wjpmr, 2022, 8(9), 38-43

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Review Article

ISSN 2455-3301

WJPMR

LARVICIDAL NANOPARTICLE TO CONTROL AEDES AEGYPTI

Nora Harminarti[1] and Jeanne Adiwinata Pawitan[1,2,3,4*]

[1]Doctoral Program in Biomedical Sciences, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[2]Department of Histology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[3]Stem Cell Medical Technology Integrated Service Unit, Cipto Mangunkusumo Central Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[4]Stem Cell and Tissue Engineering Research Center, Indonesia Medical Education and Research Institute (IMERI), Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

***Corresponding Author: Dr. Jeanne Adiwinata Pawitan**

Doctoral Program in Biomedical Sciences, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.

Article Received on 30/06/2022 **Article Revised on 20/07/2022** **Article Accepted on 10/08/2022**

ABSTRACT

Arboviruses, which cause diseases such as yellow fever, dengue, chikungunya, and zika, were transmitted by *Aedes aegypti*. Control of *Aedes aegypti* mosquitoes might involve various attempts, but mostly uses chemical insecticides. The use of the chemicals causes resistance and not safe for the environment. Therefore, another alternative is needed such as the use of natural product. Many studies developed silver nanoparticles (AgNPs) as *Aedes aegypty* larvicida using various plant-extracts as a reducing agent, such as extract from apple, *D. trifoliata*, *B. kewensis*, *A. nilagirica*, *L. Aspera*, *H. suaveolens*, and *S. mammosum*. These plants were used to avoid chemical use that might cause pollution of the environment. Various parts of plants have been used to produce AgNPs, and characterization of the biosynthesized AgNPs was done by various methods, such as UV-visible spectra, XRD, EDS, SEM, and HR-TEM.

KEYWORDS: larvicidal, nanoparticle, *Aedes aegypti*, bioimaging.

INTRODUCTION

Infectious diseases are still a health problem globally, among which are infectious diseases due to the arbovirus group that is transmitted by *Aedes aegypti* mosquitoes. Vector-borne diseases (VBDs) such as malaria, dengue, schistosomiasis, leishmaniasis, Chagas disease, yellow fever, and Japanese encephalitis are the cause of death of more than 700 000 each year. More than 80 % of the global population lives in areas at risk of at least one major vector-borne disease. Vector-borne diseases might take an immense toll on economies and can impede both rural and urban development. Around the world, dengue is common in more than 100 countries. Forty percent of the world's population, or 3 billion people, live in areas with a risk of dengue, and every year, up to 400 million people get infected with dengue. From those infected, approximately 100 million people get sick from the infection.[1, 2]

Control of *Aedes aegypti* mosquitoes might involve various attempts, such as using chemical insecticides. The use of chemical insecticides started to report many cases of resistance and disturbance of the biological system environment that caused resurgence in mosquito populations. Therefore, another alternative is needed to overcome the resistance to chemical insecticides. Larvicide is a material that is used to kill mosquito larvae such as *Aedes aegypti*. The use of chemicals to control larvae, which live in water, will pollute the surrounding water environment. Therefore alternatives to replace chemicals with natural materials are needed. In order to use natural materials more efficiently, currently many studies using nanoparticles are underway. Several studies on larvicidal nanoparticles used bioimaging methods to see the characteristics of these nanoparticles.[3] Several reports on nanoparticle manufacturing techniques showed the development of nanotechnology to produce nanoparticles, especially plant derived silver nanoparticles (AgNPs) since they have been proven to be useful in parasitology as pest control.[4-6] Field experiments showed the effectiveness of plant derived mosquito larvicidal nanoparticles. This paper aimed to provide information on AgNP mechanism of action, manufacture of *Aedes aegypti* larvicide nanoparticles using natural materials from several plants, and their characterization using various bio-imaging tools.

AGNP MECHANISM OF ACTION

It has been hypothesized that the bio-toxicity of AgNP against mosquito larvae may be related to the ability of the nanoparticles to penetrate through the exoskeleton.

Once in the intracellular space, nanoparticles can bind to sulfur moiety of proteins or to phosphorus from DNA, leading to rapid denaturation of enzymes and destruction of organelles. Subsequently, a decrease in membrane permeability and disturbance in proton motive force may cause loss of cellular function and cell death (Figure 1).[6]

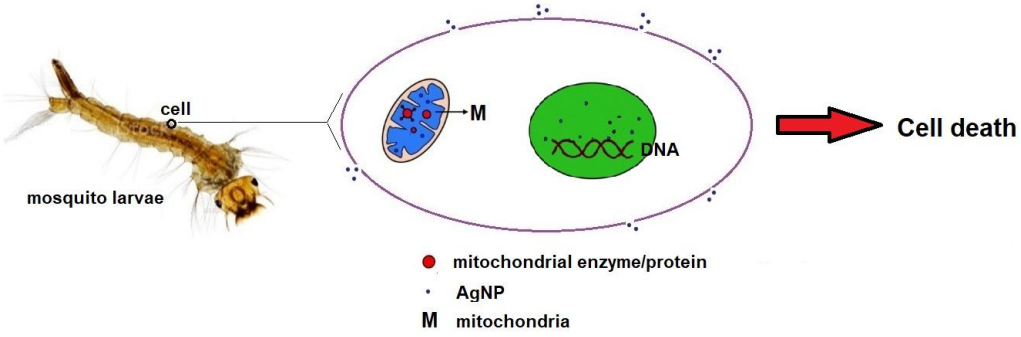

Figure 1: AgNP mechanism of action as a larvicide.

MANUFACTURE OF AEDES AEGYPTI LARVICIDAL NANOPARTICLES FROM PLANTS

Silver is classified as a precious metal. Nanoparticles are the smallest material, and their dimension range from 1nm to 100 nm in size. They are at least one dimension smaller than 1 micron and their size is as small as atomic and molecular length scales (up to 0.2 nm).[4,5] AgNPs can be synthesized through three different methods: physical, chemical, and biological synthesis. Biological methods are preferred and are thus well studied. Biological methods are alternatives to chemical synthesis, which might cause environmental pollution, to manufacture silver nanoparticles. The application of biological synthesis is cost-effective, simple, and environmentally friendly as it does not use hazardous or toxic chemicals. This method has been given much attention as it produces a high yield of AgNPs and it replaces chemicals with plant, plant extracts, bacteria, or small bioactive molecules such as amino acids.[5]

AgNPs can be produced using reducing agents that might be derived from plants. Plants may contain phytochemicals such as flavones, carboxylic acids, terpenoids, ketones, aldehydes, and amines. Flavones, organic acids and quinones may act as reducing agents and can be used in reduction of Ag ions. Whole plant or its extract can be used, but the concentration of the reducing agent is higher in the extract compared to the whole plant. The method of nanoparticle (NP) synthesis involves the mixing of a plant extract with a metal salt aqueous solution. This process is conducted at room temperature and may take minutes to few hours to complete.[7]

The benefits of using plants to manufacture nanoparticles are that plants are easily obtained, safe to handle, and contain various reducing agents that can be used in the reduction of Ag ion. Moreover, the use of plant derived materials is ecofriendly, low in cost, and not harmful to human health. Various plant parts like roots, stem, latex, leaves, and seeds have been used in AgNP synthesis. Plant derived AgNPs show a wide spectrum of pharmacological activities, effective, environmentally safe, eco-friendly, non-toxic, bio-degradable, and recyclable.[8-12]

Limitation of AgNPs is their quick reaction with oxygen, which forms a strong ionic bond that leads to oxidation. Oxidation of AgNPs will alter their structure, thus changing their physicochemical properties. Another limitation of AgNPs is the tendency to form aggregates.[5] To overcome these limitations, oxidation and aggregate formation can be prevented by adding human serum albumin (HSA) to stabilize AgNPs. Using HSA as a stabilizing agent in AgNP production is a practical solution to overcome issues with AgNP stability and is effective in a range of nanoparticle sizes. However, HSA forms a corona around the AgNP that interferes with its uptake into cells.[13]

Method of preparation of *Aedes aegypti* larvicidal nanoparticles from plants

Various plant extracts, which were derived from apple, *D. trifoliata*, *B. kewensis*, *A. nilagirica*, *L. aspera*, *H. suaveolens*, *S. mammosum*, and *H. antidysenterica*, were used in the synthesis of AgNPs that are potential larvicidal agents against dengue vector *Aedes aegypti*.[10,11,14-17] A study on the synthesis of AgNPs used apple extract. The extract was made from about 100 g of apple that was cut into pieces, put in 200 ml of de-ionized water, and heated for 1 hour at 80°C. The extract was then filtered using a filter paper, and the filtrate was used as a reducing agent for AgNP production. Production of AgNPs was carried out by mixing 50 ml of the apple extract with 50 ml of 0.1 M AgNO3 aqueous solution. Two types of AgNPs were produced: by heating (referred as AgNPs-T) and non-heating (referred as AgNPs-RT) methods. To produce AgNPs-RT, the mixture was left for 24 hour and the initial colorless mixture turned into dark-brownish solution. To produce

AgNPs-T, the mixture was heated at 80°C for 60 minutes, and black-brownish precipitation of AgNPs was formed at the bottom of the flask. In both procedures the precipitates were rinsed with de-ionized water for few times, and dried in an oven at 100°C. Before use, characterization and anti-larvicidal test were conducted.[14] The same procedure can be applied to produce AgNPs using another plant extract (Figure 2).

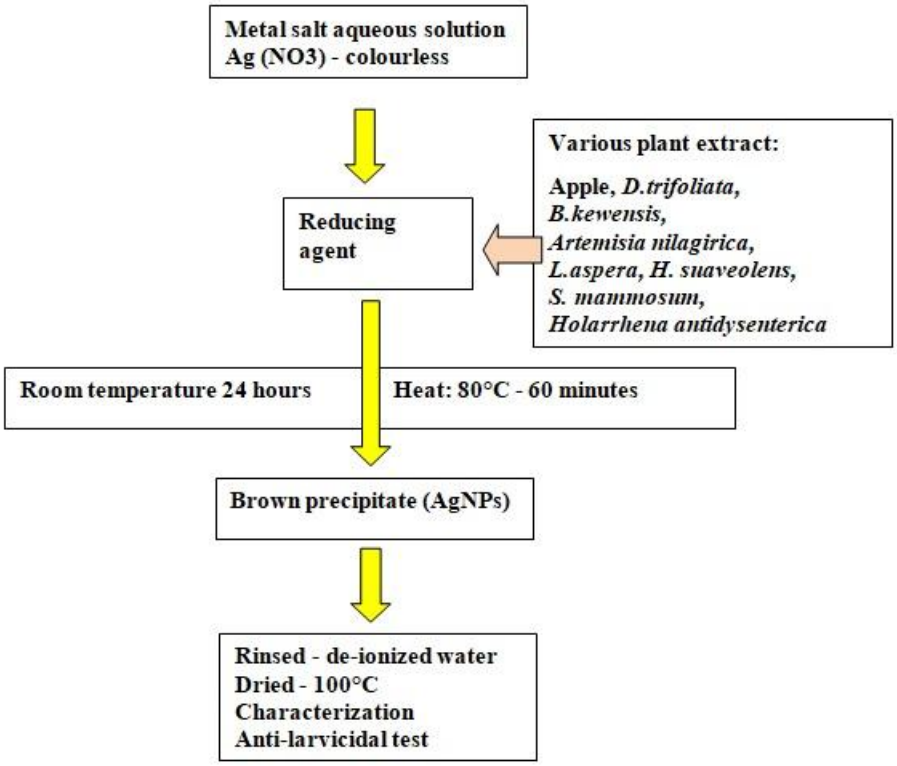

Figure 2: AgNP larvicide production using plant extracts.

CHARACTERIZATION OF LARVICIDE AGNPS

Characterization of larvicide AgNPs can be conducted by various techniques, including spectroscopic, microscopic and diffraction techniques, such as UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, energy dispersive spectroscopy (EDS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD) to determine their size, morphology, porosity, crystalline arrangement, and phase of the nanomaterials.[18,19]

UV-Visible Spectrophotometers

A UV-visible spectrophotometer measures the intensity of light that is passing through a sample. It is widely used to study particles and biochemical processes. It enables quantification of substances in micro-molar concentrations. It has a broad application as most biochemical compounds absorb light in the UV–visible region. However, this technique often results in interferences during the determination of the compound/particle of interest, but the interferences can be minimized in the presence of strong background absorption or by using differential spectrophotometry.[20]

UV-visible spectrophotometers can be used to evaluate the stability and characteristics of AgNPs. Besides particle size, absorption of AgNPs also depend on the medium that forms their chemical environment that might be a poor conductor, but an efficient supporter of electrostatic fields.[5] Compared to the bulk material, nanomaterials have a highly reduced size to nano-scale particles ranging from 2 to 100 nm, which causes a shift towards a shorter absorption wavelength due to surface plasmon resonance (SPR).[19]

Many studies on larvicidal nanoparticle used UV–visible spectroscopy to monitor color changes of the mixture in wavelength region of 400–500 nm due to SPR. Synthesis of mosquito larvicidal silver nanoparticles using plant reported that formation of silver nanoparticles exhibited change of color from light yellow to dark brown. The studies mixed a silver nitrate solution and commercially available plant powders, such as *Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis*. Silver ions were reduced to silver nanoparticles after 1, 24 and 48 hours of reaction, and change in color of the solution indicated the formation of silver nanoparticles.[5,21] In a study, the formation of AgNPs

using *Annona glabra* (Annonaceae) aqueous extract was observed by shift in absorption due to SPR at 435 nm using a UV-visible spectrophotometer.[22]

Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy can be used to characterize various chemical bonding in nanomaterials and observe molecular interactions using electromagnetic radiation at wavelengths of 400–4000 cm^{-1}.[5]

Fourier transform infrared spectrophotometer provides an infrared (IR) spectrum that is emitted from a source, and subsequently passes through an interferometer where the spectral encoding takes place. The combination of beams with different path lengths that passes the interferometer creates constructive and destructive interference, which is called an interferogram. When the beam enters the sample compartment, the sample absorbs specific frequencies of energy from the interferogram, which are unique characteristics of the sample. Then, the detector measures the interferogram signals in energy versus time for all frequencies simultaneously. In the meantime, a beam is superimposed to serve as a reference (background) of the instrument. Finally, the desirable spectrum is obtained by automatically subtracting the background from the sample spectrum by Fourier transformation computer software.[23]

A study reported synthesis of AgNPs using *Azadirachta indica* aqueous leaf extract, and to identify the compound that was responsible for reduction of silver ions, the functional groups that were present in the plant extract were investigated by FTIR. FTIR results concluded that some of the bioorganic compounds from A. indica extract formed a strong coating/capping on AgNPs. The extract sample showed a wide and strong peak with a maximum intensity at 553 cm^{-1}. The results were in good agreement with those found in previous studies.[24] Another study using *Annona glabra* extraxt to form AgNPS showed that FTIR spectrum of the extract sample had three prominent peaks at 1739 cm^{-1}, 1366cm^{-1} and 1217 cm^{-1}, which indicated the characteristic functional groups of phytochemicals. This findings confirmed that the AgNPs were coated by the phytochemicals of *A. glabra*.[22]

Scanning Electron Microscope (SEM) and Energy dispersive X-ray (EDX) Analysis

Scanning electron microscopy (SEM) uses electron beams, which are scattered across the surface of a particle, and the emitted secondary electron beams are detected. A more sophisticated SEM may use a cathode to emit electrons under a very high electric field to give better images that is called field emitter (FE) SEM.[19] SEM, which has a magnification of 10 – 300.0000 times, depth of field 4 – 0.4 mm, and resolution of 1 – 10 nm, can be used to investigate the surface morphology, measure the size of AGNPs, and count their number using a specific software.[5] Moreover, SEM is valuable to determine the purity and aggregation state of AgNPs. However, the method that needs sample drying and the electron beam can cause degradation of AgNPs, so that analysis of surface morphology, size and counting the number should be done on a limited sample. A newer technique, which combines SEM and energy dispersive X-ray spectroscopy (EDX/EDS), does not require sample drying; thus prevents degradation of the sample and allows measurement of AgNP image at larger depths.[19]

A study used *Annona glabra* (Annonaceae) aqueous leaf extract to synthesize AgNPs (An-AgNPs), which showed a strong larvicidal activity, against dengue vector mosquitoes, *A. aegypti* and *A. albopictus*. SEM was used to analyze the surface morphology and particle distribution of AgNPs and the results showed that An-AgNPs were roughly spherical, and were a mixture of several sizes.[22]

X-ray Diffraction (XRD) Analysis

X-ray Diffraction (XRD) analysis involves projection of X-ray beam into a sample to study its scattering pattern by the sample atoms. In case of AgNPs, the scattered X-rays from AgNPs interfere with each other to produce a characteristic diffraction pattern that gives insights on the structure of AgNPs. XRD analysis may easily detect defects in AgNP crystal, its resistance level to stress, its texture, size and degree of crystallinity, and any other variables that are related to AgNP basic structure.[19,25] XRD analysis has been extensively used for AgNP analysis to measure its crystallinity degree at atomic scale. Moreover, XRD analysis can be used for compounds identification, and to determine structure imperfections.[5, 19]

High Resolution Transmission Electron Microscopic (HR-TEM) Analysis

Transmission electron microscopy (TEM) is a microscope that can visualize an object up to two million times of magnification. Advantages of TEM for AgNP analysis are in its high resolution capacity (up to 0.5 nm) and the diffraction pattern can be obtained along with the image. The diffraction pattern provides detail lattice parameters of the crystal structure and defects in the structure. High resolution TEM (HR-TEM) uses phase contrast imaging that combines both transmitted and scattered electrons to construct images. HR-TEM has been used to characterize AgNP's size, morphology, crystal structure, and lattice parameters.[19]

A study used *Azadirachta indica* aqueous leaf extract to synthesize AgNPs, and TEM was used to characterize them, to identify AgNP's size, shape and morphology. The results showed that the AgNPs were well dispersed and mostly spherical in shape, while only some of them were of irregular shape.[24] Another study used *Solanum mammosum L.* (Solanaceae) fruit extract to synthesize AgNPs, and the results showed that they were highly dispersed in solution, that confirmed the stabilizing

activity of the fruit extract in addition to as a reducing agent.[17]

CONCLUSION

Many studies developed AgNPs as *Aedes aegypty* Larvicida using various plant-extracts as a reducing agent, such as extract from apple, *D. trifoliata, B. kewensis, A. nilagirica, L. Aspera, H. suaveolens,* and *S. mammosum*. Various parts of plants have been used to avoid chemical use that might cause pollution of the environment. Characterization of the biosynthesized AgNPs was done by various methods, such as UV-visible spectra, XRD, EDS, SEM, and HR-TEM.

ACKNOWLEDGMENT

This work was supported by a research grant from Ministry of Education and Culture and Ministry of Research and Technology of the Republic of Indonesia, Hibah PRN-BOPTN 2021, contract no. PKS-186/UN2.INV/HKP.05/2021.

REFFERENCES

1. WHO. WHO takes a position on genetically modified mosquitoes. 2020. https://wwwwhoint/news/item/who-takes-a-position-on-genetically-modified-mosquitoes
2. CDC. Dengue. 2021. https://wwwcdcgov/dengue/
3. Benelli G, Mehlhorn H. Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. Parasitol Res, 2016; 115(5): 1747-54.
4. Buzea C, Pacheco I. Nanomaterial and Nanoparticle: Origin and Activity. In: Ghorbanpour M, Manika K, Varma A (eds.). Nanoscience and Plant–Soil Systems. Cham; Springer: 2017; 71-112.
5. Salleh A, Naomi R, Utami ND, Mohammad AW, Mahmoudi E, Mustafa N, et al. The Potential of Silver Nanoparticles for Antiviral and Antibacterial Applications: A Mechanism of Action. Nanomaterials (Basel), 2020; 10(8): 1566.
6. Benelli G. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res, 2016; 115(1): 23-34.
7. Jorge de Souza TA, Rosa Souza LR, Franchi LP. Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity. Ecotoxicol Environ Saf, 2019; 171: 691-700.
8. Ahmad S, Munir S, Zeb N, Ullah A, Khan B, Ali J, et al. Green nanotechnology: a review on green synthesis of silver nanoparticles - an ecofriendly approach. Int J Nanomedicine, 2019; 14: 5087-107.
9. Kumar VA, Ammani K, Jobina R, Subhaswaraj P, Siddhardha B. Photo-induced and phytomediated synthesis of silver nanoparticles using Derris trifoliata leaf extract and its larvicidal activity against Aedes aegypti. J Photochem Photobiol B, 2017; 171: 1-8.
10. Bhuvaneswari R, Xavier RJ, Arumugam M. Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (Anopheles stephensi and Aedes aegypti). Journal of King Saud University, 2016; 28(4): 318-23.
11. Nalini M, Lena M, Sumathi P, Sundaravadivelan C. Effect of phyto-synthesized silver nanoparticles on developmental stages of malaria vector, Anopheles stephensi and dengue vector, Aedes aegypti. Egyptian Journal of Basic and Applied Sciences, 2017; 4(3): 212-8.
12. Rafique M, Sadaf I, Rafique MS, Tahir MB. A review on green synthesis of silver nanoparticles and their applications. Artif Cells Nanomed Biotechnol, 2017; 45(7): 1272-91.
13. Kennedy DC, Qian H, Gies V, Yang L. Human serum albumin stabilizes aqueous silver nanoparticle suspensions and inhibits particle uptake by cells. Environmental Science: Nano., 2018; 5(4): 863-7.
14. Ali ZA, Roslan MA, Yahya R, Wan Sulaiman WY, Puteh R. Eco-friendly synthesis of silver nanoparticles and its larvicidal property against fourth instar larvae of Aedes aegypti. IET Nanobiotechnol, 2017; 11(2): 152-6.
15. Kumar VA, Ammani K, Jobina R, Subhaswaraj P, Siddhardha B. Photo-induced and phytomediated synthesis of silver nanoparticles using Derris trifoliata leaf extract and its larvicidal activity against Aedes aegypti. J Photochem Photobiol B, 2017; 171: 1-8.
16. Elumalai D, Hemavathi M, Deepaa CV, Kaleena PK. Evaluation of phytosynthesised silver nanoparticles from leaf extracts of Leucas aspera and Hyptis suaveolens and their larvicidal activity against malaria, dengue and filariasis vectors. Parasite Epidemiol Control, 2017; 2(4): 15-26.
17. Pilaquinga F, Morejón B, Ganchala D, Morey J, Piña N, Debut A, et al. Green synthesis of silver nanoparticles using Solanum mammosum L. (Solanaceae) fruit extract and their larvicidal activity against Aedes aegypti L. (Diptera: Culicidae). PLoS One, 2019; 14(10): e0224109.
18. Jafer FS, Annon MR. Larvicidal Effect of Pure and Green-Synthesized Silver Nanoparticles against Tribolium castaneum (Herb.) and Callosobruchus maculatus (Fab.). Journal of Global Pharma Technology, 2018; 10(03): 448-54.
19. Khare T, Oak U, Shriram V, Verma SK, Kumar V. Biologically synthesized nanomaterials and their antimicrobial potentials. Comprehensive Analytical Chemistry, 2019; 87: 263-89.
20. Rojas FS, Pavón JMC. SPECTROPHOTOMETRY. Biochemical Applications. In: Worsfold P, Townsend A, Poole C (eds.). Encyclopedia of Analytical Science. 2nd ed., San Diego; Elsevier: 2005; 366-72.
21. Velayutham K, Ramanibai R, Umadevi M. Green synthesis of silver nanoparticles using Manihot esculenta leaves against Aedes aegypti and Culex quinquefasciatus. The Journal of Basic & Applied Zoology, 2016; 74: 37-40.

22. Amarasinghe LD, Wickramarachchi PASR, Aberathna AAAU, Sithara WS, De Silva CR. Comparative study on larvicidal activity of green synthesized silver nanoparticles and Annona glabra (Annonaceae) aqueous extract to control Aedes aegypti and Aedes albopictus (Diptera: Culicidae). Heliyon, 2020; 6(6): e04322.
23. Mohamed MA, Jaafar J, Ismail AF, Othman MHD, Rahman MA. Fourier transform infrared (FTIR) spectroscopy. In: Hilal N, Ismail AF, Matsuura T, Oatley-Radcliffe D (eds.). Membrane Characterization. Amsterdam; Elsevier: 2017; 3-29.
24. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences, 2019; 9(1): 1-7.
25. Bunaciu AA, Udriştioiu EG, Aboul-Enein HY. X-ray diffraction: instrumentation and applications. Crit Rev Anal Chem, 2015; 45(4): 289-99.