RESEARCH ARTICLE

BIO EFFICACY OF SPATHODEA CAMPANULATA P.BEAUV.(BIGNONIACEAE) HEXANE LEAF EXTRACT AGAINST DENGUE AND ZIKA VIRUS VECTOR Aedes Aegypti (DIPTERA: CULICIDAE).

Pravin Y and Mohanraj R.S.

PG and Research Department of Zoology, Government Arts, College, Coimbatore. 641018, Tamilnadu ,India.

Abstract

Mosquitoes are popularly referred to as ‘flying syringes’, ‘tiny buzzing vampires’, ‘wing devil’ and ‘tiny assassins’. They are responsible for the transmission of many medically important pathogens and parasites such as viruses, bacteria, protozoa and nematodes, which cause serious disease such malaria, dengue, yellow fever, filariasis and zika. Aedes aegypti exhibits ecological plasticity. Till date, specific medications and vaccinations are not available commercially for treating dengue fever; therefore, the only way of reducing the incidence of this disease is mosquito control. Many control strategies for mosquitoes have been suggested since the ancient times. Chemical insecticides are commonly considered to be the most effective control strategy against mosquito. The blind use of insecticides has resulted in the increased selection pressure on the mosquitoes leading to the development of insecticide resistance in them. During the last decade, various studies on natural plant products against mosquito vector them as possible alternative to synthetic chemical insecticides. The present study was therefore carried out to evaluate qualitative phytochemical and GC-MS analysis, larvicidal, ovicidal properties of Spathodea campanulata against Aedes aegypti. Qualitative phytochemical analysis of the plant extract were carried out according to the methodology of Harbone (1984) and Trease and Evans (1989). Bioassay test are carried out for testing the efficacy of hexane leaf extract of S.campanulata on Ae.aegypti at different stages of development viz I, II, III and IV instars and pupae. Instructions of WHO (1960) as detailed by Pampana (1963) for conducting bioassay experiment with mosquito larvae were carefully followed. Effect of hexane leaf extract of S.campanulata on the hatchability of Ae.aegypti eggs were determined and hatching rate was calculated on the basis of non-hatchability of eggs (Sahgal and Pillai, 1993). The qualitative phytochemical analysis revealed the presence of different phytochemicals such as carbohydrates, tannins, flavonoids, alkaloids, terpenoids, phenols, coumarins, and phytosteroids Considerably low LC$_{50}$/LC$_{90}$/24,48 hours values of hexane leaf extract S.campanulata against different instar (I, II, III, IV and pupae) stages of Ae. aegypti obtained during the present study proved the larvicidal, ovicidal property of the plant. Young larvae were found to be relatively more susceptible then the older ones. The
hatchability of *Ae. aegypti* eggs was decreased when placed in media of hexane leaf extract. The reduction in percent hatch was inversely proportional to the concentration of hexane leaf extract used. As the plant of the present study is widely distributed, the commercial exploitation could provide an important step in the development of new plant based insecticide as one of the alternative to expensive and environmentally harmful chemical insecticides.

---

**Introduction:**

Arthropods are dangerous vectors of deadly pathogens and parasites, which may spread as epidemics or pandemics in the increasing world population of humans and animals (Mehlhorn, 2008, Mehlhorn et al., 2012). Mosquitoes transmit a variety of parasitic (e.g., malaria and lymphatic filariasis) and viral (e.g., dengue, zika, and yellow fever) diseases that pose serious public health challenge worldwide. The three genera of mosquitoes are *Culex*, *Anopheles*, and *Aedes* which transmit major mosquito based vector borne diseases. *Ae.aegypti* is a medium- sized blackish mosquito easily recognized by a silvery- white Iyre- shaped pattern of scales on its scutum. The colouration of both males and females is similar. *Ae.aegypti* preferentially breeds and develops in artificial or domestic containers such as cisterns, flasks, bottles, earthen pots, flower vases, tin cans, jars, over-head tanks, discarded automobiles tyres, unused water closets, rain barrels, sagging roof gutters and in natural sites such as coconut shells, snail shells, leaf axils and treeholes (Anitha and Ijumba,2013).

*Ae.aegypti* play an important role in the transmission of dengue fever, chikungunya, yellow fever, filariasis, zika, Japanese encephalitis, and several diseases which are today the greatest health problems in the world. Dengue fever virus (DENV) belongs to RNA virus of the Flaviviridae family and it was first isolated from Japan in 1942 by Hotta (Izabela et al., 2010). Three structural proteins are present in mature virion: (1) capsid protein C (2) membrane protein M (3) envelope protein E. C protein forms viral nucleocapsid. Chikungunya is an infection caused by the chikungunya virus. Chikungunya virus, also referred to as CHIKV, is a member of the alphavirus genus, and Togaviridae family. It is an RNA virus with a positive-sense single-stranded genome of about 11.6kb (Weaver et al., 2012). Zika virus (ZIKV) is a member of the virus family Flaviviridae and the genus Flavivirus, transmitted by day time-active Aedes mosquitoes, such as *Ae. aegypti*. It has been estimated recently that 3.9 billions of people in 128 countries are at risk of acquiring dengue and 390 million dengue infections occur every year, of which 294 millions clinically manifest the symptoms. The increase in dengue cases is considered to be a reflection of the rampant development towards massive infrastructure as well as urbanization, which is a favourable factor for breeding of *Ae.aegypti* (Azami et al., 2011 and Gubler, 2002).

Epidemic outbreaks of dengue fever have also been reported in India. More recent and systemic data are now available because of the NVBDCP. The data on the web site of NVBDCP show that dengue has been endemic in 16 states since the beginning: Andhra Pradesh, Goa, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal, Chandigarh, Delhi and Puduchery (NVBDCP, 2014).

**Epidemic outbreaks of Dengue fever:**

| Sl.No | Year | Infected persons | Deaths | Reference |
|-------|------|------------------|--------|-----------|
| 1     | 1980 | 4601*            | Nil    | Park and Park, 1987 |
| 2     | 2001 | 2000**           | 16     | The Hindu, 2001 |
| 3     | 2006 | 5710*            | 103    | The Hindu, 2006 |
| 4     | 2010 | 28.292*          | 110    | NVBDCP, 2011 |
| 5     | 2012 | 9000**           | 50     | The Hindu, 2012 |
| 6     | 2013 | 75,454*          | 167    | The Dinamani, 2013 |
| 7     | 2014 | 1400**           | Nil    | The Dinamalar, 2014 |
| 8     | 2015 | 2000**           | Nil    | The Dinakaran, 2015 |
| 9     | 2016 | 1751**           | 65     | The Dinakaran, 2016 |
| 10    | 2017 | 23,035**         | 83*,1**| NVBDCP,2018 |
| 11    | 2018 | 40868*,2175**    |        |           |
uito, which is the larval stage. Larviciding is a successful way of reducing mosquito densities in cal control, using ide resulted in several problems such as environmental hazards, elimination of 1); acetone extract of 2), 2015); acetone extract of , 2015); acetone extract of , 2015); aqueous extract of 2015); aqueous extract of 2006). Some of the plant leaves extracts 2017); ethanolic extract of 2017); methanol, benzene and acetone extracts of 2004); aqueous extract of 2004); aqueous extract of 2009); petroleum ether extract of 1997). These and other pitfalls have compelled scienti

At present, no effective vaccine is available for these diseases; therefore, the only way of reducing the incidence of this disease is mosquito control (Sarita et al., 2012). The control methods should aim at the weakest link of the life cycle of the mosquito, which is the larval stage. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated than they are in the adult stage (Rutledge et al., 2003).

Many control strategies for mosquitoes have been suggested since the ancient times. Among the various control measures, viz., mechanical control by source of reduction (Mazzarri and Georghiou, 1995); biological control, using endopathogenic bacteria, Bacillus thuringiensis (Seleena et al., 1995 and Mulla et al.,1999; Tabashnik et al., 1994); larivorous fish (Gluckman and Hartney, 2000) as well as predatory arthropods (Bohidar and Mohapatra, 2000 ) and chemical control (Laird and Miles, 1983). The chemical insecticides, including organophosphates, organochlorines and pyrethroids are being utilized for the control of vector and mosquito populations (Govindarajan et al., 2013). Repeated use of chemical insecticide resulted in several problems such as environmental hazards, elimination of natural enemies, toxic residues in food, and also produced insecticidal resistance in major vector species (Macedo et al., 1997). These and other pitfalls have compelled scientists to advocate for a refocus on botanicals, in Integrated Mosquito Management (IMM) protocols.

Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Naturally products are generally preferred because of the innate biodegradability. More than 2000 plant species have been known to produce chemical factors and metabolites of value in the pest control programmes (Ahmed et al., 1984) and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes (Sukumar et al.,1991). The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility and smoke toxicity (Elimam et al., 2009a,b). Some of the plant leaves extracts are tested for their d 2013). These and other pitfalls have compelled scientists to advocate for a refocus on botanicals, in Integrated Mosquito Management (IMM) protocols.

### Epidemic outbreaks of Chikungunya:

| Sl.No | Year | Infected persons | Deaths | Reference |
|-------|------|------------------|--------|-----------|
| 1 | 1965 | 3-laks* | Nil | Park and Park, 1987 |
| 2 | 2006 | 13-laks*, 63,000** | Nil | The Hindu, 2006 |
| 3 | 2013 | 500** | Nil | The Dinamani, 2013 |

At present, no effective vaccine is available for these diseases; therefore, the only way of reducing the incidence of this disease is mosquito control (Sarita et al., 2012). The control methods should aim at the weakest link of the life cycle of the mosquito, which is the larval stage. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated than they are in the adult stage (Rutledge et al., 2003).

Many control strategies for mosquitoes have been suggested since the ancient times. Among the various control measures, viz., mechanical control by source of reduction (Mazzarri and Georghiou, 1995); biological control, using endopathogenic bacteria, Bacillus thuringiensis (Seleena et al., 1995 and Mulla et al.,1999; Tabashnik et al., 1994); larivorous fish (Gluckman and Hartney, 2000) as well as predatory arthropods (Bohidar and Mohapatra, 2000 ) and chemical control (Laird and Miles, 1983). The chemical insecticides, including organophosphates, organochlorines and pyrethroids are being utilized for the control of vector and mosquito populations (Govindarajan et al., 2013). Repeated use of chemical insecticide resulted in several problems such as environmental hazards, elimination of natural enemies, toxic residues in food, and also produced insecticidal resistance in major vector species (Macedo et al., 1997). These and other pitfalls have compelled scientists to advocate for a refocus on botanicals, in Integrated Mosquito Management (IMM) protocols.

Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Naturally products are generally preferred because of the innate biodegradability. More than 2000 plant species have been known to produce chemical factors and metabolites of value in the pest control programmes (Ahmed et al., 1984) and among these plants, products of some 344 species have been reported to have a variety of activities against mosquitoes (Sukumar et al.,1991). The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility and smoke toxicity (Elimam et al., 2009a,b). Some of the plant leaves extracts are tested for their d
As far as our literature survey is concerned that there was no information available on larvicidal, ovicidal effects of the hexane leaf extract of the S.campanulata. The present study was therefore carried out to evaluate the larvicidal, ovicidal effects of S.campanulata hexane leaf extract against the vector mosquito, Ae.aegypti.

*Spathodea* is a monotypic genus in the flowering plant family Bignoniaceae. It contains the single species, *Spathodea campanulata*, which is commonly known as the African Tulip Tree, Flame-of-the forest in English, Rugtoora in Hindi, Patadi in Tamil. It is a tree that grows between 7-25 m (23-82ft) tall and native to tropical Africa. This tree is planted as ornamental tree throughout the tropics and much appreciated for its very showy reddish argane (or) crimson (rarely yellow), campanulated flowers. It is commonly planted as a street tree in south Tamil Nadu. The tree is considered evergreen but it sheds leaves in dry summers and hence it is a dry season deciduous tree. *S.campanulata* commonly employed to control epilepsy. This species has many uses in folk medicine. The flowers are employed as diuretic and anti-inflammatory while the leaves are used against kidney diseases, urethra inflammation and as a antidote against animal poisons. The leaves have furnished Spathodol, caffeic acid and other phenolic acids and flavonoids. The plant leaf is used for anti-plasmodial activity, anti-microbial activity and anti - larvicidal activity (Kowti et al., 2010; Kowti et al.,2011; EI – Hela , 2001).

The aim of the present study is therefore to find out:
1. Phytochemical and GC-MS analysis of hexane leaf extract of S.campanulata,
2. Estimate the toxicity of the hexane leaf extract of S.campanulata to the larvae and pupa of Ae.aegypti,
3. Ovicidal activity of the hexane leaf extract S. campanulata on Ae.aegypti egg.

Materials and methods:-

**Colonization of Ae.aegypti:**
The eggs of *Ae.aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore (Dt), Tamil Nadu, India. The eggs were then brought to the laboratory and transferred to enamel trays containing water and kept for larval hatching. They were hatched, reared and have been still maintained for many generations in the laboratory. The eggs and larvae obtained from this stock were used for different experiments. The larvae were reared in plastic cups. They were daily provided with commercial fish food *ad libitum* (Lymio et al.,1992). Water was changed alternate days. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes. The pupae were collected from culture trays and were transferred into plastic cups containing water with help of a sucker. The pupae containing plastic cups were kept inside mosquito cage for adult emergence. The females were fed by human arm every alternate day (Judson,1967; Briegel,1990). Both females and males were provided with 10% glucose solution on cotton wicks (Villani et al.,1983). An egg trap (plastic cup) lined with filter paper containing water was always placed at a corner of the cage.

**Collection of plant materials**
*S. campanulata* P. Beauv. (Family :Bignoniaceae) leaves were collected from Government Arts college campus, Coimbatore, Southern India. The identification of the plants was authentified at BSI (Botanical Survey of India), Coimbatore.

**Preparation of plant extract**
The fresh leaves of the plant *S. campanulata* were collected in our college campus area. Then the leaves brought to the laboratory. The plant leaves were observed carefully for any kind of diseases or infection and if found any, those parts were separated and not used for the experiment. The selected leaves washed with distilled water in order to clean dust or any particle stuck to them. Then the leaves kept for drying under shade at room temperature (27± 2°C) for about 2 weeks till they dried completely. The leaves were finely powdered using electric blender. 100 g of leaf powder was dissolved in 1000 ml of hexane in airtight wide mouth bottle and kept for 4 days with periodic shaking. After that, the extract was filtered using Whatman No.1filter paper and kept in Petri dishes for drying at room temperature (Kongkathip ,1994). Dried extract was used for the preparation of stock solution.

**Preparation of stock solution and different concentrations of leaf extract**
1g of the concentrated extract of leaves of *S. campanulata* was dissolved in 100 ml of acetone and kept as stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.
Phytochemical analysis of the plant extract:
Qualitative phytochemical analysis of the plant extract were carried (Harbone, 1984; Trease & Evans, 1989).

Gas Chromatography- Mass Spectrometry (GC-MS) Analysis:
The GC-MS analysis was conducted at South Indian Textile Research Association, Coimbatore. 1 µl of hexane leaf powder was injected into a Thermo GC –Trace ultra ver: 5.0, Thermo MS DSQ 11. The chromatography was performed by using the DB 35- MS capillary standard non-polar column. Helium flow was 1ml/ min. The oven temperature was increased at 70ºC /min to 250ºC.

Scanning Electron Microscope analysis
Ovicidal changes of the treated egg were studied and recorded further compared to the control larvae after treatment of the hexane, ethyl acetate, Iso-propanol, dichloromethane, chloroform extracts for Scanning electron microscope study, subsequently spurted with 45nm gold, attached to the stubs and viewed under Scanning Electron Microscope (FEI Quanta 250, Bharathiar University, Coimbatore, Tamilnadu India)

Larvicidal, pupicidal assay test:
Bioassay test are carried out for testing the efficacy of hexane leaf extract of S. campalulata on Ae.aegypti at different stages of development viz I, II, III and IV instars and pupae (Pampana, 1963 ). Mortality rates of larvae were recorded after 24 and 48 hours. Five or more concentrations of a test compound giving between 0 and 100% mortality for larvae at different instar stages were tested. In recording the percentage mortalities for each concentration, the moribund and dead larvae in three replicates were combined. The values of LC 50 /24, 48hrs and their 95% confidence limit of upper confidence limit (UCL) and lower confidence limit (LCL), regression and chi-square values were calculated using probit analysis (Finney, 1971). The SPSS 17.0 (Statistical Package of Social Sciences) used for statistical analysis.

Ovicidal assay:
Effect of hexane leaf extract of S.campanulata on the hatchability of Ae.aegypti eggs were determined (Judson & Gojrati, 1967). Hatching rate was calculated on the basis of non-hatchability of eggs (Sahgal & Pillai, 1993). The data were statistically examined using Student’s t-test.

Results and discussion:-
Phytochemical analysis of the hexane leaf extract of S.campalulata:
The qualitative phytochemical analysis revealed the presence of different phytochemicals such as carbohydrates, tannins, flavonoids, alkaloids, terpenoids, phenols, coumarins, and phytosteroids (Table-1).

Gas Chromatography- Mass Spectrometry (GC-MS) analysis of the hexane leaf extract of S.campalulata:
Important compounds identified in the GC-MS analysis of hexane leaf extract of S.campanulata. 3-Methyl-6-isopropylcyclohex-3-en-1-one, 2-Hexadecanol-1 ol,3,7,11,15,tetramethyl1-[R*-R*-(E)]-(CAS) Phytol Isomer, Hexane 1,6-dicyclocexyl, 2-Pentadecanone 6,10,14-trimethyl-, Neophytadine, Nonacosane (CAS), 13-Docosenamide, Phthalic acid, di(2-propylpentyl)ester, Hexanoic acid 4-hexadecyl ester, 4-Ethoxyisophosphamamide, 2(4H)-Benzofuranone 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-(CAS), Docosane (CAS),Octacosane(CAS), 2,6,10,14,18,22-Tetracosahexane 2,6,10,15,19,23-hexamethly-(CAS), Hexadecanoic acid, methyl ester(CAS), Tricosane(CAS), Juniperencamphor, Neopentyl bromoacetate, Phytol acetate, Palmitaldehyde, dially acetal (CAS), Ligularine, Hexadeacane(CAS), Octadecane (CAS) (Table-2).

Toxicity of hexane leaf extract of S.campanulata to the developmental stages of Ae.aegypti:
1. Bioassay test were conducted to find out the toxicity of hexane leaf extract to I, II, III, IV instars and pupae of the mosquitoes of Ae. aegypti. The data were subjected to Finney’s method of probit analysis. The results expressed in terms of LC 50 /LC 90 /24 .48 hour.
2. LC 50 /LC 90 /24 hour values of hexane leaf extract of S.campanulata to I instar larvae was 0.12%, 0.41% (24hrs), and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance to the hexane leaf extract of S.campanulata as evident from the relatively higher LC 50 /LC 90 /24 hour values 0.65% , 0.011 % (Table-3).
3. LC 50 /LC 90 /48 hour values of hexane leaf extract of S.campanulata to I instar larvae was 0.011% , 0.23 %(48hrs), and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance
Effect of hexane leaf extract of *S. campanulata* hatching of *Ae. aegypti* eggs:

Freshly laid eggs obtained from the general stock of mosquitoes were tested for their hatching ability in relation to the different concentrations of hexane leaf extract of *S. campanulata*. Percent hatch of eggs placed in control medium was 93 % where as in 0.1, 0.3, 0.5, 0.7 and 0.9% concentrations it was 76.51, 43 and 26. 0.9 % dose completely arrested hatching eggs. The decrease in hatchability was found to be dose dependent (Table-4). The results showed that the hexane leaf extract of *S. campanulata* possesses significant larvicidal properties against *Ae. aegypti*. The findings agree with some of the previous reports. Methanolic leaf extract of *S. campanulata* were found most effective with *LC*$_{50}$/24hour values of 1.343, 1.607, 1.981, 2.165 and 2.432 against I, II, III, IV and pupae of *An. stephensi* respectively (Aarthi et al., 2010); *LC*$_{50}$/24 hour values of ethanolic leaf extract of *Leucas aspera* against 1$^{st}$, 2$^{nd}$ instar larvae and pupae of *An. stephensi* was 4.31, 4.46, and 8.94 % (Sivapriyajothi et al., 2013); the *LC*$_{50}$/24 hour values of ethanolic leaf extract of *Delonix elata* to 1, 2, 3, 4 instar larvae of *Ae. aegypti*, were 4.91,5.16, 5.95 and 6.87% (Vasugi et al.,2013); *LC*$_{50}$/24 hrs values of methanol, aqueous, ethyl acetate, chloroform and petroleum ether leaf extracts of *Erythrina indica* were tested against the fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were 126.76,170.44, 155.87, 188.22, and 376.89, 411.92, 435.27, 466.13,565.94 ppm respectively (Rathi Sre et al.,2013); the LC$_{50}$/24 hour values of acetone leaf extract of *Rhodomyrtus tomentosa* against the first to fourth instar larvae and pupae of *Ae. aegypti* were 312.17, 352.65, 407.21, 471.06 and 523.91 ppm (Muthu et al.,2018); the *LC*$_{50}$/24/48 hour values of *Rhodomyrtus tomentosa* leaf methanolic extract (RTME) against the first to fourth instar larvae and pupae of *Ae. aegypti* were; 263.82 ppm (I), 299.83 ppm (II), 331.64 ppm (III), 386.64 ppm (IV) and 419.56 ppm (pupae); was 609.83 ppm (I), 677.22 ppm (II), 746.13 ppm (III), 858.83 ppm (IV) and 908.72 ppm (pupae) respectively (Kasinathan et al., 2018); the *LC*$_{50}$/ values of the aqueous and ethanolic extracts of *Artemisia annua* against 4$^{th}$ instar larvae of *Ae. aegypti* were recorded as 100.38, 45.25 and 120.37, 14.29 ppm and after 24 and 72 hours respectively (Alanazi, 2018); the methanolic leaf extract of *Lippia adoensis* with *LC*$_{50}$ = 94.71 ppm was revealed to be the most effective against 4$^{th}$ instar larvae of *An. gambiae* compared to *Hyptis suaveolens* *LC*$_{50}$ = 132.01 ppm and *Chenopodium ambrosoides* *LC*$_{50}$ = 204.56 ppm extracts 24 hours post-treatment (Oumarou et al., 2018); the 4$^{th}$ instar larvae of *An. stephensi* showed maximum larvicidal activity at a *LC*$_{90}$/48 hour value of 58.2 ppm for ethyl acetate extract. Above 90% mortality was observed for ethyl acetate extract at *LC*$_{50}$/48 hour of 62.2 ppm and 65.7 ppm for *Cx. quinquefasciatus* and *Ae. aegypti* respectively (Gandhimathi and Jeyasankar, 2018); the LC$_{50}$/24 hour values of the methanolic leaf extract of *Senna alata* on 1$^{st}$ instar larvae of *Ae. aegypti* was 0.011% and this was found to gradually increase with age of larvae. The pupae showed the highest resistance to the methanolic leaf extract of *Senna alata* as evident from the relatively higher LC$_{50}$/24 hour value 0.041% (Karthiyayni et al., 2018).

The effectiveness of this plant could be attributed to the presence of phytochemical compounds that act as insecticides (Abayomi,1993). *S.alata* leaves have furnished carbohydrates, tannins, flavonoids, terpenoids, coumarins, phenols, alkaloids, phytosteroids. These compounds were previously reported to have mosquito larvicidal activity (Faroq et al.,1999). These compounds may jointly (or) independently contribute to larvicidal activity against *Ae.aegypti*. The phytochemicals interfered with functioning of mitochondria (Usta et al.,2002) and primarily effect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae (Rey et al.,1999; David et al.,2003). Tannins are toxic by blocking the digesting of foods causing growth disturbances (Lumowa & Nova, 2014), also causing a water-absorption disorder in the larvae, thus causing death to the larvae (Steyaningsih & Pasmianti,2015). Flavonoids have larvicidal effects due to their mechanism of action to inhibit the respiratory system and disrupt the electron transport process in the larval body thus decreasing ATP production and reducing the use of oxygen by mitochondria (David et al.,2003). Alkaloids are nitrogenous compounds that show insecticidal properties at low concentration and the mode of action on insect vectors varies with the structure of their molecules, but many are reported to affect acetylcholinesterase (AChE) or sodium channels as inhibition of acetylcholinesterase activity is responsible for terminating the nerve impulse transmission through synaptic pathway (Rattan, 2010; Liu et al., 2012). Alkaloids work by constricting blood vessels and depressing autonomic nervous system activity thereby contributing to the insecticides effectiveness in killing the larvae of mosquitoes and disrupting the life cycle of the mosquito (Simon-Oke et al., 2015). Terpenoids are known to possess insecticidal properties (Mann & Kaufman, 2012). neurotoxic and mediate their toxic action via acting on acetylcholinesterase and octopaminergic system (Khanikor et al.,2013).
The findings of the present investigation were comparable with other ovicidal studies and revealed that the *S.campanulata* hexane leaf extract possesses ovicidal activity against the eggs of *Ae. aegypti*. Aqueous leaf extract of *Calotropis procera* treatment at 1000 ppm *Cx. tritaeniorhynchus* and *Cx. gelidus* eggs resulted into 100% ovicidal activity (Kumar et al., 2012); ovicidal activity with ethyl acetate, aqueous solution, ethanol leaf extracts of *Nerium oleander* against *An. stephensi* at 100, 150, 200, 250 and 300 ppm were calculated. With each extract at a concentration of 100 ppm, the percentage of hatchability was very high and nil hatchability was recorded when the concentration of extract was increased to 300 ppm in the case of aqueous and ethanol extracts (Roni et al., 2013); the percent hatch of the eggs placed in the control medium was 93 and 88% where as in 0.01, 0.05, 0.010 and 0.015% ; 0.1, 0.010, 0.020 and 0.030 concentrations of methanolic leaf extract of *Senna alata* and *Callistemon citrinus* it was 81.65, 71.65, 40 and 10; 72, 63, 45 and 20 respectively. 0.020 and 0.40% does completely arrested the hatching of the *Ae. aegypti* eggs (Karthiyayni et al., 2018; Palanikumar et al., 2017); number of *Ae.aegypti* eggs hatched reduced with increased concentration of aqueous extract of *Duranta erecta* and ranged between 81-22% against 92% for control (Julia et al., 2018); both acetone plant extracts exhibited excellent oviciding effect as 92.33% of *Ae. albopictus* eggs failed to be hatch when treated with 705.0 mg/L of *Melanochyla fasciculiflora* and 86.67% with 562.5 mg/L of *Gluta renghas* (Zuharah et al., 2015); ovicidal activity of methanol leaf extract of *Taddalia asiatica* was tested against the eggs of *Ae. aegypti*. It was observed that methanol extract concentration at 400ppm produced 99.3%, 94.00% and 100.00% ovicidal activity against *Ae. aegypti* (Saranya et al., 2018); in the case of ovicidal activity, exposure of freshly laid eggs was more effective than that of the older eggs (Miura et al., 1976). The hexane leaf extract of *S.campanulata* treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion (Rajkumar et al., 2011). Any compound that can cause permeability or a disruption to the chorionic layers in order to effectively deliver compounds that can terminate embryogenesis can be considered for development of effective ovicides. Our compound disrupted chorionic layers (Plate. 1c,1e,) so, it can be consider for development of effective ovicides. When eggs were directly exposed to high concentrations of the compounds, more chemicals entered the egg shell, which affected the embryogenesis; similarly, longer exposure periods also facilitated the increased penetration of the compounds into the shells, thus increasing their effectiveness (Broadbent & Pree, 1984). The mechanism through which the embryos died may not be known as this was not investigated by the present study but it is speculated it could have been due to blackage of the micropyle apparatus (Plate.1c) thereby interfering with gaseous exchange or by interfering with purity of the gases within the operculum preventing the embryo from accessing air and thus ultimately dying (Bhatnagar & Sharma, 1994).

**Conclusion:**

The present investigation revealed that hexane leaf extract of *S.campanulata* possesses remarkable larvicidal, ovicidal effects against dengue vector mosquito. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants. Further investigations about the mode of action of the constituent, effect on non-target organisms and field evaluation are necessary prior to commercialization.

**Table1:** Qualitative phytochemical analysis of *S.campanulata* hexane leaf extract.

| PHYTOCHEMICAL CONSITUTANTS | RESULTS |
|---------------------------|---------|
| Carbohydrates             | +       |
| tannins                   | +       |
| saponins                  | -       |
| flavonoids                | +       |
| alkaloids                 | +       |
| quinones                  | -       |
| glycosides                | -       |
| cardiacglycosides         | -       |
| terpenoids                | +       |
| triterpenoids             | -       |
| phenols                   | +       |
| coumarins                 | +       |
| steroids                  | -       |
| phytosteroids             | +       |
| Stages of development (Instars) | Number of larvae/trail | LC₅₀/24 hour(%) | Confidence limit LCL(%) | UCL(%) | Regression Equation | R Value | Slope | Chi-Square | Degrees of Freedom |
|-------------------------------|------------------------|----------------|--------------------------|--------|---------------------|--------|-------|------------|-------------------|
| I                             | 20                     | 0.12           | 0.094                    | 0.27   | Y=270.94x+18.861    | 0.874  | 100.1  | 0.487      | 3(16.26)          |
| II                            | 20                     | 0.31           | 0.28                     | 0.35   | Y=235x-19.5         | 0.991  | 94.6   | 0.615*     | 3(16.26)          |
| III                           | 20                     | 0.49           | 0.41                     | 0.53   | Y=138.33x           | 0.831  | 200    | 0.862*     | 3(16.26)          |
| IV                            | 20                     | 0.53           | 0.48                     | 0.56   | Y=213x-56.3         | 0.975  | 195    | 0.848*     | 3(16.26)          |
| Pupae                         | 20                     | 0.65           | 0.61                     | 0.69   | Y=205x-75           | 0.993  | 205    | 0.891*     | 3(16.26)          |

1. LC₅₀ – lethal concentration that kills 50 % of the exposed larvae and pupae.
2. LCL – lower confidence limit.
3. UCL – upper confidence limit.
4. R-value – regression value.
5. *, P< 0.001 level of significance of chi-square values.
Table 2:- Important compounds identified in the GC- MS analysis of hexane leaf extract of *S.campanulata.*

| S.No | Retention Time | Area % | Molecular Name | Molecular Formula | Molecular Weight |
|------|----------------|--------|----------------|------------------|-----------------|
| 1    | 3.05           | 24.17  | 3-Methyl-6-isopropylcyclohex-3-en-1-one | C_{10}H_{16}O | 152             |
| 2    | 25.19          | 11.45  | 2-Hexadecen-1-ol,3,7,11,15,tetramethyl1-[R-[R*-R*-E]]-(CAS) Phytol Isomer | C_{20}H_{34}O | 296             |
| 3    | 37.01          | 7.88   | Hexane, 1,6-dicyclohexyl | C_{16}H_{34} | 250             |
| 4    | 19.89          | 7.35   | 2-Pentadecanone, 6,10,14-ethyl- | C_{18}H_{32}O | 268             |
| 5    | 18.66          | 7.02   | Neophytadine | C_{20}H_{38} | 278             |
| 6    | 36.23          | 5.91   | Nonacosane (CAS) | C_{29}H_{60} | 408             |
| 7    | 39.11          | 5.90   | 13-Docosenamide | C_{32}H_{60}NO | 337             |
| 8    | 33.19          | 4.57   | Phthalic acid, di(2-propylpentyl)ester | C_{24}H_{36}O_{4} | 390             |
| 9    | 30.22          | 3.16   | Hexanoic acid, 4-hexadecyl ester | C_{22}H_{42}O_{3} | 340             |
| 10   | 23.06          | 2.81   | 4-Ethoxyisophosphamide | C_{24}H_{24}N_{4}O_{4}P | 312             |
| 11   | 17.17          | 2.03   | 2(4H)-Benzoquinone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-(CAS) | C_{11}H_{16}O_{2} | 180             |
| 12   | 38.78          | 1.81   | Docosane(CAS) | C_{29}H_{62} | 408             |
| 13   | 32.60          | 1.78   | Octacosane(CAS) | C_{32}H_{68} | 394             |
| 14   | 36.65          | 1.76   | 2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-hexamethyl-(CAS) | C_{38}H_{50} | 410             |
| 15   | 21.7           | 1.29   | Hexadecanoic acid, methyl ester(CAS) | C_{17}H_{32}O_{2} | 270             |
| 16   | 34.2           | 0.95   | Triocosane(CAS) | C_{32}H_{56} | 324             |
| 17   | 17.91          | 0.90   | Junipercamphor | C_{15}H_{25}O | 222             |
| 18   | 15.75          | 0.82   | Neopentyl bromoacetate | C_{10}H_{19}BrO_{2} | 208             |
| 19   | 35.07          | 0.81   | Phytol, acetate | C_{22}H_{42}O_{2} | 338             |
| 20   | 26.19          | 0.74   | Palmitaldehyde, diallyl acetol (CAS) | C_{22}H_{42}O_{3} | 338             |
| 21   | 28.14          | 0.73   | Lligularine | C_{28}H_{43}NO_{9} | 465             |
| 22   | 13.30          | 0.66   | Hexadecane(CAS) | C_{16}H_{34} | 226             |
| 23   | 14.74          | 0.53   | Octadecane (CAS) | C_{18}H_{38} | 282             |

Table 3:- LC_{90}/24 hour values of hexane leaf extract of *S.campanulata* to the pre-adult stages (I,II,III,IV instar and pupae) of *Ae.aegypti*

| Stages of development (Instars) | Number of larvae/trial | LC_{90}/24 hour(%) | Confidence limit | Regression Equation | R Value | Slope | Chi-Square | Degree of Freedom |
|---------------------------------|------------------------|--------------------|-----------------|---------------------|---------|-------|------------|------------------|
| I                               | 20                     | 0.41               | 0.391           | 0.45                | Y=120x+31       | 0.862 | 108        | 3(16.26)         |
| II                              | 20                     | 0.57               | 0.54            | 0.61                | Y=130x+12       | 0.832 | 89         | 3(16.26)         |
| III                             | 20                     | 0.75               | 0.72            | 0.79                | Y=111.96x       | 0.844 | 213        | 3(16.26)         |
| IV                              | 20                     | 0.89               | 0.86            | 0.93                | Y=-9.8959x+83.957 | 0.030 | 190        | 3(16.26)         |
| Pupae                           | 20                     | 0.011              | 0.009           | 0.16                | Y=-0.505        | 0.897 | 200        | 3(16.26)         |
1. LC$_{90}$ – lethal concentration that kills 90 % of the exposed larvae and pupae.
2. LCL – lower confidence limit.
3. UCL – upper confidence limit.
4. R-value – regression value.
5. *, P< 0.001 level of significance of chi-square values.

**Table 3:** LC$_{90}$/48 hour values of hexane leaf extract of *S.campanulata* to the pre-adult stages (I,II,III,IV instar and pupae) of *Ae.aegypti*

| Stages of development (Instars) | Number of larvae/trail | LC$_{90}$/48 hour(%) | Confidence limit | Regression Equation | R Value | Slope | Chi-Square | Degrees of Freedom |
|-------------------------------|------------------------|----------------------|------------------|---------------------|---------|--------|------------|------------------|
| I                             | 20                     | 0.23                 | LCL 0.204 UCL 0.256 | Y=196.5x+32.3 06   | 0.709   | 98.3   | 0.479     | 3(16.26)         |
| II                            | 20                     | 0.35                 | LCL 0.313 UCL 0.392 | Y=210x+7          | 0.747   | 102.8  | 0.613     | 3(16.26)         |
| III                           | 20                     | 0.54                 | LCL 0.521 UCL 0.587 | Y=140.74x          | 0.689   | 192    | 0.867     | 3(16.26)         |
| IV                            | 20                     | 0.61                 | LCL 0.584 UCL 0.648 | Y=190x-41         | 0.690   | 205    | 0.833     | 3(16.26)         |
| Pupae                         | 20                     | 0.82                 | LCL 0.796 UCL 0.863 | Y=-6.6792+75.021  | 0.003   | 199    | 0.881     | 3(16.26)         |

1. LC$_{90}$ – lethal concentration that kills 50 % of the exposed larvae and pupae.
2. LCL – lower confidence limit.
3. UCL – upper confidence limit.
4. R-value – regression value.
5. *, P< 0.001 level of significance of chi-square values.
4. R-value – regression value.
5. *, P< 0.001 level of significance of chi-square values.

**Table 4:** Alternation in the hatchability of *Ae. aegypti* eggs exposed to different concentrations of hexane leaf extract of *S. campanulata*

| Parameters                        | Control | Concentrations (%) |
|-----------------------------------|---------|--------------------|
|                                   |         | 0.1    | 0.3    | 0.5    | 0.7    | 0.9    |
| Number of eggs introduced         | 20      | 20     | 20     | 20     | 20     | 0      |
| Mean number of eggs hatched*      | 18.66   | 15.33  | 10.33  | 8.666  | 5.333  | 0      |
| S.D                               | ± 0.761 | ± 0.361| ± 0.452| ± 0.319| ± 0.539| 0      |
| Percent hatchability              | 93      | 76*    | 51*    | 43*    | 26*    | 0      |
| Percent reduction over control    | 18.27   | 45.16  | 53.76  | 72.04  | 100    |

1. , Mean (±SD) of 5 replicates.
2. #, Significantly different from control, P<0.001 %.

Scanning electron microscope (SEM) images of control and treated eggs of *Ae. aegypti*.

**Plate 1:** Control egg of *Aedes aegypti*,
**Plate 1a:** Damaged exo chorionic surface of *Ae. aegypti* egg treated with hexane leaf extract of *S. campanulata*. 
Plate 1b:- Micropylar apparatus (MPA) of the control Ae. Aegypti egg.

Plate 1c:- Damaged micropylar apparatus (DMPA) and damaged micropylar pore (DMPP) of Ae. Aegypti egg.

Plate 1d:- Posterior region of control Ae. Aegypti egg.

Plate 1e:- Damaged posterior region of Ae. Aegypti egg.

References:
1. Mehlhorn, H. (2008). Encyclopedia of parasitology (3edn). Springer, Heidelberg.
2. Mehlhorn, H., Al-Rasheid, K., Al-Quraishy, S. & Abdel-Ghaffar, F. (2012). Research and increase of expertise in arachno-entomology are urgently needed. Parasitol. Res., 110, 259-265.
3. Anitha, P. & Ijumba, J.N. (2013). Preferred breeding habitats of Aedes aegypti (Diptera:Culicidae) mosquito and its public health implications in Dares Salaam, Tanzania. J. Environ. Res. Manage., 4, 0344-0351.
4. Izabela, A., Zybert, R. & Wilschut, J. (2010). Dengue virus life cycle: viral and host factors modulating infectivity. Cell. Mole. Life Sci., 67, 2773-2786.
5. Weaver, A., Scott, C., Osorio., Jorge, E., Livengood Jill A., Chen Rubing., Stinchcomb. & Dan T. (2012). Chikungunya virus and prospects for a vaccine. Exp. Rev. Vacc., 11, 1087-1101.
6. Azami, M.N.A., Salleh, S.A., Neoh, H.M., Zakaria, S.Z.S. & Jamal, R. (2011). Dengue epidemic in Malaysia: not a predominantly urban disease anymore. BMC Res. Notes., 4,216.
7. Gubler, D.J. (2002). Epidemic dengue /dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol., 10, 100-103.
8. National Vector Borne Disease Control Programme (NVBDCP). 2014. National vector borne disease control programme. Dengue/dengue haemorrhagic fever. https://www.nhp.gov.in/nvbdcp.
9. Park, J.E. & Park, K. (1987). Text book of preventive and social medicine. XI ed. M/s Banarsides Bhanot, India, 217-221.
10. The Hindu, 2001. The Hindu daily. 18th October.
11. The Hindu, 2006. The Hindu daily. 18th October.
12. National Vector Borne Disease Control Programme (NVBDCP). 2011. Dengue cases and Deaths in the country since 2007. Available at: http://nvbdcp.gov.in/den.cd.html.
13. The Hindu, 2012. The Hindu daily. 15th July.
14. The Dinamani, 2013. The Dinamani daily. 17th August
15. The Dinamalar, 2014a. The Dinamalar Daily. 16th November.
16. The Dinamalar, 2014. The Dinamalar Daily. 10th December.
17. The Dinakaran, 2015. The Dinakaran Daily. 17th February.
18. The Dinakaran, 2016. The Dinakaran Daily. 27th August.
19. The Dinakaran, 2017. The Dinakaran daily. 1st October.
20. National Vector Borne Disease Control Programme (NVBDCP). (2018). Dengue cases and Deaths in the country since 2007. Available at: http://nvbdcp.gov.in/den.cd.html.
21. Sarita, K., Naim, W., Monika, M. & Radhika, W. (2012). Evaluation of 15 local plants species as larvicidal agents against an Indian strain of dengue fever mosquito, Aedes aegypti L. (Diptera: Culicidae). Front. Physiol, 3, 1-6.
22. Rutledge, C.R., Clarke, F., Curtis, A. & Sackett, S. (2003). Larval mosquito control. Tech. Bull. Florida Mosq. Control Assoc, 4, 16-19.
23. Mazzarri, M.B. & Georgiou, P. (1995). Characterization of resistance to organophosphate, carbamate and pyrethroid insecticides in field populations of Aedes aegypti from Venezuela. J. Am. Mosq. Control Assoc, 11, 315-322.
24. Seelena, P., Lee, H.L. & Lecadet, M.M. (1995). A new Serovar of Bacillus thuringiensis possessing 28a28c flagellar antigenic structure: Bacillus thuringiensis Serovar Jegathesan selectively toxic against mosquito larvae. J. Am. Mosq. Control Assoc, 13, 471-473.
25. Mulla, M.S., Su, T.Y., Thavara, U., Tawatsin, A., Nagamsuk, W. & Pan Ural, P. (1999). Efficacy of new formulations of the microbial larvicide Bacillus sphaericus against polluted water mosquitoes in Thailand. J. Vec. Eco, 24, 99-110.
26. Tabashnik, B. E. (1994). Evolution of resistance to Bacillus thuringiensis. Anu. Rev. Entomol, 39, 47-79.
27. Gluckman, T.L. & Hartney, K.B. (2000). A tropic analysis of mosquito Fish, Gambusia affinis Breder, inhabiting blue holes on Andras island, Bahamas. Car. J. Sci, 36, 104-111.
28. Bohidar, K. & Mohapatra, S. (2000). Predation efficacy on the water bug Sphacrodema annulatum Fab. against mosquito larvae. Insect Environ, 5, 173-174.
29. Laird, M., & Miles, J.W. (1983). Integrated mosquito control methodologies. Academic Press Inc. (London) Ltd., London.
30. Govindarajan, M., Sivakumar, R., Rajeswary, M. & Yogalakshmi, K. (2013). Chemical composition and larvicidal activity of essential oil from Ocimum basilicum (L.) against Culex tritaeniorhynchus, Aedes albopictus and Anopheles subpictus (Diptera: Culicidae). Exp. Parasitol, 134, 7-11.
31. Macedo, M.E., Consoli, R.A., Grandi, T.S., Anjosdos, A.M., de Oliveira, A.B., Mendes, N.M., Queiroz, R.O. & Zani, C.L. (1997). Screening of asteraceae (Compositae) plant extracts for larvicidal activity against Aedes fluviatilis (Diptera: Culicidae). Mem. Inst. Oswaldo. Cruz, 92, 565-568.
32. Ahmed, S., Graivey, M., Hylin, J.M., Mitchell, M.C. & Listinger, J.A. (1984). Some promising plant species for use as pest control agents under traditional farming system. In: Schnutterer A. and Ascher K.R.S., (eds) Proc. 2nd International neem conference, Rauischolzhausen, Germany; 565-580 GTZ, Eschborn, Germany.
33. Sukumar, K., Perich, M.J. & Boobar, L.R. (1991). Botanical derivatives in mosquito control: a review. J. Am. Mosq. Control Assoc, 7, 210-237.
34. Elimam, A.M., Elmalik, K.H. & Ali, F.S. (2009a). Efficacy of leaves extract of Calotropis procera Ait. (Asclepiadaceae) in controlling Anopheles arabiensis and Culex quinquefasciatus mosquitoes. Saudi. J. Biol. Sci, 16, 95-100.
35. Elimam, A.M., Elmalik, K.H. & Ali, F.S. (2009b). Larvicidal, adult emergence inhibition and oviposition deterrent effects of foliage extract from Ricinus communis L. against Anopheles arabiensis and Culex quinquefasciatus in Sudan. Trop. Biomed, 26, 130-139.
36. Prempree, P. & Sukhapangth, N. (1990). Phytochemical toxicity of the crude extract from Derris elliptica Bentham on mosquito larvae. J. Sci. Soc. Thailand, 16, 133-139.
37. Ojewole, J.A.O., Rahim, S. & Shode, F.O. (2000). Mosquito larvicidal properties of aqueous extract of Senna didymobotrya. Nig. J. Nat. Prod. Med, 4, 46-47.
38. Singh, M., Kumari, S., Attri, R. & Kumar, S. (2015). Impact of Calotropis procera leaf extracts on the survival, morphology and behavior of dengue vector, Aedes aegypti L. DU. J. Under. Res. Innova, 1, 96-107.
39. Rajkumar, S. & Jebanesan, A. (2004). Ovicidal activity of Solanum trilobatum L. (Solanaceae) leaf extract against Culex quinquefasciatus Say and Culex triaeniorhynchus Giles (Diptera: Culicidae). Int. J. Trop. Ins. Sci., 24, 340-342.
40. Khanna, V.G. & Kannabiran, K. (2007). Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre and Eclipta prostrata against Culex quinquefasciatus mosquito larvae. African. J. Biotech, 6, 307-311.
41. Govindarajan, M. (2009). Bioefficacy of Cassia fistula Linn. (Leguminosae) leaf extract against Chikungunya vector Aedes aegypti (Diptera: Culicidae). Eur.Rev.Med. Pharmacol.Sci, 13, 99-103.
42. Okeke, R.N., Okeke, J.J. & Madu, N.C. (2010). Larvicidal effects of Azadirachta indica, Ocimum gratissimum and Hyptis suaveolens against mosquito larvae. J. Agri. Tech, 6, 703-719.
43. Ramanibai, R., Mahavaran, A. & Boomilingam, M. (2011). Larvicidal efficacy of Leucas aspera (Wild) extracts against the mosquito larvae of Culex quinquefasciatus. J. Pharma. Res, 4, 4424 - 4425.
44. Swathi, S., Muruganathan, G., Ghosh, S. K. & Pradeep, A. S. (2012). Larvicidal and repellent activities of ethanolic extract of Datura stramonium leaves against mosquitoes. Int. J. Phar. Phyto. Res, 4,25-27.
45. Saranya, M., Mohanraj, R.S. & Dhanakkodi, B.(2013a.).Oviposition and oviposition deterrence activity of aqueous leaf extract of Spathodea campanulata P. Beauv. (Bignoniaceae) against Aedes aegypti (Diptera: Culicidae). Int. J. Rec. Sci. Res,4, 28-34.
46. Saranya, M., Mohanraj, R.S. & Dhanakkodi, B.(2013b). Larvicidal, pupicidal activities and morphological deformities of Spathodea campanulata aqueous leaf extract against the dengue vector Aedes aegypti. Euro. J. Exp. Bio,3,203-213.
47. Saranya, M., Mohanraj, R.S. & Dhanakkodi, B.(2013c).Adult emergence and prolong larval duration effects of Spathodea campanulata aqueous leaf extract against the dengue vector Aedes aegypti. Advance Appl. Sci. Res, 4,372-380.
48. Karthika Devi, K., Mohanraj, R.S. & Dhanakkodi B.(2013). Mosquitocidal activities of Spathodea campanulata methanolic leaf extract against the dengue vector Aedes aegypti. Asian J. Plant Sci. Res, 3,138-149.
49. Pravin, Y., Saranya, M., Sivakumar, T., Mohanraj, R.S. & Dhanakkodi, B.(2014).Oviposition deterrent activity of acetone leaf extract of Spathodea campanulata P.Beauv. (Bignoniaceae) against chikungunya vector Aedes aegypti (Diptera: Culicidae). Am. J. Pharm. Health Res, 2, 1-16.
50. Mahendran, S., Pravin, Y., Saranya, M., Sivakumar, T., Mohanraj, R.S. & Dhanakkodi,B.(2015). Ovipositional deterrence, ovicidal and developmental effects of Pithecellobium dulce (Roxb.) Benth. (Fabaceae) aqueous leaf extract against dengue vector mosquito Aedes aegypti (Diptera: Culicidae). Int. J. Curr. Res. Biosci. Plant Biol,2, 79-91.
51. Pravin, Y., Saranya, M., Sivakumar, T., Mahendran, S., Mohanraj, R.S. & Dhanakkodi, B.(2015). Larvicidal, pupicidal, ovicidal activity and GC-MS analysis of Spathodea campanulata P.Beauv. (Bignoniaceae) acetone leaf extract against the dengue vector mosquito Aedes aegypti (Diptera: Culicidae). Int. J. Curr. Res. Aca. Rev, 3,92-111.
52. Navaneethan, M., Pravin, Y., Mohanraj, R.S. & Dhanakkodi B.(2016). Tecoma stans (L.) Juss. Ex Kunth (Bignoniaceae) a prospective mosquito repellent in the management of zika virus vector mosquito Aedes aegypti (Diptera: Culicidae). Int. J. Microbiol. App. Sci, 5, 869-889.
53. Palanikumar, M., Pravin, Y., Navaneethan, M., Mahendran, S., Mohanraj, R.S. & Dhanakkodi, B.(2017). Callistemon citrinus (Myrtaceae) methanolic leaf extract: a potent mosquitocidal agent for controlling dengue vector mosquito Aedes aegypti (Diptera: Culicidae).J.Entomol. Zool. Stud, 5,1051-1059.
54. Mashlawi, A.M. & Ali, M.K.H.(2017). Evaluation of the biological activity of crude latex and ethanolic leaves extracts of Calotropis procera (Asclepiadaceae) against the mosquito vector Culex quinquefasciatus (Diptera: Culicidae). Int. J. Mosq. Res. 4, 01-06.
55. Karthikeyani, N., Pravin, Y., Palanikumar, M, Mohanraj, R. S. & Sujatha. P. S.(2018). Mosquitocidal efficacies of the medicinal plant Senna alata methanolic leaf extract against dengue and zika virus vector Aedes aegypti Int.J.Pharma.Bio Sic, 9(3),B) 244-255.
56. Lawi, Y., Saria, J. & Kidakuli, A.W.(2018). Brine shrimp cytotoxicity, phytochemical screening and larvicidal activities of Plectranthus barbatus barbatus extracts. Res. Rev. Insights, 2, 1-4.
57. Sebastian, A., Jhon, D.M., George, R. & Omanakuttan, S.(2018). Comparative study on the effect of aqueous and ethanol extracts of Carica papaya and Cereus pterogonus on Aedes aegypti. Int. J. Recent Scienti. Res, 9, 26697-26699.
58. Dhanasekaran, S., Bharanitharan, M., Muthulingam, M., Senthilmurugan, S., Jayakumar, A. & Sawicka B.(2018). Studies on the impact of medicinal plants in relation to malarial vector control against Anopheles stephensi. Innorginal Int. J. Sci, 5, 12-14.
59. Rathnasakar, K. & Thiyyagaraj, A.(2018). Larvicidal activity of Lantana indica and Vitex negundo on Culex quinquefasciatus. Asian J. Pharm. Clin. Res., 11: 414-418.
60. Iqbal, J., Ishtiaq, F., Algarni, S. A. & Owayss, A. A.(2018). Evaluation of larvicidal efficacy of indigenous plant extracts against Culex quinquefasciatus (Say) under laboratory conditions. Turk J. Agric. For, 42, 1-9.
61. Kowti, R., Harsha, R., Ahmed, M.G., Hareesh, A.R., Gowda, T.S.S. & Dinesha, R. (2010). Antimicrobial activity of ethanol extract of leaf and flower of Spathodea campanulata P. Beauv. Res. J. Pharm . Biol & Chem. Sci, 3,691 – 698.
62. Kowti, R., Joshi, V., Dabadi, P., Gowda, T.S.S., Sathish, B.P. & Dinesha R. (2011). Antioxidant activity of Spathodea campanulata in prevention of T – BOOH and H2O2 induced DNA damage. Int. J. Curr. Pharm. Res, 3 : 87 - 89.
63. El – Hela, A.A.( 2001). A phenolics from Spathodea campanulata Beauv. Leaves. Al – Azar J. pharm. Resi, 27, 152 – 162.
64. Lymio, E.O.W. & Koella, J.C.(1992). Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of Anopheles gambiae. Entomol. Exp. Appl, 63, 265-271.
65. Judson,C.L. (1967). Alternation of feeding behavior and fertility in Aedes aegypti by the chemosterilant apholate. Entomol. Exp. Appl,10, 387-394.
66. Briegel A.(1990). Metabolic relationship between female body size, reserves, and fecundity of Aedes aegypti. J.Insect physiol,36, 165-172.
67. Villani, F., White, G.P., Curtis, C.F. & Miles, S.J. (1983). Inheritance and activity of some estrases associated with organophosphate resistance in mosquitoes to the complex of Culex pipiens L. (Diptera: Culicidae) . Bull.Ent. Res,73, 153-170.
68. Kongkathip,N.(1994).Chemistry and extraction method of neem- Bankok : 3rd Workshop in the using neem leaf extracts for control and eradicate insects.
69. Harbone, J.B.(1984). Phytochemical methods. A guide to modern technique of plant analysis.Chapman and Hall, London, 49-188.
70. Trease, G.E. & Evans, W.C.(1989). Ed. Pharmacognosy, 11th Edition, Brailliar Tridel and Macmillan Publishers, London.
71. Pampana, E.(1963). A text book of malaria eradication. Oxford University Press, London, pp:459-462.
72. Finney, D.J.(1971). Probit Analysis.Cambridge University, London, 68-78pp.
73. Judson, C.L. & Gojhati, H.A.N.(1967).The effects of various oxygen tensions on embryogeny and larval responses of Aedes aegypti. Entomol.Exp. Appl,10, 181-188.
74. Sahgal,Ak Pillai, M.K.K.(1993).Ovicidal activity of permethrin and deltamethrin on mosquitoes.Entomon, 17,149-154.
75. Aarthi, N. & Murugan, K.(2010). Larvicidal and smoke repellent activities of Spathodea campanulata P.Beauv.against the malarial vector Anopheles stephensi Lis. (Diptera: Culicidae)J. Phyto. 2: 61-69.
76. Sivapriyajothi, S., Murugan, K., Mary, C., Roni, M. & Suganya, N.A.(2013). Effect of Leucas aspera leaf extract against the malarial vector, Anopheles stephensi. Int. J. Curr. Trop. Med. Heal. Res, 1, 019-025.
77. Vasugi, C., Kamalakannan, S. & Murugan, K.(2013). Toxicity effect of Delonix elata (Yellow Glumohr) and predatory efficiency of Copeod, Mesocyclops aspercornis for the control of dengue vector, Aedes aegypti. Asian. Pac.J. Trop. Dis,3, 119-126.
78. Rathi Sre, P.R., Reka, M., Elumalai, D., Kaleena, P.K. & Murugesan, K.(2013). Comparative study of larvicidal activity of leaf and root extracts of Erythrina indica lam against Aedes aegypti and Culex quinquefasciatus.Am.J.Pharma. Tech. Res, 3, 357-367.
79. Muthu, K., Jayapal, S., Elanchezhiyan, C., Samivel, K. & Mayakrishnan, V.(2018). Mosquitocidal potential of Rhodomyrtus tomentosa leaf extracts against Dengue and Zika virus vector , Aedes aegypti. Int. J. Entomol. Res, 3,95-100.
80. Kasinathan, M., Subramaniam, J., Elanchezhiyan, C., Kanthammal, S. & Vijay .M. ( 2018).Mosquitocidal potential of Rhodomyrtus tomentosa leaf extracts against dengue and zika virus vector , Aedes aegypti. Int. J. Entomol. Res. 3,95-100.
81. Alanazi A. N. (2018). Larvicidal effect of Artemisia annua (Asterales: Asteraceae) against the dengue fever mosquito vector Aedes aegypti (Diptera: Culicidae). Int. J. Mosq. Res, 5: 35-38.
82. Oumarou, K. M., Younoussa, L. & Nukenine, N.(2018). Toxic effect of Chenopodium ambrosoides, Hyptis suaveolens and Lippia adoensis leaf methanol extracts against fourth instar larvae of Anopheles gambiae (Diptera: Culicidae). Int. J. Mosq. Res, 5, 61-66.
83. Gandhimathy, S. & Jeyasankar, A.(2018). Larvicidal properties of Psychotria octosulcata (W. A.Talbot,) (Rubiaceae) crude extracts on human vector mosquitoes Aedes aegypti (Linn.), Culex quinquefasciatus (Say.) and Anopheles stephensi Liston. J. Entomol. Zool. Stud. 6,1190-1195.
84. Abayomi, S.(1993). Historical review of traditional medicine in Africa. Ibadan: Spectrum Book Ltd, 3, 9-25.
85. Farooq, A.M., Waseem, B., Muzaffar, R., Tirupathi, J., Tharani, M. & Sharma, M . ( 2014). A comparative study of phytochemical investigation of Xanthium stramarium medicinal plant. Int. J. Pharma. Che, 4, 96-100.
86. Usta, J., Kreydiyyeh, S., Bakajian, K. & Chmaissee, H.N.(2002). In vitro effect of eugenol and cinnamaldehyde on membrane potential and respiratory complexes in isolated rat liver mitochondria. Food. Chem. Toxicol, 40, 935-940.
87. Sudjari, D.H,, Agustin, I.M.K. & Telussa, A.S.(2008). Pengaruh deok daun mint (Mentha arvensisvar Javanica) sebagaim larvaisida nabati nyamuk Anopheles sp. di Pantai Balekambang, Kecamantan Bantur, Kabupaten Malang [Online Journal].Download in 26 September 2012.]Available at: http://elibrary.ub.ac.id
88. Rey, D., Pautou, M.P & Meyran, J.C.(1999). Histopathological effects of tanic acid on the midgut epithelium of some aquatic dipteran larvae. J. Invertebrate Pathol, 73 , 173-181.
89. David, J.P., Rey, D., Pautou M.P. & Meyran, J.C. (2003). Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. J. Invertebrate Pathol, 75, 9-18.

90. Lumowa, S.V. & Nova, T.P. (2014). Larvicidal effect of Syzygium polyanthum W. Leaf extract against Aedes aegypti L. larvae. Prog Health. Sci, 5, 102-6.

91. Steyaningsih, N.M., & Pasmitai, I.K. (2015). Efektitias Ekstrak Etanol Daun Salam, (Syzygium polyanthum) sebagai larvasida terhadap Larva Nyamuk Aedes aegypti. Unud, 2015.

92. Rattan, S. R. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection, 29, 913-920.

93. Liu, Z. L., Liu, Q. Z., Du, S. S. & Deng, Z. W. (2012). Mosquito larvicidal activity of alkaloids and limonoids derived from Evodia rutaecarpa unripe fruits against Aedes albopictus (Diptera: Culicidae). Parasitol. Res, 111, 991-996.

94. Simon-Oke, L. A., Afolabi, O. J. & Ajayi, O. T. (2015). Larvicidal activity of a perennial herb, Solanum xanthocarpum against the larvae of culicine species. Futa J. Res. Sci, 1, 152-156.

95. Mann, R.S & Kaufman, P.E. (2015). Natural product pesticides: their development, delivery and use against insects insect vector. Mini. Rev. Org. chem, 9, 185-202.

96. Khanikor, B., Parida, P., Yadav, R.N.S., & Bora, D. (2013). Comparative mode of action of some terpene compounds against octopamine receptor and acetyl cholinesterase of mosquito and human system by the help of homology modelling and Docking studies. J. Appl. Pharm. Sci, 3, 6-12.

97. Kumar, G., Karthik, L., Venkata, B.R.K., Kirthi, A.V, Jayaseelan, G., & Rahuman, A. (2012). Phytochemical composition, mosquito larvicidal, ovicidal and repellent activity of Calotropis procera against Culex tritaeniorhynchus and Culex gelidus. Bangladesh J. Pharma, 7, 1-5.

98. Roni, M., Murugan, K., Mary, C., Sivapriyajothi, S., Sugana, S., Dinesh, N.A. & Vivek, S. (2013). Ovicidal and adulticidal activity of Nerium oleander extract against Anopheles stephensi Liston (Diptera: Culicidae). Int. J. Innovat. Res, 1, 12-18.

99. Julia, A. K., Gregory, O. E., Felicia, E., Michael, U., Godwin, N. & Chinelo E. (2018). Bioefficacy of Duranta erecta leaf extract on yellow fever dengue vector, Aedes aegypti Linn. in Nigeria. J. Med. Plants Res, 2: 124-132.

100. Zuharah, W. F., Ling, C. J., Zulkifly, N. & Fadzly, N. (2015). Toxicity and sub-lethal effect of endemic plants from family Anacardiaceae on ovipoaition behavior of Aedes albopictus. Asian Pac. J. Trop. Biomed, 5, 612-618.

101. Saranya, J., Kasinathan, I. D. & Elumalai, K. (2018). Bio-efficacy of five plant extracts against Callosobruchus maculatus (Stored pest), Spodoptera litua (Field pest) and Aedes aegypti (vector). World J. Pharma. Res, 7, 1189-1203.

102. Miura, T., Schaefer, C.H, Takahashi, R.M. & Mulligan, F.S. (1976). Effects of the insect growth inhibitor, dimilin on hatching of mosquito eggs. J. Econ. Entomol, 69, 655-658.

103. Rajkumar, S., Jebanesan, A. & Nagarajan, R. (2011). Effect of leaf essential oil of Coccinia indica on egg hatchability and different larval instars of malarial mosquito Anopheles stephensi. Asian Pac. J. Trop. Med, 4, 948-951.

104. Broadbent, A.B & Pree, D.J. (1984). Effects of diflubenzuron and bay SIT 8514 on the oriental fruit moth and oblique bended leaf roller. J. Econ. Entomol, 77, 194-197.

105. Bhatnagar, A. & Sharma, V.K. (1994). Insecticidal and ovicidal activities of plant extracts against maize stem borer, Chilo partellus (Swinhoe). Plant Protect. Bull, 46, 12-16.