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Assessment of larvicidal and antioxidant activities of crude extract and synthesised silver nanoparticles from leaves and stem of *Ranunculus sceleratus* L.

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The present study evaluates the efficacy of crude extract and synthesised silver nanoparticles (AgNPs) of leaves and stem of *Ranunculus sceleratus* L. against immature larval stages of *Culex quinquefasciatus.* Different concentrations of crude extract of both plant parts were tested against 1st to 4th instar larval stages, and synthesized AgNPs were tested against 3rd instar larvae of *Cx. quinquefasciatus*. The characterisation of synthesized silver nanoparticles (AgNPs) was also carried out. The antioxidant activity of the methanolic extract of both plant parts was also explored. A 100% mortality was observed in 1% crude extract and 5 ppm of synthesized AgNPs of leaves and stem of *R. sceleratus* against 1st and 2nd instar larvae, respectively. The UV–Vis spectrophotometer confirmed the synthesis of AgNPs where the absorption maximum (A0) was recorded at 450 nm and 440 nm, respectively, in leaves and fruits of *R. sceleratus*. The scanning electron micrographic (SEM) of the AgNPs indicates that almost all the particle's surface is rough in shape. The core shell morphology of synthesised AgNPs were identified by FTIR measurements. 80 µL concentration of methanol extract of both the plant parts almost completely inhibited DPPH with IC_{50} values of 44.93 and 22.17 µL, respectively, in the leaves and stem of *R. sceleratus*. It is concluded that both the plant parts of *R. sceleratus* showed excellent mosquito larvicidal activities and good free radical scavenging activity.

Keywords: Anti-oxidant activity, *Culex quinquefasciatus*, Insecticidal activity, Phytochemical analysis, *Ranunculus sceleratus*, Silver nanoparticles

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Introduction

The medium-sized mosquito *Culex quinquefasciatus*, often known as the domestic mosquito, is mostly prevalent in tropical and subtropical countries around the globe[1]. It transmits the nematode *Wuchereria bancrofti*, responsible for the disfiguring disease, elephantiasis. Besides *W. bancrofti,* avian malarial parasite, Western equine encephalitis virus, St. Louis encephalitis virus, and West Nile virus are also transmitted by this vector. Worldwide, lymphatic filariasis is thought to affect 120 million people, 44 million of whom have the disease's main chronic symptom[2]. The quality of human and animal health is negatively impacted by the use of chemical insecticides on a routine basis, which in turn causes the expansion of resistance among vectors and other issues like environmental pollution and biomagnification[3]. More than 700 million individuals worldwide contract mosquito-transmitted diseases each year, which continue to be a major cause of human mortality[4].

An insecticide should be safe for the environment and other non-target organisms rather than having a high mortality rate among its target organisms. Many plants have been shown to have insecticidal action, making the hunt for new biocontrol agents from natural sources like secondary metabolites from plants appealing among researchers in nations with a strong herbal heritage[5]. Insect control potential of phytochemicals from various chemical groups, including steroids, alkaloids, terpenes, and phenolic components, has been previously examined and found promising[6-8]. There is a renewed effort to find natural products because they are considered more environmentally benign due to their inherent biodegradability and cause minimum toxicity to other non-target organisms[9]. Nowadays, the most efficient method of preventing diseases spread by vector mosquitoes has been considered the radical approach to mosquito control management. The majority of

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insecticides available in the market are synthetic insecticides with unintended consequences, including the development of resistant mosquito strains, ecological disparity, and severely affecting the non-target organisms in the environment[10]. The necessity to employ environmentally friendly synthesised silver nanoparticles has been identified as viable replacements for synthetic chemical pesticides.

New paths for research into the efficacy of mosquitocidal activity are opened up by the direct administration of plant extract to mosquito larvae and the use of various metal nanoparticles (NPs) created by green chemical synthesis. Nanotechnology aims to produce nanoparticles with different sizes and shapes through experiments and controlled discrepancies. This offers effective control over a wide range of chemical and physical properties, which have a variety of possible uses in the pharmaceutical and medical industries. Besides silver and gold, noble metals such as lead (Pb) and platinum (Pt) are used most frequently to create metallic nanoparticles. Among these, silver (Ag) is preferred in the field of biological systems. Green synthesis using plant parts is an alternate way of synthesising silver nanoparticles. Plant extracts are discovered to be the most practical and appropriate reducing agents[11]. It is necessary to utilise these environmentally friendly AgNPs for the control of vector mosquitoes. AgNPs are a feasible replacement for man-made chemical insecticides that are less likely to cause environmental pollution. This study aims to find possible plant-based mosquito larvicides to control the filarial vector *Culex quinquefasciatus* as an alternative source with minimal environmental and human health effects.

Ranunculus sceleratus L. is also known as celery-leaved buttercup or celery-leaf buttercup. The annual plant *R. Sceleratus*, a member of the Ranunculaceae family, grows along the banks of ponds and streams. It is an annual plant that grows to 20 to 60 cm tall, branches regularly, prefers damp environments, tolerates brief droughts, and has achenes, simple, broad leaves, and yellow flowers. The leaves have tiny, strongly lobed blades that are mostly glabrous (hairless) or divided into three leaflets[12]. They have lengthy petioles that carry them. Numerous allelochemicals, including phenolics, flavonoids, alkaloids, saponins, fatty acids, organic acids, and essential oils, have been documented to be produced by *Ranunculus* species and may offer protection against chronic illnesses for people[13]. In traditional medicine, several species of *Ranunculus* have been used to cure a variety of ailments and symptoms, including rheumatism, jaundice, gout, nebula, oedema, malaria, asthma, pain, skin inflammation, cancer, and hypertension[14].

The principal objective of the present study was to evaluate the mosquito larvicidal potency of crude and synthesised AgNPs of the leaves and stem of *R. sceleratus* against *Cx. quinquefasciatus*. The study also includes the antioxidant activity of methanol solvent extract of leave and stem of *R. sceleratus*. Qualitative phytochemical analysis was also carried out to know the core-shell morphology of synthesised AgNPs. Characterisation of synthesised AgNPs has also been done. The research findings offer insight into the usage of environment friendly plant-based mosquito control and natural antioxidant agents.

Materials and Methods

Study area

The present study was carried out at the Laboratory of Parasitology, Vector Biology, and Nanobiotechnology of the University of Gour Banga, Malda, West Bengal, India, from February to July 2023.

Collection of plant material

R. sceleratus plants were collected from the bank of the Mahananda River, also known as Malda Bandh. The plant (Voucher no-01) was identified and authenticated by Prof. Monoranjan Chowdhury, Herbarian In-charge, North Bengal University, West Bengal, India.

Collection of mosquito larvae

Cx. quinquefasciatus mosquito larvae were collected from the drains surrounding the University of Gour Banga campus in a plastic bucket (5 L). Then, they were transferred into a plastic tray (45 cm x 0.7 cm x 30 cm). To maintain the larvae, 500 mL of water was added to the tray, and the larvae were fed with a mixture of dog biscuit and yeast. The cultures were kept free from any pathogens and maintained at a temperature of 25–27°C, humidity 75–85% and photoperiod 14L:10 D.

Preparation of crude extract

The fresh leaves and stems of *R. sceleratus* were washed with tap water several times to remove the filth and dust. According to Rawani *et al*.,[15] 10 g of both plant parts were ground by an electric grinder machine to get a fine paste, and then a cotton towel

with a 20 µm mesh size was used to squeeze out the extracts. Both extracts were collected separately in a container and stored at 4°C in the refrigerator for further use.

Synthesis of silver nanoparticles

10 g of both plant parts were measured and kept in a 500-mL beaker, and then 100 mL of double-distilled water was used to prepare an aqueous solution. Then, a 10^{-3} M silver nitrate ($AgNO_3$) solution was prepared. 88 mL of the stock solution of $AgNO_3$ was mixed with 12 mL of the aqueous solution of both leaf and stem extracts of *R. sceleratus* separately. Both mixtures were stirred with a vortex stirrer for 5 minutes and then heated at 60°C for 10 minutes. The colour change occurs from light green to dark brown, resulting in a nano-colloidal solution. Then, the repeated centrifugation (two times) of the nano-colloidal solution was carried out in a Remi research centrifuge instrument at 10,000 rpm for 15 min. After centrifugation, pellets were collected from both samples, dried in a vacuum desiccator, and stored for further experimentation. From these synthesised AgNPs, different test concentrations for larvicidal bioassay were prepared[16,17].

Mosquito larvicidal bioassay

The larvicidal bioassay was evaluated following the WHO procedure[18] with some suitable changes. 1st to 4th instar larvae of *Cx. quinquefasciatus* was used for the bioassay with a crude extract of the leaves and stem. Twenty-five larvae of each instar were separately transferred into different beakers containing 100 mL of water, and 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mL concentrations of crude extract from both plant parts were added to each beaker separately. Mortality of larvae was recorded after 24, 48, and 72 hours of exposure. A control set-up was also there with only 100 mL of water and larvae. The experiment was repeated three times (n = 3) for both plant parts, and mortality rates were recorded up to 72 hours.

For the bioassay with synthesised silver nanoparticles, 3rd instar larvae of *Cx. quinquefasciatus* was used for this experiment following the protocol of Rawani[19]. Twenty-five 3rd instar larvae were separately transferred into different beakers containing 100 mL of water. Different test concentrations (1–5 ppm) of synthesised silver nanoparticles from both plant parts were mixed with dimethyl sulfoxide (DMSO) separately and added to each beaker. A control set-up was also used, containing 100 mL of water, DMSO, and twenty-five 3rd instar larvae. After 24 hours, the mortality rate was recorded against each concentration AgNPs of both plant parts. The experiments were repeated three times on three different days. The calculated percentage mortality (% M) was corrected using Abbott's formula[20].

Characterisation of synthesised silver nanoparticles

The SPR absorption spectra and the kinetics of nanoparticle formation of the water-dispersed, ultra-centrifuged pellet of AgNPs were examined using a UV–vis spectrophotometer (Shimadzu 2450, Japan) set to 1 nm resolution and 10 mm optical length over a wavelength range of 190–700 nm. The synthesised AgNPs show a characteristic surface plasmon resonance (SPR) absorption[21] in the UV-visible region.

After centrifugation, the purified AgNPs were powdered and analysed by X-ray diffraction, through which the crystallinity of the AgNPs could be confirmed[22].

The Transmission Electron Microscopy (TEM) study was done to determine the shape and size of synthesised AgNPs. The TEM images were captured using a Technai 20 Philips TEM instrument operated at 200 kV with a beam current of 104.1 µA. Both samples for this analysis were cast onto 300 mesh size copper grids separately, allowing them to dry overnight at 25°C in a vacuum[17].

The surface topography of the synthesised AgNPs in both samples was investigated by scanning electron microscopy (SEM). After centrifugation, the dry synthesised AgNPs were used for SEM analysis. Before analysis, both samples were made conductive by a gold coating. Then, the samples were characterised through SEM at a voltage of 5 KV.

The core shell morphology of synthesised AgNPs of both samples can be studied by a Perkin Elmer Lx10-88873 FT-IR spectrometer. During the FTIR measurements, the scanning range was 40 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Antioxidant scavenging methodology

The antioxidant scavenging activity of both plant parts was examined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay described by Mahdi-Pour *et al.*[23] and Zuraini *et al.*[24] with slight modification. Concisely, 50 mL of leaf and stem extracts of *R. sceleratus* in concentrations

ranging from 20 to 500 µL and 5 mL of a 0.004% (w/v) solution of DPPH were added to the universal bottle. After the prepared mixture was vortexed, it was let to stand at room temperature for thirty minutes in a dark area and then read using a spectrophotometer at 517 nm. Methanol at 80% (v/v) made up the blank. Vitamin C, or ascorbic acid, was utilised as a comparator. The measurements were made three times. The following formula was used to determine the DPPH scavenging effect:

DPPH scavenging effect (%) = {A0-A/ AO} x 100

Where A0 is the absorbance of the negative control (0.004% DPPH solution), and A is the absorbance in the presence of extract.

Phytochemical analysis

The leaves and stems of *R. sceleratus* were collected and then washed properly with tap water. After two weeks of shade drying at room temperature, the plant parts were ground into a powder. The powder of both plant parts was extracted with distilled water, methanol, and ethanol, and these were further used for the qualitative determination of phytochemical constituents according to Trease and Evans[25], Choudhuri *et al.*[26] and Rawani[27].

Statistical analysis

Abbott's formula[20] was used to correct the percentage of larval mortality (M%). The computer software Statplus v5 was used for the probit analysis, adopting the Finney method[28]. MS Excel 2021 was used to find out the average larval mortality. All values are found to be statistically significant at $P \leq 0.05$.

Result and Discussion

Characterisation of silver nanoparticle

UV-VIS spectrum analysis

The bio reduction of Ag^+ ions to Ag^0 takes place when $AgNO_3$ solution is mixed with fresh leaves and stem aqueous extract from *R. sceleratus,* and physically, the formation of AgNPs is evident from a notable change in colouration from pale to brown and then reddish-brown. Further, it is confirmed by a UV-Vis spectrophotometer, where synthesised AgNPs were analysed in a range of wavelengths of 320–700 nm. In the present study, the characteristic surface plasmon resonance (SPR) band at 450 and 440 nm for the leaves and stem of *R. sceleratus*, respectively, defines the formation of AgNPs (Fig. 1 and 2). Earlier studies by Arjuman *et al.*[29] and Gope *et al.*[30] supported the findings.

X-ray diffraction analysis

The XRD analysis confirms the elemental composition and the crystalline nature of the nanoparticles. Fig. 3 and 4 show the XRD pattern observed for the synthesised AgNPs of both plant parts of *R. sceleratus*. The diffraction peaks at 2ɵ values of 38.29, 44.34, 64.06, and 76.17° of leaf AgNPs and 34.17, 45.06, 67.04, and 76.17° of stem AgNPs correspond to (1 1 1), (2 0 0), (2 2 0), and (3 1 1) four main facets known for zero-valent FCC silver crystal planes. Thus, the XRD pattern confirms

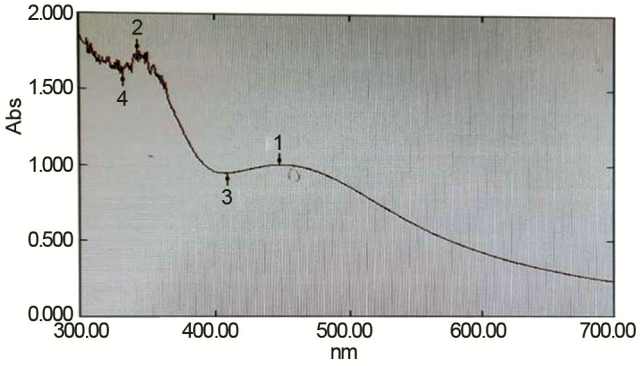

Fig. 1 — UV–vis spectra showing absorption maxima of green-synthesised silver nanoparticles at 450 nm (leaf of *R. sceleratus*).

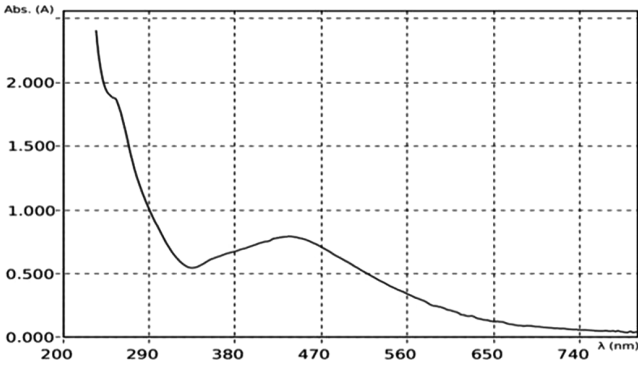

Fig. 2 — UV–vis spectra showing absorption maxima of green-synthesised silver nanoparticles at 440 nm (Stem of *R. sceleratus*).

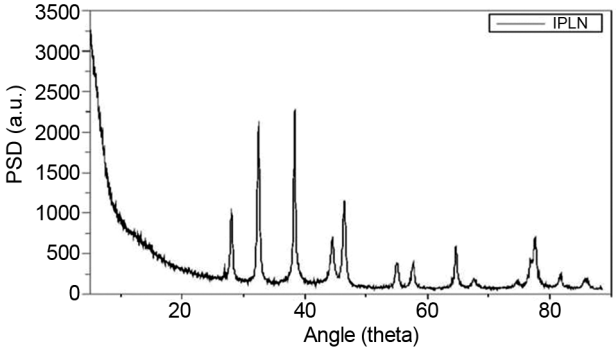

Fig. 3 — XRD pattern of the biosynthesised silver nanoparticles using leaves of *R. sceleratus*.

that leaf and stem AgNPs are crystalline, homogenous, and have an organic coat. The result is consistent with the findings of Kumar *et al.*[31] and Ghosh *et al.*[32].

FESEM Analysis

The field emission scanning electron microscope (FESEM) is used to know the surface topography of synthesised AgNPs. The SEM micrographs of the synthesised leaf AgNPs (Fig. 5) indicate that almost all of the particle's surface is rough in texture. The SEM micrographs of stem AgNPs (Fig. 6) clearly show that the outer surface of the synthesised AgNPs is rough and crystalline. Thus, the scanning electron micrographs of both types of AgNPs clearly show that most of the AgNPs were clumped and agglomerated, and a few free nanoparticles were also observed. Earlier, the surface morphology of An-AgNPs was reported by Elechiguerra *et al.*[33] using SEM, demonstrating that the majority of AgNPs had a rough surface. The studies of Malla *et al.*[11]. and Dey *et al.*[34] also support the findings of the present study.

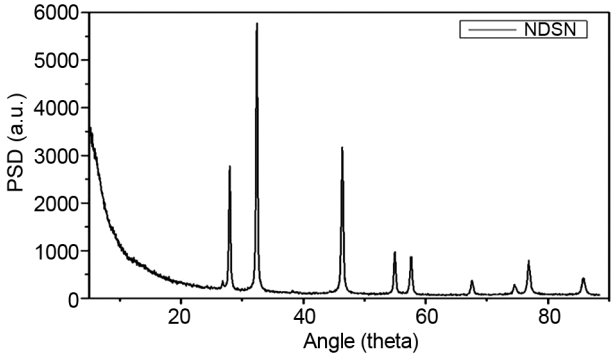

Fig. 4 — XRD spectra of green-synthesised silver nanoparticles using stem of *R. scleretus*.

HRTEM analysis

Transmission electron microscopy (TEM) is an analytical technique used to visualise shape and size as well as size distribution of the synthesised AgNPs. Fig. 7 show a representative TEM micrograph of the

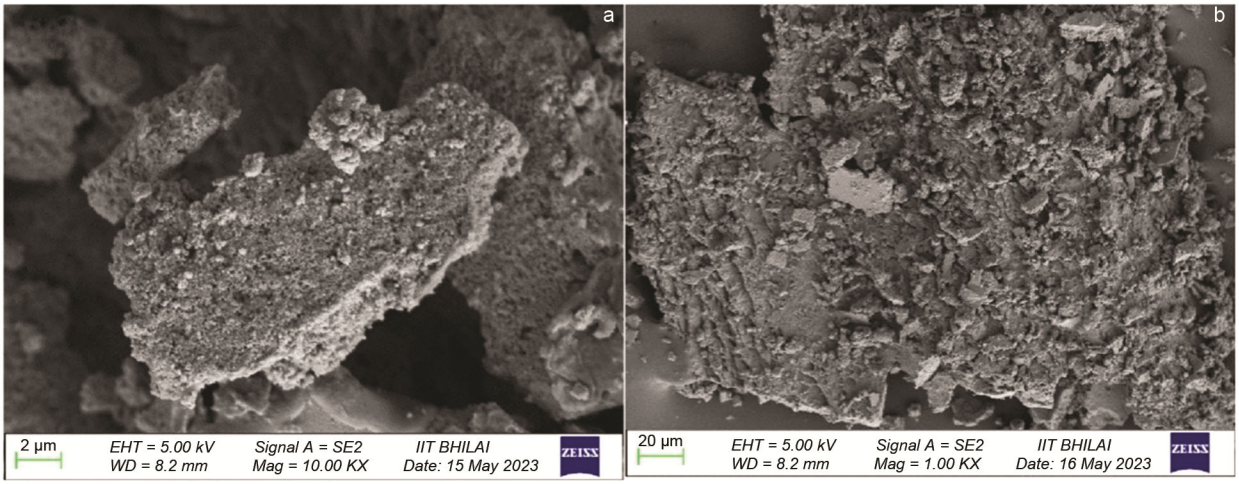

Fig. 5 — SEM image of synthesised silver nanoparticle from the leaf of *R. sceleratus.*

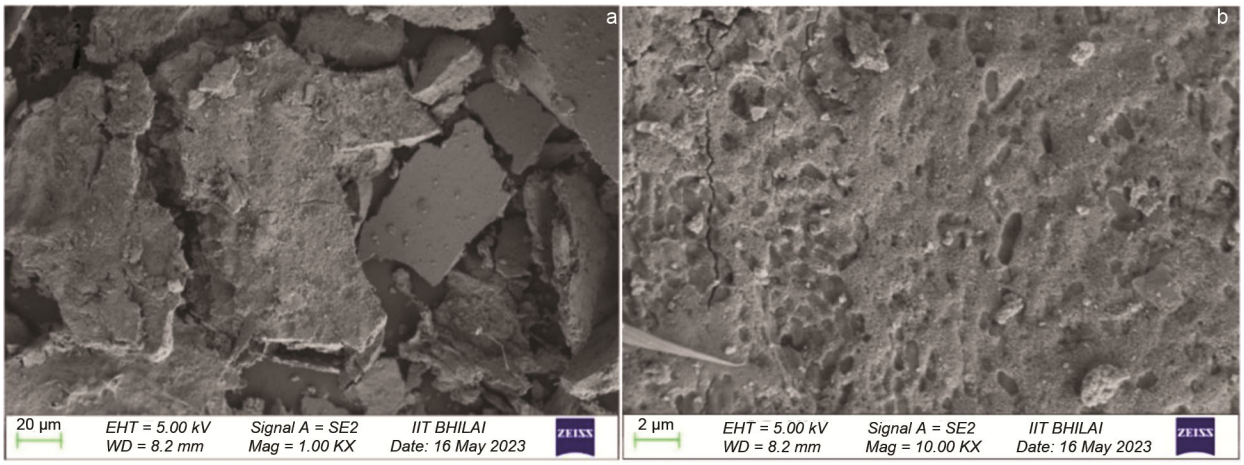

Fig. 6 — SEM image of synthesised silver nanoparticle from the stem of *R. sceleratus*.

formed AgNPs from the leaves of *R. sceleratus*, where most of the synthesised nanoparticles were spherical and quasi-spherical in shape. The average size of the synthesised AgNPs calculated was 56.67 nm. Fig. 8 show a representative TEM micrograph of the formed AgNPs from the stem of *R. sceleratus*; here, the synthesised silver particles were oval and quasi-spherical. The average size of the synthesised AgNPs calculated was 92.5 nm. The synthesised AgNP from the fresh bud of *P. tuberosa* showed most of the formed AgNPs were spherical or quasi-spherical in shape, and the average size of the synthesised AgNPs calculated was 56.67 nm[19]. Halder *et al.*[17] and Gope *et al.*[30] also described that the major population of AgNPs are found within the 25 to 50 nm size.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy analysis was done to study the core-shell morphology of AgNPs[35]. FTIR measurements were used to pinpoint the functional groups involved in the particular reduction of Ag^{+} ions and capping of the reduced AgNPs produced by the extracts of both plant parts. Fig. 9 and 10 depict the FTIR spectra of synthesised AgNPs from the leaf and stem of *R. sceleratus,* respectively. According to Rodriguez *et al.*[36] the bulk of the peaks were related to the phytochemicals present in the plant extract. Phenols, triterpenoids, alkaloids, steroids, and tannins are sufficiently prevalent in *R. sceleratus* leaves and stem extract and aid in the creation of AgNPs. The bands from both spectra were interpreted in Table 1.

Mosquito larvicidal activity of crude extract of leaves and stem of *R. sceleratus*

Table 2, shows the larvicidal activity of the leaves and stem crude extract of *R. sceleratus* against 1st to 4th instar larvae of *Cx. quinquefasciatus* in different concentration ranges from 0.5 to 1 mL. 100% mortality was observed in 1% crude extract of leaves of *R. sceleratus* against 1st instar larvae after 24 h of exposure. In contrast, the stem crude extract showed

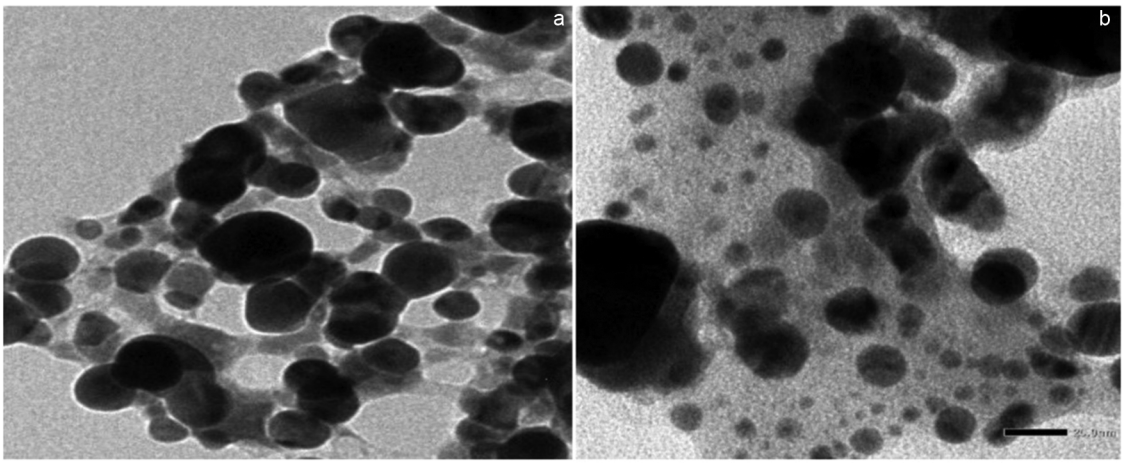

Fig. 7 — TEM image of synthesised silver nanoparticles from leaves of *R. sceleratus*.

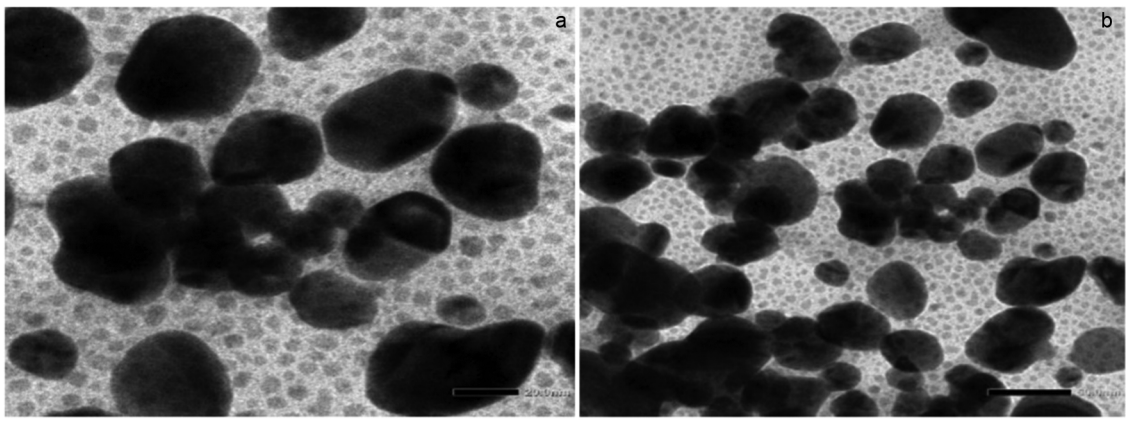

Fig. 8 — TEM photograph of the synthesised AgNPs from stems *R. sceleratus*.

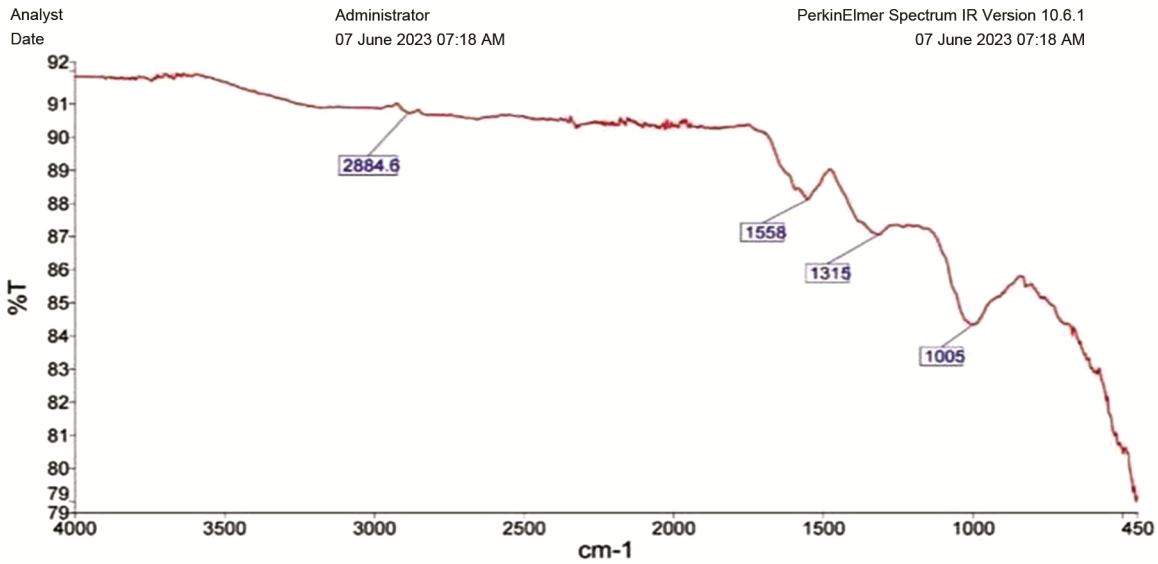

Fig. 9 — FTIR spectra of green-synthesised silver nanoparticles from leaves of *R. sceleratus*.

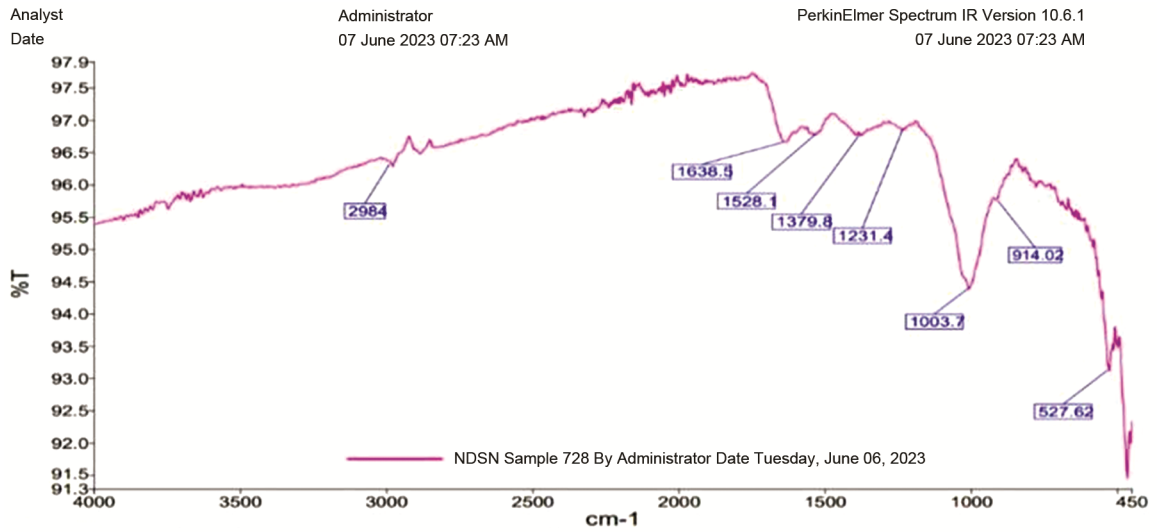

Fig. 10 — FTIR spectra of the silver nanoparticles from the stem of *R. scelereteus*.

Table 1 — FTIR absorption spectra and their probable functional group of AgNPs of leaves and stem of *R. sceleratus*

Plant parts	Absorbance Bands	Probable functional group
Fresh leaf	2884.6 cm^{-1}	alkenes, C-H stretch
	1558 cm^{-1}	nitro compound, N=O stretch
	1315 cm^{-1}	acyl group, C-O stretch
	1005 cm^{-1}	alkoxy group in alcohols, C-O
Dry stem	2984 cm^{-1}	alkanes, C–H stretching
	1638.5 cm^{-1}	amide, C=O stretching
	1528.1 cm^{-1}	Nitro compounds, N〓O
	1379.5 cm^{-1}	Nitro compounds, N〓O
	1231.4 cm^{-1}	acyl group, C—O
	1003.7 cm^{-1}	alkoxy in alcohols, C—O
	914.02 cm^{-1}	alkenes, CH groups

100% mortality of 2nd instar larvae after 72 h of exposure. The LC_{50} values and LC_{90} values of mortality rates of *Cx. quinquefasciatus* were prensented in Table 3. The lowest LC_{50} value observed was 0.33 mL and 0.45 mL of crude leaves and stems against the first and second instar larval stages after 72 hours of exposure, respectively. Calculated lowest LC_{90} values were 0.76 and 0.79 mL of crude leaves and stems against 1st and 3rd instar larvae, respectively, after 72 hours of contact. The regression equation depicts the positive correlation between the independent variable, i.e., concentration, and the dependent variable, i.e., mortality. The regression coefficient value was also found to be the nearest one. In the present study, 100% mortality was

Table 2 — Mean larval mortality of leaf and stem crude extract of *R. sceleratus* against 1st to 4th instar larvae of *Cx. quinquefasciatus*

Larval instar	Concentration (mL)	Mean mortality(%)±SE 24 hours		Mean mortality (%)±SE 48 hours		Mean mortality(%)± SE 72 hours	
		Leaf	Stem	Leaf	Stem	Leaf	Stem
1st	0.5	46.6±1.20	42.0±1.15	50.0±1.00	45.33±0.88	60.0±1.00	55.67±1.20
	0.6	66.6±2.33	61.67±0.88	73.3±1.66	68.67±0.88	80.0±0.00	75.33±0.88
	0.7	70±1.15	66.33±1.45	76.6±0.88	71.00±0.58	83.0±1.20	81.67±1.17
	0.8	80±1.00	75.00±0.58	83.6±0.88	79.00±1.15	93.3±0.33	88.00± 0.58
	0.9	83.3±0.66	88.67±0.88	90.0±0.00	86.67±1.76	96.6±0.33	91.00±1.00
	1.0	100±0.00	93.00±0.58	100±0.00	96.33±1.45	100±0.00	98.00±1.15
	Control	00±00	00±00	00±00	00±00	00±00	00±00
2nd	0.5	56.6±0.33	51.0±0.58	63.3±0.33	59.0±1.15	70.0±0.57	62.0±0.58
	0.6	63.3±1.33	58.67±0.88	66.6±1.66	61.33±0.88	76.6±1.20	69.67±1.45
	0.7	66.6±1.20	62.33±1.20	73.3±1.45	68.67±0.88	80.0±1.15	75.67±0.88
	0.8	76.6±2.18	72.0±1.15	80±1.15	76.0±1.15	86.6±1.15	81.33±0.88
	0.9	80.0±1.15	77.0±0.58	83.3±0.88	85.0±0.58	90.0±0.57	85.33±0.88
	1	93.3±0.66	88.67±0.88	93.3±0.66	89.0±1.15	100±0.00	100.0±0.00
	Control	00±00	00±00	00±00	00±00	00±00	00±00
3rd	0.5	40.0±0.57	35.67±0.88	60.0±1.00	56.0±1.15	66.6±1.20	62.33±1.20
	0.6	60.0±1.52	55.0±0.58	66.6±1.20	61.0±0.58	73.3±0.88	68.33±0.88
	0.7	73.3±0.33	68.33±0.88	76.6±0.33	71.67±0.88	83.3±0.33	78.67±0.88
	0.8	83.3±0.66	79.0±1.15	90.3±0.33	85.33±0.88	96.3±0.33	91.67±0.88
	0.9	90.0±0.33	86.0±0.58	93.3±0.33	89.0±0.58	93.3±0.33	94.33±0.58
	1	93.3±0.33	90.0±0.58	96.6±0.33	92.0±1.15	99.6±0.33	99.0±0.58
	Control	00±00	00±00	00±00	00±00	00±00	00±00
4th	0.5	23.3±0.88	18.33±0.88	26.6±1.20	21.67±1.20	36.6±1.20	32.0±1.15
	0.6	33.3±0.33	29.33±1.45	40.0±0.57	35.33±0.88	50.0±1.00	45.0 ±0.58
	0.7	43.3±0.66	38.67±0.88	46.3±0.33	41.67±0.88	56.6±0.66	51.33±0.88
	0.8	46.3±0.33	41.0±0.58	50.0±1.33	46.0±1.15	60.0±1.52	55.33±0.88
	0.9	50.0±1.52	47.33±0.88	56.6±1.85	51.0±0.58	70.0±1.52	67.33±0.88
	1	66.6±1.20	62.0±1.15	80.0±1.00	75.67±0.88	93.3±0.33	88.0±0.58
	Control	00±00	00±00	00±00	00±00	00±00	00±00

(Mean of experiments, SE= Standard Error)

Table 3 — Probit analysis and regression analysis of mortality rates of *Cx. quinquefasciatus* from crude extract from mature leaves and stem of *R. sceleratus*

Instar	Hours of exposure	LC_{50} (ml)		LC_{90}		Regression equation		R value	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1st instar	24 h	0.52	0.55	0.87	0.98	Y= 9.33x+ 0.67	Y= 98.48x+ 2.74	0.77	0.98
	48 h	0.44	0.52	0.90	0.94	Y=6.76x+ 2.87	Y= 90.57x+ 6.57	0.63	0.96
	72 h	0.33	0.46	0.76	0.82	Y= 4.95x+ 4.95	Y= 75.71x+ 24.83	0.52	0.95
2nd instar	24 h	0.48	0.50	1.06	1.02	Y= 6.05x+ 1.30	Y= 90.01x+ 7.19	0.66	0.99
	48 h	0.49	0.47	1.09	1.13	Y= 7.23x+ 1.79	Y= 65.24x+ 24.33	0.88	0.99
	72 h	0.40	0.45	0.88	0.93	Y= 5.81x+ 3.80	Y= 67.89x+ 27.91	0.66	0.98
3rd instar	24 h	0.49	0.58	0.97	0.98	Y= 8.38x+ 1.16	Y= 107.23x+ 11.43	0.64	0.97
	48 h	0.44	0.49	0.86	0.95	Y= 6.95x+ 2.84	Y= 99.33x+ 16.33	0.68	0.98
	72 h	0.37	0.47	0.84	0.79	Y= 4.76x+ 4.87	Y= 78.38x+ 23.59	0.56	0.98
4th instar	24 h	0.79	0.88	1.67	1.99	Y= 9.14x+ 2.41	Y=78.48x+19.42	0.81	0.98
	48 h	0.67	0.79	1.37	1.63	Y= 9.33x+ 1.44	Y= 91.81x+ 23.63	0.76	0.95
	72 h	0.57	0.67	1.08	1.30	Y= 9.34x+ 0.35	Y= 100.28x+ 18.71	0.75	0.97

Fig. 11 — Microscopic view, a) Normal untreated larva of *Cx. quinquefasciatus*; b) dead larvae treated with crude leaf extract of *R. scleratus*; and c) dead larvae treated with crude stem extract of *R. scleratus*.

achieved at a very low concentration, which showed the excellent efficacy of both plant parts compared to other similar studies by Karmakar *et al.*[37], Saha *et al.*[38], Gope and Rawani[39], and Rawani *et al.*[40] where different concentrations of crude extracts of plant parts such as stems and leaves were tested against different instars of *Cx. quinquefasciatus*. During the treatment, general behaviour changes were shown in the tested mosquito larvae. Larvae become inactive within a few hours of treatment. The compound microscopy study (Fig. 11) of treated (leaf extract) 2nd instar of the *Cx. quinquefasciatus* showed that the cuticle was completely disrupted and the digestive system was also destroyed, which indicates it acts as a stomach poison (Fig. 11b). Fig. 11c showed that, in stem crude extract treated 2nd instar larvae, the cuticle is completely disrupted, which proves its mechanism of action as a contact poison. Fig. 11a showed the normal untreated 2nd instar larvae of *Cx. quinquefasciatus*. These findings were supported by the earlier reports of Karmakar *et al.*[40] and Gope *et al.*[30], as *R. scleratus* showed good larval mortality in each tested concentration comparable to the former reported plant parts, it can be said that both plant parts of *R. scleratus* have potent mosquito larvicidal properties and could be selected for further studies about their effect on the growth and development of mosquitoes.

Mosquito larvicidal activity of synthesised silver nanoparticles (AgNPs) of leaves and stem of *R. scleratus*

Table 4 shows the larvicidal activity of synthesised AgNps of leaves and stem of *R. scleratus* against 3rd instar larvae of *Cx. quinquefasciatus* in different concentrations ranges from 1 ppm to 5 ppm (Table 4). 80 and 60% mortality of 3rd instar larvae were observed at 1 ppm concentration of leaf and stem AgNPs of *R. scleratus*, whereas 96.6 and 100% mortality occurred at 5 ppm concentration of leaves and stem AgNPs. The lowest LC_{50} value observed was 0.10 ppm of AgNPs of leaves of *R. scleratus* after 24 hours of exposure (Table 5). Here, like crude extract, the regression equation reveals the positive relation between concentration (independent variable) and mortality (dependent variable). The calculated lowest LC_{50} values, 0.10 and 0.23 ppm of synthesised AgNPs of both plant parts, revealed good efficacy against the 3rd larval stage of *Cx. quinquefasciatus* compared to a study by Sutthanont *et al.*[41], who reported LC_{50} and LC_{90} values of silver nanoparticles synthesised from leaves of *Curcuma zedoaria* were 36.32 and 85.11 ppm, respectively, against 3rd instar larvae of *Culex quinquefasciatus*. Gope *et al.*[30] found LC_{50} values of 2.90 ppm and 1.97 ppm in leaf and fruit AgNPs of *Phyllanthus acidus* against 3rd instar

Table 4 — Larvicidal activity of synthesised leaf and stem AgNPs of *R. scleratus* against 3rd instar larvae of *Culex quinquefasciatus*

Larval instar	Concentration (ppm)	Mean mortality (%) + SE (24 hours)	
		Leaf	Stem
3rd	1	80±0.57	60.00±0.00
	2	83.3±0.88	66.6±0.88
	3	86.6±0.33	73.3±2.18
	4	93.3±0.33	76.6±0.88
	5	96.6±0.33	100.00±0.00
	Control	00±00	00±00

Table 5 — Probit analysis and regression analysis of mortality rates of *Culex quinquefasciatus* from synthesised silver nanoparticles from leaves and stem of *R. scleratus*

Plant parts	Hours of exposure	LC_{50} (ppm)	LC_{90}	Regression equation	R value
Leaves	24	0.10	3.24	Y= 0.30x+ 7.90	0.90
Stem	24	0.23	20.48	Y= 0.53X+5.93	0.86

larvae of *Cx. quinquefasciatus.* The tested larvae of *Cx. quinquefasciatus* were also observed under a 10X lens of a compound microscope. Microscopic studies (Fig. 12) revealed that 3rd instar of *Cx. quinquefasciatus* treated with the synthesised AgNPs of the stem of *R. sceleratus* died due to clogging of the spiracles as well, and AgNPs were found to be deposited in the gut of the mosquito (Fig. 12b), whereas in the leaf AgNps treated larvae showed that the delicate larvae died due to the penetration of AgNPs through the membrane of the larva (Fig. 12a). These observations were further supported by the view that AgNPs may bind to the epithelial membrane of the midgut, where the enzymes of midgut become inactive and produce peroxide effect, which causes the death of the mosquito larvae[42,43]. Thus, the synthesised AgNPs of both plant parts *of Ranunculus sceleratus* showed the greatest mortality in a very minimal concentration, proving its potential as an excellent vector mosquito larvicidal agent.

Phytochemical analysis

Qualitative phytochemical analysis of the leaf and stem of *R. sceleratus* revealed the presence of only seven phytochemicals, i.e. alkaloids, anthraquinones, flavonoids, phlobatanin, terpenoids, tannins, and proteins in leaf extracts. In the stem extract, only nine phytochemicals were present; these were carbohydrates, phenolic compounds, glycosides, phlobatannin, tannin, saponin, steroids, proteins, and terpenoids (Table 6). The result indicates that both parts of the plant offer a much broader array of phytochemicals. Phytochemical analysis of plants was needed to discover and extend novel therapeutic agents with improved efficiency. The therapeutic properties of plants depend on the substances that plants store as secondary metabolites. Plant extract has a variety of physiological effects on pests, such as repellent, larvicidal, pupicidal, adulticidal, and ovicidal effects[16,44-46]. This might be because different phytochemicals in plants interact with one another to create these kinds of reactions. Shaalan *et al.*[47] reported several secondary metabolites, such as phenolics, alkaloids, phytosteroids, flavonoids, tannins, glycosides, and terpenoids, from a wide range of plants that were reported previously for their pesticide or insecticide properties. These phytochemicals also play a certain role as a capping agent of synthesised AgNPs[11]. In the present study, phlobatannin, terpenoids, tannins, and proteins are present in both plant parts of *R. sceleratus.*

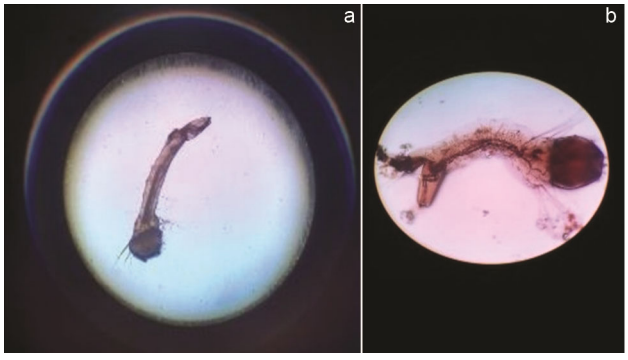

Fig. 12 — Microscopic view of dead larvae (3rd instar) of *Cx. quinquefasciatus*, a) dead larvae treated with synthesised AgNPs of the leaf of *R. sceleratus*; and b) dead larvae treated with synthesised AgNPs of the stem of *R. sceleratus*.

Antioxidant analysis

The 80 µL concentration methanol extracts of both plant parts of *R. sceleratus* were found to be the most efficacious DPPH radical scavengers. Investigated seven methanol extracts inhibited DPPH absorption at the percentage respectively (20 µL- 19.73%, 30 µL - 42.05%, 40 µL - 48.05 %, 50 µL- 67.10 %, 60 µL- 71.06 %, 70 µL 75.00 %, 80 µL-90.13 %). While stem of *R. sceleratus* inhibited DPPH absorption at the percentage respectively (20 µL - 46.05%, 30 µL - 53.28%, 40 µL - 62.34%, 50 µL- 69.73%, 60 µL- 71.05 %, 70 µL 75.26%, 80 µL-80.13%). The resulting solution always has some yellowish colour once the reaction is finished. Therefore, its absorption inhibition in comparison to a colourless methanol solution can't reach 100%, so these percentages can be thought of as a partial absorption inhibition of DPPH. Out of the experimental plant extracts at 80 µL concentration of methanol extract, both the plant parts

Table 6 — Result of qualitative phytochemicals analysis of crude leaf and stem extract of *R. sceleratus*

Phytochemicals	*R. sceleratus*	
	Leaves	Stem
Alkaloids	++	--
Anthraquinone	++	--
Glycosides	--	++
Flavonoids	++	--
phenol	--	++
Phlobatannin	++	++
Protein	++	++
Saponin	--	++
Steroid	--	++
Tannin	++	++
Terpenoid	++	++
Carbohydrate	--	++

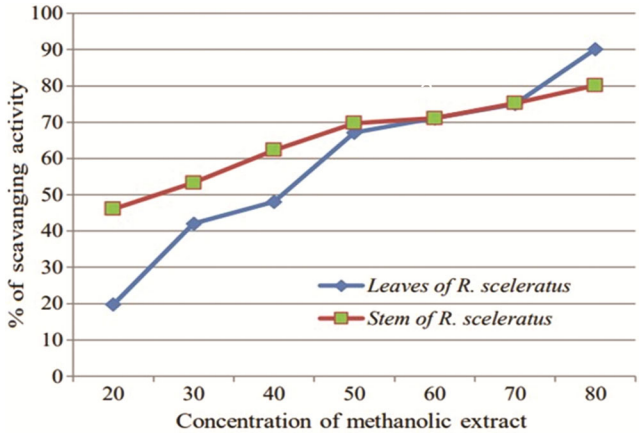

Fig 13 — Comparative antioxidant activity from the methanolic extract of leaves and stem of *R. sceleratus* at various concentrations.

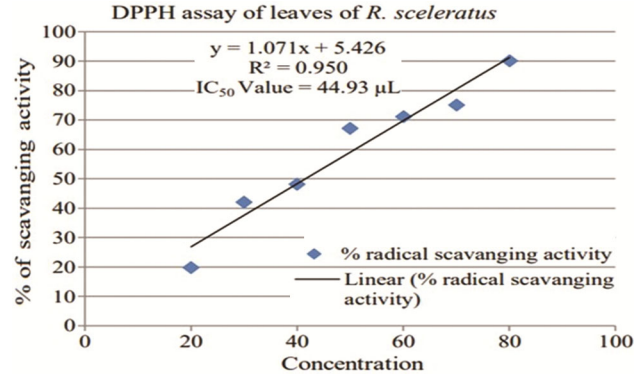

Fig. 14 — Scatter plot of DPPH scavenging assay of leaves of *R. sceleratus* showing IC_{50} value.

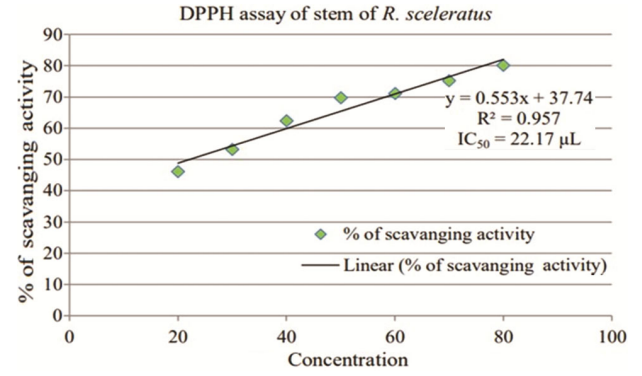

Fig. 15 — Scatter plot of DPPH scavenging assay of the stem of *R. sceleratus* showing IC_{50} value.

have the higher inhibited DPPH absorption, i.e. 90.13% in leaves and 80.13% (Fig. 13) in the stem of *R. sceleratus* with IC_{50} values of 44.93 µL (Fig. 14) and 22.17 µL (Fig. 15) respectively.

Antioxidant qualities of Ranunculaceae are generally well known. Many active substances that have been proven to possess antioxidant action have been identified from these species. The antioxidant-rich plants *Ranunculus acris*, *Ranunculus platanifolius*, *Ranunculus repens*, and *Ranunculus serpents* were shown to contain high quantities of hexadecanoic acid, phytol, octadecadienoic, and octadecatrienoic acids[48]. In this study, 80 µL methanol extracts of both plant parts of *R. sceleratus* were the strongest radical scavengers among the other concentrations screened. So, it can be said that both plant parts of *R. sceleratus* have promising free radical scavenging properties and could have application possibilities.

Conclusion

The current study offers an affordable, safe, and environmentally acceptable way to synthesise silver nanoparticles. The present study evaluated the great potentiality of crude extracts and synthesised AgNPs from the leaf and stem of *R. sceleratus* as larvicidal agents against *Cx. quinquefasciatus.* In summary, synthesised silver nanoparticles derived from *R. sceleratus* leaves and stem extract showed promising results for mosquito larvicidal activity against *Cx. quinquefasciatus* larvae. However, more thorough research is required to assess its effects in the field before endorsing it as a commercial mosquito control agent. Additionally, the present study showed that the plant has good free radical scavenging activity. Phytochemicals present in both plant parts play a role in bioreduction and capping agents for synthesised AgNPs. In conclusion, it may be said that leaves and stems of *R. sceleratus* can be excellent choices as mosquitocidal agents, and they can also be further tested for identifying active components.

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Conflict of interest

It is asserted that the authors have no conflicting interests.

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