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RESEARCH ARTICLE

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

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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* hexane leaf extract 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

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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.

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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 lympatic 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**	Nil	The Dinakaran, 2016
10	2017	23,035**	65	The Dinakaran 2017
11	2018	40868*,2175**	83*,1**	NVBDCP,2018

		(till September)		

1. India.
2. Tamil Nadu.

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

1. India.
2. Tamil Nadu.

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 diverse insecticidal properties on the medically important mosquitoes: methanolic extract of *Derris elliptica* (Prempree and Sukhapanth,1990); aqueous extract of Senna didymobotrya (Ojewole et al., 2000); aqueous extract of *Solanum nigrum* (Singh et al., 2001); acetone extract of *Solanum trilobatum* (Rajkumar and Jebanesan, 2004); aqueous extract of *Gymnema sylvestre* and *Eclipta prostrate* (Khanna and Kannabiran, 2007); methanol, benzene and acetone extracts of *Cassia fistula* (Govindarajan, 2009); petroleum ether extract of *Azadirachta indica, Ocimum gratissimum* and *Hyptis suaveolens* (Okigbo et al., 2010); aqueous and chloroform extracts of *Leucas aspera* (Ramanibai et al., 2011); ethanolic extract of *Datura stramonium* (Swathi et al., 2012); aqueous extract of *Spathodea campanulata* (Saranya et al., 2013a, b, c); methanolic extract of *Spathodea campanulata* (Karthika Devi et al., 2013); acetone extract of *Spathodea campanulata* (Pravin et al., 2014); aqueous extract of *Pithecellobium dulce* (Mahendran et al., 2015); acetone extract of *Spathodea campanulata* (Pravin et al.,2015); aqueous extract of *Tecoma stans* (Navaneethan et al., 2016); ethanolic extracts of *Callistemon citrinus* (Palanikumar et al., 2017); ethanolic extract of *Calotropis procera* (Mashlawi and Ali, 2017); methanolic leaf extract of *Senna alata* (Karthiyani et al., 2018); methanolic extract of *Plectranthus barbatus* (Lawi et al., 2018); aqueous and ethanolic extracts of *Carica papaya* and *Cereus pterogonus* (Sebastian et al., 2018); methanolic extracts of *Seasamum indicum, Pungamia pinnata* and *Croton bonplandianum* (Dhanasekarn et al., 2018) petroleum ether, ethyl acetate and aqueous extract of *Lantana indica* (Rathnasagar and Thiyagaraj , 2018); aqueous extract of *Cinnamomum tamala, Aloe vera, Datura alba, Allium sativum, Allium cepa, Zingiber officinale* and *Ocimum basilicum* (Iqbal et al.,2018).

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 ($27 \pm 2^{o}C$) 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 and further compared to the control larvae after treatment of the hexane, ethyl acetate , Iso-propanal, 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. campanulata* 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 $LC_{50,}LC_{90}$/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.campanulata*:
Important compounds identified in the GC- MS analysis of hexane leaf extract of *S.campanulata*. 3-Methyl-6-isopropylcyclohex-3-en-1-one, 2-Hexadecen-1 ol,3,7,11,15,tetramethyl1-[R-[R*,R*-(E)]]-(CAS) Phytol Isomer, Hexane 1,6-dicyclohexyl, 2-Pentadecanone 6,10,14-trimethyl-, Neophytadine, Nonacosane (CAS), 13-Docosenamide, Phthalic acid, di(2-propylpentyl)ester, Hexanoic acid 4-hexadecyl ester, 4-Ethoxyisophosphamide, 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), Junipercamphor, 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

to the hexane leaf extract of *S.campanulata* as evident from the relatively higher $LC_{50,}LC_{90}$ / 48 hour values 0.41%, 0.082%.(Table-3).

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.campaulata*. 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 1st , 2 ndinstar 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. quniquefasciatus* were 126.76,170.44, 145.42, 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},LC_{90}/24 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), 299.83ppm (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 4th 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 4th instar larvae of *An. gambiae* compared to *Hyptis suaveolens* LC_{50} = 132.01 ppm and *Chenopodiuum ambrosoides* LC_{50} = 204.56 ppm extracts 24 hours post-treatment (Oumarou et al., 2018); the 4th instar larvae of *An. stephensi* showed maximum larvicidal activity at a LC_{50}/48 hour value of 58.2 ppm for ethyl acetate extract. Above 90% mortality was observed for ethyl acetate extract at LC_{50}/48 hour62.2 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 1st 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, alkalodis, phytosteroids. These compounds were previously reported to have mosquito larvicidal activity (Farooq 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 & Pasmiati,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 acetylecholinesterase 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 hatched 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	+

phylobatannis	-
anthraquinones	-

Table 3:-LC_{50}/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/trail	LC_{50} /24 hour(%)	Confidence limit		Regression Equation	R Value	Slope	Chi-Square	Degrees of Freedom
			LCL(%)	UCL(%)					
I	20	0.12	0.094	0.27	Y=270.94x+18.861	0.8746	100.14	0.487*	3(16.26)
II	20	0.31	0.28	0.35	Y=235x-19.5	0.9915	94.6	0.615*	3(16.26)
III	20	0.49	0.41	0.53	Y=138.33x	0.8319	200	0.862*	3(16.26)
IV	20	0.53	0.48	0.56	Y=213x-56.3	0.9755	195	0.848*	3(16.26)
Pupae	20	0.65	0.61	0.69	Y=205x-75	0.9935	205	0.891*	3(16.26)

1. LC_{50}– 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	$C_{20}H_{40}O$	296
3	37.01	7.88	Hexane, 1,6-dicyclohexyl	$C_{18}H_{34}$	250
4	19.89	7.35	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	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	$C_{22}H_{43}NO$	337
8	33.19	4.57	Phthalic acid, di(2-propylpentyl)ester	$C_{24}H_{38}O_4$	390
9	30.22	3.16	Hexanoic acid, 4-hexadecyl ester	$C_{22}H_{44}O_2$	340
10	23.06	2.81	4-Ethoxyisophosphamide	$C_9H_{19}C_{12}N_2O_3P$	312
11	17.17	2.03	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-(CAS)	$C_{11}H_{16}O_2$	180
12	38.78	1.81	Docosane(CAS)	$C_{29}H_{60}$	408
13	32.60	1.78	Octacosane(CAS)	$C_{28}H_{58}$	394
14	36.65	1.76	2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-hexamethly-(CAS)	$C_{30}H_{50}$	410
15	21.7	1.29	Hexadecanoic acid, methyl ester(CAS)	$C_{17}H_{34}O_2$	270
16	34.2	0.95	Tricosane(CAS)	$C_{23}H_{48}$	324
17	17.91	0.90	Junipercamphor	$C_{15}H_{26}O$	222
18	15.75	0.82	Neopentyl bromoacetate	$C_7H_{13}BrO_2$	208
19	35.07	0.81	Phytol, acetate	$C_{22}H_{42}O_2$	338
20	26.19	0.74	Palmitaldehyde, dially acetal (CAS)	$C_{22}H_{42}O_2$	338
21	28.14	0.73	Ligularine	$C_{23}H_{31}NO_9$	465
22	13.30	0.66	Hexadeacane(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/trail	LC_{90}/24 hour(%)	Confidence limit		Regression Equation	R Value	Slope	Chi-Square	Degrees of Freedom
			LCL(%)	UCL(%)					
I	20	0.41	0.391	0.45	Y=120x+31	0.8623	108	0.484*	3(16.26)
II	20	0.57	0.54	0.61	Y=130x+12	0.8325	89	0.619*	3(16.26)
III	20	0.75	0.72	0.79	Y=111.96x	0.8442	213	0.860*	3(16.26)
IV	20	0.89	0.86	0.93	Y=-9.8959x+83.957	0.0303	190	0.842*	3(16.26)
Pupae	20	0.011	0.009	0.16	Y=-	0.505	200	0.897	3(16.26

					63.172x+98.648	4		*	)

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_{50}/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_{50}/48 hour(%)	Confidence limit		Regression Equation	R Value	Slope	Chi-Square	Degrees of Freedom
			LCL(%)	UCL(%)					
I	20	0.11	0.009	0.0135	Y=2688.2x+20.129	0.8626	103	0.477*	3(16.26)
II	20	0.12	0.0018	0.024	Y=370x+38.2	0.1216	91	0.605*	3(16.26)
III	20	0.20	0.0091	0.0142	Y=355.52x	0.7238	207	0.872*	3(16.26)
IV	20	0.35	0.31	0.385	Y=-164.53x-5.2326	0.9369	187	0.838*	3(16.26)
Pupae	20	0.41	0.38	0.446	Y=164.53x-5.2326	0.9369	218	0.881*	3(16.26)

1. LC_{50}– 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 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
			LCL(%)	UCL(%)					
I	20	0.23	0.204	0.256	Y=196.5x+32.306	0.7094	98.3	0.479*	3(16.26)
II	20	0.35	0.313	0.392	Y=210x+7	0.7475	102.8	0.613*	3(16.26)
III	20	0.54	0.521	0.587	Y=140.74x	0.6895	192	0.867*	3(16.26)
IV	20	0.61	0.584	0.648	Y=190x-41	0.6902	205	0.833*	3(16.26)
Pupae	20	0.82	0.796	0.863	Y=-6.6792+75.021	0.0037	199	0.881*	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.

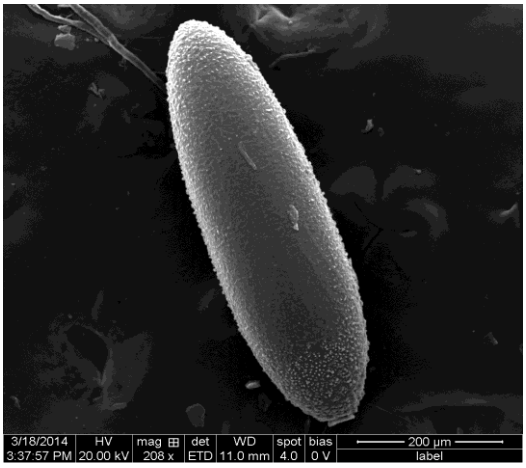
4. R-value – regression value.
5. *, P< 0.001 level of significance of chi-square values.

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

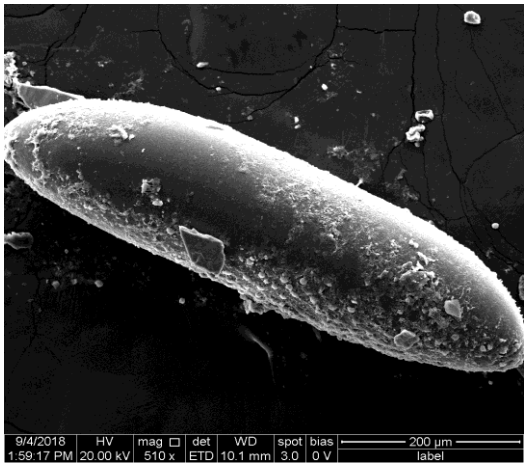
Parameters	Control	Concentrations (%)				
		0.1	0.3	0.5	0.7	0.9
Number of eggs introduced	20	20	20	20	20	0
Mean number of eggs hatched*	18.66	15.33	10.33	8.666	5.333	0
S.D	± 0.761	± 0.361	± 0.452	± 0.319	± 0.539	0
Percent hatchability	93	76#	51#	43#	26#	0
Percent reduction over control		18.27	45.16	53.76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



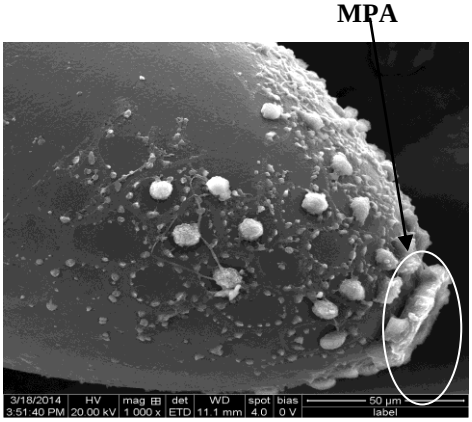
1



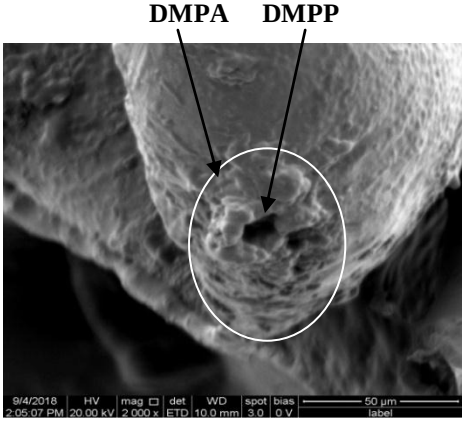
1a

Plate 1:-Control egg of *Aedes aegypti*,

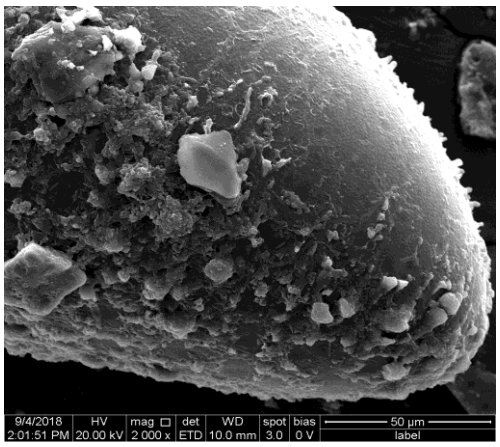
Plate 1a:-Damaged exo chorionic surface of *Ae. aegypti*egg treated with hexane leaf extract of *S.campanulata*.

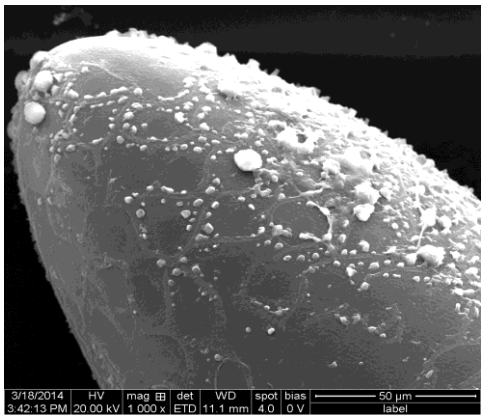


1b



1c





1d **1e**

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.

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