Research Article

Antibacterial and Larvicidal Activity of Biologically Synthesized Silver Nanoparticles from *Bambusa arundinaceae* Leaves Extract

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ABSTRACT

The increased morbidity and mortality due to the resistance developed in the pathogenic microorganism against antibiotic drugs and mosquito-borne diseases is an emerging issue in medical research. We need to find the new antimicrobial compound and effective biocontrol agent to reduced transmission of pathogenic infection. Due to diverse chemical and mechanical properties, silver nanoparticles produced by the green synthesis method are increasing demand for various pharmaceutical activities. In the present study, the aqueous extract of *Bambusa arundinaceae* leaves were used for the synthesis of silver nanoparticles. The synthesized (*Bambusa arundinaceae* derived silver nanoparticles) BA-AgNPs were characterized using UV-vis spectroscopy, FTIR, SEM and their antibacterial along with larvicidal potential was evaluated. Antioxidant capacity was measured using the DPPH method. SEM analysis revealed that BA-AgNPs were predominantly spheroidal shape with particle size distribution in a range of 20 - 80 nm. Lower IC_{50} value (0.71 mg/ml) of biosynthesized AgNPs showed higher antioxidant activity compared to *B. arundinaceae* leaf extract (0.92 mg/ml) alone. BA-AgNPs were tested against mosquito larvae (*Aedes aegypti* and *Cx. quinquefasciatus*) and their mortality was examined. In larvicidal bioassay, biologically synthesized AgNPs were more toxic (LC_{90} = 50.8 mg/L and 100.8 mg/L) than silver nitrate (LC_{90} = 79.0 mg/L and 146.0 mg/L) to fourth instars larvae of *A. aegypti* and *Cx. quinquefasciatus* respectively. BA-AgNPs demonstrated the highest mortality in fourth instars larvae of *A. aegypti* then in *Cx. quinquefasciatus*. The biosynthesized BA-AgNPs showed a strong antimicrobial activity by causing inhibition of growth with a well diffusion assay. BA-AgNPs showed considerably higher antimicrobial activities against *Escherichia Coli (E.Coli)* when compared with both $AgNO_3$ and streptomycin alone. The results of this experiment suggest that biologically synthesized BA-AgNPs are a quite ideal candidate for the development of new antimicrobial drugs. The collective effect of BA-AgNPs with streptomycin was higher as compared to BA-AgNPs alone which indicates the synergistic effect of these components.

Key words: Antibacterial, Antioxidant, *Bambusa arundinaceae*, Larvicidal, Silver nanoparticles

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INTRODUCTION

The prevalence of bacterial resistance to conventional antibiotics has become one of the main health problems around the globe and increasing the economic burden on the health sector. Pollution, mutation and altered environmental condition are possible factors increase the number of multi-drug resistant bacterial strains.[1] *Aedes aegypti* acts as a vector of parasites that cause dengue fever, chikungunya, zika fever and other disease agents in human beings. Mosquitoes like *Culex* species (*Culex quinquefasciatus*) are a principal vector of bancroftian filariasis transmitted to humans.[2] Synthetic insecticide has limited in used due to harmful effects on human

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health and due to environmental sustainability.[3] To eradicate this problem, scientists are trying to develop drugs for the treatment of such microbial infection as well as diseases spread by mosquitoes. The results of studies indicated the effectiveness of many metals and metal nanoparticles in inhibiting the growth of many infectious bacteria. The use of Silver metal in medicine is the most effective against more than 650 pathogens.[4]

Silver nanoparticles (AgNPs) have obtained considerable attention due to their unique physical, biological and chemical properties. The biological method has clear advantages over chemical and physical methods for the synthesis of silver nanoparticles due to decreased toxicity and environment friendly.[5] Currently, the nanoparticles are coated on the medical devices, food packing sheets and for efficient drug delivery. Metal nanoparticles are being used for various purposes in industrial and pharmaceutical applications.[6] Plant extract mediated reduction of silver particles can be beneficial compared with other biological processes as it does not require the process of maintaining the cell cultures and aseptic environments.[7] A plant extract used to synthesized silver nanoparticles is easy accessibility, safe and non-toxic secondary metabolites help in the reduction of silver ion.[8] Due to smaller in size and large surface area, AgNPs are always in the center of attention due to its strong antimicrobial activity against various microorganisms. The presence of different polyphenolic, ketones, aldehydes, amides and flavonoids acts as a reducing agent and also helps in stabilizing the agent.[9,10] Nanoparticles synthesized from the natural source have been reported to exhibit a variety of bioactivities like as anti-bacterial, anti-larvicidal and antioxidant activities.[11-13] Anti-fungal,[14] anti-inflammatory[15] and anti-viral properties[16] of biologically synthesized silver nanoparticles are well reported. In addition to this, AgNPs have also exhibited a strong inhibitory effect on antibiotic-resistant bacteria such as methicillin-resistant *S. aureus* (MRSA) and drug-resistant *E. coli*. AgNPs synthesized from leaf extracts of *Leucas aspera* were tested for larvicidal activity against the fourth instar larvae of *A. aegypti*.[17] Larvicidal activities of silver nanoparticles from *Parthenium hysterophorus* root extract was studied on *C. quinquefasciatus*.[18] Several plants like *Coleus aromatics*, *Ambrosia arboescen, Ficus racemosa, Colocasia esculenta* have been attempted for green synthesis of their silver nanoparticles (AgNPs) and evaluate their larvicidal activity.

Various bioactive components of a plant extract help efficiently in reducing and stabilizing the nanoparticles. Bamboo leaves have been utilized in the China as a conventional drug for treating hack, fever and sickness. It is likewise been clinically appropriately in the treatment of hypertension, arteriosclerosis and malignancy. *Bambusa arundinacea* has been recognized that bamboo extract has antioxidant activities and anti-inflammatory effects.[19] Antimicrobial, antiulcer, antifertility, mitigating and hypoglycemic action of bamboo leaves has been accounted.[20] The medicinal value of bamboo leaves is mostly attributed to their proactive polyphenol constituents. The real phytochemical comprises present in bamboo leaves are protein, gluteline, contains lysine, methionine, betain, cholin, proteolytic compound, nuclease, glycosides, urease.[21] Ethanolic leaves extract of *Bambusa arundinacea* demonstrates hypoglycemic in diabetic animals[22] and also reported to possess antiulcer, antioxidant and antifertility activity. Leaves mainly contain protein, gluteline, lysine, methionine, betain, cholin, proteolytic enzyme, nuclease, urease.[21]

In the present work, we have synthesized silver nanoparticles using an aqueous leaf extract of *Bambusa arundinaceae* and analyze for the synergistic effect of bio-synthesized silver nanoparticles with antibiotics against pathogenic microbes. This study also attempted to evaluate the larvicidal potential of BA-AgNPs.

MATERIALS AND METHODS

Collection, identification and extraction method

Fresh leaves of *Bambusa arundinaceae, Bambusa vulgari* and *Bambusa ventricosa* were collected from Directorate of Medicinal and Aromatic Plants Research, Boriavi during the month of July 2019. The plant was taxonomically identified and authenticated with the voucher number (MPSC-023) at the herbarium of botany department, M. B. Patel Science College, Anand. The plant leaves were air-dried for five days and pulverized. The pulverized plant materials (200 g) were extracted with distilled water successively using a soxhlet extractor for 24 hr. The freshly prepared aqueous extract was used for the synthesis of silver nanoparticles. The percentage yield of the extract was calculated using the formula yield (%) = (weight of solventless extract/weight of the initial sample taken) × 100

Phytochemical screening

Aqueous leaf extract of all selected three species of *Bambusa* was subjected to preliminary phytochemical screening for the detection of various plant constituents.[23,24]

Determination of total phenolic and flavonoid content

Total phenolic content of bamboo leaf extract was determined by using Folin-Ciocalteu (FC) reagent

method with some modification.[25] The absorbance was measured at 725 nm after 90 min. A calibration curve was prepared by using gallic acid as standard. A result was expressed as mg Gallic Acid Equivalents (GAE) per g of plant extract. The total flavonoid was determined by using aluminium chloride colorimetric assay.[26] 150 µl of 96% ethanol was added to 50 µl of extracts or standard solution and followed by 10 µl of 10% the aluminium chloride solution. 1.0 M sodium acetate (10 µl) was added to the mixture in a 96 well plate and incubates for 40 min at room temperature. The absorbance was measured at 415 nm with a microplate reader. A calibration curve was prepared by using quercetin as standard.

Green synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$; molar mass 169.87 g mol^{-1}, boiling point 440°C) was used as a source of silver. An aqueous solution of $AgNO_3$ (100 ml of 1.0 mM) with continuous stirring was taken for the synthesis of silver nanoparticles. 25 ml of *Bambusa arundinaceae* leaf extract was added dropwise into the solution of silver nitrate. Reduction of silver ions to silver nanoparticles can identify by observing color change from whitish to yellowish-brown color. The synthesized AgNPs obtained from the solution was purified by repeated centrifugation at 12,000 rpm for 20 min using REMI cooling centrifuge C-24. The collected pellets were washed with milliQ water and dried at room temperature. Due to light-sensitive reaction of silver nanoparticle synthesis, all glassware covered with aluminium foil.

Characterization of AgNPs

The optical properties of purified AgNPs were initially analyzed for UV spectra between the wavelengths from 300–600 nm by using UV visible spectroscopy (Systronics Double beam UV-Visible-2202). The solution of synthesized nanoparticles was diluted twofold in an equal volume of distilled water before UV–Vis analysis. The stability of the bioreduction of silver ion monitored periodically.[27] The dried nanopowder of silver was used for Fourier transform infrared spectroscopy (FT-IR) analysis according to the method of Daisy and Saipriya[28] to determine the possible functional groups involved in the synthesis of AgNPs. FTIR measurements were carried out using Perkin-Elmer model spectrometer. The scanning range was 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. Both samples were directly placed in the KBr crystal and the spectrum was recorded in the transmittance mode.

Morphological study using microscopic methods

Transmission Electron Microscopy of AgNPs was performed at Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Vallabh Vidhyanagar, Gujarat. Samples of the BA-AgNPs were dropped on the carbon-coated copper grids and kept overnight under vacuum desiccation to dry before loading them on to a specimen holder.

DPPH radical scavenging activity

Antioxidant potential of aqueous leaf extract of *Bambusa arundinaceae* and biosynthesized silver nanoparticles were analyzed by DPPH assay.[29] 3.0 ml of DPPH (0.1 mM) prepared in methanol was mixed with each leaf extract (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml) of *Bambusa arundinaceae*. The reaction mixture was shaken and allowed to stand for 30 min at room temperature. Absorbance was measured at 517 using Uv spectroscopy. The percent inhibition of activity was calculated as [(Ao - Ae)/Ao]*100 (Ao = absorbance without extract; Ae = absorbance with extract) and determined IC_{50} value.

Antibacterial activity

The silver nanoparticles synthesized from aqueous extract of *Bambusa arundinaceae* leaves were tested for their antimicrobial activity in combination with streptomycin by well diffusion method against pathogenic organisms like *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The pure culture of all tested strains of the pathogens were subculture on nutrient broth and then transferred to the surface of the individual agar plates using a sterile cotton swab with each bacterial strain. 6 mm wells were made using sterile borer. 25 µl of each sample (bamboo leaves extract mediated AgNPs, streptomycin+ bamboo leaves extract mediated AgNPs, streptomycin, silver nitrate solution only) were introduced into each well and well filled with deionized water was used as control. All the plates were kept in an incubator at 37° for 24 h. The assays were carry out in triplicate and mean inhibition zone diameters were measured.

Larvicidal activity

The strain of *Ae. aegypti* and *Cx. quinquefasciatus* larvae used for this work was collected in Sept 2019 from the Malarial Research Centre, Nadiad. The larvae reaching the 4th instar were used for experimentation. To investigate the larvicidal activity of the aqueous extract and the BA-AgNPs, we followed the protocol proposed by the WHO[30] as per the method of Velayutham *et al.*[31] The larvicidal activity of bamboo leave extract and BA-AgNPs were tested against *Ae. aegypti* and

Cx. quinquefasciatus larvae. Standard insectary conditions (28 ± 1°C temperature, 80 ± 10% relative humidity and 12 h light/12 h darkness) photoperiod was maintained during the bioassays. The late 4th instar larvae of each ***strain*** were tested in four batches of 25 larvae, with a final total of 100 larvae for each concentration. Each batch of both strains was transferred in 100 ml of deionized water and 100 ml of the desired concentration of bamboo leaves extract (10-100 ppm), AgNPs (1-10 ppm) and BA-AgNPs (1-10 ppm) for a total of five concentrations. The control and untreated groups were included using $AgNO_3$ solution and deionized water, respectively. Mortality was evaluated 24 hr after the beginning of each bioassay to determine the acute larvae toxicities. The 50% and 99% lethal concentration (LC_{50} and LC_{99}, respectively) were derived.

Statistical analysis

Results with $P<0.05$ were considered to be significantly significant calculated using ANOVA.

RESULTS

The phytochemical screening was done in an aqueous extract of three different species of Bambusa including *Bambusa arundinaceae, Bambusa vulgaris* and *Bambusa ventricosa* leaves. The results of the phytochemical analyses are represented in Table 1.

The result showed the absence of alkaloids, saponins and cyanogenic glycosides in species *Bambusa arundinaceae* and *Bambusa vulgaris* investigated, whereas leaves of *Bambusa ventricosa* contains alkaloids and saponins.

Figure 1 shows the total phenolic and flavonoid content present in the aqueous extract of three species (*Bambusa vulgaris*, *Bambusa arundinaceae, Bambusa ventricosa)* of bamboo leaves. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of leaves extract. Among the three species, the highest phenolic content was found in *Bambusa arundinaceae* (14.5 mg GAE/g dry wt.) followed by *Bambusa vulgaris* (12.5 mg GAE/g dry wt.) and *Bambusa ventricosa* (8.8 mg GAE/g dry wt.) Regarding to the phenolic content, there were no statistical differences ($p>0.05$) between *Bambusa arundinaceae* and *Bambusa vulgaris* varieties, with the exception of variety '*Bambusa ventricosa*' which showed a statistically significant ($p<0.05$) lower phenolic content. Flavonoid compounds of an aqueous leaf extract of *Bambusa arundinaceae* (5.80±0.10 mg QAE/g wt) showed significantly ($p<0.05$) higher value than *Bambusa vulgaris* (0.5 mg QAE/g dry wt.) and *Bambusa ventricosa* (2.8 mg QAE/g dry wt.)

The semitransparent white color of silver nitrate solution was changed to light brown color after the addition of leaves extract within 30 min indicates the formation of Ba-AgNPs depicted in Figure 2.

UV–vis absorption spectrum of the BA-AgNPs was recorded and shown in Figure 3.

Table 1: Qualitative analysis of phytochemical constituents present in the leaves of *Bambusa arundinaceae, Bambusa vulgaris* and *Bambusa ventricosa*.

Type of compound	Test for conformation	*Bambusa arundinaceae*	*Bambusa vulgaris*	*Bambusa ventricosa*
Flavonoids	Shindo's test	++	+	+
Saponins	lead acetate method	-	-	+
Cyanogenic glycosides	*Liebermann's Test.*	-	-	-
Alkaloids	Dragendorff's test	-	-	+
Anthraquinones	Borntrager's test	+	+	+
Sterols	H_2SO_4 test	+	+	+
Terpenoids	Knollar's test	+	+	+
Anthocyanins	NaOH test	++	+	++
Quinones	HCl test	+	+	+
Tannins	Ferric chloride method	++	++	++

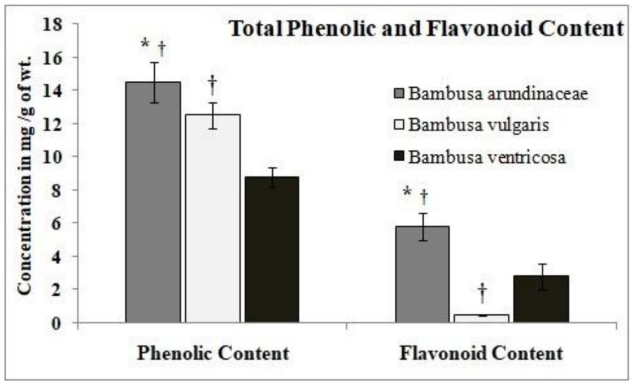

Figure 1: Total phenolic and flavonoid content present in the aqueous leaf extract of three *Bambusa* species.

Figure 2: Yellowish-brown color of synthesized silver nanoparticles.

In the case of bamboo leaves extract mediated AgNPs; the optical absorption band peaking was recorded at 425 nm. The structure and size of the nanoparticles was investigated using Transmission Electron Microscopy (TEM) which is represented in Figure 4.

TEM image indicate that shape of BA-AgNPs ware are polydisperse, spherical with few exceptional as ellipsoidal seen in the micrograph with typical sizes in the range between 8-15 nm. Dried powder of BA-AgNPs was evaluated using FT-IR spectroscopy. FTIR analysis was carried out to determine the role of various phyto-constituents of *Bambusa arundinaceae* which might be responsible for synthesis and stabilization of BA-AgNPs. FTIR analysis of the BA-AgNPs revealed the presence of peaks at 3426, 2825, 1631, 1384, 1126, 618 cm^{-1}. (Figure 5)

Antioxidant activity of BA-AgNPs and aqueous leaf extract using DPPH assay is presented in Figure 6. The percentage of inhibition of free radicals was increased in a dose-dependent manner. BA-AgNPs (IC_{50} = 0.71 mg/ml) exhibited more effective free radical scavenging activity then *Bambusa arundinaceae* leaf extract alone (IC_{50} = 0.92 mg/ml) but not higher then vitamin C (IC_{50} = 0.25 mg/ml) used as standard.

The antibacterial activity of BA-AgNPs was examined against gram-negative bacteria *E. coli* and gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* using the agar well diffusion assay. The zone of inhibition was observed in each N-agar plate and measured in mm presented in Figure 7.

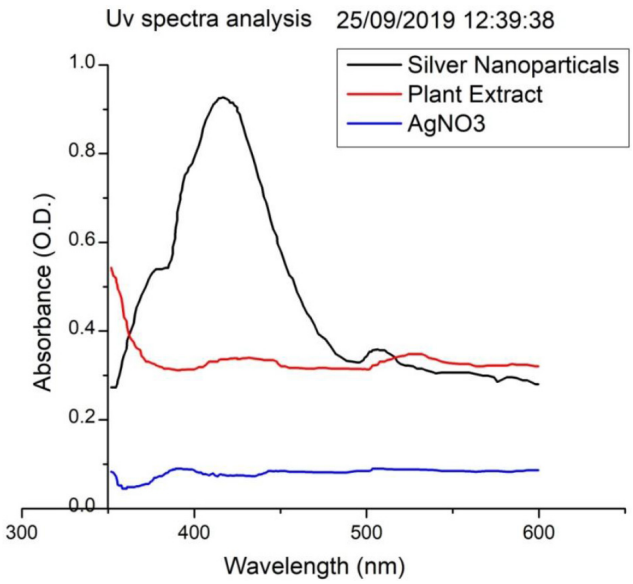

Figure 3: UV-VIS spectroscopy for silver nanoparticles (BA-AgNPs), $AgNO_3$ solution and leaf extract of *Bambusa arundinaceae*.

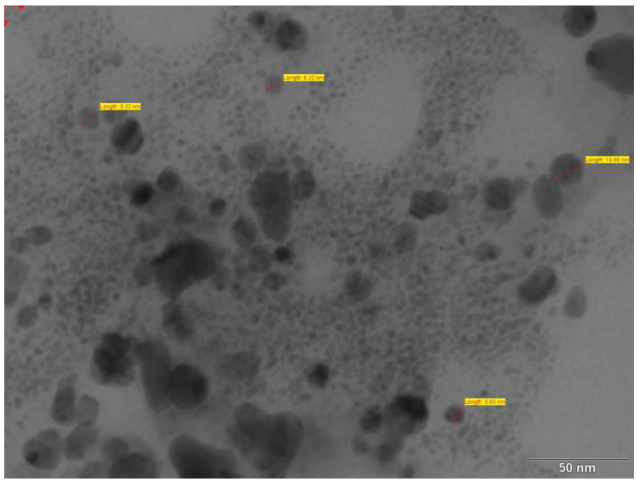

Figure 4: Transmission Electron Microscopy (TEM) showing polydisperse and spherical shape of BA-AgNPs synthesized by using the leaf extract of *Bambusa arundinaceae*.

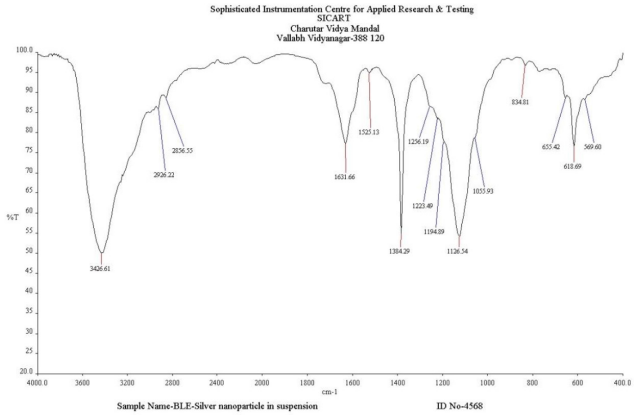

Figure 5: FTIR spectrum of silver nanoparticles synthesized by using the leaf extract of *Bambusa arundinaceae*.

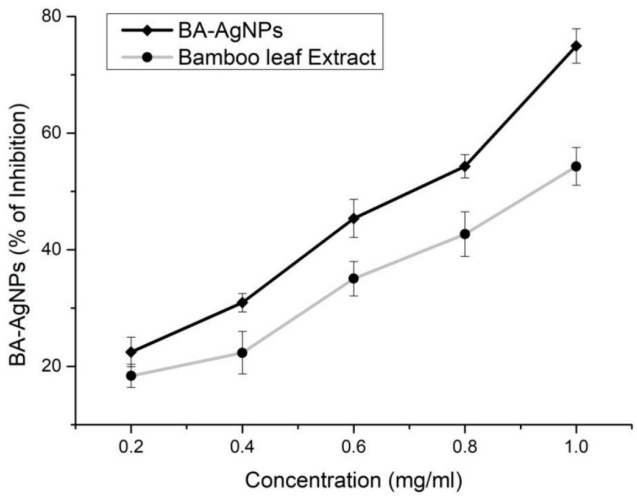

Figure 6: Comparative antioxidant potential in the form of % inhibition of free radical by biologically synthesized AgNPs and aqueous leaf extract of *Bambusa arundinaceae* (DPPH radical scavenging assay).

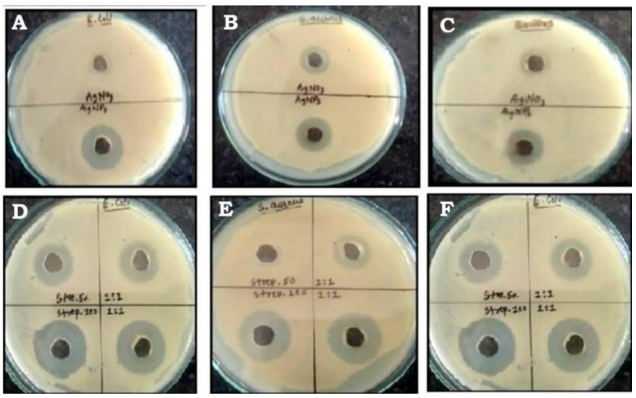

Figure 7: Antibacterial activity of A. *Escherichia coli* B. *Bacillus subtilis* C. *Staphylococcus aureus* of AgNPs and $AgNO_3$ Synergistic effect streptomycin with AgNPs on D. *Escherichia coli* E. *Bacillus subtilis* F. *Staphylococcus aureus.*

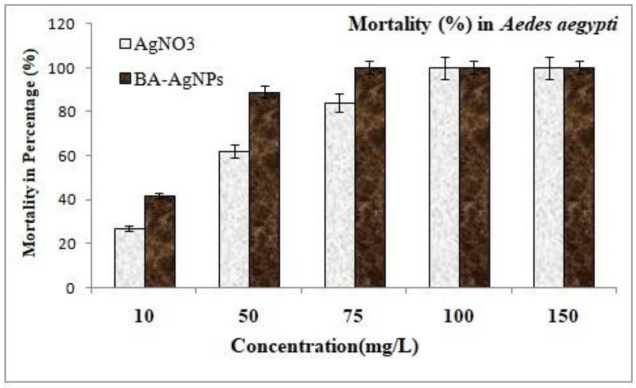

Figure 8: Larvicidal activity of silver nanoparticles synthesised using leaf extract of *Bambusa arundinaceae* against *Aedes aegypti*.

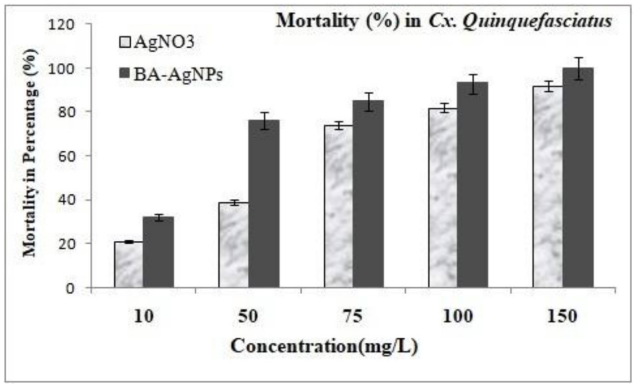

Figure 9: Larvicidal activity of silver nanoparticles synthesised using leaf extract of *Bambusa arundinaceae* against *Cx. quinquefasciatus*.

Table 2: Synergistic effect of streptomycin with and without biosynthesized AgNPs against pathogens.

No.	Microorganisms	Zone of inhibition (in mm)			
		$AgNO_3$	BA-AgNPs	Streptomycin	Streptomycin + BA-AgNPs
1.	*Escherichia coli*	8	22	16	24
2.	*Bacillus subtilis*	8	19	15	19
3.	*Staphylococcus aureus*	11	20	12	22

Antibiotics streptomycin used as a positive control. Among the all tested pathogenic microbes, the growth of gram-negative *E. coli* was greatly inhibited with a maximum zone of inhibition 21.0 mm for BA-AgNPs compared to $AgNO_3$ (8.0 mm). Gram-positive *S. aureus* was found to exhibit greater zones of inhibition of 20.0 and 11.0 mm compared to *Bacillus subtilis* with 19.0 and 8.0 mm zone of inhibition for BA-AgNPs and $AgNO_3$ respectively (Table 2).

The nanoparticles synthesized from *Bambusa arundinaceae* were subjected to larvicidal bioassay on fourth instars larvae of *Ae. aegypti* and *Cx. quinquefasciatus* larvae. The percentage of mortality of *Ae. aegypti* and *Cx. quinquefasciatus* larvae with different concentration are depicted in Figure 8 and 9 respectively.

The LC_{50} and LC_{90} values of silver nitrate only and BA-AgNPs are presented in Table 3.

Table 3: LC_{50} and LC_{90} of biosynthesized silver nanoparticles for larvicidal activity determined by probit analysis and regression analysis on 4th instars larvae of *Ae. aegypti* and *Cx. quinquefasciatus* larvae.

Mosquitoes species	LC_{50} (mg/L)		LC_{90} (mg/L)		R^2 value
	$AgNO_3$	BA-AgNPs	$AgNO_3$	BA-AgNPs	
Aedes aegypti	40.5	28.8	79.0	50.8	0.954
Cx. quinquefasciatus	64.4	33.6	146.0	100.8	0.968

DISCUSSION

The results of the present study indicated the presence of coumarins, flavonoids, tannins polyphenols and terpenoids in all three species of Bambusa. Cardiac glycoside is absent in aqueous extract of bamboo leaves suggest that it can be used as safe herbal supplement. Tannins are reported to exhibit antiviral, antifungal, antibacterial, anti-tumor activities. The presence

of anthraquinones in bamboo leave extract indicates the purgative, anti-inflammatory, moderate antitumor activity. An alkaloid compounds which are basically contains nitrogen atom naturally present in the various plants having antibacterial and antifungal activity.[32] Many researchers reported that natural phenolic compounds isolated from plants demonstrate to have numerous of biological effect including anti-inflammatory, antimicrobial, antiangiogenic, anticancer activities.[33] Flavonoids isolated from various medicinal plants have been traditionally used for the treatment of microbial diseases including pneumonia, cough, abdominal pains and diarrhea.[34,35] Several experimental studies exhibited pharmacological properties of flavonoids especially protection against pathogenic bacteria, inflammation and having antioxidant and anti-tumor effects.[36] The intensity of the brown color of silver nanoparticles depends on the incubation period. The stability of synthesized BA-AgNPs was further confined by UV-vis spectrometer and FT-IR analysis. Yellowish-brown color of synthesized silver nanoparticles in water is the physical characteristic due to Surface Plasmon vibration. Nanoparticles were synthesized through the reduction of $AgNO_3$ using an aqueous extract of *Bambusa arundinaceae* as a source of reducing agents. The Surface Plasmon resonance (SPR) band in UV-visible spectra of AgNPs colloidal suspension appeared between 410 and 440 nm with the desired size. The presence of free electrons in many metal nanoparticles produced a SPR due to the mutual vibration of electrons in resonance with lightwave.[37,38] Electron spectra of silver nanoparticles depend on the shape and size. It can be assumed from the above results that the bioactive component present in the bamboo leaves extract induce the reduction of silver ions to silver nanoparticles. Previous studies demonstrated that the smaller the size of silver nanoparticles increases the efficacy in inhibiting the growth of bacteria.[12] The size of the nanoparticles synthesized using *Bambusa arundinaceae* leaf extract ranged from 8-15 which could have a potential effect on the antimicrobial properties of the particles. The absorption bands at 3426 and 2825 cm^{-1} indicate the presence of O–H and C–H stretchings. A Peak at 1631 cm^{-1} attributed to the N-H bond of amines. The absorption bands at 694 cm^{-1} peak confirm the presence aromatic ring which indicates the presence of the phenolic groups on silver nanoparticles. CH_3-CH bond of alkanes and alkyl groups gave short peaks around 1384 cm^{-1}. Finally, the absorption peak at 1126 cm^{-1} is attributed to the C-O-C vibration stretching. Data of FTIR analysis suggested the presence of various phytocompound and amino acid present in bamboo leaves extract act as a capping agent in synthesized silver nanoparticles. This capping agent on silver nanoparticles may enhance efficacy in the various bioassay. The results of FITR suggest that bioactive molecules attached with AgNPs have free and bound amide groups. The amide group present in the aromatic ring could be polyphenols which attached with AgNPs.[39] DPPH assay was performed with different concentrations of leaf extract and biosynthesized silver nanoparticles to determined IC_{50} value. Increased antioxidant activity of biosynthesized silver nanoparticles compared to leaf extract could be attributed to phenolic and flavonoid compounds adhered to them which were originated from the leaf extract. It seems quite possible that the phenolic compound present in the bamboo leaves play a significant part in the reduction of silver ions to AgNPs. As the concept of antioxidant action of phenolic compounds from bamboo leaves are not new.[19,21] Silver nanoparticles bind efficiently with negative charge surface protein on the cell membrane and cell wall leads to disturb cell permeability and finally cell death.[40] The bactericidal activity of silver nanoparticles depends on size and dose.[41,42] The increased antimicrobial activity of BA-AgNPs compared to silver nitrate is due to the larger surface area whichincreases the efficacy for the attachment with microorganism. Several authors explained this increased antibacterial activity of biosynthesized silver nanoparticles is the synergistic effect between particles and natural bioactive compounds. Capping of silver nanoparticles with phenolic compound increased lipophilic characters which enhanced their interaction with the cell membrane of microbes.[43] The results revealed that antibiotics in association with AgNPs exhibited better bactericidal activity and produced a higher zone of inhibition as compared to AgNPs and antibiotics alone. Antibacterial activity against *E. coli* and *S. aureus* increased 1.23 and 1.11 fold respectively in the present study. Combination of silver nanoparticles with antibiotics and its synergetic effect has been observed in the previous study.[44,45] Regression coefficient values clearly indicate the positive correlation between mortality rate and concentration of BA-AgNPs. Plant-synthesized AgNPs showed a dose-dependent toxic effect against *Ae. aegypti* and *Cx. quinquefasciatus* larvae. Larvicidal activity of BA-AgNPs was produced more lethality in both the strains Ae. *aegypti* and *Cx. quinquefasciatus* larvae compared to $AgNO_3$. Based on these reports, the BA-AgNPs synthesized in this present study showed lethality against both mosquito vectors. The larvicidal activity observed in synthesized AgNPs using bark aqueous extract of *Ficus racemosa* against

Culex quinquefasciatus and *Culex gelidus* was (LC_{50}=12.00 and 11.21 mg/L) respectively.[31] The LC_{50} value for fourth instar larvae of *An. subpictus* and *Cx. quinquefasciatus* of synthesized AgNPs using aqueous leaves extract of *Tinospora cordifolia* were 6.43 and 6.96 mg/L respectively.[46] *Gymnema sylvestre* display strong larvicidal activity against the forth-instar larvae of *C. quinquefasciatus* with LC_{50} value 15.92 ppm.[47] Silver nanoparticles can penetrate through larval membrane and bind to sulphur-containing proteins which denature enzyme function.[48] The Silver nitrate solution was less toxic than the green synthesized silver nanoparticles against two tested mosquito species. Biological mechanisms of lethality towards the strains *Ae. aegypti* and *Cx. quinquefasciatus* larvae are not clearly understood, but it seems quite possible that the attachment of phenolic and flavonoid capped on nanoparticles play important role in toxicity.

CONCLUSION

Aqueous leaf extract of *Bambusa arundinaceae* can be used as an efficient capping as well as a reducing agent for the green synthesis of silver nanoparticles. It needs to further investigate the biochemical mechanism of the mode of action as a toxic agent towards studied mosquito species for effective control over the vectors. The result of the present study indicates that the synthesis of BA-AgNPs may apply as nanomedicine as an antimicrobial agent and good insecticide for the control of mosquito vectors.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in the subject matter or materials discussed in this manuscript.

ABBREVIATIONS

BA-AgNPs: *Bambusa arundinaceae* derived silver nanoparticles; **$LC_{50:}$** Lethal Concentration 50; **IC50:** half maximal inhibitory concentration; **FTIR:** Fourier-transform infrared spectroscopy; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl.

SUMMARY

This study reports the potent bactericidal and larvicidal activity of silver nanoparticles synthesized from aqueous leaf extract of *Bambusa arundinaceae*. We select *Bambusa arundinaceae* for the synthesis of silver nanoparticles due to its higher phenolic and flavonoid content. Biologically synthesized AgNPs has been characterized by UV-Vis Spectroscopy, Tunnelling Electron Microscopy (TEM) and FTIR. We also reports higher antioxidant activity of silver nanoparticles than bamboo leaf extract. Higher mortality rate of mosquito vectors by silver nanoparticles indicated larvicidal activity. The combination of antibiotics with AgNPs demonstrated an overall percentage increase in average fold-area of zone of Inhibition in this study.

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