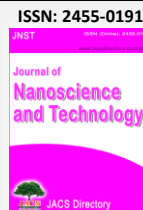




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Biosynthesis of Metallic Nanoparticles from Medicinal Plants: A Review

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ABSTRACT

Biosynthesis of nanoparticle from various medicinal plants has been effectively used in controlling the various diseases. Secondary metabolites which are derived from various plant parts such as leaves, stems, roots, shoots, flowers, barks and seeds through extraction used as reducing agents for bioreduction reaction. The secondary metabolites present in the plants such as terpenoids, flavones, ketones, aldehydes, alkaloids, tannins, phenolics and saponins used in the biological synthesis of nanoparticles which are a less toxic and non-hazardous method as compare to the other physical and chemical biosynthesis. Various metals such as cobalt, copper, silver, gold, palladium, platinum, zinc oxide and magnetite are used for nanoparticle synthesis. There are different techniques which are used to characterize nanoparticles such as UV-visible (vis) spectrophotometer, dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDS) and Fourier transform infrared spectrometer (FTIR). Plant derived nanoparticles shows various pharmacological applications such as anti-bactericidal activities, anti-fungicidal activities, antiplasmodial activity, anti-inflammatory action, anticancer studies, antiviral effects, antidiabetic and antioxidant activities.

1. Introduction

Ethnobotanical data exist in Indian medicine is known for its richness across the globe. Ethnobotanical surveys are done in order to collect information and identification of unexplored herbal plants on the basis of traditional knowledge that helps in healthcare programs. Traditional information was obtained through ethnobotanical surveys which include interviews of traditional healers. Medicinal important plant species are documented on basis of their medicinal values. Indian medicinal plants in large number have been used in the treatment of urolithiasis with fewer side effects [1]. The medicinal use of the documented plants provides a basis for further research focused on pharmacological studies and conservation of the most important plant species. Some important tribal communities from Manipur (India) provide important information about plants which are useful against stone formation in the urinary system [2]. Identification of these plants may be novel and reported for the first time. Indian rich biodiversity and easy availability of medicinal plant have been explored for the synthesis of nanoparticles. Nanoparticles are gaining much importance in a pharmaceutical study. Plants which are collected and documented through surveys can be used in nanoscience for nanoparticle biosynthesis. Synthesis of noble metallic nanoparticles from a particular plant has become a major research area in the field of nanotechnology. Medicinal plants are needed to develop clean, nontoxic, simple and eco-friendly procedures for the synthesis of nanoparticles [3]. Monodispersed nanoparticles biogenic syntheses with specific sizes and shapes have been a challenge in biomaterial sciences. Nanoparticles have remarkable advantages in the pharmacological industry to cure various bacterial and viral diseases [4]. In ancient times, metals like gold and silver were used in different forms to improve human health. Use of metals like gold and silver metal in nanoparticles biosynthesis is considered as a metal-based antimicrobial technique. Metallic nanoparticles such as silver nanoparticle have extremely large surface area which provides better attachment to the cell membrane of bacteria. This leads to penetration into the bacteria cell membrane and after penetration, silver nanoparticles interact with proteins in the cell as well as with the phosphorus-containing compounds like DNA. They show good antibacterial properties due to their

large surface-area- to-volume ratio [5]. During the last few decades, various chemical and physical methods available for the synthesis of gold nanoparticles like ion sputtering, reverse micelle, chemical reduction, hydrothermal and sol gel [6]. Plant extract from various plant parts having secondary metabolite is the latest technique which is used in green chemistry for nanoparticle biosynthesis. Generally, Techniques for the synthesis of nanoparticles are classified into three categories: Physical, Chemical and Biological techniques. But methods like physical and chemical are not considered as a reliable system to obtain metallic nanoparticles because they require enormous consumption of energy to maintain high pressure and temperature. Chemical procedures involved in the synthesis of nanoparticles use toxic solvents which could generate unsafe and hazardous byproducts and involve high energy consumption [7]. Synthesis of nanoparticles through green processes deals with the safe and ecofriendly methods which are an alternative method for conventional physical and chemical methods. In biological methods, nanoparticle synthesis from plant extracts is the most adopted method as compared to fungi and bacteria. They require comparatively longer incubation time in the growth media for reducing a metal ion. As compared to other methods like chemical and physical method, plant-based synthesis of nanoparticles works at low temperatures and requires modest and environmentally safe components [8].

2. Biosynthesis of Metallic Nanoparticles

Different parts of plants used to produce metallic NPs such as leaves, stem, fruit, flowers and roots. Plant crude extract may contain novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids which are mainly responsible for the bioreduction of ionic into metallic nanoparticles formation [9]. Biosynthesis of metallic nanoparticles using plant extract involve three important parameters, they are (1) metal salt (2) reducing agent (3) stabilizing or capping agent. Metal salts comprising of metal ions such as silver, gold and zinc are first reduced to atoms by means of reducing agents. Reducing agents such as proteins/enzymes, amino acids, carbohydrates, alkaloids, terpenoids, tannins, saponins, phenolic compounds, reducing sugar and vitamins have the ability to reduce metal ions. The stabilizing or capping agent control size of nanoparticles and prevent their aggregation [10]. Biosynthesis of the metallic nanoparticle can be altered by a range of metal concentration

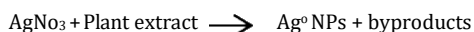
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and amount of plant extract in the reaction medium. It may also transform the shapes and size of the nanoparticles [11,12].

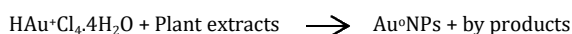
2.1 Biochemical Reactions Mechanism

There are different types of nanoparticle synthesis from plant resources such as silver, gold, copper, platinum and zinc oxide.

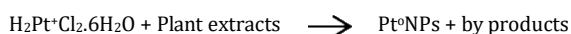
Silver: Reduction reaction of AgNO_3 with plant extract leads to the formation of the silver nanoparticle as shown in reaction [13].



Gold: Plant extract contains metabolites which are involved in bioreduction process reduce Au^+ ions into metallic Au^0 nanoparticle [14] as shown in reaction.



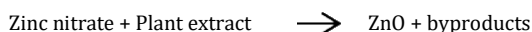
Platinum: It is involved in the following reduction process such as [15].



Copper: Copper nanoparticles are synthesized from plant extracts and their bioreduction mechanism was proposed as [16].



Zinc oxide: A typical procedure was followed in ZnO nanoparticles production. Here, zinc nitrate was dissolved in the aloe plant extract to produce the nanoparticles. This method was followed as shown in reaction [17].



2.2 Preparation of Nanoparticles

Silver nanoparticles (Ag NP) are prepared by addition of leaf extract to an aqueous solution (1 mM) of silver nitrate (AgNO_3). This reaction results in a colour change of the mixture from faint light to yellowish brown to reddish brown to colloidal brown. Then the color change is monitored periodically (time and color change are recorded along with periodic sampling and scanning by UV-visible spectrophotometry) for maximum of 30 min [18].

Table 1 Summary of plants derived nanoparticles

S.No.	Plant Name	Nanoparticle	Plant Part used	Size (nm)	Ref.
1.	<i>Acalypha indica</i>	Ag, Au	Leaves	20-30	[19]
2.	<i>Aloe vera</i>	In_2O_3	Leaves	5-50	[20]
3.	<i>Alternanthera sessilis</i>	Ag	Whole	40	[21]
4.	<i>Andrographis paniculata</i>	Ag	Leaves	67-88	[22]
5.	<i>Argemone mexicana</i>	Ag	Leaves	20-50	[23]
6.	<i>Artemisia nilagirica</i>	Ag	Leaves	70-90	[4]
7.	<i>Azadirachta indica</i>	Au	Leaves	5-7	[24]
8.	<i>Cassia fistula</i>	Au	Stem	55-98	[25]
9.	<i>Cinnamon zeylanicum</i>	Ag	Leaves	45	[26]
10.	<i>Citrullus colocynthis</i>	Ag	Calli	5-70	[27]
11.	<i>Citrus sinensis</i>	Ag	Peel	35	[28]
12.	<i>Catharanthus roseus</i>	Ag	Leaves	48-67	[29]
13.	<i>Doipyros kaki</i>	Pt	Leaves	2-12	[30]
14.	<i>Euphorbia prostrata</i>	Ag	Leaves	52	[31]
15.	<i>Eucalyptus camaldulensis</i>	Au	Leaves	5.5-7.5	[24]
16.	<i>Embllica officinalis</i>	Ag,Au	Fruit	10- 20 and 15-25	[32]
17.	<i>Mentha piperita</i>	Au, Ag	Leaves	90-150	[33]
18.	<i>Mirabilis jalapa</i>	Au	Flower	100	[34]
19.	<i>Mangifera indica</i>	Ag	Leaves	20	[35]
20.	<i>Ocimum tenuiflorum</i>	Ag	Leaves	25-40	[36]
21.	<i>Pinus resinosa</i>	Pt	Bark	6-8	[37]
22.	<i>Jatropha curcas</i>	Ag	Seed	15-50	[38]
23.	<i>Syzygium cumini</i>	Ag	Seed	73-92	[26]
24.	<i>Tinospora cordifolia</i>	Ag	Leaves	34	[39]
25.	<i>Withania somnifera</i>	Ag	Leaves	5-40	[40]

3. Techniques for Characterization of Metallic Nanoparticles

Several experimental techniques are applied for observation and characterization of metallic nanoparticles.

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3.1 UV-Visible Spectroscopy (UV-VIS)

It is used to quantify the light that is absorbed or scattered by a sample and to examine the size and shape of nanoparticles in aqueous suspension [41]. Copper, silver and gold metal nanoparticle show distinct and prominent absorption peaks in the visible region. The optical absorption spectra of noble metallic nanoparticles exhibit the unique optical property of surface plasmon resonance (SPR). SPR of metallic nanoparticle aggregates will be redshifted to a longer wavelength as compared with SPR of the individual nanoparticle. SPR effect is determined by oscillation of the conducting electrons aligned in resonance to the wavelength of the irradiated light [42]. According to Mie's theory, a single SPR band is expected in the absorption spectra of spherical nanoparticles. In contrast, anisotropic nanoparticles give rise to two or more SPR bands depending on the shape of the particles. Increase in a number of SPR peaks shows that symmetry of the nanoparticle decrease [43]. Change in color of plant extract is due to the reduction of the metal ions to metal [44].

3.2 Scanning Electron Microscopy

In this electron microscopy technique, the sample is exposed to a high energy focused beam of electrons. After scanning it measure commonly secondary electrons, backscattered electrons and X-ray photons. Three dimensional appearances can be obtained due to the high depth of field in SEM. The samples are coated with gold or graphite to maintain the conductivity of the sample. Samples are maintained under ultra-high vacuum. High vacuum prevents interaction of secondary electrons with gas molecules. The substrate is typically a filter membrane or a conducting grid [45].

3.3 Transmission Electron Microscopy (TEM)

In TEM the electron beam is transmitted through a very thin specimen on a conducting grid such as a copper grid. Electron beam has to interact with the particles and non-absorbed electrons are focused onto an imaging detector such as fluorescence screen or CCD camera. Electron diffraction depends on the orientation of planes of atoms in a crystal. In TEM absorbance of the electron beam is a function of thickness and electron density of the elements in a particle. The organic matter having light elements needs to be stained by a heavy metal cocktail in order to be visible. High-resolution TEM is a method that is used for sub-nanometer resolution and it is used to study atomic structure in material sciences. HR-TEM is a very useful and demanding as well as time-consuming method to detect nanoparticle [46,47]. TEM shows high detailed and powerful magnification of element and compound structure. But it is very laborious in sample preparation and large and expensive. TEM micrograph visualization also reveals distribution, dispersion, exfoliation, intercalation and orientation of nanoparticles [48].

3.4 Fourier Transform Infrared (FTIR)

FTIR spectroscopy technique is used to evaluate chemical bonds in surface atoms and functional atoms on the surface of nanoparticles. The identification of functional groups leads to determine the reducing agent and the capping agent which is responsible for the synthesis and stability of nanoparticles [50]. FTIR can identify and detect changes in secondary structures of protein which help to resolve between similar components [51]. FTIR analysis of the dried silver nanoparticles carried through the potassium bromide (KBr) pellet.

3.5 Energy Dispersive X-ray (EDX)

EDX spectroscopy technique is used for identification and elemental composition of the nanoparticles. This technique is used in conjugation with SEM. Its characteristic X-rays are used to identify composition of the sample. This technique is used to observe the mapping of samples [48].

3.6 X Ray Diffraction (XRD)

Size of the nanoparticles can be analyzed by XRD. The narrow and strong diffraction peaks indicate the crystalline nature of synthesized nanoparticles. The mean size of nanoparticles is measured from the broadening of the diffraction peaks corresponding to the most intensive reflections. Scherrer equation [51] was used to determine the size of nanoparticles from XRD diffraction pattern.

$$d = K\lambda / B \cos\theta$$

where: D= the average dimension of crystallites in nanometers, λ = the wavelength of the X-ray radiation; K=Scherrer constant (shape factor, usually taken as 0.94); B=line width at half-maximum height (FWHM) in radians and θ = the Bragg angle, (the position of the diffraction peak maximum).

3.7 Dynamic Light Scattering (DLS)

The particle size distribution and zeta potential analysis of NPs can be evaluated by dynamic light scattering (DLS). The particle size distribution spectra of the metallic nanoparticles are recorded as the diameter (nm) versus frequency (%/nm) spectra. Graph showing diameter (nm) on x-axis and frequency (%/nm) on the y-axis. Zeta potential values reveal about the surface charge and predict long-term stability of the dispersion of metallic nanoparticles [12]. The zeta potential spectra of the nanoparticle recorded as zeta potential versus intensity spectra that is zeta potential (mV) on x-axis and intensity (a.u) on the axis. Nanoparticles having zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. They have a large negative or positive zeta potential. They will tend to repel each other and there will be no tendency of nanoparticles agglomerate formation. The nanoparticles having low zeta potential values then there will be no force to prevent from the particles coming together and flocculating [52,53]. Zeta potential of nanoparticles depends on pH and electrolyte concentration of the dispersion [54].

4. Pharmacological Applications of Metallic Nanoparticles

4.1 Anti-Bactericidal Activities of Metallic Nanoparticles

Metallic nanoparticles such as AgNPs have the ability to disrupt polymer subunits of the cell membrane in pathogenic organisms. The action of nanoparticles shows its action by breaking of the cell membrane of bacteria and disturbs its protein synthesis mechanism [55]. As concentrations of silver nanoparticles increase, they show faster membrane permeability than the lower concentrations and consequently rupture the cell wall of bacteria [56]. Reduced metallic silver nanoparticles show a low number of the bacterial colony in the culture plate as compared with AgNO₃ treated cells. Metallic silver and gold nanoparticles interact with the active site of the cell membrane of bacteria which leads to inhibit the cell cycle functions [57]. The biosynthesis of silver nanoparticles by using *Citrus sinensis* peel extract reduced silver nanoparticles effectively and the antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* (gram-negative) and *Staphylococcus aureus* (gram positive) has been proved [28]. Also, it is reported that *Acalypha indica* plant leaf synthesized silver nanoparticles effectively control pathogenic bacteria [19]. Similarly, synthesis of plant mediated silver nanoparticle using PAPAYA fruit extract and evaluation of their anti-microbial activities [58].

4.2 Anti-Fungicidal Activities of Metallic Nanoparticles

Metallic silver nanoparticles are developed as a novel and effective drug against microbes. Reduced metallic nanoparticles have a fungicidal mechanism which shows more potential than commercial antibiotics such as fluconazole and amphotericin. The plant derived silver nanoparticles have clearly shown the membrane damage in *Candida* sp. and damage in fungal intercellular components. It leads to disruption in cell function [59]. Generally, commercial antifungal agents have limited recovery application from the microbial disease but there is more adverse effect as compared to their benefits. Commercial drugs induce side effects such as renal failure, increased body temperature, nausea, liver damage, and diarrhea. The multifunctional AgNPs have a toxic activity against spore producing fungus and they can effectively destroy the fungal growth. The fungal cell membrane shows significant structural changes when treated with metallic nanoparticles [60].

4.3 Antioxidant Mechanisms of Plant Derived Nanoparticles

Free radicals are regulated by antioxidant agents and these are enzymatic and non-enzymatic in nature. These free radicals are generated by reactive oxygen species (ROS) such as superoxide dismutase, hydrogen peroxides and hydrogen radicals. Free radicals are responsible for causing cellular damage, brain damage, atherosclerosis and cancer [61]. The scavenging power of various nanoparticles is useful for the management of various chronic diseases such as diabetes, cancer, AIDS, nephritis, metabolic and neurodegenerative disorders. Silver nanoparticles show a stronger antioxidant effect than commercial antioxidant standards such as ascorbic acid [62].

4.4 Anticancer Studies on Plant Mediated Nanoparticles

Cancer is an uncontrolled proliferation of cells. These cancerous cells change their biochemical and enzymatic properties. Overexpression of cellular growth can be arrested and regulated by plant-based nanoparticles [63]. Plant mediated nanoparticles show a cytotoxic effect against cancer cell lines such as Hep 2 and Hela cell lines. The improved cytotoxic activity against the tumour cell is due to the secondary <https://doi.org/10.30799/jnst.277.19050502>

metabolites and other non-metal composition present in the synthesizing medium [64,65]. Gold nanoparticles derived from the plant have ability to induce apoptosis mechanism in malignant cells [66]. The metallic nanoparticles have their applications in the medical field to diagnose and treatment of various cancerous cell lines. The plant-based nanoparticles are new way to treat malignant cells and without interfering the normal cells [67].

4.5 Antidiabetic Management of Metallic Nanoparticles

Diabetes Mellitus (DM) is a group of metabolic dysfunctions which is characterized by a complete lack of insulin, a relative lack of insulin, or insulin resistance. Diabetes Mellitus (DM) leads to an uncontrolled sugar level in blood. There are many current challenges in diabetes management such as optimizing the use of the already available therapies to ensure adequate glycemic, blood pressure, and lipid control. At present several kinds of research have been focusing on nanoparticle mediated management options for diabetes. Hence, biosynthesis of nanoparticles from plants could be considered as alternative drugs to cure the diabetes mellitus. Gold nanoparticles against diabetic mice show a reduction in level of liver enzymes such as alanine transaminase, alkaline phosphatase, serum creatinine and uric acid [25]. Similarly, Gold nanoparticles treated diabetic mice also showed a reduction of HbA (glycosylated hemoglobin) level which is the same as the normal cell level [68]. Nanoparticles synthesized from *Sphaeranthus amaranthoides* able to inhibit α -amylase and acarbose sugar in diabetic animal model system. Ethanolic extract of *S. amaranthoides* contains α amylase inhibitory components [69]. It is showed [70] that the nanoparticles have potential therapeutic ability to control diabetes with few side effects.

5. Conclusion

Biosynthesis of metal nanoparticles using plant derivatives is a most trending area in the last two decades. As a prospect, the eco-friendly synthesis of nanoparticles using plant crude extracts and purified metabolites are novel substrates for reduction of metal to produce metallic nanoparticle at large scale. The plant mediated nanoparticles used in various fields such as pharmaceuticals, therapeutics, sustainable and renewable energy. Plant mediated nanoparticles are also used for commercial products such as waste water treatment, cosmetics and food industry. The plant derived metallic nanoparticles show the impact on diagnosis and treatment of various diseases. In the future, the plants have wide potential for the synthesis of metallic nanoparticles in healthcare and commercial products. There are two areas which need more exploration for biological production of nanoparticles such as phytochemical compounds (active metabolites) which are involved partially/fully in the reduction of the metallic nanoparticle and more explanation is needed about the functional mechanism of metallic nanoparticles against the pathogenic organisms.

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