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Review Article

NEEM (*AZADIRACHTA INDICA* A. JUSS) AS A SOURCE FOR GREEN SYNTHESIS OF NANOPARTICLES

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ABSTRACT

Nanoscience has found various applications in different biomedical fields. The synthesis of nanoparticles (NPs) has become a vast area of research due to its potential applications. These particles can be prepared by different chemical, physical, and biological approaches. In recent years, green synthesis of NPs using plant extracts has gained much interest due to non-toxicity and very low cost of synthesis. The plant extracts act both as reducing agent as well as capping agent. Neem (*Azadirachta indica* A. Juss) is a well-known medicinal plant and has been studied for the biosynthesis of NPs. *A. indica* has various phytochemicals identified that can reduce the metal ions. The bioreduction of NPs from neem extract is an eco-friendly, low cost, and green synthesis method and these NPs are reported to exhibit good antimicrobial, mainly antibacterial, activity.

Keywords: *Azadirachta indica*, Neem, Nanoparticles, Antimicrobial activity.

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INTRODUCTION

Nanotechnology is an emerging interdisciplinary field which mainly concerns with the fabrication of nanoparticles (NPs) with specific requirements in terms of size, shape, and controlled dispersity. The field of nanotechnology is one of the most active research areas in modern material science. Over the last few decades, the syntheses of novel metal NPs have been the subject of many applied researches due to their unique properties [1]. "Nano" is used to indicate 1 billionth of a meter (or) 10^{-9} [2]. In general, particles with a size <100 nm are referred to as NPs. The "Nano" is derived from the Greek word for dwarf and is also related to the Spanish word Nino [3]. In nanotechnology, a nanoparticle is defined as a small object that behaves as a whole unit in terms of its transport and properties [4]. This technology presently has attracted various branches of application due to the dissimilar behavior of the bulk material when reduced to its nanosize [5].

The distinctive properties of nanomaterials are mainly influenced by size, shape, interactions with stabilizers, media, and methods of synthesis [1,6]. Therefore, controlled synthesis of NPs with respect to their size, shape, and stability is a key challenge to reach their better applied characteristics [1]. In general, metal NPs can be prepared and stabilized by chemical, physical, and biological methods; the chemical approach, such as chemical reduction, electrochemical techniques, photochemical reduction [7-9], and pyrolysis [10]; and physical methods such as arc discharge and physical vapor condensation [11] are used. However, many of these nanoparticle synthesis methods involve the use of hazardous chemicals or high energy requirements, which are rather difficult and include wasteful purifications [12]. Biological systems such as the use of plant materials provide an innovative, eco-friendly alternative for the production of NPs [13]. Biosynthesis using plant sources offers several advantages such as cost-effectiveness, eco-friendliness, and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods [14]. Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents [15]. The plant extracts contain various organic compounds such as terpenoids that aid nanoparticle synthesis. Terpenoids are surface-active molecules that help to stabilize the NPs [16].

Microorganisms can also be utilized to produce NPs, but the rates of syntheses are slow compared to routes involving plants mediated synthesis [12]. Many bacterial cultures were used for different kind of NPs such as gold NPs using *Shewanella algae*, a marine bacterium [17] and actinomycetes *Thermomonospora* sp., [18] and *Streptomyces viridogenes* strain HM10 [19], silver NPs (AgNPs) by cyanobacteria *Plectonema boryanum* [20] and by fungi *Aspergillus fumigatus* [21], *Trichoderma asperellum* [22], and *Fusarium oxysporum* [7,23], *Fusarium udum,* and *Stemphylium vericans* [23]; cadmium NPs biosynthesis was done by *Clostridium thermoaceticum*, magnetite NPs by *Actinobacteria* sp., and uranium NPs by *Shewanella oneidensis* [24], titanium dioxide NPs using bacteria *Lactobacillus* sp., [25] and *Bacillus subtilis* [26].

Biosyntheses of NPs using extracts from various plants such as *Aloe vera* [27], *Jatropha curcas* [28], *Acalypha indica* [29], *Sesuvium portulacastrum* [30], *Garcinia mangostana* [31], *Ocimum tenuiflorum* [32,33], *Abutilon indicum, Melia dubia* [34], *Solanum trilobatum* [35], *Musa balbisiana* [33], *Crataegus douglasii* [36], *Beta vulgaris* (beet root) [37], Tagetes erecta (marigold) [38], *Ziziphora tenuior* [39], *Carica papaya* [40], *Digitaria radicosa* [41], and *Lantana camara* [42], have been reported in literature with nanoparticle size ranging from 5 to 20 nm. However, the potential of higher plants as source for this purpose is still largely unexplored.

NPS SYNTHESIS USING NEEM EXTRACT

Extracts from neem (*A. indica*) plant have been used for greener synthesis of NPs [16,43,44]. The major advantage of using the neem leaves is that it is a commonly available medicinal plant and known to have antimicrobial activity [45].

Gold NPs were synthesized using neem leaf extract and the synthesis was confirmed by color changes and characterized by ultraviolet (UV)-visible spectroscopy [46,47]. Gold NPs were synthesized by mixing aqueous extract of neem leaves and 10^{-3} M aqueous chloroauric acid ($HAuCl_4$). Isomorphic spherical gold NPs of 15–18 nm in size were synthesized [48]. Gold NPs were synthesized using neem leaf extract and 2% auric chloride solution ($AuCl_4$) and were characterized [43].

Using neem leaf extract, researchers produced platinum NPs from chloroplatinic acid. The solution color changed from light yellow to black on heating, indicating the formation of platinum NPs. The NPs were between 5 and 50 nm in diameter. These platinum NPs are potentially useful in the bioelectronics and chemical industries for various medicinal and catalytic applications [16].

Zerovalent iron NPs were synthesized from the leaf extract of the neem plant. The diameter of iron NPs was within the range of 50–100 nm [49]. Spherical-shaped iron oxide particles ranging in size from 10 to 20 nm were synthesized by simple coprecipitation method. The synthesized NPs were stabilized by biocapping with aqueous leaf extract of neem. The antimicrobial activity of neem extract capped magnetite NPs was evaluated against *Bacillus* sp., *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas* sp., by agar well diffusion method. The capping agents on the surface of magnetite NPs were inefficient to inhibit the growth of bacteria [50].

Zinc oxide nanoparticle (ZnONPs) without calcinations were developed by green synthesis method using aqueous leaf extracts of *A. indica.* The formed NPs were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectrometer (FTIR), and energy dispersive analysis of X-rays (EDAX) for its morphology, size, crystallinity, and percentage composition. The results confirmed the formation of nanoflowers of 51 nm. This ZnONPs could be used as an inexpensive and effective adsorbent for the removal of arsenic ions from aqueous solution [51]. ZnO nanotubes were successfully fabricated using neem leaf extract. ZnO nanotubes were characterized by SEM and XRD. The final product was highly crystalline with the size of 25 nm. ZnONPs showed a significant antibacterial activity against *E. coli* with zone of inhibition of 12 mm [52]. ZnO is non-toxic and used in industrial sectors including environmental, synthetic textiles, food, packaging, medical care, healthcare, as well as construction and decoration [53].

A. indica plant-mediated process was developed for the synthesis of titanium NPs from titanium dioxide [54] and titanium isopropoxide [55]. The titanium NPs were spherical in shape and the size ranged from 15 to 42 nm [54].

Synthesis of AgNPs using neem extract

Silver is one of the most commercialized nanomaterials with 500 tons of AgNPs production per year [56] and is estimated to increase in next few years. Including its profound role in the field of high sensitivity biomolecular detection, catalysis, biosensors, and medicine; it has been acknowledged to have strong inhibitory and bactericidal effects along with the antifungal, anti-inflammatory, and anti-angiogenesis activities [31,57]. AgNPs have unique optical, electrical, and thermal properties that play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, and tissue engineering [58].

A number of techniques are available for the synthesis of AgNPs such as ion sputtering, chemical reduction, and sol-gel. [37,38,40,59]. However, these methods also result in contaminants during the synthesis procedures or in later applications with associated limitations. AgNPs can be produced at low concentration of leaf extract without using any additional harmful chemical/physical methods. This greener synthesis of AgNPs is one-step, cost-effective, environment-friendly, and relatively reproducible and often results in more stable materials [60]. This eco-friendly method could be a competitive alternative to the conventional physical/chemical methods used for the synthesis of silver nanoparticle and has a potential to be used in biomedical applications and will play an important role in optoelectronics and medical devices in near future.

AgNPs, due to their antimicrobial properties, have been used most widely in the health industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams, and a number of environmental applications [61]. Since ancient times, elemental silver and its compounds have been used as antimicrobial agents and was used to preserve water in the form of silver coins/silver vessels [38,62]. Antibacterial properties of silver are documented since 1000 B.C. when silver vessels were used to preserve water [63,64].

Silver ions (Ag+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity toward animal cells [65]. Bacterial cells exposed to the Ag+ ions activate stress response that leads to the condensation of DNA in the center of the cell, and this condensation of DNA prevents cell replication by preventing the DNA from being accessed by transcriptional enzymes such as DNA polymerase. It can also cause cell membrane detachment from the cell wall, cell wall damage, and electron dense granules composed of silver outside and, in some instances, inside the cell [66]. AgNPs anchor to and penetrate the cell wall of bacteria [67], suggesting that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane, and ultimately cell death. Silver inactivates proteins by binding to sulfur-containing compounds [68]. It was also reported that treating cells with silver leads to cell shrinkage and dehydration [69]. It has also been proposed that the antibacterial mechanism of AgNPs is related to the formation of free radicals and subsequent free radical-induced membrane damage [70,71]. However, it has been reported that AgNPs are non-toxic to humans though most effective against virus, bacteria, and other eukaryotic microorganisms even at low concentrations without any side effects [72].

AgNPs are synthesized by reducing silver ion using reducing agents present in neem extract and are characterized by UV-visible spectrophotometer, particle size analyzer (DLS), SEM, transmission electron microscopy, energy-dispersive spectroscopy, XRD analysis, nanoparticle tracking analyzer analysis, and FTIR analysis [5,33,73-75]. Transformation of silver ions into AgNPs during exposure to the neem leaf extract is followed by color change from pale yellow to dark yellowish brown color in aqueous solution [1]. Studies have indicated that the reducing phytochemicals in the neem leaf consisted mainly of terpenoids. It was found that these reducing components also served as capping and stabilizing agents in addition to reduction as revealed from FTIR studies [76].

Antimicrobial activity of neem synthesized AgNPs

Studies showed that the surgical masks coated with AgNPs had antibacterial properties. Nanoparticle-coated masks were capable of 100% reduction in viable *E. coli* and *S. aureus* cells after incubation. In addition, the study reported no signs of skin irritation in any of the persons wearing the masks [77]. Biosynthetic production of AgNPs by aqueous extract of neem leaves (20% w/v) and 0.01 M silver nitrate ($AgNO_3$) solution in 1:4 mixing ratio and its bactericidal effect in cotton cloth against *E. coli* by disk diffusion method were studied. The results demonstrated the antibacterial activity, and thereby possible use of cotton cloths incorporated with biologically synthesized AgNPs for wound dressing [78].

The growth of gentamycin and ampicillin-resistant *Klebsiella pneumoniae*, gentamicin and piperacillin-resistant *Salmonella typhi*, fluconazole-resistant *Candida albicans,* and multiple drug-resistant *E. coli* was inhibited by neem synthesized AgNPs. The average size of AgNPs synthesized using neem leaves extract was 43 nm [73]. Neem-mediated biosynthesized NPs, in the size of around 70–80 nm, showed significant inhibition of urinary tract infection (UTI) pathogens such as *S. aureus, Proteus* sp., *Pseudomonas* sp., and *E. coli* [79]. Green synthesized AgNPs from 1 mM $AgNO_3$ solution through aqueous leaf extract of neem showed antibacterial activity against *S. typhi* and *Klebsiella pneumoniae* [80]. AgNPs were prepared using aqueous neem leaf extract and $AgNO_3$ solution at room temperature. The synthesized NPs were coated on polyurethane foams (PU) by overnight exposure to the aqueous solution. Several rounds of washing and air drying resulted in stable PU foam with uniform coating. This silver nanoparticle-coated PU was capable of removing the complete load of bacteria (*E. coli*, *S. aureus,* and *Pseudomonas aeruginosa*) from water samples with initial load of 1×10^5 and 1×10^6 CFU/ml, after a contact time of 30 min. The water treated with the control sample (pure PU) showed substantial growth on the plates. Even after the treatment of 30 min, the AgNPs were found to have stable binding with PU and did not mix with water [5].

AgNPs synthesized from neem leaves were tested for their antimicrobial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. AgNPs rendered high antimicrobial efficacy and the zone of inhibition observed against *S. aureus* and *E. coli* was around 10 mm and 12 mm for 50μg/ml and 13 mm and 15 mm for 100 μg/ ml, respectively [58]. AgNPs synthesized using aqueous leaf extract of neem and aqueous solution (1 mM) of $AgNO_3$ were effectively accessed against Gram-positive (*Bacillus* sp.) and Gram-negative (*E. coli*) bacteria. The inhibition zones obtained indicated maximum antibacterial activity of the prepared NPs over the plant extract or $AgNO_3$ solution used individually [33]. AgNPs using leaf extracts of *A. indica* were synthesized and estimation of antimicrobial activity was conducted *in vitro* by disc diffusion method against *E. coli* (MTCC 448). It was observed that NPs exerted higher zone of inhibition than the $AgNO_3$ solution [43]. AgNPs were synthesized using neem leaves extract and $AgNO_3$ (1 mM) solution. The synthesized AgNPs were found to possess an effective antibacterial property against *E. coli* [15]. AgNPs were synthesized using neem leaf extract and its antibacterial activity against disease-causing bacteria *B. subtilis*, *Klebsiella planticola*, *K. Pneumoniae, Staphylococcus* sp., and *E. coli* was analyzed. At 90 µl concentration synthesized AgNPs exhibited significant antibacterial activity against all the tested bacteria which was better than ampicillin [81].

AgNPs synthesized from 1 mM $AgNO_3$ solution and aqueous leaf extract of neem showed zone of inhibition against Gram-positive (*Micrococcus* sp., *Bacillus* sp., and *Staphylococcus* sp.) and Gram-negative (*Klebsiella* sp. and *E. coli*) bacteria. The activity observed was similar to that of the standard antibiotics tested. The size of synthesized silver nanoparticle was 146 nm [76]. AgNPs were synthesized using aqueous extract of neem leaves and silver salt. Maximum zone of clearance of 6 mm was observed at 12 mg/ ml of AgNPs [44].

CONCLUSION

Synthesis of NPs using neem extract provides the single-step process for biosynthesis of NPs. This method is environment-friendly and commercially economic. Green synthesized NPs, especially the neem synthesized AgNPs, are found to have enhanced antibacterial activity. Due to the enhanced antimicrobial activity, it could be effectively used in the field of medicine as well as in food and cosmetic industries. However, further, progresses are desirable for the rational practical approach.

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