Selvarani et al., J Nanomater Mol Nanotechnol 2016, 5:2
http://dx.doi.org/10.4172/2324-8777.1000179

Journal of Nanomaterials & Molecular Nanotechnology

Research Article

A SCITECHNOL JOURNAL

Ocimum Kilimandscharicum Leaf Extract Engineered Silver Nanoparticles and Its Bioactivity

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Abstract

The present study designed to engineer highly active silver nanoparticle from ELE of *Ocimum kilimandscharicum* and evaluated its larvicidal potential against *Aedes aegypti.* The ELE was used for the synthesis of nanoparticle and the synthesized nanoparticle was subjected to instrumental analysis such as UV-Vis spectrum, SEM, EDX and XRD. It was observed from the present study that, the nanoparticles (Ok-AgNps) was spherical, polydispersed, 10 to 50 nm in size, negatively charged and stable in nature. The Ok-AgNps and ELE was studied for its larvicidal efficiency against *Ae. aegypti* and found promising activity by Ok-AgNps (LC_{50} value 0.009ppm) than ELE (LC_{50} value 46.61 ppm). It could be further analyzed for its suitability in mass scale evaluation and toxicity to non-target organisms.

Keywords

Silver nanoparticle; *Ocimum kilimandscharicum*; GC-MS; *Aedes aegypti;* Ethanol leaf extract

Introduction

Mosquitoes, an arthropod pest (Diptera: Culicidae) are vectors of major killer diseases such as dengue, malaria and yellow fever which are now prevailed in developing countries [1]. Among them, dengue [2] is a viral infection caused by the biting of flavivirus infected *Aedes aegypti.* According to the Bhatt et al. [3] 390 million dengue infections were reported per year. Brady et al. [4] estimated that in 128 countries, 3.9 billion people are at the risk of dengue viral infection. In India, according to National Vector Borne Disease Control Programme [5] about 28, 292 dengue infection cases and 108 deaths were reported during 2010. In order to reduce the dispersal of vector borne diseases, different campaign have been introduced like chemical pesticide application, but in their high frequency, it loaded not only toxic residue in various ecosystems but also develop resistant to it [6]. Despite recent advances for potential vaccines and new therapeutic options, the control of vector-borne diseases remains difficult [7]. Subarani et al. [8] have reported that the plant extracts are effective against vector control.

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Received: November 17, 2015 **Accepted:** March 14, 2016 **Published:** March 19, 2016

As these strategy consumes enormous time and dose, the need of environmental benign technology that support the rapid eradication in considerably low dose usage is emerged. In this line, nanoparticles (Nps) have been found suitable and consistent replacement for the mosquito control program. As nanotechnology is a unique and interesting branch of science, manufacturing novel biomaterials in various size and shape [9] for the welfare of human beings. In order to reduce toxic chemical synthesis procedures involved in nanoparticle synthesis, currently different kind of biological organisms and plant extracts are beings used [10]. Of the metal nanoparticles used, such as silver, copper, titanium, zinc [11], gold, magnesium [12], silver nanoparticle had been found effective towards bacteria, eukaryotic microorganisms and viruses [13]. Currently, silver nanoparticles (AgNps) [14,15] and gold nanoparticle [16] have been greatly employed for the control of mosquito larvae. It has been identified that, photochemical (extracts) of plants [17-19] and algae [20] have assisted in synthesis of highly active nanoparticles. In this line, use of one of the family of the sacred and holy herb, *Ocimum kilimandscharicum* would definitely become a potential precursor for the synthesis of silver nanoparticles with broad bioactivity. Pragadheesh et al. reported that, the leaf of *O. kilimandscharicum* exhibited linalool, as a major constituent, followed by camphor and 1,8, *cineole*. Besides, *O. kilimandscharicum* reported to exhibiting excellent anti-oxidant potentials. It has been known that, triterpenes flavonoids and eugenol are important compounds found in leaf extract of basil [21]. de Lima et al. [22] reported that, the leaves of *O. kilimanscharicum* comprise 45 components with a predominance of monoterpenes. Hence, in the present investigation, such an excellent phtyochemical factory of *O. kilimanscharicum* was used for as precursor for the synthesis of stable and highly active nanoparticles.

Materials and Methods

Chemicals and reagents

Analytical grade silver nitrate and phytochemical screening chemicals were purchased from Reachem laboratory chemicals, India for the present investigation. Whatman No.1 filter paper was purchased from Hi-Media, India. Ok-AgNps characterization was performed at Karunya University, Coimbatore, Tamil Nadu, India.

Collection of *Ocimum kilimandscharicum*

Ocimum kilimandscharium plants were collected from vaigai river bed, korippalayam Madurai district, Tamil Nadu, India. The plants were brought to the laboratory after proper identification.

Preparation of Ethanol Leaf Extract (ELE) of *Ocimum kilimandscharicum*

Fresh leaves of *O. kilimandscharicum* were collected and shade dried. It was powdered by using a mixer (Preethi, India) and sieved. The ground plant material was subsequently used for extraction. 5 gm of fine powered sample was weighed and soaked with 100 ml of ethanol and allowed to stand for 7 days at ambient room temperature. The soaked plant powder was filtered by passing through a whatman No.1 filter paper (Hi-Media, India) and crude extract was stored for phytochemical analysis.

Phytochemical screening

The initial screening of the phytochemicals present in the ethanol leaf extract (ELE) was carried out by using method followed by Harborne [23]. Thin layer chromatography separation was performed to identify compounds present in the ELE. The ethyl acetate and benzene (1:1) solvent system was used for separation. The plate was air dried and sprayed with perchloric acid [24] for fraction development. After the successful development, the plate was examined under the UV Chamber at 366 nm. The observed fraction was subjected to Gas Chromatography - Mass Spectrometry (GC/MS) analysis (GC-MS; QP-5050, Schimadzu, Japan) [25].

Synthesis of silver nanoparticles

1ml of ELE was taken from ELE stock (100 mg of ELE of *O. kilimandscharium* dissolved in 10 ml of distilled water) and added to 99 ml of 1 mM (0.017 mg/100 mL) of silver nitrate ($AgNO_3$) (Reachem Laboratory Chemicals, India). It was then allowed to constant stirring in an Orbit shaker (Neolab, Neolab instruments, Mumbai, India) and the colour change was noticed. The samples were taken at different period of time (3, 6, 9, 12 miniutes) interval by using 1000 µl pipette and its absorbance was observed in UV-Visible Spectrophotometer (LabKit, Hongkong) at a resolution of 1 nm between 200 and 800 nm. The sample containing silver nanoparticle was centrifuged and the pellet was collected, which was then rinsed with double distilled water and centrifuged. Finally, the pellet was collected and was mixed with 1 ml of double distilled water for further use. The nanoparticle synthesized (Ok-AgNps) was characterized by using Scanning Electron Microscope (Hitachi S-4500 SEM machine) for its physical morphology and size, EDX (Hitachi S-340 N) for the presence of elemental silver and the crystalline characteristics of silver nanoparticle were determined from the width of the XRD peaks, using the Debye-Scherrer formula,

$$D = 0.94\lambda / \beta \cos\theta$$

Where, D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X- ray wave length and β is the full width at half maximum and θ is the diffraction angle. The capping agent or active substance along with silver nanoparticle was studied by using Fourier Transform-Infra Red (FTIR). The stability and charge of the nanoparticle was also studied by using DLS.

Larvicidal bioassay

Larvae of *A. aegypti* were collected from Indian Council of Medical Research, (ICMR) Madurai and reared in the laboratory by supplementing crushed fine powder 3:1 ratio of dog biscuit and yeast extract. The bioassay was performed by using the method followed by WHO [26]. Bioassay was conducted with five different concentrations of ELE (50, 100, 150, 200 and 250 ppm) and Ok-AgNps (0.01, 0.02, 0.03, 0.04 and 0.05ppm). Triplicate was maintained for each concentration. For the bioassay I, II, III and IV instars larvae of (25 number) were transferred into 1000 mL glass beaker (Borosil®) containing different concentration of ELE and Ok-AgNps. The set-up was maintained at 28 ± 2°C and 79 ± 2% RH. The distilled water alone maintained as control group. The mortality of larvae was recorded at 24-hr intervals at both control and experimental group.

Statistical analysis

The Duncan's Multiple Range Test (DMRT) was performed against per cent cumulative mortality data to identify the best treatment. The mortality date was subjected to probit analysis by using Statistical Packages for Social Science (SPSS 11.5) to estimate the lethal concentrations (LC_{50}; LC_{90}) and Chi-square value.

Results

Phytochemical composition of *Ocimum kilimandscharicum*

The present study investigated the nanoparticles synthesis from *Ocimum kilamandscharicum* and evaluated its larvicidal activity against dengue vector, *Aedes aegypti*. The phytochemical composition of ELE resulted that, the ELE was endowed with carbohydrates, terpenoids, flavoids, alkaloids and reducing sugars (Table 1). It was observed from the chromatogram of GC-MS that, the ELE was enormous composition of terpenes of 14 types (Figure 1). The percentage of peak area represents the quantity of individual presence of terpenes in the fraction. The most prevailing major compounds 3,5-di-terta-butyl-methyl phenol as *terpene*-derived compounds, tetradecanoic acid, hexadecanoic (palmitic) acid, tetradecanoic acid of sesquiterpenes, dodecanoic acid and benzaldehyde of monoterpenes heneicosane and octadecanoic acid, diterpenes obtained from *O. kilimandscharicum* leaves.

Characterization of Silver nanoparticles (Ok-AgNps)

It was observed from the Figure 1 is that the terpene rich ethanol extract was used for the synthesis of silver nanoparticle (Ok-AgNps) and was measured in UV-Vis spectrum (Figure 2A). Ok-AgNps formation in the experimental solution was confirmed qualitatively by the change in the color of solution from yellow to brown and it was well supported by the plasmon band at 410 nm, which was indicating the presence of spherical Ag nanoparticles in the UV-Vis spectrum. The spherical shape with particle size range from 5 to 40 nm of the Ok-AgNps was confirmed in SEM analysis (Figue 2B). The elemental analysis of the silver nanoparticles was performed using EDX (Figure 2C) and the peaks around 2.8 KeV correspond to the binding energies of Ag, which indicating the reaction product was composed of Ag nanoparticles. The FTIR spectra (Figure 2D) of silver nanoparticles synthesized from ethanol leaf extract of *Ocimum kilimandscharicum* detailed that, it have had different vibrations possessing different functional groups. The FTIR spectra of silver nanoparticles synthesized from ethanol leaf extract of *Ocimum kilimandscharicum* detailed that, it have had different vibrations possessing different functional groups. A symmetric stretching vibration of the carboxylate group was found at the peak around 1570.06^{-1}. A peak at 1631.78^{-1} indicates the amine N-H bending. Similarly, peak at 2931.8^{-1} indicates the R-CH_2-R, a C-H stretching, a functional aliphatic methylene group. The peak at 3410.15^{-1} indicates

Table 1: Phytochemicals analysis of ethanol leaf extract of *Ocimum kilimandscharicum*.

S.No	Phytochemical tests	*Ocimum kilimandscharicum*
1	Carbohydrate	+
2	Sterols, Phytosterols, Triterpenoidalsapogenins	+
3	Coumarins	-
4	Xanthoprotein	-
5	Aromatic acid	+
6	Essential Oil	+
7	Tannins	-
8	Flavanoids	+
9	Alkaloids	+
10	Saponins	-
11	Reducing sugar	+

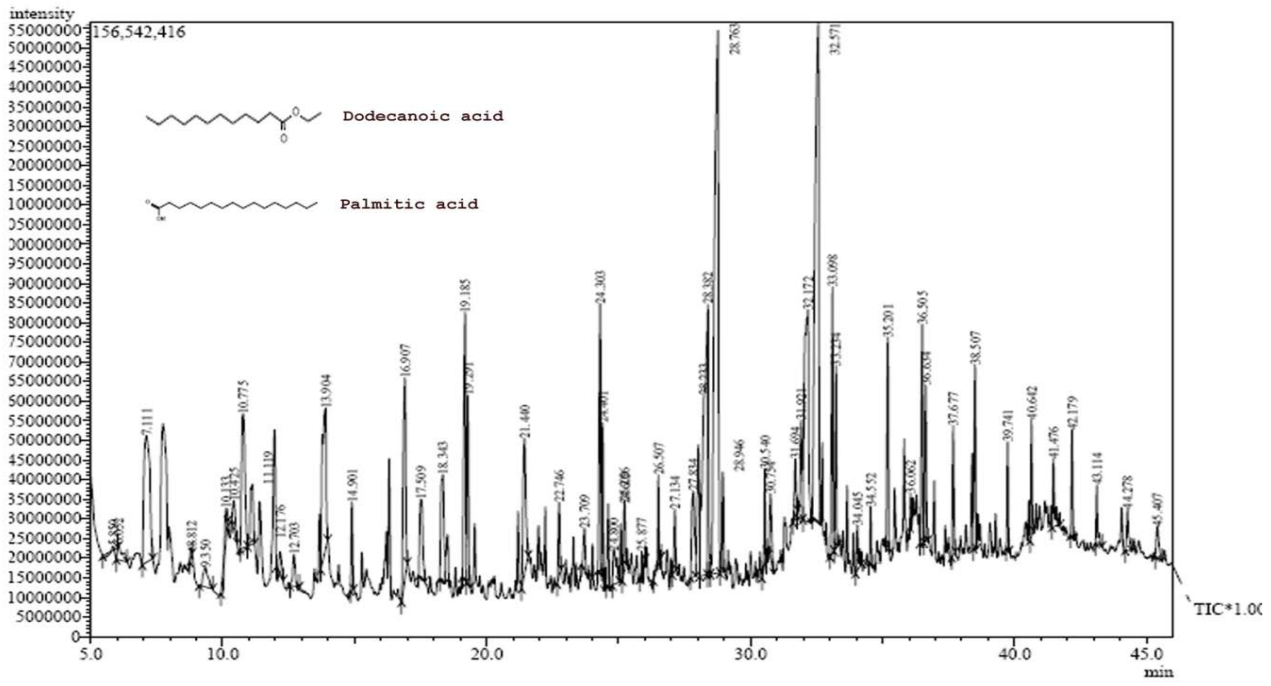

Figure 1: Gas Chromatography-Mass Spectra of ELE of *Ocimum kilimandscharicum.*

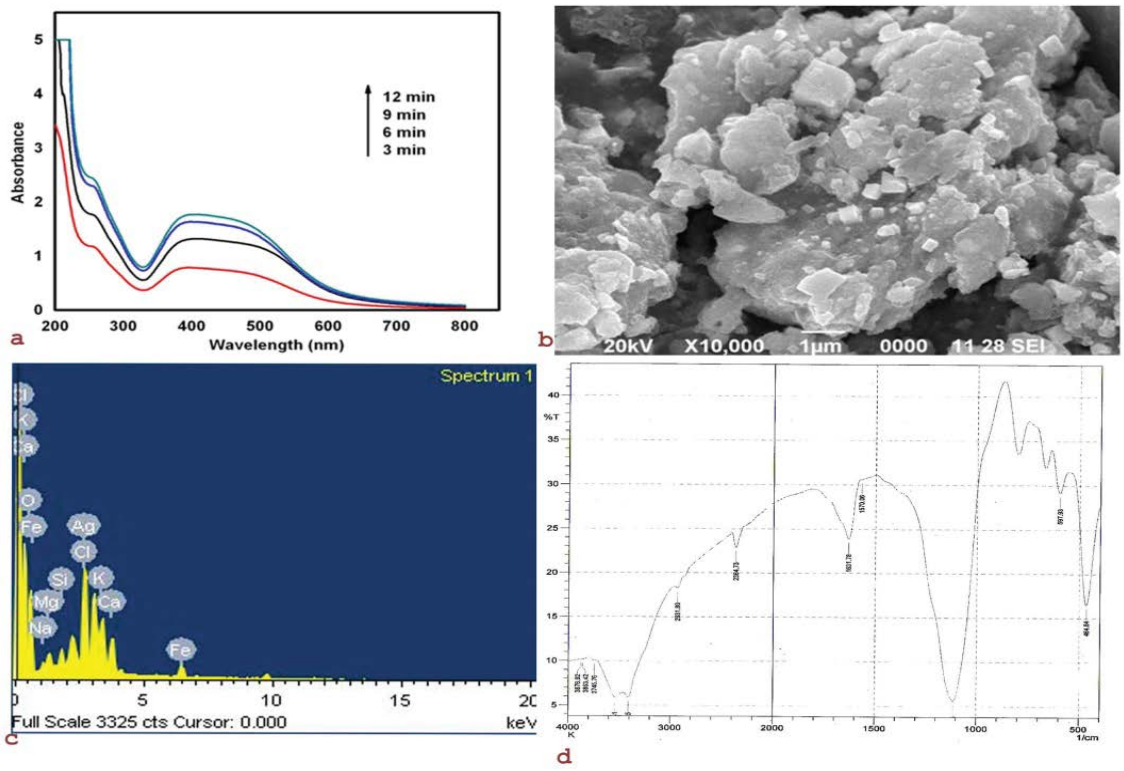

Figure 2: Structural elucidation of silver nanoparticle synthesized by using leaf extract of *Ocimum kilimandscharicum*.
2A: UV-Vis spectrum of silver nanoparticle (Ok-AgNps) during different period of intervals
2B: Scanning Electron Microscopic (SEM) view of silver nanoparticle (Ok-AgNps)
2C: Energy dispersive X-ray spectroscopy (EDX) of silver nanoparticle (Ok-AgNps); 2d. Fourier Transform-Infra Red (FTIR) spectra of silver nanoparticle (Ok-AgNps) synthesized by using leaf extract of *Ocimum kilimandscharicum*

the hydroxyl group stretching. This clearly evidenced the capping of carboxylate group of decacoic acid bound chemically over the silver nanoparticles. The diffraction intensity of the Ok-AgNps was recorded from 10° to 90° at 2 theta degrees (XRD) and the results revealed that, 27.84°, 32.24°, 46.21°, 55.72°, 66.78° and 77.32° corresponding to (111), (200), (220), (311) a set of lattice planes were observed which may be indexed based on the face-centered cubic structure of silver (Figure 3). It was evidenced from the particle size analysis that, the size of the nanoparticles ranged from 10 to 50 nm, in which 30 and 40 nm size nanoparticle had higher percentage (~50%). The surface of the nanoparticle was coated with negatively charged (-18.7 mV) and their stability was also high, which was clearly identified from zeta potential measurements (Figure 4).

Bioassay

In the present study, the larvicidal activity of ELE and nanoparticles (Ok-AgNps) synthesized from *O. kilimandscharicum* was performed against instars dengue vector, *Aedes aegypti* and the results were presented in Tables 2 and 3. The results revealed that, silver ELE yielded a maximum per cent cumulative mortality (PCM) (95.08%) against I instars larvae of *Ae. aegypti* followed II (88.34%) and III instar larvae (77.08%), while activity of Ok- AgNps revealed 97.08% and 90 % per cent cumulative mortality against I and II, III as well as IV instars larvae. of *Ae. aegypti.* The probit analysis revealed that, (LC_{50} and LC_{90} value) the Ok-AgNps (0.009ppm) showed excellent larvicidal efficiency against I instar larvae of *Ae. aegypti* which was followed by II and III instars larvae with respect to 0.015 and 0.018 ppm accordingly, while LC_{50} of ELE revealed only 46.61 ppm against I instar larvae of *Ae. aegypti* followed by II and III instars larvae with respect to 71.63 and 126.75 ppm.

Discussion

Mosquitoes control program composed and merely consumed vast number of efficient technologies and are still taunting errands to biomedical scientist all over the world. In the recent decade, use of viable and promising technologies over mosquito control has been raised considerably. In this line, plant extracts and its metabolites are not only fulfilling the medicinal values but also they potent precursor or capping agent [20,27] for nanoparticle synthesis, the central dogma of nanotechnology which have now been employed for the efficient control of mosquito borne disease [18,19,27,28].

In the present investigation, leaf extract of the one of the member of basil family, *Ocimum kilimanscharicum* was used for the synthesis of nanoparticles. Dwivedi et al. [29]; Patil et al. [30]; Philip and Unni [31] have synthesized silver nanoparticles from foliage while Ahmad et al. [32] used stem and root. The use *O. kilimanscharicum* was introduced for the synthesis of silver nanoparticle in the present investigation. Phytochemical of plants (Borah et al. [33] and algae [20] are of greater importance in nanoparticle synthesis, as they are potent chemical doses, capable of generating nanoparticles useful for the welfare of human beings. From the present investigation, it was evidenced that, the ELE of *O. kilimandscharicum* was reservoir of terpenes namely 3, 5-di-terta-butyl-methyl phenol, tetradecanoic acid, hexadecanoic (palmitic) acid, tetradecanoic, benzaldehyde and octadecanoic acid. It was strongly supported by de Lima et al. [22], who reported that, the leaf of *O. kilimandscharicum* have been endowed with enormous amount of terpenes and Shim et al. [34] who have used individual chemicals of palmitic and dodecanoic acid for the synthesis of silver nanoparticles and provided clear evidence that, the conversion of silver nitrate into silver palimate followed by silver nanoparticle. The role of terpenoids in silver reduction and generation of nanoparticles was studied by Mukhlesur Rahman et al. [35].

Such a terpene enriched extract (ELE) was used for the synthesis of silver nanoparticles. The nanoparticle synthesized from *O. kilimandscharicum* exhibited a plasmon band at 410 nm, in support of our study almost similar excitation (406 nm) by the silver nanoparticle synthesized from *O. sanctum* leaf was observed by Rao et al. [36]. The present study was well supported by Dwivedi et al. [29] who observed surface plasmon resonance (SPR) excitation between 410 and 480 nm. Similar to the result observed by Subba Rao et al. [36], the present investigation also found nanoparticle of varied size (10-50 nm), shape and polydispersed using SEM analysis. A similar investigation was also done by Shankar et al. [37] who observed polydispersed spherical and flat like nanoparticles of 5-35 nm size. Derivatives of terpene have also synthesized monodispersed nanoparticles [34]. Besides, Mukhlesur Rahman et al. [35] strongly supported the present study that, the emanation of nanohexagons and triangles was due to the binding of aldehydes/ketones of terpenes. Krishnaraj et al. [38] observed 20-30nm size nanoparticles synthesized from *Acalypha indica.* The SEM coupled EDX analysis revealed that, in addition to strong silver excitation peak, O, Fe and Al peaks were also observed, which was due to nanoparticles bound terpenes such as palmitic acid, dedecanoic acid on the surface of the Ok-AgNps. It was strongly supported by Subba Rao et al. [36] who observed oxygen and aluminium besides AgNps. Role of proteins, phenolics and carbohydrates in metal nanoparticle synthesis was demonstrated by Kumar and Yadav [39]. The FTIR spectra also clearly suggests that the carboxylate group of the decanoic acid had acted as capping agent over the surface of the silver nanoparticles and it was highly supported by Pahari et al. [40]. The zeta potential study revealed that, the nanoparticle synthesized from *O. kilimandscharicum* was negatively charged and are of highly stable. This indicates the repulsive/attractive force exists in the nanoparticle and this can be used to measure the stability.

The nanoparticle (Ok-AgNps) synthesized from ELE of *O. kilimandscharicum* revealed least LC_{50} values 0.009 and 0.015 ppm against *Ae. aegypti*. Compared to Suresh et al. [18] and Chitra et al. [17], who have recorded LC_{50} value of 6.2 (III Instar) and 0.211 ppm

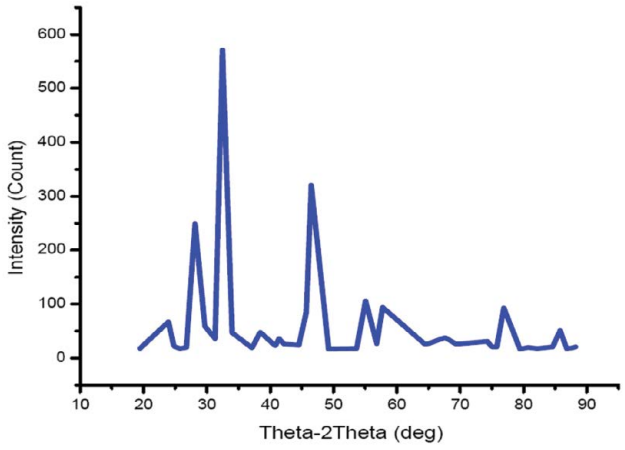

Figure 3: X- Ray Diffraction (XRD) spectra of silver nanoparticle (Ok-AgNps) synthesized by using leaf extract of *Ocimum kilimandscharicum*.

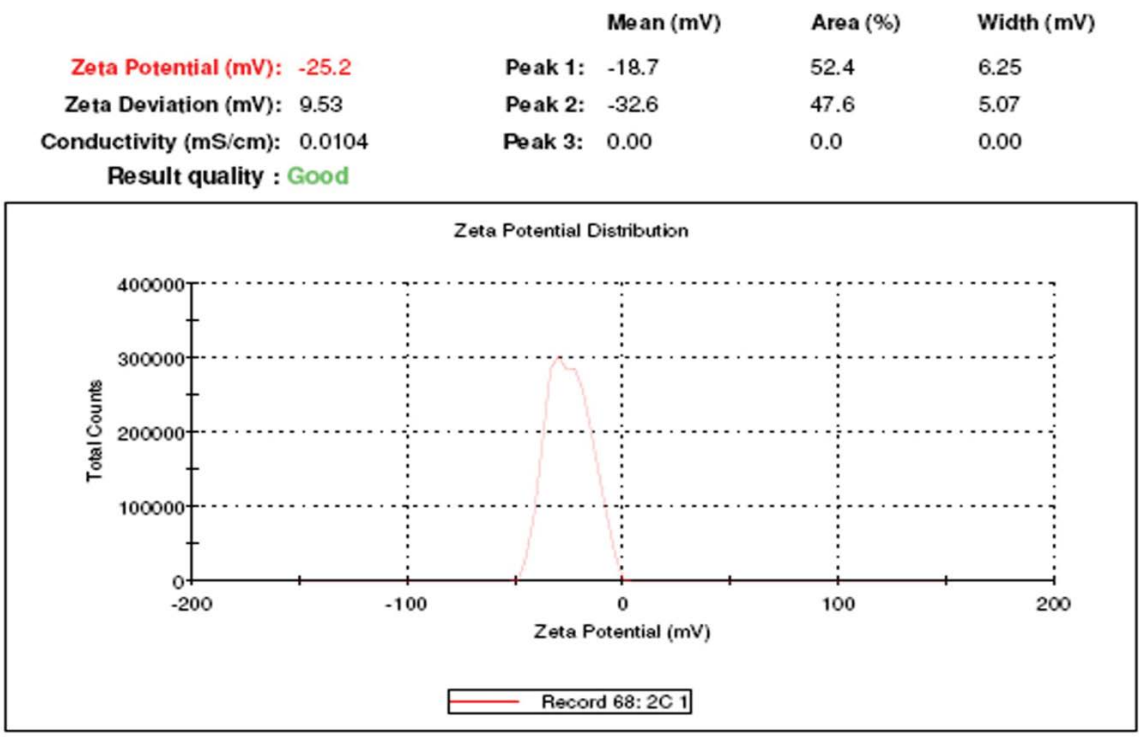

Figure 4: Zeta potential of silver nanoparticle (Ok-AgNps) synthesized by using leaf extract of *Ocimum kilimandscharicum.*

Table 2: Larvicidal activity of ethanol leaf extract (ELE) of *Ocimum kilimandscharium* against *Aedes aegypti.*

	Percent cumulative mortality (%) of *Culex quinquefasciatus*					LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2
	50	100	150	200	250			
Control	-	-	10.00^{e}	10.00^{e}	10.00^{e}	-	-	-
I	39.23^{b}	53.73^{b}	67.21^{a}	77.08^{a}	95.08^{a}	46.61 (-93.5693.146)	230.82 (181.59- 385.6)	0.827**
II	53.73^{a}	56.79^{a}	63.43^{b}	71.56^{b}	88.34^{b}	71.63 (-88.21-120.04)	298.1 (232.6- 648.7)	2.04**
III	33.21^{b}	42.13^{c}	50.73^{c}	67.21^{c}	77.08^{b}	126.75 (52.68- 173.2)	346.8 (262.3- 694.8)	0.293**
IV	30.00^{c}	33.21^{d}	47.87^{d}	60.00^{c}	77.08^{b}	185.60 (136.3 - 293.2)	427.6 (309.7-1043.07)	0.22**

Larval stages-I, II, III & IV Instars; LC50: Lethal concentration required to kill 50% population; LC90: Lethal concentration required to kill 90% population; Concentrations are in ppm; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit: Percent cumulative mortality values are mean of three replications; Duncan's Multiple Range Test: (DMRT) a-good: e-poor treatment * - significant; **- not significant (P<0.05).

Table 3: Larvicidal activity of silver nanoparticle (Ok-AgNps) synthesized from ethanol leaf extract (ELE) of *Ocimum kilimandscharium* (Ok) against *Aedes aegypti*

	Percent cumulative mortality (%) of *Aedes aegypti*					LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	X^2
	0.01	0.02	0.03	0.04	0.05			
Control	-	-	-	-	-	-	-	-
I	33.21^{b}	50.76^{b}	63.43^{a}	77.21^{c}	97.08^{b}	0.009 (0.007-0.022)	0.017 (0.034-0.055)	0.911**
II	36.27^{a}	53.73^{a}	63.43^{a}	77.08^{a}	90.00^{a}	0.015 (0.008-0.020)	0.034 (0.029-0.044)	0.484**
III	30.00^{c}	39.23^{b}	60.00^{b}	71.56^{b}	90.00^{a}	0.021 (0.016-0.025)	0.038 (0.033-0.048)	1.013**
IV	30.00^{c}	36.27^{d}	45.00^{c}	56.79^{d}	90.00^{a}	0.026 (0.020-0.031)	0.049 (0.041-0.0635)	3.99**

Larval stages-III Instars; LC50: Lethal concentration required to kill 50% population; LC90: Lethal concentration required to kill 90% population; Concentrations are in µL/mL-1; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit: Percent cumulative mortality values are mean of three replications; Duncan's Multiple Range Test: (DMRT) a-good: d-poor treatment; **- not significant (P<0.05).

(IV-Instars) against *Ae. aegypti* treated with silver nanoparticles synthesized from leaf extract of *Phyllanthus niruri* and *Mukia maderaspatana* respectively the present study report, least LC_{50} value was recorded against *Ae. aegypti*. The lethal concentration (0.009 ppm) level of AgNps synthesized in the present study was comparatively higher than the lethal concentration (20.27 ppm) of AuNps synthesized from *Cymbopogon citrates* [16]. Patil et al. [30] recorded LC_{50} value of 1.49 and 1.82 ppm with respect to II and IV instars larvae of *Ae. aegypti* treated with AgNps synthesized from *Murray koenigii,* which was much higher (200 times) the LC_{50} value recorded in the present investigation. It was observed from the study by Veerakumar et al. [19] that, the AgNps synthesized from leaf extract of *Sida acuta* had higher LC_{50} value recorded (23.96 and 26.13 µg/mL) against *Ae. aegypti* and *Cx. quinquefasciatus* compared to the present investigation. On the basis of the larvicidal activity performed against *Ae. aegypti* it was derived from the present study that the nanoparticle synthesized from *O. kilimandschardicum* could be used as potent vector control agent. It was also clearly evidenced that, the terpenes present in the leaf extract of *O. kilimandscharicum* played a potential precursor for the synthesis of such a highly active nanoparticle. Shim et al. [34] clearly stated the pictorial representation of synthesis of silver nanoparticles by palmitic acid and dedecanoic acid, the compounds present in the ELE of *O. kilimandscharicum*. Besides, the nanoparticle capped with bioactive compounds of leaf extracts would serve as additional support for its enhanced larvicidal efficiency. In order to reduce the mosquito borne diseases, this eco-friendly approach would be further administered for cytotoxity and field evaluation.

Conclusion

The terpene such as palmitic acid and dodecanoic acid etc. rich ELE of *Ocimim kilimandscharicum* has engineered a biologically active spherical shaped, negatively charged, polydispersed and stable nanoparticls. The potential larvicidal Ok-AgNps was encompassing of terpene precursor and this could be further analysed for its suitability in mass scale evaluation in the field and toxicity to non-target organisms.

Conflict of Interest

Authors have no conflict of Interest.

Acknowledgement

Author SS thank the president, principal and Head, department of Zoology, Thiagarajar College, Madurai , Tamil Nadu, India for their support in laboratory work.

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