Review article

The factors affecting optimisation of phytosynthesis of silver nanoparticles using Indian medicinal plant species and their biological applications: A review

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ABSTRACT

Recently, silver nanoparticles (AgNPs) hold a centre stage in the vast arena of research in nanomedicine. Biological methods of synthesis of nanoparticles are being adopted to avoid the use of chemicals that are toxic and hazardous and used in chemical synthetic methods. The choice of the plant extracts used for the synthesis of AgNPs is based on their phytochemicals and pharmaceutical properties. Another importance of the phytosynthesis method is that it is simple, easy, fast, reliable and cost effective. The phytochemical components of the herbal extracts not only cause reduction of Ag^{+1} to Ag^{0}, but also increase the biological activities of the nanoparticles. This article briefly describes the research on green synthesis of AgNPs using Indian medicinal plant extracts. We aim to provide a systemic depth of information regarding the effect of various reaction factors during the synthesis of AgNPs such as the concentration of silver salt and phytoextract, light intensity, time, temperature, pH of the reaction mixture. The characterizations of nanoparticles are done by X-ray diffraction, ultraviolet- visible spectroscopy, Fourier-transform infrared spectra, transmission electron microscopy, dynamic light scattering and zeta potential analysis. The phytochemicals and pharmaceutical activities present in the plant extracts are also reported here. The articles from 2011 to 2022 were selected and studied in detail to get in-depth knowledge about the phytosynthesis of AgNPs and their biological activities.

Keywords: phytosynthesis; silver nanoparticles (AgNPs); Indian medicinal plants; pharmacological activities; biological activity.

INTRODUCTION

Around 20% of the world-wide plant species (45,000 species) are found in the Indian sub-continent and among them 6000- 7000 different plants are used in traditional and documented medicine systems like Siddha, Unani, Ayurveda and Homeopathy. For their unique properties and multipurpose applicability, medicinal plants have been gaining importance in various developing areas of research and development (1). Nanoparticles (NPs) occupy a special place in the modern research arena due to their large surface area and nanoscale dimension (1-100 nm) (2). Nanoparticles can be smaller than the size of human cells, and so, can be used for drug delivery into cells. NPs can be delivered by all established methods of administration and has the ability to go deeper to the cellular nucleus (3). Among the various types of NPs, silver nanoparticles (AgNPs) play a key role in nanoscience and technology, especially in nanomedicine (2). Besides that, AgNPs have increased potential for exposure and interaction with terrestrial and aquatic environments. This may lead to toxicity to human health (4). NPs are synthesized using chemical, physical or biological processes. Physical synthesis is cost effective, whereas, the chemical methods involve the use of toxic chemicals and have adverse effects, as some of these toxic chemicals remain adsorbed on the surface of the NPs (5). To eliminate or reduce the toxicity, various biological processes of AgNPs synthesis have been developed. NPs can be synthesized using microbes, enzymes, fungi and plants. The standardisation of these environment friendly methods for the NPs synthesis, especially that of AgNPs, has emerged as a key discipline of nanotechnology, which has diverse applications (6). The method for the synthesis of AgNPs using plant extracts seems to be the best because the methods are simple, easy, faster, reliable, and cost effective (7). Plant extracts contain phytochemicals- such as flavonoids, phenolics, terpenoids followed by polysaccharide, polymers, enzymes and proteins that mainly act as capping, stabilizing and reducing agents (8). It allows the interaction of the green synthesized NPs with microorganisms and also enhances their antimicrobial activity (9). AgNPs synthesized using *Azadirachta indica* showed no adverse effect when administered orally at 10 mg/kg up to 28 days (10). In the present article, the phytosynthesis and biological activities of silver nanoparticles utilising Indian medicinal plants are discussed, that could help the researchers in their future work involving the phytosynthesis of AgNPs.

Method of phytosynthesis of AgNPs using plant extracts

Synthesis of AgNPs using plant extracts have been gaining focus because of the wide range of bioactive

compounds and reducing metabolites that can lead to the production of desirable NPs with predefined characteristics (11). During synthesis, plant phytochemicals reduce silver ions from Ag^+ to Ag^0, which helps in the formation of NPs. A schematic diagram of phytosynthesis using plant extract is provided in Fig. 1.

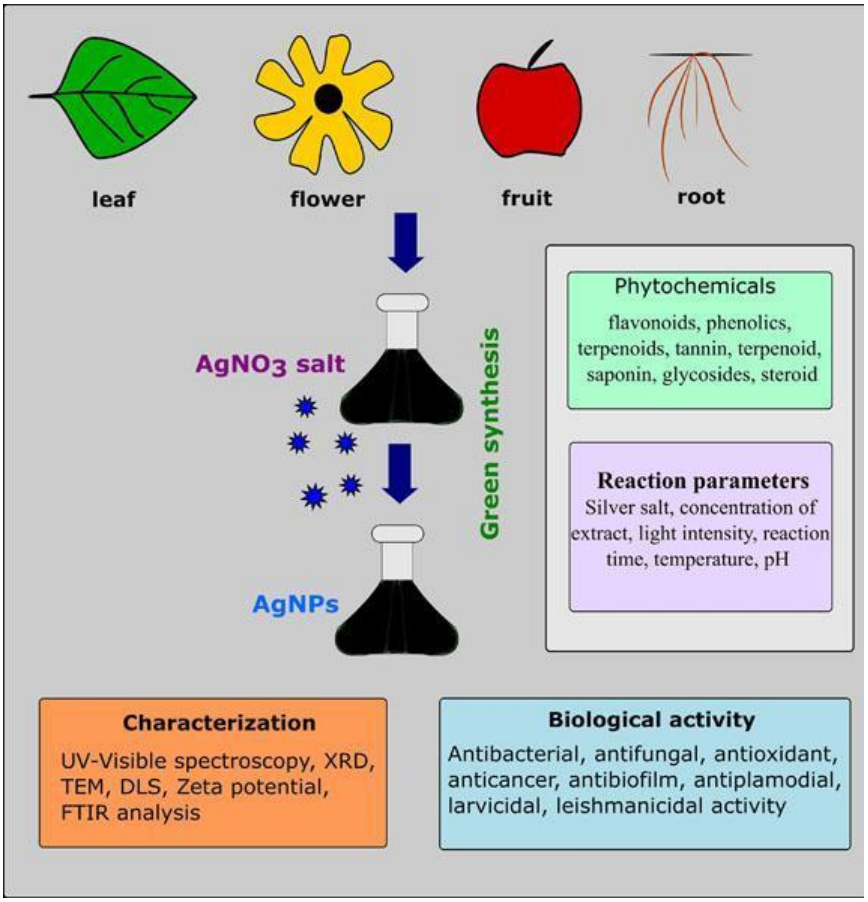

Fig. 1: A schematic diagram of plant mediated synthesis of silver nanoparticles

Factors affecting phytosynthesis

Plant mediated production of AgNPs is dependent on different conditions of the reaction mixture: molarity of silver salt, concentration of phytoextract, light intensity, time of reaction, temperature, pH at which the plant extracts can be mixed with metal precursor solution. These reaction parameters also affect the dimensions and morphology of the synthesized silver nanoparticles. In most cases, the colour of the reaction mixture turns dark brown/ yellowish brown/ blackish brown due to formation of nanoparticles. The different reaction conditions which affect AgNPs synthesis are described below.

Molarity of silver salt as a factor affecting AgNPs phytosynthesis

Most of the researchers have used 1 mM silver nitrate salt for the synthesis of AgNPs due to less toxicity (2,12). Whereas some researchers have used 1-5 mM silver precursors and found larger sized NPs were synthesized with increased salt concentration. The size of nanoparticles increased from 12 ± 2 to 20 ± 3.3 nm when the salt concentration used was 5 mM (13). Different concentrations of $AgNO_3$, ranging from 2 to 100 mM (14-16) were used.

Effect of plants metabolites

Studies reveal that extract quantity is also proportional to the increase of the absorbance peak (17). AgNPs synthesized using *Cassia auriculata* flower extract showed an intense narrow peak at 435 nm in UV-Vis spectroscopy (18), whereas, in case of AgNPs synthesized using different concentrations of *Mentha piperita* extract, different morphologies of the synthesized NPs were observed such as spherical, nanotriangles and irregular (19). Concentration of the plant metabolites can also affect the zeta potential value of the synthesized AgNPs. Zeta potential values of −20.7, −21.3, −12.0 and −11.3 mV were found when 1%, 5%, 50% and 100% tea extract was used for the synthesis. These studies also indicate that the stability of AgNPs suspensions is proportional to increasing extract concentration (20).

Effect of pH

pH, another vital factor, influences the dimensions and morphology of phytosynthesized silver NPs. A change in pH of the reacting solution can change electrical charges of quercetin, a biomolecule present in Tulsi extracts, which can alter their reducing and capping capacity and the subsequent growth of nanoparticles. It was also observed that the peak shifted towards the higher wavelength with an

increase in pH of the reaction mixture, which indicates an increased reaction rate (21). Absence of the yellow-brown colour was found at a lower pH, whereas basic condition is found to be favourable for phytosynthesis (15,16).

Effect of reaction time

Studies reveal that AgNPs can be synthesized within 15 mins and the solution remains stable after 24 h. To get higher concentration of AgNPs, reaction time should be increased. By increasing the reaction time, higher concentration of NPs was obtained (16,19,22) and the peak became sharper (21).

Effect of temperature

Temperature is another important factor that affects the size of the hydrate particle. As the temperature increases, the reaction rate also increases and more silver ions get accumulated. It was observed that the size of tea leaf extract synthesized AgNPs were 91 nm, 129 nm, and 175 nm at 25˚C, 40˚C, and 55˚C respectively (20). Alterations in the intensity of plasmon band were recorded with varying temperature from 5-50°C in case of the AgNPs synthesized using *Allium cepa* and *Musa acuminate*. A sharply increased peak was observed with temperature increase up to 40°C, while a sharp reduction was observed when reaction temperature was raised to 50°C. The effect of temperature was also indicated by a change of colour of reaction mixture to deep brown at 40°C and whitish yellow and yellow at other temperatures also indicate the influence of temperature on green nanoparticle synthesis. Optimum nanoparticle synthesis at a specified temperature may be due to optimum conditions of reduction and interaction between the flavonoids and terpenoids present in the extract (15). By increasing reaction temperature from 25°C to 150ºC, a sharper peak was found. The size of the synthesized NPs determines the intensity of the absorbance peak. At elevated reaction temperature, the reaction rate increases and particle size decreases, resulting in sharpness of plasmon resonance band of AgNPs (21).

Effect of light intensity

When AgNPs was prepared using Durian rind extract, under different light intensities ranging from 0 (dark place); to 2,810; 8,120 and 13,430 lux; the peak intensity increased with increasing light intensity and an increased number of particles were synthesized due to continuous exposure to light of higher intensity (23). Similar kind of phenomenon was found when AgNPs were synthesized using *Fusarium oxysporum*. Absorbance peak increased with the light intensity and showed complete reduction of silver ions in presence of sunlight. Light intensity at 190.7 and 141.3 lux showed a symmetric graph with narrow size, but it required more time for silver ions to be reduced completely as light intensity was not enough. When light intensity was at 15.5 lux, no evidence of synthesis was found even after 72 hrs (24).

Study of the properties of AgNPs

Different characterization techniques such as, dynamic light scattering (DLS), UV–visible spectrophotometry (UV-Vis), zeta potential analysis, X-ray diffraction (XRD), energy dispersive spectroscopy (EDX), scanning electron microscopy (SEM), transmission electron microscopy (TEM), photoluminescence (PL), Fourier transform infrared spectroscopy (FTIR), atomic force microscopy are done to determine the properties and dimensions of the synthesized NPs. A few of the most important characterisation techniques are discussed in this review.

UV–visible spectrophotometry

The formation of AgNPs is primarily examined by UV–visible spectrophotometry - a very reliable technique for physical characterization of nanoparticles. The spectra give an idea about the synthesis of AgNPs by showing peaks at a range of 400 - 480 nm which is dependent on their size, shape, and morphology (21). The shifting of the UV-Vis spectrum of synthesized NPs are due to the changes in concentration of salt solution, temperature, pH and other reaction conditions.

Analysis of X-ray diffraction (XRD)

The crystalline structure and surface morphology of phyto-synthesized AgNPs is ascertained by X-ray diffraction (XRD) pattern analysis in the range of 10–90° at 2θ angles. The XRD pattern confirms the size as well as the presence of silver crystallites. Further confirmation of the result can be done by transmission electron microscopy (TEM). Increase in pH of the reaction mixture results in a decreased width of XRD peak, indicating an increased size of synthesized AgNPs (25).

Fourier transform infrared (FTIR) analysis

The biomolecules which are involved in the complete reduction and stabilization of silver ions can be identified using FTIR analysis (20, 25). The FTIR spectra is recorded at resolution of 4 cm−1 in the transmission mode 4000– 400 cm−1 using a FTIR spectrophotometer. The absorption peak indicates the groups present in the phytochemicals. The functional groups present in the leaf extracts such as amides, primary and secondary amines, alcohols, alkanes, alkynes, aldehydes, carboxylic acids ketones, ethers, esters, alkyl bromides or aliphatic amines are probably involved in AgNPs synthesis (26). An analysis of FTIR data of extracts of medicinal plants and that of phytosynthesized AgNPs is presented in Table 1.

Table 1: Analysis of FTIR data of extracts of selected medicinal plants and phytosynthesized AgNPs

Name of the plant	FTIR absorption bands (cm^{-1})		Functional group	Ref.
	Plant extract	Nanoparticles		
Curcuma longa	3295	3329	-OH	(27)
	2923	2920	-NH, -CH	
	1638	1643	-C=O	
	1375	1420	$–NO_3$	
Amaranthus sp.	3339	-	O-H stretch (phenol group)	(6)
	2935	-	aromatic C–H stretching	
	1602	1647	C=C stretch	
	1410	1454	O–H bend of polyphenol	
	1072	1129, 1192	C–O stretching (polyphenol)	
Phyllanthus emblica	1621	1637	amide I vibrations	(28)
	1384	1384	C–H symmetric vibrations	
	1238	1219	C–C stretching vibration	
Ricinus communis	3435	3443	alcohol O–H stretch	(8)
	2046	2054	C = O stretch	
	1634	1631	N−H bond of amine groups	
Terminalia chebula	3380	3377	-OH stretching of phenolic group	(7)
	1714, 1615	-	carbonyl group	
	1032	-	-C-O	
	1448	1383	Aromatic -CH	
Tribulus terrestris	3488.76	3419.18	O-H	(29)
	2811.25, 2727.52	2811.83, 2726.33	C-H vibrational mode	
	2164.11	2171.34	C=C	
	1613.12	1613.12	carboxyl group of C-C	
	1348, 1125.13	1348.66, 1125.13	C-N aromatic and aliphatic amine	
Ginkgo biloba	3557.98	3428	N−H stretching, amides	(30)
	1446.61	1379.97	C−N stretching mode of the aromatic amine/ -C-O stretching modes.	
Ceratonia siliqua	3309, 3421	3410	stretching of the -NH band of amino groups/ bonded -OH hydroxyl group	(11)
	1712, 1581	1608	CO, C-O, and O-H groups	
	1581, 1446	1558	Carbonyl (C=O) and amine (−NH) stretching	
	1,446	1408	methylene scissoring vibrations from the proteins	

Transmission electron microscopy (TEM)

The shape of the synthesized AgNPs and their size range is determined by TEM. The TEM image also shows that the synthesized AgNPs are well capped. Selected area electron diffraction (SAED) images confirm that the synthesized AgNPs are polycrystalline (25). An expected change in size distribution of nanospheres was detected by TEM when the concentration of salt and extract was changed as discussed above.

Zeta Potential and Dynamic Light Scattering (DLS) Analysis

DLS data reveals hydrodynamic size of synthesized AgNPs. The difference in the particle size by TEM and DLS may be due to the aggregation during the sample preparation. Zeta potential values determine the stability of the synthesized AgNPs. Zeta potential, in the range between −20 and −23 mV indicates the synthesis of stable nanoparticles. The stabilizing ability is due to the phytochemicals present in the plant extracts (19). It is assumed that salt concentration, temperature and pH can affect the zeta potential value (20). A summary of phytosynthesis of AgNPs using Indian medicinal plants extracts is provided in Table 2.

Table 2: A summary of phytosynthesis of silver nanoparticles using extracts of Indian medicinal plants

Name of the plant and part used	Techniques used for characterization	Size of NPs (nm)	$AgNO_3$ (mM)	SPR (nm)	Ref.
Centella asiatica (Thankuni) leaves	UV-Vis, NTA, DLS, TEM	40±19	1	426	(31)
Catharanthus roseus (bright eyes) leaves	UV-Vis, SEM, EDX, XRD	35-55	1	390 to 410	(32)
Azadirachta indica (Neem) leaves	UV-Vis, SEM, FTIR	45	1	430	(10)
Ipomoea batatas (Kolmi) leaves	UV-Vis, XRD, FTIR, SEM, TEM, EDX	10- 50	1	410	(33)
Aegle marmelos (Bael) leaves	UV-Vis, DLS, FE-SEM, XRD, HR-TEM, EDS, XRD	60	1	422	(34)
Leptadenia reticulata (Dori) leaves	UV-Vis, XRD, SEM	50-70	0.5	420	(35)
Trigonella foenum-graecum (Medoghna) seed	UV-Vis, SEM, XRD, FTIR, EDAX	33.93	1	420	(36)
Acalypha indica (Indian Mercury) leaves	UV-Vis, SEM, XRD, EDS, HRTEM	20–30	1	420	(37)
Mangifera indica (Mango) leaves	UV-Vis, XRD, PSA, SEM with EDS	32 ± 2	1	393	(38)
Carica papaya (Papaya) leaves	UV-Vis, XRD, SEM-EDX, TEM, FTIR	50-250	1	470	(39)
Memecylon edule (Iron wood tree) leaves	UV-Vis, SEM, TEM, FTIR, EDAX	50-90	1	475	(40)
Murraya Koenigii (curry tree) leaves	UV-Vis, TEM, XRD, FTIR	10	1	411	(41)
Dioscorea bulbifera (Air potato) tubers	UV-Vis, TEM, HRTEM, EDS, XRD, FTIR, DLS	8-20	1	450	(42)
Diospyros malabarica fruit	UV-Vis, XRD, FTIR, DLS, Zeta potential, FESEM, EDX, TEM, PL	17.4	1	430	(9)
Momordica cymbalaria fruit and tuber	UV-Vis, SEM, EDS, XRD, FTIR	20.35, 60.85	1	446, 432	(43)
Asparagus officinalis root	UV-Vis, SEM, TEM, XRD, FTIR	16	3	425	(44)
Euphorbia nivulia stem	UV-Vis, SEM, FTIR	20-90	1	432	(3)
Hemigraphis colorata flower	UV-Vis, FTIR, SEM, TEM, XRD	10-20	0.1	360	(4)
Buchanania lanzan gums/poly-saccharides	UV–Vis, FTIR, zeta potential and particle size analysis, SEM, TEM, atomic force microscopy	14.74–19.86	0.5	415–440	(45)

Use of green synthesized silver nanoparticles in our daily lives and industry

The potential biological activities of AgNPs have been reported in several studies. AgNPs have been used in various industries including the textile industry. The antimicrobial activity of nano silver and its promising role in wound healing (39) has made it a key component in textiles and other industries. AgNPs have been extensively used for coating on medical and contraceptive devices, medical textiles, diagnosis, treatment and drug delivery (27). AgNPs are reported to possess anti-fungal, antiviral, anti-inflammatory, antiplatelet, anti-angiogenesis activity (38). AgNPs are also used in home water purification systems, electronics, cosmetics, nanodevice fabrication, medicine, drug delivery, biosensing, imaging, mosquito larvicidal and treatment of brucellosis, chronic ulcers etc. (44). AgNPs are now used in wound dressings, bond prostheses, surgical instruments and artificial heart valves. In addition, AgNPs are used in water cleaners, food storage containers, room sprays and laundry additives (9). NPs also aided in plant growth and germination by sequestering nutrients for them and could hence be implemented for agricultural purposes. Antibacterial effect of AgNPs was evaluated against the bacteria present in sewage water samples by spread plate method and a significant decrease of the bacterial load was found (12).

Antibacterial activity

A plethora of studies have revealed that AgNPs have good antibacterial activity against pathogenic bacteria. The inhibition studies were performed against *Escherichia coli*, *Staphylococcus aureus* (13), *Bacillus cereus*, *Micrococcus luteus*, *Klebsiella pneumoniae* (16). AgNPs may exert its potential to kill the bacteria by any of the following means: (i)

release of silver ions and generation of reactive oxygen species (ROS); (ii) accumulation in the cell membrane, thus affecting membrane permeability; (iii) interaction with membrane proteins which affects their function; and (iv) entry into the cell where it can lead to ROS generation and release of silver ions, which in turn, can interact with DNA (16).

Smaller NPs are found to be more toxic to bacteria, due to their easier uptake and larger surface area. Furthermore, the potential for the silver ion release is also increased with the decreasing size of AgNPs (46). The silver ions released from AgNPs are certainly responsible for its high bactericidal activity. Interaction of cell membranes with the released silver ions causes increased membrane permeability. In *E. coli*, a Gram-negative bacterium, the diameter of the zone of inhibition due to AgNPs is higher compared to that of Gram-positive bacteria, which may be due to fact that Gram-negative bacterial cell wall contains a thinner peptidoglycan layer (20). A schematic diagram is provided in Fig. 2.

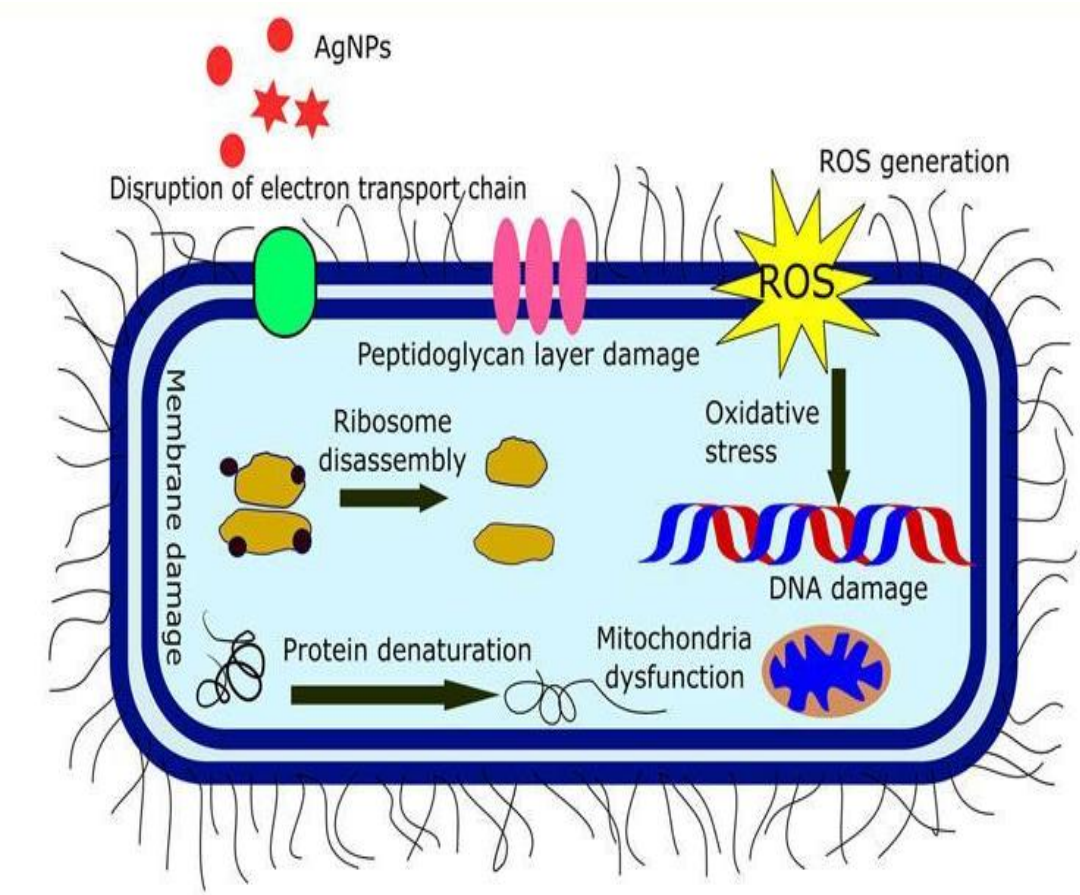

Fig. 2: Effect of phytosynthesized AgNPs on bacterial cells: possible mechanisms

Antifungal activity

Use of nano-sized AgNPs has become more prevalent, as their production has become more economical. Since AgNPs exert their inhibitory activity on microorganisms in multiple ways, they can be utilized for the management of diseases of plants. Besides this, it will be safer compared to synthetic fungicides in controlling various plant pathogens (16). It is found that *A.cepa* synthesized AgNPs prevent the growth of *Ceci neri* and *Vigna radiata* seedlings by attacking *Fusarium oxysporum* (15). AgNPs synthesized from *Amaranthus gangeticus* inhibits the growth of plant pathogenic fungi *Sclerotinia sp.* by producing a hollow zone. It damages the cell envelope by piercing the cell and then binds to the DNA (25).

Antiviral activity

Green synthesized AgNPs have antiviral activity. AgNPs synthesized using *A. paniculata, P. niruri* and *T. cordifolia* possess antiviral properties against chikungunya virus (CHIKV). The AgNPs synthesized from *A. paniculata* and *T. cordifolia* showed excellent antiviral activity against CHIKV when tested on Vero cells (2).

Antioxidant activity and radical scavenging activity

For appropriate determination of antioxidant capacity, the extraction technique, its conditions, solvent used, and particular assay methodology are important (48). In living systems, the formation of oxygen free radicals and uncontrolled accumulation of H_2O_2 cause major oxidative stress in cell

membranes (49). The different functional groups adhering to the surface of phytosynthesized nanoparticles are responsible for their antioxidant property. These functional groups are contributed by the medicinal plant extracts during the green synthesis process (Table 3). These groups also increase the biological activity of the NPs. Antioxidant activity of AgNPs from different medicinal plants, e.g., *Hygrophila auriculata* (12), *Bauhinia tomentosa* (26), *Nyctanthes arbor-tristis* (22), *Basella alba* (14) have been reported.

Table 3: Phytochemical components and pharmacological activities of herbal phytosynthesized silver nanoparticles

Plant name	Phytochemicals	Pharmacological activities	Ref.
C. roseus	alkaloids, terpenoids, flavonoids	antimalarial, antibacterial, antidiabetic, antioxidant, anticancer, antioxidant activity	(32)
C. asiatica	flavonoids	ant filarial, antibacterial, antioxidant, anti-inflammatory activity	(31)
C. papaya	phenols, terpenoids, flavonoids	antibacterial, antioxidant, immunostimulant activity	(39)
H. auriculata	alkaloids, glycosides, alkaloids	anticancer, hypoglycemic, aphrodisiac, antimicrobial, antioxidant, hepatoprotective, hematopoietic activity	(12)
N. arbor-tristis	flavonoids tannins, glycosides	antimicrobial and antioxidant activity	(22)
B. alba	ascorbic acid, carbohydrates, proteins, flavonoids, phenols	antibacterial, anti-oxidant, antifungal, anti-inflammatory and analgesic activity	(14)
A. marmelos	flavonoids, phenol, isoflavones, catechins	antimicrobial, antioxidant, anti-inflammatory, antidiabetic activity	(34)
P. emblica	alkaloid, tannin, phenol, flavonoid, sterol, saponin	anti-inflammatory, antidiabetic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective activity	(28)
M. Koenigii	alkaloid, flavonoid, polyphenol	lipidemic, antioxidant, antimicrobial activity	(41)
D. bulbifera	flavonoid, phenolic, glycoside	antibacterial, antioxidant and antidiabetic properties	(42)
D. malabarica	gallic acid, flavonoid, anthocyanin, saponin, alkaloid, tannins, triterpenes	antioxidant, antiprotozoal, antihelminthic, antiviral and anticancer activity	(9)
M. cymbalaria	flavonoids, quercetin, saponin, glycosides	hypoglycaemic activity	(43)
A. officinalis	shatavarin	antimicrobial, insecticidal, anti-cancerous, anti-oxidative activity	(44)
H. colorata	flavonoids, saponins, carboxylic acid, carbohydrate	antimicrobial, anti-inflammatory, antidiabetic activity	(4)

Anticancer activity

Plant extracts are the best sources to evaluate the anticancer activity with least or no side effects for safe diagnosis (50). The anticarcinogenic activity of *B. tomentosa* synthesized AgNPs was established by the MTT assay on lung A549 carcinoma cell line. 50% of A549 cells were inhibited at 28.125 µg/mL conc. of synthesized AgNPs (26). Some researchers suggest that the phytochemicals present in the extracts may be related with the anti-cancer property of the green synthesized NPs. *Trigonella foenum-graecum* seed synthesized AgNPs have anticancer properties which can be attributed to the presence of secondary metabolites such as flavonoids, alkaloids and other phenolic compounds present in the fenugreek seeds (36). Each individual property of AgNPs makes it an option for cancer treatment. The cytotoxic activity of AgNPs from various medicinal plants, such as *L. reticulata* (35), *Diospyros malabarica* (9) have also been reported.

Antibiofilm activity

Phyllanthus emblica synthesized AgNPs demonstrated a significant antibiofilm activity against pathogen Ao strain RS-2 of rice bacterial brown stripe (28). Studies reveal that *Mangifera indica* synthesized AgNPs can prevent the formation of bacterial colonies in the medicated part of the teeth and can restore the dental structure (38).

Anti-plasmodial activity

Antimalarial activity of *Catharanthus roseus* synthesized AgNPs was observed at different concentrations of AgNPs. 20.0% inhibition rate of parasites was observed in parasitaemia at 25 g/mL concentration of the AgNPs (32).

Larvicidal activity

The larvae killing activity of *Ipomoea batatas* synthesized AgNPs was observed at various concentrations against the first to fourth-instar larvae of the mosquito vectors *Aedes albopictus*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The mortality rate at 25 µg/mL concentration was 34.3% and it increased to 81% when the concentration was increased to 125 µg/mL, which reveals that the mortality rate is proportional to the conc. of AgNPs used (33).

Catalytic activity

Presently, natural pigments and synthetic organic dyes are directly discharged into water and soil, which can affect the plants and animals indirectly.

Studies reveal that the green synthesized AgNPs exhibit excellent performance for the catalytic reduction. The catalytic action of AgNPs synthesized using different plants, such as. *Cassia auriculata* (18), *Amaranthus gangeticus* (25), *Terminalia chebula* (7), *Diospyros malabarica* (9) have been reported.

Leishmanicidal activity

AgNPs showed good leishmanicidal activity compared to pentostam. It is found that *Leishmania donovani* can infect macrophages at 79% without any treatment, while when AgNPs synthesized using *Fusarium graminarum* (2.5 µg/mL) and pentostam (100 mg/mL) was used as a treatment, reduction of infection was found and percentage of infected macrophages with promastigotes were only 22% and 35%, respectively. These results prove that the effect of *Fusarium* AgNPs on macrophage infection with promastigote was greater than that of pentostam, when compared with the control group (47). Similar kind of activity was found in the case of AgNPs synthesized from *Commiphora myrrh*. It showed a marked and significant growth inhibition of *Leishmania major* promastigotes. The higher concentrations of AgNPs (100 and 150 µL) had a significantly greater inhibitory effect for the treatment on the promastigote growth compared to the pentostam 150 (46).

CONCLUSION

Plant mediated green synthesis of silver nanoparticles using extracts of Indian medicinal plants can be done by a simple, eco-friendly and cost-effective technique. As the Indian subcontinent is renowned for its greeneries, extending from the sea level to world's highest mountain Himalaya, more studies are required to explore the other plants that can be used for the synthesis of AgNPs. This work also suggests that phytochemicals may control the shape, size and yield of the NPs and also their biological activities. Although intensive research is needed to identify the exact phytocompound which is mainly responsible for the synthesis of AgNPs. Here, in this review, the optimization, characterization and biological activity of the AgNPs were critically discussed that could benefit young researchers working in this area.

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CONFLICT OF INTEREST

Authors have no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

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