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Review Article

Biological applications of biosynthesized silver nanoparticles through the utilization of plant extracts

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Abstract

Widespread uses of metallic nanoparticles, especially silver nanoparticles (AgNPs) in biology, pharmaceuticals, and medicine lead to the development of biosynthesis methods that are in turn utilized to prepare these nanoparticles. Among the biosynthesis methods, which are used to prepare nanoparticles, the plant-mediated methods have gained great attention due to several advantages such as cost-effectiveness, availability, eco-friendliness and nontoxicity of plants. Moreover, plant extracts are rich in different compounds which act as inhibitory and capping agents. For these reasons, plant-mediated methods can be potentially used for large-scale production of nanoparticles with different properties. The present article focuses on plant-mediated AgNPs using various plants and their biological applications such as antimicrobial, antioxidant, anticancer, anti-inflammatory, hepatoprotective and antilarvicidic properties.

Keywords: Silver nanoparticles, Plant extract, Antimicrobial, Antioxidant, Anticancer

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Introduction

Over the last few years, a variety of inorganic nanomaterials such as nanoparticles, nanowires, and nanotubes have been created or modified in order to obtain superior properties with greater functional versatility. The nanoscale science and technology significantly contribute to the development of new strategies for the synthesis of uniform nanomaterials with the controlled size and shape. In particular, nanoparticles in the range of 1–100 nm, have been investigated due to their size for their uses as tools for a new generation of technological devices. Moreover, due to the similarity of their dimensions and shapes to several biological structures (e.g., membrane cell genes, proteins, and viruses), they have been proposed for investigating biological processes as well as for sensing and treating diseases (1).

Many studies have focused on silver nanoparticles (AgNPs) due to their wide variety of applications (2). The AgNPs have widespread biological activities such as antimicrobial (3), anthelmintic (4), antilarvicidic (5), antioxidant (6), anticancer (7), anti-inflammatory (8), hepatoprotective (9), and wound healing activities (10).

The review of literature in the time span between 1987 to 2017 using silver nanoparticles as a keyword in "Scopus" database resulted in 23,312 articles (Figure

1) from which 3,169 articles deal with medicinal issues (Figure 2). Furthermore, the search results were refined by several keywords such as plant extracts, biosynthesis, green synthesis and biological applications which resulted in 1797, 2428, 5705 and 7302 articles, respectively.

Green chemistry refers to the activity of designing chemical products and processes that reduce or eliminate the use or generation of substances which are hazardous for human health and environment. Therefore, green chemistry protects the environment, not by cleaning up, but by introducing new chemical processes that do not pollute the environment. In this review, the green synthesis of AgNPs using plant extracts and their biological applications has been discussed.

Conventional Methods of AgNPs Synthesis

Several methods, such as chemical synthesis (11), electrochemical (12), radiation (13, 14), photochemical (15) and biological synthesis (16-19) have been used for the synthesis of AgNPs. Chemical, electrochemical, radiation and photochemical methods are expensive, energy consuming, harmful for both human beings and environment, and not suitable for biological applications. In comparison with these methods, biological methods used for synthesis of AgNPs are cost-effective, safe and environmental friendly. Consequently, these methods have been preferred as green chemistry methods.

Plant-mediated AgNPs Synthesis

Nowadays, the development of green synthesis of metallic nanoparticles and their applications are considered to be among the most important areas of research. Among the biological methods, the use of plant extracts for the synthesis of AgNPs is simple and cost-effective. Moreover, the synthesized particles are stable. On the other hand, plants possess secondary metabolites which lead to the reduction of metal nanoparticles in the easiest way (20). Recently, a rapid, energy-efficient, green and economically scalable room temperature method for the synthesis of stable AgNPs by the use of tannic acid (a polyphenolic compound derived from plant extract) was developed by Sivaraman et al (21). The proposed mechanism for biosynthesis of AgNPs has been illustrated in scheme 1.

Various plant species and their distinct components have been reported for the synthesis of AgNPs. The data indicated in Table 1 concern the plant species and that part of them which has been used, average size, shape and application of biosynthesized AgNPs. As it can be observed, most biosynthesized AgNPs are spherical in shape and have an average size less than 100 nm.

Applications of Biosynthesized AgNPs

Several reports demonstrated that the antimicrobial activities of AgNPs depend on the size, shape and stabilizing agents of nanoparticles. The antibacterial activities increase as the size of AgNPs is reduced (22, 23). Aggregation of nanoparticles reduces antibacterial activities of AgNPs. Therefore, combination of nanoparticles with stabilizer agents prevents the aggregation and leads to the maintenance of antibacterial activities of AgNPs.

Antimicrobial Properties

Shankar et al., used the *Rhomdomyrtus tomentosa* acetone extract (RAE) as reducing and capping agents for the synthesis of gold, silver and gold-silver-alloy nanoparticles. The obtained nanoparticles were in the range of 10-100 nm. Fourier-transform infrared spectroscopy (FT-IR) spectra demonstrated that compounds in RAE were capped on the nanoparticles. This phenomenon leads to the extension of the antibacterial activity of RAE towards Gram-negative bacteria (39).

The antibacterial activity of biosynthesized AgNPs using *Anredera cordifolia* extract was investigated against several Gram-positive strains (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumonia*). The results showed that the inhibition zones for six organisms were as follow: *E. coli* (16.33±0.58 mm), *B. cereus* (15.33±0.58 mm), *S. aureus* (14.67±0.58 mm), *K. pneumonia* (13.67±0.58), *P. aeruginosa* (13.33±0.58), and *P. vulgaris* (13.00±0.00). The antibacterial activity of biosynthesized AgNPs from aqueous extract of *A. cordifolia* was compared with the standard drug, gentamicin, which indicated that the biosynthesized AgNPs have significant antibacterial activity against six organisms (42).

Another study indicated that the diameters of the inhibition zones of biosynthesized AgNPs using

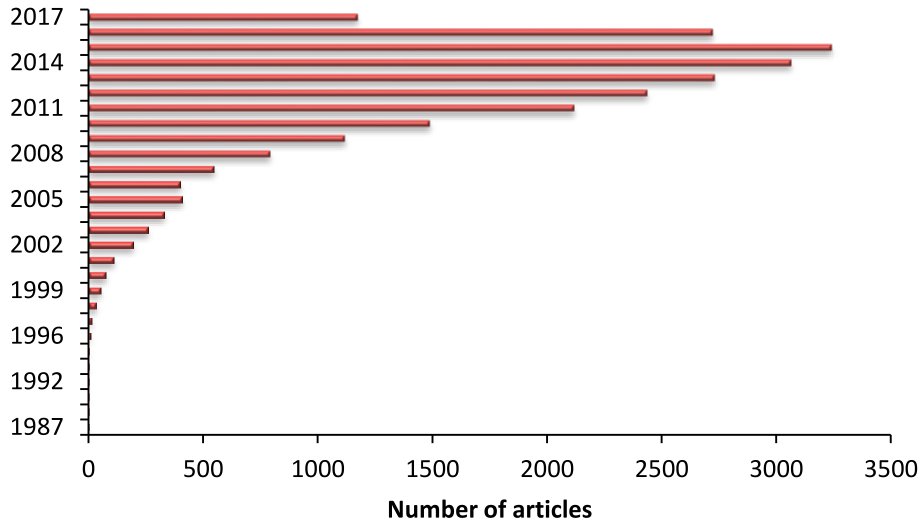


Figure 1. Number of articles in the time span between 1987 to 2017 using silver nanoparticles as keyword..

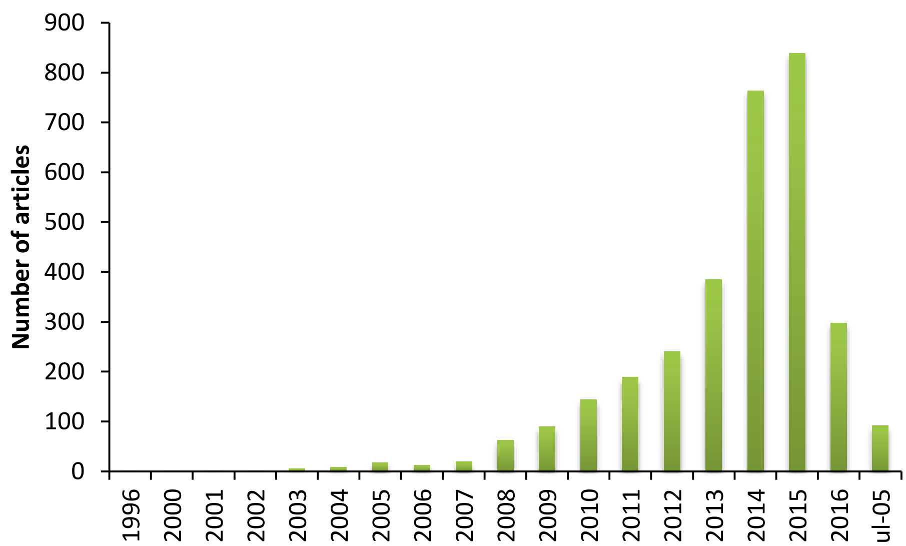


Figure 2. Number of articles dealing with medicinal topics in the time span between 1996 to 2017 using silver nanoparticles as keyword.

Tamarindus indica fruit extract against *B. cereus*, *S. aureus*, *Micrococcus luteus*, *Bacillus subtilis*, and those of *Enterococcus sp*. are 15, 16, 14, 18, and 16 mm respectively and *Pseudomonas aeruginosa*, *Salmonella typhi*, *E. coli*, and *K. pneumonia* are 22, 15, 15, and 10 mm respectively. In this study, the diameter of the inhibition zones of biosynthesized AgNPs were compared with $AgNO_3$. The results showed that the antibacterial activity of biosynthesized AgNPs against studied organisms was greater than that of $AgNO_3$ (43).

The biosynthesized AgNPs using corn leaf waste of *Zea mays* were evaluated for their antibacterial activity against foodborne pathogenic bacteria (*B. cereus*, *Listeria monocytogenes*, *S. aureus*, *E. coli* and *Salmonella Typhimurium*) by Patra el al., (46). The AgNPs at 50 µg/disk exhibited a moderate level of antibacterial activity against all five pathogenic bacteria with the diameter of inhibition zones of 9.26–11.57 mm. Moreover, synergistic antibacterial activity of biosynthesized AgNPs, together with the standard antibiotics, kanamycin and rifampicin, were examined against the above-mentioned five foodborne pathogenic bacteria. The results demonstrated that the use of antibiotic and biosynthesized AgNPs together enhanced the antibacterial activity against all pathogens.

Rajakumar et al., reported that leaf extract of *Millettia pinnata* and biosynthesized AgNPs possess antibacterial potential against E. coli, *Pseudomonas aeruginosa, P. vulgaris, S. aureus and K. pneumonia*. This study suggests that the antibacterial activity of AgNPs could be fulfilled in three mechanisms (49). First, the AgNPs could adhere to bacterial cell wall and deactivate the cellular enzyme due to their fine size and large surface area. Therefore, permeability of the cell membrane of bacteria increased which in turn led to cell death (51). Second, interactions of AgNPs with the thiol group of L-cysteine protein may lead to enzymatic dysfunction (52). Finally, the AgNPs may facilitate the release of reactive oxygen species (ROS) which lead to cell death (53).

Antioxidant Activity

The antioxidant activity of biosynthesized AgNPs using *Anredera cordifolia* leaf extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH). In comparison with gallic acid as a standard case, antioxidant activity of biosynthesized AgNPs exhibited an effective inhibitory effect. The IC_{50} value of AgNPs was equal to 48.32 μg/mL (42).

In another study, the antioxidant potential of biosynthesized AgNPs using corn leaf waste of *Zea mays* was determined by *in vitro* assays of DPPH radical scavenging against vitamin C as standard. The AgNPs showed a moderate DPPH radical scavenging potential of 34.09% at 100 μg/mL, whereas vitamin C as the reference standard showed higher DPPH scavenging activity of 42.41% at 100 μg/mL (46). However, results for biosynthesized AgNPs are satisfactory.

Anticancer Activity

Sathishkumar et al., proposed the biosynthesis of AgNPs using an aqueous leaf extract of *Alternanthera tenella.* The phytochemical monitoring results showed that flavonoids act as inhibitory and capping agents. The average size of the nanoparticles was found to be 48 nm. The energy-dispersive X-ray spectroscopy (EDX) results demonstrate the AgNPs formation with average size of 40 nm. The biosynthesized AgNPs are used for the treatment of Human breast adenocarcinoma (MCF7) cells. The IC_{50} value of the AgNPs was calculated to be 42.5 μg/mL. The AgNPs showed a significant reduction in the migration of MCF-7 cells (34).

In another study, the HCT116 cell lines were treated with different concentrations of biosynthesized AgNPs (50, 100,150, 200, 250, 300 and 350 μg/L) using *Actinidia deliciosa* fruit extract. It is believed that intracellular ROS generation could enhance the anti-cancer activity via nanoparticles. They are dose dependent. The AgNPs treated HCT116 cells showed 78% viability at highest concentration (350 μg/mL) which confirmed the anti-cancer activity of biosynthesized AgNPs (40).

MTT assay results indicated that *Mentha arvensis*-mediated AgNPs could have remarkable cytotoxicity in breast cancer cells (MCF7 and MDA-MB-231). Cell cycle analyses of MCF7 cells exhibited a considerable rise in sub-G1 cell population, confirming the cytotoxicity of AgNPs. On the other hand, human peripheral blood lymphocytes exhibited noticeably less cytotoxicity compared with MCF7 and MDA-MB-231 cells when treated with the same dose. Expression patterns of proteins revealed that AgNPs caused caspase 9-dependent cell death in both cell lines. The Ames test indicated that AgNPs were nonmutagenic in nature (41).

In another study AgNPs were synthesized using *Anthemis atropatana* extract to evaluate their antimicrobial and cytotoxic impacts. The biosynthesized AgNPs have spherical shapes with an average size of 38.89 nm. The MTT results demonstrate the dose dependence of cytotoxic impacts of biosynthesized AgNPs on colon cancer cell lines (HT29). The maximum cytotoxicity impact for biosynthesized AgNPs on HT29 cancer cell line was obtained at 100 μg/mL concentration which was remarkable in terms of statistics when compared with control cells ($p<0.001$). Moreover, real time PCR and flow cytometry results approved the apoptotic impacts of AgNPs. The results suggest that the green synthesis of AgNPs is an eco-friendly and cost effective approach which could lead to the development of new anticancer and antibacterial agents (44).

Rajakumar et al. examined four different concentrations including; 11.11, 33.33, 100 and 300

Table 1: Properties and applications of biosynthesized AgNPs using plant extract.

Plant	Plant part	Size (nm)	Shape	Application	Ref.
Euphorbia prostrata	Leaves	10-15	Spherical	Leishmanicidal	(24)
Red ginseng	Root	10-30	Spherical	Antibacterial	(25)
Azadirachta indica	Leaves	41–60	Spherical	Biolarvicidal	(26)
Nigella sativa	Leaves	15	Spherical	Cytotoxicity	(27)
Pistacia atlantica	Seeds	27	Spherical	Antibacterial	(28)
Anogeissus latifolia	Gum powder	5.5-5.9	Spherical	Antibacterial	(29)
Tagetes erecta	Flower broth	10-90	Spherical, hexagonal and irregular	Antibacterial and antifungal	(30)
Plumeria rubra	Latex	32-220	Spherical	Mosquito larvicides	(31)
Murraya koenigii	Leaf	20-35	Cubic and spherical	Mosquito larvicides	(32)
Citrullus colocynthis	Calli cells	31	Spherical	Anticancer	(33)
Alternanthera tenella	Leaf	48	Spherical	Anticancer	(34)
Mimosa pudica	Leaf	25-60	Spherical	Antiparasitic	(35)
Olea europaea	Leaves	90	Spherical	Anticancer	(36)
Oak	Fruit hull	40	Spherical	Anticancer	(37)
Solanum tuberosum	Fruit	10	Spherical	Interaction with HSA[a]	(38)
Rhodomyrtus tomentosa	Leaves	10-100	Spherical	antibacterial	(39)
Actinidia deliciosa	Fruit	25-40	Spherical	antioxidant, anticancer and bactericidal	(40)
Mentha arvensis	Leaf	2.8-9.9	Spherical	Anticancer	(41)
Anredera cordifolia	Leaf	40-60	-	Antioxidant and antibacterial	(42)
Tamarindus indica	Fruit	10	Spherical	Antibacterial	(43)
Anthemis atropatana		39	Spherical	Anticancer	(44)
Ipmoea nil	Leaf	-	-	Antibacterial	(45)
Zea mays	Corn leaf waste	45	-	Antioxidant and antibacterial	(46)
Syzygium aromaticum	Flower	5-40	Spherical	Anticancer	(47)
Andrographis paniculata	Leaves	60-70	Hexagonal and spherical	Anticancer	(48)
Millettia pinnata	Flower	49	Spherical	Antibacterial and cytotoxic	(49)
Dunaliella salina	-	15	Spherical	Anticancer	(50)

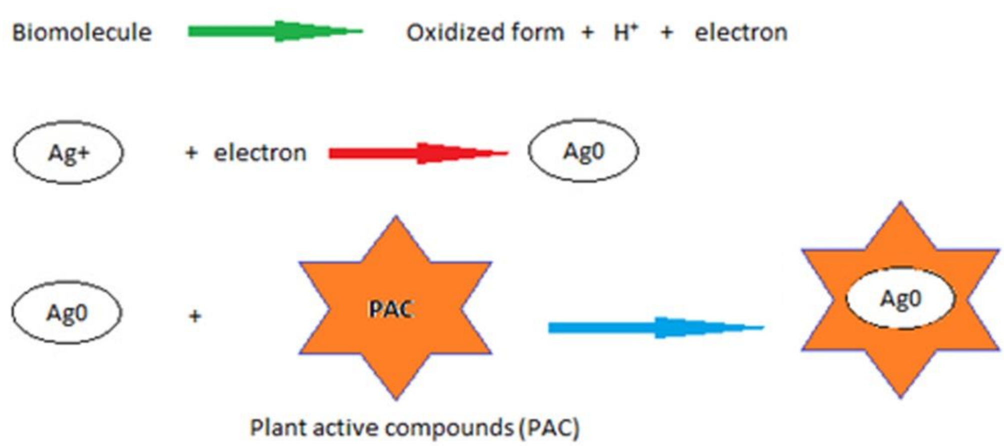
HSA; human serum albumin

mg/mL of synthesized AgNPs and *Millettia pinnata* flower extract to find their cytotoxicity using brine shrimp (*artimia salina*) lethality assay. LD_{50} value of *M. pinnata* aqueous flower extract was found up to 84.32 mg/mL. The low LD_{50} value brine shrimp (36.41 mg/mL) exhibited the higher cytotoxic effect of AgNPs compared to *M. pinnata* extract. Cytotoxic effects of AgNPs on shrimp's larvae could be linked with anticancer activity and result in the introduction of an alternative source of anticancer drugs (49).

Anti-inflammatory Capacity

Steroids and non-steroidal anti-inflammatory drugs (NSAIDs), as the main therapeutic agents in inflammation, have serious side effects. Therefore, it is essential to develop new drugs likely to have promising results without serious side effects. Likewise, the anti-inflammatory properties of AgNPs using European black elderberry fruits extract were investigated as in vitro on HaCaT cells exposed to UVB radiation and in vivo on acute inflammation model and in humans on psoriasis lesions. The results indicated that the biosynthesized AgNPs had a significant anti-inflammatory impact both in vitro and in vivo. In the case of in vitro, the anti-inflammatory effect was confirmed by the reduction of cytokines production and also by keeping their low level after UVB irradiation. In the case of in vivo, the pre-administration of AgNPs led to decline of the level of cytokines in the paw tissues. In the comparison with hydrocortisone, the local treatment of psoriasis vulgaris skin lesions confirmed the significant anti-inflammatory impact of biosynthesized AgNPs (54).

The production of ROS, such as superoxide anion, is one of the key factors in inflammation mediated cell damage. In order to examine whether ethanolic petals extract of *Rosa indica* and synthesized AgNPs could have inhibitory effect against H_2O_2 stimulated superoxide anion generation, macrophages were pre-treated with either ethanolic petals extract of *R. indica* and biosynthesized AgNPs. Superoxide anion



Scheme 1. The proposed mechanism for biosynthesis of AgNPs using plant extract.

generation in untreated control was observed, which indicates H_2O_2 stimulated ROS generation in rat peritoneal macrophages. Pre-treatment with both ethanolic extract and synthesized AgNPs significantly inhibited superoxide anion generation in macrophages. The significant reduction was observed in the generation of strong inflammatory mediators such as nitric oxide and superoxide anion upon exposure to synthesized AgNPs which confirm the anti-inflammatory activity of biosynthesized AgNPs (55).

Hepatoprotective Activity of Biosynthesized AgNPs

The hepatoprotective activity of biosynthesized AgNPs using aqueous extracts of *Andrographis paniculata* leaves and *Semecarpus anacardium* nuts against diethylnitrosamine (DEN) induced liver cancer in mice model was investigated. The results of this study revealed that end-capped biomolecules on AgNPs had a potential hepatoprotective impact against DEN induced liver cancer and could be utilized as an efficient anticancer nanodrug (48).

Antilarvicidic Activity

Mosquitoes are capable of transmitting acute human diseases resulting in millions of deaths every year. Synthetic insecticides were utilized to control vector mosquitoes but the result was physiological resistance and pernicious environmental impacts. Furthermore, these synthetic insecticides have high operational cost. Consequently, it is extremely necessary to introduce insecticides with natural sources for vector control.

The results exhibited by the study by Poopathi et al. indicated that biosynthesized AgNPs that use *Azadirachta indica* have significant larval control over *Aedes aegypti* and *Culex quinquefasciatus*. In this study, the highest effective mortality at LC_{50} and LC_{90} levels was observed in *A. aegypti* larvae treated with very low dosage of neem-based AgNPs (LC_{50} and LC_{90} equal to 0.006 and 0.04 mg/L, respectively) (26).

Moreover, larvicidal activity of synthesized AgNPs using an aqueous extract from *Eclipta prostrate* was observed in crude aqueous and synthesized AgNPs against *Cx. quinquefasciatus* ($LC_{50} = 27.10$ and 4.56 mg/L; $LC_{90} = 70.389$ and 13.14 mg/L) and *Anopheles subpictus* ($LC_{50} = 27.85$ and 5.14 mg/L; $LC_{90} = 71.45$ and 25.68 mg/L), respectively (56). In this study, LC_{50} and LC_{90} values for biosynthesized AgNPs were higher than the previous report (26). This phenomenon can be attributed to the distiction in end-capping molecules in two AgNPs.

In another research, the activity of biosynthesized AgNPs using *Plumeria rubra* plant latex against of *Aedes aegypti* and *Anopheles stephensi* was evaluated. The results indicated that the biosynthesized AgNPs from *P. rubra* latex were much more toxic compared with crude latex extract in both mosquito species. The LC_{50} values for biosynthesized AgNPs after 24 h of exposure were 1.49, 1.82 mg/L against *A. aegypti* and 1.10, 1.74 mg/L against *A. stephensi*, respectively. On the other hand, these values for crude latex extract were 181.67, 287.49 mg/L against *A. aegypti* and 143.69, 170.58 mg/L against *A. stephensi*, respectively. This study was the first report on mosquito larvicidal activity of latex synthesized AgNPs (31).

The activity of biosynthesized AgNPs using *Murraya koenigii* plant leaf extract against the first to the fourth instars larvae and pupae of *A. stephensi* and *A. aegypti* was determined. The results indicated that biosynthesized AgNPs from *M. koenigii* leaf were much more toxic compared with crude leaf ethanol extract in both mosquito species. Larvae were exposed to different concentrations of biosynthesized AgNPs and ethanol leaf extract for 24 h. The LC_{50} values for biosynthesized AgNPs and ethanol leaf extract of *M. koenigii* against *A. stephensi* were 10.82 and 279.33 mg/L, respectively. Moreover, LC_{50} values for biosynthesized AgNPs and ethanol leaf extract of *M. koenigii* against *A. aegypti* were 13.34 and 314.29 mg/L, respectively. These results demonstrate that biosynthesized AgNPs using *M. koenigii* can be used as a rapid and eco-friendly bio-pesticide which can be developed as a novel approach to produce effective biocides for controlling the target vector mosquitoes (32).

Conclusion

Literature review indicated that the shape and size of biosynthesized AgNPs changed with the variation of extract compounds which could influence the biological applications of these NPs. Plants are rich in medicinally important bio-molecules which act as capping and inhibitory agents for the biosynthesis of AgNPs. The use of plant extract for the synthesis of AgNPs is simple, cost-effective, green and safe. Unlike the chemically synthesized AgNPs, plant-mediated AgNPs are more stable and suitable for biological applications. Biosynthesized AgNPs have significant biological applications, hence, they could contribute to the development of novel drugs for various diseases.

Conflict of Interest

The authors declare that they have no conflict of interest.

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