WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 1623-1633. Research Article ISSN 2277– 7105

LARVICIDAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM *VITEX NIGUNDO* LEAF AGAINST DENGUE VECTOR *AEDES ALBOPICTUS* (SKUSE)

Yuvo Singh[1, 2], Swarnagowreeswari G[1], Sathyavathi R[3], Ramachandra Reddy P[2] and B. Reddya Naik*[1]

[1]Department of Zoology, Osmania University Hyderabad. Telangana. India.
[2]Department of Botany, Osmania University Hyderabad. Telangana. India.
[3]Sandor Life Sciences, Hyderabad, Telangana, India.

Article Received on
14 October 2014,

Revised on 05 Nov 2014,
Accepted on 27 Nov 2014

***Correspondence for Author**
B. Reddya Naik
Department of Zoology,
Osmania University
Hyderabad. Telangana.
India.

ABSTRACT

In the present study we reported the larvicidal activity of silver nanoparticles synthesized from aqueous leaf extract of Vitex Nigundo against the larvae of Aedes albopictus which is prime vector for Dengue transmission. The characterization of synthesized silver nanoparticles was carried out using UV-vis spectrophotometer and transmission electron microscopy (TEM) Fourier Transform Infrared (FTIR). To find out presence of compounds which are responsible for larvicidal activity, we performed GC-MS analysis. The parasite larvae were exposed to varying concentrations of aqueous extract of and synthesized silver nanoparticles for 24 h as per WHO protocols. From the results it is observed that the mortality rate is two orders of Magnitude higher for silver nanoparticles compared to plant extract. By this approach, it is suggestive that this rapid synthesis of nanoparticles would be proper for developing a biological process for mosquito control.

KAYWORDS: Silver nanoparticles, Aedes albopictus, Vitex nigundo, FT-IR, GC-MS.

1. INTRODUCTION

There are a number of diseases borne by mosquitoes few of them are malaria, filaria, dengue, Japanese encephalitis and yellow fever. Dengue is a mosquito-borne viral infection causing a severe illness and fever, sometimes causing a potentially lethal complication called dengue haemorrhagic fever (DHF). The incidence of dengue has increased 30-fold over the last 50

years. [1] Up to 50-100 million infections are now estimated to occur annually in over 100 endemic countries, putting together almost half of the world's population at risk. [2] Dengue poses the greatest risk in highly populated regions with rainy seasons where there are large populations of *Aedes albopictus* with a high degree of contact between the mosquitoes and humans. [3]

It is imperative that the effective anti-larval measures can reduce the burden of mosquito menace to a greater extent, and applications of such anti-larval measures with the extracts or essential oils from plants are potential alternatives for mosquito larval control, as they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for use in control of mosquito larvae. [4] In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae. [5, 6] But, its application in the field is yet to be ascertained. However, biological reduction of metal would be a boon for the development of clean, nontoxic, and environmentally acceptable metal nanoparticles; the formed silver nanoparticles are hydrophilic in nature, disperse uniformly in water, highly stable, and had significant mosquito larvicidal activity. [7]

There are very few reports are available so far involving novel approaches to synthesize nanoparticles of various sizes by controlling larvicidal activity. Plant mediated bio-synthesis of nanoparticles using for larvicidal activity is to be viable alternative to chemical methods. Currently, there is a growing need to develop environmentally benevolent nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol. [8] Many biological systems, such as that of fungi [9] algae, [10] bacteria [11] and plants [12] have been studied for the biosynthesis of silver Nanoparticles. However, plant-based nanoparticles syntheses can be advantageous over other biological methods for mosquito larvicidal activity. The reaction rate for the synthesis of nanoparticles is very high, eco-friendly and not harmful to Non-target organisms. [13]

2. MATERIAL AND METHODS

2.1. Plant material: *Vitex nigundo* plant leaves was collected from Vikarabad forest, Ranga Reddy district, Andhra Pradesh, India (17° 19′ 48″ N, 77° 54′ 0″ E). *Vitex nigundo* (Verbinaceae family) was taxonomically identified by Prof. P. Ramachandra Reddy, Taxonomist, Department of Botany, Osmania University, Hyderabad, India. A voucher specimen was deposited in the Department of Zoology, Osmania University.

2.2. Processing of plant material

The Leaves of *Vitex nigundo* was shade dried at room temperature, for 10-15 days. The dried plant material was grinded to powder with an electrical stainless steel blender. The powder was socked with ethanol (99.5%) solvent and extracted using Soxhlet apparatus for 8hrs. The extract was concentrated to paste with rotary evaporator at 55ºC for 4 hours and the residue obtained was stored at 4ºC in an airtight bottle until further use.

2.3 Preparation of stock solution of plant Extracts

1gm of extract was taken and dissolved in 1000ml of double distilled water and filtered using Whatman filter paper No 1. It was stored at room temperature for further experiments as stock solution (1000ppm).

2.4 Synthesis of Silver Nanoparticles (AgNPs)

In a typical synthesis of silver (Ag) nanoparticles, the leaf extract (1.0ml) was added to 20 ml of 10^{-3} M AgNO3 (99.99%) aqueous solution and kept at room temperature. The experiment was done in triplicate for reproducibility. After 1 hour the colour of the solution changed from colourless to honey brown indicating the formation of silver nanoparticles and this is confirmed by UV-visible spectroscopy.

3. CHARACTERIZATION OF SILVER NANOPARTICLES

3.1 UV-Vis Absorption Spectrometry

The bio reduction of pure Ag+ ions was monitored by measuring the UV-visible spectra of the reaction medium. UV-visible spectral analysis was carried out with a SHIMAZDU 2600 – (TCC) UV-visible absorption spectrophotometer with a resolution of 1nm between 200nm and 700nm. A small aliquot of 300 µL. of the sample is diluted 10 times with Millipore water to avoid errors due to high optical density of the solution.

3.2 X-Ray Diffraction

The crystalline nature of the nanoparticles was measured using X-Ray Diffractometer (XRD) by depositing thin film of the Silver nanoparticles was made by dipping a glass plate in the solution and carried out the X-ray studies.

3.3 FTIR

For FTIR measurements, the AgNPs solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/

enzymes that are not capping the AgNPs. The samples were dried and grinded with KBr pellets and analysed on a Bruker Optics (Germany made) Tensor 27model in the diffuse reflectance mode operating at a resolution of 0.4 cm−1.

3.4 Transmission Electron Microscopy

The size and shape of AgNPs are visualized through the 200 kV Ultra High Resolution Transmission Electron Microscope (JEOL-2010). TEM grids are prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp.

3.5 Gas-Chromatography Mass Spectrometry (GC-MS)

GC-MS analysis was done by the SHIMAZDU QP2010, an oven temperature from 50 to 280 °C at 4°C/min and held at this temperature for 5min; inlet and interface temperatures were 250°C and 280 °C, respectively. Carrier gas was *He* at a flow rate of 1.0ml/min (constant flow). 0.2ml of sample was injected under split of 20:1. EIMS: electron energy, 70 eV.

Identification of compounds

Interpretation of mass spectrum GC-MS was conducted using data base of NIST, having more than 62,000 patterns. The spectrum of the known compounds was compared with the NIST library.

3.6 Dose-preparation

Based on the preliminary screening results the leaf extract of *Vitex nigundo* and synthesized AgNPs were subjected to dose-response bio-assay for larvicidal activity of *Ae. albopictus* larvae**.** The synthesis of leaf mediated silver Nanoparticles of *V. nigundo* was estimated at a concentration of 0.25ppm (Lc_{50}) and 1ppm (Lc_{90}). Whereas in solvent extraction with ethanol was estimated at a concentration of 25 ppm, 50ppm, 75ppm and 100ppm. [14]

3.7 Bioassay: According to WHO (2005) bioassay test was performed with different concentration to assess the larvicidal activity. [15]

3.8 Statistical analysis: The average larval mortality data were subjected to Probit analysis (FORTRAN) for calculating Lc_{50} and Lc_{90}. [16]

4. RESULTS AND DISCUSSION

At the larval stage can provide many associated benefits to vector control. Since silver nanoparticles are considered to be potential agents for various biological applications

including antimicrobial, its application as a mosquito larvicidal agent was investigated and found to be a highly effected. *Vitex nigundo* leaf solvent (Ethanol) extract was subjected to synthesis of Silver nanoparticles and the visible colour change (figure 1) indicates the formation of nanoparticles which is confirmed by UV-Visible absorption spectroscopy. The progress of the reaction between metal ions and the leaf extracts were monitored by UV–visible spectra of silver nanoparticles in aqueous solution with different reaction times that are shown in Figure 2. It was observed from the figure that the peak blue shifted in the absorption spectrum from 420 nm to 436 nm with increasing reaction time from 30 min to 120min. It took two hours to complete the reaction to form stable nanoparticles.

Figure 1: Shows the photograph (a—leaf extract, b—silver nanoparticles after 1 hour) of leaf extract of *Vitex nigundo.*

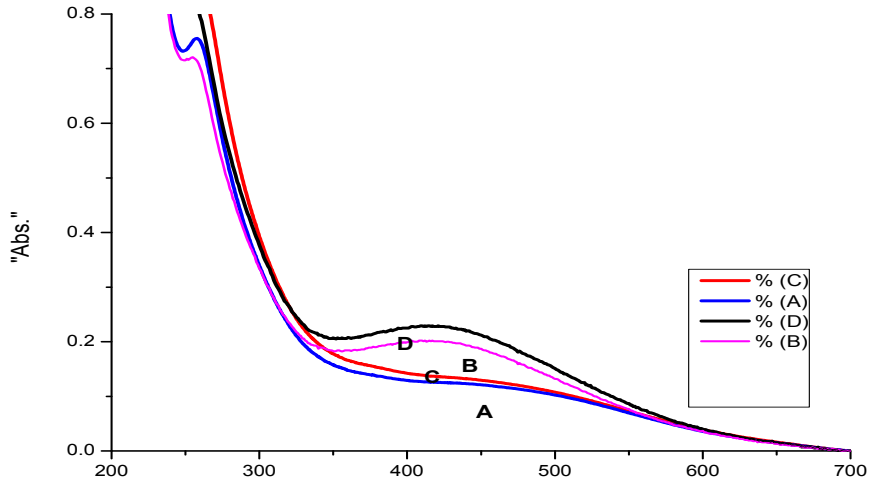


Figure 2: UV-visible spectra recorded as a function of time of reaction of an aqueous solution of 10−3 M AgNO3 with the leaf extract (a) 30 min (b) 60 min (c) 90 min and (e) 120 min.

The crystalline nature of Silver nanoparticles was confirmed by the X-ray diffraction analysis. Figure 3 shows the XRD pattern with the diffraction peaks at 38.0, 45.5 and 66.50 corresponding to the (111), (200) and (220) facets of the Face Centred Cubic (FCC) crystal structure. The broadening of the Bragg peaks indicates the formation of nanoparticles. In addition to the Bragg peaks representative of face centred cubic silver Nano crystals, additional, and yet unassigned, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles.

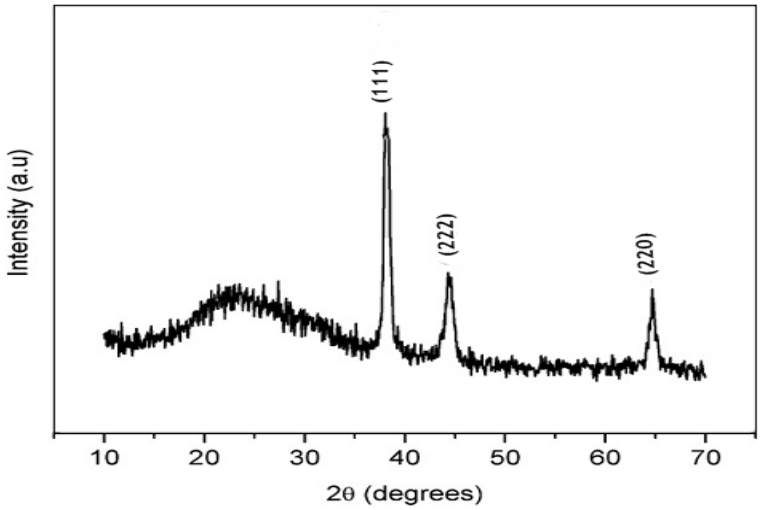


Figure 3: XRD pattern of as synthesized silver nanoparticles of *Vitex nigundo* leaf extract.

FTIR spectroscopy analysis were carried out to identify the potential bio molecules in the leaf extract responsible for the reduction and also the capping reagent responsible for the stability of the bio reduced silver nanoparticles. A typical FTIR spectrum of the obtained Silver nanoparticles is shown in Figure 4, the absorption bands at 3359, 2925, 2854, 1736, 1604, 1454, 1232and 1010 cm-1. The intense band at 3359 is corresponds to O-H stretching, 2925 is =C-H Medium stretching, 2854 Carboxylic acid O-H stretching, 1736 is due to stretch vibration of –C=O, the band at 1604 is corresponds to amide I, arising due to carbonyl stretch in proteins. The bands at 1454 and 1232 correspond to C-O stretch (phenolic) and ester phenolic compound respectively. The weak band at 1100 is –C-O- stretch. It is observed from the spectra of Silver nanoparticles the appeared bands at 1642 and 3359 which are due to hydroxyl group and amide-I that are responsible for reducing the Ag+ ions to atoms and suppressed bands at 1736, 1454 and 1232 are responsible for stabilizing the Nanoparticles.

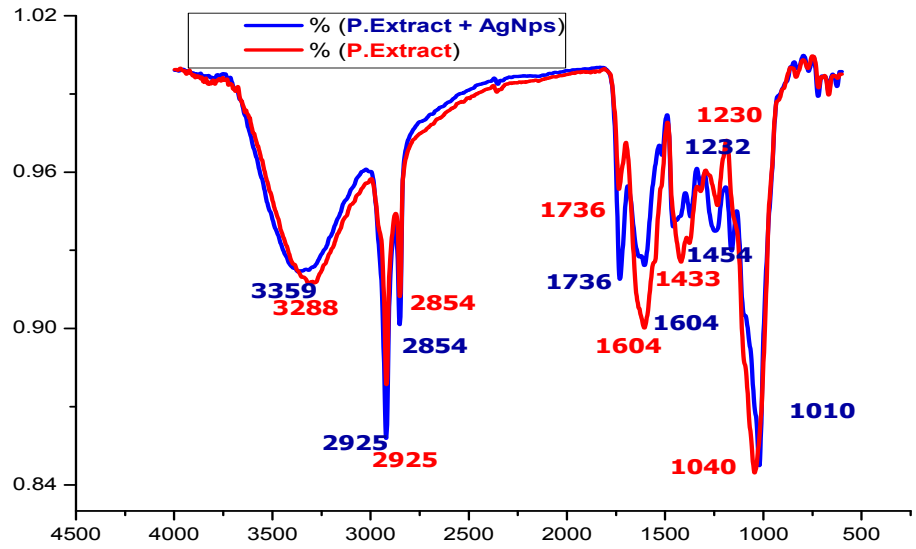


Figure 4: FT-IR spectrum of (a) Ag nanoparticles prepared from the extract and (b) fresh *Vitex nigundo* leaf extract.

TEM technique was employed to visualize the size and shape of Silver nanoparticles. TEM grids were prepared by placing a drop of the particle solution on a carbon coated copper grid and dried under lamp. Figure 5. Shows the typical bright –field TEM micrograph of synthesized by reduction of Ag^{+} ions with 1g biomass are predominantly are of the spherical in shape. It is evident that there is variation in particles sizes and average size estimated was 20nm and the particles size ranged from 10 nm to 80 nm.

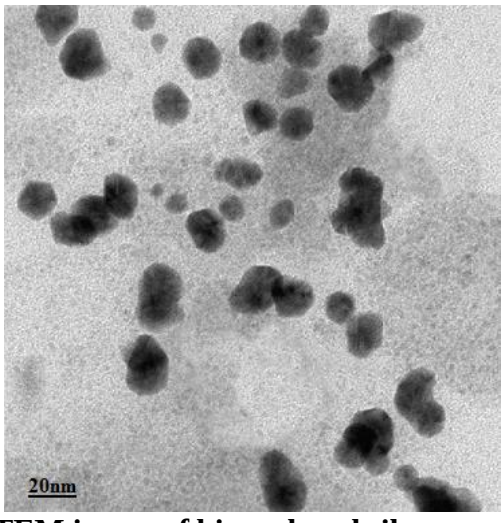


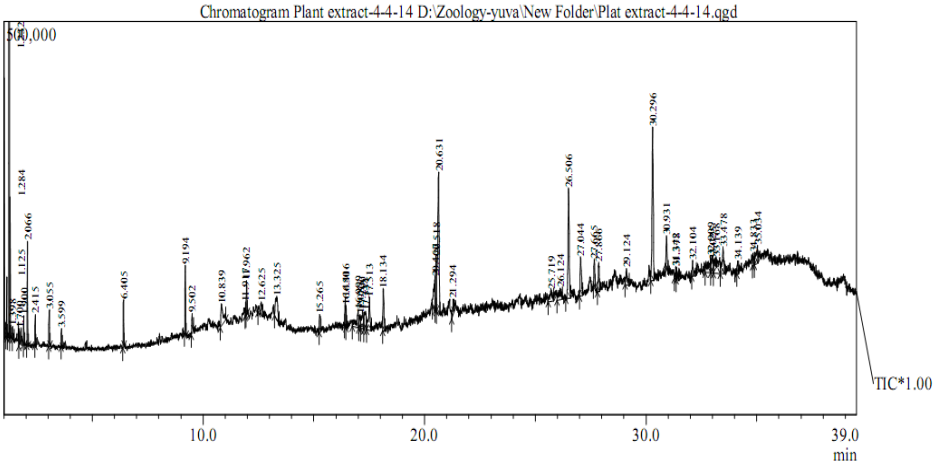
Figure 5. TEM image of bio-reduced silver nanoparticles.

The chromatogram of Gas Chromatography - Mass Spectrometry (GC-MS) shows that the active ingredients in synthesized silver nanoparticles of the leaf extraction (Table 1) was analysed and found that there were approximately 55 active compounds recorded within 25

minutes of retention time. However, there were two prominent compounds found at different peaks in chromatogram are (at peak number 30 and 39) namely **9-Octodecenoic acid (Z) (C18 H34 O2)** and **Tetracosanoic acid (C25 H50 O2)** with a maximum peak area i.e. 5.44% and 5.31% respectively and both are having larvicidal property, [17, 18] Therefore, it is derived that 9-Octodecenoic acid (Z) and Tetracosanoic acid are responsible as potential larvicide at Nano scale which are environmentally benign. The larvicidal activity of AgNPs against the larvae of Dengue vector (*Aedes albopictus)* was given in Table 2. From the results it was observed that the leaf extraction has shown 100 % mortality of the larvae at 100 ppm whereas AgNPs has 100% mortality rate is achieved at 1 ppm within 24 hours of exposure. It means almost 10^2 order magnitude higher mortality rate with AgNPs. The mechanism which causes the death of the larvae could be explained due to the nanoparticles can enter through oral cavity as well as body membrane of the Mosquito larvae. These AgNPs in the intracellular space can bind to sulphur containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes, subsequently, the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and finally cell death. Moreover in the present case, it is evident from the GC-MS spectra that the active compounds 9-Octodecenoic acid and Tetracosanoic acid which are having high larvicidal activity are also contributing towards the high mortality rates with less concentration of AgNPs. Similar findings were noticed in the Chloroxylon swietenia, Pongamia pinnata. [13, 17]

Table 2: Shows Larvicidal activity of the *Vitex nigundo* leaf extract and synthesis of Silver nanoparticles against *Aedes aegypti* Mosquito Larvae.

Extracts	Concentration (in ppm)	Mortality %	LCL-UCL (95%confidence limit)
Control	0	0	0
Plant Extract	25	20±1	18.864-21.131
	50	50±2.00	47.736-52.263
	75	75±2.64	72.006-77.993
	100	95±1.00	93.868-96.131
Control	0	0	0
Synthesized AgNPs	0.1	33±3.605	28.920-37.079
	0.25	51.66±2.081	49.311-54.022
	0.5	78±2.00	75.736-80.263
	0.75	87±1.00	85.868-88.131
	1	100±0.00	100±0.00



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	1.125	1.100	1.150	63576	0.45	76014	0.95	0.84	3-Pentanol, 2,3,4-trimethyl- (CA
2	1.232	1.150	1.258	7074184	50.06	5765703	72.38	1.23	Ethane, 1,1-diethoxy- $$ Acetald
3	1.284	1.258	1.317	145088	1.03	179479	2.25	0.81	3-Penten-2-one, (E)- (CAS) TRA
4	1.398	1.383	1.458	33741	0.24	16214	0.20	2.08	Propanoic acid, 2-hydroxy-, penty
5	1.700	1.675	1.717	21842	0.15	20244	0.25	1.08	Propane, 1,1-diethoxy- (CAS) 1,
6	1.900	1.875	1.942	44292	0.31	36819	0.46	1.20	2,4-Dimethyl-1-heptene
7	2.066	2.033	2.117	139402	0.99	132681	1.67	1.05	Propane, 1,1-diethoxy-2-methyl-
8	2.415	2.400	2.433	38533	0.27	39628	0.50	0.97	Propane, 1-(1-methylethoxy)-
9	3.055	3.017	3.092	94885	0.67	46229	0.58	2.05	1,1-diethoxy pentane $$
10	3.599	3.583	3.642	28246	0.20	23208	0.29	1.22	Pentane, 2,4-dimethyl-
11	6.405	6.367	6.433	97451	0.69	59871	0.75	1.63	Undecane
12	9.194	9.150	9.233	159195	1.13	88450	1.11	1.80	Hexadecane
13	9.502	9.475	9.558	62208	0.44	24787	0.31	2.51	Tetradecane, 1-chloro-
14	10.839	10.767	11.025	182423	1.29	27277	0.34	6.69	Benzoic acid, 4-hydroxy- (CAS)
15	11.917	11.900	11.933	29346	0.21	16292	0.20	1.80	Cyclohexene, 1-methyl-4-(1,5,9-t
16	11.962	11.933	12.000	96506	0.68	42592	0.53	2.27	pentadecane $$
17	12.625	12.467	12.692	102876	0.73	17279	0.22	5.95	Methanone, (1-hydroxycyclohexy
18	13.325	13.233	13.433	187855	1.33	29144	0.37	6.45	Methanone, (1-hydroxycyclohexy
19	15.265	15.233	15.317	71228	0.50	21779	0.27	3.27	DECANE, 2,3,5,8-TETRAMET
20	16.416	16.375	16.442	80331	0.57	36367	0.46	2.21	(7a-Isopropenyl-4,5-dimethyloct
21	16.450	16.442	16.492	43093	0.30	27973	0.35	1.54	1,2-Benzenedicarboxylic acid, di
22	16.999	16.733	17.033	150171	1.06	22834	0.29	6.58	6,9,12-Octadecatrienoic acid, ph
23	17.083	17.033	17.117	63261	0.45	17228	0.22	3.67	1,3-Dioxan-4-one, 2-(1,1-dimethy
24	17.200	17.117	17.250	123770	0.88	23073	0.29	5.36	Hexanoic acid, 2-methyl- (CAS)
25	17.334	17.250	17.375	136868	0.97	23229	0.29	5.89	Pentanoic acid, methyl ester (CA
26	17.513	17.375	17.600	209281	1.48	42109	0.53	4.97	Hexadecanoic acid, methyl ester
27	18.134	18.108	18.217	177198	1.25	53961	0.68	3.28	1,2-Benzenedicarboxylic acid, bu
28	20.467	20.350	20.483	198400	1.40	40534	0.51	4.89	N-[3-(dimethylamino)-3-ethoxy-
29	20.518	20.483	20.542	201911	1.43	69363	0.87	2.91	11,14-Eicosadienoic acid, methyl
30	20.631	20.542	20.700	769268	5.44	179758	2.26	4.28	9-Octadecenoic acid (Z)-, methyl
31	21.294	21.225	21.333	54945	0.39	19276	0.24	2.85	(2r,3r)-2,3-epoxyoctadec-4-yn-1-
32	25.719	25.583	25.825	112982	0.80	15251	0.19	7.41	Phosphine oxide, triphenyl- (CAS
33	26.124	25.975	26.233	112104	0.79	15112	0.19	7.42	Benzeneacetic acid, 4-methoxy-.a
34	26.506	26.367	26.608	546697	3.87	140274	1.76	3.90	Docosanoic acid, methyl ester
35	27.044	26.992	27.150	213401	1.51	49906	0.63	4.28	4-Oxazolemethanol, 4,5-dihydro-
36	27.665	27.625	27.708	102292	0.72	34950	0.44	2.93	1,2,3-Benzotriazin-4(3H)-one, 3-
37	27.866	27.800	27.917	136341	0.96	37135	0.47	3.67	Heneicosanoic acid, methyl ester
38	29.124	29.058	29.250	95753	0.68	20644	0.26	4.64	A-Norcholestan-3-one, 5-ethenyl-
39	30.296	30.225	30.367	750084	5.31	195828	2.46	3.83	Tetracosanoic acid, methyl ester
40	30.931	30.892	31.000	112505	0.80	39942	0.50	2.82	Triacontanoic acid, methyl ester
41	31.342	31.283	31.358	33817	0.24	15634	0.20	2.16	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9
42	31.378	31.358	31.475	48673	0.34	15518	0.19	3.14	Acetophenone, 2-phenyl-2'-(trimc
43	32.104	32.067	32.267	81684	0.58	19891	0.25	4.11	3H-Pyrazol-3-one, 4-[[4-(diethyla
44	32.909	32.667	32.925	148273	1.05	20816	0.26	7.12	Severine $$ Benzamide, N-[2-[4-
45	33.025	32.925	33.042	102881	0.73	15876	0.20	6.48	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9
46	33.168	33.042	33.358	263214	1.86	20306	0.25	12.96	Benzoic acid, 2,4-bis[(trimethylsi
47	33.478	33.358	33.542	180613	1.28	34136	0.43	5.29	Nonadecanoic acid, methyl ester
48	34.139	34.100	34.175	42643	0.30	16311	0.20	2.61	1,1,3,3,5,5,7,7,9,9,11,11,13,13-1
49	34.833	34.817	34.892	42023	0.30	15535	0.20	2.71	Nonyl-phenol mix of isomers $$
50	35.034	34.892	35.058	125432	0.89	23136	0.29	5.42	1,3,5,7,9-Pentaethylbicyclo[5.3.1
				14132786	100.00	7965596	100.00		

Table 1: GC-MS analysis of *Vitex nigundo* leaf extract synthesis with AgNPs.

REFERENCES

1. http://www.medindia.net/patients/patientinfo/mosquito.htm#ixzz33f4oCQZt.
2. Whitehorn J, Farrar J. Dengue. *Br. Med. Bull*, 2010; 95:161–73. doi:10.1093/bmb/ldq019. PMID 20616106
3. http://www.nature.com/scitable/topicpage/dengue-transmission-22399758.
4. Amer, A. and Mehlhorn, H. Persistency of Larvicidal Effects of Plant Oil Extracts under Different Storage Conditions. Parasitology Research, 2006; 99: 473-477. http://dx.doi.org/10.1007/s00436-006-0183-2.
5. Amer, A. and Mehlhorn, H. Larvicidal Effects of Various Essential Oils against Aedes, Anopheles, and Culex Larvae (Diptera: Culicidae). Parasitology Research, 2006; 99: 466 472. http://dx.doi.org/10.1007/s00436-006-0182-3.
6. Abdul Rahuman, A., Gopalakrishnan, G., Venkatesan, P. and Geetha, K. Larvicidal Activity of Some Euphorbiaceae Plant Extract against Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). Parasitology Research, 2008; 102: 867-873. http://dx.doi.org/10.1007/s00436-007-0839-6
7. Subarani S, Sabhanayakam S and Kamaraj C. Studies on the Impact of Biosynthesized Silver Nanoparticles (AgNPs) in Relation to Malaria and Filariasis Vector Control against Anopheles stephensi Liston and Culex quinquefasciatus Say (Diptera: Culicidae). Parasitology Research, 2013; 112:487-499. http://dx.doi.org/10.1007/s00436-012-3158-5.
8. G M Whitesides Nat. Biotechnol., 2003; 21: 1161–1165. Published online: 30 September 2003 | doi:10.1038/nbt872
9. D. S. Balaji, S. Basavaraja, R. Deshpande, D. B. Mahesh, B. K. Prabhakar, A. Venkataraman, Extracellular biosynthesis of functionalized silver nanoparticles by strains of Cladosporium cladosporioides fungus. Colloids and surfaces B: bio interfaces, 2009; 68: 88-92.
10. H.M. El-Rafie Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. Carbohydrate Polymers, 2013; 96: 403–410.
11. C. Krishnaraj, E G Jagan, S Rajasekar and P Selvakumar, P T Kalaichelvan, N Mohan, Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces, 2010; 76: 50-56.
12. Philip D, Green synthesis of gold and silver nanoparticles using Hibiscus Rosa sinensis. Physica. 2010; 42: 1417–1424.

13. B. Bannoth Reddya Naik, G. Swarna Gowreeswari, Yuvo Singh, R. Satyavathi, S. S. Daravath, P. Ramachandra Reddy Bio-Synthesis of Silver Nanoparticles from Leaf Extract of Pongamia pinnata as an Effective Larvicide on Dengue Vector Aedes albopictus (Skuse) (Diptera: Culicidae). Advances in Entomology, 2014; 2: 93-101 http://www.scirp.org/journal/ae http://dx.doi.org/10.4236/ae.2014.22016.
14. WHO (2013) http://www.who.int/mediacentre/factsheets/fs117/en/index.html
15. WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/WHOPES/GCDPP, 2005; 13: 10-12.
16. Reddy P J, Krishna D, Murthy U S and Jamil K. A Microcomputer FORTRAN Program for Rapid Determination of Lethal Concentration of Biocides in Mosquito Control. Computer Applications in the Biosciences, 1992; 8: 209- 213.
17. Reddya Naik Bannoth, Yuvo Singh, Swarnagowreeswari, G., Satyavathi, R and 3 Ramachandra Reddy, P Silver Nanoparticles synthesis From Leaf Extract Of *Chloroxylon swietenia* DC As An Effective Larvicide On Dengue Vector *Aedes albopictus* (Skuse) (Insecta: Diptera: Culicidae) International Journal of Recent Scientific Research, 2014; 5(3): 580-584.
18. Sivakumar R, Jebanesan A, Govindarajan M, Rajasekar P. Larvicidal and repellent activity of tetradecanoic acid against Aedes aegypti (Linn.) and Culex quinquefasciatus (Say.) (Diptera: Culicidae). Asian Pac J Trop Med, Sep 2011; 4(9): 706-10. doi: 10.1016/S1995.