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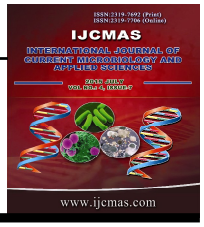


Original Research Article

GC-MS Analysis and Larvicidal Activity of *Andrographis paniculata* (Burm.F) Wall. Ex Nees. against the Dengue Vector *Aedes aegypti* (L) (Diptera: Culicidae)

M. Thangavel, S. Umavathi*, Y. Thangam, A. Thamaraiselvi and M. Ramamurthy

PG and Research Department of Zoology, J.K.K. Nattraja College of Arts and Science, Kumaraplayam, Namakkal District, Tamil Nadu, India
**Corresponding author*

Keywords

Andrographis paniculata, *Aedes aegypti*, Dengue, Larvicides, phytochemicals and GC-MS

A B S T R A C T

The present study aimed to evaluate the larvicidal activity of acetone extract of *Andrographis paniculata* against the dengue vector *Aedes aegypti.* I, II III, IV instar larvae and pupae of *A. aegypti* were exposed to various concentrations (100, 150, 200, 250 and 300 ppm) of acetone extract of *Andrographis paniculata* for 24 hrs. The concentration and age based larvicidal and pupicidal effect was noticed in this study. Alkaloid, flavonoids, steroids, tannin, chlororogenic acid and phenolic compounds were present in acetone extract of *A. paniculata* were qualitatively and quantitatively estimated. Based on the quantitative studies the maximum amount of flavonoid (44.6%) was noticed in acetone extract of *A. paniculata* followed by phenol (32.2%), alkaloid (22.2%), steroid (20.5%), chlorogenic acid (5.3%) and tannin (3.7%). In addition 19 chemical components were identified in the acetone extract of *Andrographis paniculat*a by Gas Chromatography-Mass Spectrometry. Results of the present study reveal the acetone extract of *Andrographis paniculata* and its phytochemicals were considered as a potent source for the production of natural larvicides.

Introduction

Mosquitoes are carriers of diseases such as malaria, dengue fever, yellow fever, filariasis etc. They are responsible for the death and illness of millions of people through the transmission of diseases. The vector-borne diseases caused by mosquito are one of the major health problems in most of the countries. It is affecting the socio economic status of many nations and it is an important pest against human causing allergy too. Mosquito is frequently found due to poor drainage system especially during rainy seasons, fish pond, irrigation ditches and rice fields. This provides a better breeding place for mosquitoes.

Aedes aegypti (L) a vector of dengue and Chickungunya is widely distributed in the tropical and subtropical zones. About two-thirds of world's populations live in areas infested with dengue vectors, mainly *Aedes aegypti*. Dengue viruses, causative agents of dengue fever and more severe dengue hemorrhagic fever (DHF/Dengue Shack

Syndrome) infect over 100 million people every year (Hahn *et al.,* 2001). There is no vaccine to prevent mosquito borne diseases, so vector control is the most commonly chosen solution available for reducing morbidity (Hakim, 1996). Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings. There are many methods used for mosquito control depending on the station, source reduction, biocontrol, larviciding (control of larvae) and adulticiding (control of adults). Though techniques of habit modification such as removing stagnant water and other breeding areas, application of aerial toxicants are practiced for mosquito control, they are not effective since the mosquito is highly domesticated and many adults rest indoors in hidden places such as closets. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water and thus it is easy to control them in this habit. Effective repeated use of controlling agents has disturbed natural biological systems and led to outbreak of insect species showing pesticide resistance.

There is provocative interest in research for larvicidal compound from natural sources. Even though chemical vector program has been carried on for long time, these mosquito vectors remain because of repeated use of synthetic products, house hold spray and insecticides for mosquito control. As a result, the mosquito develops their resistance. Hence, there is a need for developing biologically active natural chemical constituents which act as a larvicidal and promising to reduce the risk to humans and harmful accumulated residues. This has necessitated the need for research and development of environmentally safe, bio- degradable and low cost indigenous method for vector control, which can be used with minimum care by individuals and communities in specific situation. This has necessitated the need for search and development of environmentally safer, low cost, indigenous methods for vector control. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Promsiri *et al.,* 2006).

Andrographis paniculata (Burm.f.)Wall. Ex Nees., (Acanthaceae) is an annual herbaceous plant and is extensively cultivated in Southern Asia, China and some parts of Europe. In traditional medicine, *A. paniculata* is widely used to get rid of body heat, dispel of toxins from the body, prevent common cold, upper respiratory tract infections including sinusitis and fever (Gabrielian *et al.,* 2002) and as an antidote against poisons of snakes and insects (Samy *et al.,* 2008). *A. paniculata* has been reported to exhibited various mode of biological activities *in vivo* as well as *in vitro* viz., antibacterial (Abubakar *et al.,* 2011), antiviral (Wiart *et al.,* 2000), anti-inflammatory (Wen *et al.,* 2010), antihuman immunodeficiency virus (HIV) (Calabrese *et al.,* 2000), immunomodulating / immunostimulatory (Iruretagoyena *et al.,* 2005) and anticancer (Li *et al.,* 2007). The characteristic secondary metabolites encountered in this plant have considerably enhanced its importance in the area of medicinal plants. Hence in the present study an attempt has been made to evaluate the larvicidal and pupicidal activity of *A. paniculat* against the dengue vector *A. aegypti.*

Materials and Methods

The leaves of *A. paniculata* were collected from Salem District (Tamil Nadu) and brought to the laboratory. The leaves were

thoroughly washed with tap water and were dried under shade at room temperature (29 ± 2^0 C) for about 20 days. The completely dried leaves were powdered and sieved to get fine powder. The leaf powder (100 gms) was extracted with 300ml acetone by using the Soxhlet apparatus for 8 hours (Vogal, 1978). The extracts were concentrated using a vacuum evaporator at 45^0C under low pressure. After complete evaporation of the solvent the concentrated extract was collected and stored in a refrigerator for further experiments. One gram of concentrated extract was dissolved in 100 ml of the respective solvent and used as a stock solution. This stock solution was used to prepare the desired concentrations (100, 150, 200, 250 and 300ppm) of the extract for exposure of the mosquito larvae. The larvicidal bioassay was done using standard WHO Protocols (WHO, 2005). Mortality of larval and pupal stages of the treated and control was observed over a period of 24hours. The percentage of larval and pupal mortality was corrected by Abbot's formula (1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

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

LC_{50}, LC_{90} were calculated from toxicity data by using probit analysis (Finney, 1971). The acetone extract of *A. paniculata* was subjected to preliminary phytochemical tests to determine the groups of secondary metabolites present in the plant materials (Harborne, 1998). Based on the preliminary phytochemical studies the quantitative estimation of alkaloid (Harborne, 1998), flavonoid (Ozsoy *et al.*, 2007), steroids (Evans, 1996), tannin (Van Burden and Robinson, 1981) and total phenolic content (Li *et al.*, 2008) were analyzed.

Phytochemical analysis by GC-MS (Gas Chromatography-Mass Spectrometry)

Plant extract was dissolved in ethanol and analyzed using GC-MS SHIMADZU QP2010 instrument with GC-MS solution version 2.53SU3software. The sample was analyzed with Elute – DB-5M column. Initially oven temperature was maintained at 70º C for 2.0 min and the temperature was gradually increased up to 300º C at 10.0/35.0 min and 4.0 µL of sample was injected for analysis. Helium gas 99.995% of purity was used as a carrier gas as well as eluent. The flow rate of helium gas was set to 1.5 mL/min. The sample injector temperature was maintained at 260ºC and the split ratio is 20 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectrum was recorded across the range 40–1000 m/z for the duration of 35 minutes. Identification of components was based on comparison of their mass spectra. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass.

The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008, WILEY8 and FAME.

Results and Discussion

In the present investigation I-IV instar larvae and pupae of *A. aegypti* were exposed to 100, 150, 200, 250 and 300 ppm of acetone extract of *A. paniculata* (Fig. 1). The larval mortalities were noticed after 24 hours and larval mortality was concentration dependent. In the present study acetone extract of *A. paniculata* showed concentration and age based effect against the developmental stages of *A. aegypti*. The maximum toxic effect was noticed against I instar larvae flowed by II>III>Pupae and IV instar larvae respectively. During the study period noticed LC_{50} values were 113.661, 149.068, 162.731, 216.888 and 93.958 ppm against the I-IV instar larvae and pupae of *A. aegypti*.

Similar kind of results were observed by Jang *et al.* (2002) have reported that the methanol extracts of *C. obtusifolia*, *C. tora* and *V. tetrasperma* exhibited more than 90% larval mortality at 200 ppm on *A. aegypti* and *Culexpipiens*. The larvicidal activity of petroleum ether, ethanolic, aqueous extracts of dried leaves and fixed oil from the seeds of *Caesalpinia bonduc* (Family: Caesalpiniaceae) showed 100% mortality in 1% concentration of petroleum ether and ethanolic extract of leaf, whereas it was 55% in 2.5% concentration of aqueous extract and 92.6% in 2.5% concentration of fixed oil against the fourth instar larvae of *Culex quinquefasciatus* (Saravanan *et al.*, 2007). In the present study IV instar larvae of *A. aegypti* showed least susceptibility than pupae against the acetone extract of *A. paniculata*. Similar type of results was observed in the studies reported by Shyamala *et al.* (2003); Murugan *et al.* (2007); Vineetha and Murugan (2009) and Umavathi and Manimegalai (2010). In this study mortality might be due to the chemical constituents present in the acetone extract *A. paniculata* that arrest the metabolic activity of the larvae, which caused the high percentage of mortality.

Similarly Mullai and Jebanesan (2007) have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *Citrullus colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92 and 118.74 ppm and 75.91, 117.73 and 171.64 ppm, respectively against *Culex quinquefasciatus* larvae. Kuppusamy and Murugan (2008) reported the morphological abnormalities of developmental stages of *Anopheles stephensi* were exposed to the ethanolic extract of *A. paniculata*. The morphological abnormalities were death during moulting, splitting of cuticle at different regions of the body, larval, pupal intermediates, loosing of appendages in pupa due to ruptured cuticle and abnormal adult emergence. Elango *et al.* (2010) reported that the hexane and chloroform extract of *A. paniculata* showed 100% egg mortality at 250ppm and at 100ppm a very low hatchability was noticed.

In the present study alkaloid, flavonoids, steroids, tannin, chlorogenic acid and phenolic compounds were identified in the acetone extract of *A. paniculata* (Table 2 and 3). The biological activity of the plant extracts might be due to the present of various phytochemical compounds (Amer and Mehlhorn, 2006). These compounds jointly or independently contribute to produce larvicidal and adult emergence inhibition activity against mosquitoes (Harborne, 1998). Plant alkaloids resulted in

a significant loss in fecundity and fertility in the adult species of mosquitoes (Saxena, 1992). Kalaivani *et al.* (2012) reported the presence of various secondary metabolites like steroids, alkaloids, phenols, catechine, flavonoids, saponins and tannins in the ethanol extract of *A. paniculata.* Earlier study revealed that the bioactive compounds in the leaves of *Acacia nilotica* were polyphenols (Ginwal *et al.*, 1997), glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins in *Jatropa curcus*, with analoids in *W. somnifera* (Jayaprakasam *et al.*, 2003), seeds of *C. colocythis*that constitute elaterin, citrullol, hentriacontane, a phytosterol and a mixture of fatty acids (Duke, 1990) and the whole plant of *Argemene mexicana* that contained alkaloids of protopine and sangainarine and long chain alcohol (Sushma and Singh, 1999).

The quantitative studies of *A. paniculata* showed22.2% of alkaloid, 44.6% of flavonoids, 20.5% of steroid, 3.7% of tannin, 32.2% of phenol and 5.3% of chlorogenic acid is present in acetone extract of *A. paniculata*. The phytochemiclas accumulate in insect brain and other nervous tissues causing neuro endocrine disruptions. These compounds at low concentrations promote effective intercellular communications and cellular metabolism but at high concentrations were known to cause serious disruption to the normal timings of events in the cell cycle (Sinha, 2000). Hence in the present study also reveals that larval mortality may be due to cell death and cellular disruptions which affected the development and survival and inflict considerable larval mortality.

The chemical components of acetone extract of *A. paniculata* were analyzed by Gas Chromatography Mass Spectrum (GC-MS). Chemical components are listed in the table 5 and figure 1. In addition 19 components were identified with the retention time were ß – terpinolene at 3.401, α – terpinene at 3.686, Ethyl iso-allocholate at 4.572, 2-Propenamide, 2-methyl-N-phenyl- at 25.215, Nonane, 1-chloro- at 25.215, 1-Hexadecanol at 25.281, Dotriacontane at 25.860, Phenol, 2,4-bis(1,1-dimethylethyl)- at 26.359, Cyclopentanetridecanoic acid, methyl ester at 26.560, Dodecanoic acid at 27.574, Ethyl Ester of Docosanoic Acid at 27.574, Ethyl Ester of Docosanoic Acid at 32.920, Neophytadiene at 33.811, D-glucose 6 O- α D galactopyranosyl at 33.944, Oxirane, hexadecyl- at 33.811, 9-Eicosyne at 33.811, Isochiapin B at 34.4198, ß–pinene at 34.715, 1,2-Benzenedicarboxylic acid, dioctyl ester at 35.876 and Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- at 38.047.

Previous studies determined the chemical profile of chloroform extracts of *A. paniculata* leaves using GC-MS (Roy *et al.*, 2010). Kalaivani *et al.* (2012) reported the presence of thirteen different phytochemical compounds namely 1, 1, 3-triethoxy-propane, Tetradecanoic acid, 3, 7, 11, 15-tetramethyl-2-hexadecan-1-ol, n-hexadecanic acid, 9, 12-octadecadienoyl chloride,(Z,Z)-, Phytol, 9, 12-Octadecadienoic acid (Z,Z), 9,12,15-Octadecatrienoic acid(Z,Z,Z), 1,2-Benzenedicarboxylic acid, diisooctyl ester, squqlene, Retionic acid methyl ester, Androstan-17-one,3-ethyl-3-hydroxy-, (5α) and β-sitosterol in ethanol extract of *A. paniculata.* The therapeutically important active principle andrographolide was observed in the aerial part of *A. paniculata* (Chandrasekaran *et al.*, 2009). Shen *et al.* (2006) reported six entlabdanediterpenoids i.e. 3-O-beta-D-glucopyranosyl-14, 19-dideoxyandrographolide, 14-deoxy-17-hydroxyandrographolide, 19-O-(beta-D-apiofuransy (1-2)-beta-D,glucopyranoyl)-3,

14-dideoxyandrographolide, 3-O-beta-D-glucopyranosyl andrographolide, 12S-hydroxyandrographolide and andragraphato side from the aerial part of the plant. Similar to our results Gannadi and Dezfuly (2011) also reported seventeen compounds from *Myrtus communis* and Chibani *et al.* (2011) characterized eighteen compounds from *Ferula communis* using GC-MS analysis. The result could encourage the search for new active natural compounds offering an alternate to synthetic insecticides from other medicinal plants.

Table.1 Larvicidal effect of acetone extract of *A. paniculata* against different larval instar and pupae of *A. aegypti* treated with 24 hours

Larval and pupal stages	% of mortality				
	100ppm	150ppm	200ppm	250ppm	300ppm
I-Instar	45.0 ± 2.6	67.7 ± 1.2	76.3 ± 1.5	85.3 ± 2.5	100 ± 0.0
II-Instar	34.3 ± 2.1	44.7 ± 3.1	71.0 ± 2.6	84.7 ± 2.5	96.7 ± 1.5
III -Instar	34.3 ± 0.6	42.0 ± 1.7	63.3 ± 1.5	77.3 ± 2.1	89.3 ± 2.5
IV-Instar	25.3 ± 2.5	26.7 ± 2.1	40.0 ± 3.6	56.0 ± 4.6	79.3 ± 3.8
Pupae	31.7 ± 2.1	42.3 ± 3.5	69.0 ± 3.6	81.0 ± 5.0	93.3 ± 1.5

Values given in each cell is the mean ± SD of three replicates

Table.2 Lethal concentration values of acetone extract of A. paniculata against different larval instar of A. aegypti treated with 24 and 48 hours

Developmental stages	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	Regression equation	Chi-square
I-Instar	113.661 (16.570-151.561)	247.062 (206.570-360.353)	Y=-1.092+0.197X	7.880
II-Instar	149.068 (134.653-161.276)	268.120 (250.637-291.812)	Y=-1.605+0.194X	3.151
III –Instar	162.731 (146.429-176.648)	309.342 (285.526-343.567)	Y=-1.422+0.184X	0.885
IV-Instar	216.888 (176.595-270.189)	389.015 (315.740-626.649)	Y=-1.615+0.145X	6.715
Pupae	93.958 (71.313-109.892)	199.132 (184.965-218.317)	Y=-1.145+0.233X	1.819

LCL- Lower Confidential LimitUCL- Upper Confidential Limit

Table.3 Qualitative analysis of phytochemicals in acetone extract *A. paniculata*

S. No	Name of the Test	Phytochemical Constituents	Acetone
1	**Alkaloid**	Mayer's test Dragendroff's test Wagner Test	+ + +
2	**Carbohydrate**	Molish Test Fehling Test Benedicts Test	- - -
3	**Flavonoids**	Ammonia test	+
4	**Saponin**	Foam Test	-
5	**Coumarin**	Sodium chloride test	-
6	**Steroids**	Libermann's test Salkowaski test	- +
7	**Tannin**	Ferric chloride test	+
8	**Chlorogenic acid**	Ammonia test	+
9	**Anthocyanin**	H_2So_4 test	-
10	**Phenol**	Phenol reagent	+
11	**Flavones**	Shinoda's Test	-
12	**Anthracene Glycoside**	Borntrager's test	-

+ Presence of compounds; - Absence of compounds

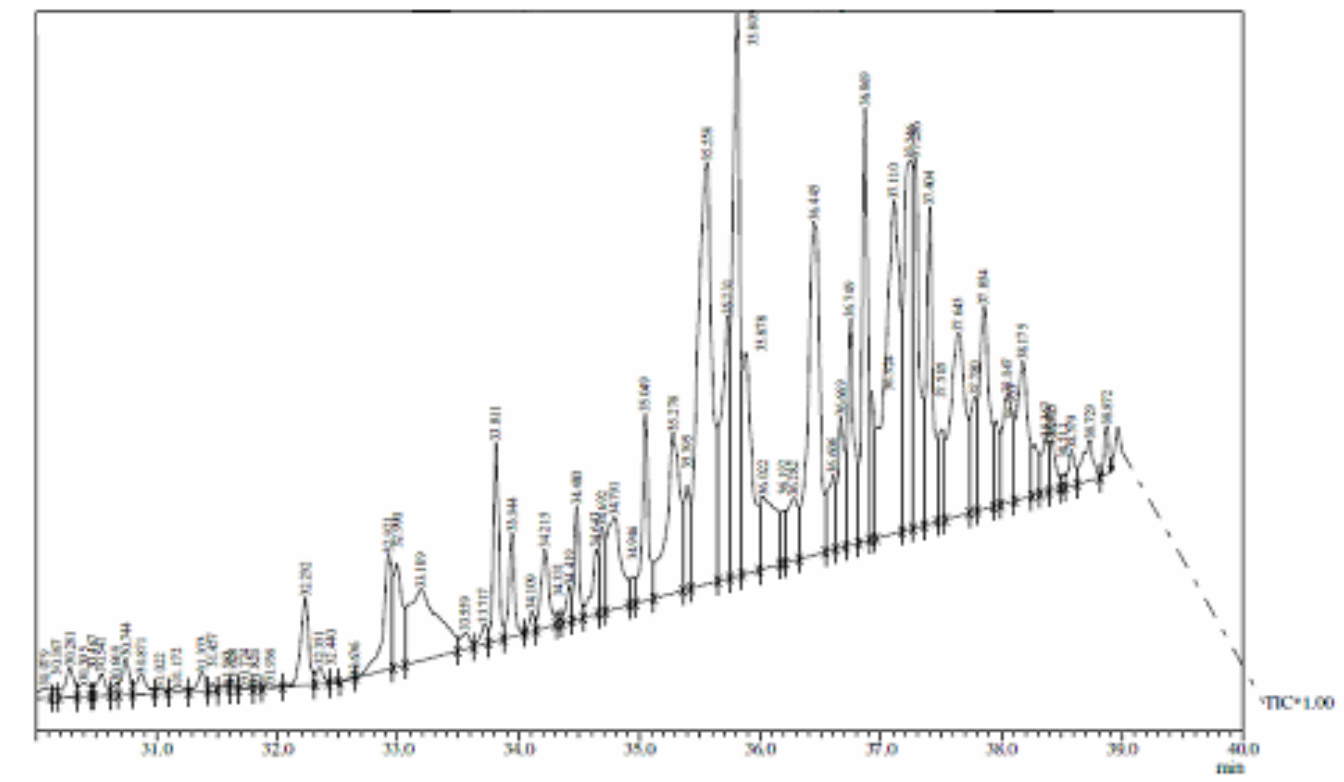
Table.4 Quantitative analysis of the phytochemicals in acetone extract *A. paniculata*

S. No	Name of the test	Acetone
1	**Alkaloid**	22.2**%**
2	**Flavonoids**	44.6**%**
3	**Steroid**	20.5**%**
4	**Tannin**	3.7**%**
5	**Phenol**	32.2**%**
6	**Chlorogenic acid**	5.3 %

Table.5 GC-MS analysis of acetone extract of *A. paniculata*

Compound Name	Retention Time	Molecular Formula	Molecular Weight	Peak area	Compound nature
ß – terpinolene	3.401	$C_{10}H_{16}$	136	0.02	Terpenoid
α – terpinene	3.686	$C_{10}H_{16}$	136	0.03	Terpenoid
Ethyl iso-allocholate	4.572	$C_{26}H_{44}O_5$	436	0.06	Steroid
2-Propenamide, 2-methyl-N-phenyl-	25.215	C10 H11 N O	161	0.36	-
Nonane, 1-chloro-	25.215	C9 H19 CL	162	0.36	-
1-Hexadecanol	25.281	C16 H34 O	242	0.12	Unsaturated fatty acid
Dotriacontane	25.860	C32 H66	451	0.17	Alkenes
Phenol, 2,4-bis(1,1-dimethylethyl)-	26.359	C14 H22 O	206	0.13	Organic compound
Cyclopentanetridecanoic acid, methyl ester	26.560	C19 H36 O2	296	0.10	Carboxylic acid amide
Dodecanoic acid	27.574	C12 H24 O2	200	0.94	Saturated fatty acid
Ethyl Ester of Docosanoic Acid	32.920	C24 H48 O2	368	1.53	-
Neophytadiene	33.811	C20 H38	278	1.47	-
D-glucose 6 O- α D galactopyranosyl	33.944	$C_{12}H_{22}O_{11}$	342	0.80	Sugar moiety
Oxirane, hexadecyl-	33.811	C18 H36 O	268	1.47	-
9-Eicosyne	33.811	C20 H38	278	1.47	-
Isochiapin B	34.4198	C19 H22 O6	346	0.27	-
ß – pinene	34.715	C10H16	136	0.88	Monoterpene
1,2-Benzenedicarboxylic acid, dioctyl ester	35.876	C24 H38 O4	390	2.78	-
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	38.047	C15H24	204	1.55	-

Fig.1 Gas chromatogram of acetone extract of *A. paniculata*



Plants have been used since ancient times to repel or kill blood-sucking insects in the human history and even now, in many parts of the world people are practicing plant substances to repel or kill the mosquitoes and other blood-sucking insects. We are all just around the corner to reinstate the chemical substances with plant-derived ones. In the present investigation, we have identified ecofriendly substances (leaf extract of *A. paniculata)* for the control of vector mosquitoes. Plants can provide safer alternatives for modern deadly poisonous synthetic chemicals. Further research regarding the effect of active principles of the medicinal plants on larvae is needed to understand the mechanism of action of active principles against the mosquito larvae. The results suggest for a possible utilization of the cheap and readily available medicinal plants for possible control of mosquitoes as a part of the integrated vector management program.

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