

Green synthesis of copper oxide nanoparticles and mosquito larvicidal activity against dengue, zika and chikungunya causing vector *Aedes aegypti*

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Sekaran Muthamil Selvan¹, Kabali Vijai Anand¹ ✉, Kasivelu Govindaraju², Selvaraj Tamilselvan², Vijayakumar Ganesh Kumar², Kizhaeral Sevathapandian Subramanian³, Malaisamy Kannan³, Kalimuthu Raja³

¹Department of Physics, Sathyabama Institute of Science and Technology, Chennai 600 119, India

²Nanoscience Division, Centre for Ocean Research, Sathyabama Institute of Science and Technology, Chennai 600 119, India

³Department of Nanoscience and Technology, Tamilnadu Agricultural University, Coimbatore 641 003, India

✉ E-mail: anandkvijai@gmail.com

Abstract: In the present study, high purity copper oxide nanoparticles (NPs) were synthesised using *Tridax procumbens* leaf extract. Green syntheses of nano-mosquitocides rely on plant compounds as reducing and stabilising agents. Copper oxide NPs were characterised using X-ray diffraction (XRD) analysis, Fourier transform infrared (FT-IR), Field-emission scanning electron microscopy with energy dispersive spectroscopy, Ultraviolet–visible spectrophotometry and fluorescence spectroscopy. XRD studies of the NPs indicate crystalline nature which was perfectly matching with a monoclinic structure of bulk CuO with an average crystallite size of 16 nm. Formation of copper oxide NPs was confirmed by FT-IR studies and photoluminescence spectra with emission peaks at 331, 411 and 433 nm were assigned to a near-band-edge emission band of CuO in the UV, violet and blue region. Gas chromatography–mass spectrometry studies inferred the phytochemical constituents of the leaf extract. Larvicidal activity of synthesised NPs using *T. procumbens* leaf extract was tested against *Aedes aegypti* species (dengue, chikungunya, zika and yellow fever transmit vector).

1 Introduction

Chemical insecticides are used to control mosquitoes but they are harmful to non-target organisms and cause human health problems. Hence, effective and eco-friendly control strategies have to be focused to control this issue effectively. Developing biopesticides with multiple mechanisms may be successful for effective mosquito control. Mosquitoes are responsible for the transmitting dreadful diseases and parasites worldwide including malaria, dengue, filariasis etc., mosquito-borne diseases are endemic and cause mortality of nearly two million people every year [1].

Aedes aegypti (*A. aegypti*) transmits viral pathogens to human which causes yellow fever and dengue [2]. Dengue fever is increasing and the human population is now at risk due to this. Effective measures are being taken for mosquito control but still, there are tough challenges including increased mosquito resistance to insecticides [3]. Larviciding is used in many vector control programs around the world and this research work aims towards the discovery of cost effective alternatives for effective control of mosquito vector. The search for cost effective and abundant sources of plant extract as mosquito larvicides and the use of plant extract is promising due to the availability and cost-effectiveness [4].

Nanoscale materials have gained research interest, due to their potential to revolutionise a wide array of applications, facing important and timely challenges in parasitology and integrated pest management. In the last few years, nano-synthesis routes have been improved using botanical and algal extracts as sources of reducing and stabilizing agents to produce metal and metal oxide nanoparticles (NPs) with a wide range of biological activities [5, 6]. The so-called ‘green fabrication’ of NPs and nano-composites has been reported as advantageous if compared with classic chemical and physical methods, since green synthesis is usually cheap, quick, and does not require high pressure, energy, temperature nor the use of highly toxic chemicals. Many studies

have shown the potential of NPs for the control of arthropod pests of agricultural and medical-veterinary importance (mainly mosquitoes and ticks) [7]. Green-synthesised NPs are usually fabricated in aqueous suspensions; following bio-reduction of metal ions by plant extract constituents soluble in water, therefore the final product is an ideal formulation to treat insect young instars living in the aquatic environment [8]. Metal oxide NPs have a major role in pharmaceutical, industrial and biotechnological applications. Copper and copper-based nanostructured materials have efficient biocidal properties which are generally used in catalysis, pesticidal formulations and several health-related applications [9, 10].

Tridax procumbens L. of family Asteraceae is a common medicinal herb used by ethno-medical practitioners [11]. It has been used in treating various ailments such as wound healing, as anti-coagulant, anti-fungal agent and in the treatment of various infectious skin diseases [12]. Hence, the usage of this plant extract for the preparation of copper oxide NPs could have great potential for various applications. Therefore, in the present study, leaf extract of *T. procumbens* was used for the preparation of copper oxide NPs for mosquito *A. aegypti* larvicidal activity.

2 Materials and methods

2.1 Materials

The material used for the preparation of copper oxide NPs was copper sulphate (Sigma-Aldrich) and was used without further purification. *T. procumbens* leaves were collected from in and around Sathyabama University, Chennai, India (latitude 12.8731°N longitude 80.2219°E).

2.2 Preparation of leaf extract and phytochemical analysis using gas chromatography–mass spectrometry (GC–MS)

The leaves of *T. procumbens* were thoroughly washed with deionised water for removing the dust particles. The preparation of leaf aqueous extract, taking 10 g of chopped leaves in a 250 ml Erlenmeyer flask with 100 mL of deionised water and boiled at 60°C for 10 min. This extract was filtered and stored for further experiments. Phytochemical constituents of *T. procumbens* leaf extract were determined using GC–MS (Shimadzu-GCMS QP2010 Ultra).

2.3 Preparation of CuO NPs

The copper sulphate solution was prepared using 1 mM of copper sulphate in 100 ml of de-ionised water. For the synthesis of CuO NPs, an optimum amount of leaf extract (40 ml) was added to 90 ml of aqueous copper sulphate solution and was kept under vigorous stirring for 4 h at 80°C until it reduced to a deep brown coloured solution. This solution was centrifuged at 5000g for 10 min. The pellet was collected and calcined in a box furnace at 400°C for 5 h.

2.4 Characterisation of CuO NPs

An X-ray diffraction (XRD) study was performed using an X-ray powder diffractometer SMART LAB Rigaku, Japan. The functional groups in the copper oxide NPs were analysed by using a Fourier transform infrared (FT-IR) spectrometer (Perkin- Elmer RX1). An energy dispersive X-ray spectrometer (SUPRA55 ZEISS with an energy dispersive X-ray spectroscopy–wavelength dispersive X-ray spectroscopy apparatus) was used for elemental analysis of copper oxide NPs. An Ultraviolet–visible (UV–vis) spectrophotometer (UV-2101, Shimadzu) was used to study the optical property of CuO NPs in the wavelength range of 200–800 nm. Photoluminescence (PL) spectra of CuO NPs were recorded using a Shimadzu fluorospectrophotometer.

2.5 Parasites rearing and collection

Larvae of *A. aegypti* were collected from the water tank, broken bottles and small water courses at Velachery, Chennai and identified in Zonal Entomological Research Centre, Vellore. The larvae were kept in plastic trays containing tap water. They were maintained and nurture in the laboratory condition as per the reported protocol [13].

2.6 Mosquito larvicidal bioassay of plant extract and copper oxide NPs

The *A. aegypti* mosquito larvicidal experiment was performed by following the procedure of World Health Organisation. For larval bioassay analysis, *A. aegypti* larvae were taken in five groups of 20 in 249 ml of tap water and 1 ml of *T. procumbens* aqueous extract and copper oxide NPs concentration. The control group was maintained with dechlorinated tap water. The numbers of larval death were counted after 24 h of treatment and mortality % was calculated from the average of five replicates. The experimental results in which 100% mortality of larvae occurs alone were selected for further dose response larval bioassay.

2.7 Larvicidal bioassay by dose-response

Based on the preliminary screening results, *T. procumbens* aqueous extract and *T. procumbens* mediated synthesised copper oxide NPs were subjected to dose response for larvicidal bioassay against the larvae of *A. aegypti*. Different concentrations ranging from 10, 25, 50, 75 and 100 mg/l (for *T. procumbens* extracts) and 1, 2.5, 5, 7.5, and 10 mg/l (for copper oxide NPs for larvicidal activity) were used. The numbers of dead *A. aegypti* larvae were calculated after 24 h of exposure and mortality % was calculated from the average of five replicates.

2.8 Statistical analysis

Average larval mortality data were subjected to probit analysis for calculating LC₅₀. Here, LC₅₀ was the lethal concentration (LC) that 50% kills. The fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL) were calculated by using the software developed by Reddy *et al.* [14]. There was a relationship between the experimental doses and death rate of *A. aegypti* larvae showed in the r^2 and all differences were considered significant $P \leq 0.05$.

3 Results and discussion

The identification and composition of the main bioactive compounds present in the leaf extract of *T. procumbens* are shown in Table 1. Nine compounds were identified by GC–MS and the main compounds were 2,6,10-trimethyl,14-ethylene-14-pentadecne; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; 2-hexadecen-1-ol, 3,7,11,15-tetramethyl; l-(+)-ascorbic acid 2,6-dihexadecanoate; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; 9,12,15-octadecatrienoic acid; pentadecane, 8-hexyl; tetrapentacontane and squalene (Fig. 1).

The crystallinity and crystal phase of synthesised CuO NPs were studied by XRD and is shown in Fig. 2i. XRD analysis show intense peaks at 32.6°, 35.5°, 38.7°, 48.6°, 53.5°, 58.2°, 61.4°, 66.2°, 67.9°, 72.5° and 75.0° corresponds to (1 1 0), (0 0 2), (1 1 1), (2 0 2), (0 2 0), (2 0 2), (1 1 3), (3 1 1), (1 1 3), (3 1 1) and (2 2 2), respectively. The observed diffraction reflections are comparable with JCPDS No. 45–0937 and are attributed to bulk CuO materials [15, 16]. All diffraction peaks can be indexed as the typical monoclinic structure and no extra diffraction peaks of other phases are observed, which indicates the high purity of the synthesised CuO NPs. Moreover, the well-defined, sharp CuO reflections observed in the XRD patterns verify the well-crystalline nature of CuO NPs. The average grain size of CuO NPs is determined to be 16 nm from the full-width at half maximum

Table 1 Phytochemicals identified from the *T. procumbens* leaf extract by GC–MS

Retention Time (RT)	Name of the compound	Molecular formula	Mol. weight (g/mol)
24.500	2,6,10-Trimethyl,14-ethylene-14-pentadecne	C ₂₀ H ₃₈	278
25.000	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296
25.379	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	C ₂₀ H ₄₀ O	296
26.872	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	652
29.152	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296
29.454	9,12,15-Octadecatrienoic acid	C ₁₈ H ₃₀ O ₂	278
30.505	Pentadecane, 8-hexyl-	C ₂₁ H ₄₄	296
35.252	Tetrapentacontane	C ₅₄ H ₁₁₀	758
36.065	Squalene	C ₃₀ H ₅₀	410

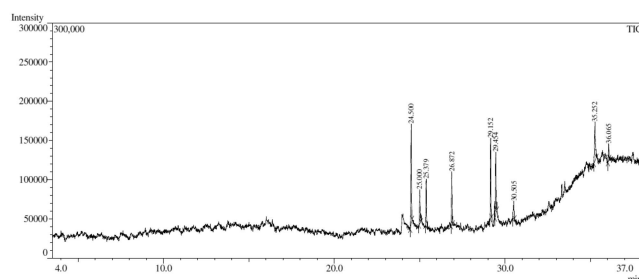


Fig. 1 GC–MS chromatogram of *T. procumbens* leaf extract

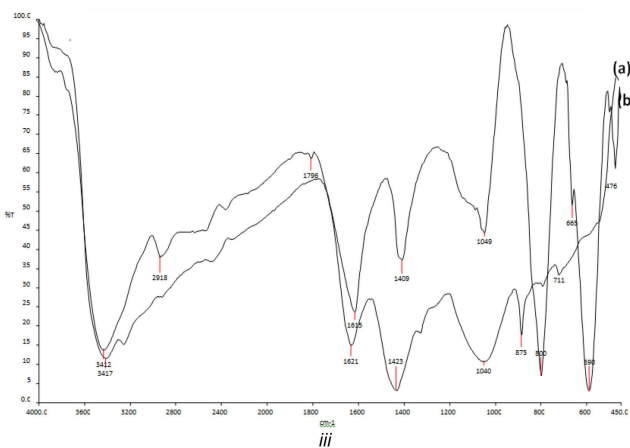
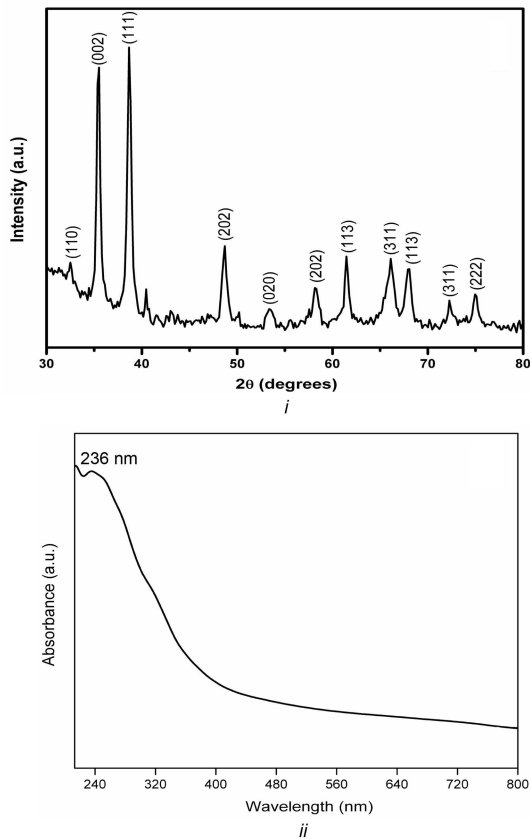


Fig. 2 Characterisation of CuO NPs (i) XRD pattern, (ii) UV-Vis absorption spectrum, (iii) FT-IR spectrum of (a) *T. procumbens* leaf extract and (b) synthesised CuO NPs

(FWHM) of the most intense peak using Debye-Scherrer's equation:

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

where λ is the wavelength of the X-ray radiation (for $\text{CuK}\alpha$ radiation, $\lambda = 1.5418 \text{ \AA}$), β is the FWHM in radians of the XRD peak and θ is the angle of diffraction.

The UV-visible absorption spectrum of green synthesised CuO NPs was obtained using *T. procumbens* leaf extracts (Fig. 2ii). CuO NPs have an optical absorbance range around 236 nm suggesting the formation of CuO NPs and are in good agreement with the previous reports [17, 18]. The bioactive molecules present in the *T. procumbens* leaf extract reduce precursor and formation of CuO NPs were confirmed by FT-IR. Fig. 2iii shows the FT-IR spectra of (a) *T. procumbens* leaf extract and (b) CuO NPs using *T. procumbens* leaf extract. The relative shifts in position and intensity were identified using FT-IR (Fig. 2iii(a)) recorded for *T.*

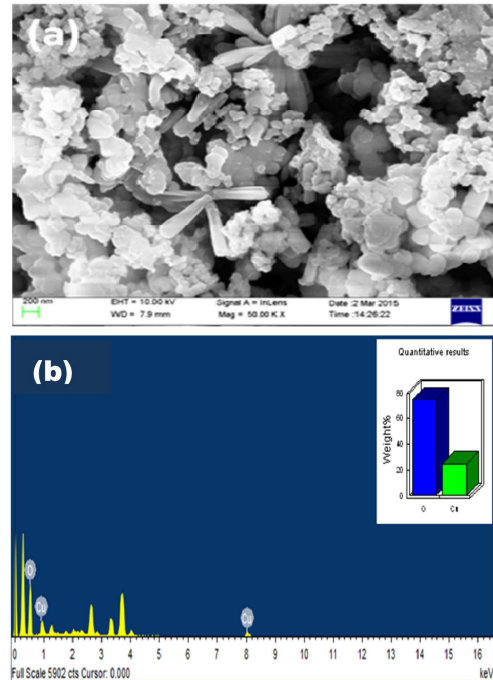


Fig. 3 Surface morphological characterisation of CuO NPs (a) FESEM, (b) EDAX spectrum of CuO NPs

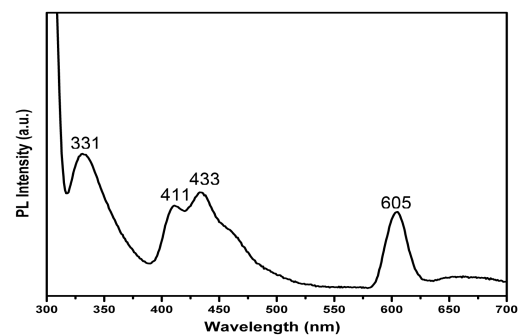


Fig. 4 PL spectrum of CuO NPs

procumbens leaf extract, where the peaks were observed at 3417 cm^{-1} (amide N-H stretching), 2921 cm^{-1} (alkane C-H stretching), 1799 cm^{-1} (anhydride C=O bending) and 1049 cm^{-1} (C-O stretching). From Fig. 2iii(b), the interaction of CuO NPs with biomolecules of *T. procumbens* leaf showed intense peaks at 3412 cm^{-1} (amide N-H stretching), 2918 cm^{-1} (alkane C-H stretching), 1796 cm^{-1} (anhydride C=O bending) and 1040 cm^{-1} (C-O stretching). The peak at 471 cm^{-1} corresponds to vibrations of Cu-O, confirms the formation of CuO NPs. Moreover, it is confirmed that the peak corresponds to cuprous oxide (Cu_2O) at around $605\text{--}660 \text{ cm}^{-1}$ is absent [15, 19].

The size and shape of green synthesised CuO NPs using *T. procumbens* leaf extract were analysed using field-emission scanning electron microscopy (FESEM), which is shown in Fig. 3a. The energy dispersive spectroscopy (EDAX) spectrum was used to analyse the elemental composition of the CuO NPs synthesised using *T. procumbens* leaf extract. In the EDAX spectrum, the presence of strong signals of Cu and O was observed (Fig. 3b) which confirms the formation of CuO NPs.

In general, emission spectra of metal oxides can be divided into two broad categories namely the near-band-edge (NBE) UV emission and deep-level (DL) defect-related visible emissions [20, 21]. Fig. 4 shows the PL spectrum of the CuO NPs recorded in the wavelength range of $300\text{--}700 \text{ nm}$ with an excitation wavelength of 300 nm at room temperature. The PL spectrum exhibits a UV emission peak at 331 nm and visible emission peaks in the violet region (411 nm), blue region (433 nm) and green region (605 nm). The sharp emission peaks appear at $331, 411$ and 433 nm are

Table 2 Dose-dependent larvicidal activity against *A. aegypti* species

Materials	Species	Conc., mg/l	% Mortality, mean \pm SD	LC ₅₀ , mg/l	UCL–LCL, mg/l	r ²	χ^2
Control (distilled H ₂ O)	<i>A. aegypti</i>	25	—	—	—	—	—
<i>T. procumbens</i> leaf extract		100	80 \pm 0.680	60.965	66.431–55.855	0.977	2.205
		75	69 \pm 0.320				
		50	37 \pm 0.190				
		25	19 \pm 1.270				
		10	11 \pm 1.020				
CuSO ₄		25	—	—	—	—	—
CuO NPs		10	100 \pm 0.00	4.209	5.622–2.651	0.993	10.101
		7.5	78 \pm 0.230				
		5	53 \pm 0.140				
		2.5	37 \pm 0.280				
		1	17 \pm 0.280				

—, Nil mortality; LC₅₀, lethal concentration that kills 50% of the exposed larvae; UCL, upper confidence limit; LCL, lower confidence limit; r², regression coefficient; χ^2 , chi square; Significant at $P < 0.05$ level.

assigned to the NBE emission band of the CuO in the UV, violet and blue region [9]. These results are in good agreement with the previous reports [22–24]. The sharp DL green emission peak at around 605 nm is assigned to the singly ionised oxygen vacancies [25]. The visible luminescence originates from the radioactive recombination of a photo-generated hole with an electron occupying the oxygen vacancy [26, 27].

The larvicidal property of *T. procumbens* leaf extract and synthesised CuO NPs against *A. aegypti* was studied (Table 2). However, the highest activity was found in *T. procumbens* leaf extract and CuO NPs against the larvae of *A. aegypti* (LC₅₀ = 60.965 and 4.209 mg/l; r² = 0.977 and 0.993).

The CuO NPs, which are likely to cause ecological damage, have been identified as a potential replacement for chemical synthetic insecticides and hence, the need to use the bio-based preparation of CuO NPs for the control of disease vectors. Biological-mediated preparation of copper oxide NPs using *Escherichia coli* has been reported and the results indicated the presence of both Cu₂O and CuO phases [28]. Fungi can also be used to synthesise metallic oxide NPs. The biogenic synthesis of copper oxides was reported using *Penicillium aurantiogriseum*, *Penicillium citrinum* and *Penicillium waksmanii* isolated from soil [29]. Gopalakrishnan *et al.* reported the synthesis of Cu₂O NPs using *T. procumbens* leaf extract and studied their antibacterial activity against *E. coli* [30]. Monodispersed, versatile and highly stable CuO NPs were synthesised using *Aloe vera* extract. This method is both eco-friendly and inexpensive [27]. Mageshwari *et al.* reported the synthesis, characterisation and antimicrobial activity of flower-shaped CuO nanostructures [15]. Sankar *et al.* reported the green synthesis of colloidal copper oxide NPs using *Carica papaya* for photocatalytic dye degradation [19]. Green synthesis of CuO NPs by aqueous extract of *Gundelia tournefortii* and its evaluation of catalytic activity for reduction of 4-nitrophenol has been reported [31]. Nasrollahzadeh *et al.* reported the *Tamarix gallica* leaf extract-mediated novel route for the green synthesis of CuO NPs and their application to *N*-arylation of nitrogen-containing heterocycles under ligand-free conditions [32]. Furthermore, CuO NPs synthesised using *Calotropis gigantea* leaf extract is used for dye-sensitised solar cells applications [16].

Recently, a green chemistry approach for the preparation of NPs using microbes, plants and plant based bioactive compounds showed potent mosquito larvicidal and pupicidal activity against a number of mosquito vectors [33].

4 Conclusions

Advanced technology for effective mosquito vector control is a need of the hour. The present study emphasises the cost effective and eco-friendly approach for the synthesis of CuO NPs using leaf extract of *T. procumbens* is reported. The larvicidal activity against dengue, chikungunya and yellow fever transmit vector *A. aegypti* species was studied using CuO NPs and the results were discussed.

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