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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)

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A B S T R A C T

Development of insect resistance to synthetic pesticides , high operational cost and environmental pollution have created the need for developing alternative approaches to control vector-borne diseases. The objective of the present study was to observe larvicidal, pupicidal and ovicidal effects of acetone extract of leaves of *Spathodea campanulata* against the dengue vector *Aedes aegypti*. Bioassay test was carried out by WHO method for larva and of pupa *Ae. aegypti* mosquito for 24hrs. Ovicidal activity was determined against *Ae. aegypti* species at various concentrations under the laboratory conditions. Different compounds of the acetone leaf extract of *S. campanulata* leaf identified by the Gas chromatography - Mass Spectrometery (GC-MS).Considerably low $LC_{50,}$ / 24, hours values of acetone 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, pupicidal property of the plant. Young larvae were found to be relatively more susceptible than the older ones. The hatchability of *Ae. aegypti* eggs was decreased when placed in media of acetone leaf extract. The reduction in percent hatch was inversely proportional to the concentration of acetone leaf extract used. From the results it can be concluded the acetone leaf extract of *S. campanulata* is an excellent natural alternative to get control over *Ae. aegypti* mosquitoes.

Introduction

Our world today is still plagued by a myriad of aliments/ diseases and a number of these diseases are caused by organism which are vector- borne. Mosquitoes which also serve

as vectors of diseases to humans have a worldwide distribution. Mosquito, living throughout the world apart from the Antarctic regions (Mullen and Durden, 2009) and is one of the most tarnished creatures in the animal kingdom with horrific reputation as highly persuasive vector of several diseases.

There are currently more than 300 mosquito species in the world grouped in 39 genera and 135 sub genera and more than a hundred species are capable of transmitting various diseases to humans (Reuda**,** 2008). Mosquito menace is particularly high in South East Asian countries (Rao *et al.,* 2008) and in recent years global warming has lead to the spread of mosquitoes into temperate countries and higher altitude regions and the people in these regions are severely affected (Nerio *et al.,* 2010)

Among all 135 sub genera, *Aedes aegypti* is a very important disease transmitting vector. *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. It is breeds in many types of household containers, such as water storage jars, drums, tanks and plant or flower containers (Harrington *et al.,* 2005). Compared to any other species of *Aedes*, *Ae. aegypti* shows more dependency on human blood (Scott *et al.,* 1993) *Ae. aegypti* breeds throughout the year. The eggs laid singly on the side of containers at or above the water line and also on the water surface. Hatching can take place in 2 or 3 days. These mosquitoes go through distinct stages of development: egg, larva, pupa and adult. The life cycle can be completed in about 10 days. The adult life-span of a mosquito is 50–55 days or approximately two months (Park and Park, 1987).

Ae. aegypti is generally known as a vector for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Pancharoen *et al.,* 2002) About two-thirds of the world's population lives in tropical and subtropical areas infested with dengue vectors, mainly *Ae. aegypti* (Hahn *et al.,* 2001).

Dengue or 'break bone' fever had been known in our country for every long time. Recently, the dengue fever virus is found in the patients of Tamilnadu, Andra Pradesh, Karnataka, Kerala and Maharastra states severely. Epidemic outbreaks of dengue fever have also been reported in India. For instance, in 1980 a total of 4,601 cases were recorded (Park and Park, 1987). In October 2001, an outbreak of dengue resulting in 16 deaths was reported in Chennai (Tamil Nadu) India (The Hindu, 2001). In October, 2006, a total of 5,710 cases were recorded in India. Delhi had the highest (1,637) patients. Tamilnadu, India had 307 patients; 103 deaths were also reported (NVBDCP, 2011). In 2010, there were a total of 28, 292 cases and 110 deaths (The Hindu, 2006). In 2012 a total of 9,000 cases and 50 deaths were reported in Madurai, Tirunelveli and Kanyakumari districts (Tamil Nadu) (The Hindu, 2012). According to the Central Health Ministry of India in 2013, 17,000 people affected by this disease, in Tamilnadu alone 4,000 affected by dengue (The Dinamani, 2013).

Chikungunya, a febrile disease is caused by Chikungunya virus which is transmitted by *Ae. aegypti*. There was an outbreak of this disease in Calcutta in 1963-1964 and another in Madras (Chennai) in 1965 which gave rise to 3,00,000 cases in Madras city alone (Park and Park, 1987).

According to Central Health Secretary of India, in 2006, 13 lakh people affected by this disease. In Tamil Nadu alone 63,000 persons were affected by this disease (NVBDCP, 2011). In 2013, a total of 500 cases were reported in Thirunelvali district (Uthakulam village) Tamilnadu, India (The Dinamani, 2013). These diseases devastate Indian economy every year (Jaswanth *et al.*, 2002).

At present, no effective vaccine is available for dengue; 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).

Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into the food chain. They even result in mutation of genes and these changes become prominent only after a few generations (Ghose, 1991). This phenomenon has triggered and urged the development of alternative techniques using natural products.

During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Mittal and Subbarao, 2003). In this regard, India has a rich flora that is widely distributed throughout the country. More than 2000 plants 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). Phytochemicals are advantageous due to their eco-safty, target- specificity, non development of resistance, reduced number of applications, higher acceptability and suitability for rural areas. Plants being rich source of bioactive chemicals (Rajkumar and Jebanesan, 2004) and so far there is no report of resistance to plant extracts (Sharma *et al.*, 1992). Botanical insecticides also have potential uses such as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility (Elimam *et al.*, 2009a,b). Some of the plant leaf extract tested for their diverse insecticidal properties on the medically important mosquitoes are: methanolic extracts of *Derris elliptica* leaves (Prempree and Sukhapanth, 1990); aqueous extract of *Senna didymobotrya* leaves (Ojewole *et al.*, 2000); acetone extract of *Solanum trilobatum* leaves (Rajkumar and Jebanesan, 2004); aqueous extract of *Gymnema sylvestre* and *Eclipta prostrate* leaves (Khanna and Kannabiran, 2007); methanol, benzene and acetone leaf extracts of *Cassia fistula* (Govindarajan, 2009); petroleum ether extract of *Azadirachta indica*, *Ocimum*

gratissimum and *Hyptis suaveolens* leaves (Okigbo *et al.,* 2010); aqueous and chloroform extracts of *Leucas aspera* leaf (Ramanibai *et al.,* 2011); ethanolic extract of *Datura stramonium* leaves (Swathi *et al.,* 2012); aqueous extract of *Spathodea campanulata* leaves (Saranya *et al.,* 2013a,b,c); methanolic extract of *Spathodea campanulata* leaves (Kathika Devi *et al.,* 2013); petroleum ether, chloroform, and ethyl acetate extracts of *Lantana camara, Bauhinia racemosa* leaves (Babita *et al.,* 2014); methanol extract of *Garcinia gummi-gutta* leaves (Dhanya and Benny, 2014); aqueous, ethanol, ethyl acetate and hexane extracts of *Gossypium hirsutum* leaf (Bt cotton) (Patil *et al.,* 2014); aqueous, methanol extracts of *Veronia adoensis* leaf (Ngule *et al.,* 2014); ethyl acetate, methanol extracts of *Ficus krishnae* leaves (Haldar *et al.,* 2014); petroleum ether, benzene, ethyl acetate, chloroform:methonal (1:1 v/v), acetone and absolute alcohol extracts of *Swietenia mahagoni* leaves (Adhikari and Chandra, 2014); methanol extract of *Annona reticulata, Pongamia pinnata* leaves (Nayak, 2014); petroleum ether, N – butanol extracts of *Cassia occidentails* leaves (Kumar *et al.,* 2014); acetone extracts of *Spathodea campanulata* leaves (Pravin *et al.,* 2014); acetone, benzene, petroleum ether, chloroform and aqueous extracts of *Nerium oleander* leaves (Fakoorziba *et al.,* 2015); methanol and water extract of *Indigofera arrecta* leaves (Neema *et al.,* 2015); water, ethanol and petroleum ether extracts of *Citrullus colocynthis* leaves (Satti and Edriss, 2015); petroleum ether extract of *Zizyphus jujube* leaves (El-Husseiny and El-Kholy, 2015); methanol and petroleum ether extract of *Mallotus repandus* leaves (Hasan, *et al.,* 2015); petroleum ether, ethyl acetate, chloroform and methanol extracts of *Rhinacanthus nasutus* leaves (Jayapriya and Shoba, 2015); methanol, ethyl acetate and hexane extracts of *Ageratum houstonianum* leaves (Samuel *et al.,* 2015); aqueous, ethanol, methanol, acetone and chloroform extracts of *Lantana camara aculeate* leaves (Hemalatha *et al.,* 2015); hexane, ethyl acetate, benzene, chloroform and methanol extracts of *Erythrina indica* leaves (Govindarajan and Sivakumar, 2015); acetone extract of *Cipadessa baccifera* leaves (Ramkumar *et al.,* 2015a); aqueous extract of *Argemone mexicana* leaves (Zeinab, 2015); aqueous extract of *Cleistanthus collinus* leaves (Arivoli *et al.,* 2015) ethanol extract of *Ixora coccinea* and *Allamanda violacea* leaves (Rahul *et al.,* 2015); isoproponal, methanol, acetone, dimethyl sulfoxide and water extracts of *Vernonia cinerea*, *Prosopis juliflora*, *Hyptis suaveolens* and *Malvastrum coromandelianum* leaves (Yadav *et al.,* 2015); acetone, ethyl acetate, methanol, chloroform and benzene extracts of *Clausena dentata* leaves (Ramkumar *et al.,* 2015b); hexane, benzene, ethyl acetate and methanol extracts of *Oxystelma esculentum* leaves (Elumalai and Krishnappa, 2015); hexane, ethyl acetate, acetone and methanol extracts of *Hippocratea excelsa, Hippocratea celastroides, Argemone mexicana, Tagetes lucida* and *Pseudosmodingium perniciosum* leaves (Ruiz- Guerrero *et al.,* 2015); hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Blumea mollis, Chloroxylon swietenia, Clausena anisata, Feronia limnonia, Lantana camera, Plectranthus amboincius* and *Tagetes erecta* leaves (Jayaraman *et al.,* 2015); methanol, acetone, hexane, chloroform and water extracts of *Excoecaria agallocha* leaves (Pradeepa *et al.,* 2015); methanol extracts of *Acalypha alnifolia* and *Vitex negundo* leaves (Kamalakannan *et al.,* 2015); water and ethanol extracts of *Phytolacca dodecandra* leaves (Owiti *et al.,* 2015).

It could be as certained from the literature survey that there was no information

available on the larvicidal, pupicidal, and ovicidal effects of the acetone leaf extract of the *S. campanulata.*

The present study was therefore carried out to evaluate mosquitocidal properties of *S. campanulata* acetone 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; 2011; El-Hela 2001).

The aim of the present study is therefore to find out:

- Toxicity of the acetone leaf extract of *S. campanulata* to the larvae of *Ae. aegypti*,
- Ovicidal activity of the acetone leaf extract of *S. campanulata* on mosquito eggs,

Materials and Methods

Colonization of *Ae. aegypti*

Collection of eggs

The eggs of *Aedes aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore, Tamil Nadu, India without exposure to any insecticide. The eggs were then brought to the laboratory and transferred to enamel trays containing water and kept for larval hatching. They were hatched and 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.

Maintenance of larvae

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 breeding medium was regularly checked and dead larvae were removed at sight. 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.

Maintenance of pupae and adult

The pupae were collected from culture trays and were transferred to glass beakers containing water with help of a sucker. The pupae containing glass beaker were kept in side mosquito cage for adult emergence. The cage was made up of steel frame wrapped

with mosquito netting. The cage had a provision (a hole) for handling of materials and animals placed inside. The hole was guarded with a sleeve which was useful to close suddenly after being used.

Blood feeding of adult *Ae. aegypti* and egg laying

The females were fed by hand every alternate day. Feeding mosquitoes on human arm for experimental purposes was suggested by Judson (1967) and Briegel (1990).

Both females and males were provided with 10% glucose solution as described by Villani *et al.*, (1983) on cotton wicks. The cotton was always kept moist with the solution and changed every day.

An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arrangement made the collection of eggs easier.

Collection of plant materials and authentication

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^{o}C) for about 2 weeks till they dried completely. The leaves were finely powdered using electric blender. 250g of leaf powder was dissolved in 200ml of acetone (as a solvent) and extracted in the Soxhlet apparatus for 8 h over a mantle heater at 55 ◦C. The acetone extract was concentrated using a vacuum evaporator at 45◦ C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in a refrigerator for later use.

Preparation of stock solution and different concentrations of leaf extract

1g of the concentrated extract of leaves of *S. campanulata* was dissolved in 100ml 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.

Gas Chromatography- Mass Spectrometry (GC/MS) analysis

The GCMS analysis was conducted at South Indian Textile Research Association, Coimbatore. 1 µl of acetone 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.Important compounds identified in the GC- MS analysis of acetone leaf extract of *S.campanulata* was presented in table-1.

Bioassay test

Bioassay tests were carried out for testing the efficacy of acetone 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.

The values of LC_{50} 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 acetone leaf extract of *S. campanulata* on the hatchability of *Ae. aegypti* eggs were determined adopting the following procedure (Judson and Gojrati, 1967).Hatching rate was calculated on the basis of non-hatchability of eggs according to Sahgal and Pillai (1993). The data were statistically examined using Student's *t*- test.

Results and Discussion

Toxicity of acetone leaf extract of *S. campanulata* to the developmental stages of *Ae.aegypti*

Bioassay tests were conducted to find out the toxicity of acetone 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} / 24 hour.

LC_{50} / 24 hour values of acetone leaf extract of *S. campanulata* to I instar larvae was 0.193%, and this was found to gradually increase with the age of larvae. Pupae showed the highest resistance to the acetone leaf extract of *S. campanulata* as evident from the relatively higher LC_{50} / 24 hour values 0.794% (Table- 2)

Effect of acetone leaf extract of *S.campanulata* on 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 acetone leaf extract of *S. campanulata*. Percent hatch of eggs placed in control medium was 90 % where as in 0.1, 0.3, 0.5, and 0.7 % concentrations it was 83, 72, 33, and 11. 0.9% dose completely arrested hatching eggs (Fig. 1). The decrease in hatchability was found to be dose dependent.

Globally, there is prompt awareness going on and always desired to use natural, ecofriendly compounds for larvicidal activity. Mosquito risk has become more acute in recent time and the death of millions of people every year due to mosquito –borne disease has resulted in the loss of socioeconomic wealth in many countries. The control of mosquito by chemical substance is not safe at present because of insecticide resistance by vector and environmental imbalance. Application of chemical or synthetic insecticides leads to deleterious effects in the long term, hence it does not provide absolute result, that is why alternative mosquito control method is need.

The results showed that the acetone leaf extract of *S. campanulata* possesses significant larvicidal properties against *Ae. aegypti*. The findings agree with some of the previous reports.

Petroleum ether leaf extracts of *Pongamia pinnata* and *Kigelia africana* were evaluated against 3rd and 4th instar larva of *Ae. aegypti*. Results showed that *Pongamia pinnata* and *Kigelia africana* posses high larvicidal acticity (LC_{50} / 24 hrs 63.24 ppm) (Savitri and Rajendar, 2013); acetone extract of

Vernonia cinerea, Prosopis juliflora and *Cassia tora* leaf had higher motality with the values of LC_{50} 81.30, 37.55 and 140.9 ppm was observed after 24hrs exposure against third instar larvae of *An. stephensi* (Varun *et al.,* 2013); the LC_{50} /24 hrs values of water, ethanol, ethyl acetate and hexane leaf extracts of *Gossypium hirsutum* against, fourth instar larvae of *An. stephensi* were 211.7, 241.64, 358.07, 401.03 (Patil *et al.,* 2014); LC_{50} /24 hrs values of ethanol leaf extract of *Heliotropium indicum* against 3rd instar larvae of *An. gambiae* was180 ppm respectively (Azokou *et al.,* 2013); LC_{50}/ 24 hrs values of hexane leaf extract of *Crotons sparciflorus* against III instar larvae and pupae of *Cx. quinquefasciatus* were 145.3, 446.9 ppm respectively (Ramar *et al.,* 2013); acetone and aqueous leaf extract of *Ocimum gratissimum* and *Solenostemon monostachyus* had higher mortality with the values of LC_{50} 52.00, 232.00, and 28.00, 200.00, ppm was observed after 72hrs exposure against 2nd instar larvae of *An. gambiae* (Chukwura and Iheukwumere, 2013); hexane, acetone, ethanol extract of *Ocimum basilicum* leaves exhibited significant larvicidal activity against third instar larvae of *An. arabiensis* with LC_{50} values of 101, 390, 705 (24hrs) 854, 284, 532 (48hrs) and 777, 228, 455 (72hrs) ppm respectively (Basheer, 2013); highest larval mortality was found in methanol extract of leaf of *Pithecellobium dulce* against the third instar larvae of *An. stephensi* and *Ae. aegypti* with LC_{50}/24hrs values 145.43, 155.78, mg/l respectively (Govindharajan *et al.,* 2013); petroleum ether extract of *Cartharanthus roseus* of LC_{50} 3.34, 4.48, 5.90 and 8.17 g/l was observed after 24hrs exposure against first to fourth instar larvae of *An. stephensi* (Panneerselvam *et al.,* 2013b); LC_{50}/ 24 hrs values of methanol, aqueous, ethyl acetate, chloroform and petroleum ether extracts of leaf of *Erythrine indica* were tested against the fourth instar larvae of *Ae. aegypti* and *Cx. quniquefasciatus* were 126.76,170.44, 145.42, 155.87, 188.22 and 187.92, 191.29, 158.60,125.55, 135.96 respectively (Rathi Sre *et al.,* 2013); the LC_{50}/24 hrs value of methanol leaf extract of *Murraya exotica* for III and IV instar larvae and pupae of *Cx. quinquefasciatus* is 135.53 ppm and 154.36 ppm and 178 ppm respectively. Likewise for *Lawsonia inermis* it is 139.05 for III instar, 163,63 for IV instar and 188.151ppm for the pupa (Dass and Mariappan 2014); *Ricinus communis* leaf ethyl acetate extract obtained as the best result where mortality among the III instar larvae of *An. arabiensis* is found to be 96 % after 24 hrs with an LC_{50} at 0.390 mg/l. 100 % mortality was observed after 48 hrs with LC_{50} at 0.284 mg/l (Basheer, 2014); the hexane extracts of *Tragia involucrate* was to be higher mortality against the III instar larvae of *Ae. aegypti* with a $LC_{50/24\ hrs}$ value of 153.51 mg / l (Ramar and Jeyasankar, 2014); the methanol extract of the leaves of *Pithecellobium dulce* was the most effective against the III instar larvae of *Cx. quinquefasciatus* with LC_{50} values 164.12 mg/l being observed after 24 hrs of exposure (Govindarajan and Rajeshwary, 2014a); the LC_{50} values of ethanol leaf extract of *Morinda citrifoila* against the first to fourth larvae and pupae of *An. stephensi* had values of LC_{50} = 152.05, 190.22, 237.43, 273.12, 305.25 mg / l at 24 hrs (Kovendan *et al.,* 2014); the highest larvicidal activity was observed in leaf methanol extract of *Impatiens balsamina* against III instar larvae of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* with the LC_{50} / 24 hrs values 98.04, 119.68, 125.06 mg/l respectively (Govindarajan and Rajeshwary, 2014b); the LC_{50}/24 hrs value of methanol extract of *Oxystelma esculentum* against 3rd instar larvae of *Ae. aegypti* was 125.82 mg/L. (Elumalai and Krishnappa, 2015); results showed that leaf powder of *Vernonia cinerea* in acetone

collected during summer showed highest efficacy against *Ae. albopictus* with LC_{50} /24 hrs value of 0.22g/l (Yadav *et al.,* 2015).

S.campanulata leaves have furnished Spathodol, caffeic acid, phenolic acids and flavonoids (Ngouela *et al.,* 1991, EI – Hela, 2001**,** EI – Hela, 2001b). These compounds may jointly (or) independently contribute to larvicidal activity against *Ae.aegypti*. The phytochemicals interfered with proper functioning of mitochondria more specifically at the porton transforming sites (Usta *et al.,* 2002) and phyto chemicals primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae (Rey *et al.,* 1999, David *et al.,* 2000). The death of treated larvae may be due to the inability of the molting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdysis or to a metamorphosis inhibiting effect of the plant extract which is possibly based on the disturbance of the hormonal regulation (Al – Sharook *et al.,* 1991).

The crude methanol and benzene leaf extract of *Cardiospermum halicacabum* exerted 100% reduction of egg hatching at 300 ppm against *Cx. quinquefasciatus* and in *Ae. aegypti* 100% reduction of egg hatching at 400 ppm (Govindarajan, 2011b); aqueous leaf extract of *Calotropis procera* treatment at 1000 ppm *Cx. tritaeniorhynchus* and *Cx. gelidus* eggs resulted in to 100% ovicidal activity (Kumar *et al.,* 2012); an acetone extract of *Solanum trilobatum* leaves was evaluated for its ovicidal activity on the *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*, by exposing eggs ranging concentrations of 50 – 200 ppm of the extract and a 100 ppm of the extract killed all the eggs from both the species (Rajkumar and Jebanesan, 2004); in the laboratory, eggs of *Cx.quinquefasciatus* and *Ae. aegypti* were tested at 1000 ppm concentration ethyl acetate extract of *Swertia chirata* leaves shows 23% egg hatchability (Balaraju *et al.,* 2009); mortality (no egg hatchability) was observed 100 percent with ethyl acetate and methanol extracts of *Andrographis paniculata, Eclipta prostrata and Tagetes erecta* leaves at 998.85 mg/l against *An. subpictus* (Elango *et al.,* 2011b); at a dose of 82.5 mg/ml the ethanolic leaf extract of *Hyptis suaveolens* completely inhibited *An. gambiae* hatching whereas the aqueous extract could inhibit only 70.42% egg hatching at the same dose (Ivoke *et al.,* 2009); hundred percent ovicidal activities were observed at 350 ppm and 450 ppm of methanol, benzene, acetone extract of *Pemphis acidula* leaves (Samidurai *et al.,* 2009); aqueous extract of *Leucas aspera* was found to be ovicidal against *Ae. aegypti, An.stephensi* and *Cx. quinquefasciatus* with hatchability values of 39.4 and 21.2; 42.4 and 27.8; 50.6 and 30.2 percent at 500 and 1000 ppm respectively (Arivoli and Samuel 2011a); ovicidal activity with ethyl acetate, aqueous solution, ethanol leaf extract 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 extract (Roni *et al.,* 2013); at 300 ppm of ethanolic leaf extract of *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis* and *Srermacoce hispida* showed 100% ovicidal activity against *An. stephensi, Ae. aegypti* and *Cx. tritaeniorhynchus* (Dhanasekaran *et al.,* 2013);

Table.1 Important compounds identified in the GC- MS analysis of acetone leaf extract of *Spathodea campanulata*

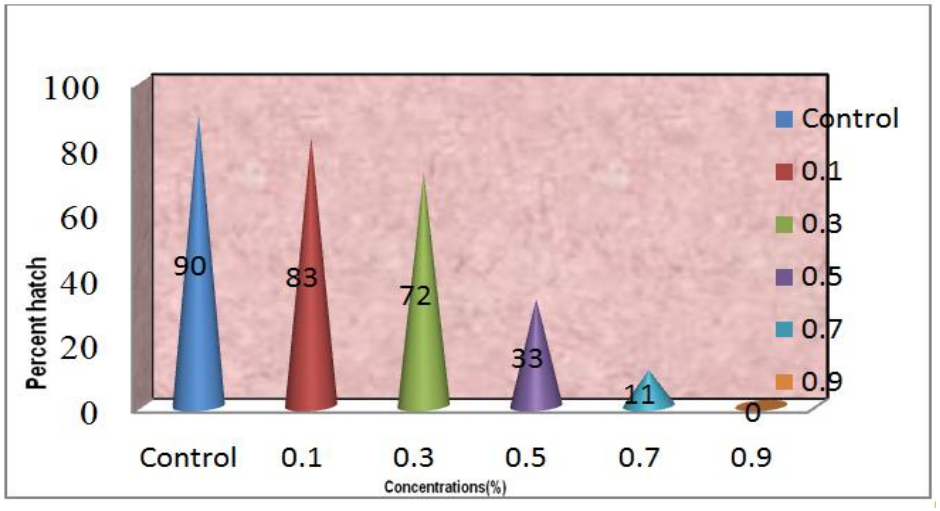
S.No	Retention Time	Area %	Chemical Formula	Compound Name	Molecular Weight
1	24.66	38.01	$C_{20}H_{40}O$	Phytol Isomer	296
2	35.31	13.82	$C_{30}H_{50}$	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-(CAS)	410
3	38.00	7.45	$C_{17}H_{17}NO_2$	2AR*,3R*,8BS*-2A,8*B-Dihydroxy-3-Phenyl-1,2,3,4-Tetrahydrocyclobut [C]Quinoline	267
4	33.07	5.53	$C_{29}H_{60}$	Nonacosane(CAS)	408
5	19.44	4.16	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-Hexadecen-1-o1	296
6	36.78	4.14	$C_{28}H_{28}Si$	12-(tert-Butyldiphenylsilyl)-,3,5,-dodecadiene,-7,9,11-triyn-2-o1	408
7	30.60	3.90	$C_{35}H_{68}O_5$	Hexadecanoic acid,1-(hydroxymethyl)-1-2-ethanediyl ester(CAS)	568
8	26.69	1.87	$C_{30}H_{50}O$	Viminalol	426
9	31.85	1.85	$C_{22}H_{20}N_4O_3$	2-Ethyl-3-[1'-phenyl 1-4'(ethoxycarbonyl)-5'-pyrazolyl]-4(3H)-quinazolinone	388
10	34.23	1.75	$C_{20}H_{38}$	Neophytadiene	278
11	27.84	1.73	$C_{42}H_{42}N_2P_2$	(R)-[4,4'- bis(dimethylamino) -6,6'-dimethylphenyl-2,2'-diyl]bis (diphenylphosphine)	636
12	9.43	1.43	$C_{19}H_{24}O_2$	1-(2-Hydroxyphenyl)-1-(3-isopropyl-2hydroxypheny) butane	284

13	17.99	1.31	$C_{11}H_{16}O_3$	(-)-Loliolide	196
14	25.16	1.27	$C_{16}H_{28}O_3$	Z-(13,14-Epoxy) tetradec-11-en-1-o1 acetate	268
15	6.97	1.25	$C_{18}H_{37}NO_3Si_3$	0,0,0-Tris-Trimethylsilyl-Epinephrine	399
16	16.00	1.21	$C_{17}H_{10}CIN_3O_2S$	3-[4'-(2"-Chlorophenyl)-2'-thiazolyl]-2,4-dioxo-1.2.3.4-tetrahydroquinazoline	355
17	4.63	1.10	$C_{37}H_{48}IN_3O_7$	22-Iodo-3a-(methoxymethoxy)-5a,8a-(4-phenyl-1,2-urazolo)cholesta-6-en-25-ol-26,23-lactone	773
18	20.29	1.04	$C_{15}H_{16}O_2$	1,2-syn-Diphenylpropan-1,3-diol	228
19	31.04	0.93	$C_{24}H_{38}O_4$	Di-(2-ethylhexyl) phthalate	390
20	35.97	0.78	$C_{23}H_{28}Br_4$	1,7- Bis (3,5-bis (bromomethyl) phenyl) heptane	620
21	14.04	0.67	$C_{11}H_{17}NO$	4-Isopropenyl-1-methylbicyclo[4.1.0] Heptan-2-one oxime	179
22	11.74	0.64	$C_{12}H_{11}Br_2NO_3$	Ethyl 3,4-dibromo-5-methoxyindole-2-carboxylate	375
23	12.44	0.61	$C_{14}H_{17}F$	1-(2'Cyclohexyl-2'-fluoroethenyl) benzene	204

Table.2 LC50/24 hour values of acetone leaf extract of *Spathodea campanulata* to the pre- adult stages (I, II, III, IV instar and pupae) of *Aedes aegypti*

Stages of development (Instars)	Number of larvae / trial	LC_{50} 24 hour (%)	Confidence limit		Regression Equation	R value	Slope	Chi-square	Degrees of freedom
			LL(%)	UL(%)					
I	20	0.193	0.156	0.235	y = 224.92x + 8.5668	0.968	225	1.073	3(16.26)
II	20	0.296	0.249	0.338	y = 200x - 7	0.980	200	0.894	3(16.26)
III	20	0.378	0.341	0.415	y = 200x - 22	0.980	200	0.867	3(16.26)
IV	20	0.592	0.548	0.639	y = 195x – 63	0.983	195	0.825	3(16.26)
Pupae	20	0.794	0.752	0.836	y = 205x – 109	0.988	205	0.913	3(16.26)

Fig.1 Changes in the hatchability (percent hatch) of *Aedes aegypti* eggs exposed to different concentrations of the acetone leaf extract of *Spathodea campanulata* and control

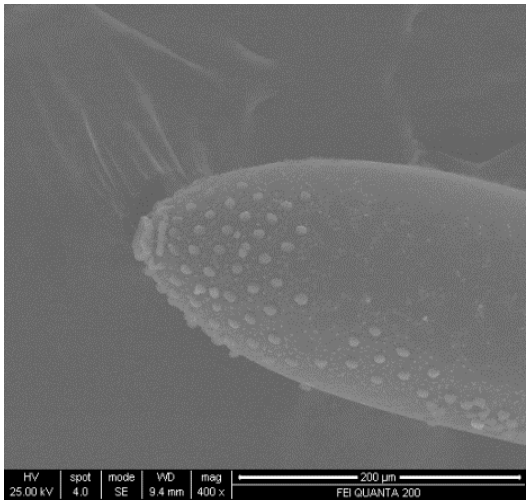


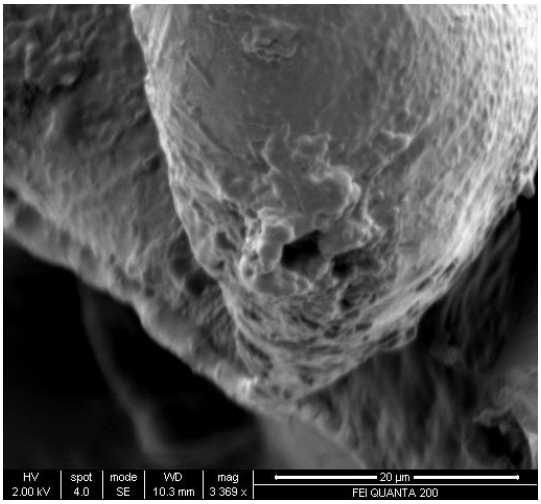
Scanning electron microscope photos

Plate.1 Anterior pole and microphylar apparatus of control *Aedes aegypti* egg

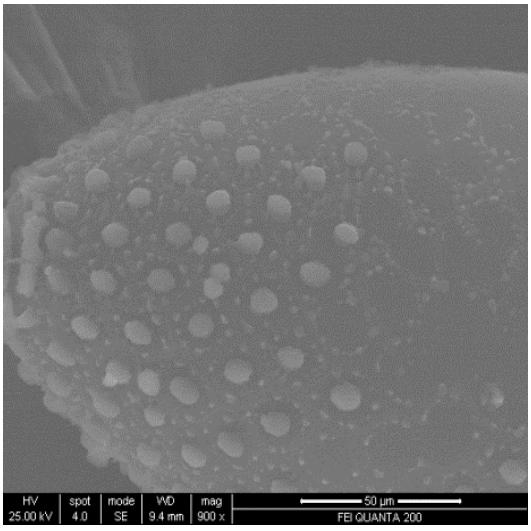
1A. Damaged anterior pole and microphylar apparatus exposed to 0.9% of acetone leaf extract of Spathodea campanulata; 1B. Outer chorionic cell and tubercel of the control *Aedes aegypti* egg; 1C. Damage outer choronic cells and tubercle exposed 0.9% of acetone leaf extract of Spathodea campanulata

1A





1B



1C

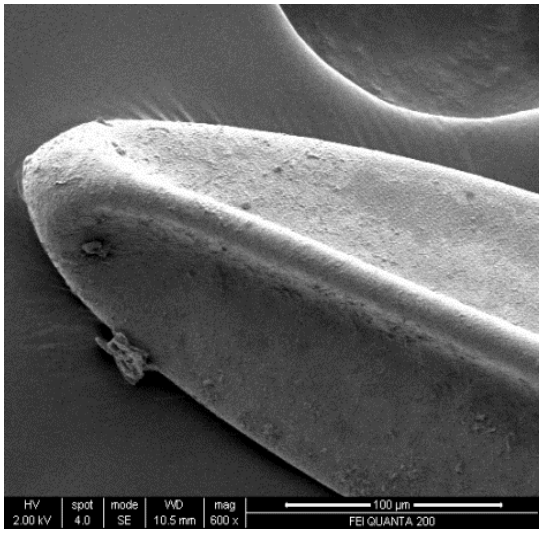
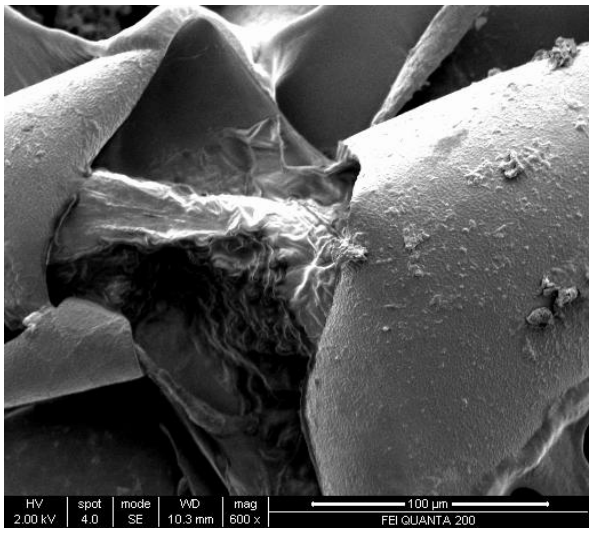


Plate.2 Larvae trapped within the shells of the *Aedes aegypti* egg exposed to 0.9% of acetone leaf extract of *Spathodea campanulata*



percent hatch of eggs placed in control medium was 80% where as in 0.1%, 0.2%,0.4% and 0.6% concentrations of aqueous leaf extract of *S. campanulata* against *Ae. aegypti* was 65, 46, 40 and 2%. 0.8% dose completely arrested hatching eggs (Saranya *et al.,* 2013b); zero percent hatchability was observed at 400 mg/l for leaf methonal extract of *Pithecellobium dulce* against *An. stephensi* eggs (Govindharajan *et al.,* 2013); the methanol extract of sea weed leaf exerted 100% egg mortality (zero hatchability) at 240,300 and 360 ppm for *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (Rajalakshmi *et al.,* 2013); about 100 % mortality was observed of the egg rafts of *Cx.quinquefasciatus* at 500 mg/l for leaf methanol extract of *Pithecellobium dulce* (Govindarajan and Rajeshwary, 2014). 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 acetone extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract (Table 1). The extract may inhibit the hatchability of the eggs by interfering with their chorion (Plates 1, 1A, 1B, 1C). Eggs and egg shells treated with plant extracts become damaged probably due to endosmosis. After the initial phase of swelling, eggs become desiccated, followed by shrinkage and death of larvae trapped within (Plate 2). It is also evident from the present study on exposure of *Ae. aegypti* eggs to the acetone leaf extract of *S. campanulata*. The treated eggs contained developed embryos the eclosion of the egg was incomplete (Miura *et al.,* 1976).

The findings of the present investigation were comparable with other ovicidal studies and revealed that the acetone *S. campanulata* leaf extract possesses ovicidal activity against the eggs of *Ae. aegypti.*

Conclusion

In conclusion, our findings showed that the plant *S. campanulata* exhibits larvicidal, pupicidal, ovicidal activity against dengue vector mosquito *Ae. aegypti.* These results could encourage the search for new active natural compounds offering an alternative to

synthetic insecticides from other plants. *S. campanulata* acetone leaf extracts may contribute greatly to save environment and to an overall reduction in the population density of dengue vector *Aedes aegypti*. These results of study also demonstrate the potential of new alternative sources of mosquito larvicides and ovicides which are generally free of adverse effects.

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