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ORIGINAL ARTICLE

Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (*Anopheles stephensi* and *Aedes aegypti*)

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KEYWORDS

Belosynapsis kewensis;
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Surface plasmon resonance

Abstract Mosquito vectors spread severe human diseases which lead to millions of deaths every year. Vector management is ultimately aimed to develop the health of every individual's life by reducing the mosquito diversity. Control of vectors in growing counties is an important issue with various aspects. The advancement of green nanotechnology will attribute the solution for vector controlling policy. To identify the larvicidal property of silver nanoparticles (AgNPs) using *Belosynapsis kewensis* (*B. kewensis*) leaf extract against the *Anopheles stephensi* (*A. stephensi*) and *Aedes aegypti* (*A. aegypti*) *in vitro* study (LC_{50} and LC_{90}) was analyzed. The synthesized AgNPs were characterized by UV–vis. (Ultraviolet–visible spectroscopy), FTIR (Fourier Transform Infra Red spectroscopy), TEM (Transmission Electron Microscopy), and XRD (X-ray Diffraction). Green AgNPs have a maximum absorption at 411 nm. The FTIR spectrum showed prominent peaks in (3863.55, 3759.02, 3361.01, 2926.81, 1575.12, 1388.16, 1034.79, 821.96, 717.07, 590.92, 534.32 and 472.42 cm^{-1}) in the region of 4000–400 cm^{-1}. The XRD peaks shown at 27.4°, 35.90°, 37.20°, 51.23° and 71.10° correspond to (3 1 1), (1 0 0), (1 0 1), (1 0 4) and (0 0 6) planes for face centered cubic (FCC). The TEM image showed the NPs with an average size of 24 ± 1.6 nm. *In vitro* larvicidal activity of AgNPs was used against the fourth instar of *A. stephensi* and *A. aegypti*. The LC_{50} and LC_{90} values of AgNPs showed to be effective against *A. stephensi* (LC_{50} = 78.4; LC_{90} = 144.7 -ppm) followed by *A. aegypti* (LC_{50} = 84.2; LC_{90} = 117.3 ppm). These results recommend that the green synthesized AgNPs have a potential to be used as a candidate for the control of *A. stephensi* and *A. aegypti* through eco-friendly and cost effective approach.
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1. Introduction

In tropical and subtropical regions mosquitoes contributing a major problem in public sectors especially, severe human diseases like malaria, dengue, Chikungunya, filariasis and yellow fever (Kovendan et al., 2012; Logeswari et al., 2013).

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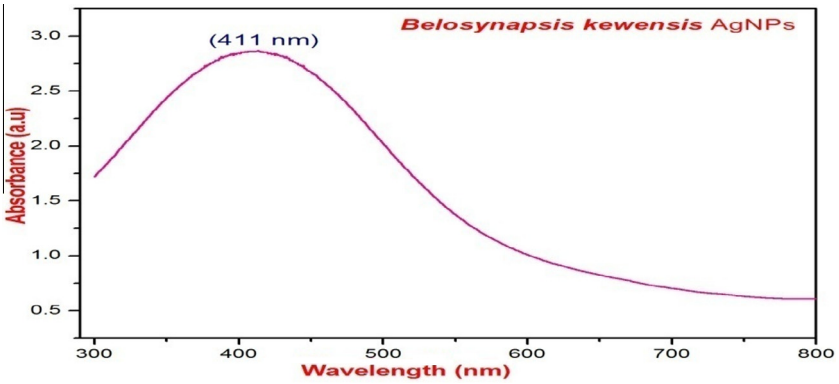

Figure 1 UV–vis spectrum of silver nanoparticles using *B. kewensis* leaf extract.

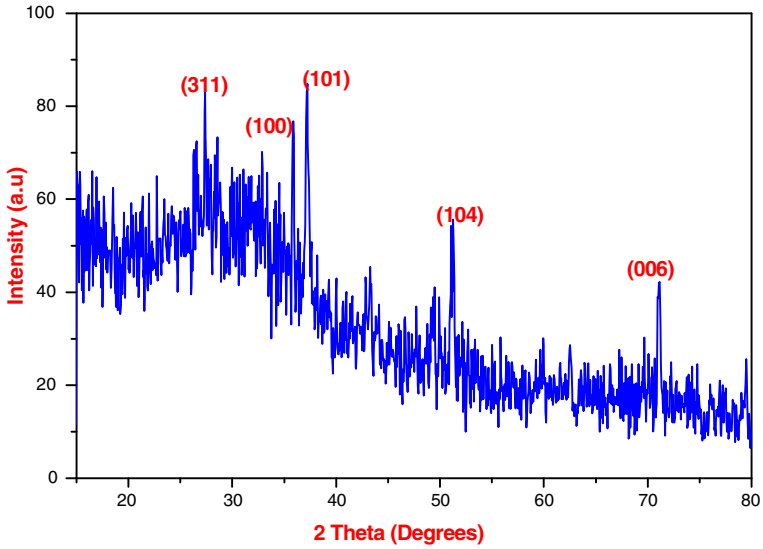

Figure 2 XRD pattern of synthesized silver nanoparticles using *B. kewensis* leaf extract.

All parts of the world population are being affected by vector borne diseases, but in the developing countries control of such vector is becoming a very big issue. So, green synthesized NPs may be a right opt source for mosquito control agent. The NPs consist of a variety of active compounds with many advantages like an eco-friendly, non-hazardous, greater surface, cost effect, inorganic nanomaterial and biocompatible (Albrecht et al., 2006; Harekrishna et al., 2009). Green synthesis of AgNPs is carried out by plants like *Dodonaea viscosa*, *Elaeocarpus ganitrus, Terminalia arjuna, Pseudotsuga menziesii, Prosopis spicigera, Ficus religiosa, Ocimum sanctum* and *Curcuma longa* (Poushpi et al., 2014) and *Syzygium cumini* (Mittal et al., 2014). Routine use of synthetic insecticidal products for control of mosquito has a problem with disturbing the biological system and cause to resurgences in mosquito populations (Chakkaravarthy et al., 2011; Srinivasan et al., 2014). *Belosynapsis kewensis* is a member of commelinaceae with high medicinal importance. Therefore, green synthesized AgNPs play a major role in the invention of larvicidal products. The present study aimed to investigate the larvicidal activity of synthesized AgNPs of *B. kewensis*.

2. Materials and methods

2.1. Chemicals and plant material collection

Silver nitrate ($AgNO_3$) and dimethyl sulfoxide (DMSO) were purchased from Merck, India. The glasswares were washed thoroughly with acid and followed by rinsing with Millipore-Milli-Q water. The leaves of *B. kewensis* were collected from Manjolai hills, Thirunelveli district, Tamilnadu, India.

2.2. Biosynthesis of AgNPs

The collected leaves were washed thoroughly with running water and followed by de-ionized water. 10 g of cleaned leaves were boiled at 80 °C for 10 min in 100 mL with deionised water and crude extract was filtered by Whatman No.1 filter paper finally the extract was stored at 4 °C for further uses (Abou El-nour et al., 2010). The obtained extract of *B. kewensis* was used for synthesis of AgNPs as a reducing as well as a stabilizing agent. To facilitate the synthesis of AgNPs 10 mL of *B. kewensis* leaf extract was mixed with 90 mL of 1 mM $AgNO_3$

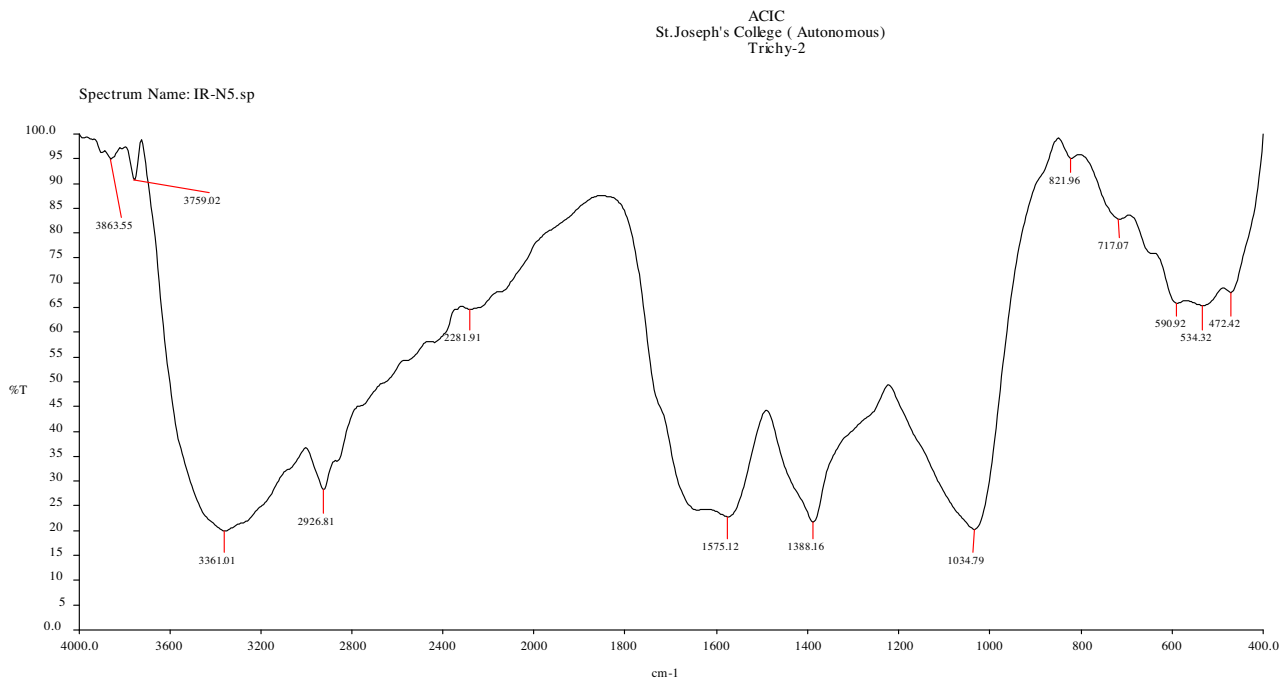

Figure 3 FTIR spectrum of synthesized silver nanoparticles using *B. kewensis* leaf extract.

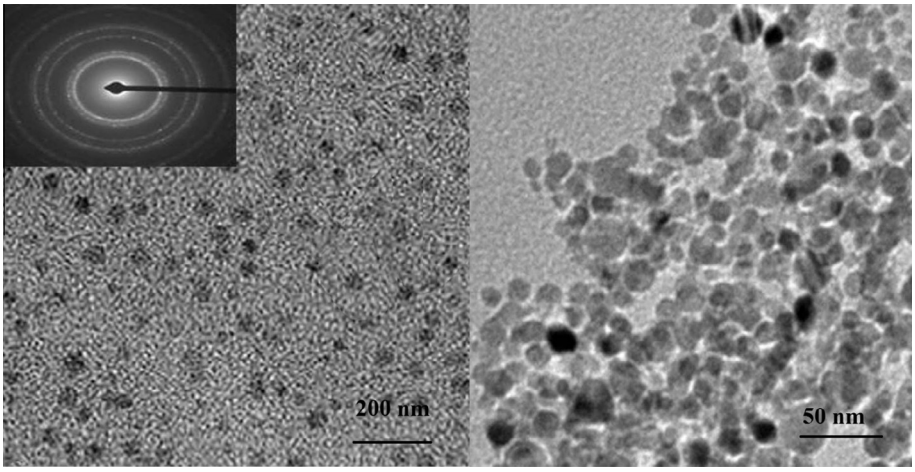

Figure 4 TEM image of 200 nm and 50 nm magnification showing the silver nanoparticles using *B. kewensis* leaf extract.

solution. This reaction mixture was kept at room temperature. Later, the color change was observed to designate the formation of colloidal AgNPs.

2.3. Characterization of AgNPs

During the incubation period, the synthesis of AgNPs was conformed by UV–vis spectroscopy (Hitatchi-2001) in a wavelength ranges between 200 and 800 nm at 1 nm resolution. Synthesized AgNPs were diluted with deionized water and centrifuged at 10,000 rpm for 15 min. The residue was dispersed with deionized water twice to remove the biological impurities. The purified residue was dried in oven at 70 °C for overnight. The AgNPs were used for FTIR analysis to identify the functional groups on a Perkin-Elmer spectrum instrument at a resolution of 4 cm^{-1} in the transmission mode of 4000–400 cm^{-1} in KBr pellets. The particles size and nature of the green AgNPs were determined by XRD using XPERT-PRO, D-8 at 30 kV, 40 mA with Cu Kα radians at 2θ angle. XRD is a rapid analytical method to identify the crystalline structure and calculate the dimensions of unit cell using Scherrer's formula ($D = K\lambda/\beta\cos\theta$). For TEM study, the sample was prepared by placing a drop of the suspension onto a carbon–coated Cu grid and allowed to evaporate. Observation was done in JEM 1011, JEOL, Japan, instrument operated at 200 kV an accelerated voltage.

Table 1 Larvicidal activity of synthesized silver nanoparticles using *Belosynapsis kewensis* leaf extract against *A. stephensi* and *A. aegypti* reared larvae.

Name of the mosquito species	Exposure period (h)	Conc. (ppm)	% mortality ± standard error	LC_{50} (LCL-UCL)[a]	LC_{90} (LCL-UCL)[a]	χ^2 (d = 4)[b]
Anopheles stephensi	12	Control	0 ± 0	92.4 (76.2–134.5)	181.6 (160.8–192.6)	8.3
		50	19.1 ± 0.18			
		100	36.6 ± 0.37			
		150	48.2 ± 0.75			
		200	62.4 ± 0.34			
		250	88.5 ± 0.75			
	24	Control	0 ± 0	78.4 (63.2–96.5)	144.7 (124.0–150.2)	1.42
		50	28.0 ± 0.98			
		100	42.1 ± 0.56			
		150	58.2 ± 0.13			
		200	88.6 ± 0.55			
		250	98.3 ± 0.87			
Aedes aegypti	12	Control	0 ± 0	104.2 (68.5–93.7)	166.7 (96.1–136.4)	4.57
		50	16.3 ± 0.28			
		100	29.4 ± 0.57			
		150	41.1 ± 0.73			
		200	54.4 ± 0.39			
		250	78 ± 0.81			
	24	Control	0 ± 0	84.2 (63.5–81.4)	117.3 (80.1–140.7)	4.6
		50	21.0 ± 0.14			
		100	32.0 ± 0.45			
		150	46.0 ± 0.38			
		200	64.0 ± 0.15			
		250	37.0 ± 0.67			

Control-nil activity, SE-standard error, LCL-lower confidence level, UCL-upper confidence level.
[a] 95% confidence interval.
[b] Degree of freedom, χ^2-Chi square value.

2.4. Larvicidal activity of AgNPs

For the larvicidal activity, the eggs of *Anopheles stephensi* and *Aedes aegypti* were collected from the field station, Center for Research in Chennai, Tamilnadu, India and they were reared under laboratory condition (25–28 °C). The larvicidal activity was studied by the standard procedure recommended by WHO (WHO, 2005). Green synthesized AgNPs were dissolved in 1 mL of methanol and prepared into different concentrations viz., 50, 100, 150, 200 and 250 ppm with double distilled water. Twenty-five reared early fourth instar stage larvae were transferred by means of the dropper to (100 mL beakers) each concentration and the experiment was performed in three replicates with the control under the laboratory conditions at 26–28°C. Mortality of the larvae was calculated at 12 and 24 h of exposure periods. During the both exposure periods, no food was supplied to the larvae and percentage of mortality was calculated. The data were presented in Mean ± Standard Deviation and all the statistical analyses were performed by SPSS version 11.5. The average of the larvae mortality LC_{50}, LC_{90} and chi-square test was calculated.

3. Results and discussions

The formation of NPs was done by reduction of Ag^+ ions into AgNPs with exposure of the leaf extract of *B. kewensis* and the formation was seen by the color change. The initial color of the suspension ($AgNO_3$ and leaf extract) was yellow, after the incubation period, the yellow was turned into dark brown in color which indicated the surface plasmon resonance (SPR) (Vijayaraghavan et al., 2012; Karuppiah and Rajmohan, 2013; Basavegowda and Rok Lee, 2014; Mansour and Robabeh, 2014). The SPR phenomenon was very sensitive to NP's nature, size and shape, which were formed by their inter particle distance and the surrounding media (Noginov et al., 2007; Vijayakumar et al., 2014). Fig. 1 represents the UV–vis spectrum of green synthesized AgNPs with higher peak level observed at 411 nm and SPR band also exposed at the same peak without any shifting. Muhammad et al. (2014) observed the same absorption band in seaweeds. Single peak indicated the synthesized particles were uniform in size and shape. So, the formation of AgNPs was attributed to hydrophilic and hydrophobic interaction, which, prevents the particles from aggregation by intermolecular forces (Shannahan et al., 2013). XRD analysis was employed to study the crystalline nature of the AgNPs. Fig. 2 shows the XRD pattern of AgNPs and peak values at 2θ degrees of 27.4°, 35.90°, 37.20°, 51.23° and 71.10° corresponding to (311), (100), (101), (104) and (006) planes of AgNPs. All the degrees of the peaks corresponded to a face centered cubic (FCC) crystalline structure. The intense peak 37.20° represented a high degree of crystallinity (Karuppiah and Rajmohan, 2013; Basavegowda and Rok Lee, 2014). FTIR analysis was carried out, to identify the functional groups of the synthesized AgNPs. FTIR spectrum indicated the clear peaks with (3863.55, 3759.02, 3361.01, 2926.81, 1575.12, 1388.16, 1034.79, 821.96, 717.07, 590.92, 534.32, 472.42 cm^{-1}) different values (Fig. 3). Above the peak values they corresponded to functional groups like, amide group (N–H stretching 3361.01 cm^{-1}), an aliphatic group (cyclic CH_2 – 2926.81 cm^{-1}), a methyl group (bend CH_2–CH_3

stretching 1388.16 cm^{-1}), aliphatic amine group (C–N stretching 1034.79 cm^{-1}), alkyl halides group (C–Cl stretching 821.96 and 717.07 cm^{-1}) and alkyl halides (C–Br stretching 590.92 cm^{-1} and 534.32 cm^{-1}). The functional groups such as alcohol, amines, amides, alkanes, methyl, aliphatic and halides confirmed their presence in AgNPs and these are the main classes in most of the functional groups. They were denoted as possible biomolecules responsible for stabilizing, capping and reducing agents of the AgNPs (Cho et al., 2005; Vijayaraghavan et al., 2012; Srinivasan et al., 2014). The terpenoid groups have a high potential to convert the aldehyde groups into carboxylic acids in the Ag^+ medium. Additionally, amide groups are also responsible for the presence of the some enzymes which, may be responsible for the synthesis of metal particles. Further, polyphenols are also proving that they have the potential to reduce the silver metals (Srinivasan et al., 2014). TEM micrograph (Fig. 4) indicated the synthesized AgNPs with 50 and 200 nm scales and most of the AgNPs were spherical in structure. The particle sizes vary from 10 to 28 nm and the average size of the AgNPs was 24 nm (Aguilare et al., 2011; Thomas et al., 2014). The Larvicidal activity of synthesized AgNPs was studied, which showed the effective larvicidal activities (Table 1) against the fourth instar larvae at 12 and 24 h of exposure time. After the 12 h of exposure time the larvicidal activity of AgNPs was LC_{50} = 147.6; LC_{90} = 285.9 ppm against *A. stephensi* and LC_{50} = 178.4; LC_{90} = 344.7 ppm against *A. aegypti*. The larvicidal activity after the 24 h of exposure time was LC_{50} = 112.7; LC_{90} = 217.3 ppm against *A. stephensi* and LC_{50} = 149.9; LC_{90} = 288.4 ppm against *A. aegypti*. The plant *B. kewensis* mediated AgNPs were expressed as 98.3% of larvicidal activity at 250 ppm. Srinivasan et al. (2014) reported the larvicidal activity of biosynthesized AgNPs of *Avicennia maria* leaf extract against *An. aegypti* (LC_{50} = 4.374 and LC_{90} = 4.928 ppm) and *A. stephensi* (LC_{50} = 7.40 and LC_{90} = 9.865 ppm). The larvicidal activity of synthesized AgNPs of *Ficus racemosa* was noted against *Culex quinquefasciatus* and *Culex gelidus*, which were LC_{50} = 67.72 and LC_{90} = 63.70 ppm. The larvicidal activity was observed in AgNPs of *Tinospora cordifolia* against fourth instar larvae of *Anopheles subpictus* and *C. quinquefasciatus* LC_{50} = 6.43 ppm. The AgNPs showed (using *Eclipta prostrata*) maximum efficacy of larvicidal activity against the fourth instar larvae of *C. quinquefasciatus* (LC_{50} = 27.49 and LC_{90} = 70.38) and *A. subpictus* (LC_{50} = 27.85 and LC_{90} = 71.45 ppm) (Velayutham et al., 2013). In the present investigation, the larvicidal activity of AgNPs showed higher activity against *A. stephensi* than *A. aegypti*. Vector management is one of the major issues due to the capacity of resistance against the usual insecticides. Therefore, an urgent need has emerged to develop the new insecticides (Krishnamoorthy et al., 2015). Hence, the invention of nanometals using *B. kewensis* as represented here, could be provided a new product to control the mosquitoes by replacing the synthetic larvicidal products and this route would make available for larvicides to prevent several dreadful diseases.

4. Conclusion

The green synthesized AgNPs using the leaf extract of *B. kewensis* offered an excellent larvicidal activity. Green route of AgNPs synthesis shows to be an eco-friendly, time consuming, cost-effective, and alternative to chemical methods of NPs production would be an appropriate for extending the biological route for large-scale production of the biolarvicidal product.

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References

Abou El-nour, K.M., Eftaiha, A., Al-Warthan, A., Ammar, R.A., 2010. Synthesis and applications of silver nanoparticles. Arabian J. Chem. 3 (3), 135–140.

Aguilare, M.A.M., Martin, E.S.M., Arroyo, L.O., Portillo, G.C., Espindola, E.S.J., 2011. Synthesis and characterization of silver nanoparticles: effect on phytopathogen *Colletotrichum gloesporioides*. Nanopart. Res. 13 (6), 2525–2532. http://dx.doi.org/10.1007/s11051-010-0145-6.

Albrecht, M.A., Evan, C.W., Raston, C.L., 2006. Green chemistry and the health implications of nanoparticles. Green Chem. 8, 417–432.

Basavegowda, N., Rok Lee, Y., 2014. Synthesis of silver nanoparticles using Satsuma mandarin (*Citrus unshiu*) peel extract: a novel approach towards waste utilization. Mater. Lett. 109, 31–33.

Chakkaravarthy, V.M., Ambrose, T., Vincent, S., 2011. Bioefficacy of *Azadirachta indica* (A. juss) and *Datura metel* (Linn.) leaves extracts in controlling *Culex quinquefasciatus* (Diptera: Culicidae). Trends Appl. Sci. Res. 8, 191–197.

Cho, K.H., Park, J.E., Osaka, T., Park, S.G., 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim. Acta 51, 956–960.

Harekrishna, B., Dipak, K.B., Gobinda, P.S., Priyanka, S., Sankar, P. D., Misra, A., 2009. Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. Colloids Surf. A 339, 134–139.

Karuppiah, M., Rajmohan, R., 2013. Green synthesis of silver nanoparticles using *Ixora coccinea* leaves extract. Mater. Lett. 97, 141–143.

Kovendan, K., Murugan, K., Vincent, S., Barnard, D.R., 2012. Studies on larvicidal and the pupicidal activity of *Leucas aspera* Willd. (Lamiaceae) and bacterial insecticide, *Bacillus sphaericus* against the malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). Parasitol Res.

Krishnamoorthy, S., Chandrasekaran, M., Adaikala Raj, G., Jayaraman, M., Venkatesalu, V., 2015. Identification of chemical constituents and larvicidal activity of essential oil from *Murraya exotica* L. (Rutaceae) against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res. http://dx.doi.org/10.1007/s00436-015-4370-x.

Logeswari, P., Silambarasan, S., Abraham, J., 2013. Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties. Sci. Iranica 20, 1049–1054.

Mansour, G.M., Robabeh, D., 2014. Plant mediated green synthesis and antibacterial activity of silver nanoparticles using *Crataegus douglasii* fruit extract. Ind. Eng. Chem. 20, 739–744.

Mittal, A.K., Bhaumika, J., Kumar, Sanjay., Banerjee, U.C., 2014. Biosynthesis of silver nanoparticles: elucidation of prospective mechanism and therapeutic potential. J. Colloid Interface Sci. 415, 39–47.

Muhammad, A.A., Najmul, H., Shaikh, M., 2014. Silver nanoparticles: green synthesis, characterization, and their usage in determination of mercury contamination in seafoods. Nanotechnology 5, 1–5. http://dx.doi.org/10.1155/2014/148184.

Noginov, M.A., Zhu, G., Bahoura, M., Adegoke, J., Small, C., 2007. The effect of gain and absorption on surface plasmos in metal nanoparticles. App. Phys. B 86, 455–460.

Poushpi, D., Shahid, S.N., Ravi, P.T., 2014. Phytofabrication characterization and comparative analysis of Ag nanoparticles by diverse biochemicals from *Elaeocarpus ganitrus* Roxb., *Terminalia arjuna* Roxb., *Pseudotsuga menzietii*, *Prosopis spicigera*, *Ficus religiosa*, *Ocimum sanctum* and *Curcuma longa*. Ind. Crops Prod. 54, 22–31.

Shannahan, J.H., Lai, X., Ke, P.C., Podila, R., Brown, J.M., Witzmann, F.A., 2013. Silver nanoparticle protein corona composition in cell culture media. PLoS One 8 (9), e74001. http://dx.doi.org/10.1371/journal.pone.0074001.

Srinivasan, B., Muthukumaraswamy, S., Mohanraj, J., 2014. Biosynthesis of silver nanoparticles from mangrove plant (*Avicennia marina*) extract and their potential mosquito larvicidal property. Parasit. Dis. http://dx.doi.org/10.1007/s12639-014-0621-5.

Thomas, S., John Britto, S., Mathew, S., Mani, B., 2014. Evaluation of antibacterial potential of silver nanoparticles (SNPS) produced using rhizome extract of *Hedychium coronarium* j. Koenig. Int. J. Pharm. Pharm. Sci. 6 (10), 92–95.

Velayutham, K., Rahuman, A.A., Rajakumar, G., Mohan Roopan, S., Elango, G., Kamaraj, C., Marimuthu, S., Santhoshkumar, T., Iyappan, M., Siva, C., 2013. Larvicidal activity of green synthesized silver nanoparticles using bark aqueous extract of *Ficus racemosa* against *Culex quinquefasciatus* and *Culex gelidus*. Asian Pac. J. Trop. Med. 6 (2), 95–101. http://dx.doi.org/10.1016/S1995-7645(13)60002-4.

Vijayakumar, P.P.N., Pammi, S.V.N., Pratap, K., Satyanarayana, K.V.V., Shameem, U., 2014. Green synthesis and characterization of AgNPs using *Boerhavia diffusa* plant extract and their antibacterial activity. Ind. Crops Prod. 52, 562–566.

Vijayaraghavan, K.S.P., Nalini, K., Udaya Prakash, N., Madhankumar, D., 2012. Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*. Mater. Lett. 75, 33–35.

WHO, 2005. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization document WHO, cds/WHO-pes/gcdpp/13.