0.33 Rf	52.6 AU	0.37 Rf	137.9 AU	13.36 %	0.40 Rf	15.9 AU	3828.8 AU	18.72 %	unknown *
10	0.47 Rf	13.5 AU	0.50 Rf	17.4 AU	1.69 %	0.55 Rf	4.0 AU	672.5 AU	3.29 %	unknown *
11	0.74 Rf	3.1 AU	0.78 Rf	62.5 AU	6.06 %	0.82 Rf	6.1 AU	1721.2 AU	8.41 %	unknown *
12	0.86 Rf	2.1 AU	0.89 Rf	31.3 AU	3.04 %	0.92 Rf	0.1 AU	612.7 AU	3.00 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Steroid	7	Terpenoid
2	Saponin	8	Flavonoid
3	Tannin	9	Steroid
4	Alkaloid	10	Alkaloid
5	Alkaloid	11	Glycoside
6	Saponin	12	Glycoside

The HPTLC fingerprinting patterns of the petroleum ether extract of *Saritaea magnifica* was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total twelve peaks (Rf -0.04, 0.01, 0.06, 0.14, 0.18, 0.22, 0.27, 0.32, 0.40, 0.55, 0.82, 0.92) were observed in the chromatogram and the components at Rf 0.06, 0.40 were present in significant level (Table 3.21; Figure 3.11).

Table 3.22. Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.05 Rf	5.1 AU	0.03 Rf	769.7 AU	53.60 %	0.08 Rf	31.5 AU	25093.4 AU	46.52 %	unknown *
2	0.09 Rf	162.2 AU	0.10 Rf	237.3 AU	16.52 %	0.16 Rf	37.5 AU	7135.4 AU	13.23 %	unknown *
3	0.18 Rf	66.7 AU	0.26 Rf	155.2 AU	10.81 %	0.34 Rf	56.2 AU	11253.8 AU	20.87 %	unknown *
4	0.34 Rf	56.3 AU	0.36 Rf	67.5 AU	4.70 %	0.40 Rf	13.1 AU	1813.6 AU	3.36 %	unknown *
5	0.56 Rf	4.9 AU	0.63 Rf	18.8 AU	1.31 %	0.66 Rf	4.0 AU	777.9 AU	1.44 %	unknown *
6	0.80 Rf	0.6 AU	0.88 Rf	187.5 AU	13.06 %	0.92 Rf	31.5 AU	7862.3 AU	14.58 %	unknown *

Peak	Assigned Compounds
1	Saponin
2	Terpenoid
3	Steroid
4	Steroid
5	Terpenoid
6	Glycoside

The HPTLC fingerprinting patterns of the chloroform extract of *Saritaea magnifica*was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total six peaks (Rf -0.08, 0.16, 0.34, 0.40, 0.66, 0.92) were observed in the chromatogram and the components at Rf 0.08, 0.34 were present in significant level (Table 3.22; Figure 3.11).

Table 3.23. Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	3.5 AU	-0.01 Rf	28.9 AU	3.19 %	0.00 Rf	23.6 AU	428.7 AU	2.20 %	unknown *
2	0.00 Rf	25.0 AU	0.03 Rf	797.2 AU	87.84 %	0.09 Rf	43.1 AU	17479.2 AU	89.69 %	unknown *
3	0.09 Rf	43.3 AU	0.10 Rf	49.5 AU	5.45 %	0.14 Rf	11.1 AU	1031.4 AU	5.29 %	unknown *
4	0.14 Rf	9.8 AU	0.16 Rf	19.5 AU	2.15 %	0.19 Rf	4.0 AU	262.4 AU	1.35 %	unknown *
5	0.63 Rf	4.2 AU	0.65 Rf	12.5 AU	1.38 %	0.68 Rf	4.4 AU	286.7 AU	1.47 %	unknown *

Peak	Assigned Compounds
1	Steroids
2	Alkaloid
3	Alkaloid
4	Tannin
5	Terpenoid

The HPTLC fingerprinting patterns of the ethanol extract of *Saritaea magnifica* was developed at 254nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total five peaks (Rf -0.00, 0.09, 0.14, 0.19, 0.68) were observed in the chromatogram and the components at Rf 0.09 was present in significant level (Table 3.23; Figure 3.11).

Figure 3.13. HPTLC three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Saritaea magnifica* at 366 nm

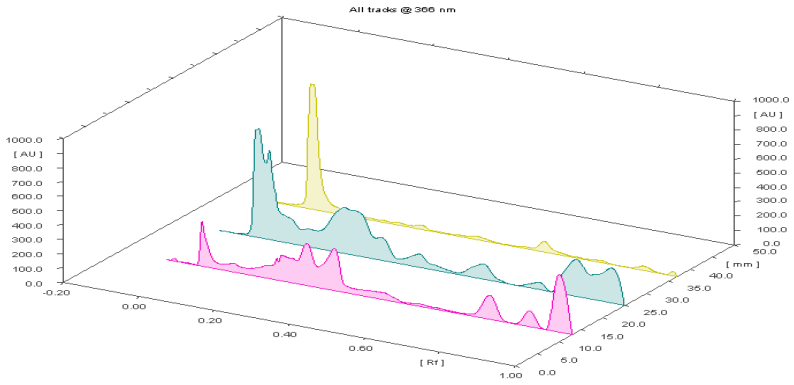


Figure-3.14: HPTLC image after derivatization observed at 366nm

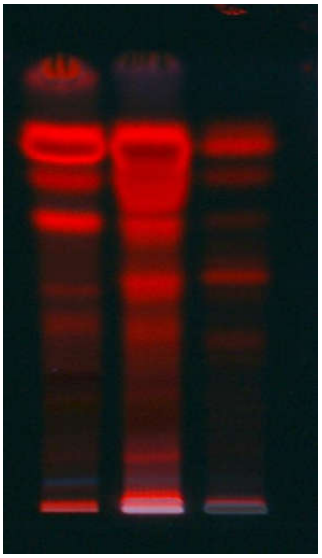


Table 3.24. Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	0.1 AU	-0.05 Rf	17.7 AU	1.08 %	-0.05 Rf	0.5 AU	141.1 AU	0.33 %	unknown *
2	-0.00 Rf	1.7 AU	0.02 Rf	308.5 AU	18.85 %	0.07 Rf	37.3 AU	4827.4 AU	11.24 %	unknown *
3	0.07 Rf	37.9 AU	0.10 Rf	61.1 AU	3.73 %	0.12 Rf	41.4 AU	1739.9 AU	4.05 %	unknown *
4	0.13 Rf	41.0 AU	0.23 Rf	177.9 AU	10.86 %	0.25 Rf	37.2 AU	7250.4 AU	16.88 %	unknown *
5	0.25 Rf	167.2 AU	0.26 Rf	169.1 AU	10.33 %	0.27 Rf	57.7 AU	2316.3 AU	5.39 %	unknown *
6	0.27 Rf	157.9 AU	0.29 Rf	289.6 AU	17.69 %	0.32 Rf	42.8 AU	7740.0 AU	18.02 %	unknown *
7	0.32 Rf	143.3 AU	0.37 Rf	290.7 AU	17.76 %	0.41 Rf	37.5 AU	9526.8 AU	22.19 %	unknown *
8	0.46 Rf	39.6 AU	0.50 Rf	47.9 AU	2.93 %	0.55 Rf	7.1 AU	2179.3 AU	5.08 %	unknown *
9	0.73 Rf	9.8 AU	0.78 Rf	164.5 AU	10.05 %	0.82 Rf	3.9 AU	4677.4 AU	10.89 %	unknown *
10	0.85 Rf	0.1 AU	0.89 Rf	109.9 AU	6.71 %	0.92 Rf	0.6 AU	2542.8 AU	5.92 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	6	Steroid
2	Saponin	7	Terpenoid
3	Terpenoid	8	Alkaloid
4	Flavonoid	9	Glycosides
5	Terpenoid	10	Glycoside

The HPTLC fingerprinting patterns of the petroleum ether extract of *Saritaea magnifica* was developed at 366nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total ten peaks (Rf -0.08, -0.05, 0.07, 0.12, 0.25, 0.27, 0.32, 0.41, 0.55, 0.82, 0.91) were observed in the chromatogram and the components at Rf 0.41 were present in significant level (Table 3.24; Figure 3.13).

Table 3.25. Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.01 Rf	9.8 AU	0.03 Rf	761.8 AU	27.12 %	0.05 Rf	32.6 AU	15777.0 AU	18.51 %	unknown *
2	0.05 Rf	535.9 AU	0.06 Rf	625.4 AU	22.27 %	0.14 Rf	10.5 AU	15739.7 AU	18.46 %	unknown *
3	0.14 Rf	110.9 AU	0.16 Rf	124.9 AU	4.45 %	0.18 Rf	22.8 AU	3012.4 AU	3.53 %	unknown *
4	0.18 Rf	123.1 AU	0.26 Rf	319.8 AU	11.39 %	0.27 Rf	37.9 AU	13920.7 AU	16.33 %	unknown *
5	0.27 Rf	307.9 AU	0.28 Rf	310.6 AU	11.06 %	0.34 Rf	45.9 AU	10621.5 AU	12.46 %	unknown *
6	0.34 Rf	146.1 AU	0.35 Rf	159.8 AU	5.69 %	0.40 Rf	30.3 AU	4293.9 AU	5.04 %	unknown *
7	0.40 Rf	30.3 AU	0.46 Rf	93.3 AU	3.32 %	0.55 Rf	12.2 AU	4626.7 AU	5.43 %	unknown *
8	0.56 Rf	12.5 AU	0.63 Rf	107.2 AU	3.82 %	0.70 Rf	0.2 AU	4503.9 AU	5.28 %	unknown *
9	0.72 Rf	0.0 AU	0.78 Rf	49.7 AU	1.77 %	0.80 Rf	0.4 AU	1431.3 AU	1.68 %	unknown *
10	0.81 Rf	1.3 AU	0.87 Rf	256.2 AU	9.12 %	0.91 Rf	37.1 AU	11313.8 AU	13.27 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	6	Steroid
2	Alkaloid	7	Flavonoid
3	Alkaloid	8	Flavonoid
4	Terpenoid	9	Flavonoid
5	Terpenoid	10	Saponin

The HPTLC fingerprinting patterns of the chloroform extract of *Saritaea magnifica* was developed at 366nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total, ten peaks (Rf -0.05, 0.14, 0.18, 0.27, 0.34, 0.40, 0.55, 0.70, 0.80, 0.91) were observed in the chromatogram and the components at Rf 0.05, 0.14were present in significant level (Table 3.25; Figure 3.13).

Table 3. 26 Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.02 Rf	8.0 AU	0.03 Rf	869.7 AU	81.22 %	0.14 Rf	16.4 AU	19777.3 AU	79.25 %	unknown *
2	0.24 Rf	9.1 AU	0.26 Rf	19.5 AU	1.82 %	0.28 Rf	11.6 AU	424.1 AU	1.70 %	unknown *
3	0.30 Rf	14.0 AU	0.32 Rf	30.8 AU	2.87 %	0.35 Rf	6.0 AU	656.6 AU	2.63 %	unknown *
4	0.43 Rf	8.0 AU	0.47 Rf	22.7 AU	2.12 %	0.52 Rf	3.9 AU	831.0 AU	3.33 %	unknown *
5	0.57 Rf	2.4 AU	0.60 Rf	12.6 AU	1.17 %	0.61 Rf	8.8 AU	245.6 AU	0.98 %	unknown *
6	0.61 Rf	9.0 AU	0.65 Rf	73.2 AU	6.84 %	0.72 Rf	5.5 AU	2013.1 AU	8.07 %	unknown *
7	0.77 Rf	3.1 AU	0.80 Rf	13.9 AU	1.30 %	0.83 Rf	0.0 AU	330.2 AU	1.32 %	unknown *
8	0.88 Rf	0.1 AU	0.91 Rf	28.5 AU	2.66 %	0.96 Rf	0.0 AU	678.4 AU	2.72 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Alkaloid	5	Saponin
2	Saponin	6	Terpenoid
3	Terpenoid	7	Terpenoid
4	Terpenoid	8	Terpenoid

The HPTLC fingerprinting patterns of the ethanol extract of *Saritaea magnifica* was developed at 366nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total eight peaks (Rf 0.14, 0.28, 0.35, 0.52, 0.61, 0.72, 0.83, 0.96) were observed in the chromatogram and the components at Rf 0.14 was present in significant level (Table 3.26; Figure 3.13).

Figure 3.15. HPTLC Three-dimensional densitogram of different solvent extracts (petroleum ether, chloroform and ethanol) of *Saritaea magnifica* at 550 nm

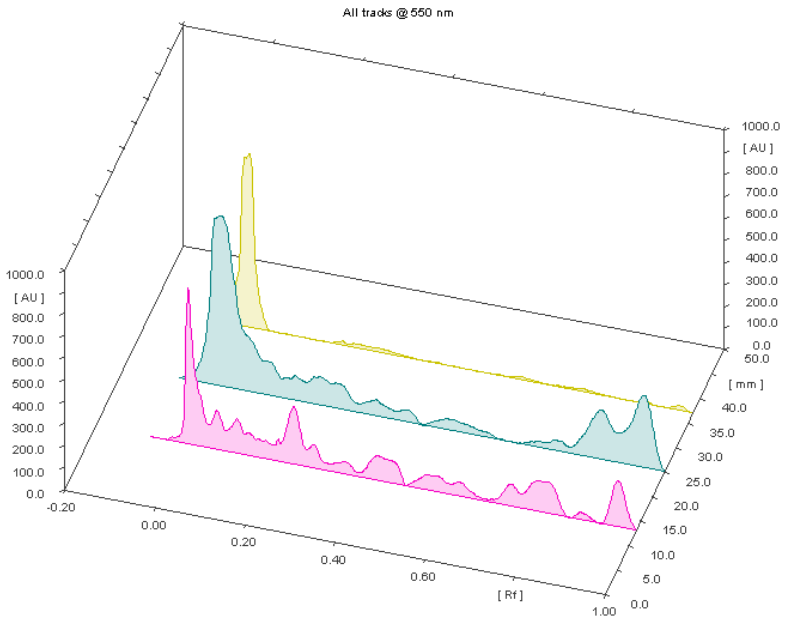


Figure 3.16: HPTLC image after derivatization observed at 550nm

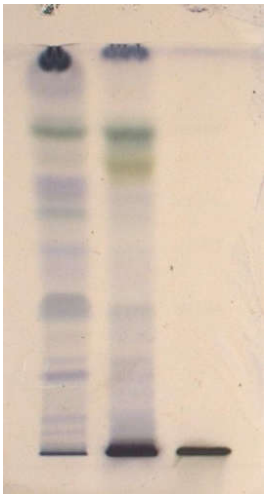


Table 3.27.Peak list and Rf values of the chromatogram of petroleum ether extract

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	17.9 AU	0.00 Rf	716.1 AU	30.63 %	0.05 Rf	34.8 AU	12613.0 AU	23.54 %	unknown *
2	0.05 Rf	106.4 AU	0.07 Rf	177.1 AU	7.58 %	0.09 Rf	34.2 AU	3158.0 AU	5.89 %	unknown *
3	0.09 Rf	94.6 AU	0.11 Rf	157.0 AU	6.72 %	0.13 Rf	34.8 AU	3440.9 AU	6.42 %	unknown *
4	0.13 Rf	95.8 AU	0.14 Rf	106.4 AU	4.55 %	0.17 Rf	76.8 AU	2258.7 AU	4.22 %	unknown *
5	0.17 Rf	77.2 AU	0.18 Rf	83.2 AU	3.56 %	0.19 Rf	39.9 AU	1151.8 AU	2.15 %	unknown *
6	0.21 Rf	81.8 AU	0.24 Rf	262.0 AU	11.21 %	0.27 Rf	76.3 AU	6430.2 AU	12.00 %	unknown *
7	0.27 Rf	77.0 AU	0.28 Rf	106.0 AU	4.54 %	0.31 Rf	33.8 AU	2075.1 AU	3.87 %	unknown *
8	0.32 Rf	33.1 AU	0.35 Rf	57.0 AU	2.44 %	0.38 Rf	19.2 AU	1768.3 AU	3.30 %	unknown *
9	0.38 Rf	19.6 AU	0.43 Rf	111.7 AU	4.78 %	0.49 Rf	2.0 AU	5414.9 AU	10.10 %	unknown *
10	0.49 Rf	3.3 AU	0.56 Rf	73.7 AU	3.15 %	0.58 Rf	48.1 AU	3137.2 AU	5.85 %	unknown *
11	0.59 Rf	47.7 AU	0.61 Rf	65.2 AU	2.79 %	0.66 Rf	10.9 AU	1882.7 AU	3.51 %	unknown *
12	0.67 Rf	11.1 AU	0.72 Rf	102.1 AU	4.37 %	0.74 Rf	34.3 AU	2710.3 AU	5.06 %	unknown *
13	0.75 Rf	64.3 AU	0.79 Rf	141.0 AU	6.03 %	0.80 Rf	38.9 AU	3742.9 AU	6.98 %	unknown *
14	0.80 Rf	139.2 AU	0.81 Rf	144.2 AU	6.17 %	0.85 Rf	0.1 AU	3051.4 AU	5.69 %	unknown *
15	0.85 Rf	0.3 AU	0.88 Rf	35.1 AU	1.50 %	0.91 Rf	2.7 AU	751.4 AU	1.40 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Saponin	9	Alkaloid
2	Alkaloid	10	Glycosides
3	Flavonoid	11	Terpenoid
4	Saponin	12	Glycosides
5	Tannin	13	Flavonoid
6	Terpenoid	14	Terpenoid
7	Terpenoid	15	Saponin
8	Tannin	-	-

The HPTLC fingerprinting patterns of the petroleum ether extract of *Saritaea magnifica* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total fifteen peaks (Rf 0.05, 0.09, 0.13, 0.17, 0.19, 0.27, 0.31, 0.38, 0.49, 0.58, 0.66, 0.74, 0.80, 0.85, 0.91) were observed in the chromatogram and the components at Rf 0.05 was present in significant level (Table 3.27; Figure 3.15).

Table 3. 28 Peak list and Rf values of the chromatogram of chloroform extract

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.07 Rf	3.6 AU	0.01 Rf	778.5 AU	43.90 %	0.11 Rf	52.4 AU	38924.7 AU	53.77 %	unknown *
2	0.11 Rf	152.4 AU	0.12 Rf	153.3 AU	8.64 %	0.15 Rf	35.6 AU	3113.6 AU	4.30 %	unknown *
3	0.15 Rf	86.2 AU	0.18 Rf	111.9 AU	6.31 %	0.20 Rf	39.7 AU	3444.6 AU	4.76 %	unknown *
4	0.20 Rf	90.0 AU	0.23 Rf	129.2 AU	7.29 %	0.26 Rf	36.7 AU	4276.6 AU	5.91 %	unknown *
5	0.26 Rf	106.9 AU	0.27 Rf	115.0 AU	6.48 %	0.32 Rf	40.0 AU	3306.5 AU	4.57 %	unknown *
6	0.32 Rf	40.1 AU	0.36 Rf	74.3 AU	4.19 %	0.40 Rf	38.9 AU	2867.3 AU	3.96 %	unknown *
7	0.41 Rf	39.2 AU	0.43 Rf	57.2 AU	3.23 %	0.46 Rf	0.1 AU	1463.5 AU	2.02 %	unknown *
8	0.47 Rf	1.1 AU	0.53 Rf	51.3 AU	2.89 %	0.63 Rf	5.3 AU	3406.5 AU	4.71 %	unknown *
9	0.68 Rf	4.5 AU	0.72 Rf	28.5 AU	1.61 %	0.73 Rf	27.0 AU	595.3 AU	0.82 %	unknown *
10	0.74 Rf	28.1 AU	0.77 Rf	49.7 AU	2.80 %	0.79 Rf	29.3 AU	1377.2 AU	1.90 %	unknown *
11	0.79 Rf	29.6 AU	0.86 Rf	224.5 AU	12.66 %	0.90 Rf	34.2 AU	9608.9 AU	13.27 %	unknown *

Peak	Assigned Compounds	Peak	Assigned Compounds
1	Flavonoid	7	Steroid
2	Flavonoid	8	Unknown
3	Terpenoid	9	Flavonoid
4	Terpenoid	10	Glycosides
5	Flavonoid	11	Saponin
6	Steroid	-	-

The HPTLC fingerprinting patterns of the chloroform extract of *Saritaea magnifica* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total eleven peaks (Rf 0.11, 0.15, 0.20, 0.26, 0.32, 0.40, 0.46, 0.63, 0.73, 0.79, 0.90) were observed in the chromatogram and the components at Rf 0.11 was present in significant level (Table 3.28; Figure 3.15).

Table 3.29. Peak list and Rf values of the chromatogram of ethanol extract

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.08 Rf	2.2 AU	0.02 Rf	796.1 AU	91.64 %	0.06 Rf	0.4 AU	20993.4 AU	93.44 %	unknown *
2	0.20 Rf	0.6 AU	0.22 Rf	20.5 AU	2.36 %	0.23 Rf	9.7 AU	292.3 AU	1.30 %	unknown *
3	0.59 Rf	5.8 AU	0.62 Rf	15.3 AU	1.77 %	0.66 Rf	6.2 AU	429.6 AU	1.91 %	unknown *
4	0.74 Rf	8.3 AU	0.76 Rf	14.7 AU	1.69 %	0.78 Rf	6.0 AU	346.9 AU	1.54 %	unknown *
5	0.94 Rf	0.1 AU	0.97 Rf	22.0 AU	2.53 %	0.99 Rf	0.3 AU	404.0 AU	1.80 %	unknown *

Peak	Assigned Compounds
1	Tannin
2	Saponin
3	Terpenoid
4	Terpenoid
5	Steroids

The HPTLC fingerprinting patterns of the ethanol extract of *Saritaea magnifica* was developed at 550nm. The binary solvent system, Toluene: Ethyl acetate (8:2 v/v) efficiently resolved the components present in the crude extract. In total, five peaks (Rf 0.06, 0.23, 0.66, 0.78, 0.99) were observed in the chromatogram and the components at Rf 0.06 was present in significant level (Table 3.29; Figure 3.15).

In *Saritaea magnifica*, the predominant compounds such as tannins, steroids, terpenoids and saponins where in the petroleum ether extract. In chloroform extract, saponins, steroids, alkaloids and flavonoids and in ethanolic extract, alkaloids and tannins were tentatively identified by comparison of Rf values with authentic published data (Yamunadevi et al. 2011; Gomathi et al. 2012).

Identification of chemical constituents by LC-Q-ToF-MS

The identification of compounds present in the ethanolic extracts of *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* with respect to their retention time (RT) are depicted in Table 3.30 to 3.32.

Most of the polar compounds responsible for their bioactivity are easily eluted by ethanolic solvents. Hence ethanolic extract was chosen for phytochemical profiling of leaf extracts using LC-Q-ToF-MS (Eloff 1998). The base peak chromatograms of the plant ethanolic extracts resulting from both positive mode and negative mode are included as Figure 3.19 to 3.21, where the peaks are numbered according to their elution order. The analysis provided data based on mass to charge (m/z) ratio, chemical formula and molecular mass of the analytes from the crude sample. Using the information provided by LC/Q-TOF/MS analysis, the set of known secondary metabolites were identified from the databases of Metlin and MassBank. The data from the LC/Q-TOF/MS are obtained in both positive and negative modes. Many peaks with corresponding mass, m/z ratio and chemical structure are utilized for the identification of present study.

LC-Q-ToF-MS of *Adenocalymma alliaceum*

The ethanolic extract of *Adenocalymma alliaceum* was subjected to LC-Q-ToF-MS analysis and the probable compounds were identified from data bases. The results are given in Table 3.30

Table 3.30 The compounds identified (tentatively) in the ethanolic extract of *Adenocalymma alliaceum* from available data base.

Peak	$T_{R(Min)}$	Ion Mode	M/Z	Molecular Formula	Tentatively Identified Compound	Class of Compounds
1	3.91	+	197.1177	$C_{10}H_{10}O_5$	Annularin I	Steroid
2	4.27	+	327..0503	$C_{21}H_{19}O_{11}$	Luteolin-6-C-Glucoside	Flavonoid
2a	4.27	+	395.1106	$C_{20}H_{19}F_3O_5$	(2s, 3's) -3, 3, 3 – Trifluoro – 2 –Methoxy – 2 - Phenyl Propionic Acid 3' – Hydroxy - 2' – Oxo – 4 '-Phenylbutyl Ester.	Flavonoid
2b	4.27	+	477.1020	$C_{15}H_{18}O_8$	P-Coumaroyl-O-Galloyl-Glucose	Tannin
2c	4.27	+	507.1126	$C_{23}H_{22}O_{13}$	Quercetin-3-O-(6"-O-Acetyl) -Beta-D-Glucopyranoside	Flavonoid
2d	4.27	+	287.0560	$C_{15}H_{11}O_6$	Cyanidin	Anthocyanin (glycoside)
2e	4.27	+	327.0511	$C_{21}H_{19}O_{11}$	Luteolin-6-C-Glucoside	Flavonoid
2f	4.27	+	463.0848	$C_{21}H_{20}O_{12}$	Quercetin-O-Glucoside	Flavonoid
2g	4.27	+	535.1442	$C_{26}H_{28}O_{14}$	6-C-B-L-Arabinopyranosyl-8-C-A-L-Arabinopyranosyl-Apigenin	Flavonoid
2h	4.27	+	639.1539	$C_{28}H_{32}O_{17}$	Isorhamnetin 3-Sophoroside	Flavonoid
3	5.54	+	331.0813	$C_{17}H_{14}O_7$	Tricin	Flavonoid
3a	5.54	+	181.1225	$C_{15}H_{22}O_3$	Hebelophyllene	Sesquiterpenes
3b	5.54	+	287.0555	$C_{15}H_{10}O_6$	Luteolin2-(3, 4-Dihydroxyphenyl) -5, 7-Dihydroxy-4-Chromenone Luteolin	Flavonoid
3c	5.54	+	317.0665	$C_{16}H_{13}O_7$	2, 5-Dimethoxy-8-Methyl1, 3, 6-Trihydroxy Xanthone	Triterpenoids
4	6.54	+	103.0754	$C_5H_{10}O_2$	3-Methylbutanoic Acid(Isovaleric Acid)	Fatty Acid

5	5.79	+	107.0490	$C_{10}H_{16}O_2$	2-Methyl-5-Isopropyl-1-Cyclopentene-1-Carboxylic Acid	Terpenoic Acid
5a	5.79	+	315.0869	$C_{17}H_{15}O_6$	Rosinidin	Flavonoid
6	7.18	+	257.1512	$C_{15}H_{22}O_2$	Dehydrobotrydienol	Terpenoids
7	7.79	+	149.0236	$C_7H_{14}O_6$	O-Methyl Pongaglabol	Terpenoids
7a	7.79	+	301.1416	$C_{15}H_{20}$	Sinularianin	Sesquiterpenes
8	7.36	+	149.0237	$C_{17}H_{26}O_3$	(6) -Paradol	Flavonoid
8a	7.36	+	337.2346	$C_{20}H_{34}O$	Geranyllinalool	Diterpenoids
8b	7.36	+	413.2668	$C_{16}H_{18}O_5$	Aculeatin	Alkaloid
9	8.02	+	400.3418	$C_{23}H_{45}NO_4$	Palmitoylcarnitine	Fatty Acid
10	3.91	-	283.0268	$C_{17}H_{14}O_6$	Dihydroxyl-Dimethoxylflavone-Sulfate	Flavonoid
10a	3.91	-	387.1286	$C_{23}H_{34}O_{15}$	Genipin 1-Gentiobioside	Precursor Of Glycosides
10b	3.91	-	563.1392	C26 H28 O14	Apigenin-Chexosidec-Pentoside	Flavonoid
9	4.27	-	475.0869	$C_{22}H_{19}O_{12}$	2-C-Glucopyranoside Of Flavokermesic Acid (DCII)	Anthroquinone Flavonoid
10	4.57	-	505.0965	$C_{23}H_{21}O_{13}$	Delphinidin-3- Acetylglucosid	Flavonoid
11	4.75	-	533.1289	$C_{25}H_{24}O_{13}$	Malonyl Glycitin	Isoflavone Glucosides

Based on the LC/Q-TOF/MS profiling and subsequent database searches, thirty compounds were identified as significant in *Adenocalymma alliaceum* (Table. 3.30). As seen in Figure 3.19, about thirty components were detected from the total ion chromatogram obtained from the extract of *Adenocalymma alliaceum*. The peak with m/z values of RT was tentatively identified as Annularin I; Luteolin-6-C-glucoside; (2s, 3s) -3, 3, 3-Trifluoro-2-methoxy-2-phenyl propionic acid 3'-hydroxy-2'- oxo - 4'-phenyl butyl ester; P - coumaroyl - O - galloyl-glucose;9-Kaempferol-7 - methoxy - 3 - O - β- D - glucopyranosyl-3'-oic acid; Cyanidin 2 - (3, 4 - Dihydroxyphenyl) chromenylium -3, 5, 7 triol;Luteolin-6-C-glucoside; Qercetin - O-glucoside; 6-C-β-L-Arabinopyranosyl-8 - C-α – L-arabinopyranosyl – apigenin; Isorhamnetin 3-Sophoroside; Tricin; Hebelophyllene; Luteolin 2-(3, 4-Dihydroxyphenyl) -5, 7-dihydroxy – 4-chromenone Luteolin; 2, 5-Dimethoxy-8-methyl1, 3, 6-trihydroxy xanthone; 3-Methylbutanoic acid (isovaleric acid) ; 2-methyl-5-Isopropyl-1-cyclopentene-1-carboxylic acid; Rosinidin; Dehydrobotrydienol; O-methyl pongaglabol; Sinularianin; (6) - Paradol; Geranyllinalool; Aculeatin; Palmitoylcarnitine; Dihydroxyl-dimethoxylflavone-sulfate; Genipin 1-gentiobioside; Apigenin – Chexoside C – pentoside; 2-C-glucopyranoside of flavokermesic acid (DCII) ; Malonyl glycitin and delphinidin-3- acetyl glucoside.

LC-Q-ToF-MS of *Pimenta dioica*

Similar to *Adenocalymma alliaceum*, the ethanolic extract of *Pimenta dioica* were subjected to LC-Q-ToF-MS analysis and the probable compounds were identified from data bases. The results are given in Table 3.31.

Table 3.31The compounds identified (tentatively) in the ethanolic extract of *Pimenta dioica* from available data base.

Peak	$t_{R(min)}$	Ion mode	m/z	Molecular formula	Tentatively identified Compound	Class of compounds
1	2.11	+	181.0489	$C_{18}H_{16}O_8$	Rosmarinic acid	Poly phenol
2	2.69	+	801.1631	*	*	*
3	2.96	+	291.0858	$C_{15}H_{14}O_6$	Epicatechin	Flavonoids
3a	2.96	+	563.1531	$C_{15}H_{10}O_5$	Genistein	Flavonoids
4	3.60	+	95.0848	$C_{16}H_{28}O_2$	Hexadecadienoic acid	Fattyacids
5	3.60	+	161.1312	$C_{30}H_{50}O_4$	Bryodulcosigenin	Triterpenoids
6	4.06	+	164.131	$C_{30}H_{46}O_3$	Micromeric acid	Triterpenoids
7	4.22	+	319.0437	$C_{15}H_{10}O_8$	Myricetin Pentosyl hexoside	Polyphenols
7a	4.22	+	465.1002	$C_{27}H_{30}O_{16}$	Rutin	Flavonoid
8	4.54	+	784.0481	*	*	*
8a	4.54	+	153.0173	$C_{15}H_{10}O_6$	Luteolin	Flavonoid
8b	4.54	+	629.1295	$C_{33}H_{25}O_{13}$	Acremoxanthone D	Anthraquinone
9	5.02	+	303.0499	$C_{15}H_{10}O_7$	Quercetin	Flavonoid
10	6.22	+	119.0478	$C_{21}H_{18}O_{12}$	Luteolin-7-o-glucuronide	Flavonoid
10a	6.22	+	147.0430	$C_{15}H_{12}O_5$	Naringenin	Flavonoid
11	6.83	+	625.4543	*	*	*
12	7.85	+	245.8783	*	*	*

13	8.12	+	874.1332	*	*	*
14	2.37	-	555.0927	$C_{30}H_{20}O_{11}$	(2r, 3s) -morelloflavone	Flavonoid
15	2.70	-	459.0942	*	*	*
16	2.97	-	289.0716	$C_{30}H_{26}O_{12}$	Procyanidin B_2	Phenol
17	3.35	-	577.1356	$C_{30}H_{26}O_{12}$	Procyanidin B_2	Phenol
17a	3.35	-	305.0698	$C_{15}H_{14}O_7$	Gallocatechin	Flavonoid
16	3.53	-	407.1225	*	*	*
17	4.27	-	463.0887	$C_{27}H_{30}O_{16}$	Quercetin 3-glucoside-7-rhamnoside	Flavonoid
18	4.47	-	300.9986	$C_{41}H_{28}O_{26}$	Galloy-bis-HHDP-β-D-glucopyranose	Tannin
18a	4.47	-	493.1361	$C_{23}H_{25}ClO_{12}$	Malvidin 3-galactoside	Anthocyanin (glycoside)
18b	4.47	-	591.1136	$C_{30}H_{20}O_{11}$	Ephedrannin	Polyphenols
18c	4.47	-	591.1136	$C_{30}H_{24}O_{13}$	Ephedrannin D2	Flavonoid
19	4.66	-	408.9871	$C_{21}H_{13}BrO_2S$	3-(2-Bromophenyl) -4-(Phenylthio) -1H-isochromen-1-one	Coumarins
19a	4.66	-	435.1307	$C_{33}H_{42}O_{19}$	Troxerutin or Eriodictyol-Neo-Rha	Flavonoid
20	4.81	-	267.0908	$C_7H_6O_3$	4-hydroxybenzoic acid	Phenol
21	5.06	-	301.0352	$C_{15}H_{10}O_7$	Quercetinii	Flavonoid
21a	5.06	-	489.1047	$C_{24}H_{22}O_{14}$	Luteolin 7-O- β-D-(6-O-	Flavonoid

					acetate) -glucopyranoside	
22	5.88	-	313.0719	$C_{21}H_{20}O_{10}$	Isovitexin	Flavonoid
23	6.26	-	119.0494	$C_{16}H_{18}O_8$	4-p-Coumaroylquinic acid	Phenol
23a	6.26	-	163.0393	$C_9H_8O_3$	P-Coumaric acid	Phenol
24	6.83	-	325.1452	$C_6–C_2–C_6$	Stilbenoids	Phenol
24a	6.83	-	487.3440	$C_{30}H_{46}O_5$	Cordialin A	Triterpenes
25	7.20	-	233.1541	$C_{15}H_{22}O$	Zerumbone	Sesquiterpene
26	7.82	-	311.1692	$C_{16}H_{24}NO_5$	Sinapine	Alkaloid
27	8.18	-	534.0152	*	*	*

* Not available in compound form

Based on the LC/Q-TOF/MS profiling and resultant searches on data bases thirty four compounds were identified as significant compounds in *Pimenta dioica*. As seen in Figure 3.20, thirty four components were detected from the total ion chromatogram obtained from the extract of *Pimenta dioica*. The peak with m/z values of RT was tentatively identified as Rosmarinic acid; Epicatechin; Genistein; Hexadecadienoic acid; Bryodulcosigenin; Micromeric acid; Myricetin Pentosyl hexoside; Rutin; Luteolin; Acremoxanthone D; Quercetin; Luteolin-7-o-glucuronide; Naringenin; (2R, 3S) -Morelloflavone; Procyanidin B_2; Procyanidin B_2; Gallocatechin; Quercetin 3-glucoside-7-rhamnoside; Galloy-bis-HHDP-β-D-glucopyranose; Malvidin 3 - galactoside; Ephedrannin; 3,5,7,3,4 -Pentshydroxy flavonone -3-O- α - L - rhamnoyranosyl-7-O-β-D-glucopyranosyl-(1-3)-O-β -D –xylopyrenoside; 3-(2-Bromophenyl) -4 - (Phenylthio) - 1H -isochromen - 1- one; Eriodictyol - Neo - Rha; 4-hydroxybenzoic acid; Quercetin II; Luteolin 7- O - β - D - (6 - O - acetate) - glucopyranoside; Isovitexin; 4 - p -Coumaroylquinic acid ; *p* - Coumaric acid; Stilbenoids; Cordialin A; zerumbone; Sinapine.

LC-Q-ToF-MS of *Saritaea magnifica*

Similar to *Adenocalymma alliaceum* and *Pimenta dioica,* the ethanolic extract of *Saritaea magnifica* were subjected to LC-Q-ToF-MS analysis and the probable compounds were identified from data bases. The results are given in Table 3.32.

Table 3.32.The compounds identified (tentatively) in the ethanolic extract of *Saritaea magnifica* from available data base.

Peak	$t_{R(min)}$	Ion mode	m/z	Molecular formula	Tentatively identified Compound	Class of compounds
1	1.64	-ve	191.0566	$C_{14}H_{16}O_{10}$	Galloyl quinic acid	tannin
2	2.24	-ve	181.0512	$C_{36}H_{56}N_2O_5$	Buxakashmiramine	Alkalloids
3	2.57	-ve	347.1374	*	*	*
4	2.79	-ve	393.1433	*	*	*
5	3.47	-ve	609.1512	$C_{27}H_{31}O_{16}$	Cyanidin-3-sophoroside	Glycoside
6	3.70	-ve	593.1569	$C_{27}H_{30}O_{15}$	Kaempferol4-glucoside7-rhamnoside	Flavonoids
7	4.15	-ve	509.1649	*	*	*
8	4.37	-ve	623.1957	$C_{34}H_{44}O_{19}$	Forsythoside A	Phenylethanoid Glycosides
9	4.62	-ve	129.0548	*	*	*
10	5.30	-ve	775.1616	*	*	*
11	6.91	-ve	469.3314	$C_{30}H_{45}O_4$	Cleistocalyxic acid A	Triterpenoids
12	7.54	-ve	255.2318	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid
13	8.05	-ve	983.4594	*	*	*
14	0.54	+ve	611.1581	$C_{27}H_{31}O_{16}^+$	Cyanidin-3-sophoroside	Anthocyanin (glycoside)
15	2.24	+ve	137.0596	$C_{10}H_{12}O_2$	Eugenol	Phenol
15a	2.24	+ve	183.0654	$C_9H_{10}O_4$	Syringaldehyde	Phenol
15b	2.24	+ve	461.1024	$C_{22}H_{22}O_{11}$	Diosmetin-7-O-β-D-glucopyranoside	Flavonoid
16	3.44	+ve	611.1616	$C_{15}H_{10}O_6$	Fisetin	Flavonoid

17	4.01	+ve	163.0393	$C_{15}H_{18}O_9$	Caffeoyl-glucose	Phenol
17a	4.01	+ve	463.1233	$C_{16}H_{13}O_6$	*Peonidin*	Anthocyanin (glycoside)
18	4.59	+ve	825.8291	*	*	*
19	5.06	+ve	681.2675	$C_{32}H_{44}O_9$	8, 12-o-diacelyl ingol 3, 7-dibenoate	Triterpenoids
20	5.28	+ve	173.3247	$C_{10}H_8O_3$	4-methoxycoumarin	Coumarins
20a	5.28	+ve	569.2520	$C_{14}H_{12}O_3$	Resveratrol (3, 5, 4′-trihydroxy-*trans*-stilbene)	Phenol
21	6.92	+ve	997.3548	*	*	*
22	7.76	+ve	413.2666	$C_{24}H_{38}O_4$	Anthroquinonol	Resin
22a	7.76	+ve	639.2412	$C_{21}H_{18}NO_4$	Toddalin C	Alkaloid

*Not available in compound form

In the case of *Saritaea magnifica*, based on the LC/Q-TOF/MS profile and on references to databases nineteen compounds were identified as significant. As seen in Figure 3.21, nineteen components were detected from the total ion chromatogram obtained from the extract of *Saritaea magnifica.* The peak with m/z values of RT was tentatively identified as Galloyl quinic acid; Buxakashmiramine; Cyanidin-3-sophoroside; Kaempfero l4- glucoside 7- rhamnoside; Dimethyl 1- Benzyl - 2-(4-methoxybenzoyl) -7- methyl – 2 – oxo - 2, 9 a - dihydrospiro[indoline3, 1-quinolizine]-3, 4-dicarboxylate; Cleistocalyxic acid A; Palmitic acid; Cyanidin-3-sophoroside; Glucuro-nidated 3, 4- or 3, 5-Dihydroxyphenylpropionic acid; DHPPA-glucuronide; Syringaldehyde; Diosmetin-7-O-β-D-glucopyranoside; Fisetin; Caffeoyl-glucose; *Peonidin,* 8, 12-o-diacelyl ingol 3, 7-dibenoate; 4-Methoxycoumarin; Resveratrol (3, 5, 4′-trihydroxy-*trans*-stilbene) ; Anthroquinonol; Toddalin C.

The phytochemical profile of the ethanolic extract was obtained on LC/Q-TOF/MS analysis followed by integrated library search. 30 constituents in *Adenocalymma alliaceum*; 34 constituents in *Pimenta dioica* and 19 constituents in *Saritaea magnifica* were identified. In *Adenocalymma alliaceum*, thirty compounds characterized can be classified into different groups such as steroid, flavonoid, tannin, anthocyanins (glycoside), fatty acid, terpenoids and alkaloid. In *Pimenta dioica*, 34 compounds characterized can be classified into different groups such as phenol, anthraquinone, coumarin, flavonoid, anthocyanins (glycoside), fatty Acid, terpenoids and alkaloid and in *Saritaea magnifica*, 19 compounds characterized may be classified into different groups such as phenol, glycolipid, flavonoid, tannin, anthocyanins (glycoside), fatty Acid, terpenoids, alkaloid and resins.

Today, the environmental safety of an insecticide is considered to be of paramount importance. Phytochemicals may serve as suitable alternatives to

synthetic insecticides in future as they are relatively safe, inexpensive and are readily available throughout of the world (Aarthi and Murugan 2010).

Certain plants are extensively reported to possess toxic effects on mosquitoes and can be utilized as a potent source of mosquito control. Earlier, few plants such as *Annona squamosa* L., *Gloriosa superba* L., *Millingtonia hortensis, Abuta grandifolia*, *Minthostachys setosa*, *Azadirachta indica*, *Ocimum gratissimum and Hyptis suaveolens* have been reported to control mosquito populations (Ciccia 2000; Chansang et al.2005; Mullai and Jebanesan 2007; Rahuman et al. 2009; Okigbo et al.2010).

In the present study, an attempt has been sought to categorize phytochemicals in the leaf extracts (petroleum ether, chloroform and ethanol) of *Adenocalymma alliaceum*, *Pimenta dioica* and *Saritaea magnifica* which can be used for mosquito control. The study observed a functional response by larval life stages of the three mosquitoes on the leaf extracts of these plant species. This biological activity was attributed to the presence of compounds in leaves, including alkaloids, phenols, terpenoids, carbohydrates, saponins, tannins, flavonoids, glycosides and steroids which together or independently produce morbidity and mortality effects in mosquitoes. The present results are in accordance with the observations of Dishani and Dhivya (2017) ; Tariwari et al. (2017) ; El-Akhal (2016) ; Hemalatha et al. (2015) ; Rajeswary and Govindarajan (2013) Kumar (2012); Chowdhury (2008) where preliminary phytochemical profiling and ovicidal potential of leaf extracts against mosquito vectors were found to be effective. The mosquito larvicidal properties of the foregoing secondary compounds, particularly saponin, alkaloids, tannins, steroids, glycosides, flavonoids and terpenoids are reported in almost all studies. Although, the actual modes of action of all ingredients were not fully elaborated, it is most likely that these chemicals interfere mainly with certain biological, ecological and physiological aspects of the

insect larvae. The lethal effects observed on mosquito larvae could be attributed to the secondary metabolites present in plants.

The chemical nature of compounds in the active fraction will contribute to their specific activity. It can be assumed that the activity of *Adenocalymma alliaceum*is due to flavonoids and terpenoids; *Pimenta dioica* due to phenol and flavonoids and *Saritaea magnifica* due to flavonoids, alkaloids, anthocyanins and terpenoids.Secondary compounds, particularly saponins, alkaloids, tannins, steroids and terpenoids are reported to attribute mosquito larvicidal properties. In addition to this, compounds like proanthocyanidins (Muema et al. 2017) ; cinnamaldehyde (Cheng et al. 2004) ; elatol- halogenated sesquiterpene (Bianco et al. 2013) ; saponin (Chapagain et al. (2008) ; limonoids-triterpenoids (Yuan et al. 2010); tectoquinone-glycosides (Cheng et al. 2009) ; alkaloids (Sung-Eun Lee 2000) are reported to have established mosquitocidal activity against medically important species.

Though larvicides play a vital role in controlling mosquitoes in their breeding sites, these could also be detrimental to the beneficial and non-target organisms due to their indiscriminate action. Hence new alternative insecticides need to be evaluated against non-target organisms which share habitats with mosquito larvae. So the plant extract employed for larvicidal activity should be ecologically safeto be used in the field condition. Moreover, extensive research needs to be carried out for bringing more selective and biodegradable mosquitocidal compounds.

Summary and Conclusion

The present findings reveal the fact that the leaf extracts of *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* contain phytochemicals of varying group responsible for mosquitocidal effect.

Chromatographic methods were attempted for the isolation of active components from the ethanolic extracts of the three plant species. The extracts were subjected to qualitative tests to get an assumption on the active ingredients responsible for larval / adult mortality. The phytochemicals such as alkaloids, phenols, terpenoids, carbohydrates, saponins, tannins, flavonoids, glycosides and steroids present in each extract were confirmed with HPTLC analysis. The compounds were tentatively identified by comparison of Rf values with authentic samples, published data and data from computer library. The peak with m/z values of RT was tentatively identified as thirty, thirty four and nineteen compounds in *Adenocalymma alliaceum*, *Pimenta dioica* and *Saritaea magnifica* respectively by LC/Q-TOF/MS profiling. In the present study, the activity of *Adenocalymma alliaceum* is assumed to be due be to flavonoids and terpenoids; *Pimenta dioica* due to phenol and flavonoids and in *Saritaea magnifica* due to flavonoids, alkaloids and terpenoids.

GENERAL SUMMARY AND CONCLUSION

Mosquitoes are principal vectors of many vector borne diseases affecting various organisms including human beings. Several mosquito species belonging to the genera Anopheles, Culex, Aedes and Armigeres are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue fever, yellow fever etc. Repeated use of synthetic insecticides for mosquito control has resulted in the development of resistance, disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures.

One of the approaches for the control of these mosquito borne diseases is the interruption of disease transmission, either by preventing mosquitoes from biting human beings (using repellents) or by effecting larval mortality in a large scale at the breeding centers of these vectors using chemicals which are not having other environmental consequences. Plants are rich sources of bioactive compounds and hence can be an alternative source of mosquito control agents. Phytochemicals associated with plants can either be used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity possessed.

In light of the above, the present study entitled “Studies on the effect of plant secondary metabolites in the control of mosquito vectors” revealed that plant extracts could be effectively utilised for the control of mosquitoes. The elucidation of the findings of the present study has also been carried out in three chapters as given below:

- **Chapter I. Laboratory trials on the rearing of mosquitoes and standardization of growth conditions.**

Mosquito larvae, collected from natural habitats were pooled in the laboratory and subjected to species level identification using standard manual. The screened larvae were reared to adults in the laboratory under controlled conditions. From these adults, the first generation larvae were produced at varying temperature and humidity. The larvae in the growth medium were fed with oats and yeast in the ratio 3:1. The number of eggs, larvae and hatch out percentages were estimated for each species of mosquito reared at a varying condition of temperature and humidity.

The result of the present study revealed that a temperature ranging from 27.19 to 27.54 ^{O}C and humidity ranging from 74.10 to 75.21% were ideal for oviposition and larval emergence in *Aedes albopictus*. Likewise a range of temperature from 27.03 to 27.05 ^{O}C and humidity from 71.65-72.74% was ideal for the growth of *Culex sitiens*. In case of *Armigeres subalbatus* a temperature of 27.59 ^{O}C and humidity of 74.51% was noted to be ideal for egg and larval production. Also the number of hatch out of larvae was correlated positively with humidity and negatively with temperature.

The condition standardized for higher production of egg and larvae were maintained through rearing studies for the production of larvae for larvicidal bioassay.

- **Chapter II. Screening for larvicidal activity of plant extracts on mosquito vectors**

The primary aim of this work was to formulate ways and means of controlling mosquito larvae using aqueous extracts of plant origin. One hundred and twenty plant species belonging to 42 varied families were screened for this purpose. Aqueous extracts of selected plants / parts were

prepared (0.5, 1.0, 2.0, 4.0 and 8.0 ml) and tested against mosquito larvae for a period of 96hours. Mortality percentages and LC_{50} were calculated as per WHO protocols and standards. Attempts were also carried out to assess the lethality of plant materials on specific mosquito species like *Aedes albopictus, Culex sitiens* and *Armigeres subalbatus*.

The present study confirmed the larvicidal efficacy of 77 plants from an array of 120 plants screened. Of seventy seven, thirty one plants were proved to be 100% efficient in exhibiting larvicidal properties. Considering literature, the present investigation provided three additional botanical agents which are having larvicidal efficiency. These three promising plants have the potential, to be included in the formulations of new and safe control products, especially in the control of mosquito species with medical importance (Aedes, Culex and Armigeres). As these plant species have a cosmopolitan distribution, they can help minimize the dependence on expensive synthetic pesticides, generate local employment and stimulate local efforts to enhance public health.

It can be concluded that these three plants can be treated as source of phytochemicals for the control of mosquito larvae belonging to the varied taxonomic groups. The forthcoming chapter is intended to discuss in detail the reasons behind the lethal effect of these three plant extracts on the survival of mosquito larvae belonging to Aedes, Culex and Armigeres.

- **Chapter III. Isolation and characterization of phytochemicals having insecticidal properties**

The present findings reveal the fact that the leaf extracts of *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* contain phytochemicals of varying group responsible for mosquitocidal effect. Chromatographic methods were attempted for the isolation of active

components from the ethanolic extracts of the three plant species. The extracts were subjected to qualitative tests to get an assumption on the active ingredients responsible for larval / adult mortality. The phytochemicals such as alkaloids, phenols, terpenoids, carbohydrates, saponins, tannins, flavonoids, glycosides and steroids present in each extract were confirmed with HPTLC analysis. The compounds were tentatively identified by comparison of Rf values with authentic samples, published data and data from computer library. The peak with m/z values of RT was tentatively identified as thirty, thirty four and nineteen compounds in *Adenocalymma alliaceum*, *Pimenta dioica* and *Saritaea magnifica* respectively by LC/Q-TOF/MS profile. In the present study the activity of *Adenocalymma alliaceum* is assumed due be to flavonoids and terpenoids; *Pimenta dioica* due to phenol and flavonoids and in *Saritaea magnifica* due to flavonoids, alkaloids and terpenoids.

In general concise the present study confirms the larvicidal efficacy of 7 plants (*Allium sativum* L., *Ricinus communis* L., *Zingiber officinale* Roscoe., *Citrus reticulata* Blanco., *Pimenta dioica*(L.) Merr., *Adenocalymma alliaceum* (Lam.) Miers. and *Saritaea magnifica* (W. Bull) Dugand.) from an array of 120 plants screened. Detailed studies were attempted on *Adenocalymma alliaceum, Pimenta dioica* and *Saritaea magnifica* as their efficiencies in controlling mosquito vectors have not been reported yet. Apart from larvicidal properties, all the three plants were noted to be effective in repelling mosquito vectors in smoke toxicity assay also, the results of which are not included in the thesis. Characterization of the compounds responsible for larvicidal efficacy with respect to *Adenocalymma alliaceum* is noted to be due to flavonoids and terpenoids; in *Pimenta dioica* due to phenols and flavonoids and in *Saritaea magnifica* due to flavonoids, alkaloids and terpenoids.

REFERENCES

Aarthi, N., and Murugan, K. (2010). Larvicidal and smoke repellent activities of *Spathodea campanulata* P.Beauv. against the malarial vector *Anopheles stephensi* Lis (Diptera: Culicidae). J Phytol. 2, 61-69.

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18, 265-267.

Abdul F, A., Venkatesan, P., and Gopalakrishnan, G. (2008). Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. Parasitology Research, 103, 1383-1390.

Abu Bakar, A., Sulaiman, S., Omar, B. and Ali, R.M. (2009). Evaluation of *Melaleuca cajuputi* Powell (Family: Myrtaceae) extract in aerosol can against dengue vectors in the laboratory. J. Trop. Med. Parasitol. 32, 58-64.

Adetuyi, A.O., and Popoola, A.V. (2001). Extraction and dyes ability potential studies of the colourant in *Zanthoxylum zanthoxyloides* plant on cotton fabric. Journal of Science Engineering Technology, 8 (2), 3291-3299.

Afolabi, O.J., Simon-Oke, L.A., and Omosalewa, O.B. (2013). Distribution, abundance and diversity of mosquitoes in Akure, Ondo Sae, Nigeria. J Parasitol Vector Biol, 5(10), 132-136.

Ahmed, B., Mamy, O., Baba, M.O., Barry, Y., Isselmou, K., Dia, M.L., Hampate, B., Diallo, M.Y., Kory, M.O.B., Diop, M., Lo, M.M., Thiongane, Y., Bengoumi, M., Puech, L., Ludovic, P.L., Claes, F., Rocque, S. and Doumbia, B. (2011). Unexpected Rift Valley fever outbreak, Northern Mauritania. Emerging Infectious Diseases, 17, 1894–1896.

Ajaegbu, E. E., Danga, S. P. Y., Ikemefuna Uzochukwu Chijoke, I. U. and Okoye, F. B.C. (2016).Mosquito adulticidal activity of the leaf extracts of *Spondias mombin* L. against *Aedes aegypti* L. and isolation of active principles. J Vector Borne Dis, 53, 17–22.

Akhila, S and Vijayalakshmi.N.G. (2015). Phytochemical studies on *Carica papaya* leaf juice. International Journal of Pharmaceutical Sciences and Research, 6(2), 880-883.

Akinmoladun, A.C, Ibukun, E.O, Afor, E., Obuotor, E.M., and Farombi, E.O. (2007). Pytochemical constituents and antioxidant activity of extract from the leaves of the *Ocimum grattisimum*. Sci. Res. Essay., 2, 163-166.

Akono, N.P., Jazet, D.P.M., Tonga, C., Kouotou, S., Kekeunou, S., and Lehman, L.G. (2015). Larvicidal activity of essential oils from pericarps of ripe fruits cultivated in Cameroon on pyrethroids sensitive and resistant strains of *An. gambiae* Giles. Journal of Entomology and Zoology Studies, 3(4), 334-339.

Akov, S. (1962). J. Insect Physiol. 8, 319.

Akram, W., Khan, H.A.A., Hafeez, F., Bilal, H., Kim., Y.K., and Lee, J.J. (2010). Potential of citrus seed extracts against dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). Pak J Bot., 42, 3343-3348.

Aktar, W., Sengupta, D., Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. Interdisc Toxicol, 2, 1–12.

Albert, Sasson. (2005). Medical Biotechnology: Achievements, Prospects and Perceptions, United Nations University press, Tokyo.

Alfonso-Parra, C., Ahmed-Braimah, Y.H, Degner, E.C, Avila, F.W., Villarreal, S.M., and Pleiss, J.A. (2016). Mating-Induced Transcriptome Changes in the Reproductive Tract of Female *Aedes aegypti*. Neglected Tropical Diseases, 10(2), 4451

Alto, B. W. and S. A. Juliano. (2001). Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae) : implications for range expansion. Journal of Medical Entomology, 38, 646-656.

Amala, S., Rajendrabhoopathy, S., Arunachalam, N., and Anuradha, V. (2011). A study on diversity of mosquitoes in Rajathanikottai village, Dindigul District, Tamil Nadu, India. Annals of Biological Research, 2(6), 496–499.

Amraoui, F., Krida, G., and Bouattour, A. (2012). *Culex pipiens*, an experimental efficient vector of West Nile and Rift Valley fever viruses in the Maghreb region. PLoS One, 7, e36757.

Ananthakrishnan, T. N. (1990). Chemical Ecology in Biological Control. Current science, 59 (24), 1319 – 1322.

Anjali Rawani., Anupam Ghosh., Subrata Laskar., and Goutam Chandra.(2009). Aliphatic Amide from Seeds of *Carica papaya* as Mosquito Larvicide, Pupicide, Adulticide, Repellent and Smoke Toxicant. Journal of Mosquito Research, 2, (2), 8-18.

Annapoorani, C.A. and Manimegalai, K. (2014). In silico molecular docking studies for identification of larvicidal compounds in *Momordica charantia* targeting the odorant binding protein of *Culex quinquefasciatus*. International Journal of Recent Scientific Research, 5(4), 764-766.

Ansari, M. A. (1993). Domestic Mosquito Breeding Places and Their Management. Bull.Env.Sci., XI, 56 – 62.

Anuskha Dishani, U. and Dhivya, R. (2017). Preliminary phytochemical profiling and ovicidal potential of *Carica papaya* leaf extracts against the filarial vector *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Mosquito Research, 4(3), 01-08.

Araujo, M., Gil, L.H, and E-Silva, A. (2012). Larval food quantity affects development time, survival and adult biological traits that influence the vectorial capacity of *Anopheles darlingi* under laboratory conditions. Malar J., 11, 261.

Arivoli, S., Tennyson, S and Martin, J.J. (2011). Larvicidal efficacy of *Vernonia cinerea* (L.) (Asteraceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Biopesticides, 4(1), 37-42.

Arnason, J. T., T. Swain, C. K. Wat, E. A. Graham, S. Partington, J. Lam and G. H. N. Towers. (1981). Mosquito larvicides from polyacetylenes occurring naturally in asteraceae. Biochem. Syst. Ecol. 9, 63- 68.

Arumugam Durga, Syed Mohammed Imthiyaz Begum, Berchmans Scholastica Mary Vithiya, and Rathinasamy Regina Mary. (2014). Larvicidal activity of *Wedelia chinensis* (Asteraceae) plant extracts against *Aedes aegypti* and *Culex quinquefasciatus*. 4, (7). 173-175.

Arunachalam, N., Mariappan, T. and Somachary, N. (1992). Seasonal Abundance of *Culex sitiens* Wiedemann 1828, in Vypeen Island Situation, Kochi, Kerala. Proceedings of the Fourth Kerala Science Congress, 290.

Avila, Varshini. R., and Kanagappan, M. (2015). Mosquito Larval Diversity in Three Rural Areas of Kanyakumari District, Tamil Nadu. International Journal of Mosquito Research, 2(3), 10-13.

Ayres, C.F.J., Romao, T.P.A., Melo-Santos, M.A.V., and Furtado. A.F. (2002). Genetic Diversity in Brazilian Populations of *Aedes albopictus*. Mem Inst Oswaldo Cruz, Rio de Janeiro, 97(6), 871-875.

Azokou, A., Kone, M.W, Koudou, B.G., and Tra Bi, H.F. (2013). Larvicidal potential of some plants from West Africa against *Culex quinquefasciatus* (Say) and *Anopheles gambiae* Giles (Diptera: Culicidae). Journal of Vector Borne Diseases, 50(2), 103–110.

Bagavan, A., Rahuman, A.A (2011). Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. Asian Pac J Trop Med., 4, 29-34.

Balandrin, M.F. (1985). Natural Plant Chemicals: Sources of Industrial and Medicinal Materials. Science, 228, 1154-1160.

Baranitharan, M., and Dhanasekaran, S. (2014). Mosquitocidal efficacies of medicinal plant of *Coleus aromaticus* Benth (Lamiaceae) leaf extracts against Chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). Int. J. Curr.Res. Chem. Pharma. Sci., 1, 61-67.

Barraud, P.J. (1934).The Fauna of British India, including Ceylon and Burma. Diptera. Vol. V. Family Culicidae. Tribes Megarhini and Culicini. Taylor and Francis, London, 1934.

Becker, N. (2010). Mosquitoes and Their Control. 9, Springer-Verlag Berlin Heidelberg .

Benjamin, M., and Pandian, S. R. (1997). Larvicidal and pupicidal action of selected synthetic pesticides and phytochemical pesticide against the Filarial vector mosquito *Culex quinquefasciatus* Say. J. of Envt. and Pollution, 4 (4), 285- 289.

Bhattacharya, K., Chandra, G. and Phago. (2014). Larvicidal and oviposition deterrence activity of *Tragia involucrata* L. (Euphorbiaceae) root extractives against vector of lymphatic filariasis *Culex quinquefasciatus* (Diptera: Culicidae). Asian Pac J of Trop Dis., 4 (Suppl 1), 226 - 232.

Bianco, E.M., Pires, L., Santos, G.K.N., Dutra, K., Reis, T.N.V., Vasconcelos, E.R.T.P.P., Cocentino, A.L.M., and Navaro, D.M.A.F. (2013). Larvicidal activity of seaweeds from North - Eastern Brazil and of a halogenated sesquiterpene against the dengue mosquito (*Aedes aegypti*). Ind. Crop. Prod. 43, 270–275.

Bishnu, P. Chapagain., Vinod, Saharan., and Zeev Wiesman. (2008). Larvicidal activity of saponins from *Balanites aegyptiaca* callus against *Aedes aegypti* mosquito. Bioresource Technology, 99, 1165–1168.

Bor, N. L., and Raizada, M. B. (1990). Some Beautiful Indian Climbers And Shrubs. Revised Second Edition, Bombay Natural History Society, Oxford University Press.

Borah, R., Kalita, M.C., Kar, A., and Talukdar, A.K. (2010). Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus.* African Journal of Biotechnology, 9(16), 2527-2530.

Briegel, H., (1990). Fecundity, metabolism, and body size in Anopheles (Diptera: Culicidae), vectors of malaria. J Med Entomol., 27, 839–850.

Briegel, H., and Horler, E. (1990). Multiple blood meals as a reproductive strategy in Anopheles (Diptera: Culicidae). Journal of Medical Entomology. 30, 975–985.

Brown, A. W. A. (1986). Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc., 2, 123-140.

Campbell, F.L., Sullivan, W.W., and Smith, L.N. (1993). The relative toxicity of nicotine, anabasine, methyl anabasine and lupinine for Culicine mosquito larvae. J. Econ. Entomol., 26, 505–509.

Carvalho, A.F., Melo, V.M., Craveiro, A.A., Machado, M.I., Bantim, M.B., and Rabelo, E.F. (2003). Larvicidal activity of the essential oil from *Lippia*

sidoides Cham. against *Aedes aegypti* L. Mem Inst Oswaldo Cruz., 98, 569-571.

Chaithong, U., Choochote, W., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., and Chaiyasit, D. (2006). Larvicidal effect of pepper plants on *Aedes aegypti* (L.) (Diptera: Culicidae). J Vector Ecol; 31, 138-143.

Chalannavar, R.K., Hurinanthan, V., Singh, A., Venugopala, K.N., Gleiser, R.M., Baijnath, H., and Odhav, B. (2013). The antimosquito properties of extracts from flowering plants in South Africa. Trop Biomed, 30, 559– 569.

Chansang, U., Zahiri, N.S., Bansiddhi, J., Boonruad, T., Thongsrirak, P., and Mingmuang, J. (2005). Mosquito larvicidal activity of crude extracts of long pepper (*Piper retrofractum* vahl) from Thailland. J Vector Ecol., 30, 195–200.

Chapagain, B. and Wiesman, Z. (2005). Larvicidal effects of aqueous extracts of *Balanites aegyptiaca* (desert date) against the larvae of *Culex pipiens* mosquitoes. African J. Biotechnol., 4(11), 1351-1354.

Chaves, L.F., Keogh, C.L., Nguyen, A.M., Decker, G.M., Vazquez-Prokopec, G.M., and Kitron, U.D.(2010). Combined sewage overflow accelerates immature development and increases body size in the urban mosquito *Culex quinquefasciatus*. J. Appl. Entomol. 135, 611–620.

Chaves, L.F., Imanishi, N., and Hoshi, T. (2015). Population dynamics of *Armigeres subalbatus* (Diptera: Culicidae) across a temperature altitudinal gradient. Bull Entomol Res., 105, 589-597.

Chen, X.G., Marinotti, O., Whitman, L., Jasinskiene, N., and James, A.A. (2007) The *Anopheles gambiae* vitellogenin gene (VGT2) promoter directs persistent accumulation of a reporter gene product in transgenic *Anopheles stephensi* following multiple blood meals. Am J Trop Med Hyg., 76, 1118–1124.

Cheng, S.S., Chua, M.T., Chang, E-H., Huang, C.G., Chen, W.J., and Chang, S.T. (2009). Variations in insecticidal activity and chemical compositions of leaf essential oils from *Cryptomeria japonica* at different ages. Bioresource Technology, 100, 465 - 470.

Choochate, W., Chaiyasit, D., Kanjanapothi, D., Rattanachanpichai, E., Jitpakdi, A., and Tuetun, B. (2005). Chemical composition and antimosquito potential of rhizome extract and volatile oil derived from *Curcuma aromatica* against *Aedes aegypti* (Diptera: Culicidae). J Vector Ecol, 30, 302-319.

Christophers, S. R. 1945. Structure of the Culex egg and egg-raft in relation to function (Diptera). Trans. R. Entomol. Sot. Lond. 95 (4), 25-34.

Chung, I.M., Seo, S.H., Kang, E.Y., Park, W.H, and Moon, H.I. (2009). Larvicidal effects of the major essential oil of *Pittosporum tobira* against *Aedes aegypti* (L.). J Enzyme Inhib Med Chem, 25, 391-393.

Ciccia, G., Coussio, J., and Mongelli, E. (2000). Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. J Ethnopharmacol., 72, 185-189.

Clements.A.N. (1992).The Biology of Mosquitoes: Development, Nutrition and Reproduction. 1, Chapman and Hall, London.

Clemons, A.C., Mori, A., Haugen, M., Severson, D., and Duman-Scheel, M. (2010). *Aedes aegypti*: culturing and egg collection. Cold Spring Harbor Protoc.

Collins, L. E. and Blackwell, A. (2000). The Biology of *Toxyrhynchites* Mosquitoes and Their Potential as Bio control Agents. Biocontrol News and Information, 21(4), 115 –116.

Conti, B., Benelli, G., Flamini, G., Luigi Cioni, P., Profeti. R., Ceccarini, L., Macchia, M. and Canale, A. (2013). Larvicidal and repellent activity of *Hyptis suaveolens* (Lamiaceae) essential oil against the mosquito *Aedes albopictus* Skuse (Diptera: Culicidae). Parasitol. Res., 110, 2013-2021.

Costa, E. Santos., E.M.M. Correia, J.C., and Albuquerque, C.M.R. (2010). Impact of small variations in temperature and humidity on the reproductive activity and survival of *Aedes aegypti* (Diptera: Culicidae). Rev Bras Entomol 54, 488–493.

Dan, M., George, V., and Pushpangadan, P. (2002). Studies on the essential oils from *Curcuma haritha* and *Curcuma raktakanta*, two endemic species of Kerala. Journal of Spices and Aromatic Crops, 11(1), 42-45.

Das, B. P., Chowdhury, D. N., Choudhury, B., Das, G. K., and Choudhury, R. T. (1996). Studies of some alkaloids for toxicity on the larvae of *Culex quinquefasciatus*. Indian Journal of Environmental Health, 38 (2), 81- 85.

Das, N.G., Goswami, D., and Radha, B. (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. J Vect Borne Dis, 44, 145-148.

Dass, K., and Mariappan, P. (2014). Larvicidal activity of *Lawsonia inermis* and *Murraya exotica* leaves extract on filarial vector, *Culex quinquefasciatus.* Int J Mosq Res, 1(2), 25-27.

de Santos, N.D.L., de Moura, K.S., Napoleao, T.H., Santos, G.K.N., Coelho, L.C.B.B., Navarro, D.M.A.F., and Paiva, P.M.G. (2012). Oviposition-stimulant and ovicidal activities of *Moringa oleifera lectin on Aedes aegypti.* PLos One 7, 44840.

Dhanalakshmi, D. (2013). In silico approaches for characterizing antimicrobials and larvicidal activity of selected plant extracts against the filarial vector *Culex quinquefasciatus*. Ph.D Thesis, Avinashilingam Deemed University for Women.

DHS Kerala. (2014). Annual reports of DHS, Government of Kerala 2011-2014 (Unpublished Document).

Dodson, B.L., Kramer, L.D. and Rasgon. J.L. (2011). Larval nutritional stress does not affect vector competence for West Nile Virus (WNV) in *Culex tarsalis*. Vector Borne Zoonot. Dis. 11, 1493–1497.

Dohutia, C., Bhattacharyya, D.R., Sharma, S.K., Mohapatra, P.K., Bhattacharjee, K., Gogoi, P., Mahanta, J., and Prakash, A. (2015). Larvicidal activity of few select indigenous plants of North East India against disease vector mosquitoes (Diptera: Culicidae). Tropical Biomedicine, 32(1), 17–23.

Douglas, E. Norris. (2004). Mosquito-borne diseases as a consequence of land use change. EcoHealth., 1, 19-24.

Dubey, N. K., Bhawana, Srivastava., and Ashok Kumar. (2008). Current status of plant products as botanical pesticides in storage pest management. Journal of Biopesticides, 1(2), 182 – 186.

Duraisamy, Gomathi., Ganesan, Ravikumar., Manokaran, Kalaiselvi., Balasubramaniam, Vidya., and Chandrasekar, Uma. (2012). HPTLC fingerprinting analysis of *Evolvulus alsinoides* (L.) L. Journal of Acute Medicine, 2(3), 77-82.

Edriss, A.E., Satti, A.A, and Alabjar, Z.A.(2013). Larvicidal properties of two asclepiadaceous plant species against the mosquito *Anopheles arabiensis* Patton (Diptera: Culicidae). Journal of the Saudi Society of Agricultural Sciences, 12, 59- 66.

EI Kamali, H. H. (2001). Larvicidal activity of crude aqueous extract of *Solenenostemma argel* against mosquito Larvae. J. of Herbs, Spices and Medicinal Plants, 8 (4), 83-86.

Elango, G., Bagavan, A., Kamaraj, C., Abduz Zahir, A. and Abdul Rahuman, A. (2009). Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against Anopheles subpictus Grassi (Diptera: Culicidae). Parasitol. Res., 105, 1567-1576.

Elof, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology, 60, 1–8.

Erlanger, T.E., Weiss, S., Keiser, J., Utzinger, J., and Wiedenmayer, K. (2009).Past, present, and future of Japanese encephalitis. Emerg Infect Dis., 15(1), 1–7.

Fabricant, D. S., Farnsworth, N. R. (2001).The value of plants used in traditional medicine for drug discovery. Environ Health Perspect.109, 69–75.

Finney, D.J. (1971). Probit analysis. Cambridge, U.K., University Press, 333.

Fouad, El-Akhal., Raja, Guemmouhc., Saad, Maniard., Khalid, Taghzoutie., Abdelhakim, and El Ouali Lalamia., (2016). Larvicidal activity of essential oils of *Thymus vulgaris* and *Origanum majorana* (lamiaceae) against of the malaria vector *Anopheles labranchiae* (Diptera: Culicidae). International Journal of Pharmacy and Pharmaceutical Sciences, 8(3).

Gamble J.S., (1935). The flora of the Presidency of Madras adland and son ltd. London.

Gerberc, E.J. (1970). Manual for Mosquito Rearing and Experimental Techniques, AMCA Bulletin No. 05. 1–91.

Ghosh, A., Chowdhury, N., and Chandra, G. (2012). Plant extracts as potential mosquito larvicides. Indian J Med Res, 135, 581-598.

Goma, L.K.H. (1966). The mosquito. Tropical Monographs. London, United Kingdom: Hutchinson

Gomathi, R., Indrakumar, I., and Karpagam, S. (2014). Larvicidal activity of *Monstera adansonii* plant extracts against *Culex quinquefasciatus*. J. Pharmacognosy and Phytochem. 3(3), 160-162.

Gonzalo, Batallan., Romina, Torre., Fernando, Flores., Brenda, Konigheim., Francisco, Luduena-Almeida., Carlos, Tonn., Marta, Contigiani., and Walter, Almiron. (2013). Larvicidal activity of crude extracts from *Larrea cuneifolia* (Zygophyllaceae) and of its metabolite nordihydroguaiaretic acid against the vector *Culex quinquefasciatus* (Diptera: Culicidae). Revista da Sociedade Brasileira de Medicina Tropical., 46(1), 84-87.

Govindarajan, M. (2010). Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes, Asian Pac. J. Trop. Med., 3. 691-695.

Govindarajan. (2011). Mosquito larvicidal and ovicidal activity of *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) leaf extract against *Culex quinquefasciatus* (say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). Eur Rev Med Pharmacol Sci. 15(7), 787-794.

Govindarajulu, B., Sirmathi, A., Bahavana, R., and Karthikeyan, J. (2015). Mosquito larvicidal efficacy of the leaf extracts of *Annona reticulata* against *Aedes aegypti*. Int. J. Curr. Microbiol. App. Sc., 4(8), 132-140.

Gubler, D.J. (1998). The global pandemic of dengue / dengue hemorrhagic fever: current status and prospects for the future. Annals of the Academy of Medicine, 27, 227–234.

Gutierrez, P.M., Antepuesto, A.N., Eugenio, Bal., and Santos, M.F.L. (2014). Larvicidal activity of selected plant extracts against the Dengue vector *Aedes*

aegypti mosquito. International Research Journal of Biological Sciences, 3(4) 23-32.

Haldar, M. K., Ghosh, P., and Chandra, G. (2012). Evaluation of target specific larvicidal activity of the leaf extract of *Typhonium trilobatum* against *Culex quinquefasciatus* Say. Asian Pac J Trop Biomed., 1(2), 199-203.

Harborne, J.B. (1973). Phytochemical methods. Chapman and Hall, Ltd, London, 49 -188.

Harish Chandra, Parul Bishnoi, Archana Yadav, Babita Patni, Abhay Prakash Mishra and Anant Ram Nautiyal. (2017). Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials-A Review, Plants, 6, 16, 1-11.

Hartzell, A., and Wilcoxon. F. (1941). A survey of plant products for insecticidal properties. Contrib. Boyce Thompson Inst., 12, 127-141.

Harwood, F. R., and M. T. James. (1979). Entomology in human and animal health. MacMillan Publishing Co., Inc., New York. 548.

Hashmat Imam., Zarnigar, Ghulamuddin, and Sofi. (2014). Mosquito larvicidal efficacy of *Acorus calamus* extracts against *Aedes aegypti* L. larvae. Asian Pac J Trop Dis; 4(1), 181-185.

Hazra, R.K., and Dash, A.P. (1998). Distribution of *Mansonioides* in Orissa, India. Trop Biomed, 15: 53-59.

Hemalatha, P., Elumalai, D., Janaki, A., Babu, M., Velu, K., and Velayutham, K. (2015). Larvicidal activity of *Lantana camara aculeate* against three important mosquito species. Journal of Entomology and Zoology Studies. 3(1), 174-181.

Hongjie, Zhang., Shengxiang, Qiu., Pamela, Tamez., Ghee, Teng Tan., Zeynep, Aydogmus., Nguyen, Van Hung., Nguyen, Manh Cuong., Cindy, Angerhofer. Doel, Soejarto D., John, M. Pezzuto., and Harry, H.S. Fong. (2002). Antimalarial Agents from Plants II. Decursivine, A New Antimalarial Indole Alkaloid from *Rhaphidophora decursiva*. Pharmaceutical Biology, 40 (03), 221–224.

Idu, M., Omonigho, S.E., and Igeleke, C.L. (2007). Preliminary investigation on the phytochemistry and antimicrobial activity of *Senna alata* L. flower. Pak. J. Biol. Sci., 10, 806-809.

Ilango.S., and Mariappan Malarvizhi. (2016). Efficacy of *Terminalia bellirica* seed extract for the control of filarial vector, *Culex quinquefasciatus* say. World Journal of Pharmacy And Pharmaceutical Sciences, 5 (3), 1007-1014.

Jackson, M. Muema, Joel, L. Bargul., Sospeter, N. Njeru., Joab, O. Onyango., and Susan, S. Imbahale. (2017). Prospects for malaria control through manipulation of mosquito larval habitats and olfactory-mediated behavioural responses using plant-derived compounds. Parasites and Vectors, 10, 184.

Jagbir Singh Kirti and Simarjit Kaur (2015). Prevalence and distrıbutıon of *Armigeres subalbatus* Coquıllett in Punjab. International Journal of Fauna and Biological Studies, 2 (3), 44-47.

Jaime, A., Cuervo-Parra., Teresa Romero Cortes., and Mario Ramirez-Lepe. (2016). Mosquito-Borne Diseases, Pesticides Used for Mosquito Control, and Development of Resistance to Insecticides, Stanislav Trdan (Ed.), InTech.

Jaswanth, A., Ramanathan, P., and Rukmini, K. (2002). Evaluation of mosquitocidal activity of *Annona squamosa* leaves against Filarial vector mosquito *Culex quinquefasciatus* Say. Ind. J. of Expt. Bio., 40, 363- 365.

Jayaprakasha, G. K., Singh, R. P., Pereira, J., and Sakariah, K. K. (1997). Limonoids from *Citrus reticulata* and their moult inhibiting activity in mosquito *Culex quinquefasciatus* larvae. Phytochemistry, 44, 843-846.

Jayapriya, G., and Shoba, G.F. (2015). Adulticidal and repellent activities of *Rhinacanthus nasutus* leaf extracts against *Aedes aegypti* Linn.and *Culex quinquefasciatus* Say. J. Ent. Zoo. Stu., 3, 154–159.

Jeeshna, M.V., Mallikadevi, T. and Paulsamy, S. (2010). Screening of the weed plant species, *Croton bonplandianum* Baill. for larvicidal activity of *Aedes aegypti*. J Biopest., 3, 192-194.

Jeyabalan, D., Arul, N., and Thangamathi, P. (2003). Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector *A. stephensi* Liston. Bioresour. Technol., 89 (2), 185-189.

Jomon, K. V., Sudharamini, S., and Thomas. T. (2009). Aedes mosquitoes in arboviral endemic prone area of Kottayam district, Kerala, India. 16(1 and 2), SB Academic Review, 171-178.

Jonathan, Cannon. Du Li, Steven G., Wood, Noel L. Owen., Alexandra, Gromova., and Vladislav, Lutsky. (2001). Investigation of Secondary Metabolites in Plants. A General Protocol for Undergraduate Research in Natural Products J. Chem. Educ., 78 (9), 1234.

Kalu, I.G, Ofoegbu, U., Eroegbusi, J., Nwachukwa, C.U., and Ibeh, B. (2010). Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. J Med Plants Res. 4, 496–498.

Kalyanasundaram, M. and Das, P. K. (1985). Larvicidal Synergic Activity of Plant Extract for Mosquito Control. Ind. J. Med. Res., 82, 19 – 23.

Kamaraj, C., and Rahuman, A.A. (2010). Larvicidal and adulticidal potential of medicinal plant extracts from south India against vectors. Asian Pacific J Trop Med., 3, 948–53.

Kamaraj, C., Rahuman, A.A., Bagavan, A., Abduz Zahir, A., Elango, G., and Kandan, P. (2011). Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). Trop Biomed, 27, 211-9.

Kannathasan, K. Senthilkumar, A., and Venkatesalu, V. (2011) Mosquito larvicidal activity of methyl-p-hydroxybenzoate isolated from the leaves of *Vitex trifolia* Linn. Acta Tropica, 120, 115–118.

Kantesh, Shidaraddi., Madhavi, Gajula., Neelesh, M.N., and Geeta, and V. Bathija. (2015). An insight into the knowledge and practices concerning mosquito borne diseases in urban slums of old Hubli. Journal of Evolution of Medical and Dental Sciences, 4(18), 3093-3100.

Kaushik, R., and Saini, P. (2008). Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. J Vector Borne Dis, 45, 66-69.

Khanna, V.G., and Kannabiran, K. (2007). Larvicidal effect of *Hemidesmus indicus, Gymnema sylvestre*, and *Eclipta prostrata* against *Culex quinquefaciatus* mosquito larvae. Afr J Biotechnol., 3, 307-311.

Kostic, M., Popovic, Z., Brkic, D., Milanovic, S., Sivcev, I., and Stankovic, S. (2008). Larvicidal and antifeedant activity of some plant-derived compounds to *Lymantria dispar* L. (Lepidoptera: Limantriidae). Bioresour. Technol. 99, 7897-7901.

Krishnaiah, D., Devi, T., Krishnaiah, A., and Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants, J. Medicinal Plant Research, 3(2), 67-72.

Krishnan, K., Senthilkumar, A., Chandrasekaran, M., and Venkatesalu, V. (2007). Differential larvicidal efficacy of four species of Vitex against *Culex quinquefasciatus* larvae. Parasitol Res, 10, 1721-1723.

Kumar, A., Dua, V.K., and Rathod, P.K. (2011). Malaria-attributed death rates in India. The Lancet, 377, 991–992.

Kumar, P. M., Murugan, K., Kovendan, K., Subramaniam, J., and Amaresan, D. (2012). Mosquito larvicidal and pupicidal efficacy of *Solanum xanthocarpum* (Family: Solanaceae) leaf extract and bacterial insecticide, *Bacillus thuringiensis*, against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research, 110: 2541-2550.

Kumar, S. M., and Manimegalai, S. (2008). Evaluation of larvicidal effect of *Lantana camara* Linn against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Advances in Biologi Res., 2(3 - 4), 39 - 43.

Kumar, S., Wahab, N., Mishra, M., and Warikoo, R. (2012), Evaluation of 15 local plant species as larvicidal agents against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). Frontiers in Physiology, 3(104), 1-6.

Kundu,M., Anjali, Rawani ., and Goutam, Chandra. (2013). Evaluation of Mosquito Larvicidal Activities of Seed Coat Extract of *Cassia sophera* L., Journal of Mosquito Research, 3(11), 76-81.

Kweka, E.J., Mosha, F., Lowassa, A., Mahande, A.M., Kitau, J., Matowo, J., Mahande, M.J., Massenga, C.P., Tenu, F., Feston, E., Lyatuu, E.E., Mboya, M.A., Mndeme, R., Chuwa, G., and Temus, E.A., (2008). Ethnobotanical study of some of mosquito repellent plants in north-eastern Tanzania. Malaria Journal, 7, 152.

Lalrotluanga, L., Ngente, L., Nachimuthu, S. K., and Guruswami, G. (2012). Insecticidal and repellent activity of *Hiptage benghalensis* L. Kruz (Malpighiaceae) against mosquito vectors, Parasitology Research, 111(3), 1007–1017.

Latha, C., Vijhayakumar, P.D., Velayudhan, S., and Joseph, A. (1999). Biological activity of indigenous plant extracts as mosquito larvicides. Indian J Exptl Biol, 37, 206–208.

Lazzari, C.R., Minoli, S.A., and Barrozo, R.B. (2004).Chemical ecology of insect vectors: The neglected temporal dimension. Trends Parasitol, 20, 506-507.

Lincy, Sara Varghese., Shehma, Shukkoor., Jaina, P. James., Beena, Habel., Aiswarya, Tom., and Shalia, Joseph. (2016). In vitro larvicidal activity of aqueous leaf extract of *Garcinia gummi gutta* Linn. against mosquito larvae. International Journal of Pharmaceutical Science and Research, 1(6), 01-04.

Little, V. A. (1957). General and applied entomology (2nd Ed.). Harper: New York, 385 – 394.

Lokesh, R., Leonard, B. E., Madhuri, P., Saurav, K., and Sundar, K. (2010). Larvicidal activity of *Trigonella foenum* and *Nerium oleander* leaves against mosquito larvae found in Vellore city, India. Curr. Res. J. Biol. Sci. 2, 154-160.

Lyimo, E.O., and Takken, W. (1993). Effects of Adult body size on fecundity and the pre-gravid rate of *Anopheles gambiae* females in Tanania. Med.Vet Entomol, 7, 328-332.

Magalhaes, L., Lima, M., Marques, M., Facanali, R., Pinto, A., and Tadei, W. (2010).Chemical composition and larvicidal activity against *Aedes aegypti* larvae of essential oils from four Guarea species. Molecule, 15, 5734-5741.

Maheswaran, R. Kingsley, and S. Ignacimuthu S. (2008) Larvicidal and repellent activity of *Clerodendron phlomides* against *Culex quinquefasciatus* Say (Diptera: Culicidae) Proc. Recent Trend Insect Pest Manage. 2(4), 240-243.

Malathi, P., and Vasugi, S.R., (2015). Evaluation of mosquito larvicidal effect of *Carica Papaya* against *Aedes Aegypti*. International Journal of Mosquito Research, 2(3), 21-24.

Maniafu, B.M., Wilber, L., Ndiege, I.O., Wanjala, C.C., and Akenga, T.A. (2009). Larvicidal activity of extracts from three Plumbago spp against *Anopheles gambiae*. Mem I Oswaldo Cruz. 104, 813–817.

Maragathavalli, S., Brindha, S., Kaviyarasi, N.S., Annadurai, B.S., and Gangwar, K. (2012). Antimicrobial activity in leaf extract of neem. Int J Sci Nat, 3 (1), 110-113.

Mass Bank. High Quality Mass Spectral Database, 2016.

Matasyoh, J.C., Wathuta, E.M., Kairuki, S.T, Chepkorir, R., and Kavulani, J. (2008). Aloe plant extracts as alternative larvicides for mosquito control. Afr J Biotech., 7, 912-5.

Mathivanan, T., Govindarajan, M., Elumalai, K., Krishnappa, K., and Ananthan, A. (2010). Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Stapf. (Family: Apocynaceae). J. Vec. Bor. Dis., 47, 178-180.

Maurya, P., Sharma, P., Mohan, L., Batabyal, L., and Srivastava, C.N. (2009). Evaluation of the toxicity of different phytoextracts of *Ocimum basilicum* against *Anopheles stephensi and Culex quinquefasciatus*. J Asia-Pacific Entomol, 12, 113-115.

Mawlouth, Diallo., Amadou, A., Sall, Abelardo., Moncayo, C., Yamar Ba., Zoraida Fernandez., Diana Ortiz., Lark, L., Coffey, C. Mathiot., Robert, B. Tesh., and Scott, C. Weaver. (2005). Potential role of sylvatic and domestic African mosquito species in dengue emergence. Am. J. Trop. Med. Hyg., 73(2), 445–449

Mehra, B. K. and Hirdhar, P. K. (2002). *Cuscuta hyalina* Roth., an Insect Development Inhibitor Against, Common House mosquito *Culex quinquefasciatus* Say. J. Env. Bio., 23 (3), 335-339.

METLIN.The original and most comprehensive ms / ms metabolite database, 2015.

Mgbemena, I. C. (2010).Comparative evaluation of larvicidal potentials of three plant extracts on *Aedes aegypti*. Journal of American Science, 6(10), 435-40.

Michael Russelle Alvarez., Francisco Heralde III., and Noel Quiming. (2016). Screening for larvicidal activity of ethanolic and aqueous extracts of selected plants against *Aedes aegypti* and *Aedes albopictus* larvae. Journal of Coastal Life Medicine, 4(2), 143-147.

Min, GiKim., Ju, HyunJeon., and Hoi, Seon, edx. (2013). Larvicidal activity of the active constituent isolated from *Tabebuia avellanedae* bark and structurally related derivatives against three mosquito species. J. Agric. Food Chem., 61, 10741−10745.

Mishra, K., Kumar Raj, D., Hazra, R.K., and Dash, A.P (2005). A simple, artificial-membrane feeding method for the radio-isotope labelling of *Aedes aegypti* polypeptides in vivo. Ann Trop Med Parasitol, 99, 803-806.

Mittal, P.K. and Subbarao, S.K. (2003). Prospects of using herbal products in the control of mosquito vectors. ICMR Bulletin, 33, 1-12.

Mohamed Yacoob Syed Ali., S. R. A. J. M. B. (2013). Mosquito larvicidal activity of seaweeds extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.* Asian. Pacific Journal of Tropical Diseases, 3, 196-201.

Mohankumar, T.K, Shivanna, K.S, Achuttan, V.V. (2016).Screening of methanolic plant extracts against larvae of *Aedes aegypti* and Anopheles stephensi in Mysore. J Arthropod Borne Dis; 10(3), 303-14.

Mullai, K., and Jebanesan, A. (2007).Larvicidal, ovicidal and repellent activities of the leaf extract of two cucurbitaceous plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Trop Biomed., 24, 1– 6.

Murthy, J.M., and Rani, P.U. (2009) Biological activity of certain botanical extracts as larvicides against the yellow fever mosquito, *Aedes aegypti*. L. J Biopesticides. 2, 72–6.

Murugan, K., Murugan, P., and Noortheen, A. (2007). Larvicidal and repellent potential of *Albizzia amara* Boivin and *Ocimum basilicum* Linn against

dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae). Biores. Technol., 98(1), 198-201.

Muslim, A. Fong, A.M.Y. R., Mahmud, Y.L. Lau., and Sivanandam, S. (2013). *Armigeres subalbatus* incriminated as a vector of zoonotic *Brugia pahangi* filariasis in suburban Kuala Lumpur, Peninsular Malaysia. Parasites and Vectors, 6, 219.

Mwine, J., van Damme, P., and Jumba, F. (2010). Evaluation of larvicidal properties of the latex of *Euphorbia tirucalli* L. (Euphorbiaceae) against larvae of Anopheles mosquitoes. Journal of Medicinal Plants Research, 4(19), 1954-1959.

Mwine, J., van Damme, P., and Jumba, F. (2011). Ethnobotanical survey of pesticidal plants used in South Uganda, Case study of Masaka district; Journal of Medicinal Plants Research, 5(7). 1155-1163.

Mwine, J., Van, Damme, P., Jumba, F., Arjun, N., Murugan, K., Madhiyazhagan, P., and Chippada, S.C. (2005). Preliminary phytochemical screening and evaluation of anti-inflammatory activity of ethanolic extract of leaves of *Indigofera tinctoria* Linn. Parasitology Research, 1(1), 250-259.

Nagasampagi, B. A. (1993). Environmentally friendly pesticides. Chemical Industry Digest, Second Quarter, 111- 115.

Nair, K. K., Ananthakrishnan, T. N. and David, B. (1976). General and applied entomology, Tata McGraw- Hill Publishing company Ltd. 280 – 281.

Nair.S.S., Vinaya, Shetty., and Nadikere, Jaya Shetty.(2014). Relative Toxicity of Leaf Extracts of *Eucalyptus globulus* and *Centella asiatica* against Mosquito Vectors *Aedes aegypti* and *Anopheles stephensi*. Journal of Insects, 6(4), 7-12.

Nandita, Chowdhury., Anupam, Ghosh., and Goutam, Chandra. (2008). Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector Stegomyia aegypti .BMC Complementary and Alternative Medicine, 8, 10.

Narayanan, G., and Narayanapillai, K. G. (1996). Larvicidal efficacy of certain plant extracts in mosquito Control. Proceedings of the Eighth Kerala Science Congress: 462-464.

Nathan, S.S. (2007).The use of Eucalyptus tereticornis sm.(Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera:Culicidae). Bioresource technology 98 (9), 1856-1860.

Nayak, J. B., and Rajani, B. (2014). Larvicidal activity of *Vitex negundo* leaf extract against *Culex quinquefasciatus* mosquito larvae. International Journal of Current Research, 6(08), 7983-7985.

Nazer, S., Ravikuma, r S., Williams, P.G., Syed Ali M., and Suganthi, P. (2009). Investigated a hundred coastal plant extracts against the *Culex quinquefasciatus* larvae of which seventeen coastal plants were posses larvicidal potential, Indian J Sci Technol, 2 (3), 24- 27.

Nikkon F., Saud, Z.A., Hossain, K., Parvin, M.S., and Haque M.E. (2009), Larvicidal effects of stem and fruits of *Duranta repens* against the mosquito *Culex quinquefasciatus*, Int. J. Pharm. Tech. Res., 1(4), 1709-1713.

Nivsarkar, M., Cherian, B., and Padh, H. (2001). Alphaterthienyl: A plant-derived new generation insecticide. Curr. Sci., 81, 667- 672.

Nkya, J.W, Erasto, P., and Chacha, M.(2014).Larvicidal against mosquito vectors and brine shrimp activities of extracts from the flowers of *Moringa oleifera* Lam. American Journal of Research Communication. 2(8), 15–29.

Nkya, T., Akhouayri, I., Poupardin, R., Batengana, B., Mosha, F., Magesa, S., Kisinza, W., and David, J.P. (2014). Insecticide resistance mechanisms associated with different environments in the malaria vector Anopheles gambiae: a case study in Tanzania. Malar J 13, 1–15.

Ogbeibu, A. E. (2001). Composition and Diversity of Diptera in temporary Pond in Southern Nigeria. Tropical ecology, 42 (2), 259-258.

Okigbo, R.N., Okeke, J.J. and Madu, N.C. (2010). Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* against mosquito larvae. Journal of Agricultural Technology, 6(4), 703- 719.

Omena, M.C., Navarro, D.M.A.F., Paula, J.E., Luna, J.S., Lima, M.R., and Sant'Ana, A.E.G. (2007). Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. Bioresour. Technol., 98, 2549-2556.

Oomah, D.B. (2003). Isolation, characterization and assessment of secondary metabolites from plants for use in human health, PBI Bull., 13-20.

Ozer, N., Ergünay, K., and Simsek F. (2007). West Nile virus studies in the Sanliurfa Province of Turkey. Journal of Vector Ecology, 32(2). 202–206.

Pandey, V., Agrawal, V., Raghavendra, K. and Dash, A.P. (2007). Strong larvicidal activity of three species of Spilanthes (Akarkara) against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies,* species C) and filaria vector (*Culex quinquefasciatus* Say). Parasitology Research, 102(1), 171–174.

Panigrahi, S.K., Barik, T.K., Mohanty, S., and Tripathy, N.K. (2014). Laboratory evaluation of oviposition behavior of field collected Aedes mosquitoes. Journal of Insects, 1-8

Park, H. M., and I. K. Park. (2012). Larvicidal activity of *Amyris balsamifera*, *Daucus carota* and *Pogostemon cablin* essential oils and their components against *Culex pipiens* pallens. J. Asia Pac. Entomol., 15, 631–634.

Park, K. (1994). Park's text book of preventive and social medicine (14th Ed.). M/S Banarsidas Bhanot Publishers.

Parthasarathy, V.A., and Kandiannan, K. (2007). Spices and condiments. Indian institute of spices research Calicut, National Science Digital Library Publisher.

Pavela, R., Harmatha, J., Barnet, M., and Vokac, K. (2005). Systemic effects of phytoecdysteroids on the cabbage aphid *Brevicoryne brassicae* (Sternorrhyncha: Aphididae). Eur. J. Entomol., 102, 647-653.

Periaswamy, Hemalatha., Devan, Elumalai., Arumugam, Janaki., Muthu, Babu., Kuppan, Velu., Kanayairam, Velayutham., and Patheri Kunyil, Kaleena (2015). Larvicidal activity of *Lantana camara aculeata* against three important mosquito species. Journal of Entomology and Zoology Studies 3 (1), 174-181.

Pierre, Jolivet. (1988). Interrelationship between insects and plants, Ist edn, 27, CRC Press, 336.

Pitasawat, B., Champakaew, D., Choochote, W., Jitpakdi, A., Chaithong, U., Kanjanapothi, D., Rattanachanpichai, E., Tippawangkosol, P., Riyong, B., Tuetun, D., and Chaiyasit, D. (2007). Aromatic plant-derived essential oil: An alternative larvicide for mosquito control, Fitoterapia, 78, 205-210.

Polaszek, A. (2006). Two worlds colliding: resistance to changes in the scientific names of animals-*Aedes* vs *Stegomyia*. Trends Parasitol, 22, 8–9.

Prabakar, K., and Jebanesan, A. (2004). Larvicidal efficacy of some cucurbitaceous plant leaf extracts against *Culex quinquefasciatus* (Say). Bioresource Technology, 95, 113-114.

Pratheeba, T., Ragavendran, C., and Natarajan, D. (2015). Larvicidal, pupicidal and adulticidal potential of *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector. International Journal of Mosquito Research, 2(2), 01-08.

Prummongkol, S., Panasoponkul, C., Apiwathnasorn, C., and Lekuthai, U. (2009). Refractoriness of *Culex sitiens* to experimental infection with nocturnal subperiodic Brugia malayi. Journal of Tropical Medicine and Parasitology, 32, 82-86.

Puggioli, A., Balestrino, F., Damiens, D., Lees, R.S., Soliban, S.M., Madakacherry, O., Dindo, M.L., Bellini, R., and Gilles, J.R.L. (2013). Efficiency of three diets for larval development in mass rearing *Aedes albopictus* (Diptera: Culicidae). J Med Entomol, 50, 819-825.

Rahuman A. A., Bagavan A., Kamaraj C., Saravanan E., Zahir A. A., Elango G. (2009b). Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res.104, 1365–1372.

Rajasekaran, A., Duraikannan, G. (2012). Larvicidal activity of plant extracts on *Aedes aegypti L*. Asian Pacific Journal of Tropical Biomedicine, S1578-S1582.

Rajendran, C. (2000). Trends in mosquito control past, present and future. In: Recent trends in combating mosquitoes. Loyola College, Chennai, 26.

Rajeswary, M. and Govindarajan, M. (2013). Repellent properties of *Ageratina adenophora* against dengue vector mosquito, *Aedes aegypti* Linn. (Diptera: Culicidae). International J. Pure Appl. Zool., 1(2), 167-171.

Rajkumar, S., and Jebanesan, A. (2005). Larvicidal and adult emergence inhibition effect of *Centella asiatica* (Brahmi, Umbelliferae) against mosquito *Culex quinquefasciatus* Say (Diptera: Culcidae). African Journal of Biomedical Research, 8, 31-33.

Ramsewak, S. R., Nair M. G., Murugesan, S., Mattson, W. J. and Zasada, J. (2001). Insecticidal fatty acids and triglycerides from *Dirca palustris*. J. Agri. Food Chemi., 49, 5852-5856.

Ramsey, J. M., Salinas, E.J., Lopez, R., Del Angel-Cabanas, G., Martinez, L.and Bown, D. N. (1988). Laboratory oviposition, fecundity and egg hatching ability of colonized *Anopheles albimanus* from Southwestern Mexico. Journal of the American Mosquito Control Association 4, 509-515.

Rana, I.S, Rana, A.S. (2012). Efficacy of essential oils of aromatic plants as larvicide for the management of filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae) with special reference to *Foeniculum vulgare*. Asian Pac J Trop Dis., 2(3), 184-189.

Raveen, R., Dhayanidhi, P., Dhinamala, K., Arivoli, and S. Tennyson, S. (2012). Larvicidal activity of *Pedilanthus tithymaloides* (L.) Poit (Euphorbiaceae) leaf against the dengue vector *Aedes aegypti* (Diptera: Culicidae). Int J Environ Biol., 2(2), 36–40.

Ravunni, Maya and Sailas, Benjamin. (2016). *Penicillium verruculosum* Strain BS3 Produces Aurantioclavine and Rugulosuvine B Alkaloids. Electronic Journal of Biology, 12(4), 484-489.

Rawani, A., Mallick Haldar, K., Ghosh, A., and Chandra, G. (2009) Larvicidal activities of three plants against filarial vector Culex quinquefasciatus Say (Diptera: Culicidae). Parasitol Res 105, 1411–1417.

Remia, K. M. and Logaswamy, S. (2010). Larvicidal efficacy of leaf extract of two botanicals against the mosquito vector, *Aedes aegypti* (Diptera: Culicidae). Indian Journal of Natural Products and Resources, 1(2), 208- 212.

Rohit kumar, Bargah. (2015). Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. Journal of Pharmacognosy and Phytochemistry, 4(1), 07- 09.

Rosenthal, P. J. (2004). Cysteine proteases of malaria parasites. International Journal for Parasitology, 34, 1489–1499.

Rozendaal, J.A. (1997). Vector control and communities. World Health Organization, Geneva.

Sabesan, S., Vanamail, P., Raju, K.H.K., and Jambulingam, P. (2010). Lymphatic filariasis in India: Epidemiology and control measures. Science Daily, 56, 232-238.

Sabu, L., Devada, K., and Subramanian, H. (2005). Dirofilariosis in dogs and humans in Kerala. Indian J Med Res., 121, 691-693.

Samuel T, Ravindran J, Eapen A, and William J. (2015).Ovicidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease, 5(3), 199-203.

Santos, N.D.L., Moura, K.S., Napoleao, T.H., Santos, G.K.N., Coelho, L.C.B.B., Navarro, D.M.A.F., and Paiva, P.M.G. (2012). Oviposition-stimulant and ovicidal activities of *Moringa oleifera* lectin on *Aedes aegypti*. PLoS ONE 7(9), 1-8.

Saraf, D.K., and Dixit, V.K. (2002). *Spilanthes acmella* Murr. study on its extract spilanthol as larvicidal compound. Asian J. Exp. Sci., 16, 9-19.

Sathish Kumar, M., and Manimegalai, S. (2008). Evaluation of larvicidal effect of *Lantana camara* Linn against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Advances in Biologi Res., 2(3 - 4), 39 - 43.

Savithramma, L. N., Linga, Rao, M. and Bhumi, G. (2011). Phytochemical screening of *Thespesia populnea* and *Tridax procumbens*. J. Chem. Pharm. Res., 3(5), 28-34.

Zareen,K.H., Shahid, Niaz Khan., Muhammad, Adnan., Hameed Ur, Rehman., Muhammad, Asim., Sana, M.A., Waheed, Javid Khan., Telawat, Khan., and Mujaddad Ur, Rehman. (2016). Larvicidal activity of medicinal plant

Eucalyptus leaf extracts against Anopheles mosquitoes collected from district. Journal of Entomology and Zoology Studies, 4(4), 696-697.

Saxena, R.C., Dixit, O.P. and Sukumaran, P. (1992). Laboratory assessment of indigenous plant extracts for anti-juvenile hormone activity in *Culex quinquefasciatus.* Indian Journal of Medical Research, 95, 204-206.

Sen-Sung, Cheng., Ju-Yun, Liu., Kun-Hsien, Tsai., Wei-June, Chen and Shang-Tzen, Chang. (2004). Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* Provenances, J. Agric. Food Chem., 52 (14), 4395–4400.

Senthilkumar, N., Varma, P., and Gurusubramanian, G. (2009). Larvicidal and adulticidal activities of some medicinal plants against the Malarial Vector, *Anopheles stephensi* (Liston). Parasitology Research, 104, 237-244.

Serena, M.M., Balasubramani, M.S., Rajan, K., and Gerald, I.A. (2010). Evaluation of the larvicidal activity of the leaf extracts of *Duranta erecta* Linn. (Verbenaceae) on the larvae of *Culex quinquefasciatus* (Say) (Culicidae). J Biopest., 3(3), 582-585.

Service, M.W. (1996). Medical Entomology for Students. 2nd ed.Chapman and Hall: London, 278.

Setiawan., Koerniasari., Ngadino., and Sri Agus Sudjarwo. (2017). Bioinsecticide effect of *Pinus merkusii* tree bark extract on *Aedes aegypti* larvae. J Young Pharm, 9(1), 127-130.

Shaalan, E.A.S., Canyonb, D., Younesc, M.W.F., Wahaba, H.A., and Mansoura, A.H. (2005). A review of botanical phytochemicals with mosquitocidal potential. Environment International, 31, 1149-1166

Shaalan, E.A.S., Canyonb, D., Younesc, M.W.F., Wahaba, H.A., and Mansoura, A.H. (2005). Larvicidal extract of *Mundulea Sericea* (Leguminosaea) plant extract against *Aedes aegypti* (L) (Diptera: Culicidae). Pharmacol. Ther., 1(3), 106-109.

Shahi, M., Hanafi-Bojd, A.A., Iranshahi, M., Vatandoost, H., and Hanafi-Bojd, M.Y. (2010). Larvicidal efficacy of latex and extract of *Calotropis procera*

(Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). J Vector Borne Dis., 47(3), 185-8.

Sharma, R.N., Gupta, A.S., Patwardhan, S.A., Hebbalker, D.S., Tare, V., and Bhonde, S.B. (1992). Bioactivity of Lamiaceae plants against insects. Indian Journal of Experimental Biology, 30, 244-246.

Sharma, V.P., (2001). Health hazards of mosquito repellents and safe alternatives, Curr. Sci., 80(3), 341-343.

Sharma, A., Kumar, S., and Tripathi, P.(2016). Evaluation of the larvicidal efficacy of five indigenous weeds against an Indian strain of dengue vector, *Aedes aegypti* L.(Diptera:Culicidae).J Parasitol Res, 1, 1-8.

Shola, K. Babatunde., Racheal, M. Adedayo., Elizabeth, A. Ajiboye., Sunday, Ojo., Israel, B., and Ajuwon, European. (2016). Phytochemical composition and larvicidal activity of *Ocimum canum* (L.) Essential Oil against *Anopheles gambiae* (Diptera: Culicidae). Journal of Medicinal Plants, 11(4), 1-7.

Shrankhla., Bhan, S., Sharma, P., Mohan, L., and Srivastava, C.N.(2012). Relative larvicidal potential of *Pseudocalymma alliaceum* and *Allium sativum* against malaria vector, *Anopheles stephensi* (Liston). J Europ Mosq Contr Assoc., 30, 83-90.

Silva, W.C., Martins, J.R., de, S., de, H.E.M. Souza., Heinzen, H., Cesio, M.V., Mato M., Albrecht, F., de Azevedo, J.L., de Barros, N.M. (2009). Toxicity of *Piper aduncum* L. (Piperales: Piperaceae) from the Amazon forest for the cattle tick *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae) Veterinary Parasitology, 164, 267-274.

Singh, A., Saxena, S.K., Srivastava, A.K, and Mathur, A. (2012). Japanese encephalitis: A persistent threat. Proc Natl Acad Sci. 82, 55–68.

Singh, K. R. P. and Brown, A. W. A. (1957). J. Insect Physiol., 1, 199 .

Singh, R.K., Dhiman, R.C, and Mittal, P.K. (2006). Mosquito larvicidal properties of *Momordica charantia* Linn (Family: Cucurbitaceae). J Vect Borne Dis; 43: 88-91.

Singha, S., Adhikari U., Ghosh A., and Chandra G. (2012), Mosquito larvicidal potentiality of *Holoptelea integrifolia* leaf extract against Japanese encephalitis vector. *Culex vishuni* group, J. Mosq. Res., 2(4), 25-31.

Singha, S., Adhikari, U., and Chandra, G. (2011), Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. Asian Pac J Trop Biomed, 1 (1), S119-S123

Singha, S., Banerjee, S., and Chandra, G. (2011) Synergistic effect of *Croton caudatus* (fruits) and *Tiliacora acuminate* (flowers) extracts against filarial vector *Culex quinquefasciatus*.Asian Pacific Journal of Trop.Biomedicine, 1, 159-164.

Sofowora, A. (1993). Medicinal plants and Traditional medicine in Africa: Spectrum Books Ltd, Ibadan, Nigeria, 191-289.

Spitzen, J., and Takken, W. (2005) Malaria mosquito rearing - maintaining quality and quantity of laboratory-reared insects. Proc Neth Entomol Soc Meet. 16, 95–100.

Sreelatha, P. and Narayana Pillai, K. G. (1996). Larvivorous Efficacy of Certain Fishes in Controlling Culex Mosquitoes in the drainages of Allappuzha, Kerala. Proceedings of the eight Kerala Science Congress. 464-466.

Sreenivasan, D., and Laxmanaperumalsamy, P. (1993). Anti bacterial activity of some medicinal plants. Bull. Env. Sci., 11, 21-24.

Suhasini, G and Ch.Sammaiah.(2014).Diversity of mosquitoes (Diptera:Culicidae) in different habitats of Warangal Urban Environment. Journal of Entomology and Zoology Studies 2(4), 7-10.

Sujatha, S., Sukumar, K.M., Perinch, M.G. and Boobur, L.R. (1988).Botanical derivatives in mosquito control. A review J. Amer. Mosquito Control Assoc., 7: 210-237.

Sukumaran, P. (1997) Growth inhibition by *Tridax procumbens* extracts in *Culex quinquefasciatus*. Poll. Res., 16(2), 129-131.

Sulaiman, S.F., Sajak, A. A.B., Supriatno, K.L.O., and Seow, E.M. (2011). Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. J. Food Compos. Anal., 24, 506-515.

Sumodan, P. (2014).Observations on nocturnal endophagy in *Aedes (stegomyia) albopictus* (skuse), 1894 from Kerala, India. J Entomol Zool Stud., 2(5), 45–47.

Sumodan, P. K. (2003). Potential of rubber plantations as breeding source for Aedes albopictus in Kerala, India. Dengue Bull, 27, 197– 198

Sung-Eun Lee. (2000). Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper- *Piper Longum*. Journal of the American Mosquito Control Association, 16(3), 245-247.

Sutthanont, W., Choochote, B., Tuetun, A., Junkum, A., Jitpakdi, U., Chaithong, D., and Riyong, B. Pitasawa. (2010). Chemical composition and larvicidal activity of edible plant-derived essential oils against the Pyrethroid - susceptible and resistant strains of *Aedes aegypti* (Diptera: Culicidae). J. Vector Ecol., 35, 106-115.

Swaroop, A., Jain, A., Kumhar, M., Parihar, N., Jain, S.(2007).Chikungungunya fever. Indian Academy of Clinical Medicine, 8, 164-168.

Takken, W. (2005). Chemical ecology of insect vectors: temporal, environmental and physiological aspects. Trends Parasitol. 21, 57.

Talontsi, F.M., Matasyoh, J.C., Ngoumfo, R.M. and Chepkorir, R. (2011). Mosquito larvicidal activity of alkaloids from *Zanthoxylum lemairei* against the malaria vector *Anopheles gambiae*. Pestic. Biochem. Phys., 99, 82-85.

Tandon, P., and Sirohi, A. (2015). A trail to minimize the impact of chemical insecticides and damage caused by *Raphidopalpa foveicollis* Lucas. Int J Sci Res Edu., 3, 4645-4649.

Tao, Yuan., Chuan-Rui, Zhang., Sheng-Ping, Yang., and Jian-Min, Yue.(2010). Limonoids and Triterpenoids from *Khaya senegalensis*. J. Nat. Prod., 73, 669–674.

Tariwari, C. N. Angaye., Godbless, N. Oyinke., Woyengidoubara, W.T. Angaye., and Victor, D. Igbeinkutu. (2017).The Comparative Phytochemical and Bio-

larvicidal Efficacy of Leaf Extracts of *Gmelina arborea* against Mosquito Larvae. International Journal of Innovative Healthcare Research, 5(1), 1-6.

Thenmozhi, V., Rajendran, R., Ayanar, K., Manavalan, R., and Tyagi, B.K. (2006). Long-term study of Japanese encephalitis virus infection in *Anopheles subpictus* in Cuddalore district, Tamil Nadu, South India. Trop Med Int Health, 11(3), 288–293.

Thomas, C.J., DE Cross., and Bogh, C. (2013). Landscape movements of *Anopheles gambiae* malaria vector mosquitoes in rural Gambia PLoS One, 8, 7-10

Tikar, S.N., Mendki, M.J., Sharma, A.K., Sukumaran, D., Veer, V., Prakash, S., and Parashar, B.D. (2011).Resistance status of the malaria vector mosquitoes, Anopheles stephensi and Anopheles subpictus towards adulticides and larvicides in arid and semi-arid areas of India. J In- sect Sci, 11, 85.

Tiwari, S., Singh, R.K., Tiwari, R., and Dhole, T.N. (2012). Japanese encephalitis: a review of the Indian perspective. Braz J Infect Dis.16 (6), 564–573.

Tiwari. Kumar, B., Kaur, M., Kaur, G. and Kaur, K. (2011). Phytochemical screening and extraction: a review, Int. Pharm. Scientia, 1 (1), 98-106.

Trease, G.E, and Evans, W.C. (1989). A Textbook of Pharmacognosy. (13th ed). Bailliere Tinall Ltd, London.

Uma Devi, R., Lakshmi, D., and N. Aarthi. (2010).Toxicity effect of *Artemisia parviflora* against malarial vector *Anopheles stephensi* Liston. Journal of Biopesticides, 3(1), 195 - 198

Vasanth Raj P., Raghu Chandrasekhar H., Dhanaraj S.A., Vijayan P., Nitesh K., and Subrahmanyan V.M. Venkata. (2009). Mosquito larvicidal activity of *Vitex negundo*. Pharmacology, 2, 975-990.

Vasudevan. K., Malarmagal Charulatha, H., Venkata Lakshmi Saraswatula., and Prabakaran, K. (2009) Larvicidal effect of Crude extracts of dried ripened fruits of *Piper nigrum* against *Culex quinquefasciatus* larval instars. J. vector Borne Dis. 46, 153-159.

Vatandoost, H., Sanei, Dehkordi. A., Sadeghi, S.M.T., Davari, B., Karimian, F., Abai, M.R., and Sedaghat, M.M. (2012).Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima* mozaffarian

essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae). Experimental Parasitology, 132(4), 470-474.

Vector control Research Centre. (1989).Technical Workshop on vector control.Mis.pubn.VCR.10.Pondicherry.

Verma, R., Khanna, P., and Chawla, S. (2014). Yellow fever vaccine: an effective vaccine for travellers. Hum. Vaccin. Immunother. 10 (1), 126–128.

Verma, S.K., Kumar, A., Ashish, V., and Das, M.D.(2014).Antimicrobial activity of endophytic fungal isolate in *Argemone maxicana*, a traditional Indian medicinal plant. Int J Innov Res Sci Eng Technol, 3(3), 10151-62.

Vignesh, A., Elumalai, D., Rama, P., Elangovan, K., and Murugesan, K. (2016). Chemical composition and larvicidal activity of the essential oil of *Glycosmis pentaphylla* (Retz.) against three mosquito vectors. International Journal of Mosquito Research, 3(2), 62-67

Vinod, Krishan., Jyoti, Uikey., and Saxena, R. C. (2008). Evaluation of mosquito larvicidal activity of bioactive saponin isolated from *Tridax procumbens* Linn. (Family: Asteraceae) against *Aedes Aegypti*. Int. J. Chem. Sci., 6(3).1504-1510.

Vogel, Arthur Israel. (1978). Textbook of quantitative chemical analysis. (4th ed. /), Longman Scientific and Technical publishers.

Vyas, Sumit M. (2008). Certain Studies on Mosquitoes of Rajkot City. PhD thesis, Saurashtra University

Vythilingam, I., Tan, S.B, and Krishnasamy, M. (2002).Susceptibility of *Culex sitiens* to Japanese encephalitis virus in peninsular Malaysia. Trop Med Int Health. 7(6), 539–540.

Waghorn, G.C., McNabb, W.C. (2003).Consequences of plant phenolic compounds for productivity and health of ruminants. Proc. Nutr. Soc.62, 383-392.

Wallace, J.R., and Merritt, R.W. (1999).Influence of microclimate, food, and predation on *Anopheles quadrimaculatus* (Diptera: Culicidae) growth and development rates, survivorship, and adult size in a Michigan pond. Environ Entomol., 28, 233–239.

Weiss, E. A. (2002). Spice Crops. CABI Publishing, New York

WHO. Technical Report Series. 1981; WHO/VB/81, 807.

Wink, M. (1993). Production and application of phytochemicals from an agricultural perspective. In: Van Beek TA, Breteler H, editors. Phytochemistry and agriculture. Oxford: Claredon Press, 171- 213.

World Health Organization. (2014).A Global Brief on Vector-Borne Diseases, World Health Organization, http://www.who.int/campaigns/ world-health-day/2014/global-brief/en/ (accessed 14.09.16)

Yadav, M., Chatterji, S., Gupta, S.K. and Watal, G. (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine Int. J. Pharm.Pharm. Sci., 6(5), 539-542.

Yadav, R., Tikar, S.N., Sharma, A.K., Tyagi, V., Sukumaran, D., Jain, A.K., and Veer, V.(2015). Screening of some weeds of larvicidal activity against *Aedes albopictus*, a vector of Dengue and Chikungunya. J. Vector Borne Dis., 52: 88–94.

Yadav, R., Tyagi, V., Tikar, S.N., Sharma, A.K., Mendki, M.J., and Jain, A.K., (2014). Differential larval toxicity and oviposition altering activity of some indigenous plant extracts against Dengue and Chikungunya vector *Aedes albopictus*. J. Arthropod - Borne Dis. 8, 174-185.

Yadav, R.N.S., and Munin, Agarwala. (2011). Phytochemical analysis of some medicinal plants. Journal of Phytology, 3(12), 10-14.

Yamunadevi, Mariswamy., Wesely, Edward Gnaraj., and Johnson, Marimuthu Antonisamy.(2012). Chromatographic fingerprint analysis on flavonoids constituents of the medicinally important plant *Aerva lanata* L. by HPTLC technique. Asian Pacific Journal of Tropical Biomedicine, 8-12.

Yang, Y.C., Lim, M.Y., and Lee, H.S. (2003). Emodin isolated from *Cassia obtusifolia* (Leguminosae) seed show larvicidal activity against three mosquito species. J. Agric. Food Chem., 51, 7629-7631.

Yogalakshmi, K., Vaidehi, J and Ramakotti, R.(2014).Larvicidal activity of the Essential Oil from *Cestrum nocturnum* (Solanacea) against three species of

vector mosquitoes. International Journal of Recent Scientific Research 5(2), 430-432.

Zhu, L., and Tian, Y. (2011).Chemical composition and larvicidal activity of *Blumea densiflora* essential oil against *Anopheles anthropophagus:* a malarial vector mosquito. Parasitol Res., 109(5), 1417-1422.

Zubairi, S. I., and Jaeis, N. I. (2014). A study of rotenone from Derris roots of varies location, plant parts and types of solvent used. The Malaysian Journal of Analytical Sciences, 18(2), 260-270.

***1. Aedes albopictus* Skuse**

Kingdom : Animalia
phylum : Arthropoda
Class : Insecta
Order : Diptera
Family : Culicidae
Genus : Aedes
Species : *Aedes albopictus*

***1b. Culex sitiens* Wiedemann**

Kingdom : Animalia
phylum : Arthropoda
Class : Insecta
Order : Diptera
Family : Culicidae
Genus : Culex
Species : *Culex sitiens*

***Armigeres subalbatus* Coquillett**

Kingdom : Animalia
phylum : Arthropoda
Class : Insecta
Order : Diptera
Family : Culicidae
Genus : Armigeres
Species : *Armigeres subalbatus*

Plate 1: Systematic position of Mosquitoes

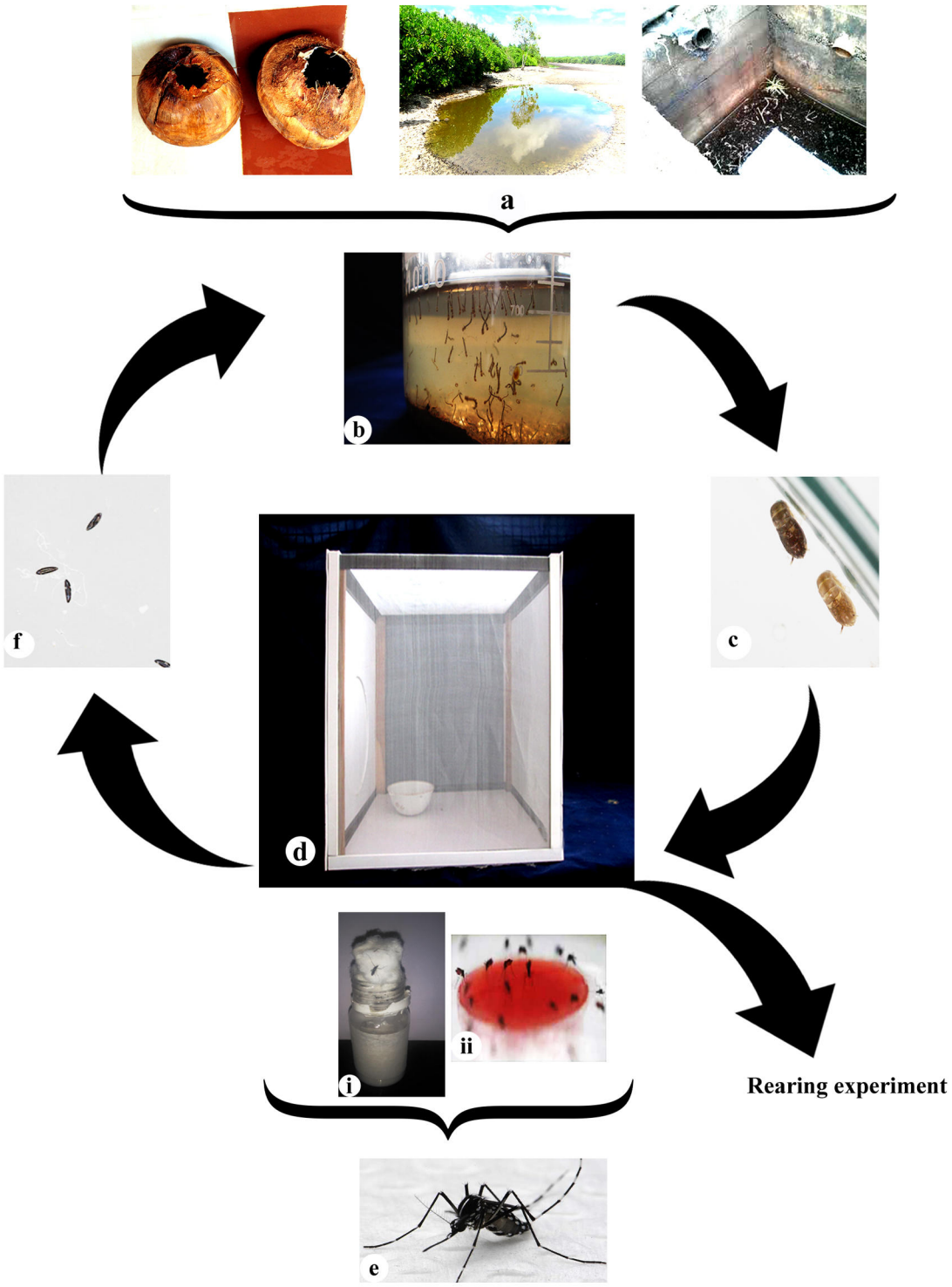


PLATE 2: Rearing procedure
(a) Breeding sites; (b) Mosquito larval stage; (c) Pupae; (d) Rearing cage; d(i) Sucrose solution; d(ii) Artificial blood feeding membrane; (e) Adult mosquito; (f) Egg

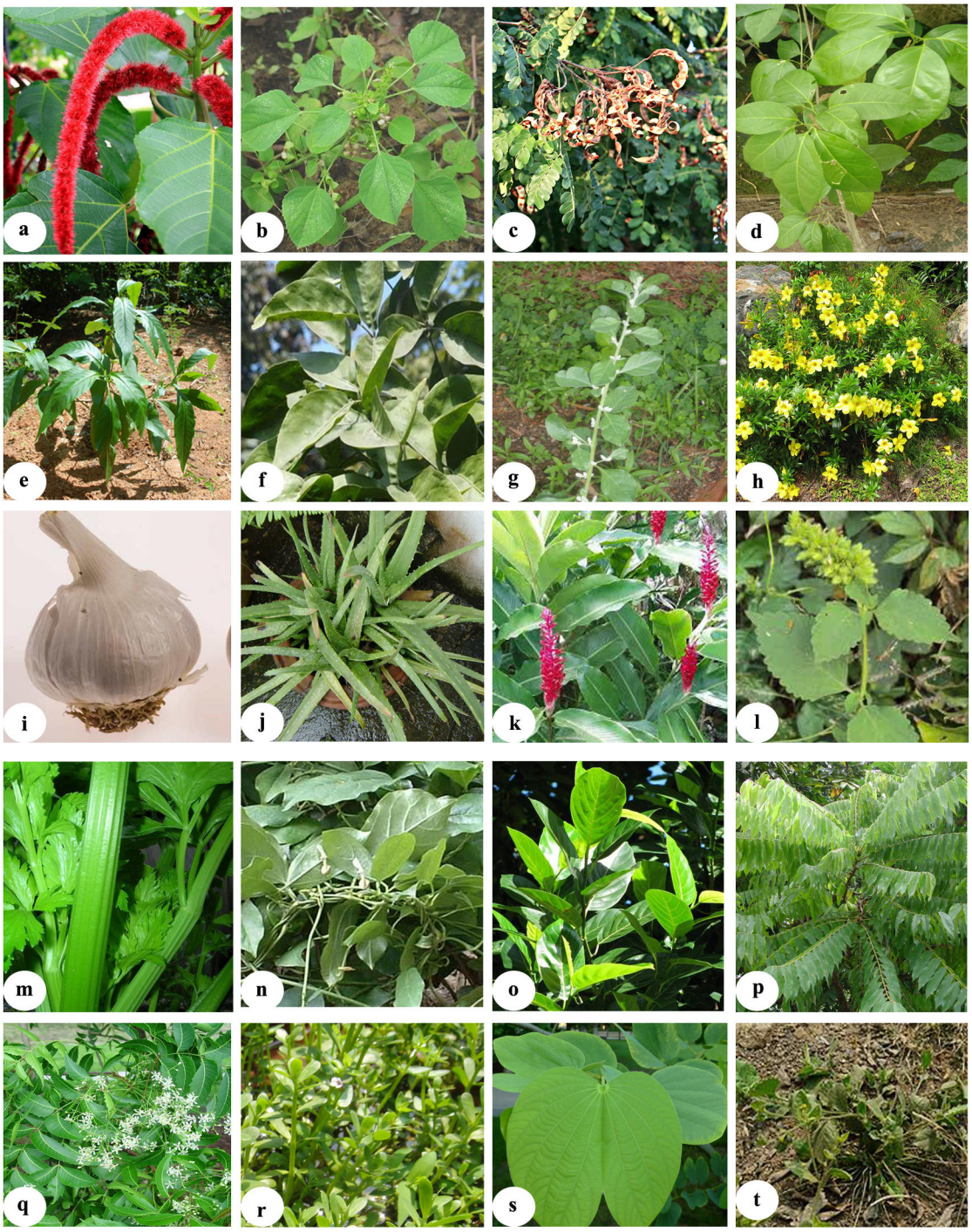


PLATE 3. Plants used for preparation of aqueous extracts
(a) ***Acalypha hispida*** **Burm.f. (Euphorbiaceae); (b)** ***Acalypha indica*** **L. (Euphorbiaceae); (c)** ***Adenanthera pavonina*** **L. (Fabaceae); (d)** ***Adenocalymma alliaceum*** **(Lam.) Miers (Bignoniaceae); (e)** ***Adhatoda vasica Nees*** **(Acanthaceae); (f)** ***Aegle marmelous*** **(L.) Corrêa (Rutaceae); (g)** ***Aerva lanata*** **(L.)Juss.(Amaranthaceae); (h)** ***Allamanda cathartica*** **(Apocynaceae); (i)** ***Allium sativum*** **L. (Amaryllidaceae); (j)** ***Aloe vera*** **(L.) Burm.f. (Asphodelaceae); (k)** ***Alpinia purpurata*** **(Vieill.) K. Schum. (Zingiberaceae); (l)** ***Anisomelos malabarica*** **(L.)R.Br.ex Sims (Lamiaceae); (m)** ***Apium graveolens*** **L. (Apiaceae); (n)** ***Aristolochia indica*** **L. (Aristolochiaceae); (o)** ***Artocarpus heterophyllus*** **Lam. (Moraceae); (p)** ***Averrhoa bilimbi*** **L (Oxalidaceae); (q)** ***Azadiracta indica*** **(A.Juss.) (Meliaceae); (r)** ***Bacopa monnieri*** **(L.) Edwall (Plantaginaceae); (s)** ***Bauhinia purpurea*** **L. (Fabaceae) (t)** ***Blumea oxyodonta*** **DC (Asteraceae)**

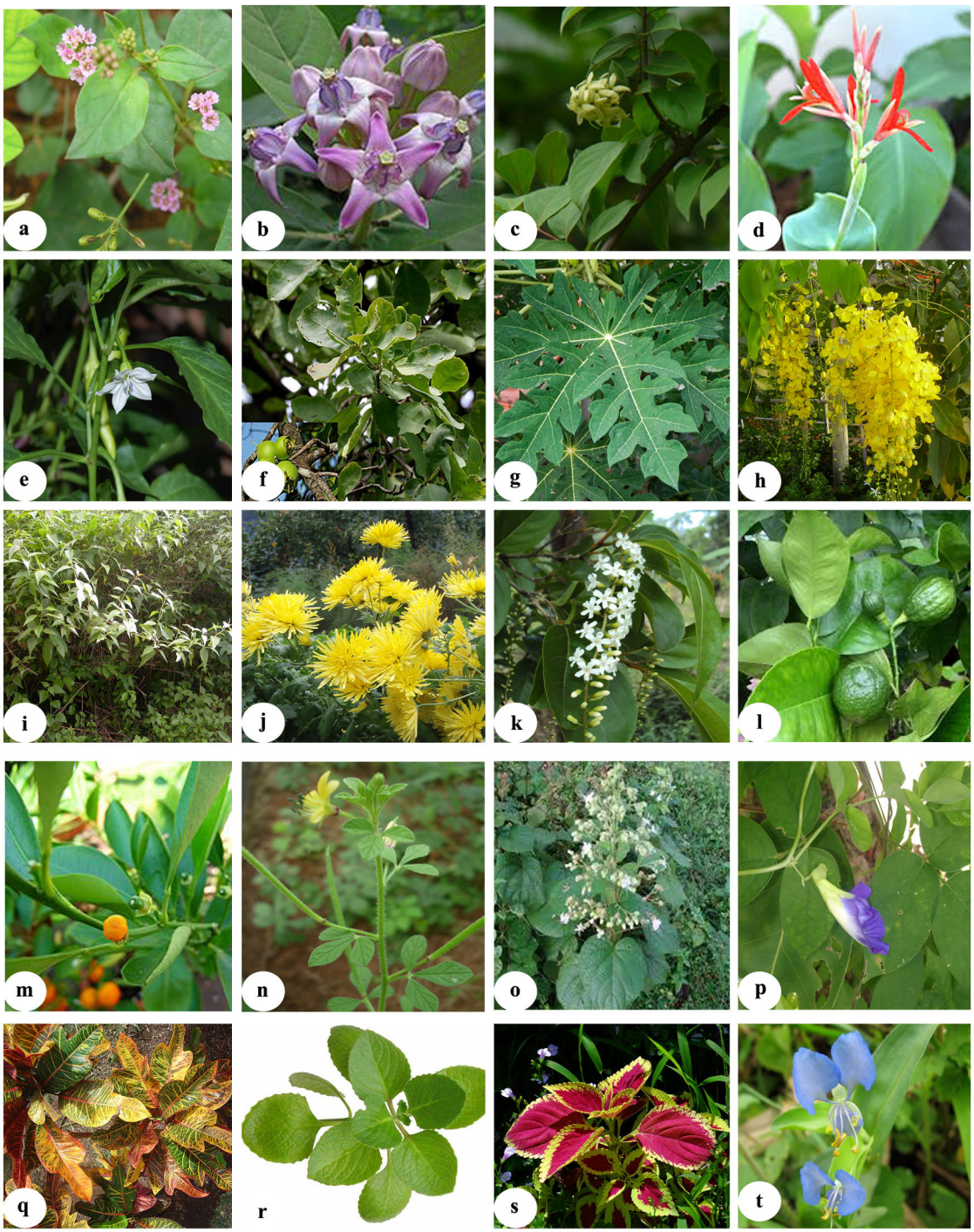


PLATE 4. Plants used for preparation of aqueous extracts

(a) *Boerhaavia diffusa* Var.hirsuta kuntze (Nyctaginaceae); (b) *Calotropis gigantea* (L.) R. Br. (Apocynaceae); (c) *Calycopteris floribunda* (Roxb.)Poir (Combretaceae); (d) *Canna indica* L. (Cannaceae); (e) *Capsicum annuum* L. (Solanaceae); (f) Careya arborea Roxb. (Lecythidaceae); (g) *Carica papaya* L. (Caricaceae); (h) *Cassia fistula* L. (Fabaceae);(i) *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae); (j) *Chrysanthemum morifolium* Ramat. (Asteraceae);(k) *Citharexylum spinosum* Kunth. (Verbenanaceae); (l) *Citrus medica* L. (Rutaceae); (m) *Citrus reticulata* Blanco (Rutaceae); (n) *Cleome viscose* L. (Cleomaceae);(o) *Clerodendron viscosum* Vent. (Lamiaceae); (p) *Clitoria ternatea* L. (Fabaceae);(q) *Codiaeum variegatum* (L.) Rumph. ex A. Juss. (Euphorbiaceae); (r) *Coleus aromaticus* Roxb.)Benth. (Lamiaceae);(s) *Coleus blumei* Benth. (Lamiaceae); (t) *Commelina diffusa* Burm.f. (Commelinaceae).

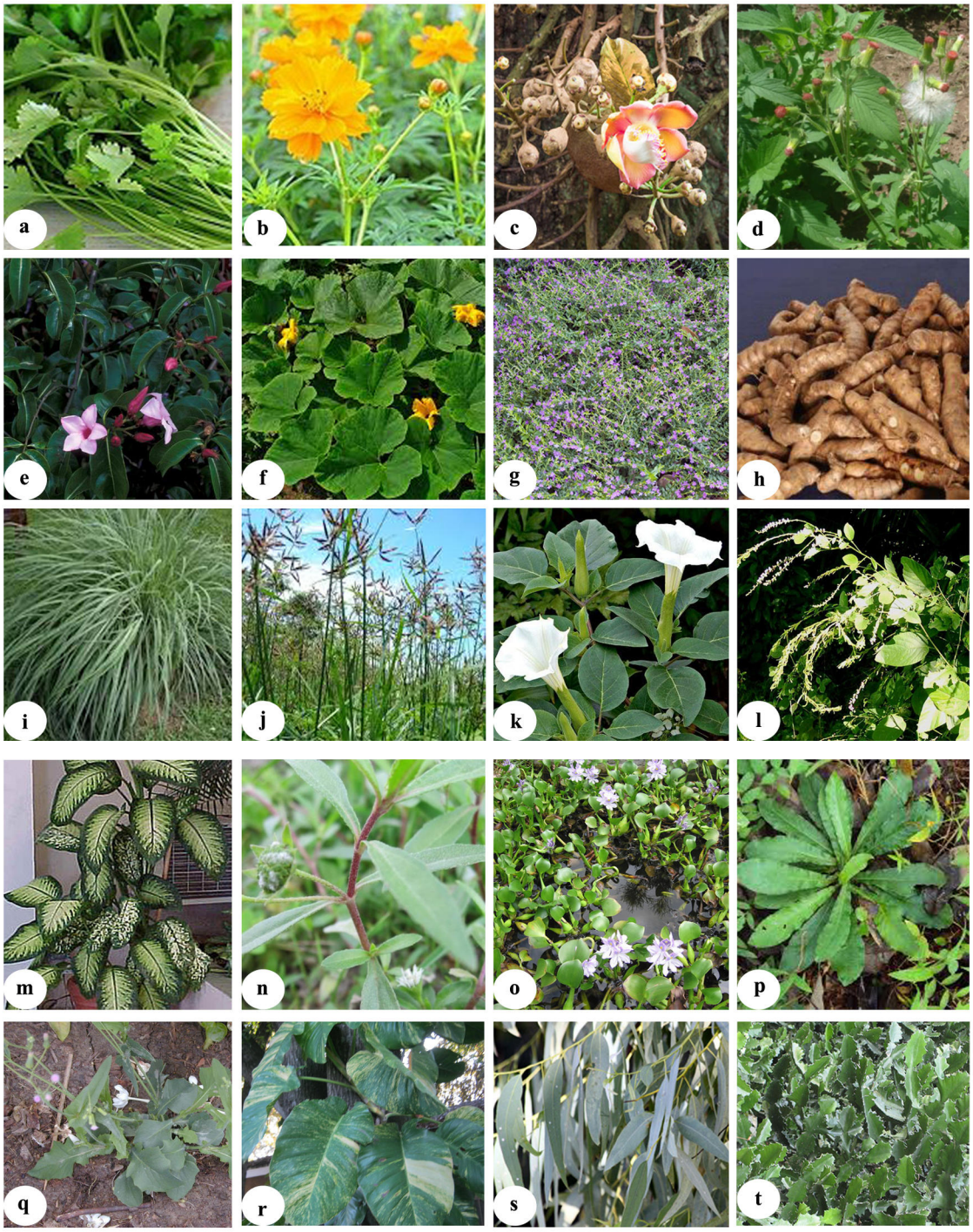


PLATE 5. Plants used for preparation of aqueous extracts
(a) *Coriandrum sativum* L. (Apiaceae); (b) *Cosmos sulphureus* (Asteraceae);(c) Couroupita guianensis Aubl. (Lecythidaceae);(d) *Crassocephalum crepidioides* (Benth.) S. Moore (Asteraceae); (e) Cryptostegia grandiflora Roxb. ex R. Br. (Apocynaceae);(f) Cucurbita pepo L. (Cucurbitaceae); (g) *Cuphea hyssopifolia* Kunth (Lythraceae);(h) *Curcuma longa* L. (Zingiberaceae);(i)Cymbopogon citratus (DC.) Stapf (Poaceae); (j) *Cyperus rotundus folatifolius* (Cyperaceae);(k)*Datura metel* L. (Solanaceae); (l) *Desmodium gangeticum* Blanco (Fabaceae); (m) *Dieffenbachia seguine* (Jacq.)Schott (Araceae);(n) *Eclipta alba* (L.) Hassk. (Asteraceae); (o) *Eichornia crassipes* (Mart.) Solms (Pontederiaceae); (p) *Elephantopus scaber* L. (Asteraceae); (q) *Emilia sonchifolia* (L.) DC. (Asteraceae); (r) *Epipremnum pinnatum* c. v. (Araceae); (s) *Eucalyptus tereticornis* Sm. (Myrtaceae); (t) *Euphorbia antiquorum* L. (Euphorbiaceae).

PLATE 6. Plants used for preparation of aqueous extracts
(a) *Gliricidia sepium* Jacq.)kunth ex Walp (Fabaceae);(b) *Glycosmis pentaphylla* (Retz.) DC. (Rutaceae); (c) *Hemidesmus indicus*(L.) R. Br. ex Schult. (Apocynaceae);(d) *Hibiscus rosa-sinensis* L. (Malvaceae); (e) *Hyptis suaveolens* (L.)Poit (Lamiaceae);(f) *Ixora coccinea* Comm. ex Lam. (Rubiaceae); (g) *Lantana camara* L. (Verbenaceae);(h) *Lawsonia inermis* L. (Lythraceae);(i) *Leucas aspera* (wild.) (Lamiaceae);(j) *Macaranga peltata* Boivin ex Baill. (Euphorbiaceae);(k) *Mangifera indica* L. (Anacardiaceae); (l) *Mentha arvensis* L. (Lamiaceae); (m) *Mesua ferrea* L. (Calophyllaceae); (n) *Millettia pinnata* (L.) Panigrahi (Fabaceae);(o) *Mimosa pudica* L. (Fabaceae); (p) *Mitracarpus hirtus* (L.) DC. (Rubiaceae); (q) *Momordica charantia* L. (Cucurbitaceae); (r) *Morinda pubescens* Sm. (Rubiaceae); (s) *Moringa oleifera* Lam. (Moringaceae); (t) *Murraya koenigii* (L.) Spreng. (Rutaceae)

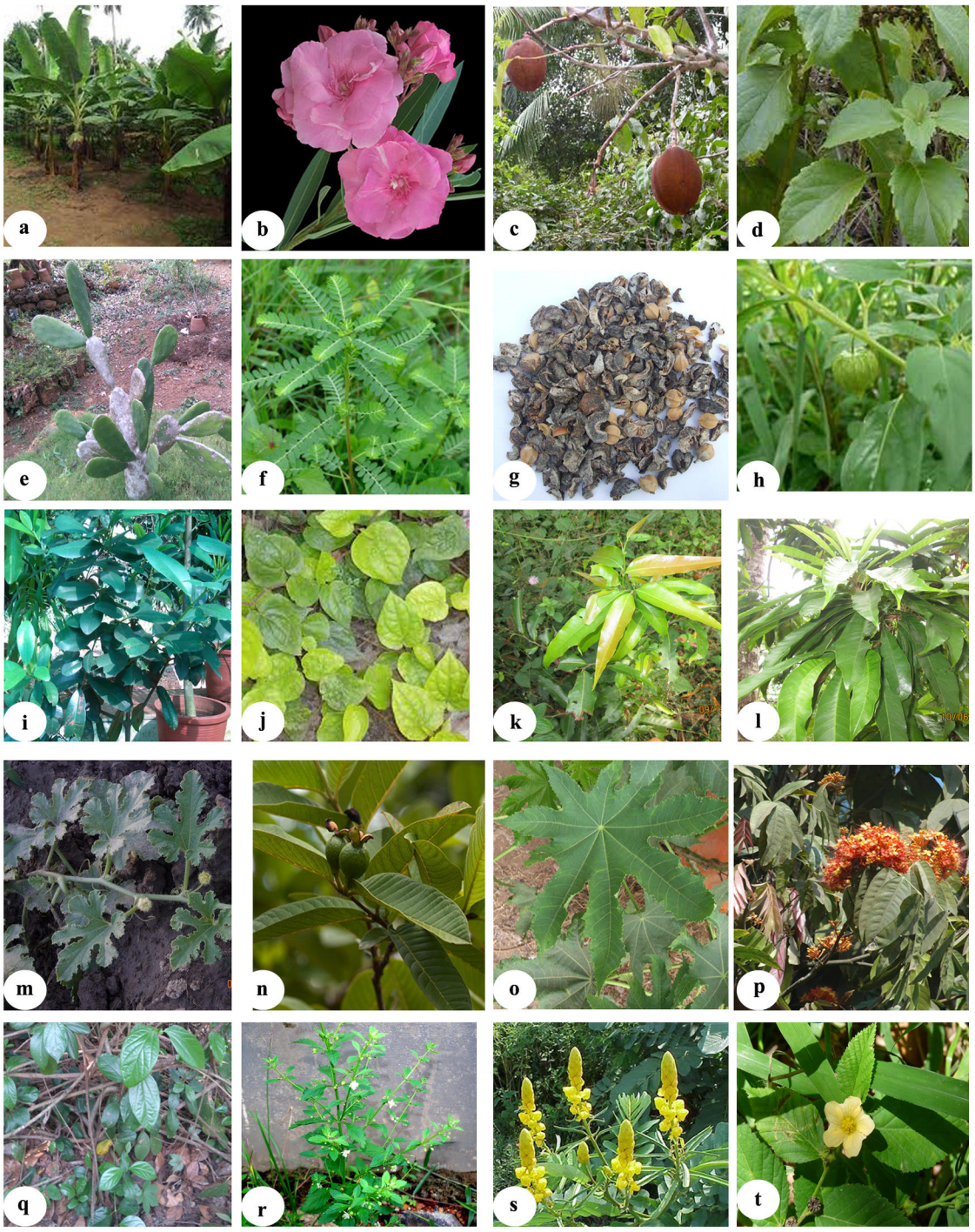


PLATE 7. Plants used for preparation of aqueous extracts
(a) *Musa* X *paradisiaca* (Musaceae); (b) *Nerium oleander* L. (Apocynaceae); (c) *Ochroma grandiflorum* Rowlee (Malvaceae); (d) *Ocimum gratissimum* L. (Lamiaceae); (e) *Opuntia dillenii* (Ker Gawl.)Haw. (Cactaceae); (f) *Phyllanthus amarus* Schumach.&Thonn. (Phyllanthaceae); (g)*Phyllanthus emblica* L. (Phyllanthaceae); (h) *Physalis minima* L. (Solanaceae); (i) *Pimenta dioica* (L.) Merr. (Myrtaceae); (j)*Piper betle* L. (Piperaceae); (k) *Polialthia longifolia* (Sonn.) Thwaites (Annonaceae); (l) *Pouteria campechiana* (Kunth)Baehni (Sapotaceae); (m) *Praecitrullus fistulosus* (stocks)Pangalo (Cucurbitaceae); (n) *Psidium guajava* L. (Myrtaceae); (o) *Ricinus communis* L. (Euphorbiaceae); (p) *Saraca asoca* (Roxb.)De Wilde (Fabaceae); (q) *Saritaea magnifica* (W. Bull) Dugand (Bignoniaceae); (r) *Scoparia ducis* L. (Plantaginaceae); (s) *Senna alata* (L.)Roxb. (Fabaceae); (t) *Sida rhombifolia* L. (Malvaceae)

PLATE 8. Plants used for preparation of aqueous extracts

(a)*Solanum nigrum* L. (Solanaceae); (b) *Spilanthes calva* DC. (Asteraceae); (c) *Synedrella nodiflora* (L.) Gaertn. (Asteraceae); (d) *Syzygium cumini* (L.) Skeels (Myrtaceae);(e) *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult. (Apocynaceae); (f) *Tagetes erecta* L. (Asteraceae) (g)*Tamarindus indica* L. (Fabaceae); (h)*Tephrosia purpurea* (L.) Pers (Fabaceae) (i) *Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae); (j)*Terminalia chebula* Ret. (Combretaceae); (k) *Thevetia neriifolia* Juss. ex Steud. (Apocynaceae);(l)*Tridax procumbens* L. (Asteraceae);(m)*Trigonella foenum* graecum L. (Fabaceae); (n)*Vernonia cinerea* (L.) Less. (Asteraceae); (o)*Vetiveria zizanioides* (L.) Nash (Poaceae); (p) *Vinca rosea* L. (Apocynaceae); (q) *Vitex negundo* L. (Lamiaceae); (r) *Wedelia trilobata* (L.)Hitchc (Asteraceae);(s) *Zingiber officinale* Roscoe (Zingiberaceae);(t) *Ziziphus jujuba* (L.) Lam. (Rhamnaceae)

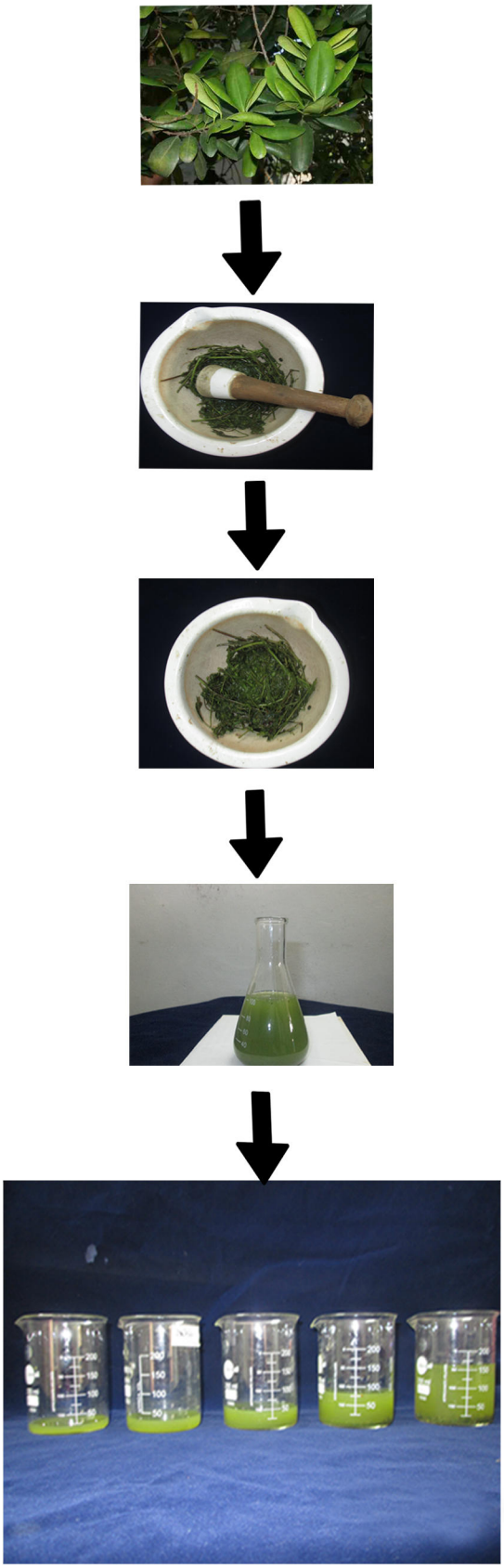


PLATE 9: Experimental procedure for larvicidal activity

Adenocalymma alliaceum **(Lam.) Miers.**

Kingdom : Plantae
phylum : Tracheophyta
Class : Equisetopsida
Order : Lamiales
Family : Bignoniaceae
Genus : Adenocalymma
Species : *Adenocalymma alliaceum* (Lam.) Miers

Pimenta dioica **(L.) Merr.**

Kingdom : Plantae
phylum : Spermatophyta
Class : Equisetopsida
Order : Myrtales
Family : Myrtaceae
Genus : Pimenta
Species : Pimenta dioica (L.) Merr.

Saritaea magnifica **(W.Bull) Dugand**

Kingdom : plantae
phylum : Spermatophyta
Class : Equisetopsida
Order : Lamiales
Family : Bignoniaceae
Genus : Saritaea
Species : *Saritaea magnifica* (W.Bull) Dugand

Plate 10. Systematic position of plants