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Larvicidal efficiency of green synthesized silver nanoparticles using selected plant extracts against malaria vector *Anopheles stephensi*

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Abstract
Nanotechnology is a growing field of materials science, with applications in numerous fields, including textiles, food storage, refrigerator surfaces, drug delivery, agriculture, military, electronics, and food production etc., Various types of nanoparticles may be synthesised using biological entities such as bacteria, fungi and plant biomass, making them an alternative to chemical nanoparticles. In this regard, the current study focused the larvicidal efficacy of silver nanoparticles synthesized using *Coleus aromaticus* and *Wrightia tinctoria* against *Anopheles stephensi* larvae. The green synthesised silver nanoparticles were characterized by UV-Vis spectroscopy, FTIR, XRD, and SEM. The 24-hour LC_{50} and LC_{90} values of *C. aromatics*, are10.11 ppm, 15.12 ppm, and 27.65 ppm, respectively. Whereas the LC_{90} values are 27.19 ppm, 46.48 ppm, and 58.70 ppm. Similarly, the LC_{50} values for *W. tinctoria* are 18.12 ppm, 21.20 and ppm, 33.26 ppm, for II, III, and IV larvae, respectively, and the LC_{90} values are 35.66 ppm, 45.32 ppm, and 64.78ppm, respectively. The results find that the green synthesized silver nanoparticles from *C. aromaticus* are effective than *W. tinctoria* nanoparticles tested.

Keywords: Green synthesised silver nanoparticle, *Coleus aromaticus, Wrightia tinctorial, Anopheles stephensi,* vector control

Introduction

Aedes, *Anopheles*, and *Culex* are important mosquito species that spread various dreadful diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, hemorrhagic fever, yellow fever, chikungunya, and Zika fever [1, 2]. There are about 3,500 mosquito species have been identified from 41 genera. Among these, *Anopheles stephensi* species are primarily responsible for the transmission of malaria [3, 4].

Thus, vector management is most important for developing countries. Control of insect using synthetic insecticides are create various side effects [5, 6].

In this situation novel strategy for enhancing the usage of effective and alternative insecticide is needed. In this line, we find that the applications of nanoparticles in various fields [7, 8]. Nano particles synthesized from biological resources, such as microorganisms and plant biomass, provide environmentally safe and alternatives to synthetic chemical insecticides [9, 10].

According to many researchers, plant-based insecticides are harmless to co-habitats, safe, low-cost, biodegradable, and eco-friendly [11, 12, 13, 14]. As a result, the current study aims to green synthesis silver nanoparticles from *C. aromaticus* and *W. tinctoria* and tested on *An. stephensi* larvae. These two plants, *C. aromaticus* and *W. tinctoria*, were chosen due to their therapeutic characteristics and active phytocompounds, which were already reported [15, 16].

Materials and methods

Collection of plant materials

Leaves of *Coleus aromaticus* (Lamiaceae) and *Wrightia tinctoria* (Apocynaceae) were obtained from Karambayam village (10.490°N, 79.300°E), Thanjavur district, Tamil Nadu, India.

Plant Extracts Preparation

C. aromaticus and *W. tinctoria* leaves were collected and washed with tap water, 25 grams of leaves were weighed, crushed, and added to 100 milliliters of sterile distilled water. The substance is filtered through Whatman No. 1 filter paper with a 25 µ pore size. Filters with a diameter of 0.6 µm were used for further filtering [17].

Preparation of Green Silver Nanoparticles

A 2 mM $AgNO_3$ solution was prepared, and 10 mL of aqueous leaf extract was added to 90 mL of the 2 mM silver nitrate solution. The solution was kept at room temperature for 24 hours to reduce Ag^+ ions. Brown solutions indicate the presence of silver nanoparticles. The samples centrifuged for 20 minutes at 5000 rpm, and the resulting suspension was redispersed in 10 mL of sterile distilled water. The procedure of centrifugation and re-dispersion was repeated three times [18]. The resultant powdered suspension is made by freeze-drying. The dried nanoparticles were used for further studies.

Green synthesised Nano Particles Characterization

UV-vis spectra analysis

The synthesized silver nanoparticles diluting with distilled water, the UV-Vis spectrum (UV-3024) of the reaction solution was measured every 5 hours to track the reduction of pure Ag^+ ions [19].

FTIR - analysis

Fourier Transform Infrared Spectra of the samples were measured by a Nicolet 520P spectrometer at 4000-400 cm^1resolution in KBr pellets [20].

XRD – Measurements
Purified dried synthesised silver nanoparticles were coated on the XRD grid and an X-ray diffractometer Rigaku Ultima III XRD used for determine the size of green synthesized silver nanoparticles. The crystallite area dimension was measured by dividing the peak width by the number of X-rays obtained, assuming that the XRD peaks are uncontaminated by non-uniform stains.

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

Where is the wavelength of the X-ray, is the full width at half maximum (FWHM), and is the diffraction angle. Here, D is the average size of crystalline domains perpendicular to the reflecting planes [21].

SEM -Analysis
The Vega 3 Tescan SEM equipment was used for scanning electron microscopic examination. A carbon-coated copper grid was used to make a thin film of the sample; the excess solution was blotted off using paper towels, and the grid was dried under a mercury lamp for 5 minutes [22].

Preparation of Stock Solution and Test Concentration
C. aromaticus and *W. tinctoria* synthesised silver nanoparticles to make different test concentrations for bioassay based on the preliminary studies (10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm, respectively).

Culture of Test Animal
The Vector Control Research Center in Madurai (VCRC), Tamil Nadu, India, provided the *An. stephensi* eggs. After hatching, the larvae were kept between 75- 85 % relative humidity and at room temperature (27 ± 2°C). Dog biscuits and yeast powder are fed to larvae at a 3:1 ratio. For bioassay, II, III, and IV instars are used [23].

Larvicidal Bioassay
The larvicidal bioassays conducted by the procedure of WHO (1992) (some procedure tweaked) (WHO, 1992) [24]. 200 ml of tap water were measured in 250 ml beakers. *C. aromaticus* and *W. tinctoria* synthesized silver nanoparticles made at different tested concentrations, such as 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm, respectively. A control was made by adding 2ml aqueous extract of *C. aromaticus* and *W. tinctoria* added in 200 ml of water in a 250 ml beaker. In the experimental bioassay, 10 larvae were used per concentration. After 24 hours of exposure to II, III, and IV instars mortality rate was recorded, and this experiment was carried out three times [25].

Statistical Analysis
Probit analysis (Finney, 1971) [26] was used to evaluate the relationship among concentration and mortality. The effects of green-synthesized silver nanoparticles on larval mortality were analyzed (ANOVA) by using SPSS (2016) [27], with $P<0.05$ being statistically significant [28].

Results
10 ml of both *C. aromaticus* and *W. tinctoria* plants aqueous solution added separately with 90 ml of 2 mM silver nitrate solution. It is kept at room temperature for 24 hours. When the color of the solution turns dark brown, it is confirmed that formation of nanoparticles. Further confirmation of the synthesis of silver nanoparticles allows spectroscopy in the form of UV-visible absorption. For *C. aromaticus*, the peak was absorbed at 441 nm, (Fig. 1) and for *W. tinctoria*, the peak was absorbed 418 nm, confirming the presence of silver nanoparticles (Fig. 2).

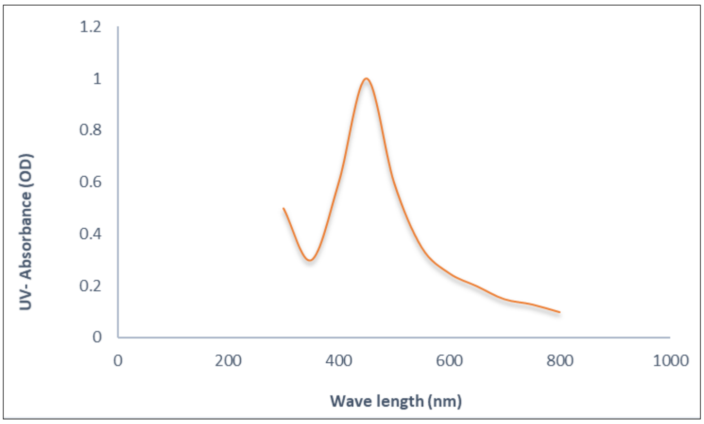


Fig 1: UV-Vis absorption spectra of silver nanoparticles synthesized by *C. aromaticus* leaves extract

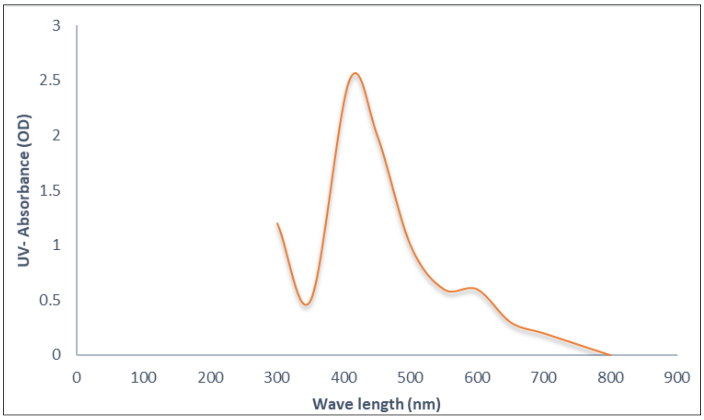


Fig 2: UV-Vis absorption spectra of silver nanoparticles synthesized by *W. tinctoria* leaves extract

FTIR analysis was carried out to identify the functional groups of the synthesized AgNPs. The FTIR spectrum of the synthesized silver nanoparticles peaks were recorded at 3336, 2922, 1612, 1389, 1016, 759, and 525 cm^{-1} for *C. aromaticus*. Similarly, *W. tinctoria* AgNPs peaks were confirmed at 2914, 2843, 2430, 2309, 2228, 2129, 1607, 1375, 1051, 822, 771, 603, and 442 cm^{-1}.

In *C. aromaticus*, the peak at 3336 cm^{-1}corresponds to the primary amine N-H medium band and stretching vibration, while the 2922 cm^{-1}band corresponds to the alkane C-H band and stretching vibration. The peak at 1389 cm^{-1}relates to asymmetric stretching of C-C bonds, and the band at 1692 cm^{-1}produced for diketone C = O groups. The peak at 1016 $cm^{-1}$was responsible for the secondary anime N-H band; the band at 759 $cm^{-1}$was responsible for a C-C stretching scissoring vibration and the peak at 525 $cm^{-1}$was responsible for C-Br stretching (Fig. 3).

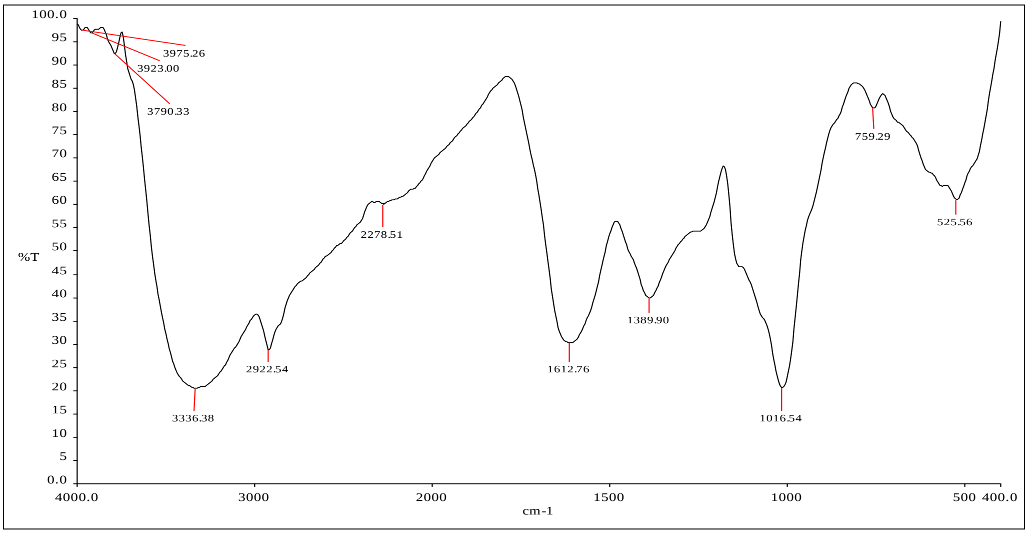


Fig 3: FTIR spectrum of silver nanoparticles synthesized by *C. aromaticus* leaves extract

Peak values relate to functional groups such as the amide group, N-H stretching 3791 cm^{-1}. The band at 2914 cm^{-1}belongs to the cyclic (CH_2-2) stretching bands of aliphatic groups. Carbonyl groups are represented by the band at 1607 cm^{-1}, methyl groups are represented by the band at 1375 cm^{-1} (CH_2 – CH_2 stretching), aliphatic amine groups are represented by the band at 1051 cm^{-1} (C-N stretching), and alkynes groups are represented by the band at 603 cm^{-1}(C-H stretching) for *W. tinctoria* (Fig. 4).

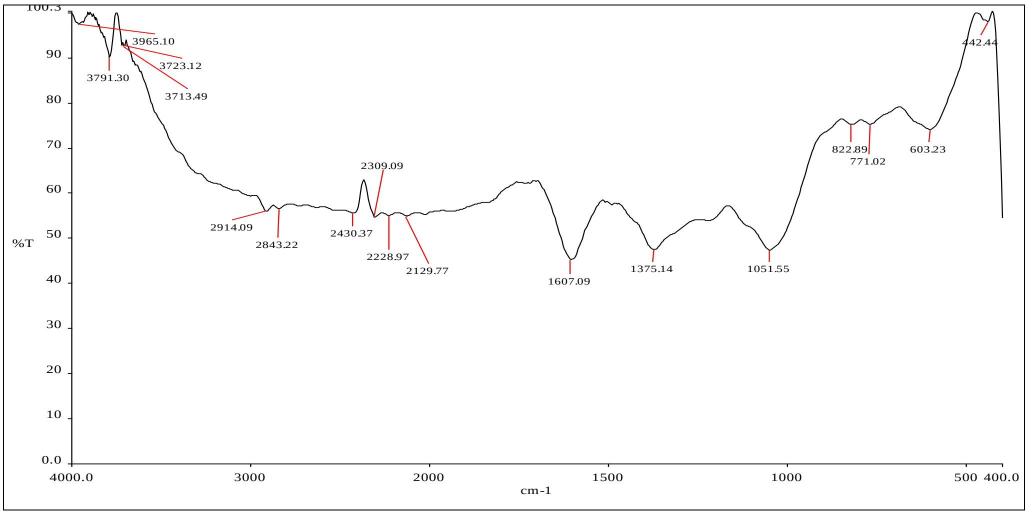


Fig 4: FTIR spectrum of silver nanoparticle synthesized by *W. tinctoria* leaves extract

The existence of alcohol, amines, amides, alkanes, methyl, carbonyl, ketone, aliphatic, and halides in AgNPs has been determined, and these are the most important functional groups. They have been identified as potential biomolecules involved in the stability, capping, and reduction of AgNPs. The findings imply that molecules attached to AgNPs have both free and bound groups.

An X-ray diffraction examination revealed the crystalline form of silver nanoparticles. The freeze-dried nanoparticles. In X-ray diffraction, the characteristic peak was exhibited between 20° and 80°, and a distinct diffraction peak was obtained at 27°, 32°, 46°, and 57° at 2θ values indexed to 220, 122, 220 and 241 respectively, for *C. aromaticus* (Fig. 5). On the other hand, for *W. tinctoria*, the peaks were obtained at 27°, 32°, 38°, 46°, and 57° for corresponding sets of latticesplanes to 220, 122, 111, 200 and 241 respectively (Fig. 6). The XRD investigation sheds light on the composition of *C. aromaticus* and *W. tinctoria* green synthesized silver nanoparticles (Tables 3, 4).

Table 3: XRD patterns of *C. aromaticus* synthesized silver nanoparticles

No.	2-theta(deg)	d(ang.)	Height(cps)	FWHM (deg)	Int. I (cps deg)	Int. W(deg)	Asym. factor
1	27.747(7)	3.2125	2904	0.278	1326	0.46	0.63
2	32.165(4)	2.7806	6550	0.283	3038	0.46	0.64
3	46.144(9)	1.9656	3624	0.335	1910	0.53	0.68
5	57.39(3)	1.6043	861	0.45	521	0.61	0.9

Table 4: XRD patterns of *W. tinctoria* synthesized silver nanoparticles

No.	2-theta(deg)	d(ang.)	Height(cps)	FWHM (deg)	Int. I(cps deg)	Int. W(deg)	Asym. factor
1	27.983(9)	3.1860	3140	0.294	1296	0.41	1.10
2	32.400(6)	2.7610	6849	0.288	2663	0.389	1.14
3	38.275(14)	2.3496	2812	0.305	1339	0.48	1.1
5	46.349(10)	1.9574	3472	0.339	1512	0.44	0.74
7	57.58(2)	1.5994	881	0.391	433	0.49	0.77

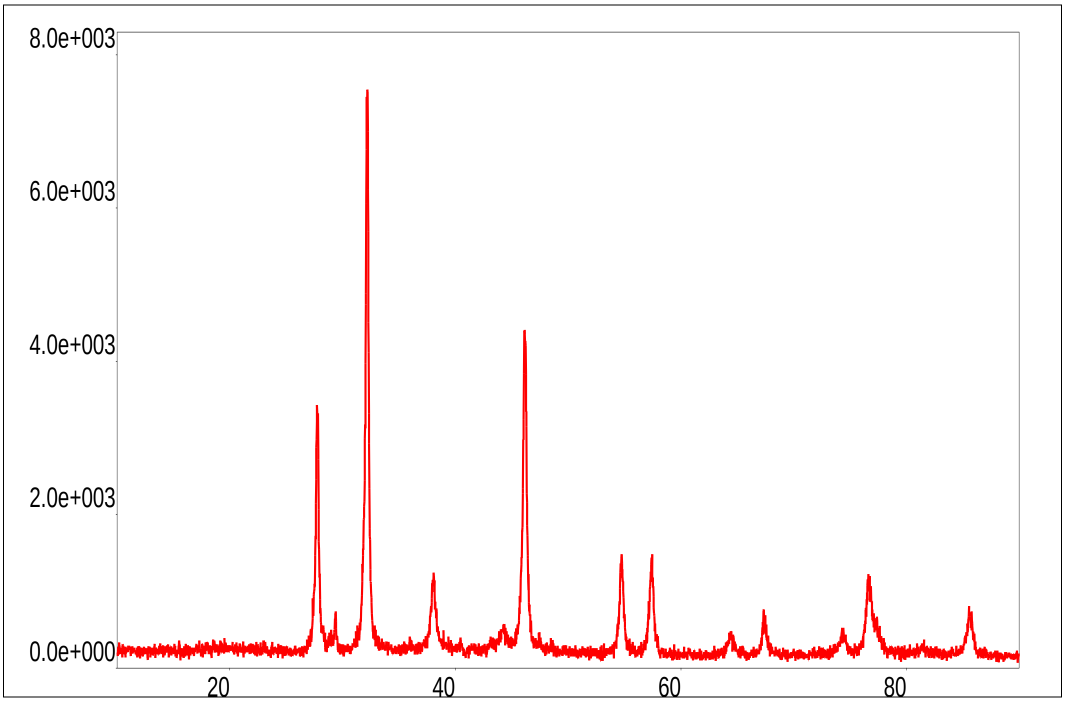


Fig 5: XRD analysis of silver nanoparticles synthesized by *C. aromaticus* leaves extract

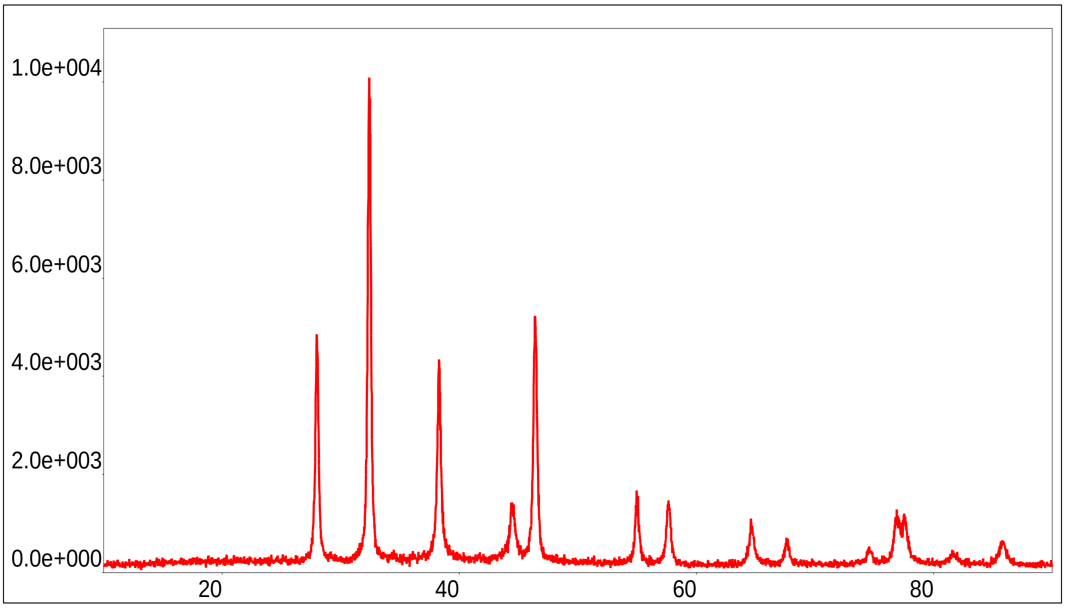


Fig 6: XRD analysis of silver nanoparticles synthesized by *W. tinctoria* leaves extract

An examination of the images made by a scanning electron microscope (SEM) revealed that AgNPs synthesized by *C. aromaticus* (79.46 nm, and 89.39nm) (Fig.7), and *W. tinctoria* (99.34nm, and 129.149nm) had a distinct form and size (Fig. 8).

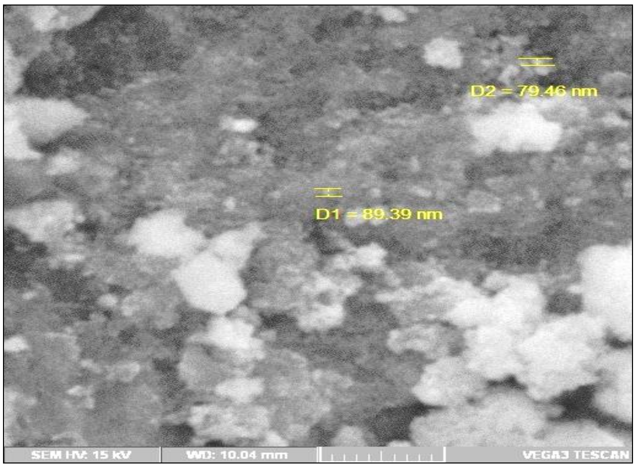


Fig. 7. SEM image of silver nanoparticles synthesized by *C. aromaticus* leaves extract

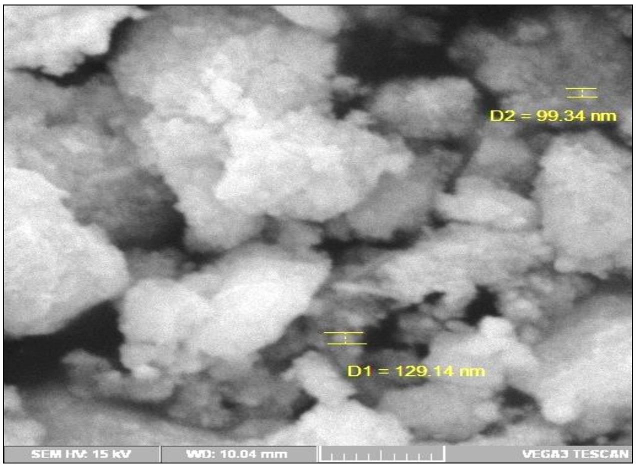


Fig 8: SEM image of silver nanoparticles synthesized by *W. tinctoria* leaves extract

C. aromaticus and *W. tinctoria* synthesized silver nanoparticles were used in larvicidal and bioassays against *An. stephensi* II, III, and IV instar. The LC_{50} and LC_{90} values of *C. aromatics*, are10.11 ppm, 15.12 ppm, and 27.65 ppm, respectively. Whereas the LC_{90} values are 27.19 ppm, 46.48 ppm, and 58.70 ppm. Similarly, the LC_{50} values for *W. tinctoria* are 18.12 ppm, 21.20 and ppm, 33.26 ppm, for second, third, and fourth instars, respectively, and the LC_{90} values are35.66 ppm, 45.32 ppm, and 64.78ppm, respectively. The LC_{50} and LC_{90} values showed that the *C. aromaticus* leaf extracts synthesized silver nanoparticles were more effective than the *W. tinctoria* silver nanoparticles used (Table 1).

Table 1: The LC_{50} and LC_{90} values of *C. aromaticus* and *W. tinctoria* synthesized silver nano-particles against the II, III, IV larvae of *An. stephensi* under 24-hour exposure

Larval stages	LC_{50} (ppm) (UCL-LCL)	LC_{90} (ppm) (UCL-LCL)	χ^2	Regression equation
Coleus aromaticus				
II instar	10.11 (18.78- 08.50)	27.19 (48.18-15.57)	2.23	Y= 1.24 + 0.10X
III instar	15.12 (21.02-12.25)	46.48 (62.08-25.90)	3.10	Y= 1.82 + 0.15X
IV instar	27.65 (40.39-18.08)	58.70 (79.98-38.70)	3.80	Y= 1.78 + 0.19X
Wrightia tinctoria				
II instar	18.12 (24.56-11.17)	35.66 (47.65–18.47)	1.80	Y= 1.25+ 0.13X
III instar	21.20 (38.21-15.93)	45.32 (71.68-31.60)	2.26	Y= 0.29 + 0.18X
IV instar	33.26 (53.60 -29.34)	64.78 (86.18-58.84)	3.11	Y= 2.66 + 1.92X

This study found that the II instar is more sensitive to *C. aromaticus* and *W. tinctoria* green synthesized silver nanoparticles compared to other instars. *C. aromaticus* synthesized silver nanoparticles more effectively than *W. tinctorial* silver nanoparticles. The analysis found a significant difference ($P < 0.05$) in larval mortality rates between the two-plant species and larval stages. This study was conducted using analysis of variance (Table. 2).

Table 2: ANOVA to test the validity of relationship in mortality (LC_{50}) as a function of green silver nanoparticles and development stages of *An. stephensi*

Source of variation	SS	*df*	F	P-Value	F-crit	Significant
Stages of development	1106.25	3	53.900	0.00347	9.566	***
Plant Species (Nanoparticles)	53.34	1	7.5731	0.0578	9.987	**
Error	19.12	3	1.9900			
Total	1175.82	7				

Discussions

UV-Vis spectroscopy is used to examine the shape and size of synthesized silver nanoparticles in aqueous solutions. The nanoparticles were characterized by using FTIR, and the data were used to identify potential active compounds involved in the stability of the synthesized silver nanoparticles. The carbon substance and metallic silver crystalline structure were confirmed by the X-ray diffraction pattern. SEM images of the shaped AgNPs with 79 -129 nm sizes revealed that the majority of the AgNPs were spherical in structure [29].

A recent review proved green synthesized silver nanoparticles to be a more sustainable, ecologically friendly, alternative to chemical pesticides, green synthesized silver nanoparticles making it an effective technique for control mosquito larvae and vector-borne diseases [30].

Gnanadesigan *et al*. (2011) [31] examined that *Rhizophora mucronata* green synthesized silver nanoparticles were tested on *Ae. aegypti* and *Cx. quinquefasciatus* larvae. The findings indicate that green-synthesized silver nanoparticles are more lethal to *Cx. quinquefasciatus* (LC_{50} = 0.585 mg/l) and *Ae. aegypti* (LC_{50} = 0.891 mg/l).

According to Borase *et al*. (2013) [32], bio-synthesized silver nanoparticles from *Jatropha gossypifolia*, *Euphorbia tirucalli*, *Padilanthus tithymaloides*, and *Alseuosmia macrophylla* are effective against the IV instar of *Ae. aegypti* and *An. stephensi*. Among the four distinct nanoparticles tested, *J. gossypifolia* was effective against

Ae. aegypti (LC_{50} = 4.44 ppm) and *An. stephensi* (LC_{50} = 4.90 ppm).

Similarly, Velayutham *et al*. (2016) [33] studied the larvicidal activity of *Annona squamosa* leaf extracts and silver nanoparticles test on dengue vector *Ae. aegypti* and the filarial vector *Cx. quinquefasciatus.* As a consequence, it has been shown that green synthesized silver nanoparticles have the potential to control dengue and filarial vectors than plant extract.

The prior research used the same plants to test silver nanoparticles against *Cx. quinquefasciatus* II, III, IV instars, and pupa. The LC_{50} values for *C. aromaticus* are 13.37 ppm, 16.26 ppm, 36.07 ppm, and 41.47 ppm. The LC_{50} values for *W. tinctoria* are 14.56 ppm, 18.77 ppm, 42.76 ppm, and 51.06 ppm, respectively [34].

There have also been reports of green synthesized silver nanoparticles utilizing *Annona glabra* leaf extract, and the findings indicate that *A. glabra* nanoparticles have the potential to eradicate *Ae. aegypti* larvae [35].

Prakash *et al*. (2022) [36] used *Aegle marmelos* and *Colocasia esculenta* green-synthesised silver nanoparticle tested on *Ae. aegypti* larvae and pupa. The LC_{50} values for *A. marmelos* and *C. esculenta* were showed the IV instar of *Ae. aegypti* have 28.42 ppm and 30.03 ppm, respectively. Dass *et al*. (2022) [37] examined methanolic leaf extracts of *C. esculenta* and *W. tinctoria* tested on the second, third, fourth, and pupa of *Ae. aegypti* and exposed that the LC_{50} values of *Ae. aegypti* second, third, fourth, and pupa were 101.17 ppm, 126.02 ppm, 161.60 ppm, and 189.28 ppm, respectively. Similarly, *W. tinctoria* LC_{50} values for II, III, IV, and pupa are 126.33 ppm, 149.90 ppm, 183.97 ppm, and 228.20 ppm. Of these two plants, the methonolic extract tested *C. esculenta* effective than that of *W. tinctoria*.

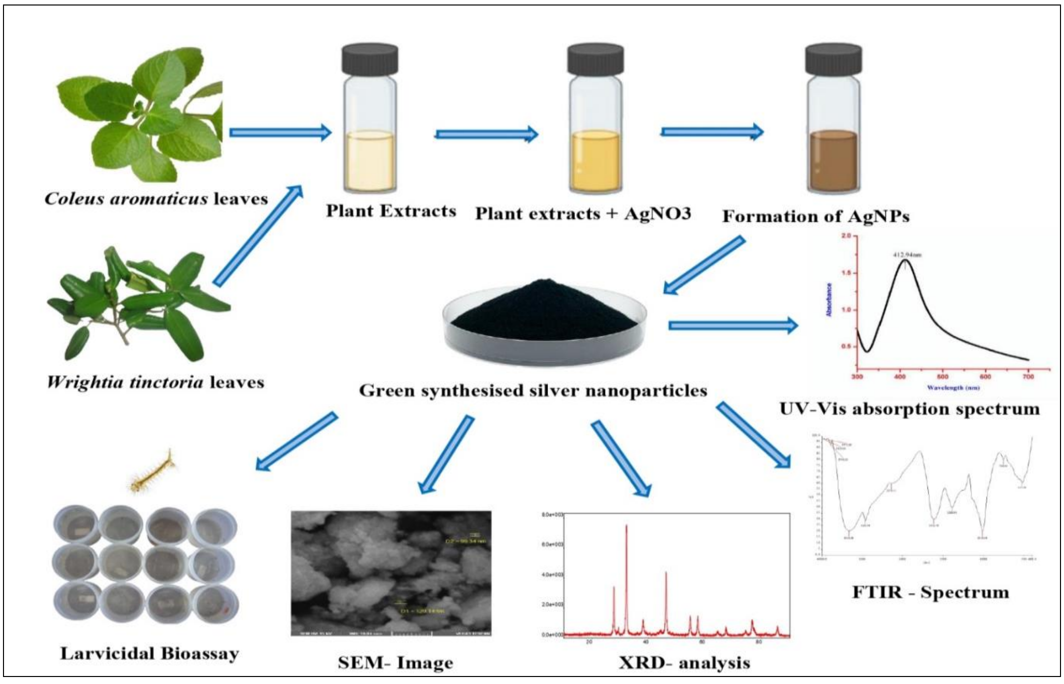
Plant of *A. marmelos, C. aromaticus, C. esculenta*, and *W. tinctoria* methanolic extracts as well as green synthesised silver nanoparticles, were studied for their capacity to predation of *Cx (L) fuscanus*, a non-target organism. Both the plant extracts and the synthesised silver nanoparticles exhibited no adverse effects on non-target organism. The results find that plant extracts and AgNPs are environmentally safe and can be used in integrated pest control programs [38].

Acacia sinuate seed silver nanoparticles showed significant larvicidal activity against 3rd instar of *Ae aegypti* (LC_{50} = 23.03 and LC_{90} =38 ppm) and *An. stephensi* (LC_{50} = 28.71 and LC_{90}=46 ppm). This work gives greater strength to my research [39].

Kumar *et al*. (2023) [40] observed that *Alternanthera sessilis* synthesised AgNPs showed significant larvicidal activity against *Ae. aegypti* (LC_{50}= 2.93 ppm and LC_{90}= 7.63 ppm). The green synthesized AgNPs were no harmful to the non-target species, *Poecilia reticulata*. This study is inexpensive, environmentally friendly, and non-toxic to the non-target organism, *P. reticulata.*

The larvicidal efficacy of *Osbeckia leschenaultiana*, green-synthesised silver nanoparticles and crude extracts tested on *Ae. aegypti*. In comparison to *O. leschenaultiana* leaf extract (LC_{50} = 85.95 µg/mL), the silver nanoparticles showed the highest mortality rate against *Ae. aegypti* (LC_{50} = 49.17 µg/mL). Lower toxicity was found when *Artemia salina*, a non-target organism, had its toxicity profile evaluated. According to the study, silver nanoparticles have larvicidal properties [41]. A Crude extract and *Ranunculus sceleratus* synthesised silver nanoparticles (AgNPs) shown to be effective against *Cx. quinquefasciatus* larvae [42].

Ae. albopictus, An. subpictus, and *Cx. tridentiorhynchus* larvae were tested against the thymol that was derived from *C. aromaticus*. The findings showed that thymol is a novel and potent natural insecticide [43] (Govindarajan *et al*., 2016). According to Lim *et al*. (2023) [44], compounds with insecticidal properties included eugenol, methyl chavicol, thymol, carvacrol, camphor, caryophyllene oxide, cineole, limonene, and myrcene. The latest study offered a useful method for controlling mosquito populations by expressing plant extracts and green synthesised silver nanoparticles as an alternative to chemical pesticides [30, 45, 46]. This current study suggests that the larvicidal efficiency of green integrated silver nanoparticles makes them an attractive as well as alternative vector control approach.



Graphical abstract

Conclusion
Aqueous extracts of *C. aromaticus* and *W. tinctoria* were used to synthesize green silver nanoparticles, and the larvicidal efficacy of these green-synthesized AgNPs was investigated against *An. stephensi* larvae. The *C. aromaticus* plant extract was more effective than the *W. tinctoria*, as evidenced by the larvicidal bioassay. The active phytochemicals such as thymol, carvacrol, eugenol, chavicol, 1,8-cineole, α-humulene, p-cymene, α-terpineol, β-selinene, β-caryophyllene, γ-terpinolene, pinene, methyl eugenol, phellandrene, chlorogenic acid, rosmarinic acid, caffeic acid in *C. aromaticus* are thought to be the main reason for its high activity than*W. tinctoria*. The possibility of using natural components to synthesize silver nanoparticles and assessing their efficiency in mosquito control programs will facilitate a more potent and ecologically friendly way to control insect pests. The use of these green synthesized plant extracts as a larvicidal agent in mosquito biocontrol should be recommended in mosquito control strategies.

Authors' contributions
K.R: Prepared the original draft of the manuscript, literature review, investigation, data analysis, editing;
P.R: Methodology, literature review;
T.J: Data analysis, Methodology;
K.D: Original draft of the manuscript, validation, supervised the work, conceptualized the study and literature review

Competing interests
Authors have declared that no competing of interests.

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