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AN ANTIMICROBIAL GREEN NANOPARTICLE SYNTHESIS: A NOVEL APPROACH IN THERAPEUTICS FIELD

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ABSTRACT

The advent of nanotechnology in the biomedical and pharmaceutical field develops the method for plant-mediated synthesis of metal nanoparticles because they produce a wide array of phytochemicals such as alkaloids, flavonoids, saponins, steroids, tannins and other metabolites which can act as a reducing and binding agent. This biological approach for the production of nanoparticles has several merits over the physicochemical methods as it is one step procedure, environment-friendly and cost-effective. This review provides insight on the role of plants and its secondary metabolites in the production of various greener nanoparticles such as copper, silver, gold, platinum, and zinc oxide which possesses antimicrobial activity and also focus on the numerous factors that affect size, shape, and yield of metal nanoparticles.

KEY WORDS

Metal nanoparticle, Phytochemicals, Secondary metabolites, Physicochemical methods, antimicrobial activity.

INTRODUCTION

An emerging area of nanotechnology is the synthesis of a nanoparticle as it has unique properties and extensive uses in various fields such as medical, nutritional and technological [1]. The green synthesis of nanoparticles has been a novel approach in the pharmaceutical industry to deal with various microbial ailments [2]. Green syntheses have more merits over other classical methods because it exploits environmentally safe procedures and medicinal plants which are readily available. The plants found in the world have been examined for their efficacy for the nanomaterials synthesis [3] and several nanotechnologists have successfully produced nanoparticles using plant materials [4]. Hence, biogenically produced nanoparticles are conceived as arsenal to control various diseases [5].

A plant produces various secondary metabolites which can be categorized as phenolic compounds, flavonoids, alkaloids and terpenoids which act as the catalyst for reduction of metal ions into metallic nanoparticles [6]. Green synthesis of nanoparticles is an environment-friendly method as it reduces environmental impact. Biological synthesis of the nanoparticle by using either live plants or whole plant extract has fascinated the researcher within the last three decades. Plant-based synthesis of nanoparticles is more advantageous because it is easy to scale up the production and cost-effective compared with the conventional method. Thus, nanoparticle production by exploiting reducing activity of phytoconstituents is more convincible and environmentally safe [7].

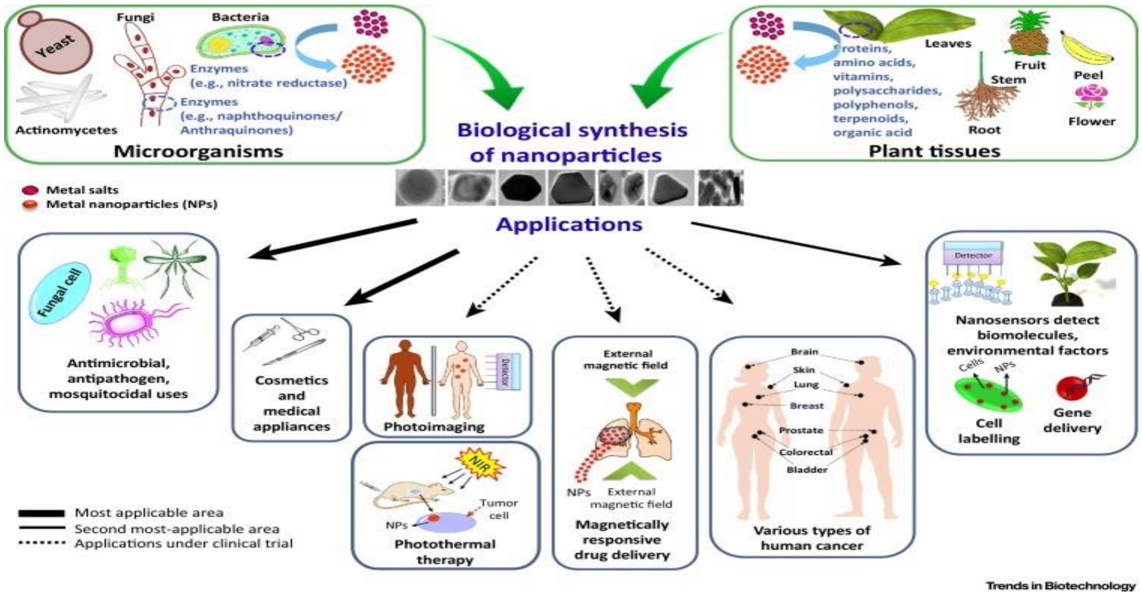
If we take a look at the history, from the thousands of year's nanomaterial has been used unknowingly. For instance, ancient people were used nanoparticles of gold to stain glasses and also prevent from certain disease. Now a day, researchers have been gradually able to perceive the size/shape dependent physiochemical properties of nanoparticles using several advanced analytical and biochemical techniques. From last decade the different applications of metallic nanoparticles have been investigated in agricultural, environmental, biomedical and physiochemical areas (Figure 1). Such as, Silver nanoparticles have been not only applied for an antimicrobial motive but also in anti-inflammatory wound treatment applications, and anticancer [8]. Likewise, titanium and zinc nanoparticles have been applied in the ultraviolet (UV)-blocking agents, cosmetic, biomedical and various cutting-edge processing applications because of its nontoxic self-cleansing, biocompatible, skin-compatible, and antimicrobial nature [9, 10]. Gold nanoparticles have been useful for the delivery of definite drugs, for instance, methotrexate, doxorubicin, and paclitaxel [11]. It has also been also for angiogenesis, tumor detection, genetic disorder diagnosis and genetic disease, photo-thermal, and photo-imaging therapy. Palladium and copper nanoparticles have offered antimicrobial activity against many pathogenic microbes [12, 13]. Iron oxide nanoparticles have been applied for drug delivery cancer therapy, tissue repair, hyperthermia, targeting and cell labeling, immunoassays, magnetic resonance imaging, detoxification of biological fluids, and magnetically responsive drug delivery therapy [14-16]. Moreover, metal nanoparticles have been applied in the spatial analysis of various biomolecules like various metabolites, nucleic acids, peptides, fatty acids, lipids, and drug molecules, for visualizing molecules [17]. Furthermore, due to its specified properties enhance, it can be used for designing biosensors and electrochemical sensors [18]. For instance, Nanosensors have been produced for the finding of mycobacteria, algal toxins, and amount mercury in drinking water [19]. Investigators also explored Nanosensors by using nanomaterials for detecting crop pests, viruses, soil nutrient levels, and stress factors and for hormonal regulation. For example, Nanosensors for identifying oxygen distribution and auxin have been developed [20].

Hence, green synthesizing nanoparticles has the benefits of reproducibility in production, easy scaling-up and nontoxicity. A definite morphology of the nanoparticles has become a new era in the production of a nanoparticle. Specifically, microbes and plants have been proved as novel resources for synthesizing nanoparticles along with significant potential. So far, numerous microflora, including bacteria, fungi, and yeast, as well as plants, have been explored for synthesis of metal nanoparticle. However the nanoparticles synthesis has been widely reviewed somewhere else [21-23], but in current review, we described an update and recent advances of the synthesis of biological nanoparticles, and update scenarios for their upcoming development and applications of green synthesis nanoparticles.

SYNTHESIS OF NANOPARTICLES

Physicochemical method for synthesis of method nanoparticles

At the beginning of the Nanotechnological era, physicochemical methods have been employed at a great extent for the production of nanoparticles (NPs) [24-28]. However, they have several demerits as they rely on a significant amount of radiation, chemically synthesized reducing agents and stabilizers that are hazardous to environmental and in turn to human health. Hence, environment-friendly procedures are required for synthesis NPs. Green synthesis of nanoparticles is one of the eco-friendly bioreduction methods as it exploits biological resources such as plant extracts, microorganisms, microalgae and enzymes [29, 30].



Trends in Biotechnology

Figure 1. Green Synthesis and Applications of Metal Nanoparticles in various Fields [31].

Green synthesis of nanoparticles

The nanomaterials synthesized by using plant materials have shown a most significant alternative of physicochemically synthesized NPs as they have generated from natural small molecules produced by plants. Different plant materials such as stem, root, fruit, leaves, and flowers have been exploited for the synthesis of various metallic nanoparticles (ZnO, Pd, Cu, Ir, Ag, Au, MnO$_3$, Fe$_3$O$_4$, Ti$_2$O$_3$, Pt and Lu$_2$O$_3$). To achieve specific size and shape of nanoparticles, the biological reaction can be modified by changing metal concentration and concentration of plant extract [1, 32].

The mechanism of green synthesis of nanoparticles

A plant produces various secondary metabolites which act as reducing agents for synthesis of various nanoparticles. The mechanism of metal nanoparticle synthesis can be divided into three phases (Figure 2). The first phase is known as a reducing phase in which metal ions react with secondary metabolites of plants and reduce to metal atoms. In the second phase, reduced metal atoms spontaneously link with each other and formed complex small nanoparticles. The third phase is the conclusive phase which is a very crucial one as it determines the final size and shape of nanoparticles [33, 34]. For instances, the mechanisms of silver, gold, platinum, copper and Zinc nanoparticles synthesis are discussed as follows:

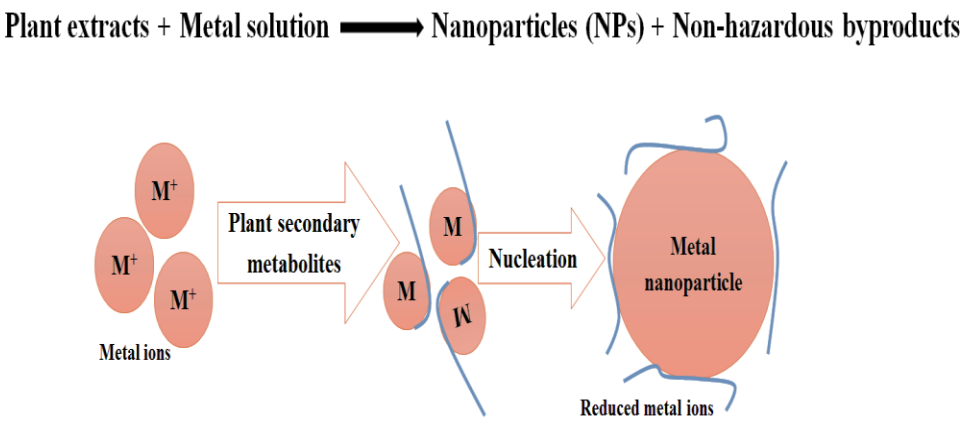


Figure 2: Schematic diagram of metal nanoparticle synthesis by using plant materials [35]

Silver nanoparticles (AgNPs): In the synthesis of AgNPs, the $AgNO_3$ reacts biochemically with plant extracts that leads to the formation of AgNPs (Reaction 2) [36].

$AgNO_3$ + Plant extract→ AgNPs + byproducts

Gold nanoparticles (AuNPs): The gold ion (Au^+) is reduced into AuNPs in the presence of plant extract [37].

$AuCl_4$ $4H_2O$ + plant extracts → AuNPs + byproducts

Platinum nanoparticles (PtNPs): The platinum is reduced by plant extracts into PtNps.

$PtCl_2$ $6H_2O$ + Plant extracts → PtNPs + byproducts

Copper nanoparticles (CuNPs): When copper containing solution mixed with plant extracts, CuNPs are synthesized by bio-reduction mechanisms [38].

$CuSO_4$ $5H_2O$ + Plant extracts → CuNPs + byproducts

Zinc oxide nanoparticles: When zinc nitrate mixed with the aloe plant extract, the nano-sized zinc oxide particles are produced [39].

Zinc nitrate + Plant extract →ZnO + byproducts

The role of plant metabolites in nanoparticle synthesis

Numerous phytochemicals such as terpenoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins, act as biological reducer compounds for the production of metal nanoparticles. Several reports showed that terpenoids are often found with phytochemically synthesized nanoparticles. Terpenoids, which have substantial antioxidant activity, are composed of isoprene units. It has been found that terpenoids, which is isolated from geranium leaves extracts, was exploited for the synthesis of silver nanoparticles and finally, it was revealed that it acts as a sequestering agent [40]. Eugenol, a purified form of terpenoid of *Cinnamomumzeylanisum* (cinnamon) extracts, showed potential reducing power for the formation of silver nanoparticles. Biochemical analysis of reducing the potential of the eugenol has revealed that oxidation reaction resulted from the hydroxyl group of it is coupled with the active reduction of metal ions which leads into nanoparticle formation [41].

Flavonoids, a polyphenolic compound, have flavone nucleus with two side aromatic rings. They have the potential to sequester metal ions into nanoparticles because flavonoids contain the hydroxyl functional group. It has been proposed that when flavonoids undergo at automatically transformation, they can produce nanoparticle by producing nascent hydroxyl group. For instance, the reduction of silver nanoparticles from Ag ions is achieved by the biotransformation of enol- form of luteolin and rosmarinic acid in *Ocimumbasilicum* (sweet basil) extracts to the keto-form [42]. Additionally, the reduction of Au^{3+} ion is also carried out by transformation of ketones to carboxylic acids in flavonoid. Furthermore, it has been reported that some flavonoids act as a metal chelating agent as they are carbonyl compound having reactive Pi-bonds. For example, quercetin has shown significant metal chelating activity (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Pb^{2+} and Co^{2+}), because structurally, it has the carbonyl group and a hydroxyl group at the third and fifth carbon positions, respectively. Probably, this potential chelating activity of flavonoids is involved in the nanoparticle formation. Moreover, it is proved that apiin, flavonoid glycosides extracted from *Lawsoniainermis*, have employed for the production of gold and silver nanoparticles [43].

Several studies on the the synthesis of the metal nanoparticles showed that plant extracts having sugars can form metal nanoparticles. It has been reported that monosaccharides such as glucose can act as reducing agents because they have an aldehyde group while monosaccharides with a keto-group like fructose had antioxidant activity when they transformed from a ketone to an aldehyde. Additionally, the reducing ability of disaccharides and polysaccharides can act as reducing agents, but they must free aldehyde group at the end of oligomers that provide interaction site for metal ions. To illustrate, maltose and lactose, the disaccharides having free aldehyde group at the open end of their chain which enhances reducing activity. In contrast to maltose and lactose, sucrose cannot be able to reduce metal ions as an open chain in its monomer is not available for reduction process [25].

Functional group analysis of plant-based nanoparticles revealed that proteins are often found with them [44]. However, the reducing capacity of different amino acids may vary depending on the free functional group. Numerous studies have been carried out to examine reducing the potential of different amino acids to form silver nanoparticles. It was proved that lysine, cysteine, arginine and methionine have significant the reduction capacity [45]. Similarly, aspartate can interact with tetrachloroauric acid to form nanoparticles. However, valine and lysine didn't show reducing activity [46]. In the recent study, all of the 20 natural α-amino acids were analyzed for their potential for nanoparticle formation [47] and it was found that tryptophan has the substantial reducing ability for Au ions, while histidine has a higher interacting capacity with Au ions. Carbonyl and amino groups of amino acids are major functional groups in interaction with metal ions [33]. For example, the hydroxyl group of tyrosine and carbonyl group of glutamine and asparagine showed their reducing power in the formation of silver nanoparticles. Similarly, thiol groups of cysteine and amino groups are also crucial for the reduction of metal ions [35].

However, the reducing power of each amino acid may change when they are polymerized into the peptide chain. Perhaps, carboxyl and amino groups become inaccessible for the interaction of with metal. However, amino acids located at free side chains can still accessible for reduction of metal ions but the amino acid sequence play major in the suitability of side chains for this interaction. It was revealed that *in vitro* synthesized peptides showed lower reduction parameters than expected. It is probably due to weak binding of amino acids of peptides to bind metal ions. Moreover, leucine, phenylalanine, and proline showed ineffective sequestering efficacy for tetrachloroauric acid anions as their inability to retain position closer to metal ions for reduction reaction [47]. Furthermore, the amino acid sequence of a protein has also a significant effect on the size, morphology, and amount of nascent nanoparticles. For instance, an *in vitro* synthesized peptide GASLWWSEKL has found to reduce metal ions rapidly into a large number of small nanoparticles of less than 10 nm in size; however, when N-and C-terminal amino acid residues were substituted in a peptide (SEKLWWGASL), it declined the reduction reaction that subsequently resulted in the formation of nanoparticles having about 40 nm in size. Hence, peptides and proteins present in the plant extract probably crucial for the determination of the size, the shape of nanoparticles and the overall yield of nanoparticles [33].

Factors affecting the formation of metal nanoparticles in plants

Factors including a different concentration of secondary metabolite and combination in plant extract which are discussed above, the pH, incubation temperature, reaction time, and electrochemical properties of a metal ion in reaction mixture have the direct effect on the generation of nanoparticles [48-51]. The pH value of a plant extract has a significant effect on the nanoparticle synthesis as it directly alter the charge of plan secondary metabolites present in the extract and subsequently affect their reducing ability which inevitable for nanoparticle synthesis [28, 52-54]. Several studies have been carried out to show the effect of pH on nanoparticle synthesis. *Avena sativa* extract at pH 2.0, 3.0 and 4.0 was explored for the production of gold nanoparticles and it was found that more aggregated particles formed at pH 2.0 compared to other pH treatment. Thus, it has been proposed that a substantial amount of nanoparticle aggregation in metal reduction and nucleation process can be achieved at very acidic pH values. This agglomeration reaction may occur due to the involvement of a smaller number of metals into nucleation processes at pH 2.0 [54]. However, it was revealed that gold Nanoplates with hexagonal and triangular structure are produced when pears extract used at alkaline pH, while nanoparticles do not form in acidic pHs [52]. Similarly, a significant number of silver nanoparticles are synthesized with the help of powder of *Curcuma longa* (turmeric) at alkaline pHs. Possibly, turmeric extracts may contain more negatively charged functional groups containing phytochemicals at alkaline pH which act as reducing agent for synthesis of silver nanoparticles [54].

Temperature also affects the formation of nanoparticles by using plant extracts [5, 55-57]. Overall, the reaction rate and efficacy of nanoparticle synthesis increase with the rising of the temperature of the reaction. For example, triangular silver nanoparticles in *M. sativa* formed only at temperatures above 30°C [56]. Similarly, lemon verbena extracts (*Aloysiacitrodora*) mediated synthesis of silver nanoparticles is efficiently elevated by increasing the reaction temperature as it increases the efficiency of the silver ion reduction [5]. Furthermore, it is also hypothesized that the nucleation reaction can be augmented by elevating the temperature. Apart from synthesis rate and nucleation rate, the structure of nanoparticles are also affected by temperature which is supported by the report of *Cassia fistula* (golden shower tree) extracts, it was revealed that silver nanoribbons and spherical nanoparticles are mainly formed at room temperature and temperatures above 60°C respectively [58]. Possibly, higher temperatures inhibit the formation of nanoribbons because it alters the interaction of secondary metabolites present in the *C. fistula* extracts with the nanoparticle surface [56].

An electrochemical property of metal ions has also a direct effect on the synthesis of metal nanoparticle since plants have a partial effect on the reduction of metal ions. Hence it was reported that ions having a significantly higher positive electrochemical potential, for example, Ag^+, could easily be reduced by a plant extract compared to ions with a low electrochemical potential such as $([Ag(S_2O_3)_2]^{3-})$ [49].

Antimicrobial activity of biosynthesized nanoparticle

The permeability of the cell membrane of pathogens is increased when they were treated with the silver nanoparticles. Subsequently, they destroy the cell membrane with osmotic pressure and also hinder the protein synthesis mechanism in the bacteria [59]. The exposure of *Rhizophoraapiculata* reduced silver nanoparticles reduced C.F.Us of bacteria, which may be due to increased membrane permeability and cell destruction as the nanoparticles are the smaller in size and have a larger surface area which may inhibit the cell cycle functions by interacting with the specific binding site of the cell membrane [60]. *Citrus sinensis* peel extract reduced silver nanoparticles showed significant antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa* (gram-negative) and *Staphylococcus aureus* (gram-positive) [61]. Similarly, the biosynthesized silver nanoparticles by using *Acalyphaindica* leaf (lower concentrations of 10 µg/ml) significantly inhibit the growth of waterborne pathogenic bacteria [62]. Additionally, the *Citrus medica* fruit mediated copper nanoparticles showed significant antibacterial activity against *E. coli*, *K. pneumoniae, P. aeruginosa, P. acne* and *S. typhi* [63].

Biosynthesized metallic nanoparticles have shown great fungicidal potential than commercially available antibiotics such as fluconazole and amphotericin. The mechanism of the fungicidal activity of plant-based silver nanoparticles involves the disruption of cell function by increasing membrane permeability in *Candida* sp. and impairment in fungal intercellular components [64]. Most of the commercial antifungal antibiotics have clinically limited applications as there are more hostile effect such as such as renal failure, increased body temperature, nausea, liver damage, and diarrhea. Nanoparticles have a promising antifungal activity because they inhibit the growth of spore-producing fungus and effectively destroy fungal growth [65]. Similarly, the sensitivity of the plant pathogenic fungi toward *Citrus medica* based copper nanoparticles was evaluated and *F. culmorum, F. oxysporum* and *F. graminearum* found to be sensitive [63]. Hence, plant-derived nanoparticles could be an alternative of antibiotic.

CONCLUSION

Plants and their extracts have been explored for the synthesis of metal nanoparticle for last twenty years as they have phytochemicals that have sequestering efficacy for the production of metallic nanoparticles. The biogenic synthesis of nanoparticle has several advantages compared to the conventional methods as it is a single step, non-hazardous and cost-effective. The plant-mediated nanoparticles have a wider application in the pharmaceutical and biochemical field as they showed significant antimicrobial activity with controlled side effects. In future, it is vital to identify the natural active molecules present in the plant extracts which are involved in the reduction reaction to scale up the production of metal nanoparticle and to explore their mode of action of antimicrobial activity.

Importantly, research gap of the chemical components accountable for the synthesis and its underlying mechanisms and stability of green nanoparticles are still open defies which make a chance to explore more about nanoparticle synthesis from the plants and micro-organisms. Mainly the term 'biocompatibility', it is vital to know about how active groups present in biological sources are bind to the nanoparticle surface and also for production of nanoparticles with higher efficacy which type of active groups are responsible. Hence, plants have been effectively and successively applied for the green synthesis of metal nanoparticles which developed more interest in the development of biological nanofactories as a rise in nanoproducts in numerous fields. Conversely, problems concerning with biomedical uses of biological nanoparticles, accounting the excretion, distribution profile and clearance of nanoparticles *in vivo* clinical trials, required to be addressed. Furthermore, advance research in the bioavailability and biocompatibility of nanoparticles are yet to be at early stages and significant research is obligatory in this field.

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