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Characterization and Evaluation of *Adhatoda vasica* and its green synthesized Nanoparticles for its efficacy in *Culex quinquefasciatus*

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Abstract

Adhatoda vasica, commonly referred to as Vasa or Vasaka, is a significant therapeutic herb in traditional ayurvedic practice. This study examines the therapeutic potential of leaves from *A.vasica* in treating Mosquito- borne diseases. The aqueous leaf extracts of *A.vasica* were employed as a reducing and capping agent for the nanoparticle synthesis. The synthesized nanoparticles were confirmed by using various characterization techniques such as Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), UV-Visible Absorbance Spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR). The analysis of plant- derived compounds in the leaf extracts using methanol, ethanol, chloroform and water showed the existence of alkaloids, terpenoids, phenols, tannins and glycosides. The leaf extracts of *A.vasica* and their nanoparticles demonstrated significant antibacterial, antioxidant and larvicidal efficacy. They also demonstrated efficient antibacterial efficacy against *S. epidermidis*, a bacterium known to cause secondary infections, in the case of Lymphatic Filariasis. The leaf extract and its AgNP's act as a good Radical Scavenging Agent. The synthesized silver nanoparticles exhibited a better larvicidal effect ($LC_{50}=34.38\text{ppm}$) when compared to the leaf extracts. Subsequent studies will be carried out to analyze the efficacy of *A.vasica* and its nanomaterial through evaluating the macrofilaricidal activity and conducting *in vitro* analysis.

Keywords: *Adhatoda vasica*, Anti-bacterial, Anti-oxidant, *Culex quinquefasciatus*, Larvicidal Activity, Phytochemical Screening, Silver Nanoparticles.

Introduction

According to [1], mosquito-borne diseases affect more than 700 million people globally and present across the world but, remain a significant cause of morbidity and mortality, particularly in tropical and subtropical regions, with substantial economic consequences such as loss of productivity and healthcare burden [2]. Among these, dengue, malaria, chikungunya, yellow fever, lymphatic filariasis, and Japanese Encephalitis (JE) are of major concern. JE is a vector-borne viral disease caused by Japanese Encephalitis Virus (JEV), a member of the *Flaviviridae* family. It is transmitted primarily by *Culex* mosquitoes, especially *Culex tritaeniorhynchus*, which breed in rice fields and irrigated landscapes and secondary species are *Cx. quinquefasciatus*, *Cx. fuscocephala* and *Cx. vishnui* [3-4]. The transmission cycle involves pigs and wild birds as virus amplifying hosts, and mosquitoes as vectors to carrying the virus, while humans are incidental, dead-end hosts [5]. JE remains one of the most important causes of viral encephalitis in Asia, with an estimated 100,000 clinical cases annually [2]. India, Nepal, China, and Southeast Asia continue to report recurrent outbreaks of JE, often with high case fatality rates 30% and high percentage of survivors who got free from JE are with severe neurological/ neuropsychiatric sequela [6-7]. Recent reports suggest that JE infection shift towards adult and Children are disproportionately affected in some regions [8]. Clinical symptoms of JE are characterized by fever, headache, seizures, altered mental status, and in severe cases, progression to coma and death [9]. Currently there is no specific antiviral treatment, but management is supportive, and vaccination remains the most effective preventive measure [10-11]. In rural settings, where vaccination rates are low, vector control and management, such as reducing *Culex* mosquito populations through larviciding and environmental management, continues to play an important role in integrated JE prevention strategies [12].

A. vasica is categorized within the Acanthaceae family. It is an up-right, ground-dwelling and everlasting perennial bush [13]. They are renowned for its efficacy in addressing respiratory ailments. The leaves are particularly recognized for their broncho-dilator property. The broad spectrum of medicinal uses of *A. vasica* is considered to its abundant concentration of alkaloids [14].

Nanotechnology is pivotal in addressing various diseases, offering numerous advantages such as eco-friendliness and lower toxicity. Unlike traditional methods, it doesn't rely on external capping or reducing agents, as the plant itself serves this purpose. Additionally, nanoparticles exhibit superior compatibility with proteins, making them a preferred option for therapeutic applications [15]. Earlier studies have also noted that nanoparticles derived from plants are utilized in managing issues such as mosquito-borne diseases [16].

Materials and Methods

Collection of Plant Material

The Leaves of *A. vasica* were collected from the Bio-Diversity Garden at Central University of Tamilnadu, Thiruvavur and authenticated by Prof .A. Ramesh Kumar, Professor & Head, Dept of Horticulture, Central University of Tamilnad uas shown in Figure 1.



Figure 1. *Adhatoda vasica*

Preparation of Leaf Extract

The fresh and healthy leaves of *A.vasica* were thoroughly washed with tap water and left to shade for dry at room temperature about 10-15 days. The dried leaves were ground into a fine powder using an electric blender. Leaf extracts were prepared by dissolving 5g of the dried leaf powder in 60 ml (12:1) of various solvents, including methanol, ethanol, chloroform, and water, using the maceration process. The mixture was left undisturbed for three days with frequent heating to enhance extraction. After 72 hours, the extract was filtered through Whatman No.1 filter paper and stored in a refrigerator for future analysis.

Qualitative Phytochemical Screening:

The leaf extract of *A.vasica* for four solvents (methanol, aqueous, ethanol and chloroform) were evaluated by qualitative determination of bioactive compounds as per the standard method described by [17].

1. Test for Alkaloids:

i. Wagner's Test

The extracts were mixed with a few drops of Wagner's reagent (Iodine-1.27g, KI-2g were dissolved in 100ml of distilled water). The formation of a reddish-brown precipitate indicates the presence of alkaloids.

ii. Mayer's Test

The extracts were mixed with few drops of Mayer's reagent (Mercuric chloride-1.36g, Potassium iodide-5g were dissolved in 100ml of distilled water). A white or green colour precipitate indicates the presence of alkaloids.

2. Test for Terpenoids

Salkowski's Test:

The crude extract was separately shaken with 2ml of chloroform and followed by the addition of concentrated H_2SO_4 , along the sides of the test tube. A reddish-brown color ring at the interface indicates the presence of terpenoids.

3. Test for Steroids

The crude extracts were dissolved in chloroform and concentrated H_2SO_4 (1ml) is added into the test tube by wall side. The color of the upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

4. Test for Phenols

Ferric Chloride Test:

To the 2 ml of the extract, add few drops of 5% Ferric chloride (FeCl_3) solution. Formation of deep blue-black color indicates the presence of phenols.

5. Test for Flavonoids:

Aluminum Chloride Test:

1ml of 1% AlCl_3 solution was added to the extract, shaken and observed for a yellow coloration. A few ml of dilute NaOH and HCL was added, that yellow colored solution turned into colorless, indicating the presence of flavonoids.

6. Test for Tannins

Ferric Chloride Test

Extract was mixed with few ml of 45% ethanol solution for 3 mins, followed by the addition of water. FeCl_3 drops changed the color of the solution to greenish black, indicating the presence of tannins.

7. Test for Saponins

1 ml of the crude extract was diluted with 4 ml of distilled water and shaken in a graduated cylinder for 15 mins. Formation of a layer of foam indicates the presence of saponins.

8. Test for Glycosides

Extract was mixed with few ml of dil. H_2SO_4 and 20% KOH solution. A mixture of 3 ml of Fehling's solution A & B was added and subjected to boiling in water bath for 5 mins. Development of brick red colored precipitate indicates the presence of glycosides.

Green synthesis of silver nanoparticles using *A.vasical* leaves extract

The aqueous extract of *A.vasica* was prepared by adding 5 grams of leaf powder was subjected to maceration with 60 ml of distilled water for 3-4 days. The extract was then filtered through Whatman No. 1 filter paper, and the resulting filtrate was used for the green synthesis of silver nanoparticles.

For the nanoparticle synthesis, 20 ml of 1mM silver nitrate (AgNO_3) solution was prepared, to which 0.2 ml of the prepared aqueous leaf extract was added. The mixture was stirred at 60°C for 30 minutes, during which a visible color change from light yellow to reddish brown confirmed the formation of silver nanoparticles.

Characterization of silver nanoparticles

The synthesized silver nanoparticles were characterized by various characterization techniques like UV-Visible Absorbance Spectroscopy, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR) and Dynamic Light Scattering (DLS).

Uv-Visible Absorbance Spectroscopy

The synthesized silver nanoparticles (AgNPs) were characterized using UV-Visible spectroscopy, following established methods [18]. Confirmation of the AgNPs was achieved by measuring the wavelength of the reaction mixture was recorded with a resolution of 1 nm over the wavelength range of 300–600 nm, using

a 2 mL quartz cuvette with a 1 cm path length. A characteristic surface plasmon resonance (SPR) peak indicated the successful synthesis of AgNPs.

Scanning Electron Microscopy (SEM) analysis

The dried pellet was examined using a SEM. SEM grids were coated with carbon to create thin films of the sample. A small amount of the sample was dropped onto the grid, excess solution was gently removed with blotting paper, and the film was allowed to dry prior to imaging [19].

Fourier Transform Infrared (FT-IR) Spectroscopy

Functional groups responsible for the reduction and stabilization of extract-synthesized AgNPs were identified using a Nicolet 6700 FTIR spectrometer. The spectra were recorded in the range of 400–4000 cm^{-1} , where different molecular bonds exhibit characteristic absorption peaks. The frequency and intensity of these absorptions provide information on the band structures and molecular geometry.

Dynamic Light Scattering (DLS)

DLS mainly measures the light scattered from a laser that travels through a colloid using Rayleigh scattering from the suspended nanoparticles. With this method, narrow particle size distributions can be determined, especially in the 2-500 nm range. Using DLS, it is possible to measure the average diameter of nanoparticles dispersed throughout liquids without causing any damage. Although it has the distinct advantage of examining several particles at once, there are certain limits that are specific to certain samples [20].

Biological assays the using aqueous leaf extracts of *A.vasica* and its nanoparticles

The therapeutic potential of *A.vasica* was evaluated through various biological assays, including anti-bacterial, antioxidant, and larvicidal activities. Proceeding to therapeutic approaches, the biological assays were carried out for the aqueous leaf extract of *A.vasica* and extract synthesized nanoparticle.

Evaluation of anti-bacterial activity

The anti-bacterial activity of the leaves extracts synthesized silver nanoparticles against *E.coli*, *Stahylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella typhi* was evaluated by well diffusion method with ampicillin (1mg\ml) as standard. The stock culture of each bacteria used was sub cultured at 37°C overnight.

Agar-well diffusion method

The medium used for this study was Muller Hinton agar medium. The medium was sterilized by autoclaving at 121°C. About 30ml of the molten nutrient agar medium inoculated with respective strains of bacterium was transferred aseptically into each sterilized plate. In each place, five wells of 5 mm diameter were made using a sterile borer. Different concentrations of test samples (plant extract- 25 mg\ml, 50 mg\ml, 75 mg\ml, 100 mg\ml & nanoparticles- 25 $\mu\text{g}\backslash\text{ml}$, 50 $\mu\text{g}\backslash\text{ml}$, 75 $\mu\text{g}\backslash\text{ml}$ and 100 $\mu\text{g}\backslash\text{ml}$) were injected into the well. The plates were maintained at room temperature for 2hr to allow the diffusion of the solutions in to the

medium. The petri-dishes used for the antibacterial screen were incubated at 37°C for 24 hr. The diameter of Zone of Inhibition (ZOI) surrounding each of the wells was recorded.

Evaluation of Anti-oxidant activity by DPPH assay

The evaluation of the anti-oxidant potential of leaf extracts and its nanoparticles to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals were assessed by standard colorimetric method. The leaves extracts and their nanoparticles concentration range from 20–100 µg/ml, and a determined amount of 0.2 mM of DPPH was added to the methanol solution for preparing DPPH working concentration. DPPH is a stable free radical, which changes its color from violet to yellow upon reduction by the process of electron donation after 30 mins of incubation. Ascorbic acid was used as the standard [21] and the absorbance was measured at 517 nm and converted into percentage Radical Scavenging Activity as follows.

$$\% \text{ Radical Scavenging Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of Control}} \times 100$$

Determination of Larvicidal Activity

Culex quinquefasciatus larvae were randomly collected from Thiruvavur district, Tamil Nadu. The field collected samples were maintained in the Vector Biology Research Laboratory (VBRL), Department of Biotechnology, CUTN, Thiruvavur. To determine the activity range for aqueous leaf extract, larvae were exposed to a range of test concentrations (1 ppm-100 ppm/100 ml) (1, 5, 10, 50, 100 ppm/100 ml and for extract-synthesized AgNPs, larvae were exposed to a range of concentrations (0.1ppm-10 ppm/100 ml) (0.1, 0.5, 1, 5, 10 ppm/100 ml). A control with no test material was maintained. The late 3rd instars larvae were selected for the assay. Food (dog biscuits and yeast powder in a 3:1 ratio) was given to the larvae. Using a strainer and dropper, 10 larvae were transferred to a test vessel containing 100 ml of water and the corresponding test solution for each trial. The test was done with triplicates with two controls. During this experiment, 14:10 light dark conditions was maintained with 25±2°C temperature for 24 hrs. Every 10 mins the knockdown was noted and damaged or unhealthy larvae were removed. To prevent any outside interference, a net was placed over the container. After 24 hours, the larvae with no movement even after slight touch with a glass rod were considered as dead. Number of dead larvae was recorded, and the mortality percentage was determined by using Abott's formula.

Statistical Analysis

All tests were conducted with triplicate, and the findings were reported with statistical analysis presented as the mean ± standard deviation. Statistical analysis was performed using a single-factor ANOVA (Analysis of Variance) test. Larvicidal activity was assessed by determining the mortality percentage using Abbot's formula. The LC₅₀ value was calculated through probit analysis. Results with a p-value ≤ 0.05 were considered statistically significant.

Results

Qualitative phytochemical screening

The phytochemical screening of the various extracts of *A. vasica* showed that the leaves are rich in alkaloids, flavonoids, terpenoids, and tannins. The presence of various phytoconstituents are confirmed by various phytochemical tests which are evident in colours using the plant extracts was observed and as shown in Figure 2-5 and Table 1.

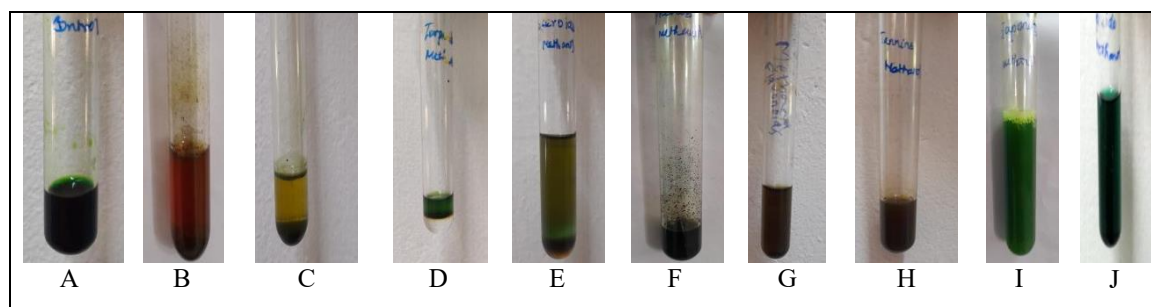


Figure 2: Phytochemical Analysis of Methanolic Extracts of *A. Vasica*

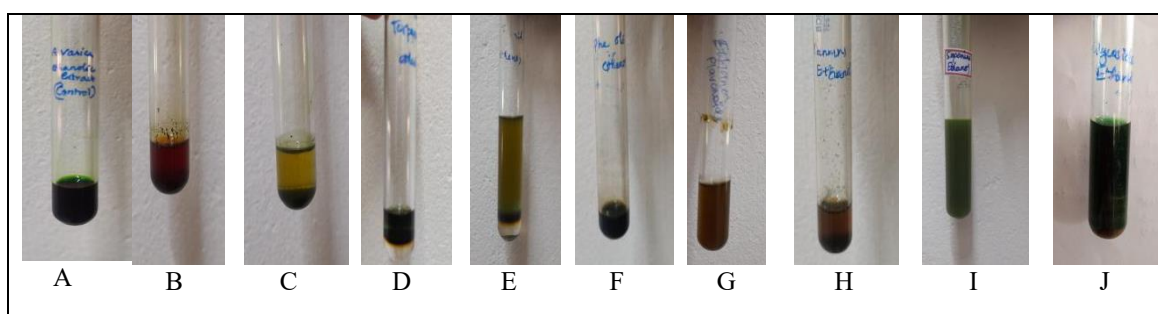


Figure 3: Phytochemical Analysis of Ethanolic Extracts of *A. Vasica*

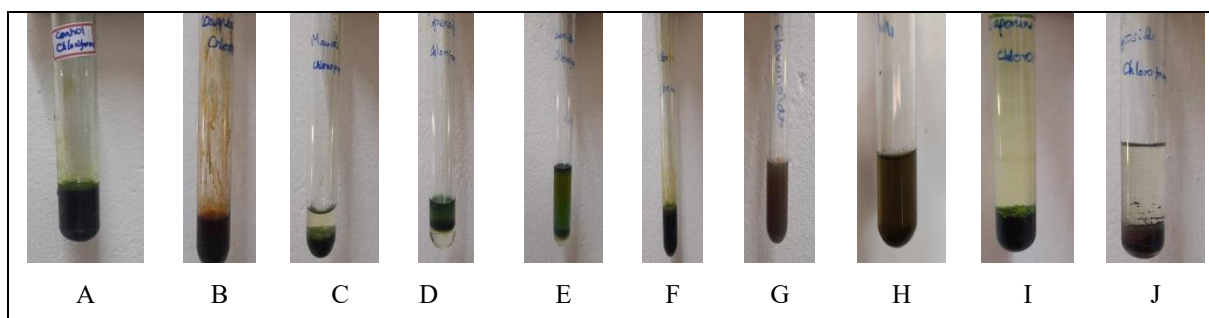


Figure 4: Phytochemical Analysis of Chloroform Extracts of *A. Vasica*

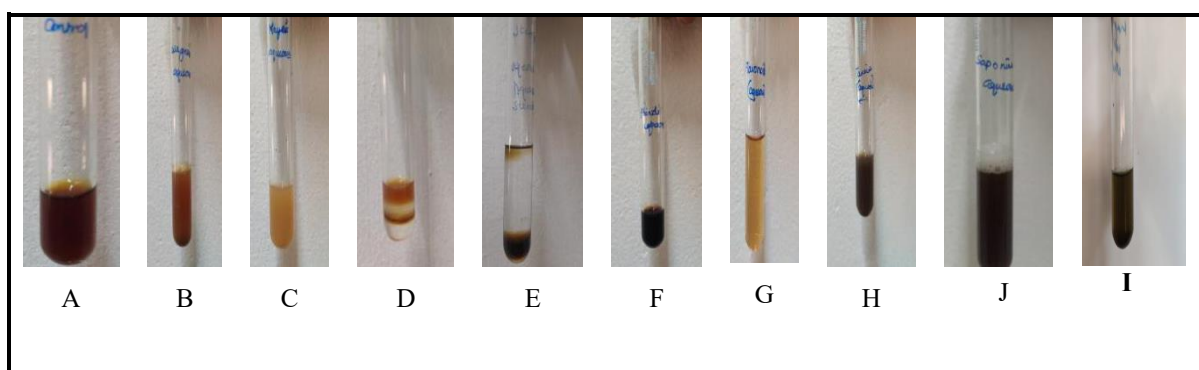


Figure 5: Phytochemical Analysis Of Aqueous Extracts Of *A. Vasica*

Table 1: Qualitative phytochemical screening of *A.vasica* leaf extracts (+positive. - negative).

Phytochemical constituents	Methanolic extract	Ethanollic extract	Chloroform extract	Aqueous extract
Wagner's test	+	+	—	+
Mayer's test	+	+	—	+
Terpenoids	+	+	—	+
Steroids	+	+	—	+
Phenols	+	+	+	+
Tannins	+	+	+	+
Flavonoids	—	—	—	+
Saponins	+	—	—	+
Glycosides	—	+	+	+

A-Control, B-Wagner's test for alkaloids, C-Mayer's test for alkaloids, D-Terpenoids, E-Steroids, F-Phenols, G-Flavonoids, H-Tannins, I-Saponins, J-Glycosides.

Aqueous leaf extract of *A.vasica* contains most of the phytoconstituents when compared to other extracts. So further the green synthesis of AgNP's, evaluation of anti-bacterial, anti-oxidant and larvicidal activities was carried out by using only the aqueous leaf extracts of *A.vasica*.

Extraction percentage yield of the leaf extract

The leaves of *A.vasica* was extracted by maceration with water and later evaporated by using rotary evaporator. The percentage yield was calculated using the following equation.

$$\text{Percentage of the yield} = \frac{(\text{Amount of the extract yield (g)})}{\text{Amount of the leaf powder (g)}} \times 100$$

The result was summarized in the Table 2.

Table:2 Percentage of yield of *A.vasica* aqueous leaf extracts

Amount of leaf powder (g)	Amount of extract yield (g)	Percentage of extract yield (%)
50	10	20

Green synthesis of AgNP's using *A.vasica* leaf extract

Green synthesis of silver nanoparticles was indicated by the virtual color change of reaction mixture from transparent to yellow after the addition of leaf extract, which indicates the green production of silver nanoparticles. Change in the colour from yellow to brown indicated the AgNP's synthesis after 30 minutes of incubation at 60°C with constant stirring as shown in Figure 6.

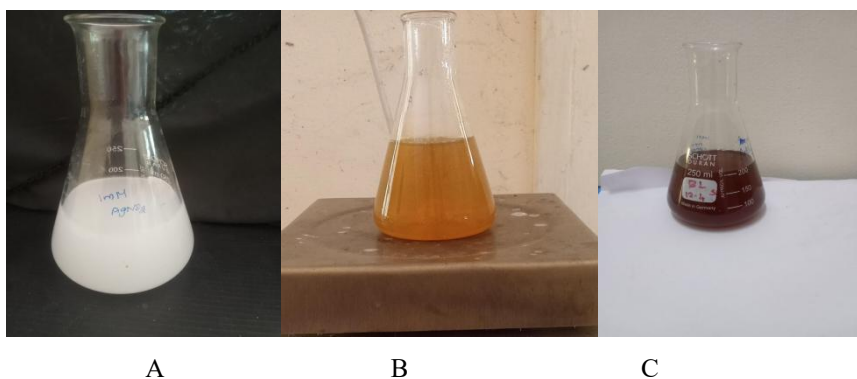


Figure 6: A- 1mM AgNO₃ solution –Transparent color, B- 1mM AgNO₃ solution+ Leaf extracts of *A.vasica* – Pale Yellow color, C- After 30 mins of incubation - Brown colour

Characterization of silver nanoparticles

The green synthesis of AgNP's from the aqueous leaf extracts of *A.vasica* was further validated by UV-visible absorbance spectroscopy within the wavelength between 250 nm to 500 nm. At precisely 420 nm, the highest wavelength was observed as shown in Figure 7.

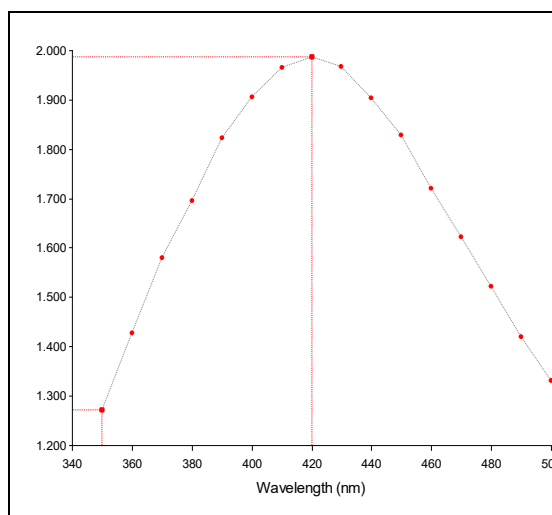


Figure 7: UV-Visible Absorbance Spectroscopy of synthesized AgNP's at 420nm

Scanning Electron Microscopy

The size and shape of the extract-synthesized AgNP's were validated by SEM technique as shown in Figure 8. It clearly shows that the synthesized nanoparticles were formed in resolution smaller in size. The image confirms that nanoparticles were in irregular sizes and shapes in 3 μ m.

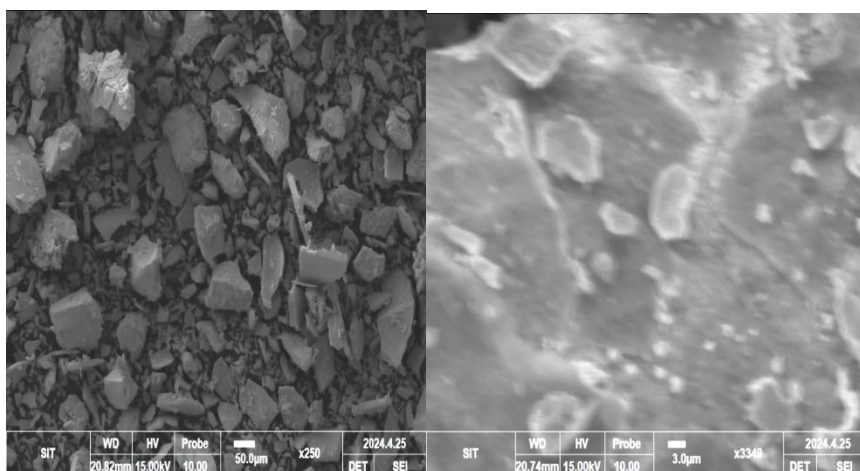


Figure 8 SEM analysis of synthesized AgNP's at 50 μ m and 3.0 μ m

Fourier Transform Infrared Spectroscopy

The FT-IR analysis was carried out to identify the possible functional groups responsible for capping and efficient stabilization of the synthesized AgNP's. The Figure 9 represents the recorded FT-IR spectra of the cell-free filtrate and the synthesized nanoparticles in the range between 400–4000 cm^{-1} . The absorption peaks for AgNP's were at 3415.93 cm^{-1} , 2854.04 cm^{-1} , and 2924.04 cm^{-1} were assigned to have O-H stretching, (alcohol), 2344.30 cm^{-1} showed O=C=O stretching (carbon-di-oxide), 2113.87 cm^{-1} showed C=C=O stretching (ketene), 1641.97 cm^{-1} showed C=O stretching (primary amide), 1462.23 cm^{-1} showed C-H bending (alkane), 1402.38 cm^{-1} and 1334.90 cm^{-1} showed O-H bending (phenol), 1243.86 cm^{-1} showed C-

N stretching (amine), 1085.97 cm^{-1} showed C-O stretching (primary alcohol), 780.02 cm^{-1} , 721.59 cm^{-1} and 517.23 cm^{-1} showed (C-cl, C-cl and C-Br) (halo compounds).

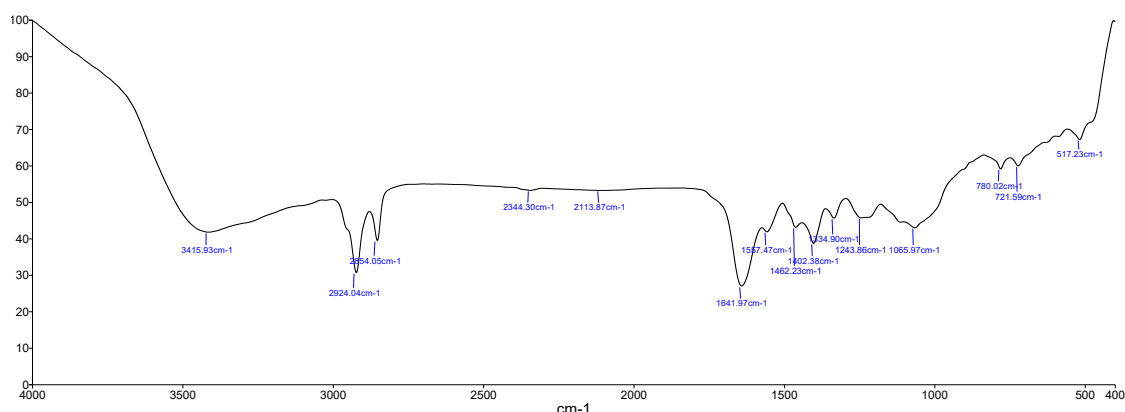


Figure 9 - FT-IR analysis of synthesized AgNP's

FT-IR analysis was carried out to identify the possible functional groups responsible for capping and efficient stabilization of the synthesized AgNP's. Figure 10 represents the recorded FT-IR spectra of the cell-free filtrate and the leaf extract of *A. vasica* in the range 400–4000 cm^{-1} range. The absorption peaks for aqueous leaf extract were at 3423.91 cm^{-1} , 2929.16 cm^{-1} , 2866.28 cm^{-1} showed O-H stretching (alcohol), 2307.75 cm^{-1} showed O=C=O stretching (CO_2), 2076.93 cm^{-1} showed N=C-S stretching (isothiocyanate), 1643.12 cm^{-1} showed C=O stretching (primary amide) , 1457.47 cm^{-1} showed C-H bending (alkane), 1417.66 cm^{-1} , 1386.03 cm^{-1} , 1336.80 cm^{-1} showed O-h bending (alcohol i.e phenols), 1111.85 cm^{-1} , C-O Stretching (secondary alcohol) , 1033.33 cm^{-1} , 1013.25 cm^{-1} , 1055.41 cm^{-1} showed C-F stretching (Fluoro compound) and 779.62 cm^{-1} and 650.88 cm^{-1} showed C-cl stretching (halo compound).

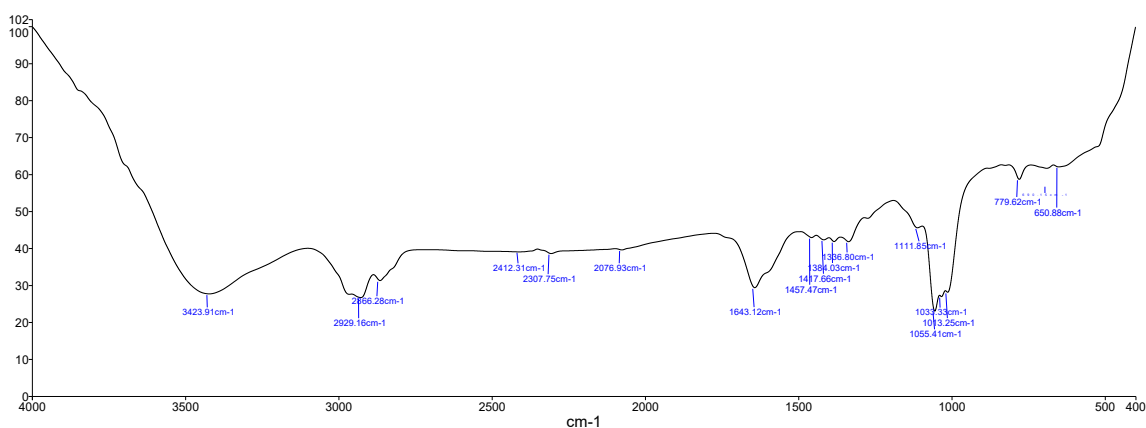


Figure 10: FT-IR analysis of aqueous leaf extracts of *A. vasica*

Dynamic Light Scattering

The DLS typically provides an intensity distribution graph, showing the intensity of scattered light as a function of particle size. The distribution result shows a single peak at 632 nm with a polydispersity index of 0.373 and average size of 283.7 nm. The results verify that silver ions were reduced to Silver (Ag) within the nanoscale dimensions, as shown in Figure 11.

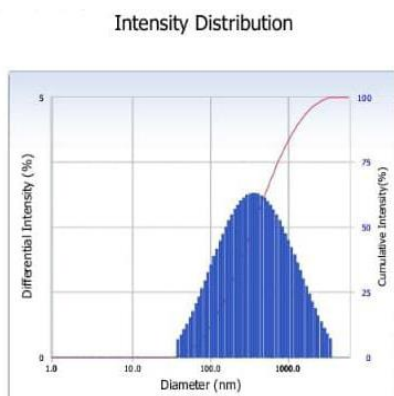
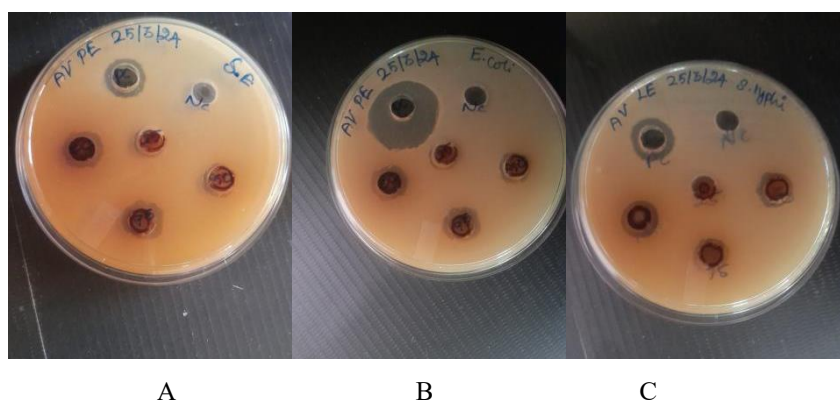


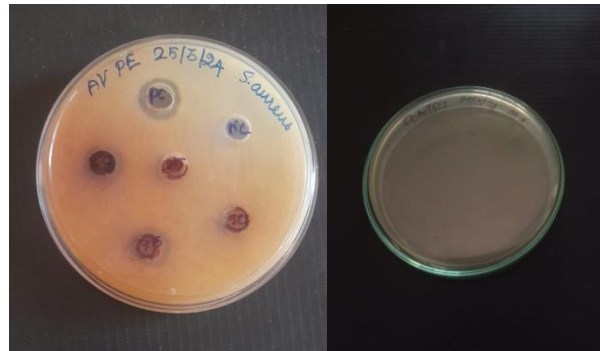
Figure 11: DLS analysis of silver Nanoparticles

Determination of biological efficacy of *A.vasica*:

Evaluation of Anti-bacterial activity

The biological potential of *A.vasica* and their nanoparticles was tested against both Gram-positive and Gram-negative bacterial strains like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi* and *E.coli*. A distinct zone of inhibition was seen around the well that contained varying concentrations of conventional antibiotics, crude aqueous extract, and its nanoparticle extract. The results were shown in Figure 12 -13 and Table 3-4

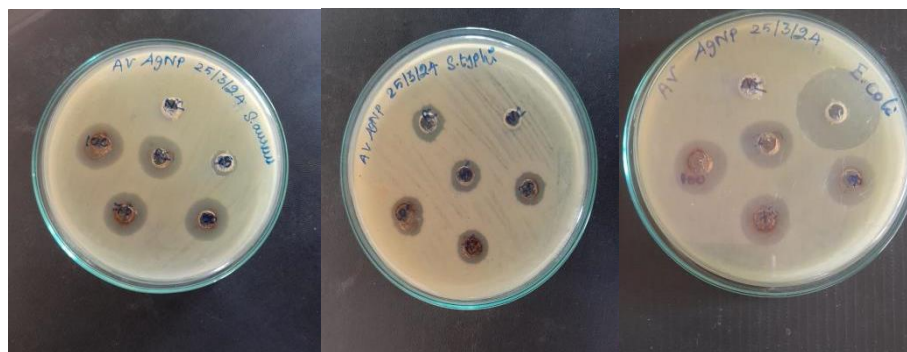




D

E

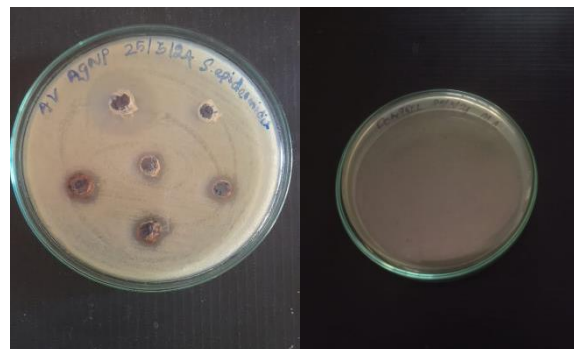
Figure 12: Antibacterial activity of *A.vasica* leaf extract of various concentrations 25, 50, 75,100 mg/ml against *Staphylococcus epidermidis*, B-Activity against *E.coli*, C-Activity against *Salmonella typhi*, D-Activity against *Staphylococcus aureus*. E- Control.



A

B

C



D

E

Figure 13: Antibacterial activity of *A.vasica* silver nanoparticles of various concentrations (25, 50, 75,100 μ g/ml) against A- *Staphylococcus aureus*, B- *Salmonella typhi*, C- *E.coli*, D- *Staphylococcus epidermidis*, E- Control.

Table: 3 Anti-bacterial activity of *A.vasica* plant extract

Con (mg/ml)	<i>Staphylococcus epidermidis</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>E.coli</i>
25	9mm	9mm	10mm	10mm
50	11mm	11mm	11mm	11mm
75	11mm	11mm	12mm	12mm
100	12mm	13mm	13mm	15mm
PC	30mm	14mm	13mm	35mm
NC	-	-	-	-

The maximum ZOI of 15 mm was observed for aqueous *N. sativa* seed extract at 100 mg/ml in *E. coli*, whereas for *S.aureus*, *S.typhi* and *S.epidermidis* 13, 13 and 12 mm respectively.

Table: 4- Antibacterial activity of *A.vasica* silver nanoparticles .

Con(µg/ml)	<i>Staphylococcus epidermidis</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>E.coli</i>
25	10mm	12mm	12mm	16mm
50	11mm	12mm	16mm	17mm
75	13mm	13mm	17mm	18mm
100	15mm	14mm	18mm	22mm
PC	23mm	12mm	18mm	32mm
NC	-	-	-	-

For extract-synthesized AgNP's, at 100 µg/ml concentrations, *E.coli* had the highest ZOI of 22 mm. It showed better results even in microgram concentrations. In spite of this, both the leaf extracts and nanoparticles of *A.vasica* inhibited the growth of *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Determination of Anti-oxidant activity

The anti-oxidant activity of aqueous *A.vasica* leaf extract and its synthesized silver nanoparticles was carried out by DPPH assay. The results were plotted as graph. The scavenging activity of compound was increased while the concentration increases and this was done with the standard reference of Ascorbic acid. Scavenging of these radicals was seen by decrease in absorbance at 517 nm by increasing the concentration of the test sample. The scavenging effect (%) was measured using the formula presented in Figure 14 and Table 5.

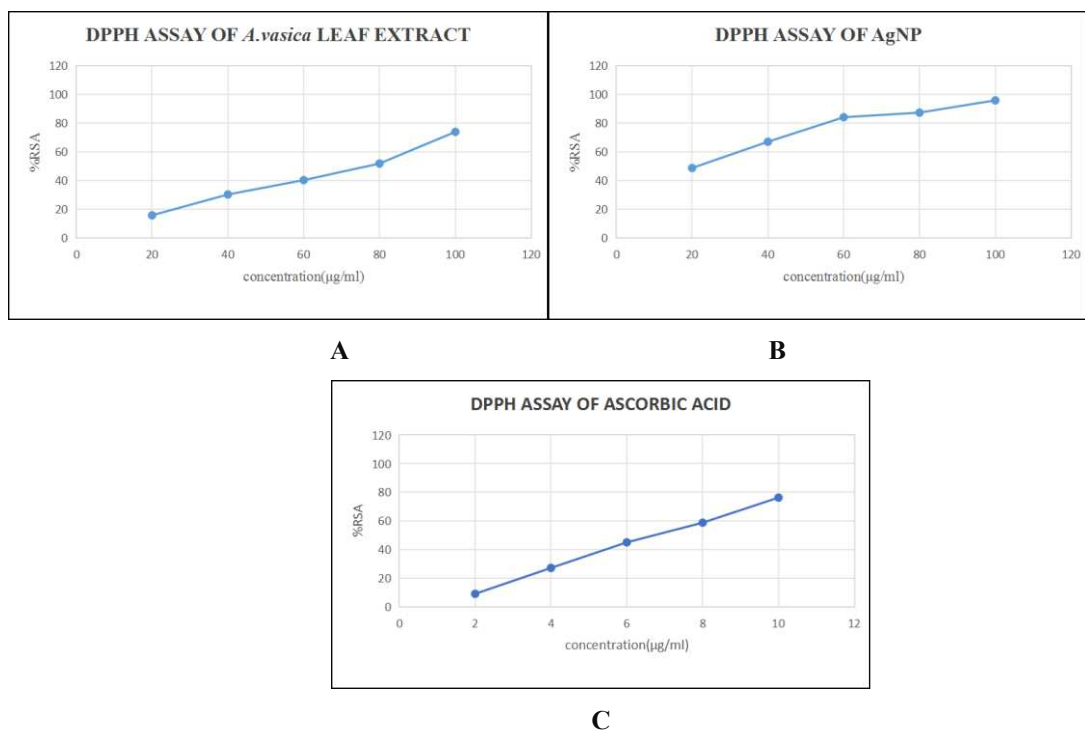


Figure 14: Determination of percentage of Inhibition of A- *A. vasica* leaf extracts, B-AgNP's and C-Ascorbic acid (Standard) by DPPH assay.

$$\text{Percentage of RSA (\%)} = \frac{[(\text{ABS of control} - \text{ABS of sample})]}{[\text{ABS of control}]} \times 100$$

Table: 5 Anti-oxidant activities by DPPH assay of ascorbic acid, aqueous *A. vasica* leaf extract and its synthesized AgNPs with IC₅₀ value.

Nature of the samples	Concentration (µg/ml)	Mean of inhibition ± SD	IC ₅₀ values
Ascorbic acid	2	9.09 ± 3.5	52.41
	4	27.19 ± 1.8	
	6	45.04 ± 2.3	
	8	58.77 ± 3.5	
	10	76.28 ± 2.1	
Aqueous <i>A. vasica</i> leaf extract	20	15.84 ± 11.5	70.93
	40	30.32 ± 2.1	
	60	40.33 ± 1.7	
	80	51.89 ± 2.2	
	100	73.95 ± 3.6	
	20	48.78 ± 2.9	

AgNPs	40	67.05 ± 2.9	13.46
	60	84.15 ± 4.8	
	80	87.36 ± 2.6	
	100	95.91 ± 1.6	

By statistical analysis, the p-values of both *A.vasica* and its nanoparticles showed Statistically significant, (p<0.005)

Evaluation of Larvicidal activity

The larvae were transferred to a test vessel containing 100ml of water and the corresponding test solution for each trial. The test was done in triplicates. The batch with the highest mortality rate was selected and test with a concentration of (for plant sample 1, 5, 10, 50, 100 ppm/100 ml & for silver nanoparticles- 0.1, 0.5, 1, 5, 10 ppm/100 ml) was carried out. Number of dead worms was recorded, and the mortality percentage was determined by Abbott's formula and the LC₅₀ value was 9.06 mg/ ml (leaf extract) and 34.38 µg/ ml (for nanoparticles) tabulated below. From this observation the leaf extract of *A.vasica* and its nanoparticles showed larvicidal activity as shown in Figure 15.-16.

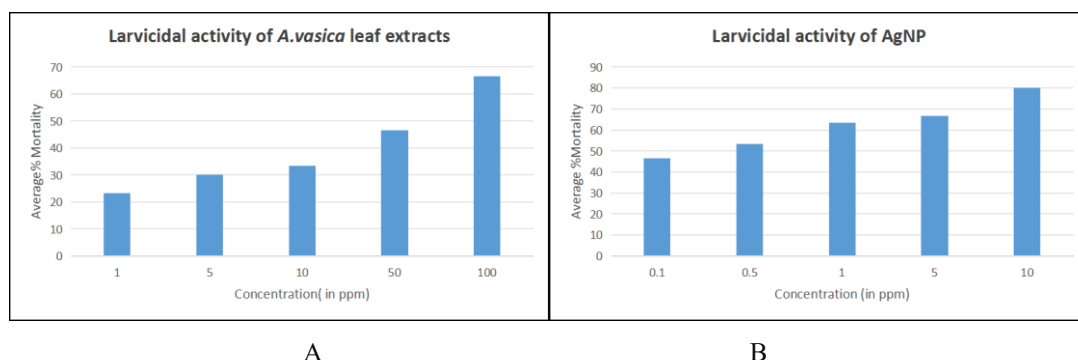


Figure 15: Evaluation of Larvicidal activity of A- *A.vasica* leaf extract, B- Larvicidal activity of AgNP.

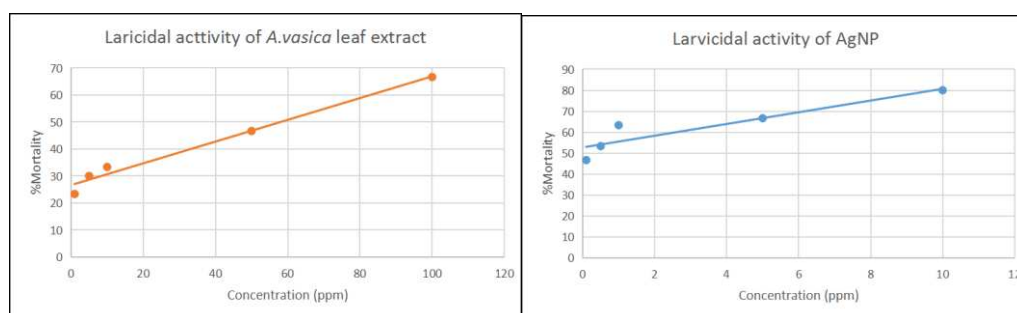


Figure 16: Dose-dependent larvicidal activity of *A.vasica* leaf extracts and its nanoparticles.

The linear regression model was statistically significant (F (1, 3) =152.18, p < 0.01) for aqueous *A.vasica* leaf extract and (F (1,3) = 16.28, p < 0.01) for synthesized AgNPs.

Discussion

The focus of the current study is to investigate the phytochemical analysis of *A. vasica* leaf extract in various solvents, such as methanol, ethanol, chloroform and water. Silver Nanoparticles were synthesized using aqueous leaf extract. Then the aqueous leaf extracts and its synthesized AgNPs were used for evaluating the larvicidal, antioxidant, and antibacterial properties. The photo-constituent analysis of *A. vasica* leaf extract in various solvents showed that the presence of alkaloids, terpenoids, steroids, tannins, phenols, saponins and glycosides. These phytochemicals may be responsible for their biological efficacy. Likewise in many plants the presence of phytosteroid compounds was highly responsible for mosquito larvicidal activity [22-23]. Green synthesis of AgNPs results in the reduction of Ag^+ to Ag^0 ions. In physical and chemical synthesis of nanoparticle, extra chemical compounds are employed to act as a reducing and capping agent. But especially in green synthesis, the plant itself is act as both reducing and capping agent. The nanoparticles itself is toxic to human health, but when it is capped with plant extract it serves as an excellent therapeutic potential. The nanoparticle synthesis is further affected by many factors like temperature; concentration of AgNO_3 and the concentration of plant extract [24]. UV- analysis of synthesized nanoparticles shows a specific peak at 420nm. This result clearly reveals the efficient reduction of Ag^+ ions. SEM analysis showed that the AgNPs are irregular in shape at $3\mu\text{m}$. Like phyto-chemical screening, the FT-IR also results in the rich content of alcohol such as phenol [25]. In case of anti-bacterial activity, both the aqueous leaf extracts and their AgNPs showed inhibition against gram positive and gram-negative bacterial strains. Among them, both aqueous leaf extract and their AgNPs showed more susceptibility against particular bacteria *S.epidermidis*, known to cause secondary skin infections. Similarly, AgNPs is more efficient against bacterial cultures even in low concentrations when compared to leaf extract. The aqueous leaf extracts of *A. vasica* and their nanoparticles shows an effective Radical Scavenging Activity. The percentage of RSA for leaf extract and nanoparticles were calculated as (73.95 ± 3.6) and (95.91 ± 1.6) respectively in $100\mu\text{g/ml}$ concentration. The methanolic leaf extract of *A. vasica* at a concentration of 250 ppm showed effective larvicidal activity against *Ae.aegypti* and *Cx.quinquefasciatus* [26]. Our results showed that the aqueous leaf extracts of *A. vasica* and their AgNP's (10 ppm) showed better larvicidal effect against *Cx.quinquefasciatus* with LC_{50} values 9.06 mg/ ml and $34.38\mu\text{g/ml}$. Similar studies was reported in 2020 with *Annona glabra* leaves (An-AgNPs) against dengue vectors such as *Ae.aegypti* and *Ae.albopictus* and the *Ae. albipictus* was more susceptible than the *Ae. aegypti* for the given concentration of An-AgNPs [27].

CONCLUSION

The qualitative phytochemical screening of the various extracts of *A. vasica* showed the presence of alkaloids, flavonoids, phenols, terpenoids, and tannins. The presence of these general phytochemicals might be responsible for their biological activities. The silver nanoparticle was synthesized from the aqueous leaf extract of *A. vasica* (12:1). The synthesized silver nanoparticles were characterized under the UV-Spectrophotometer. The peak emerge in the range between 400-40 nm confirms the formation of AgNPs. The aqueous extract of *A. vasica* and its silver nanoparticles was characterized using FT-IR (Fourier Transform Infrared Spectroscopy). The infrared spectrum indicated the presence of O-H stretching, $\text{O}=\text{C}=\text{O}$ stretching (carbon dioxide), $\text{C}=\text{C}=\text{O}$ stretching (ketene), $\text{C}=\text{O}$ stretching (primary amide), C-H bending (alkane), O-H

bending (phenol), C-N stretching (amine), C-O stretching (primary alcohol), (C-cl, C-cl and C-Br) (halo compounds).The morphology of the synthesized silver nanoparticles were measured using SEM. The image obtain by SEM reveals the irregular shape and size range in 3µm.The antibacterial activity of crude leaf extracts and its AgNPs against different strains shows a clear zone of inhibition around in both the tests and samples. Antioxidant activity of plant extracts and its AgNPs was analyzed by DPPH assay. Increase in the percentage of Scavenging level reveals the efficiency. Larvicidal activity for both plant extracts and AgNPs were analyzed. LC₅₀ values were found to be 9.06 mg/ ml for plant extracts and 34.38 µg/ ml for AgNPs.

Abbreviations

AgNPs- Silver Nanoparticles, ***A.vasica***- *Adhatoda vasica*, **SEM**- Scanning Electron Microscopy, **DLS**- Dynamic Light Scattering, **DPPH**- 1,1-diphenyl-2-picrylhydrazyl, **RSA**- Radical Scavenging Activity, **IC₅₀**- Inhibitory Concentration 50, **LC₅₀**- Lethal Concentration 50, **ppm**- particle per million.

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Conflict of Interest

The authors declare that they do not have any conflict of interests

Author Contributions

“All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr Anand, Vikas Chandra Roy and Prince leeban The first draft of the manuscript was written by Vikas Chandra Roy Prince leeban, Dr Jayalakshmi Krishnan Dr Rajalakshmi Anbalagan and Anand S B and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

Ethics approval

Not applicable

Consent to participate

Not applicable. This study did not involve human participants.

Consent to publish

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