Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Synergistic antibacterial and mosquitocidal effect of *Passiflora foetida* synthesized silver nanoparticles

Efeito sinérgico antibacteriano e mosquitocida de nanopartículas de prata sintetizadas passiflora foetida

D. S. Palanisamy[a,b], B. S. Gounder[c], K. Selvaraj[a,b], S. Kandhasamy[b], T. Alqahtani[d], A. Alqahtani[d], K. Chidambaram[d]*, K. Arunachalam[e], A. M. Alkahtani[f], Harish C. Chandramoorthy[f,g], N. Sharma[h], S. Rajeshkumar[i] and L. Marwaha[j]

[a]Bharathiar University, Research and Development Centre, Coimbatore, India
[b]Nandha Arts and Science College, Department of Biotechnology, Erode, India
[c]Government Arts College, Department of Botany, Salem, India
[d]King Khalid University, College of Pharmacy, Department of Pharmacology, Abha, Saudi Arabia
[e]Mulungushi University, School of Science, Engineering and Technology, Department of Science and Mathematics, Kabwe, Zambia
[f]King Khalid University, College of Medicine, Department of Microbiology & Clinical Parasitology, Abha, Saudi Arabia
[g]King Khalid University, College of Medicine, Centre for Stem Cell Research, Abha, Saudi Arabia
[h]Maharishi Markandeshwar University, Research and Development Centre, Solan, Himachal Pradesh, India
[i]Saveetha Institute of Medical and Technical Sciences, Department of Pharmacology, Chennai, India
[j]Lovely Professional University, School of Bioengineering and Bio Sciences, Department of Zoology, Punjab, India

Abstract

Silver nanoparticles are opted to have various applications in different fields ranging from traditional medicines to culinary items. It is toxic and most effective against bacteria, fungi viruses, parasites, parasite carrying vectors such as mosquitoes and their larvae and other eukaryotic microorganisms at low concentration without any side effects and toxicity to humans. In view of these data, the present research has been investigated by synthesizing silver nanoparticles using 1mM silver nitrate and aqueous extract of *Passiflora foetida*. The variation of nanoparticles in size and shape concerning the concentration of extract prepared were analysed. The formation of silver nanoparticles was confirmed by colour changing from yellowish green to reddish-brown implicating the surface plasmon resonance. Further, it was concluded by obtaining an absorbance peak at 420 nm using UV-Visible spectrophotometer analysis. FTIR analysis was used to identify the capping ligands, which included alkanes, aromatic groups and nitro compounds. The average grain size of ~12 nm to 14 nm with crystalline phase was revealed by X-ray Diffraction studies. The SEM images depicted the surface morphology with agglomeration; TEM studies showed the shape of nanoparticles as spherical and hexagonal with sizes ranging from 40 nm to 100 nm and EDAX analysis confirmed the presence of elemental silver as the principal constituent. The characterized silver nanoparticles were then tested for synergistic antibacterial effects with tetracycline, and the results show that they are more active against E. coli and S. aureus, but moderately effective against B. cereus and K. pneumoniae . It also had a strong larval and pupal toxic effects on the dengue vector, Aedes aegypti with the highest mortality. As a result, silver nanoparticles could be a viable alternative for a variety of applications.

Keywords: *Passiflora foetida*, silver nanoparticles, characterization, synergistic antibacterial, *Aedes aegypti*, larval and pupal toxicity.

Resumo

Os nanopartículos de prata são optados por ter várias aplicações em diferentes áreas que variam de medicamentos tradicionais a itens culinários. É tóxico e mais eficaz contra bactérias, vírus de fungos, parasitas, parasitas que transportam vetores como mosquitos e suas larvas e outros microorganismos eucarióticos em baixa concentração, sem efeitos colaterais e toxicidade para os seres humanos. Em vista desses dados, a presente pesquisa foi investigada sintetizando nanopartículas de prata usando nitrato de prata de 1 mm e extrato aquoso de Passiflora foetida. Foi analisada a variação de nanopartículas em tamanho e forma relativa à concentração de extrato preparado. A formação de nanopartículas de prata foi confirmada pela mudança de cor de verde amarelado para marrom-avermelhado, implicando a ressonância plasmônica da superfície. Além disso, foi concluído pela obtenção de um pico de absorvância a 420 nm usando análise do espectrofotômetro visível por UV. A análise do FTIR foi usada para identificar os ligantes de captura, que incluíam alcanes, grupos aromáticos e compostos nitro. O tamanho médio

*Corresponding author: kumarappan@kku.edu.sa
Received: April 24, 2022 – Accepted: October 26, 2022

dos grãos de ~ 12 nm a 14 nm com fase cristalina foi revelado por estudos de difração de raios-X. As imagens SEM retratavam a morfologia da superfície com aglomeração; Os estudos de TEM mostraram a forma das nanopartículas como esféricas e hexagonais, com tamanhos variando de 40 nm a 100 nm e a análise EDAX confirmou a presença de prata elementar como constituinte principal. As nanopartículas de prata caracterizadas foram então testadas quanto a efeitos antibacterianos sinérgicos com tetraciclina, e os resultados mostram que são mais ativos contra E. coli e S. aureus, mas moderadamente eficazes contra B. cereus e K. pneumoniae. Ele também teve um forte efeito tóxico larval e pupal no vetor de dengue, Aedes aegypti com a maior mortalidade. Como resultado, as nanopartículas de prata podem ser uma alternativa viável para uma variedade de aplicações.

Palavras -chave: Passiflora foetida, nanopartículas de prata, caracterização, antibacteriano sinérgico, Aedes aegypti, toxicidade larval e pupal.

1. Introduction

Nanotechnology focuses on the development and use of structures and devices with organisational features at the intermediate scale between individual molecule. The particles at about 100 nm, where novel properties emerge when compared to bulk and is expected to open up new avenues for disease treatment and prevention by tailoring materials at the atomic scale. (Hoseinzadeh et al., 2017). For the past decades, neumerous inorganic nanoparticles have been newly developed to provide efficient material properties

Nanoparticle synthesis is of interest because of its useful physical, chemical, optical, electronic, and catalytic capabilities, as well as their greater surface-area-to-volume ratio. (Reiss et al., 2011). With the increasing need for environmentally friendly materials and synthetic technologies, there is growing interest in nanoparticle biosynthesis as a new feature of the interface between nanotechnology and biotechnology. More effort has been put into the biosynthesis of inorganic material, especially metal nanoparticles using microorganisms and plants. Plant-derived synthesis of nanoparticles is preferred as it is safe for humantherapeutic application (Roy, 2021). The use of plant extracts to synthesise nanoparticles is receiving attention in recent times because of its simplicity, less expensive, and maybe suitably scaled up for large scale biosynthesis in a controlled manner according to their size, shape and sensitivity (Gnanadesigan et al., 2011). In the synthesis of nanoparticles, plant extracts may act as both reducing and stabilizing compounds. There are a variety of plant extract mediated synthesis of nanoparticles that have been reported in the literature. These include the synthesis of silver nanoparticles using the leaves of Bamboo, *Annona reticulata Ocimum sanctum* and *Cassia auriculata,* (Yasin et al., 2013; Rajeshkumar and Bharath, 2017). Silve nanoparticles have been recognised as having inhibitory effect on bacterial strains and other microorganisms present in medical and industrial fields (Singh et al., 2015).

Metallic silver is notably inert and poorly absorbed via means of mammalian or bacterial cells. As compared to other various heavy metals, silver is toxic to microorganisms by interfering with mitochondrial transport systems and damaging the function of DNA (Paladini and Pollini, 2019). Nanosilver has been used for embedding the fabrics in sporting equipment as an antimicrobial agent, applied in-home water purification systems, cosmetics, electronics, household appliances, medical devices and implants prepared with silver-impregnated polymers, skin ointments and creams to prevent infection of burns and open wounds and has many other medical applications (Bruna et al., 2021; Tiwari et al., 2011). Silver nanoparticles were discovered to have a potent antibacterial effect, which might be enhanced further when combined with an antibiotic.

In such a way, synergistic activity has been aimed with determining the combined effect of silvernanoparticles with antibiotics in the current research. By the way, the development of antibiotic resistance mechanisms could also be reduced. Mosquitoes cause a major public health problem whcih act as transmitters of human diseases such as malaria, filariasis, japanese encephalitis, dengue fever, chikungunya, and yellow fever. (Baskar and Ignacimuthu, 2012; Huang et al., 2014). Mosquito control is a serious task in developing countrieslike India due to the lack of general awareness, developmentof resistance, and socioeconomic reasons (Muthukumaran et al., 2015). Though the extracts from plants were used as the sources of mosquito larval control agents, the use of silver nanoparticles could be a new alternative and find its better way in controlling the spread of mosquitoes. (Govindarajan and Sivakumar, 2012; Kovendan et al., 2012). Recently, numerous works have been reported on the mosquitocidal effect using silver nanoparticles (Roopan et al., 2013).

Traditionally, fresh or dried whole plants and their preparations are accepted for medicinal use in European countries for thr treatment of nervous anxiety where leaves of the plant are utilised as a folk medicine to treat anxiety, stress and insomnia. It is also effective in the treatment of hysteria, skin inflammation, cough, and fever. and the chemical constituents in *P. foetida* include hydrocyanic acid, flavonoids, and harman alkaloids (Wang et al., 2014 and 2015) which favour the medicinal property to the plant. Utilizing *P. farcta* leaves for the green synthesis of metal nanoparticles is a low-cost, energy-free process. Since *P. farcta* has many naturally occurring secondary metabolites, combining AgNPs with P. farcta may result in the development of a promising antimicrobial and mosquitocidal agent that is effective against serious drug-resistant infectious strains while causing miminum cytotoxicity. The leaves of *P. foetida* were utilized for the synthesis of silver nanoparticles to determine the bioefficacy against Aedes aegypti mosquitoes and other bacterial pathogens due to the plant's well-established medicinal benefit and the lack of research on green synthesis of silver nanoparticles. The current study is the first to investigate green synthesis and characterization of silver nanoparticles using an aqueous extract of *P.foetida*.

2. Materials and Methods

2.1. Screening and selection of plants

The plant materials of *P.foetida* (Figure 1) were collected in Erode District, TamilNadu. The plant materials were taxonomically identified and authenticated by the Botanical survey of India, Tamil Nadu Agricultural University, India. The voucher specimen was deposited there for future reference and registered with no: BSI/SRC/5/23/2014-15/Tech-1085. The plants were surface cleaned with tap water followed by sterile distilled water. Initial screening was performed to synthesise the silver nanoparticles from aqueous extracts of plant leaves, stem and fruits. Leaves showed the rapid synthesis of silver nanoparticles. Due to this reason, Leaves had been chosen for the study.

2.2. Preparation of aqueous extract

Fresh plant materials of *P. foetida* were collected for the preparation of the extract.The aqueous extract of the sample was made in two batches by weighing 2g (low concentration throughout the experiment) and 5g (high concentration throughout the investigation) of fresh and surface cleaned leaves, cutting them into pieces with a sterilised knife, and warming them in 100ml of sterile distilled water at 60°C for about 5 minutes.The extract was then filtered through Whatman no.1 filter paper and stored at 4ºC for further experiments.

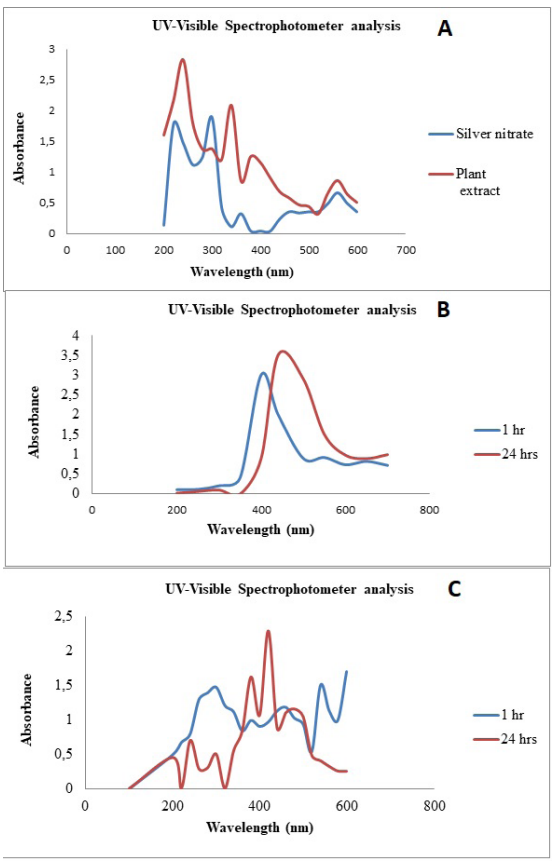

Figure 1. A. UV- Visible Spectroscopy Analysis of Silver nitrate and plant extract (Control). **B.** UV- Visible Spectroscopy Analysis of Silver nanoparticles synthesized using high concentration of plant extract. **C.** UV- Visible Spectroscopy Analysis of Silver nanoparticles synthesized using Low concentration of plant extract.

2.3. Screening for phytometabolites

The presence of preliminary phytochemicals in the aqueous extract of *P. foetida* was tested. Alkaloids,coumarins, flavonoids, glycosides, oils and fats, phenol,phlobatannin, resin, sterol, steroids, saponins, tannins, terpenoids and quinines were screened to analyse the possible phytochemical responsible for the bioreduction process (Aiyegoro and Okoh, 2010).

2.4. Synthesis of silver nanoparticles

The chemical, $AgNO_3$ was purchased from HiMedia Laboratories Pvt. Limited, Mumbai, India and was used as received. For the synthesis of silver nanoparticles, various plant extracts were investigated, but the aqueous extract revealed to be the most consistent and effective. So, in the typical synthesis of silver nanoparticles, 10ml of the aqueous extract from two sets of extracts prepared was added separately to 90ml of 1mM (10^{-3}M) solution of silver nitrate to2 different 250ml Erlenmeyer flasks, respectively. The reaction was carried out at room temperature with the orbital shaker spinning at 120 rpm. Suitable controls were kept in place throughout the experiment. To standardise the data, the protocol was repeated three times at regular time interval.

2.4.1. Characterisation of ecofriendly synthesised silver nanoparticles

The bioreduction of Ag^+ in aqueous solution was monitored by periodic sampling of aliquots (0.2ml) of the suspension, then diluting the samples with 2ml deionised water and subsequently measured UV-Visible spectra at the wavelength of 200 to 600 nm in Elico microprocessors (µp) based UV-Visible Spectrophotometer. UV-Vis spectra were obtained at intervals of between 1 hour and 24 hours for both sets of studies in order to detect the band pattern or difference in the synthesis rate due to a change in the concentration of extract used for the synthesis. The dried Ag nanoparticles were subjected to FTIR analysis for analysing the capping ligand of silver nanoparticles which act as a stabilising agent. X-ray Diffractometer determined the phase nature. The diffracted intensities were recorded from 10° to 90° of 2θ angles, and the values were used to calculate the grain size by using the Scherrer's formula (Equation 1)

$$D = (0.9\lambda) / \beta \frac{1}{2} \cos\theta \quad (1)$$

The size and surface morphology of the Green synthesised silver nanoparticles were determined using a scanning electron microscope. Components including the voltage employed, the magnification used, and the size of the images' contents all were implanted on the photographs themselves. The elemental composition of the particles in the sample was determined using EDAX spectrum analysis. Finally, TEM examination was performed

on the nanoparticle sample to determine the shape and size of the particles synthesised with an aqueous extract of *P.foetida*.

2.5. Synergistic antibacterial efficacy of synthesised silver nanoparticles

Silver nanoparticles synthesised using aqueous extract of *P. foetida* were tested for its potential antibacterial activity against a few bacterial pathogens such as *E. coli* (MTCC 1698*), Klebsiella pneumoniae*(MTCC 10309), *Staphylococcus aureus* (MTCC 3160) and *Bacillus cereus*(MTCC 430) purchased from Microbial Type culture collection, IMTECH,Chandigarh. Agar Well diffusion assay method was followed, which involved the swabbing of 18 hours test inoculums in pre-sterilised nutrient agar plates. Wells were created, and each well was loaded with 100 µl of the solutions in the following order: water as control, aqueous extract of *P. foetida,* silver nitrate solution and silver nanoparticle solution. To test the synergy of silver nanoparticles and antibiotic, tetracycline discs and tetracycline discs loaded with silver nanoparticles were utilized.Then the sample loaded Mueller Hinton agar plates were incubated at 37^0C for 24 hrs. Then the formation of a zone of inhibition was observed (Ibrahim, 2015).

2.6. Larvicidal and pupicidal efficacy of methanol extract of passiflorafoetida and silver nanoparticles

2.6.1. Mosquitoes

Aedes aegypti mosquitoes were reared properly and the larvae were fed on yeast powder and dog biscuits in the 1: 3 ratio. Adults were fed blood through a parafilm membrane and provided with 10% sucrose solution. Mosquitoes were held at 28±2 °C temperature, 70–85% relative humidity, with 12-h light/12-h dark photoperiod. The protocols were followed as per the method described by Muthukumaran et al., 2015 with slight modification.

2.6.1. Activity

The efficacy of the methanol extract of *P. foetida* and silver nanoparticles as Larvicidal and pupicidal against the dengue vector *A.aegypti* mosquito was evaluated with a concentration of 20 - 80ppm and 5 - 25ppm, respectively,according to the guidelines of the World Health Organization (WHO)protocol (2005). In a 500-mL glass beaker containing 249 mL of dechlorinated water, 1 mL of desired amounts of silver nanoparticles, and the plant extract were tested against twenty numbers of 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} instar larvae and pupa.Each test included a set of control groups (silver nitrate and distilled water) with five replicates for each concentration. Five replicates of analysis were carried out for each concentration with a total of 100 larvae. Larval mortality was observed at 24 h after exposure, during which no food was given to the larvae.

2.7. Statistical analysis

The average larval mortality was calculated. The data were subjected to probit analysis for calculating LC50, LC90, and other statistics at 95% confidence limits of upper and lower confidence limits, and chi-squared values were calculated using the Statistical Package of Social Sciences 12.0 software The results with $p<0.05$ will be considered to be statistically significant.

3. Results

3.1. Screening for phytometabolites

The phytochemical screening had shown for the presence of alkaloids, coumarins, flavonoids, glycosides, oils and fats, phenols, resin, sterols, steroids, saponins, tannins and quinines.

3.2. Synthesis of silver nanoparticles

The synthesis of silver nanoparticles were performed in an eco-friendly manner with the aid of plant extracts. The addition of *P. foetida* aqueous leaf extract to a 1mM solution of silver nitrate resulted in the presence of dark reddish-brown colour in the formulation synthesised with a higher amount of the extract. In contrast, synthesis of silver nanoparticles with low concentration of extract shown light-reddish brown colour.

3.2.1. UV-visible spectrophotometer analysis

The rate of silver nanoparticles synthesis was quick, comparable to the chemical method of synthesis. Also, the comparison was done by minimising the leaf broth concentration and silver nitrate concentration. The rate of synthesis was shown to increase as the concentration of leaf broth increased, resulting in a wider peak, whereas the peak was narrowed when the concentration of plant extract lowered, as shown in the figure (Figure 1A, 1B and 1C). The UV-Vis absorption spectrum recorded for the formulation revealed the distinctive surface Plasmon resonance band for synthesized silver nanoparticles in the range of 420-440 nm, which was acceptable to the literature report (Lopez-Lorente and Mizaikoff, 2016).

3.2.2. FTIR analysis

The FTIR spectrum of synthesised nanoparticles was depicted (Figure 2). The peak was found to be prominent at 2883.68 cm^{-1}, 1383.01 cm^{-1}, 1784.21 cm^{-1},1660.77 cm^{-1},1514.17 cm^{-1}, 1548.89 cm^{-1}, 773.48 cm^{-1} and 823.63 cm^{-1} where the functional active groups were discovered to be alkanes or nitrocompounds or aldehydes or amides or secondary amines or halogens or aromatic groups respectively.

3.2.3. SEM analysis

Scanning electron microscopic examination of the reduced form of silver nitrate was observed. It was crystal clear that the heat dried silver particles from the bio-reduced colloidal suspension showed the well-dispersed nanoparticles in both the set of samples. It revealed the surface morphology as the spherical structure with high agglomeration in the nanoparticles synthesised using a high extract concentration (Figure 3A and 3B).

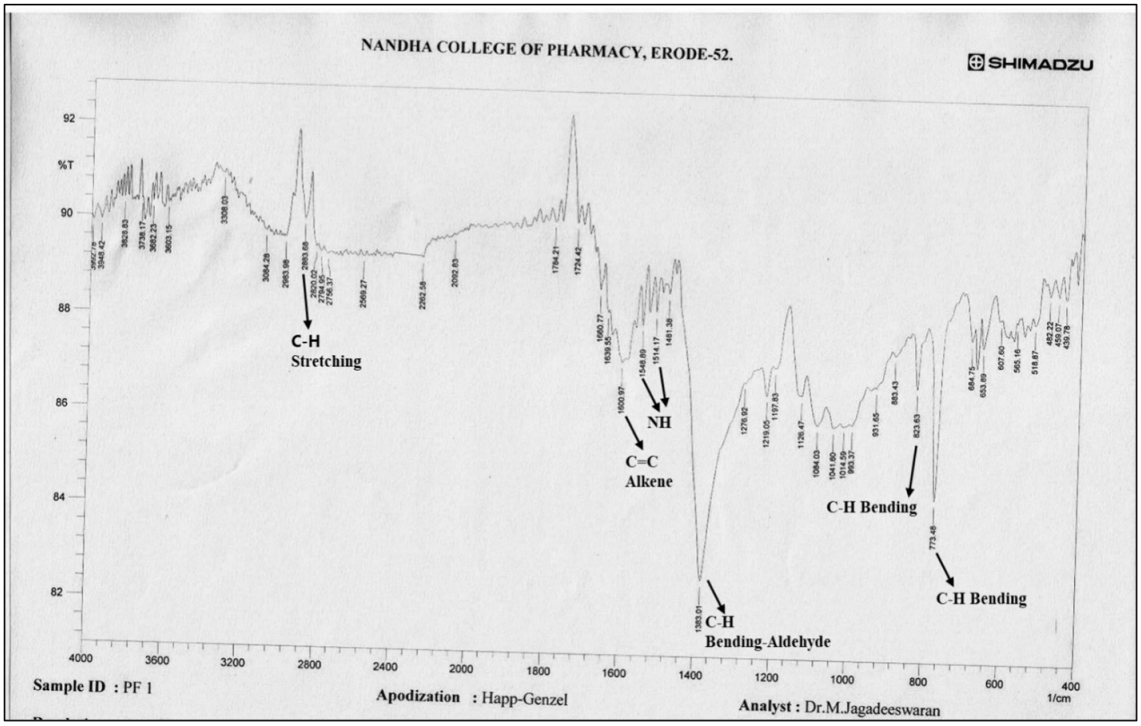

Figure 2. FTIR Spectrum of synthesized nanoparticles of plant extract.

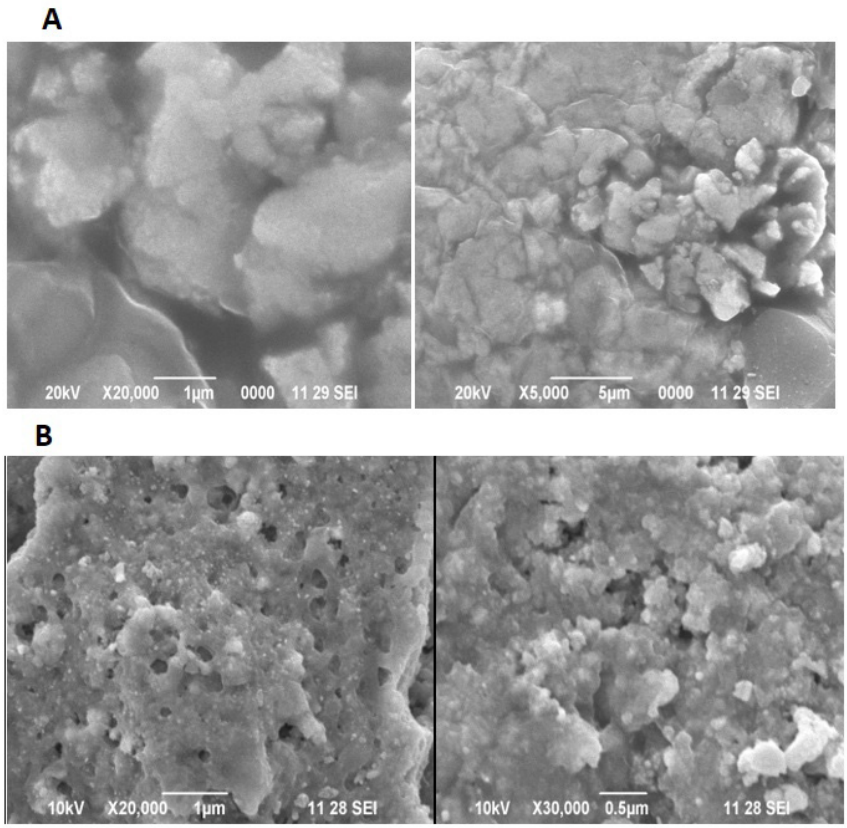

Figure 3. A. SEM images of silver nanoparticles synthesized using high concentration of *Passiflora foetida* extract. **B.** SEM images of silver nanoparticles synthesized using low concentration of *Passiflora foetida extract.*

3.2.4. EDAX analysis

EDAX evaluation shown the qualitative and quantitative analysis of silver nanoparticles synthesis, confirming the reduction of silver ions into silver elements and revealing the presence of elemental silver as the major component. The presence of elemental silver can be observed in the graph obtained from EDAX analysis which confirmed the elemental components of silver (37.57%), chloride (7.82%),silicon (0.75%), Calcium (1.23%) and oxides (49.40%) respectively (Figure 4).

3.2.5. X-Ray diffraction analysis

XRD analysis showed three distinct and strong diffraction peaks at 37.7°, 64.2° and 77.3°. These Braggs reflections revealed the presence of (111), (220) and (311) sets of lattice planes which could be indexed as the face-centred cubic structure of silver respectively concerning JCPDS file no.65-2871(Figure 5). Using Scherrer's formula, the average grain size of the silver nanoparticles produced during the bioreduction process was estimated to be around 11-14.6 nm.

3.2.6. TEM analysis

The formulated silver nanoparticles were examined through Transmission Electron Microscope to observe the morphology, structure and size. The observed TEM images clearly revelaed that few synthesized particles are hexagonal; some are oval, spherical and irregular shaped with size viz., 40nm,56.9nm, 61.5nm, 67.9nm, 68.5 nm, 70.6 nm, 80nm and 100.7nm (Figure 6).The majority of the particles fallen in size range of approximately 60 nm.

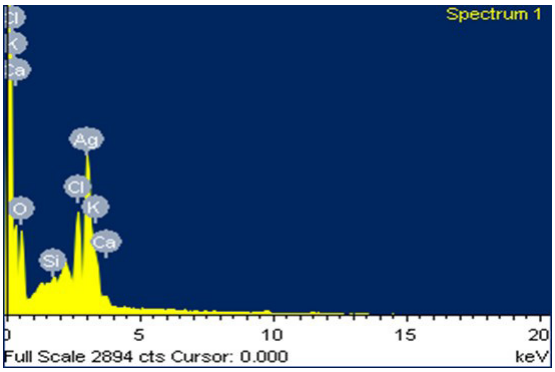

Figure 4. EDAX Spectrum of silver nanoparticles synthesized using low concentration of *Passiflora foetida extract.*

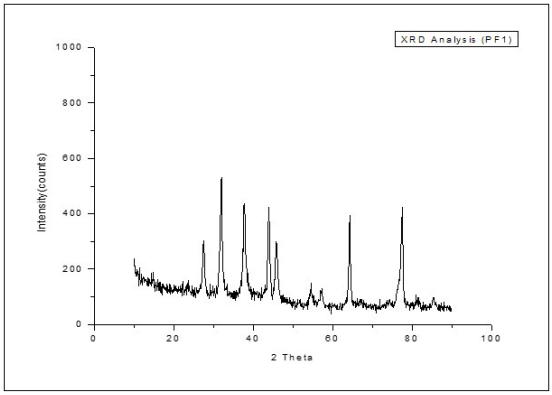

Figure 5. XRD Profile of silver nanoparticles synthesized using low concentration of *Passiflora foetida extract.*

3.3. Synergistic antibacterial efficacy of synthesised silver nanoparticles

The current study also demonstrated the antibacterial potential of silver nanoparticles and their synergetic action with tetracycline antibiotic by the well diffusion method. It was reported (Table 1 and 2) and shown the efficacy with graphical representation (Figure 7, 8A and 8B).The antibacterial activity of silver nanoparticles was higher against *E.coli* and *Staphylococcus aureus* and moderate against *Bacillus cereus* and *Klebsiella pneumoniae* than the salt of silver. As the silver nanoparticles possess extremely larger surface area, the antibacterial effect bactericidal activity was visualised to be higher for silver nanoparticles. In contrast, no zone of inhibition was identified for the aqueous extract of *P. foetida* due to the inadequate antibacterial components.

3.4. Larvicidal and pupicidal efficacy of silver nanoparticles

The Larvicidal and pupicidal effect of methanol extract of *P. foetida* and the plant synthesized Ag NPs was carried out against one to four instarsand pupal stage of *A. aegypti* larvae (Figure 9A and 9B). The mortality rates were observed and shown in Tables 3 and 4. Both plant extract and silver nanoparticles showed concentration-dependent toxic effect against *Aedes aegypti* larvae and also noted that the mortality percentage was decreased for each concentration concerning the developmental stages of larvae which means that each concentration of sample chosen had

Table 1. Screening of secondary metabolites in aqueous extract of *Passiflora foetida.*

S.No.	Secondary metabolite	Result
1	Alkaloids	+
2	Coumarins	+
3	Flavonoids	++
4	Glycosides	+
5	Oils and fats	+
6	Phenols	+
7	Phlobatannin	-
8	Resin	+
9	Sterols	+
10	Steroids	+
11	Saponins	++
12	Tannins	+
13	Terpenoids	-
14	Quinones	+

Table 2. Synergistic Antibacterial Efficacy of Silver nanoparticles synthesized using *Passiflora foetida* with Tetracycline antibiotic.

S.No.	Category of Bacteria	Test Microorganisms	Volume(µl)	Zone of inhibition in Diameter (mm)					
				Control	Aqueous extract	$AgNO_3$	AgNPs	Tetracycline 30mcg/disc	AgNPs with Tetracycline (30mcg)
1	Gram Positive bacteria	*Bacillus cereus (MTCC 430)*	100	0	0	13 ± 0.23	15±	20±	22±
2		*Staphylococcus aureus (MTCC 3160)*	100	0	0	15±	16±	20±	22±
3	Gram Negative bacteria	*Escherichia coli (MTCC 1698)*	100	0	0	16±	18±	23±	25±
4		*Klebsiella pneumoniae (MTCC 10309)*	100	0	0	10±	13±	20±	23±

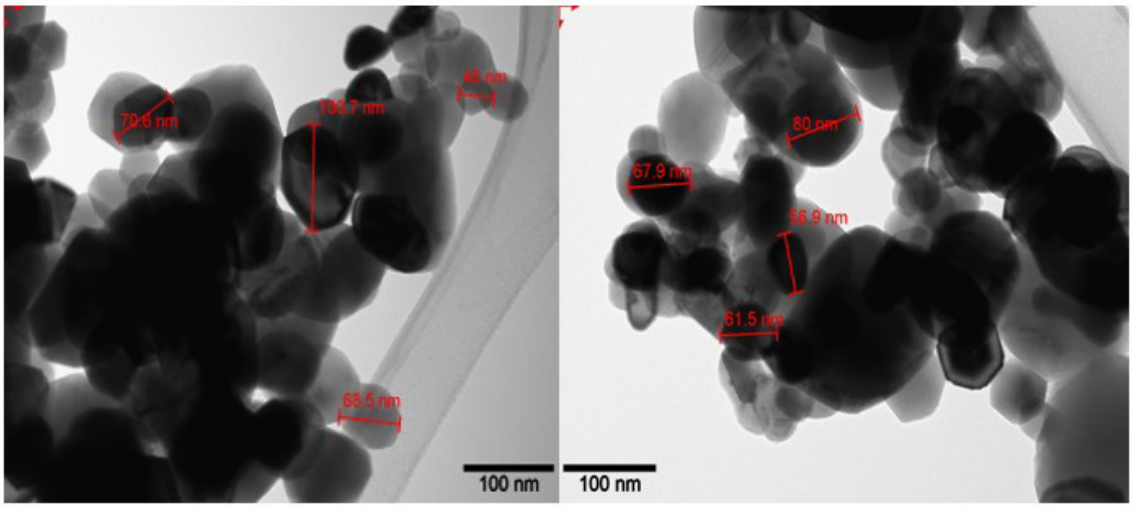

Figure 6. TEM images of silver nanoparticles synthesized using low concentration of *Passiflora foetida* extract.

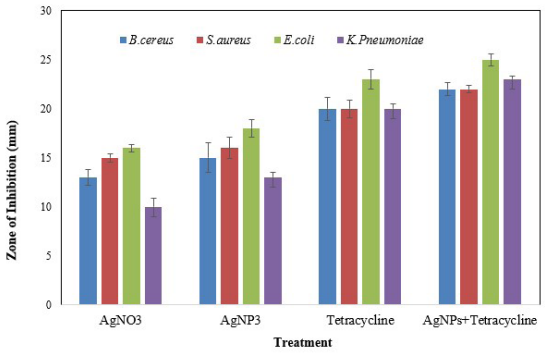

Figure 7. Synergistic antimicrobial action of Silver Nanoparticles with tetracycline

shown higher mortality against first instar larvae and was subsequently decreased against the second, third, fourth instar larvae,and pupal stage of *A.aegypti* and no mortality was observed in the control groups.

The mortality rates for methanol extract of *P. foetida* at 100ppm (higher most concentration used) was found to be 89.2% for I instar, 83.2% for II instar, 75.2% for III instar,66.2% for IV instar and 59.4% for pupa. Simultaneously, the mortality rate of silver nanoparticles at 25ppm (the higher most concentration used) by using were found to be 100% for I instar,97.2% for II instar,92.2% for III instar,87% for IV instar and 80.2% on pupa.Here in the study, the concentration, 20ppm was common for both methanol extract and silver nanoparticles. On comparing the mortality at 20ppm, only the silver nanoparticles had shown the highest mortality against all the four instars and pupae. Moreover, it was found that the silver nanoparticles had shown higher mortality even at reduced concentrations ranging from 5ppm-25ppm. In contrast, the mortality against the larvae and pupa was higher only from the concentration ranging from 20 ppm to 100 ppm for methanol extract of *P. foetida.*

The LC_{50} and LC_{90} values of the methanol extract was 47.847 ppm and 105.143ppm for first instar

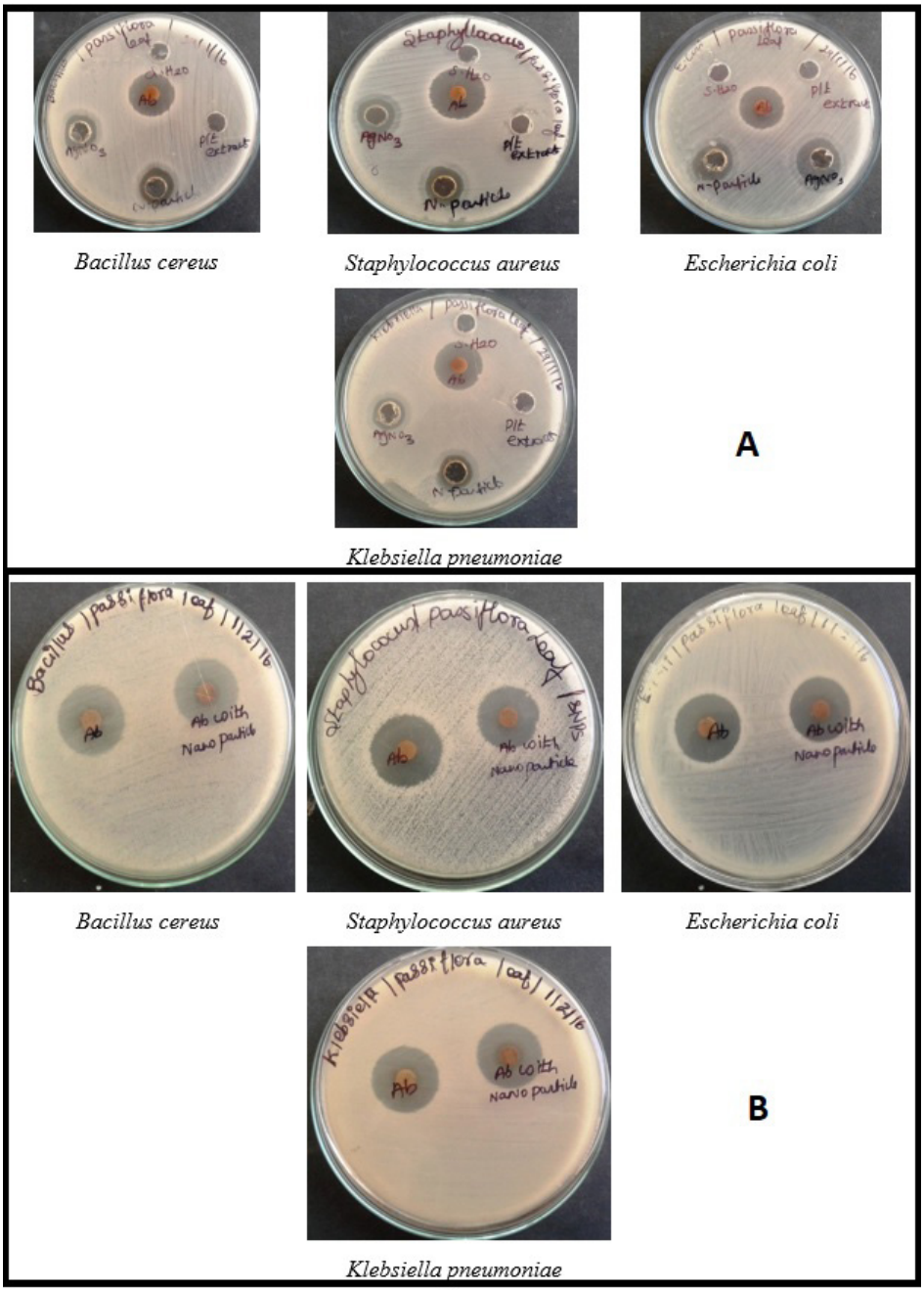

Figure 8. A. Antimicrobial efficacy of silver nanoparticles of *Passiflora foetida* extract. **B.** Synergistic antimicrobial efficacy of silver nanoparticles with tetracycline

larvae,54.36 ppm and 116.59 ppm for II instar, 61.64 ppm and 130.45 ppm for III instar,72.66ppm and 148.11 ppm for IV instar, 88.43 ppm and 168.71 ppm for Pupal stage respectively. Following, the silver nanoparticles had shown 7.1 ppm and 19.3 ppm for I instar, 8.5 ppm and 22.4 ppm for II instar, 9.9 ppm and 25.45 ppm for III instar, 12.2 ppm and 28.1 ppm for IV instar, 14.9 ppm and 30.9ppm for Pupal stage respectively.The LC_{50} and LC_{90} values were also higher for the plant synthesised silver nanoparticles than the methanol extract.

4. Discussion

The phytochemical screening of an aqueous extract of *P.foetida* flavonoids as the major component in the plant, which could be attributed to the environmentally friendly synthesis of silver nanoparticles and may be implicated as a major component in reducing silver nitrate to nanoparticles. The earlier investigation says that the water-soluble components contributed a significant role in reducing silver ions and proved to be effective capping and

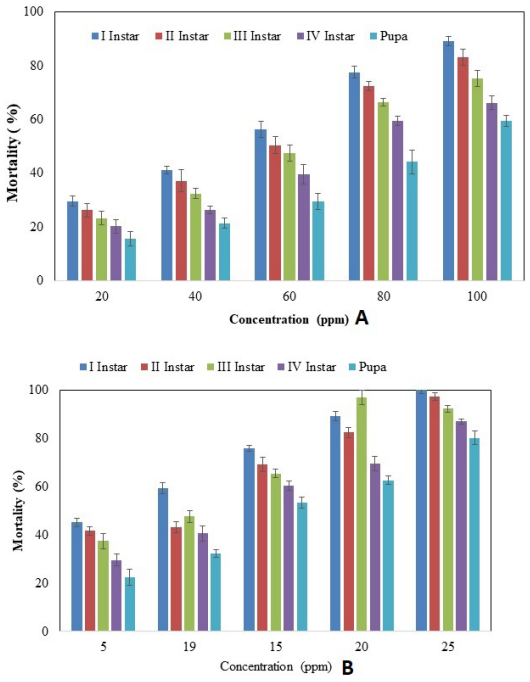

Figure 9. A. Larval and Pupal toxicity effect of methanol extract of *Passiflora foetida.* **B.** Larval and Pupal toxicity effect of silver nanoparticles (AgNPs).

stabilising agents for silver nanoparticles (Valodkar et al., 2010). The extract's varying concentrations were also found to be effective in the synthesis of nanoparticles of the desired size (Bharadwaj et al., 2021)

During the formulation of silver nanoparticles, the pale yellow colour appeared immediately after mixing the aqueous plant extract with silver nitrate. It started to transform the colour from greenish yellow to pale reddish brown within 1 hour. It was completed in about 24hrs with a reddish-brown colour, which was comparable to the synthesis of silver nanoparticles using an aqueous extract of *Eclipta prostrata* (Rajakumar and Abdul Rahuman, 2011) and also found to be incubation dependent. This result was reported to be correlated to a paper that was published on a synthesis employing *Cinnamon camphora* leaves and bark extract of *Cinnamon zeylanicum* (Sathishkumar et al., 2009).The synthesis of silver nanoparticles was observed to be decreased by decreasing the silver nitrate concentration to 0.5mM concerning the studies of Pimprikar et al. (2009). Temperature and pH played an major part in the synthesis of silver nanoparticles, supporting the earlier report (Ibrahim, 2015).The shaking effect also made an effective control over rapid synthesis. The seasonal result was checked where the synthesis was found to be faster in summer and comparatively slower in the winter. The level of phtoconstituents in the plant extract was also analysed to be very crucial in reducing the silver ions (Ahmad et al., 2003), designing the size and maintaining stability without any agglomeration of the particles. The result showing dark brown formation may indicate that the amount of phytocomponents was high, where the synthesis rate was faster, so the particles were found agglomerated.When the concentration was reduced, the synthesis was slightly slower and produced the particles with distinct structures and shown to have less agglomeration. The remarkable events were detected so that the reaction was faster for the leaf broth prepared from matured leaves, very slow for the young leaves and found to be moderate with stability for the combination of both grown and young leaves. Only the freshly prepared aqueous extract showed rapid synthesis,where the synthesis rate was slow for the stored extracts. Moreover, the results from this study had obeyed to the sayings that silver nanoparticles display a UV–Visible absorption maximum in the range of 400–500nm. As a result, this investigation was highly significant for the speedy synthesis of silver nanoparticles. The narrow absorption band observed at 420-440nm is a characteristic of monodispersed AgNPs similar to the earlier report (Maity et al., 2020).

The bioreduction of Ag+ in the aqueous extract was recorded by regular sampling of the synthesis mixture using UV-Vis spectroscopy. Figure 5A shown the UV-Vis spectra recorded from the synthesised solution of silver nitrate and aqueous extract, which served as control and Figure 5. B. revealed the spectrum of synthesised silver nanoparticles using a high concentration of extract, and Figure 5C depicted the spectrum ofsilver nanoparticles synthesised using low extract concentration.The peak observed at a wavelength of 420 to 440 nm detected broader and blunt while synthesis of silver nano particles using high concentration where the peak was sharp and narrow for the particles synthesised using low concentration. Parallely, the control did not show any characteristic peak at 420 to 440nm which indicated the absence of nanoparticles synthesis.

In FTIR spectrum, the strong absorption peak at 1084.03 cm^{-1} can be attributed to –C-O stretching vibrations in the carboxyl group and denoted stretching vibrational bands responsible for compounds like flavonoids (Siddiqui et al., 2000) be held responsible for efficient capping and stabilisation of formed AgNPs. The sharp band at 823.63 cm^{-1} was due to an aromatic ring (Anjana et al., 2021).The C=O stretch at 1660.77 cm^{-1} represented the presence of amide. The strong,broader, and highest absorption peaks referring to N=O bend at 1383.01 cm^{-1} was attributed to the nitro compounds.The stretching vibrations at 2883.68 cm^{-1} indicated the presence of alkane groups.The short C=O stretching vibration at 1784.21 cm^{-1} corresponded to either aldehydes or ketones or ester group. The peak at 1514.17cm^{-1} and 1548.89 cm^{-1} corresponding to the N-H bond indicated the presence of secondary amines. The strong absorption peak at 773.48 cm^{-1}confirmed the presence of a halogenated group (Chlorine). These identified functional groups were the possible biomolecules stabilising the biosynthesised silver nanoparticles.

SEM analysis had shown the clear morphology of silver nanoparticles. The spherical particles were visible in the nanoparticles prepared with the reduced concentration of the extract and agglomeration was reduced with respect to it. It was demonstrated that the concentration of the extract, which in turn implies the amount of phytochemicals, played a vital role in the size-controlled synthesis and stabilisation

Table 3. Larval and pupal toxicity of Methanol extract of *Passiflora foetida*.

Targeted Instars	Larval and pupal mortality					LC_{50} and (LC_{90})	95% confidence Limit		Regression equation	Chi square value
	Concentration of Methanol extract (ppm)						LC_{50} (LC_{90})			
	20	40	60	80	100		LCL	UCL		
I	29.6±2.19	41.4±2.40	56.4±1.14	77.6±0.89	89.2±1.48	47.847 (105.143)	41.766 (95.675)	53.210 (118.658)	X=0.022 Y=-1.070	1.584 n.s
II	26.2±1.64	37.2±1.78	50.4±1.51	72.4±1.14	83.2±1.78	54.369 (116.590)	48.303 (105.261)	60.052 (133.268)	X=0.021 Y=-1.120	1.322 n.s
III	23.2±1.30	32.4±1.51	47.4±2.07	66.4±2.40	75.2±1.64	61.647 (130.459)	55.349 (116.320)	68.092 (152.209)	X=0.019 Y=-1.148	0.797 n.s
IV	20.2±1.64	26.4±1.67	39.6±0.89	59.4±1.51	66.2±2.38	72.665 (148.116)	65.812 (129.996)	80.904 (177.440)	X=0.017 Y=-1.234	1.569 n.s

Mortality rates are means ± SD of five replicates; No mortality was observed in the control; Within each row, means followed by the same letter(s) are not significantly different (P<0.05); LC_{50} = lethal concentration that kills 50% of the exposed organisms=-0987654321`ff c LC_{90} = lethal concentration that kills 90% of the exposed organisms; LCL = lower confidence limit; UCL = upper confidence limit;χ^2 = chi-square value; *d.f.* = degrees of freedom; n.s. = not significant.

Table 4. Larval and pupal toxicity of Silver nanoparticles.

Targeted Instars	Larval and Pupal mortality in %					LC_{50} and (LC_{90}) in ppm	95% confidence Limit		Regression equation	Chi square value (χ^2)
	Concentration of Synthesized AgNps (ppm)						LC_{50} (LC_{90})			
	5	10	15	20	25		LCL	UCL		
I	45.2±2.58	59.4±2.60	75.8±2.28	89.2±2.16	100±0.00	7.186 (19.344)	1.832 (16.098)	10.061 (26.197)	X=0.105 Y=-0.758	5.415 n.s
II	41.6±2.07	53.2±1.78	69.2±2.58	82.4±1.14	97.2±1.48	8.503 (22.479)	6.580 (20.428)	10.003 (25.397)	X=0.092 Y=-0.780	4.478 n.s
III	37.4±1.14	47.6±1.94	65.4±2.07	76.8±1.92	92.2±1.48	9.920 (25.459)	8.001 (22.981)	11.455 (29.103)	X=0.082 Y=-0.818	2.151 n.s
IV	29.6±1.81	40.6±2.07	60.4±1.51	69.6±2.30	87.0±1.87	12.269 (28.197)	10.601 (25.404)	13.731 (32.344)	X=0.080 Y=-0.987	1.552 n.s
Pupa	22.4±1.14	32.2±1.30	53.4±2.19	62.6±0.89	80.2±1.30	14.936 (30.995)	13.443 (27.865)	16.425 (35.676)	X=0.080 Y=-1.192	1.267 n.s

Note: Mortality rates are means ± SD of five replicates, No mortality was observed in the control; Within each row, means followed by the same letter(s) are not significantly different (P<0.05); LC_{50} refers to lethal concentration that kills 50% of the exposed organisms; LC_{90} refers to lethal concentration that kills 90% of the exposed organisms; LCL implies lower confidence limit; UCL = upper confidence limit; χ^2 = chi-square value; and n.s. represent not significant.

of the silver nanoparticles and confirmed the agglomerated condition of the particles could be due to the differences in preparative condition (Dheeban Shankar et al., 2014). (Figures 7A and 7B). The morphological difference in the synthesised nanoparticle could be the reason for the difference in optical properties (Chen et al., 2004). The major sharp signal was noted at 3 keV for silver, which is a key feature for the absorption of crystalline nature of biosynthesized AgNPs (Muthukrishnan et al., 2015; Kanipandian et al., 2014).These results were proved to be supported by the earlier reports of Bhakya et al. (2015).

In X ray diffraction analysis, apart from the Bragg peaks, unassigned peaks observed had indicated the crystallisation of bioorganic phase in the extract that occurred on the surface of the nanoparticles. It was similarly reported in AgNPs synthesized using the leaves of *Catharanthus roseus* (Ponarulselvam et al., 2012) and *Cocos nucifera* coir extract (Roopan et al., 2013). Hence XRD pattern thus clearly showed that synthesized AgNPs in the present study are in the crystalline phase. The AgNPs were also capped by some organic molecules, which make them highly stable. The stabilising molecules are none other than the phytochemicals in *P. foetida* used for the synthesis. The particle size of the AgNPs was observed to be in the range between 40 to 100.7 nm.

Moreover, silver nanoparticles act as stoarge for the Ag^+ bactericidal agent. Several studies had been reported that AgNPs might attach to the surface of the cell membrane thereby disturbing permeability and respiratory functions of the cell. It was observed that the smaller particles with larger surface area for interaction would give more bactericidal effect than the larger particles (Kvitek et al., 2008). It is possible to silver nanoparticle's interaction with the surface of the membrane and thereby penetrate inside the bacteria (Roe et al., 2008). Additionally, through interacting with DNA, proteins, and other phosphorus and sulfur-containing cell elements, silver nanoparticles can dmagae bacterial cells (Marambio-Jones and Hoek, 2010). Finally, it generates an amplified biocide effect by releasing silver ions, which is size and dose-dependent (Liu et al., 2010). As synthesized AgNPs have an effective antibacterial property,they can be applied as antibacterial agents in various applications, from disinfecting medical devices and home appliances to water treatment (Cho et al., 2005). Interestingly in the current investigation, the effects of antibiotics were enhanced with observable effect against all the microbes tested by combining to silver nanoparticles, which might be due to synergistic effect. Similar studies were reported on synergistic action for the antibiotic, Levofloxacin with AgNPs against various types of microorganisms and observed to have 1.26% to 1.30% enhancement in the activity because of synergism (Ibrahim, 2015).

Several studies have been stated on the larvicidal effect of medicinal plants against *A. aegypti* and the comparison with plant synthesised silver nanoparticles have only a fewer report. The larvicidal nature of *P. foetida* using n-hexane, ethyl acetate and ethanol extract against fourth instar larvae of *A. aegypti* was previously reported (Hastutiek et al., 2017) and was observed to show an effective LC_{50} value as 440ppm for n-hexane but was identified to be low when it was tested using methanol extract of *P. foetida* which shown 72.6ppm.

When the methanol extractof *P. foetida* and silver nanoparticles were subjected to mosquiticidal activity,both had played effectively to show mortality. In contrast, silver nanoparticles had an appreciable value of 12.2 ppm, and on following, $\chi 2$ value was not significant at $p \leq 0.05$ level. To the reliability in using nanoparticles as mosquitocidal and aseco friendly, it has found that doses of plant-based AgNPs which resulted in lethality to several species of mosquito larvae and shown nontoxic on other non-target species including aquaticarthropod species and fish (Haldar et al., 2013; Rawani et al., 2013). Similar studies were reported by Govindarajan and Karuppannan (2011) where they used the methanolic extract of *Eclipta alba* against third instar larvae, obtaining an LC_{50} of 127.64 ppm. Sukhthankar et al. (2014) used a methanolic extract of the leaves of *Chromolaena odorata*, obtaining an LC_{50} of 138 ppm, and Tennyson et al. (2007) tested hexane and ethyl acetate extracts of *Ageratum houstonianum,* obtaining LC_{50} values of 8889.13 ppm and 1952.12 ppm, respectively. On the whole, silver nanoparticles had shown better activity than the plant extracts in acceptanceof the previous report on larvicidal activity.The silver nanoparticles had an effective larvicidal action which was comparable to the earlier reports (Muthukumaran et al., 2015). It was known that potential ingredients of the plant protect it from herbivores and are called as secondary metabolites where the insects feed on these phyto-metabolites, potentially interesting toxic constitutents with comparatively non-specific action on a wide range of molecular targets such as enzymes, receptors, signaling molecules, ion-channels, structural proteins, nucleic acids, biomembranes, and other cellular components in case of plant extracts, whereas the structure and functionality of mosquitoes were altered by permeation of the particles into insects cells and thereby binding to macromolecules such as proteins and DNA (Subramaniam et al., 2015; Jadoun et al., 2020). Therefore, the current research had found a better solution and it is visible that AgNPs may possibly be a right choice against mosquito larvae and pupae to make the society with good health.

5. Conclusion

P. foetida grows along roadsides and isn't grazed by cattle, left as huge mass. So an idea to exploit it for a various medical applications to the society induced to have a green method of AgNPs. The currently study had clearly shown that the silver nanoparticles could be a very good choice for antibacterial property with synergistic role where it could be applied in various forms in medical industry. As with current Covid-19 issues, the AgNPs would be recommended to be coated over the masks which may purify the incoming air and may fight against the primary entry of the infectious particles. Also, the current study had made us to initiate the use of AgNPs for mosquitocidal property which may enhance its usage to eliminate or prevent the dengue infection by showing mortality to mosquito. As a result, the research findings presented in

this report have been proven to be an effective approach of investigating natural resources for the purpose of producing useful products for a better society.

Acknowledgements

The authors are thankful to the Department of Biotechnology, Nandha Arts and Science College, Erode, TN, India for providing the facilities to carryout the research work. The research was funded by the Deanship of Scientific Research, King Khalid University, Abha, Saudi Arabia via grant number R.G.P.1/211/41.

References

AHMAD, A., MUKHERJEE, P., SENAPATI, S., MANDAL, D., KHAN, M.I., KUMAR, R.R. and SASTRY, M., 2003. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. *Colloids and Surfaces. B, Biointerfaces*, vol. 28, no. 4, pp. 313-318. http://dx.doi.org/10.1016/S0927-7765(02)00174-1.

AIYEGORO, O.A. and OKOH, A.I., 2010. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. *BMC Complementary and Alternative Medicine*, vol. 10, no. 1, pp. 21. http://dx.doi.org/10.1186/1472-6882-10-21. PMid:20470421.

ANJANA, V.N., JOSEPH, M., FRANCIS, S., JOSEPH, A., KOSHY, E.P. and MATHEW, B., 2021. Microwave assisted green synthesis of silver nanoparticles for optical, catalytic, biological and electrochemical applications. *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 49, no. 1, pp. 438-449. http://dx.doi.org/10.1080/21691401.2021.1925678. PMid:34009083.

BASKAR, K. and IGNACIMUTHU, S., 2012. Antifeedant, larvicidal and growth inhibitory effects of ononitol monohydrate isolated from Cassia tora L. against Helicoverpa armigera (Hub.) and Spodoptera litura (Fab.) (Lepidoptera: noctuidae). *Chemosphere*, vol. 88, no. 4, pp. 384-388. http://dx.doi.org/10.1016/j.chemosphere.2012.02.051. PMid:22436590.

BHAKYA, S., MUTHUKRISHNAN, S., SUKUMARAN, M. and MUTHUKUMAR, M., 2015. Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. *Applied Nanoscience*, vol. 6, no. 5, pp. 755-766. http://dx.doi.org/10.1007/s13204-015-0473-z.

BHARADWAJ, K.K., RABHA, B., PATI, S., SARKAR, T., CHOUDHURY, B.K., BARMAN, A., BHATTACHARJYA, D., SRIVASTAVA, A., BAISHYA, D., EDINUR, H.A., ABDUL KARI, Z. and MOHD NOOR, N.H., 2021. Green synthesis of gold nanoparticles using plant extracts as beneficial prospect for cancer theranostics. *Molecules (Basel)*, vol. 26, no. 21, pp. 6389. http://dx.doi.org/10.3390/molecules26216389. PMid:34770796.

BRUNA, T., MALDONADO-BRAVO, F., JARA, P. and CARO, N., 2021. Silver nanoparticles and their antibacterial applications. *International Journal of Molecular Sciences*, vol. 22, no. 13, pp. 7202. http://dx.doi.org/10.3390/ijms22137202. PMid:34281254.

CHEN, S., WEBSTER, S., CZERW, R., XU, J. and CARROLL, D.L., 2004. Morphology effects on the optical properties of silver nanoparticles. *Journal of Nanoscience and Nanotechnology*, vol. 4, no. 3, pp. 254-259. http://dx.doi.org/10.1166/jnn.2004.034. PMid:15233085.

CHO, M., CHUNG, H., CHOI, W. and YOON, J., 2005. Different inactivation behaviors of MS-2 phage and Escherichia coli in TiO2 photocatalytic disinfection. *Applied and Environmental Microbiology*, vol. 71, no. 1, pp. 270-275. http://dx.doi.org/10.1128/AEM.71.1.270-275.2005. PMid:15640197.

DHEEBAN SHANKAR, P., ANANTHI, P. and NIRUPAMA DEVI, K.H., 2014. Bioinspired synthesis and characterization of silver nanoparticles using an aqueous extract of *Artabotrys hexapetalus*. *Journal of Nanoscience and Nanotechnology*, vol. 2, pp. 66-70.

GNANADESIGAN, M., ANAND, M., RAVIKUMAR, S., MARUTHUPANDY, M., VIJAYAKUMAR, V., SELVAM, S., DHINESHKUMAR, M. and KUMARAGURU, A.K., 2011. Biosynthesis of silver nanoparticles by using mangrove plant extract and th,ir potential mosquito larvicidal property. *Asian Pacific Journal of Tropical Medicine*, vol. 4, no. 10, pp. 799-803. http://dx.doi.org/10.1016/S1995-7645(11)60197-1. PMid:22014736.

GOVINDARAJAN, M. and KARUPPANNAN, P., 2011. Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (*Asteraceae*) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: culicidae). *Asian Pacific Journal of Tropical Medicine*, vol. 4, no. 1, pp. 24-28. http://dx.doi.org/10.1016/S1995-7645(11)60026-6. PMid:21771410.

GOVINDARAJAN, M. and SIVAKUMAR, R., 2012. Adulticidal and repellent properties of indigenous plant extracts against *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: culicidae). *Parasitology Research*, vol. 110, no. 5, pp. 1607-1620. http://dx.doi.org/10.1007/s00436-011-2669-9. PMid:22009267.

HALDAR, K.M., HALDAR, G. and CHANDRA, G., 2013. Fabrication, characterization and mosquito larvicidal bioassay of silver nanoparticles synthesized from aqueous fruit extract of putranjiva, *Drypetes roxburghii* (Wall.). *Parasitology Research*, vol. 112, no. 4, pp. 1451-1459. http://dx.doi.org/10.1007/s00436-013-3288-4. PMid:23338978.

HASTUTIEK, P., SUNARSO, A. and PRASETYO, R.H., 2017. Permot (*Passiflora foetida* Linn.) leaf extracts as bioinsecticide again,st *Aedes aegypti* larvae. *The Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 48, pp. 1169-1174.

HOSEINZADEH, E., MAKHDOUMI, P., TAHA, P., HOSSINI, H., STELLING, J., KAMAL, M.A. and ASHRAF, G.M., 2017. A review on nano-antimicrobials: metal nanoparticles, methods and mechanisms. *Current Drug Metabolism*, vol. 18, no. 2, pp. 120-128. http://dx.doi.org/10.2174/1389200217666161201111146. PMid:27908256.

HUANG, S.H., XIAN, J.D., KONG, S.Z., LI, Y.C., XIE, J.H., LIN, J., CHEN, J.N., WANG, H.F. and SU, Z.R., 2014. Insecticidal activity of pogostone against Spodoptera litura and Spodoptera exigua (Lepidoptera: noctuidae). *Pest Management Science*, vol. 70, no. 3, pp. 510-516. http://dx.doi.org/10.1002/ps.3635. PMid:23966130.

IBRAHIM, H.M.M., 2015. Green Synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of Radiation Research and Applied Sciences*, vol. 8, no. 3, pp. 265-275. http://dx.doi.org/10.1016/j.jrras.2015.01.007.

JADOUN, S., ARIF, R., JANGID, N.K. and MEENA, R.K., 2020. Green synthesis of nanoparticles using plant extracts: a review. *Environmental Chemistry Letters*, vol. 19, no. 1, pp. 355-374. http://dx.doi.org/10.1007/s10311-020-01074-x.

KANIPANDIAN, N., KANNAN, S., RAMESH, R., SUBRAMANIAN, P. and THIRUMURUGAN, R., 2014. Characterization, antioxidant and cytotoxicity evaluation of green synthesized silver nanoparticles using *Cleistanthus collinus* extract as surface modifier. *Materials Research Bulletin*, vol. 49, pp. 494-502. http://dx.doi.org/10.1016/j.materresbull.2013.09.016.

KOVENDAN, K., MURUGAN, K. and VINCENT, S., 2012. Evaluation of larvicidal activity of Acalypha alnifolia Klein ex Willd. (Euphorbiaceae) leaf extract against the malarial vector,

Anopheles stephensi, dengue vector, Aedes aegypti and Bancroftian filariasis vector, Culex quinquefasciatus (Diptera: culicidae). *Parasitology Research*, vol. 110, no. 2, pp. 571-581. http://dx.doi.org/10.1007/s00436-011-2525-y. PMid:21748350.

KVITEK, L., PANACEK, A., SOUKUPOVA, J., KOLAR, M., VECEROVA, R., PRUCEK, R., HOLECOVA, M. and ZBORIL, R., 2008. Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). *Journal of Physical Chemistry*, vol. 112, pp. 5825-5834.

LIU, J., SONSHINE, D.A., SHERVANI, S. and HURT, R.H., 2010. Controlled release of biologically active silver from nanosilver surfaces. *ACS Nano*, vol. 4, no. 11, pp. 6903-6913. http://dx.doi.org/10.1021/nn102272n. PMid:20968290.

LOPEZ-LORENTE, A.I. and MIZAIKOFF, B., 2016. Recent advances on the characterization of nanoparticles using infrared spectroscopy. *Trends in Analytical Chemistry*, vol. 84, pp. 97-106. http://dx.doi.org/10.1016/j.trac.2016.01.012.

MAITY, G.N., MAITY, P., CHOUDHURI, I., SAHOO, G.C., MAITY, N., GHOSH, K., BHATTACHARYYA, N., DALAI, S. and MONDAL, S., 2020. Green synthesis, characterization, antimicrobial and cytotoxic effect of silver nanoparticles using arabinoxylan isolated from Kalmegh. *International Journal of Biological Macromolecules*, vol. 162, pp. 1025-1034. http://dx.doi.org/10.1016/j.ijbiomac.2020.06.215. PMid:32599238.

MARAMBIO-JONES, C. and HOEK, E.M.V., 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research*, vol. 12, no. 5, pp. 1531-1551. http://dx.doi.org/10.1007/s11051-010-9900-y.

MUTHUKRISHNAN, S., BHAKYA, S., KUMAR, T.S. and RAO, M.V., 2015. Biosynthesis, characterization and antibacterial effect of plant-mediated silver nanoparticles using *Ceropegia thwaitesii*: an endemic species. *Industrial Crops and Products*, vol. 63, pp. 119-124. http://dx.doi.org/10.1016/j.indcrop.2014.10.022.

MUTHUKUMARAN, U., GOVINDARAJAN, M. and RAJESWARY, M., 2015. Mosquito larvicidal potential of silver nanoparticles synthesized using Chomelia asiatica (Rubiaceae) against Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus (Diptera: culicidae). *Parasitology Research*, vol. 114, no. 3, pp. 989-999. http://dx.doi.org/10.1007/s00436-014-4265-2. PMid:25544703.

PALADINI, F. and POLLINI, M., 2019. Antimicrobial silver nanoparticles for wound healing application: progress and future trends. *Materials (Basel)*, vol. 12, no. 16, pp. 2540. http://dx.doi.org/10.3390/ma12162540. PMid:31404974.

PIMPRIKAR, P.S., JOSHI, S.S., KUMAR, A.R., ZINJARDE, S.S. and KULKARNI, S.K., 2009. Influence of biomass and gold salt concentration on nanoparticle synthesis by the tropical marine yeast Yarrowia lipolytica NCIM 3589. *Colloids and Surfaces. B, Biointerfaces*, vol. 74, no. 1, pp. 309-316. http://dx.doi.org/10.1016/j.colsurfb.2009.07.040. PMid:19700266.

PONARULSELVAM, S., PANNEERSELVAM, C., MURUGAN, K., AARTHI, N., KALIMUTHU, K. and THANGAMANI, S., 2012. Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 7, pp. 574-580. http://dx.doi.org/10.1016/S2221-1691(12)60100-2. PMid:23569974.

RAJAKUMAR, G. and ABDUL RAHUMAN, A., 2011. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. *Acta Tropica*, vol. 118, no. 3, pp. 196-203. http://dx.doi.org/10.1016/j.actatropica.2011.03.003. PMid:21419749.

RAJESHKUMAR, S. and BHARATH, L.V., 2017. Mechanism of plant-mediated synthesis of silver nanoparticles: a review on biomolecules involved, characterisation and antibacterial activity. *Chemico-Biological Interactions*, vol. 273, pp. 219-227. http://dx.doi.org/10.1016/j.cbi.2017.06.019. PMid:28647323.

RAWANI, A., GHOSH, A. and CHANDRA, G., 2013. Mosquito Larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of *Solanum nigrum* L. (Solanaceae: solanales). *Acta Tropica*, vol. 128, no. 3, pp. 613-622. http://dx.doi.org/10.1016/j.actatropica.2013.09.007. PMid:24055718.

REISS, P., COUDERC, E., DE GIROLAMO, J. and PRON, A., 2011. Conjugated polymers/semiconductor nanocrystals hybrid materials-preparation, electrical transport properties and applications. *Nanoscale*, vol. 3, no. 2, pp. 446-489. http://dx.doi.org/10.1039/C0NR00403K. PMid:21152569.

ROE, D., KARANDIKAR, B., BONN-SAVAGE, N., GIBBINS, B. and ROULLET, J., 2008. Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *The Journal of Antimicrobial Chemotherapy*, vol. 61, no. 4, pp. 869-876. http://dx.doi.org/10.1093/jac/dkn034.

ROOPAN, S.M., ROHIT., MADHUMITHA, G., RAHUMAN, A.A., KAMARAJ, C., BHARATHI, A. and SURENDRA, T.V., 2013. Low-cost and ecofriendly phyto-synthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. *Industrial Crops and Products*, vol. 43, pp. 631-635. http://dx.doi.org/10.1016/j.indcrop.2012.08.013.

ROY, A., 2021. Plant derived silver nanoparticles and their therapeutic applications. *Current Pharmaceutical Biotechnology*, vol. 22, no. 14, pp. 1834-1847. http://dx.doi.org/10.2174/1389201021666201027155708. PMid:33109040.

SATHISHKUMAR, M., SNEHA, K., WON, S.W., CHO, C.W., KIM, S. and YUN, Y.S., 2009. Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. *Colloids and Surfaces. B, Biointerfaces*, vol. 73, no. 2, pp. 332-338. http://dx.doi.org/10.1016/j.colsurfb.2009.06.005. PMid:19576733.

SIDDIQUI, B.S., AFSHAN, F., GHIASUDDIN, FAIZI, G., NAQVI, S.N.H. and TARIQ, R.M., 2000. Two insecticidal tetranortriterpenoids from *Azadirachta indica. Phytochemistry*, vol. 53, no. 3, pp. 371-376. http://dx.doi.org/10.1016/S0031-9422(99)00548-8. PMid:10703059.

SINGH, R., SHEDBALKAR, U.U., WADHWANI, S.A. and CHOPADE, B.A., 2015. Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. *Applied Microbiology and Biotechnology*, vol. 99, no. 11, pp. 4579-4593. http://dx.doi.org/10.1007/s00253-015-6622-1 PMid:25952110.

SUBRAMANIAM, J., MURUGAN, K., PANNEERSELVAM, C., KOVENDAN, K., MADHIYAZHAGAN, P., KUMAR, P.M., DINESH, D., CHANDRAMOHAN, B., SURESH, U., NICOLETTI, M., HIGUCHI, A., HWANG, J.S., KUMAR, S., ALARFAJ, A.A., MUNUSAMY, M.A., MESSING, R.H. and BENELLI, G., 2015. Ecofriendly control of malaria and arbovirus vectors using the mosquitofish *Gambusia affinis* and ultra-low dosages of *Mimusops elengi*-synthesized silver nanoparticles: towards an integrative approach? *Environmental Science and Pollution Research International*, vol. 22, no. 24, pp. 20067-20083. http://dx.doi.org/10.1007/s11356-015-5253-5. PMid:26300364.

SUKHTHANKAR, J., KUMAR, H., GODINHO, M. and KUMAR, A., 2014. Larvicidal activity of methanolic leaf extracts of plant, *Chromolaena odorata* L. (*Asteraceae*) against vector mosquitoes. *International Journal of Mosquito Research*, vol. 33, pp. 33-38.

TENNYSON, S., RAVINDRAN, J., EAPEN, A. and WILLIAM, J., 2007. Larvicidal activity of *Ageratum houstonianum* Mill. (*Asteraceae*) leaf extracts

against *Anopheles stephensi*, *Aedes aegypti* and Melanotaeniidae). *Journal of Economic Entomology*, vol. 100, pp. 61-65.

TIWARI, P.M., VIG, K., DENNIS, V.A. and SINGH, S.R., 2011. Functionalized gold nanoparticles and their biomedical applications. *Nanomaterials (Basel, Switzerland)*, vol. 1, no. 1, pp. 31-63. http://dx.doi.org/10.3390/nano1010031. PMid:28348279.

VALODKAR, M., BHADORIA, A., POHNERKAR, A.J., MOHAN, M. and THAKORE, S., 2010. Morphology and antibacterial activity of carbohydrate-stabilized silver nanoparticles. *Carbohydrate Research*, vol. 345, no. 12, pp. 1767-1773. http://dx.doi.org/10.1016/j.carres.2010.05.005. PMid:20591419.

WANG, D.M., XU, Y.F., CHEN, Z., HUANG, L.F. and CHEN, S.L., 2015. UPLC/Q-TOF-MS analysis of iridoid glycosides and metabolites in rat plasma after oral administration of Paederia scandens extracts. *Chinese Journal of Natural Medicines*, vol. 13, no. 3, pp. 215-221. http://dx.doi.org/10.1016/S1875-5364(15)30007-8. PMid:25835366.

WANG, L., JIANG, Y., HAN, T., ZHENG, C. and QIN, L., 2014. A phytochemical, pharmacological and clinical profile of Paederia foetida and P. scandens. *Natural Product Communications*, vol. 9, no. 6, pp. 879-886. http://dx.doi.org/10.1177/1934578X1400900640. PMid:25115105.

YASIN, S., LIU, L. and YAO, J., 2013. Biosynthesis of silver nanoparticles by bamboo leaves extract and their antimicrobial activity. *Journal of Fiber Bioengineering and Informatics*, vol. 6, no. 1, pp. 77-84. http://dx.doi.org/10.3993/jfbi03201307.