Research Article

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Larvicidal potential of *Cipadessa baccifera* leaf extract-synthesized zinc nanoparticles against three major mosquito vectors

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**Abstract:** Mosquitoes are important vectors, which transmit many communicable diseases throughout the world. Synthetic insecticides, such as organophosphate and pyrethroids, are commonly used for their control in the vector control program. Insecticidal compounds from natural sources, notably from plants and synthesized nanoparticles (NPs) are promising tools for managing such vectors. Hence, the study aimed to analyze the insecticidal potentiality of leaf extract of *Cipadessa baccifera* and synthesized ZnNPs against three major mosquito vectors. The results recorded from UV-Vis spectroscopy show the peak absorption spectrum at 420 nm. In FTIR, the maximum peak value is 562.85 cm⁻¹ assigned to the N–H group (amide group). The EDAX analysis shows a peak around 63.29, which confirms the binding intensity of selenium. In the scanning electron microscopy analysis, the synthesized ZnNPs sizes were ranging from 49.21 to 65.43 nm. The synthesized ZnNPs produced high mortality against *Culex quinquefasciatus* LC₅₀ = 0.04963 mg mL⁻¹; LC₉₀ = 0.9842 mg mL⁻¹, *Anopheles stephensi* (LC₅₀ = 0.053421 mg mL⁻¹ and LC₉₀ = 0.027761 mg mL⁻¹), and *Aedes aegypti* LC₅₀ = 0.55214 mg mL⁻¹ and LC₉₀ = 0.7456 mg mL⁻¹. These results suggest that the *C. baccifera* leaf extract-mediated biosynthesis of ZnNPs has the potential to be used as an ideal eco-friendly approach toward the control of mosquito vectors at early stages.

**Keywords:** botanical extracts, bionanopesticides, *Cipadessa baccifera*, vector control

1 Introduction

Mosquitoes are threatening the populations of humans and animals in the ecosystem as an important vector of various diseases [1–3]. Globally, millions of deaths occur every year due to vector-borne diseases [4–6]. Chemical insecticides are the only choice in most of vector control programs. However, alteration in the frequency of insecticide exposure has led to resistance in mosquitoes and other insect pests [7]. The frequent and indiscriminate use of chemical pesticides may also threaten the natural enemies and the surrounding environments [8–10] and is responsible for insecticide resistance [11]. Avoidance of resistance in mosquito species is achieved by using alternatives to chemical insecticides, such as the use of biopesticides and secondary metabolites of numerous plants because of their bioavailability and degradability nature [12–15]. Studies have shown that many plant secondary compounds have possessed good mosquitocidal, larvicidal, repellent, and insect growth inhibitor activity [16–18]. Apart from plant-derived compounds, green-synthesized nanoparticles (NPs) from various plant parts have been considered a novel tool for the management of the mosquito population in an eco-friendly and environmentally safe manner [19]. Phytochemicals have a major role in mosquito control programs. The bioactive plant ingredient(s) can be obtained from the whole plant or from a specific part by extraction with different types of polar and nonpolar solvents, such as petroleum ether, benzene, chloroform, ethanol, absolute alcohol, acetone, and so on. Nanotechnology is the new emerging field in the biology for various
applications, and NPs from various nanomaterials, such as Au, Ag, Cu, Fe, Al, Co, Ti, and Zn, have been frequently used in recent days. Zinc oxide (ZnO) NP is also known for its low toxicity and high UV absorption, making it a good candidate to be used in the biomedical field. ZnO NP also has a hard and rigid structure, making it useful in the ceramic industry. One of the advantages of using ZnO NP in the biomedical field is that they act as a good surface material. ZnO NP is naturally known for the strong resistance of microbes [20], and ZnO NPs have low toxicity and are biodegradable. ZnO NPs are highly toxic compared to other metal oxide NPs, they are used in different fields as antimicrobials due to their high stability under harsh processing conditions, these are excellent choices as nanocarriers in the delivery of different drugs, such as DOX, paclitaxel, and curcumin, ZnO NPs are highly toxic compared to other metal oxide NPs, and they are used in different fields as antimicrobials due to their high stability under harsh processing conditions [21]. Among the ZnNPs have gained the attention to the entomologist for the control of many insect pests including mosquitoes. Very few studies have shown the toxicology properties of plant-synthesized ZnNPs with good and moderate activity on insecticidal, antibacterial, antimicrobial, and anticancer activities [22, 23]. Cipadessa baccifera Miq. (Meliaceae) is an important medicinal plant dispersed in North Circas, Deccan, and Western Ghats of India. The plant leaves and fruits are traditionally used as medicine for the treatment of diabetes, diarrhea, and headache. Apart from its medicinal uses, C. baccifera shows an antidote effect on insect, snake, and scorpion bite [24]. Apart from its medical uses, the crude extract of C. baccifera has possess the ovicidal, larvicidal, and egg membrane alteration in Helicoverpa armigera and Culex mosquito [25–27]. Our previous study has revealed the effective adulticidal activity of C. baccifera on Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi [28]. This present study investigated the larvicidal potential of C. baccifera leaf extracts and synthesized ZnO NPs on An. stephensi, Cx. quinquefasciatus, and Ae. aegypti larvae.

2 Materials and methods

2.1 Mosquito larvae collection

The first instar larvae of An. stephensi, Cx. quinquefasciatus, and Ae. aegypti were obtained from National Center for Disease Control, Mettupalayam, Tamil Nadu, India. The larvae were reared in the insectariums at 25 ± 1°C with 75–80% humidity for 13:11 photoperiodic conditions. During the larval maintenance, they were not exposed to any pesticides and were fed with yeast granules and dog biscuits at a 3:1 ratio.

2.2 Plant collection

The disease-free fresh leaves of C. baccifera (Figure 1) were obtained from Chandra Pillai Valasu, Kalrayan Hills, which lies between 10°12′–11°7′ N, 76°77°56′ E and altitude of 1,300 m situated at Eastern Ghats of Southeast Tamil Nadu in the Salem district.

2.3 Plant leaf extracts preparation

Collected samples of C. baccifera plant leaves were immediately brought to the laboratory and washed with running tap water and distilled water several times. After washing, the leaves were subjected to shade dry for 2 weeks. The dried leaves were placed in the mechanical mixer grinder and converted into the powder form. The 300 g of leaf powder was placed in the Soxhlet apparatus and extraction was carried out with 400 mL of polar (acetone, methanol, ethyl acetate) and nonpolar (chloroform and petroleum benzene) solvents boiling point range 55–75°C for 10 h. The extract was concentrated under low temperature at 45°C and pressure 20–25 mmHg. The final crude extract was kept in refrigeration for further use.

2.4 Biosynthesis of ZnO NPs

The extract prepared from the leaf was mixed with 2.5 mL of 1.0 mM zinc nitrate (HiMedia, India) solution and was stirred for 1.5 h at room temperature (RT = 37 ± 1°C). The sample was further boiled at 65°C for 5 h with gentle shaking until the
brown-yellowish precipitate was appeared. Furthermore, the mixture was stirred continuously at RT for 24 h [29].

2.5 Characterization of ZnO NPs

The biosynthesized ZnO NPs and crude extract samples were analyzed by UV spectrum absorption at a wavelength ranging from 250 to 650 nm, using a UV-Vis double beam spectrophotometer (Systronics-2203, Uttar Pradesh, India). EDAX analysis was carried out for analyzing the percentage of elements using EDAX-30 operating at 15–25 keV. FTIR was carried out at the spectral range of 4,000–380 cm\(^{-1}\) with 4 cm\(^{-1}\) resolution. Furthermore, the size of ZnNPs was studied with scanning electron microscopy (SEM) Quanta FEG 250 (FEI). TEM analysis was performed to study the particle size and crystal structure of the synthesized ZnNPs.

2.6 Bioefficacy of crude methanol leaf extracts and synthesized ZnNPs on mosquito larvae

World Health Organization [30] larval bioassay method was followed with some slight modifications. *Cx. quinquefasciatus*,

Figure 2: Biosynthesis of zinc oxide nanoparticles from methanol leaf extract of *C. baccifera*.

Figure 4: EDAX analysis of ZnNPs biosynthesized from leaf extract of *C. baccifera*.

Figure 3: UV-Vis absorption spectrum of ZnNPs and crude methanol extract of *C. baccifera*.

Figure 5: SEM analysis of *C. baccifera* synthesized ZnNPs.

Figure 6: TEM analysis of *C. baccifera* synthesized ZnNPs.
An. stephensi, and Ae. aegypti larvae were released in a paper cup in deionized water. After that, different concentrations of various solvent leaf extracts of C. baccifera and ZnNPs were tested in 250 mL paper cups containing 100 mL of distilled water. Bioassays were performed separately at six different concentrations of crude extracts (0.1, 0.3, 0.5, 1.0, and 2.0 mg·mL⁻¹) and synthesized ZnNPs (0.1, 0.3, 0.5, 1.0, and 2.0 mg·mL⁻¹). Controls were not received any test concentration (water only). Three replicates were kept for each test and 25 larvae were released in each concentration. The larval mortality was observed after 24 h of incubation and the larvae were considered to be dead when do not show any response to the physical disturbance. The mortality in the control was adjusted by Abbott’s formula [31].

2.7 GC-MS analysis of crude methanol leaf extract

The methanol fraction was subjected to GC-MS analysis with respect to Cheng et al. [32] method, which used only one type of column (preferably Polaris Q Ion Trap GC/FID). The temperature of the oven was set at 55°C for 20 min and then improved to 300°C for 5 min at the rate of 10°C·min⁻¹. For carrier gas helium was used at the flow rate of 1.0 mL·min⁻¹. The injector temperature was set at 240°C, with a split ratio of 10:1 and the injection size was 1.0 µL. Perkin Elmer mass gold turbo detector was used as a detector system. The mass spectrum was obtained at a 70 eV ionization voltage. The identification of individual compounds was done using the Wiley/NBS Registry.
of the mass spectral database, the NIST (version 3.0) database. Furthermore, the retention time and Kovats index (KI) values of several authentic reference compounds were compared with isolated compounds for identification.

2.8 Statistical analysis

The mortality in the larval bioassay was subjected to the probit analysis [33]. The lethal concentration for 50%, 90% (LC₅₀, LC₉₀), and 95% of confidence limits, chi square, and degree of freedom was calculated using the SPSS 13.0 statistical package (Version 13.0).

3 Results and discussion

3.1 ZnNP UV-Vis analysis

Green synthesis of ZnNPs from ZnO was confirmed by UV-Vis spectra studies, and based on the color change (Figures 2 and 3), the absorption spectrum of methanol crude extract and ZnNPs dissolved in distilled water was read at 360 nm. Similar results were reported by Soni and Dhiman [34] in ZnNPs’ spectral absorption at 360 nm. Divya et al. [35] showed the ZnNPs’ absorption range between 358 and 370 nm.

3.2 EDAX spectra analysis

The EDAX spectrum 1 of synthesized ZnNPs is given in Figure 4. The result shows that the presence of different elements, among which Zn showed the highest value of 63.29 weights and 27.29% atom at 20 keV, revealed the conformation of synthesized NP. Agreeing with our result, Demissie et al. [36] also reported a 59.43% weight of ZnNPs synthesized from the leaf extract of Lippia adoensis. A similar study was reported by Dhavan and Jadhav [37] in Lumnitzera racemosa-fabricated ZnO nanorods.

3.3 TEM and SEM observations

The size and shape of synthesized ZnNPs were obtained by TEM and distribution was also confirmed by SEM. SEM

| S. no | Retention time (min) | Area (%) | Compound name | Activity                      |
|-------|----------------------|----------|---------------|------------------------------|
| 1     | 12.883               | 1.203    | Benzene-1,1,5-dimethyl-4-hexenyl-4-methyl | Insecticidal activity          |
| 2     | 19.440               | 0.926    | Pyridine      | Insecticidal and antibacterial activity |
| 3     | 22.844               | 1.332    | 5-(2'-Dibromo-phenyl)-2'-biphenyl 3-al   | Anti-microbial activity        |
| 4     | 23.312               | 1.735    | 2H-1-Benzopyran-2-one-6-(1H)-pyrazolo[3,4-c]-pyridine | Insecticidal activity          |
| 5     | 23.312               | 1.735    | 2H-1-Benzopyran-2-one-6-(1H)-pyrazolo[3,4-c]-pyridine | Anti-microbial activity        |
| 6     | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Insecticidal activity          |
| 7     | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
| 8     | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
| 9     | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
| 10    | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
| 11    | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
| 12    | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
| 13    | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
| 14    | 24.307               | 1.735    | 3-Methyl-2-(2,2-dimethyl-4-methylphenyl) pyridine | Anti-microbial activity        |
image shows the spherical-shaped polydisperse NPs with an average size range of 41.48 nm (Figure 5). Gitahi et al. [38] also reported the similar results. As shown in Figure 6, the polydisperse nature of spherical-shaped (49.21–65.43 nm) synthesized ZnNPs was seen in the TEM image.

### 3.4 FTIR analysis

The result of ZnNPs is shown in Figure 7. Various bands were visualized at 562.85, 1,026.07, 1,628.05, and 1,935.25 cm\(^{-1}\). The broad spectra exhibited around 562.85 ratios because of the straightening of alcohols and phenols with hydroxyl groups and 1,647.24 cm\(^{-1}\) assigned to O–H stretching carboxylic acids. The weaker band at 555.41 cm\(^{-1}\) corresponds to C–C stretch in the alkenes group. Yuvakkumar et al. [39] reported the same peak range to Zn–O, which confirms synthesized ZnNPs from the C. baccifera extract.

### 3.5 GC-MS analysis of a crude sample from C. baccifera

The occurrence of insecticidal molecules in the crude methanol leaf extract of C. baccifera was analyzed using GC-MS. Results revealed 14 compounds in the sample and 5 belong to major peaks (Figure 8). The composition and KI values of the compound eluted from the DB-5ms capillary column are given in Table 1. In addition to these findings, our bioassay result strongly suggests the potential of C. baccifera. Plants’ secondary metabolites, such as sesquiterpenoid and benzenoid compounds, from many origins possess significant insecticidal activities [40]. A previous report by Jang et al. [41] revealed that the methanol extract from Cassia obtusifolia, Cassia tora, and Vicia tetrasperma produced 90% of Ae. aegypti larval mortality at 200 ppm [42,17]. Based on the above findings, our result indicates a very less LC\(_{50}\) value upon tested insects. Hence, the methanol leaf extract of C. baccifera could become a better source for the management of filariasis and malaria vectors.

### 3.6 Insecticidal efficacy of C. baccifera extracts and synthesized ZnNPs

The insecticidal activity of crude leaf extract of C. baccifera and synthesized ZnNPs was assessed toward An. stephensi, Cx. quinquefasciatus, and Ae. aegypti. Various polar and nonpolar solvents were used for the extraction of phytochemicals from C. baccifera and larvicidal activity was observed after 24 h incubation. The results produced a high LC value for Cx. quinquefasciatus (LC\(_{50}\) = 2.2907 mg·mL\(^{-1}\); LC\(_{90}\) = 5.7136 mg·mL\(^{-1}\)), An. stephensi

### Table 2: Larvicidal activity of C. baccifera leaf extracts against fourth instar larvae of Cx. quinquefasciatus

| Species          | Sample    | \(n^a\) | LC\(_{50}\) LCL-UCL (95% confidence limit) (mg·mL\(^{-1}\)) | LC\(_{90}\) LCL-UCL (95% confidence limit) (mg·mL\(^{-1}\)) | \(\chi^2\) | df |
|------------------|-----------|---------|-------------------------------------------------------------|-------------------------------------------------------------|----------|----|
| Cx. quinquefasciatus | Acetone   | 375     | 3.5533 (1.15990–5.886752)                                    | 7.3228 (5.0028–9.1099)                                       | 1.771    | 3  |
|                  | Ethyl acetate | 375   | 5.291811 (3.08903–8.95671)                                   | 12.0963 (9.3373–14.3408)                                      | 2.668    | 3  |
|                  | Methanol   | 375     | 2.23907 (1.06427–3.88945)                                    | 5.7136 (3.89143–11.1821)                                     | 12.743   | 3  |

\(n^a\) – number of animals, LC\(_{50}\) – lethal concentration 50% mortality, LC\(_{90}\) – lethal concentration 90% mortality, LCL – lower confidence limits, UCL – upper confidence limits, \(\chi^2\) – chi square, df – degrees of freedom, significant at \(p < 0.05\).

### Table 3: Larvicidal activity of C. baccifera leaf extracts against fourth instar larvae of An. stephensi

| Species          | Sample    | \(n^a\) | LC\(_{50}\) LCL-UCL (95% confidence limit) (mg·mL\(^{-1}\)) | LC\(_{90}\) LCL-UCL (95% confidence limit) (mg·mL\(^{-1}\)) | \(\chi^2\) | df |
|------------------|-----------|---------|-------------------------------------------------------------|-------------------------------------------------------------|----------|----|
| An. stephensi    | Acetone   | 375     | 3.41783 (2.1281–4.9317)                                     | 12.5884 (9.98558–14.3890)                                   | 3.647    | 3  |
|                  | Ethyl acetate | 375   | 2.26036 (1.0202–3.45257)                                    | 7.61976 (5.5024–10.2100)                                     | 12.413   | 3  |
|                  | Methanol   | 375     | 1.74348 (1.16165–4.1915)                                    | 6.7399 (5.101697–9.6364)                                    | 5.919    | 3  |
|                  | Chloroform | 375     | 2.73707 (1.8502–4.0816)                                     | 8.29254 (6.91736–11.9419)                                   | 5.495    | 3  |

\(n^a\) – number of animals, LC\(_{50}\) – lethal concentration 50% mortality, LC\(_{90}\) – lethal concentration 90% mortality, LCL – lower confidence limits, UCL – upper confidence limits, \(\chi^2\) – chi square, df – degrees of freedom, significant at \(p < 0.05\).
Table 4: Larvicidal activity of *C. baccifera* leaf extracts against fourth instar larvae of *Ae. aegypti*

| Species          | Sample     | n*  | LC₅₀ LCL-UCL (95% confidence limit) (mg·mL⁻¹) | LC₉₀ LCL-UCL (95% confidence limit) (mg·mL⁻¹) | χ²  | df |
|------------------|------------|-----|-----------------------------------------------|-----------------------------------------------|-----|----|
| *Ae. aegypti*    | Acetone    | 375 | 8.5487 (6.8546–12.8101)                       | 13.8426 (7.0158–16.9612)                      | 2.165 | 3  |
|                  | Ethyl acetate | 375 | 7.9224 (6.1664–8.3415)                       | 9.3321 (9.1101–12.0454)                      | 1.721 | 3  |
|                  | Methanol   | 375 | 0.65145 (0.6327–0.91945)                     | 4.8593 (3.3943–5.1121)                      | 2.82  | 3  |
|                  | Chloroform | 375 | 4.3580 (3.192–4.9001)                        | 7.9125 (5.9881–9.2516)                      | 3.80  | 3  |

n* = number of animals, LC₅₀ = lethal concentration 50% mortality, LC₉₀ = lethal concentration 90% mortality, LCL – lower confidence limits, UCL – upper confidence limits, χ² = chi square, df = degrees of freedom, significant at p < 0.05.

Table 5: Larvicidal activity of *C. baccifera* ZnNPs against fourth instar larvae of three mosquitoes

| Species                  | Sample | n*  | LC₅₀ LCL-UCL (95% confidence limit) (mg·mL⁻¹) | LC₉₀ LCL-UCL (95% confidence limit) (mg·mL⁻¹) | χ²  | df |
|-------------------------|--------|-----|-----------------------------------------------|-----------------------------------------------|-----|----|
| *Cx. quinquefasciatus*  | ZnNPs  | 375 | 0.05321                                       | 0.02761                                       | 1.82 | 3  |
| *An. stephensi*         | ZnNPs  | 375 | 0.04963                                       | 0.9842                                        | 2.89 | 3  |
| *Ae. aegypti*           | ZnNPs  | 375 | 0.55214                                       | 0.7456                                        | 2.30 | 3  |

n* = number of animals, LC₅₀ = lethal concentration 50% mortality, LC₉₀ = lethal concentration 90% mortality, χ² = chi square, df = degrees of freedom, significant at p < 0.05.

(LC₅₀ = 1.74348 mg·mL⁻¹; LC₉₀ = 6.7399 mg·mL⁻¹), and *Ae. aegypti* (LC₅₀ = 0.65145 mg·mL⁻¹; LC₉₀ = 4.8593 mg·mL⁻¹), which were induced by the methanolic extract of *C. baccifera* leaves (Tables 2–4). Supporting our data, Famuyiwa et al. [43] reported high activity of fruit extract of *Thevetia neriifolia* LC₅₀ 1.60 ± 0.05 mg·mL⁻¹ and the leaf extracts of *Calotropis procera* LC₅₀ 2.05 ± 0.16 mg·mL⁻¹. Similarly, the methanolic leaf extract of *M. atropurpureum* and *E. astringens* showed good larvicidal activity after 24 and 48 h LC₅₀ = 11.10 and 9.68 ppm [44,45]. Similar studies investigated that the biosynthesized NPs from leaf extract of *Leucas aspera* showed potential larvicidal activity against *Ae. aegypti* larvae [46]. NP-conjugated plant extracts are highly toxic to mosquito larvae and at the same time do not show any toxicity to nontarget aquatic species [42]. *Spergularia rubra* and *Pergularia daemia*-synthesized AgNPs did not exhibit any evident toxicity effect against fishes, after 48 h of exposure [47]. AgNP-synthesized *Barleria cristata* extracts were not toxic against the nontarget organisms [43]. Larvicidal activity of synthesized ZnNPs showed good susceptibility and highest LC values against the mosquito species (LC₅₀ = 0.049653 mg·mL⁻¹; LC₉₀ = 0.9842 mg·mL⁻¹; LC₅₀ = 0.053421 and LC₉₀ = 0.027761 mg·mL⁻¹; LC₅₀ = 0.55214 and LC₉₀ = 0.7456 mg·mL⁻¹) (Table 5). Gitahi et al. [38] assessed the *M. citrifolia* aqueous root extract-synthesized TiO₂ NPs on three major mosquito vectors. Bhan et al. [48] determined the effect of *A. flavus* (entomopathogenic fungus), with the combination of *C. reflexa* petroleum ether extract on malaria and filariasis vectors.

4 Conclusion

The current data conclude that the methanol leaf extract of *C. baccifera* exhibits strong larvicidal activity on *Cx. quinquefasciatus* followed by *An. stephensi* and *Ae. aegypti*. Furthermore, the *C. baccifera*-synthesized ZnNPs produced high mortality against three species than crude extract. The importance of the present study lies in the possibility that the next-generation NP-conjugated plant bioactive molecules may be more effective control of mosquito agents. Further investigations of the mode of action of the NP-conjugated plant extract effect on non-target organisms and field evaluation are necessary prior to commercialization.

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