

Green Synthesis of Silver Nanoparticles (Ag-NPs) Using Plant Extract For Antimicrobial and Antioxidant Applications : A Review

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ABSTRACT

Development of environmental friendly technique for the synthesis of nanoparticles has emerged as a significant step in the field of nanotechnology. Nanotechnology is the branch of science that deals with the framing of materials at atomic level to achieve unique properties, which can be then manipulated for desired applications. Out of all the metallic nanoparticles silver nanoparticles grabs more attention because of its unique physical, chemical and biological properties. To overcome the limitation of conventional methods used for synthesizing nanoparticles green chemistry has emerged as an alternative. Use of plants in synthesis of nanoparticles among all the green methods available is by far considered as most suitable methods because of wide variability of bio-molecules present in them which not only act as reducing but as stabilizing/capping agents and thus increases the rate of reaction. Moreover unlike microbial culture they are easy to handle, widely distributed and easily available. The present review explores a wide variety of plants to be used for rapid and single step protocol for synthesis of silver nanoparticles and also describes its antimicrobial and antioxidant properties.

Keywords : *Silver nanoparticle synthesis, Bioreduction, Plant extract, Antimicrobial, Antioxidant*

I. INTRODUCTION

Nanotechnology is the field of science that deals with the synthesis, manipulation and use of particles ranging in size 1 to 100 nm. Such particles are termed as nanoparticles. Nanoparticles show a unique and significantly modified physical, chemical and biological properties, as compared to their macroscaled counterparts, which make them of particular interest. Day by day increasing incidence of microbial challenges, multiple drug resistance (MDR) micro-organisms, poor dietary intake and serious health hazardous drugs call on new site for researchers to work on prominent antimicrobial active metabolites with good antioxidant activity to boost metabolism of an individual and overcome the problem of clinically significant microorganisms including MDR microorganisms. With this respect silver and silver based compounds has been long known for its toxicity against microorganisms including bacteria and fungi. For over centuries, silver and silver based compounds have been used as non-hazardous, inorganic, and antibacterial agents in many applications like wood preservatives or for water purification in hospitals because of their biocidal properties. The recent advances in the field of nanotechnology have a strong impact in many scientific areas and the synthesis of silver

nanoparticles has also followed this tendency. Various literatures depict many ways to synthesize silver nanoparticles which include physical, chemical, and biological methods. The physical and chemical methods used for the synthesis of nanoparticles are not only energy consuming but also non eco-friendly due to the use of toxic solvents and stringent techniques. Thus efforts has been made for the development of eco-friendly and cost effective technique for synthesizing nanoparticles. So, use of plant extracts is the most adopted green and rapid method for nanoparticle synthesis because they are widely distributed, easy and safe to handle and contain several metabolites required for reduction and stabilization of nanoparticles.

This review article draws the attention regarding the potential of plant metabolites for the biosynthesis of silver nanoparticles and provides a database for the future researchers in the field of biosynthesis of nanoparticles using plant extracts and also describes its antimicrobial and antioxidant properties.

II. METHODS FOR SYNTHESIS OF NANOPARTICLES

Many techniques for the synthesis of metallic nanoparticles are now available. Synthesis of nanoparticles generally involve either “top to bottom” approach or “bottom to up” approach [1]. In top down method of synthesis, the nanoparticles are produced by size reduction from an appropriate starting material [2]. Variety of physical and chemical treatments are used for the achievement of size reduction, physical approaches include techniques such as evaporation-condensation and laser ablation whereas chemical approaches include chemical reduction by use of organic and inorganic reducing agents. Top down fabrication methods introduce imperfections in the surface structure of the product and this is a major drawback because the surface chemistry and the other physical properties of nanoparticles are greatly dependent on the surface structure [3].

In bottom up method of synthesis, the nanoparticles are built from smaller entities, for example by fusion of atoms, molecules and smaller particles [4]. In bottom up synthesis, the nanostructured building blocks of the nanoparticles are produced first and then assembled to manufacture the final particle [3]. The bottom up synthesis mostly achieved by chemical and biological methods.

The biological methods can be used to synthesize nanoparticles without the use of any harsh, toxic and expensive chemical substances. Out of all the biological methods used for the synthesis of nanoparticles, the methods based on microorganisms have been frequently reported [5,6]. Various advantages of microbial synthesis is that its readily scalable, environment friendly and compatible with the use of the product for medical applications but production of microorganisms is often more expensive than the production of plant extracts. Plant mediated nanoparticles synthesis using whole plant extract or by living plants was also reported in literature [7,8]. Different methods for synthesis of metallic nanoparticles were depicted in “Fig.1”.

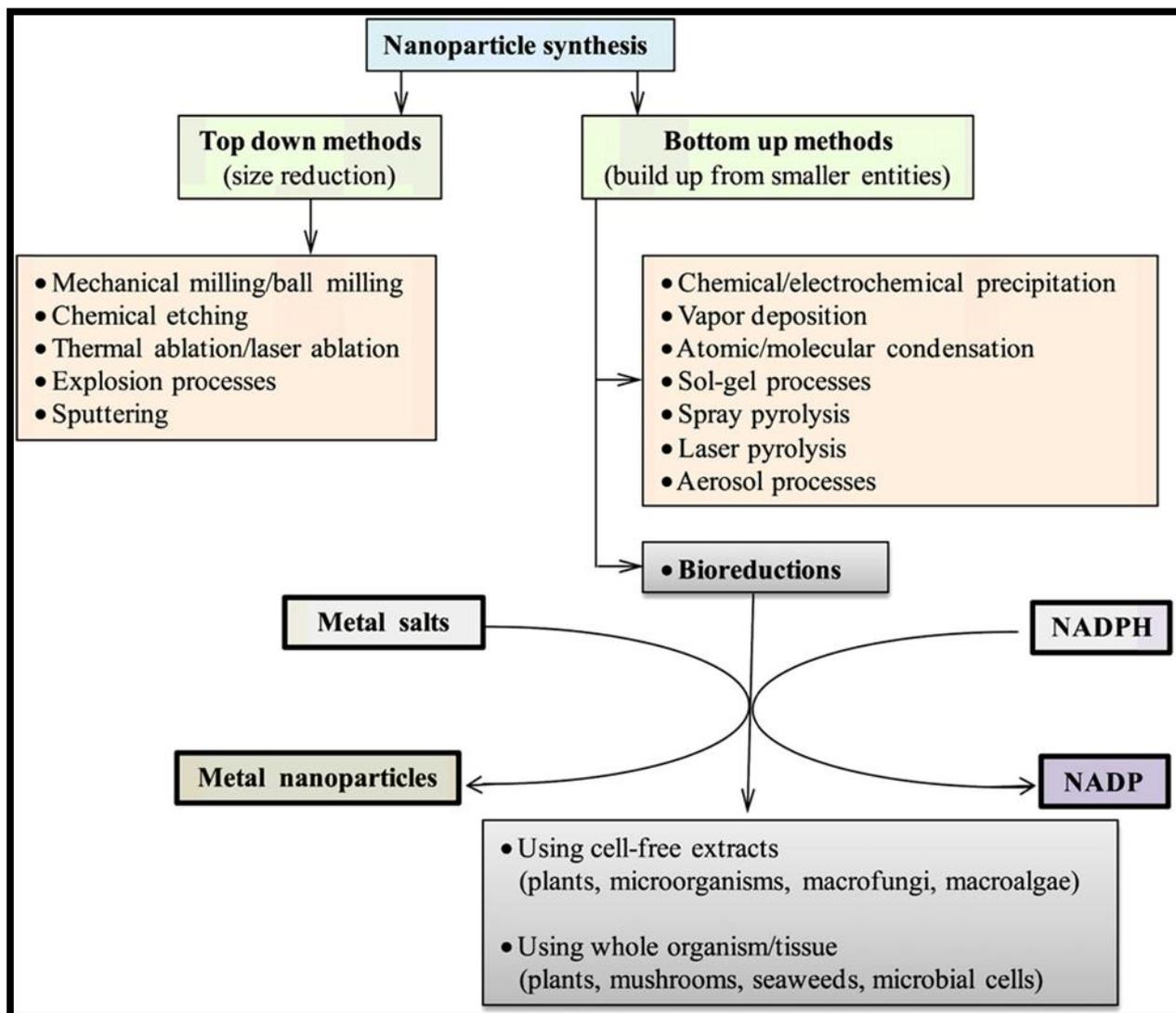


Figure 1: Different Approaches for Nanoparticle Synthesis

III. USE OF PLANT EXTRACTS IN SYNTHESIS OF SILVER NANOPARTICLES (Ag-Nps)

The use of plants extracts in the synthesis of silver nanoparticles has drawn attention, because of its rapid, environment friendly, non-pathogenic, economical protocol and providing a rapid technique for the biosynthetic processes. Moreover plant extracts are easy and safe to handle unlike microbial cultures. The reduction and stabilization of silver ions is achieved by a combination of variety of biomolecules such as amino acids, proteins, enzymes, polysaccharides, alkaloids, phenolics, tannins, saponins, terpenoids and vitamins which are already present in the plant extracts having medicinal values and are environment friendly, yet chemically complicated structures [9]. A large number of plants and plant extracts are reported to facilitate silver nanoparticles synthesis are mentioned in “Table 1” and are briefly discussed in the present review.

The general protocol for silver nanoparticle synthesis using plant extracts involves the following steps:

1. **Collection of the plant material:** Plant part of interest is collected and washed thoroughly with tap water to remove necrotic parts, dirt and epiphytes, followed by sterile distilled water to remove debris if any. The fresh and clean plant part is then shed dried for 10-15 days and powdered with the help of blender.
2. **Plant extract preparation:** The dried powdered is mixed with desired solvent in 1:10 ratio and boiled for few minutes. The infusion is then filtered through Whatmann filter paper to remove the insoluble material from the extract.
3. **Precursor preparation:** Precursor for silver nanoparticle synthesis is prepared as 1mM AgNO₃ solution.
4. **Synthesis of Ag-Nps:** 1mM AgNO₃ solution is added to few ml of the plant extract which leads to the reduction of Ag(I) ions to Ag(0).
5. **Confirmation:** Synthesis of Ag-Nps can be confirmed by measuring the UV–visible spectra of the solution at uniform intervals [10]. The absorption maxima of Ag-Nps ranges between wavelength of 400–450 nm [11]. After synthesis the nanoparticles are characterized by using various techniques. At last after affirmation of formation of nanoparticles for its application it needs to be separated and purified from the plant extract. A generalized methodology for silver nanoparticles synthesis using plant extract is depicted in “Fig.2”.

Factors affecting the rate of production of the nanoparticles, their quantity and other characteristics includes nature of the plant extract and its concentration ,the pH, the concentration of the metal salt temperature and contact time. [12]. “Fig.3” shows the probable chemical constituents present in the plant extract responsible for the bioreduction of metal ions, their growth and stabilization.

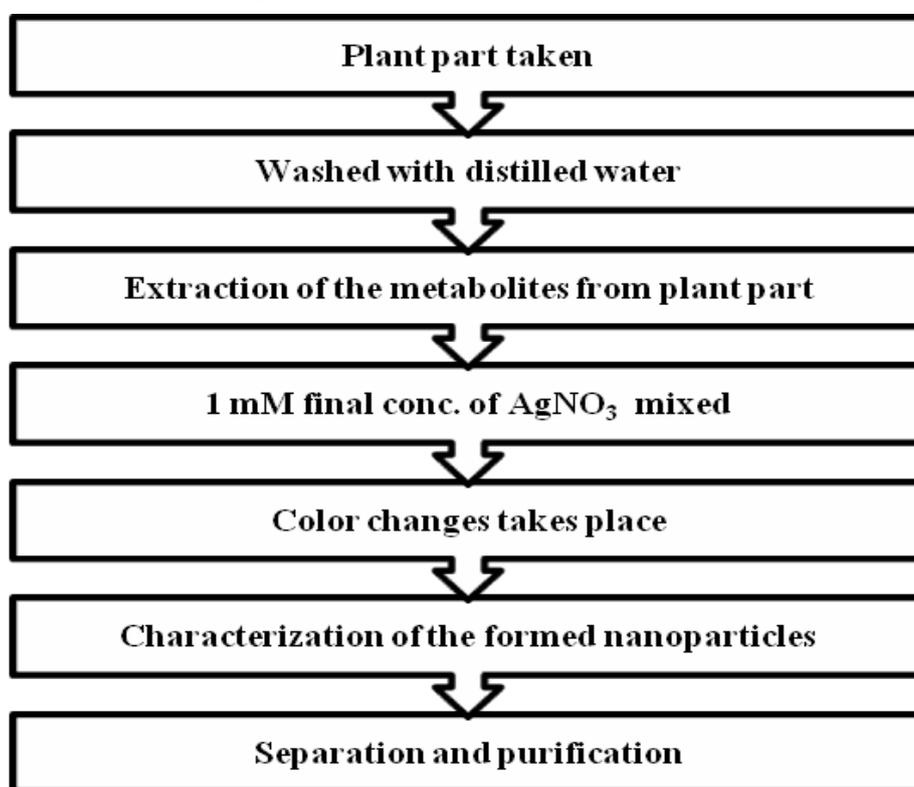


Figure 2: Generalized Methodology for Silver Nanoparticles Synthesis Using Plant Extract

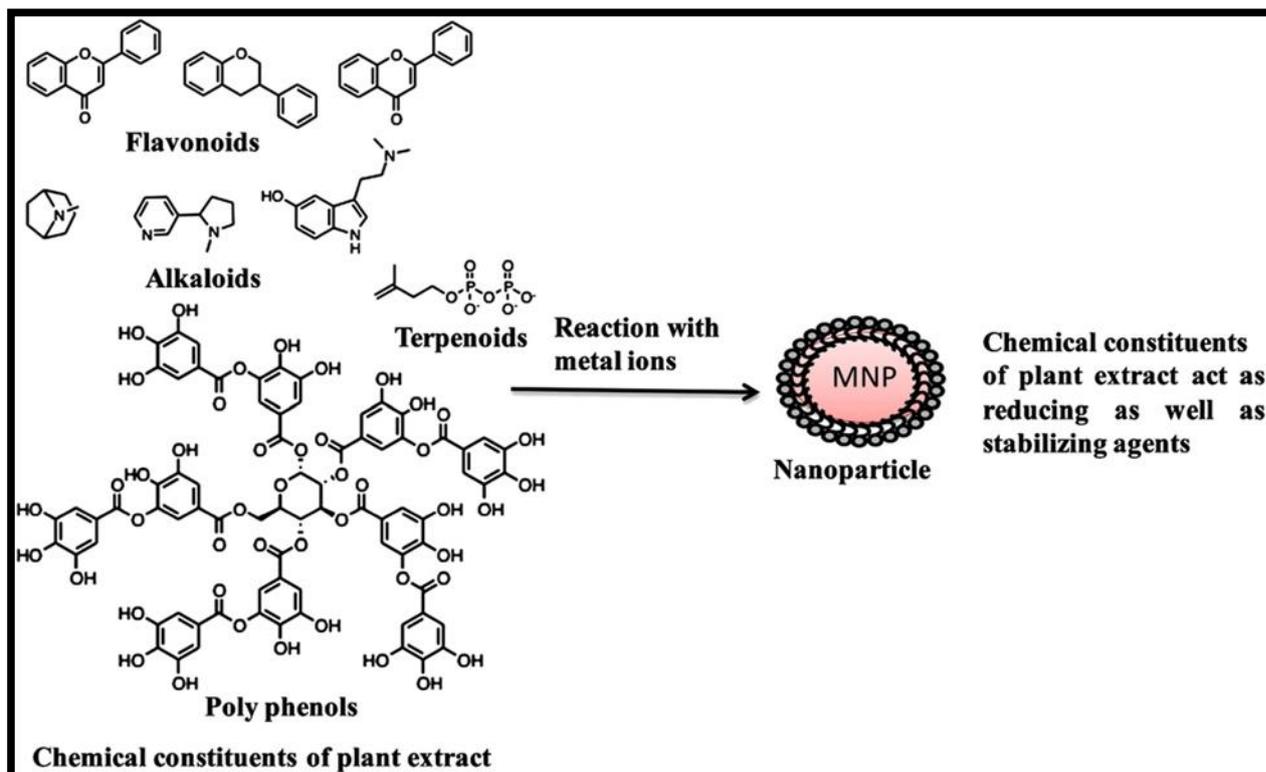


Figure 3: Possible Chemical Constituents of Plant Extract Responsible for the Bioreduction of Metal ions [13,14].

IV. CHARACTERIZATION OF NANOPARTICLES

Nanoparticles are generally characterized by their size, shape, surface area, and dispersity [15]. The uniformity of these properties of nanoparticles is important in many applications. Ample of evidences shows that physicochemical properties, such as size and surface chemistry, can affect nanoparticle behavior noticeably in biological systems and might, to some extent, determine the biodistribution, safety and efficacy of a particle. The nanoparticles are commonly characterized by the following techniques : UV–visible spectrophotometry, scanning electron microscopy (SEM), dynamic light scattering (DLS) transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), energy dispersive spectroscopy (EDS) and powder X-ray diffraction (XRD) [16,1,17]. Primarily the nanoparticles are characterized by UV–visible spectroscopy and it is the most commonly used technique [18]. Light wavelengths between 300–800 nm are generally used for characterizing various metal nanoparticles in the size range of 2 to 100 nm [16]. Silver nanoparticles are characterized by spectrophotometric absorption measurements in the wavelength ranges of 400–450 nm [11]. Characterization on the basis of the surface charge and the size distribution of the particles suspended in a liquid can be done by using dynamic light scattering (DLS) technique [15].

An additional commonly used method of characterization of nanoparticles is electron microscopy [19]. Morphological characterization at the nanometer to micrometer scale can be achieved by using Scanning electron microscopy and transmission electron microscopy [20]. The transmission electron microscopy has a

1000 times higher resolution as compared to the scanning electron microscopy as it uses more powerful beam of electrons [21]. TEM provides greater detail at the atomic scale, such as information about the crystal structure and granularity of a sample. FTIR spectroscopy is helpful for characterizing the surface chemistry [22]. Organic functional groups (e.g. hydroxyls, carbonyls) attached to the surface of nanoparticles and the other surface chemical residues can be detected by using FTIR. It is based on the fact that the subatomic particles in a molecule do not remain in intact position and transist some other position due to vibrations. If there is a periodic alteration in the dipole moment, then such mode of vibration is Infrared (IR) active by molecular vibrations. Each functional group has significant range of vibrational frequencies and sensitive to the physiochemical environment thus providing valuable information regarding the presence of certain functional groups in the specific sample.

The phase identification and characterization of the crystal structure of the nanoparticles can be done by using XRD technique [23]. X-rays penetrate deep into the nanomaterial and the resulting diffraction pattern is compared with standards to obtain structural information. For characterization on the basis of elemental composition of metal nanoparticles, the commonly established technique called energy dispersive spectroscopy (EDS) is used [24].

V.ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING PLANT EXTRACTS

Antimicrobial property of silver owes to the fact that microbes cannot build up resistance against it as they are doing against conventional and narrow-target antibiotics, because the metal attacks a broad range of targets in the organisms, which means that they would have to develop a host of mutations simultaneously to protect themselves [18].

Cubic silver nanoparticles of an average particle size 15 nm were synthesized by using *Carica papaya* which were found to be highly toxic against different multi drug resistant human pathogens [25].

Silver nanoparticles (20–30 nm) were synthesized utilizing a leaf concentrate of *Acalypha indica*. The nanoparticles were appeared to be antimicrobial against water borne pathogens, for example, *E. coli* and *Vibrio cholera* [26]. Circular silver nanoparticles (40–50 nm) have been created utilizing a leaf concentrate of *Euphorbia hirta* [27].

Extract of banana (*Musa paradisiaca*) peels were employed for the synthesis of silver nanoparticles and found that these nanoparticles exhibit antifungal activity against the yeasts *Candida albicans* and *Candida lipolytica*, and antibacterial activity against *Escherichia coli*, *Shigella sp.*, *Klebsiella sp.* and *Enterobacter aerogenes* [28].

Silver nanoparticles were synthesized by employing leaf extract of *Ocimum sanctum*, the particles formed were in the size range of 4-30 nm and were tested for antimicrobial activity. The study showed that the synthesized nanoparticles exhibit increased antimicrobial activity on gram-negative bacteria than gram-positive ones also have stronger activity than silver nitrate and standard antibiotic ciprofloxacin which were used as positive control [29].

Silver nanoparticles created utilizing peel concentrate of *Citrus sinensis* were found to have a wide range antibacterial movement. The particles framed at 60 °C had a normal size of around 10 nm yet lessening the response temperature to 25 °C expanded the normal size to 35 nm [30].

A tuber concentrate of *Dioscorea bulbifera* was utilized to create gold also, silver nanoparticles of different shapes [31,32]. These nanoparticles in mix with antibiotics were found to have a synergistic antibacterial action against test microorganisms, especially against *Pseudomonas aeruginosa*, *Escherichia coli* furthermore, *Acinetobacter baumannii* [32]. Utilization of antibiotics in blend with silver nanoparticles has been accounted for powerful control of generally antibiotic resistant safe microorganisms.

Dried fruit body extract of *Tribulus terrestris* was employed for the formation of silver nanoparticles, size range of 16-28 nm was achieved with antibacterial property against multi-drug resistant bacteria such as *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* [33].

Leaf concentrate of *Calotropis gigantean* was utilized to create silver nanoparticles and these nanoparticles showed antibacterial activity against *Vibrio alginolyticus* [34].

Profoundly stabilized silver nanoparticles (25–40 nm) were delivered utilizing a leaf concentrate of *Ocimum tenuiflorum*. The particles were antibacterial towards Gram-negative and Gram-positive microorganisms [35,36].

The plant extract of *Boerhaavia diffusa* was utilized for the synthesis of silver nanoparticles, an average particle size of 25 nm was obtained. These nanoparticles were tested for their antibacterial activity against three fish pathogens namely *Pseudomonas fluorescens*, *Aeromonas hydrophila* and *Flavobacterium branchiophilum* and found to have highest activity against *F. branchiophilum* [37].

Spherical silver nanoparticles of an average diameter 8-10 nm were synthesized by using fruit extract of cucumber (*Cucumis sativus*) and analyzed their photocatalytic and antimicrobial activity. The Photo catalytic study suggests the efficiency of these biosynthesized nanoparticles in degrading organic dyes methylene blue under solar radiation. And moreover, the result of antibacterial assay (tested against- *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*) showed that these nanoparticles possess effective bactericidal property [38].

Table 1. Plants used by various researchers in the biosynthesis of silver nanoparticles

S.No.	Name of the plant	Size of nanoparticles	Reference
1.	<i>Dioscorea bulbifera</i>	8-20 nm	[32]
2.	<i>Tribulus terrestris</i>	16-28 nm	[33]
3.	<i>Boerhaavia diffusa</i>	25 nm	[37]
4.	<i>Musa paradisiaca</i>		[28]
5.	<i>Calotropis gigantean</i>	6-12 nm	[34]
6.	<i>Ocimum tenuiflorum</i>	25-40 nm	[35,36]
7.	<i>Acalypha indica</i>	20-30 nm	[26]
8.	<i>Euphorbia hirta</i>	40-50 nm	[27]
9.	<i>Citrus sinensis</i>	10 nm at 60 °C and 35 nm at 25 °C	[30]
10.	<i>Syzygium cumini</i>	93 nm	[39]
11.	<i>Hyacinthus orientalis</i> and	-	[40]

	<i>Dianthus caryophyllus</i>		
12.	<i>Morinda pubescens</i>	-	[41]
13.	<i>Chenopodium murale</i>	30-50 nm	[42]
14.	<i>Piper longum</i>	46 nm	[43]
15.	<i>Carica papaya</i>	15 nm	[25]
16.	<i>Ocimum sanctum</i>	4-30 nm	[29]
17.	<i>Allium cepa</i>	33.6 nm	[44]
18.	<i>Aloe vera</i>	15.2 ± 4.2 nm	[45]
19.	<i>Cucumis sativus</i>	8-10 nm	[38]
20.	<i>Cinnamon zeylanicum</i>	50-100 nm	[46]
21.	<i>Aloe vera</i>	70 nm	[47]
22.	<i>Cinnamomum camphora</i>	10-20 nm	[14]
23.	<i>Chenopodium album</i>	12 nm	[12]
24.	<i>Datura metel</i>	16-40 nm	[48]
25.	<i>Embllica officinalis</i>	10-70 nm	[49]
26.	<i>Citrus limon</i>	10-30 nm	[50]
27.	<i>Capsicum annum</i>	30-70 nm	[51]
28.	<i>Mentha piperita</i>	90 nm	[52]
29.	<i>Azadirachta indica</i>	< 30 nm	[53]
30.	<i>Desmodium triflorum</i>	5-20 nm	[54]
31.	<i>Zingiber officinale</i>	10 nm	[55]
32.	<i>Eucalyptus hybrid</i>	50-150 nm	[13]
33.	<i>Moringa oleifera</i>	57 nm	[56]
34.	<i>Nelumbo nucifera</i>	25-80 nm	[57]
35.	<i>Cymbopogon citratus</i>	32 nm	[58]

VI. ANTIOXIDANT ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING PLANT EXTRACTS

Many scientists studied the antioxidant activity of plant extract mediated synthesized silver nanoparticles at different time. Concentrate of *Syzygium cumini* (Jambul) seeds were utilized to synthesize silver nanoparticles. The seed extract showed antioxidant property in-vitro. The nanoparticles formed using the extract were found to have elevated antioxidant activity compared with the seed extract. This may have been due to a better adsorption of the antioxidant material from the extract onto the surface of the nanoparticles [39]. Extract of ornamentals plants such as *Hyacinthus orientalis* and *Dianthus caryophyllus* were utilized to produce silver nanoparticles and reported that the nanoparticles formed has higher antioxidant activity as compared to the normal plant extracts [40]. In-vitro evaluation of antioxidant and anticancer potential of *Morinda pubescens* synthesized silver nanoparticles was studied and found that the nanoparticles possess high antioxidant capacity and thus can be used as potential radical scavengers against deleterious damages caused by the free radicals [41]. *Chenopodium murale* leaf extract was employed for the synthesis of silver nanoparticles, in-vitro antioxidant and antimicrobial activity of the formed nanoparticles was evaluated and found that AgNPs containing leaf extract showed a higher antioxidant activity compared to *C. murale* leaf extract alone or silver nitrate [42]. The fruit extract of *Piper longum* was utilized for synthesis of silver nanoparticles, an average size of 46 nm was formed and they showed a potent antioxidant properties in in-vitro antioxidant assays [43].

VII. CONCLUSION

The green synthesis of silver nanoparticles using plants extracts have many advantages over other methods as they are cost effective, easily scaled up and environment friendly. It is especially suited for making nanoparticles that must be free of toxic contaminants as required in therapeutic applications. Green synthesized silver nanoparticles have noteworthy aspects of nanotechnology through unmatched applications and synthesis of nanoparticles using plants can be beneficial over other other biological methods because plant products are easier and safer to handle, widely distributed and easily available. The present review by conferring various literatures reported recently has showed the importance of plant extract mediated synthesis of silver nanoparticles and describes them as a promising antimicrobial and antioxidant agent.

VIII. ACKNOWLEDGEMENT

The author, Jasmeet Kaur Sohal gratefully acknowledges all the teaching and non-teaching staff of Faculty of Biological and Chemical Sciences, MATS University, Raipur (C.G.)

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