

REVIEW PAPER

A Review on Green Synthesis of Silver Nanoparticles by Employing Plants of Acanthaceae and its Bioactivities

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ABSTRACT

Nanotechnology has immense number of applications in all contemporary fields of science. Silver nanoparticles form an important part of metal nanoparticles, with wider range of applications in diverse areas of science. Plant mediated green synthesized silver nanoparticles are usually preferred due to its advantages over other metal nanoparticles viz. non toxicity, lesser energy consumption, cost effectiveness and lesser pollution. Plants belonging to Acanthaceae family are medicinally as well as pharmacologically relevant. They contain many important phytochemicals, which can reduce, stabilize and act as capping agents in the nanoparticle synthesis. Various mechanisms to synthesize silver nanoparticles are explored here to analyse its formations with the most efficient method. Different phytochemicals with various functional groups assisting the formation of nanoparticles during green synthesis are also summarized here. The synthesized silver nanoparticles show effective antimicrobial properties along with many other potential bioactivities. The mechanisms of antimicrobial properties of silver nanoparticles are also assessed. Various bioactivity studies connected with green synthesized AgNPs comprising of antimicrobial, antifungal, biocompatibility, anti-inflammatory, anticancer, antioxidant, larvicidal, effect on Seed germination and growth are briefly outlined in this article.

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INTRODUCTION

It is a well-known fact that nanotechnology emerges as one of the most important interdisciplinary fields of science with wide range of applications. It involves the synthesis and manipulation of particle structure with a dimension of less than 100 nanometers in diameter [1]. Nanoparticles possess diverse physio-chemical properties such as optical, magnetic, catalytic properties [2], and biological applications in antimicrobial [3, 4], antioxidant, anti-inflammatory, antiviral, cardiac protection, wound dressing and anti-cancerous activities [5]. Nanoparticles (NPs) result in superior chemical reactivity, biological activity, and catalytic behaviour than their own larger particles [2, 6]. They are employed in major industries like food, chemical, feed, health, space,

cosmetics etc. The industries preferably employ green synthesized nanoparticles for manufacturing its products [2, 7]. Even though there are many metal NPs were investigated [8], silver nanoparticles (AgNPs) are considered to be the best due to the fact that the disadvantages of AgNPs are negligible as compared to its advantages [9].

Silver nanoparticles are included in a set of zero dimensional substances that demonstrate natural structure under its dimension extending from 1 to 100 nm [10]. AgNPs manifest peculiar thermal conductivity, chemical stability, and Raman scattering phenomena [11, 12]. AgNPs also possess optical, electronic, chemical photo-electrochemical, magnetic, catalytic and biological properties [13, 14] (Fig.1). This is in continuation with the application of silver metal being employed in preserving food products [15], and manufacturing

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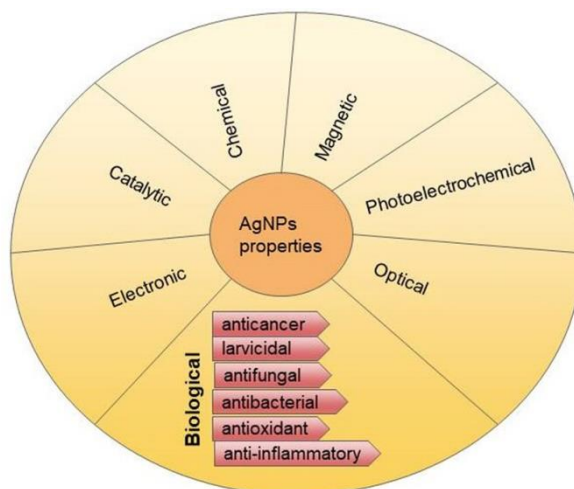


Fig. 1: Some properties of silver nanoparticles

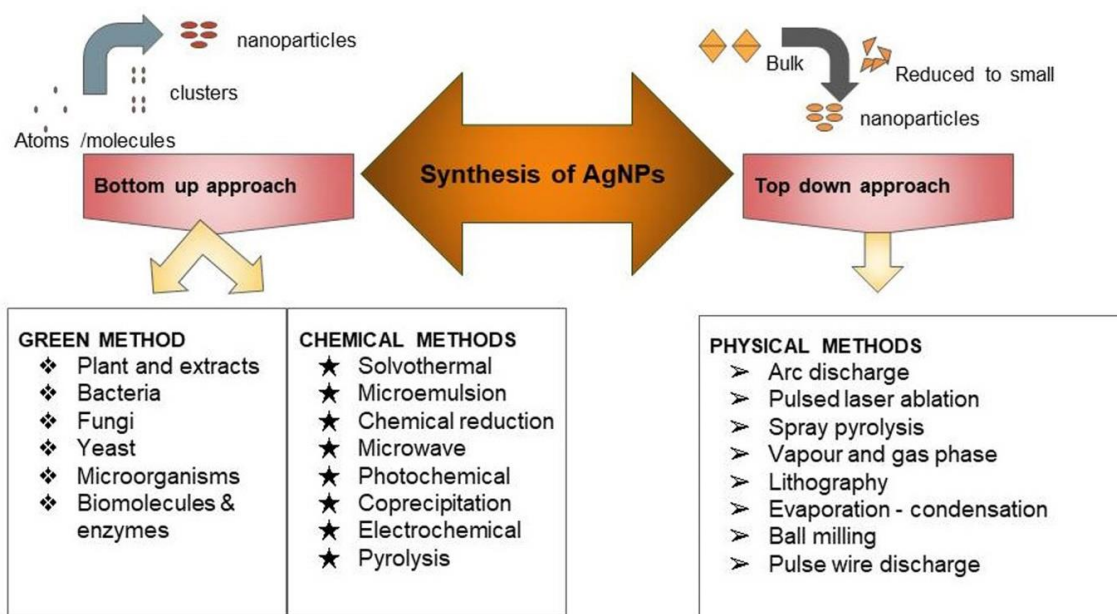


Fig. 2: Common methods of synthesis of silver nanoparticles

utensils from prehistoric time onwards [16]. Now in the 21st century, AgNPs are used in broad range of applications, such as biomedical engineering, drug delivery, food industries, antimicrobial, textile industries, agriculture, water treatment (as an antioxidant), anticancer agent, larvicides, cancer cell therapy, component of ointments etc. [14]. Among the reported antimicrobial properties of different metal NPs, it was found that AgNPs exhibited prominent bactericidal and fungicidal activity [17, 18].

Method of synthesis of silver nanoparticles

Broadly speaking, there are Top-down and Bottom-up approaches for the synthesis of metallic nanoparticles (Fig. 2). Top down method is the method of synthesizing nanoparticles in nanoscale level by plastic deformation from large sized patterns [17]. In bottom-up method nanoparticles are formed by the self-assembly of miniaturized atomic components [19, 20]. Although numerous methods are envisaged for silver nanoparticle synthesis, the most conventional ones are physical

and chemical methods [21]. These procedures provide productive output; that are coupled with constraints such as the utilization of harmful reagents, great running costs, more requirement of energy etc. [17]. Some other disadvantages of these methods include energy consumption, slow synthesis and requirement of high concentration [22-24].

Nowadays green synthesis of nanoparticles is accepted widely because of its simple, non-toxic, rapid, stable nature and also less expensive [18]. Green synthesis utilizes environmentally eco-friendly raw materials like plant extract, algae, bacteria, fungi, and enzymes [17]. The major advantage of using plant extract includes large scale production under non aseptic condition and absence of cell culture for the production of nanoparticles [14]. Although a considerable number of plants as well as plant parts are used for the synthesis, here we summarize the reports on various synthesis protocols and bioactivities of silver nanoparticle using plant extract of Acanthaceae.

Phytochemicals characterized from members of Acanthaceae

Acanthaceae, a family of Angiosperms covers over 4,000 plant species and about 230 genera of plants present across the globe, which largely grow in subtropical and tropical places as primary diversity centres like the South American, Indo-Malay, African (comprising Madagascar), and Mexican-Central American areas [25]. Members of Acanthaceae family are medicinally important due to the presence of various phytochemicals like glycosides, flavonoids, phenolic compounds, naphthoquinone, benzoids, triterpenoids etc. [42-49]; because of the presence of these phytochemicals, they show antibacterial [26, 27, 28], antifungal [29, 30], cytotoxic [31], anti-inflammatory [32], antipyretic [33, 34], antiviral [35, 36], antioxidant [18], hepatoprotective [37], insecticidal [38], immunomodulatory [39], and anti-platelet aggregation properties [40, 41].

Green Synthesis of silver nanoparticles - Acanthaceae family members

There are different methods of AgNPs synthesis protocols which allow interaction between plant extract and metal salt either by mixing, heating, incubating, stirring or shaking. This interaction facilitates the formation of nanoparticles. The

major functional groups present in the Acanthaceae family members play an important role in this synthesis of AgNPs.

AgNPs having a size ranging from 10-20 nm were synthesized using *Justicia glauca* phytochemicals possessing functional groups like -C-O-C- of carboxylic group, O-H stretching of hydroxyl group, carbonyl group of aldehydes or ketonic group [51]. Moreover, these nanoparticles were formed due to the major contribution from C-O-C of phenolic compounds [50]. Dried leaf extract of *Indoneesiella echioides* was utilized for the creation of silver nanoparticle with a mean size consisting of ~25 nm. The production was assisted due to various functional groups present in phytochemicals revealed by FTIR analysis like the occurrence of aromatic primary amine (C-N stretch), secondary amine (N-H bend), carbonate ions, organic nitrates and aliphatic fluoro compounds (C-F stretch, C-O and C-O stretching modes), carboxylic group, C-O-C, C-OH vibrations, -C-O and -C=C stretch vibrations and Amide II band [52]. Oxidation of aldehydes to carboxylic acid of enzymes/proteins found in plant distillate resulted in nanoparticle formation. Silver nanoparticles were formed using *Nilgiranthus ciliates* leaf broth having hydroxyl group, carbonyl group, alpha CH₃ methyl group of aldehydes and ketones and are characterized by UV-Vis spectra, ultra high resolution SEM, FTIR, DLS [28].

In another method, the aqueous fresh leaf extract of *Thunbergia grandiflora* with alkene, amine, alkyne, alcohol and aromatic compounds were used in the formation AgNP with an average size of 2.39 nm [26]. Fresh leaves of *Justicia adhatoda* yielded AgNPs of average size 20 nm and characterized by UV-visible spectra analysis as well as TEM analysis [53]. Leaf extract of *Barleria prionitis* containing amine, alkane, alkene and ether groups resulted by FTIR analysis, yielded 10-20 nm sized AgNP [54].

Treating aqueous leaf extract of *Lepidagathis cristata* having functional groups like secondary amide, carbonyl group and amide resulted in the formation of AgNPs of average particle size of 30 nm. The amide group of tryptophan present in the leaves significantly contributed in the reduction of the metal ion to nanoparticle [55]. The mixture of *Justitia adathoda* leaf extract composed of functional group like C-C in alkene ring and C = C of aromatic ring, ether linkage and C-N of amine and O-H of secondary alcohol resulted

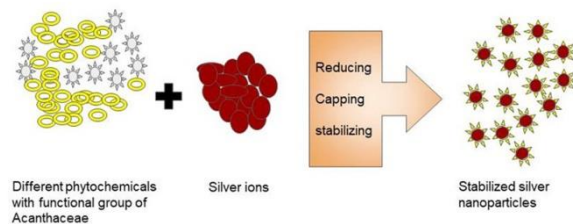


Fig. 3 : Role of Acanthaceae phytochemicals in AgNP green synthesis

in the formation of nanoparticle of size 18 nm. Furthermore, presence of phenolic and aromatic compounds helped in the stabilization of NPs [56].

Fresh leaf extracts of *Asystasia gangetica* containing functional groups like alkyl halide, aromatic amine, primary amine, OH group of alcohol and phenols, CN bond of nitride, C=O bond of carbonyl group were exploited in the assembly of AgNP having a dimension of 40 – 60 nm. [57]. Using methanolic leaf extract of *Clinacanthus nutans*, AgNPs were synthesized with an average size 46.4 - 53.8 nm here, carbonyl group contributed by different flavonoids involved in the formation of AgNPs [58].

Green synthesis of nanoparticle is the synthesis of nanoparticle using plants. Various plant parts can be used for the synthesis viz. stem, leaves, root, rhizome, flower, seed etc. Plants produce diverse bioactive secondary metabolites with different functional groups. These functional groups metal ions resulting in the generation of nanoparticles. The FTIR analysis revealed that different functional groups were associated with these processes [59]. Phenolic compounds hold carboxyl and hydroxyl groups which can disable iron ions by chelating and further inhibiting the superoxide driven Fenton reaction. This is presumed to be the most crucial supply of reactive oxygen species (ROS). Therefore, plants with a high content of phenolic compounds are one of the best candidates for nanoparticle synthesis [60].

The research by Jha et al revealed that phytochemicals particularly glutathiones, polyphenols, ascorbates and metallothioneins were certainly responsible for the generation of nanoparticles [61]. Shankar et al communicated the biosynthesis of pure metallic silver nanoparticles and they were stabilized by reducing sugars and/or terpenoids contained in the leaf broth of *Azadiracta indica* [62]. Polyols, as for example, terpenoids, flavones and polysaccharides in the

leaves of *Cinnamomum camphora* were considered as the main agents to reduce of silver as well as chloraurate ions [63]. Li et al reported that proteins with amine groups and vitamin C present in the extract of *Cinnamomum annuum* L. were accountable for the Ag nanoparticles formation and rapid precipitation [64]. High density of highly steady silver nanoparticles were quickly synthesized using leaf extract of *Datura metel* which comprised of biomolecules like alkaloids, polysaccharides, amino acid, proteins/enzymes, and alcoholic compounds which could be employed as scaffolds to accelerate the formation of silver nanoparticles in solution phase [65]. Quinol (alcoholic compound) and chlorophyll pigment resulted in the reduction of silver ions and normalization of nanoparticles [66]. Purely natural constituents and its utilization help in the reduction and stabilization of metal nanoparticles (Fig.3) is still in exploration phase [60].

Some other factors responsible for the nanoparticle formation are reaction and incubation temperature. Rise in temperature boosts the speed of generation of nanoparticles. High temperature along with increased concentration of plant extract helped the formation of small sized NPs [67]. A compacted report of silver nanoparticle synthesis of Acanthaceae is reported in Table 1.

Bioactivity of AgNPs – Antibacterial

Silver ions and silver salts were utilized for therapeutic application starting from the period of human urbanization. Silver ions and silver salts are now utilized in the form of antibacterial agent especially for wound dressing [68]. Among all other metals, silver shows higher antimicrobial activities [17]. It works against 650 microorganisms, including bacteria, fungi, virus, and eukaryotic microorganisms [58]. A summary of the findings is reported in Table 2.

It was observed such that gram positive bacteria

Table 1: Silver nanoparticles synthesis mediated by members of Acanthaceae

Name of the Plants	Plant part used	Size(nm)	Shape	Applications	References
<i>Adhatoda vasica</i>	Dried leaves	22.1-29.1	Spherical	Antimicrobial effect	[1]
<i>Adhatoda vasica</i>	Fresh leaves	10-15	spherical	Antibacterial Biocompatibility	[79]
<i>Andrographis echiooides</i>	Fresh leaves	68.06	Cubic	Anticancer antibacterial	[77]
<i>Andrographis paniculata</i>	Fresh Leaves	40-60	Cubic	Antibacterial effect	[14]
<i>Andrographis serpyllifolia</i>	Dried lesves	2-20	Cubic	Antimicrobial effect Anti oxidant effect	[18]
<i>Andrographis serpyllifolia</i>	Fresh Leaf	3.4-71.6	Spherical	Antibacterial Effect Larvicidal Activity	[27]
<i>Asystasia gangetica</i>	Fresh leaves	40-60	spherical	Antibacterial effect	[57]
<i>Barleria cristata</i>	Dried leaves	38-41	Circular	Mosquitocidal potential Biototoxicity studies	[75]
<i>Barleria prionitis</i>	Dried leaves	10-20	spherical	-	[54]
<i>Blepharismadera spatensis</i>	Dried leaves	-	spherical	Antimicrobial effect	[80]
<i>Clinacanthus nutans</i>	Methanolic leaves extract	46.4-53.8	spherical	Antimicrobial effect	[58]
<i>Hygrophila auriculata</i>	Dried leaves	40.96	spherical	Antioxidant effect Antimicrobial effect	[81]
<i>Hygrophila auriculata</i>	Seed extract	10-80	spherical	Anti bacterial	[82]
<i>Indoneesiella echiooides</i>	Dried leaves	-29	spherical	Antioxidant effect Antimicrobial effect Cytotoxicity effect	[52]
<i>Justicia adhatoda</i>	leaves	5-50	Spherical Agglomerate	Antimicrobial effect	[53]
<i>Justicia adhatoda</i>	Fresh leaves	18	spherical	Cytotoxicity studies	[56]
<i>Justicia glauca</i>	Fresh leaves	10-20	Spherical	Anti microbial effect	[51]
<i>Justicia gendarussa</i>	Dried leaf powder	12-30	Irregular spherical	Anti oxidant Anti bacterial	[78]
<i>Justicia jendaracia</i>	callus	50-65	spherical	Anti bacterial Ecotoxicity studies	[76]
<i>Justicia spicigera</i>	Dried l p	86-100	Spherical	Anti bacterial Anti fungal	[29]
<i>Lepidagathis cristata</i>	Dried leaves	20-50	spherical	Antimicrobial studies	[55]
<i>Nilgirianthus ciliates</i>	leaves	117	crystalline	Antibacterial effect	[28]
<i>Rhinacanthus nasutus</i>	Dried leaves	22-11.5	spherical	Antibacterial effect Antifungal activity	[29]
<i>Strobilanthes flaccidifolius</i>	Fresh leaves	6-54	spherical	Antibacterial studies	[83]
<i>Thunbergia grandiflora</i>	Flower	Nanoscale	Crystalline cubic	Catalytic action on congo red dye	[84]
<i>Thunbergia grandiflora</i>	Leaf	2.39	Spherical	Antibacterial effect Effect on Seed germination and growth	[26]

are much immune towards AgNPs compared to gram negative bacteria. Since gram negative bacteria are coated with lipopolysaccharides, they show negative charge and therefore get attached with positively charged silver ions. Gram-positive bacteria are enveloped by thick layer of peptidoglycans and linear polysaccharides which offer rigidity to the cell and thus avoid the binding

of NPs to its surface. In case of gram-negative bacteria, AgNPs penetrate into the cell by forming holes in the cell wall of the bacteria [69, 70].

Bioactivity of AgNPs – Antifungal

A few reports of antifungal activities are reported on green synthesis of AgNPs employing Acanthaceae family members; for example, AgNPs

Table 2: Antimicrobial activity of green synthesized silver nanoparticles using members of Acanthaceae family

Plants	CON. AgNP	METHOD	Microorganisms with zone of inhibition in mm	References
<i>Adhatoda vasica</i>	Not specified	Disk diffusion method	<i>Escherichia coli</i> (19 ± 10) <i>Bacillus thuringiensis</i> (18.30 ± 1.50) <i>Klebsiella pneumonia</i> (12 ± 0.3) <i>Pseudomonas aeruginosa</i> (24.3 ± 1.5) <i>Staphylococcus aureus</i> (19 ± 0.01)	[1]
<i>Andrographis serpyllifolia</i>	75 µg/ml	Well diffusion method	<i>Staphylococcus aureus</i> (14.66 ± 0.57) <i>Staphylococcus pneumonia</i> (21.33 ± 0.88) <i>Pseudomonas fluorescens</i> (20.00 ± 1.45) <i>Escherichia coli</i> (19.00 ± 0.52)	[27]
<i>Andrographis paniculata</i>	25 µL	Well diffusion method	<i>Pseudomonas aeruginosa</i> (21.3 ± 0.4) <i>Escherichia coli</i> (16.6 ± 0.3) <i>Vibrio cholera</i> (15.4 ± 0.3) <i>Shingella flexneri</i> (15.2 ± 0.1) <i>Bacillus subtilis</i> (18.6 ± 0.4) <i>Staphylococcus aureus</i> (13.7 ± 0.2) <i>Micrococcus luteus</i> (17.6 ± 0.3)	[14]
<i>Clinacanthus nutans</i>	10 µL	Disk diffusion method	<i>Bacillus subtilis</i> (11.50 ± 1.22) <i>Enterococcus faecalis</i> (8.33 ± 0.47) <i>Staphylococcus aureus</i> (8.67 ± 0.82) <i>Escherichia coli</i> (8.50 ± 0.50) <i>Pseudomonas aeruginosa</i> (9.00 ± 0.94) <i>Proteus vulgaris</i> (8.80 ± 0.45)	[58]
<i>Hygrophila auriculata</i>	15 µL (20 mg/ml)	Agar diffusion method Kriby disc diffusion method	<i>Staphylococcus aureus</i> (16 ± 0.25) <i>Bacillus cereus</i> (17 ± 0.10) <i>Pseudomonas aeruginosa</i> (18 ± 0.15) <i>Escherichia coli</i> (13 ± 0.35)	[81]
<i>Justicia spicigera</i>	100 mg/ml	disc diffusion method	<i>Bacillus cereus</i> (10.0 ± 0.2) <i>Klebsiella pneumonia</i> (8.3 ± 0.6) <i>Enterobacter aerogenes</i> (7.1 ± 0.4)	[29]
<i>Lepidagathis cristata</i>	50 µg/ml	Agar diffusion method	<i>Escherichia coli</i> (16 ± 0.5) <i>Pseudomonas aeruginosa</i> (15 ± 0.5) <i>Streptococcus pneumonia</i> (16 ± 0.5)	[55]
<i>Rhinacanthus nasutus</i>	50 µg/mL	Disk diffusion method	<i>Staphylococcus aureus</i> (17.66 ± 0.57) <i>Bacillus subtilis</i> (15.66 ± 1.15) <i>Pseudomonas aeruginosa</i> (17.33 ± 1.52) <i>Escherichia coli</i> (17.33 ± 1.52) <i>Klebsiella pneumonia</i> (17.66 ± 1.52)	[30]
<i>Strobilanthes flaccidifolius</i>	5 mg/ml	Agar diffusion method	<i>Proteus mirabilis</i> (26 ± 0.06) <i>Klebsiella pneumonia</i> (26 ± 0.08) <i>Escherichia coli</i> (20 ± 0.14) <i>Salmonella paratyphi</i> (26 ± 0.80) <i>Pseudomonas aeruginosa</i> (34 ± 0.14)	[83]
<i>Thunbergia grandiflora</i>	30 µl of 25 %	Disk diffusion assay	<i>Klebsiella planticola</i> (11.36 ± 0.3)	[26]

synthesized using *Rhinacanthus nasutus* barred the growth of *Aspergillus niger* and *Aspergillus flavus* with the zone of inhibition 18.66 ± 1.52mm and 17.66 ± 1.52mm respectively [30]. *Justicia spicigera* assisted AgNPs synthesis (using a concentration of 100 mg/ml) showed antifungal activity towards *Macrophomania phaseolina* (ZoI = 80.95 ± 1.35

mm), *Alternaria alternate* (ZoI = 62.12 ± 4.50 mm), *Fusarium solani* (ZoI = 35.60 ± 3.55 mm) *Colletotrichum sp.* (ZoI = 40.16 ± 2.35 nm) in PDA medium. AgNPs initiates various changes in the hyphae, cell wall and the germination pattern of fungus [71]. The Ag⁺ ions released by AgNPs get attached with thiol group of cystein proteins

of fungus leading to the death of fungus [72]. Antifungal activity decreases with increase in complexity of cellular organization of organisms, which interrupt the movement of the AgNPs into the cells [73, 74].

Bioactivity of AgNPs – Biocompatibility

AgNPs synthesized from *Barleria cristata* were tested for its safety towards non-target organisms like *Diplonychus indicus*, *Anisops bouvieri*, and *Gambusia affinis*, which are mainly insect predators in ponds. Treatment resulted in negligible toxicity against organisms tested [75]. *Justicia jendaracia* mediated AgNPs study revealed that they did not cause any distinct effect on soil enzyme activity, soil macronutrients, soil microbial population and plant growth parameters of *Vinga mungo* [76]. Thus, the AgNPs are very safe to use; as it is less toxic to useful organisms, as well as it does not alter favourable conditions of plant growth.

Bioactivity of AgNPs – anti-inflammatory activity

There are limited reports of anti-inflammatory activities on green synthesis of AgNPs by employing Acanthaceae family members. *Hemigarphis colorata* mediated synthesis of AgNPs, exhibited anti-inflammatory activity or inhibition of protein denaturation through tests namely, inhibiting heat induced albumin denaturation and membrane stabilizing test of RBC. Denaturation of protein causes inflammation. AgNPs synthesized using *Hemigarphis colorata* plant extract showed higher protein inhibition compared to its original plant extract. Moreover, results of membrane stabilization test also demonstrated that AgNPs could show higher values in the inhibition of hemolysis. Both tests demonstrated that AgNPs could act as an effective anti-inflammatory drug [32].

Bioactivity of AgNPs – Anticancer

In this case also, a few reports are available, for example, AgNPs formed from *Andrographis echiodides* demonstrated anticancer property towards the human breast adenocarcinoma cancer cell line (MCF-7) by creating free radicals like reactive oxygen species or boosting intracellular oxidative stress [77]. AgNPs synthesized by utilizing *Justicia adhatoda* exhibited anticancer property towards HeLa cell lines of cancer cells [56]. Anticancer property of AgNPs was established towards human lung adenocarcinoma cancer cells (A549) based on

AgNPs derived from *Indoneesiella echiodides* [52].

Bioactivity of AgNPs – Antioxidant effect

Indoneesiella echiodides mediated AgNPs show antioxidant property using tests namely ABTS scavenging assay and DPPH scavenging assay [52]. AgNPs synthesized by employing *Justicia gendarussa* demonstrated significant antioxidant property. They were tested for ABTS scavenging assay, DPPH scavenging assay and against nitric oxide [78]. AgNPs formed using *Andrographis serpyllifolia* presented strong antioxidant activity in assays like DPPH, NO and H₂O₂ [18]. AgNPs act as a natural antioxidant or efficient free radical scavenger against the free radicals which damages cell by means of oxidative stress, which in turn triggers injuries or apoptosis in organisms [52].

Bioactivity of AgNPs – larvicidal potential

Andrographis serpyllifolia leaf extract assisted synthesis of AgNPs showed prominent larvicidal effect on *Culex quinquefasciatus*. It possessed lower LC₅₀ and LC₉₀ values and higher mortality rate of larvae [27]. AgNPs synthesized from *Barleria cristata* displayed strong larvicidal property towards *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* [75]. AgNPs, synthesized utilizing *Nilgiranthus ciliates* were experimented on *Aedes aegypti*, which presented higher efficiency and mortality rate. The suggested mechanism for the effective larvicidal property was due to the penetrating capability of AgNPs, which resulted in molting arrest as well as morphological deformation [28].

Bioactivity of AgNPs – Effect on Seed germination and growth

AgNPs prepared using *Thunbergia grandiflora* resulted in higher rate of germination in seeds of *Vigna radiata* with a concentration of 75% AgNPs. AgNPs also exhibited higher rate of shoot and root growth on treating with 25% and 50% AgNPs concentration respectively [26].

CONCLUSION

Synthesis of silver nanoparticles is effectively established using plants belong to Acanthaceae family. The phytochemicals present in the family members serve as both stabilizing and capping agents of the synthesized nanoparticles. They are best in their biological activities especially antibacterial action against broad spectrum of bacteria. Future

studies will revolve the best mechanism of action against bacteria and the occurrence of particular phytochemicals of the family best suitable for the synthesis of nanoparticles.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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