



GREEN SYNTHESIS OF NANOPARTICLES USING PLANT EXTRACTS OF *PUNICA GRANATUM* AND *MURRAYA KOENIGII* –A REVIEW

M. Padmaa Paarakh^{1*} and Preethy Ani Jose²

¹Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore 560 068, Karnataka, India.

²Department of Pharmaceutics, The Oxford College of Pharmacy, Bangalore 560 068, Karnataka, India

***Corresponding Author: M. Padmaa Paarakh**

Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore 560 068, Karnataka, India.

Article Received on 28/11/2017

Article Revised on 19/12/2017

Article Accepted on 09/01/2018

ABSTRACT

Research in the field of nanotechnology is an expanding research in the synthesis of metal nanoparticles due to its potential applications for the development of novel technologies including Medicine. The present review gives the different methods of green synthesis of nanoparticles using plant extract of *Punica granatum* and *Murraya koenigii* and their application till date. Both the plants have been known in the traditional system of medicine for treatment of number of diseases.

KEYWORDS: *Punica granatum*; *Murraya koenigii*; Green synthesis; Nanoparticles; review; application.

INTRODUCTION

The research in biosynthesis of inorganic nanoparticles including metallic nanoparticles, oxide nanoparticles, sulfide nanoparticles, and other typical nanoparticles has expanded drastically. Different methods of synthesis of nanoparticles with reference to control the size/shape and stability of particles have been studied as biosynthesized nanoparticles has wide potential areas including targeted drug delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science and magnetic resonance imaging (MRI).^[1]

Nanoparticle having particle size of 100 nm or less- has attracted attention due to their unusual properties, with various applications, over the particles with larger size. Silver based compounds are highly toxic to microorganisms which make it an excellent choice for multiple roles in the medical field. The action of silver metal on microbes is not fully known. It is hypothesized that silver nanoparticles will cause cell lysis or inhibit cell transduction by releases of ionic silver that inhibiting the thiol group of bacterial enzymes thereby halting bacterial DNA replication, depleting levels of intracellular adenosine triphosphate (ATP) and finally cause cell death. Due to nanosize, the surface area is increased which improves their contact with microorganisms thereby exhibiting biocidal effect.

To synthesize nanoparticles a wide number of physical, chemical, biological, and hybrid methods are available. As such physical and chemical methods are more

popular for nanoparticle synthesis but the toxicity had limited their use. The development of safe eco-friendly methods of biogenetic production are now of more interest due to the simplicity of the procedures and versatility.

To overcome this problem, synthesizing nanoparticles by employing biological methods of using microbes and plants. One of them is the synthesis using plant extracts eliminating the elaborate process of maintaining the microbial culture and often found to be kinetically favorable than other bioprocesses. Biomolecules have a significant advantage over their counterparts as protecting agents.

Several groups have shown success in the synthesis of Ag, Au, and Pd nanoparticles using organisms like bacteria and fungi as well as extracts from plant parts, e.g., geranium leaves lemon grass, neem leaves, aloe vera and coriander fruits.^[2] The spectacular success in this field has opened up the prospect of developing bio-inspired methods of synthesis of metal nanoparticles with tailor-made structural properties.

In the present review, we have selected two plants which viz., *Punica granatum* and *Murraya koenigii* are commonly used by the people for regular use as food and which are found to have number of traditional uses for treatment of diseases. The aim of the study is to give different method of synthesis and application of nanoparticles synthesized by these 2 plants. The abstract of various articles published are given below.

***Punica granatum* Linn**

Abuelmagd MA and Rehab MA have synthesised both silver and gold nanoparticles using pomegranate peel extract (PPE) in alkaline media. The resulted nanoparticles had particle size of 50 nm and 20 nm for gold and silver nanoparticles respectively. The nanoparticles produced by this method are extremely fast, ecofriendly and stable.^[3]

Yang et al have used a unique way to prepare AgNP composites with pomegranate peel extract to reducing Ag⁺ under mild conditions. UV-Vis, XRD, FTIR and TEM were employed to detect the forming of AgNPs and the particle size of the composites was 15 - 35 nm with spherical shapes at pH 10. It had strong antibacterial activity against gram positive bacteria and gram negative bacteria. This provided a useful clue to further study the AgNP biosynthesis mechanism.^[4]

Dash et al have demonstrated the use of the juice of pomegranate for the synthesis of gold nanoparticles (AuNPs) at room temperature under very mild conditions. The synthesis of the AuNPs was completed in few minutes and size of the nanoparticles could be controlled by varying the concentration of the fruit extract. The AuNPs were characterized by surface plasmon resonance spectroscopy, TEM, FTIR and X-ray diffraction studies. Catalytic activity of the synthesized colloidal AuNPs has also been demonstrated.^[5]

Tokton et al have aimed to access the anti-tyrosinase activity of ellagic acid rich pomegranate peel extract (EPP; 12 % w/w) and prepared the EPP loaded nanostructure lipid carriers (NLCs) using a warm micro emulsion technique for topical application. The EPP possessed a strong anti-tyrosinase activity with IC₅₀ values of 28.54 ± 1.34 µg/ml. Lyophilized EPP loaded NLCs showed spherical particles of 200 nm, poly dispersity index of 0.2 and zeta potential of -34 mV. The *in vitro* release study showed a prolonged release of ellagic acid from the NLCs upto 12 h following the Higuchi's model. The cream containing EPP loaded NLCs clearly promoted the active compound penetration when compared to EPP cream in *ex vivo* model. The prepared EPP showed strong anti-tyrosinase activity that suitable for use in cosmetics.^[6]

Ahmad et al have biosynthesised silver and gold nanoparticles using Pomegranate peel extract at room temperature by using the bio waste of the fruit. The morphology of the NPs was determined from UV-Vis spectroscopy, transmission electron microscopy (TEM), selected area electron diffraction (SAED) and X-ray diffraction (XRD) spectra. The average particle size of 5 ± 1.5 nm and 10 ± 1.5 nm were obtained for silver and gold nanoparticles.^[7]

Nisha et al synthesized nanoparticles from aqueous extract of pomegranate leaves which were confirmed by UV-visible spectra at 437 nm, SEM with EDAX, FTIR

and XRD. Antibacterial activity was evaluated against *Pseudomonas*, *Bacillus cereus*, *Staphylococcus albus* and *Proteus* pathogens.^[8]

The reduction of pure Ag⁺⁺ ion monitored by the Vennila et al by using pomegranate leaf, flower and fruit extracts. The UV- Spectrum showed sharp bands between 350 and 400 nm in leaf, 450 and 490 nm in flower and fruit. The antibacterial assays against Gram positive (*Staphylococcus aureus*) and Gram negative strains (*Pseudomonas*) and two pathogenic fungi namely *Candida albicans* and *Aspergillus* were also performed by standard disc diffusion method.^[9]

Kaur et al biologically synthesized Copper nanoparticles (CuNPs) using peel extract of *Punica granatum*. The synthesized nanoparticles were characterized with FTIR and TEM. This showed the particles were in the range of 15 to 20 nm. Antibacterial activity against *Micrococcus luteus* MTCC 1809, *Pseudomonas aeruginosa* MTCC 424, *Salmonella enterica* MTCC 1253 and *Enterobacter aerogenes* MTCC 2823 *in vitro* were determined.^[10]

Shanmugavadivu et al synthesized silver nanoparticles using pomegranate peel extract which was characterized by UV-Vis spectrum [371 nm], FT-IR and SEM analysis with the average particles size ranges about 5-50 nm. Antibacterial activities of AgNPs were determined against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* pathogens.^[11]

Shalini et al in their work phytofabrication of silver nanoparticles using pomegranate fruit seeds demonstrated green synthesis of silver nanoparticles from the extract of seed of pomegranate fruit. The morphology of synthesized nanoparticles was determined from TEM and XRD method. The synthesized nanoparticles were found to highly toxic against different multi-drug resistant human pathogens.^[12]

Gnana Jobitha et al demonstrated the synthesis of silver nanoparticle using pomegranate fruit extract which was confirmed by UV-vis spectra peak at 460 nm and were characterized by SEM with EDAX, XRD and FTIR. Antibacterial activity against *Bacillus subtilis*, (Gram positive) and *Klebsiella planticola* (Gram-negative) were demonstrated.^[13]

Nadagouda et al described synthesis of Ag and Au nanoparticles using antioxidants from blackberry, blueberry, pomegranate, and turmeric extracts. The synthesized particles were characterized using XRD, TEM, high-resolution TEM (HR-TEM), particle size analysis, UV-Vis spectroscopy and thermo gravimetric analysis.^[14]

Shirode et al described the bioactivity assessment of novel formulations of poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) NPs loaded with pomegranate extract (PE) or individual polyphenols such

as PU or EA which were prepared by the double emulsion–solvent evaporation method. Uptake of Alexa Fluor-488-labeled NPs was evaluated in MCF-7 breast cancer cells over a 24-hour time showed that it was effectively taken up by the cells within 24 hrs. 2- to 12-fold enhanced effect on cell growth inhibition was found with PE-, PU-, and/or EA-loaded NPs on MCF-7 and Hs578T breast cancer cells which proved its anticancer activity in breast cancer cells.^[15]

Elia et al have prepared Gold nanoparticles (GNPs) using four different plants extracts [*Salvia officinalis*, *Lippiaci triodora*, *Pelargonium graveolens* and *Punica granatum*] which were measured using dynamic light scattering, nanoparticle-tracking analysis and analysis of scanning electron microscopy images. Biocompatibility was examined by L-cell growth in the presence of different concentration of GNPs which was found to be stable.^[16]

Murraya koenigii

Philip et al showed that *Murraya koenigii* leaf-assisted rapid green synthesis of silver and gold nanoparticles at 373 K. The nanoparticles were characterized using UV–vis, TEM with 10 and 20 nm particle sizes for silver and gold nanoparticles, XRD and FTIR analysis.^[17]

Bonde et al showed the biosynthesis of silver nanoparticles by the leaf extract of *Murraya koenigii* (Indian curry leaf tree) which were determined by UV–visible peak at 420 nm, FTIR and SEM showed spherical Ag NPs in the range of 40–80 nm. The bactericidal activity against pathogenic bacteria, viz., *Escherichia coli*-JM-103 (ATCC 39403), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (MTCC 424) of the standard antibiotics were significantly increased in the presence of Ag NPs with maximum activity against *E. coli* (increase in fold area –4.06), followed by *P. aeruginosa* (1.11) and *S. aureus* (0.09), while tetracycline showed maximum activity against *S. aureus* (2.16) followed by *P. aeruginosa* (0.24) and *E. coli* (0.21). The Ag NPs thus obtained demonstrated remarkable antibacterial activity against three human pathogenic bacteria when used in combination with commercially available antibiotics.^[18]

Subha et al demonstrated green synthesis of iron particles by an aqueous extract of *Murraya koenigii* fruit bulb. The spectroscopic analysis of Iron nanoparticles showed broad peak between 275 -500 nm. The iron nanoparticle was characterized by SEM, XRD and FTIR.^[19]

Sajesh et al prepared curry leaf extract from fresh curry leaves by boiling it for 3 minutes, 5 minutes and 10 minutes separately and were mixed with silver nitrate at room temperature to synthesis of silver nanoparticles. The nanoparticles were characterized by UV-Vis spectrometry with peak at 435 nm, SEM [146 nm particle sized] and EDX measurements. Antibacterial activity of nanoparticles against *Bacillus* species, *E. coli*,

Staphylococcus species, *Klebsiella* species and *Micrococcus* species were performed which showed antibacterial activity on both Gram positive and Gram negative bacteria.^[20]

Jackson et al showed that Silver nanoparticles of *Murraya koenigii* were synthesised using acacia gum within 30 minutes at 60°C. UV readings at 430 nm also confirmed the formation of nanoparticles. Antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed significant ($P < 0.05$) sensitivity of the nanoparticle and crude extract.^[21]

Krishnaveni et al described the synthesis of silver nanoparticles using plant extract of *Murraya koenigii* (Green Curry Leaves), *Ocimum teniflorum* extract and polysaccharides-Chitin and Chitosan. The antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumonia* showed satisfactory inhibitions.^[22]

Suganya et al has shown that activity of silver nanoparticles (AgNPs; 5, 10, 20, 30, and 40 ppm) synthesized using *Murraya koenigii* plant leaf extract (50, 200, 350, 500, and 650 ppm) against first to fourth instars larvae and pupae of *Anopheles stephensi* and *Aedes aegypti* for 24 hrs The synthesized AgNPs were highly toxic than crude leaf ethanol extract in both mosquito species. The maximum mortality was observed in synthesized AgNPs, and ethanol leaf extract of *M. koenigii* against *A. stephensi* had LC₅₀ values of 10.82, 14.67, 19.13, 24.35, and 32.09 ppm and 279.33, 334.61, 406.95, 536.11, and 700.16 ppm and LC₉₀ values of 32.38, 42.52, 53.65, 63.51, and 75.26 ppm and 737.37, 843.84, 907.67, 1,187.62, and 1,421.13 ppm. *A. aegypti* had LC₅₀ values of 13.34, 17.19, 22.03, 27.57, and 34.84 ppm and 314.29, 374.95, 461.01, 606.50, and 774.01 ppm and LC₉₀ values of 36.98, 47.67, 55.95, 67.36, and 77.72 ppm and 777.32, 891.16, 1,021.90, 1,273.06, and 1,509.18 ppm, respectively which suggest that the use of silver nanoparticles as effective biocides for controlling the target vector mosquitoes.^[23]

Sharmila et al synthesised and characterized silver nanoparticles, and their antimicrobial effect on bacteria were studied. The synthesized silver nanoparticles loaded with activated carbon is used in adsorption studies and obtained good results. The synthesized silver nanoparticles (AgNPs) were characterized by Ultraviolet-visible (UVVis) spectrometer, Fourier Transform Infrared spectroscopy (FTIR), X-ray diffraction study (XRD), SEM (Scanning Electron Microscopy). Zone of inhibition from the range of 13.2 mm to 23.4 mm were obtained.^[24]

Ashtaputrey et al demonstrated the synthesis of CuNPs using the leaves extracts of the plant *Murraya koenigii* (Curry leaves) which were characterized by UV-visible spectroscopy at 340 nm, Scanning Electron Microscopy (SEM) and FTIR.^[25]

Raju et al prepared curry leaf extract from fresh curry leaves by boiling it for 3 minutes, 5 minutes and 10 minutes separately and synthesized silver nanoparticles. The silver nanoparticles showed inhibitory effects against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. The zone of inhibition was found to be 13 mm, 13 mm, 15 mm and 9 mm respectively. They are characterized using UV-Vis spectrometry, SEM and EDX measurements.^[26]

Divyapriya et al established the synthesis of zinc oxide nanoparticles using *Murraya koenigii* plant extract which were characterized by SEM. The antibacterial activity of nanoparticles was demonstrated.^[27]

Indhumathi carried out synthesize silver nanoparticles from the leaves of *Murraya koenigii*. The silver nanoparticle synthesized showed highest activity against *Trichoderma* and *Rhizopus* and mild activity against *Aspergillus niger* and *Aspergillus flavus*. The highest zone of inhibition was observed in *Trichoderma*. But the silver nanoparticle was more active against *Rhizopus* than the standard antibiotic Amphotericin and plant extract.^[28]

Laura et al have investigated the biological synthesis of silver nanoparticles using *Murraya koenigii* leaf extract. The effect of broth concentration and particle size were reported. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) analysis showed that the synthesized silver nanoparticle varied from 10-25 nm and have the spherical shape. It was found that the increasing broth concentration increases the rate of reduction and decreases the particle size.^[29]

Vijaya et al have employed the novel approach for the synthesis of carbon nanoparticles (CNPs) using *Murraya Koenigii* (curry tree) shoots in the presence of castor oil. The prepared Carbon nano particles (CNPs) were characterised using X-ray diffraction (XRD), Scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR) and Energy dispersive X-ray (EDAX) analysis.^[30]

Vinoth Kumar et al have investigated the synthesis of silver (Ag) nanoparticles using leaf extract of *Murraya koenigii* for 48 h at 300°C under shaking condition. Synthesized nanoparticles were characterized using UV Vis spectroscopy, XRD and SEM. The silver nanoparticles were predominately spherical in shape and polydispersed in nature with an average size of 57 nm.^[31]

Singaravelu et al have demonstrated method to impregnate silver nanoparticles (AgNPs) into microcrystalline cellulose (MCC) by curry leaf (*Murraya koenigii*) extract. Transmission electron microscopy (TEM) analysis showed silver particle were in the range of 10 - 25 nm with a spherical shape. Solvent casting of poly (lactic-acid) was used to produce composite films

containing silver impregnated MCC and were aimed for antimicrobial applications.^[32]

Sushmita Deb have described the synthesis of silver nanoparticles using plant extract of *Murraya koenigii*(Green Curry Leaves), *Zea mays* (Baby corn).The synthesized AgNPs have been characterized by UV-Vis spectroscopy and scanning electron microscopy (SEM; 10-20 nm in range). The antibacterial activity of the nanoparticles against *Escherichia coli* and *Staphylococcus aureus* were evaluated. Ag nanoparticles inhibited the growth of *E.coli* at low concentration but had mild effect against *S. Aureus*.^[33]

CONCLUSION

Various chemical, physical and biological synthetic methods have been used in production of metal nanoparticles. All these methods have experienced stability and aggregation of nanoparticles, control of crystal growth, morphology, size and size distribution. Moreover, separation of produced nanoparticles for further applications is still an important issue. Development of non-toxic, biocompatible and eco-friendly method for synthesis of nanoparticles is the need of hour. It was shown that the metal nanoparticles produced by plants are more stable in comparison with those produced by other organisms. Plants (especially plant extracts) are able to reduce metal ions faster than fungi or bacteria. For industrial production of well-dispersed metal nanoparticles, plant extracts are better than plant biomass or microorganisms. Researchers have focused their attention on understanding the biological mechanisms and enzymatic processes of nanoparticle biosynthesis as well as detection and characterization of biomolecules involved in the synthesis of metallic nanoparticles. Many biomolecules in plants such as proteins/enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins could be involved in bio reduction, formation and stabilization of metal nanoparticles. Understanding the biochemical processes/pathways involved in plant heavy metal detoxification, accumulation, and resistance will help to improve nanoparticle production.^[2]

Silver nanoparticles using plant extracts provides benefits over chemical and physical method as it is economical, energy efficient, cost effective; provide healthier work places and communities, protecting human health and environment leading to lesser waste and safer products. This eco-friendly method can potentially be used in various areas, including pharmaceuticals, cosmetics, foods, and medical treatment. The present review given the details of nanoparticles synthesised using 2 plants and their activity against different disease causing agents. Further the authors are working towards synthesizing nanoparticles and study different pharmacological activity as this two plants are regularly used and cost of procuring the raw material is also low so that society less burden for the treatment and becomes free from diseases.

ACKNOWLEDGEMENT

The authors are grateful to Chairman, Children's Education Society Bangalore and Rajiv Gandhi University of Health Sciences, Bangalore for the facilities and fund provided respectively.

FUNDING

The project is funded by Research Grant from Rajiv Gandhi University of Health Sciences, Bangalore.

ETHICAL ISSUES

There is none to be applied.

CONFLICT OF INTEREST

None to be declared.

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