Review Paper:

Green Synthesis of Nanoparticles from Natural Resources; Their Study and Applications

Sharma Porshia[1*], Rathore Pragya[2], Pathak Richa[2] and Verma Ayushi[2]
1. Institute for Innovative Learning and Research (IILR) Academy, Indore, Madhya Pradesh, INDIA
2. Softvision Institute of Biotechnology and Science, Indore, Madhya Pradesh, INDIA
*venice12051999@gmail.com

Abstract

Green synthesis of nanoparticles via nanotechnology has many potential applications in various fields. The main aim of green synthesis is to decrease the use of toxic chemicals. Nanotechnology is considered as the revolution in the field of science and technology. It involves the manipulation of materials and synthesizes structures of different atoms and molecules on nano-scale having similar chemical composition to larger synthesized nanoparticles which had never been as easy as it has become with the "green-synthesis" methodologies. Using plants and micro-organisms such as bacteria, fungi have thus become biologically safe, cost-effective and environmental-friendly.

In this review, we have discussed about green-synthesis and the different techniques that are involved for formation of nanoparticles and focused mainly on gold and silver nanoparticles including their sources and various anti-bacterial, anti-fungal, anti-viral, anti-cancerous, anti-inflammatory, anti-fibrotic properties.

Keywords: Gold Nanoparticles (Au-NPs), Nanoparticles (NPs), Silver Nanoparticles (Ag-NPs), Transmission Electron Microscopy (TEM).

Introduction

Synthesis of nanoparticles from micro-organisms and plants through green synthesis technology (nanotechnology) is simple, cost effective, less time consuming, environment friendly, consumes less energy and is biologically safe process. Nanotechnology when combines with biology forms nano-biotechnology that includes both prokaryotic and eukaryotic living commodities such as actinomycetes, algae, cyanobacteria, bacteria, viruses, fungi and plants. Not all bio-organisms are able to produce nanoparticles because of enzymatic activities and intrinsic metabolic processes within them. Biological entities or extracts from them are used for the green synthesis of metallic nanoparticles through bio reduction of metal ions into their elemental form of size ranging between 1-100 nm. Nanoparticles play a significant role in various fields like diagnostic biomarkers, cell labelling, anti-microbial agents, drug delivery and cancer therapy.

The synthesis process is very eco-friendly that involves no toxic or harmful chemicals and also consumes less energy with usage of high ecological solvents for the production of nanoparticles. 'Green synthesis' process is required to avoid the production of unwanted or harmful by-products which is achieved from the build-up of reliable, sustainable and eco-friendly synthesis techniques. Green synthesis of metallic nanoparticles has been taken into consideration to accommodate various biological sources like bacteria, fungi and plant extracts. Utilization of plant extracts is rather simple and easy process to produce nanoparticles at large scale as compared to bacteria and/or fungi mediated synthesis. These synthesized products are collectively known as bio-genic nanoparticles.

Methodologies of green synthesis are based on biological precursors depending on various reaction parameters such as solvent, pressure, temperature and pH conditions (acidic, basic, or neutral). For the synthesis of nanoparticles, plant biodiversity has been broadly taken into consideration due to the presence of effective phytochemicals from various plant extracts, especially from leaves such as ketones, amides, aldehydes, carboxylic acids, phenols, flavones, terpenoids and ascorbic acids. These components have potential of reducing metallic salts into metal nanoparticles. Nanotechnology is designing, developing or manipulating at nanometer on a billionth of a meter scale. The dealing object size must be less than hundred nanometers at least in one dimension to call something to be nanotechnology. There are two approaches designed in nanotechnology which are known as Top-down and Bottom-up.

These are two different ways by which metallic nanoparticles can be formed and both of these approaches are useful in different applications. The former deals with the reduction in size of current technological devices whereas the latter performs an opposite role, which is responsible for building of even more complex molecular devices on an atomic arrangement. On the other hand, the top-down approach deals with the production of technological structures in a far reached order and for connecting macroscopic devices whereas the bottom-up is beneficial for the production and arrangement of short-range order at the nano-scale aspect.

Biosynthesis of nanoparticles from plants: Phyto-nanotechnology has gained attention over time as it comprises of eco-friendly, cheap and rapid processes for the synthesis of nanoparticles.

Plants have the potential to accumulate certain amounts of heavy metals in their different parts. Consequently, various biosynthesis techniques have gained consideration because of being simple, efficient, cost-effective and feasible methods as well as an excellent alternative to conventional methods for the production of nanoparticles. There are various plants which can be utilized to reduce and stabilize the metallic nanoparticles in synthesis process. Many researchers have employed green synthesis process for preparation of metallic nanoparticles via plant leaf extracts to further explore their various applications.

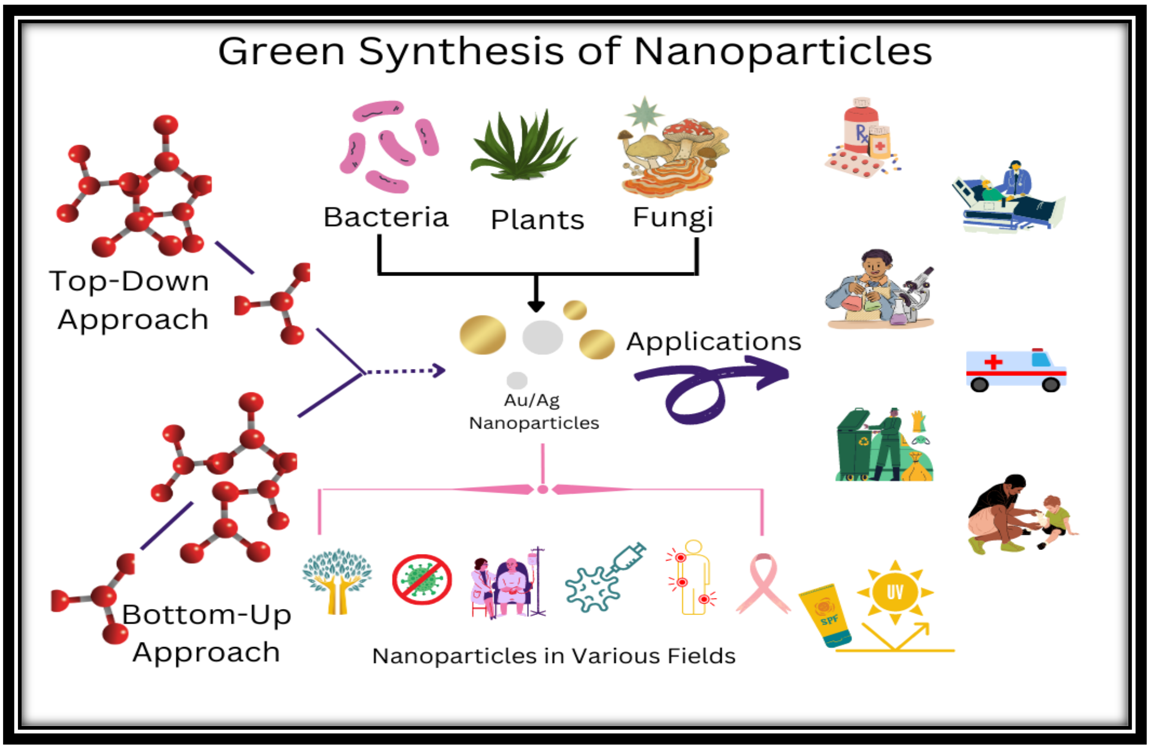

Figure 1: Representation of sources, techniques and applications of NPs.

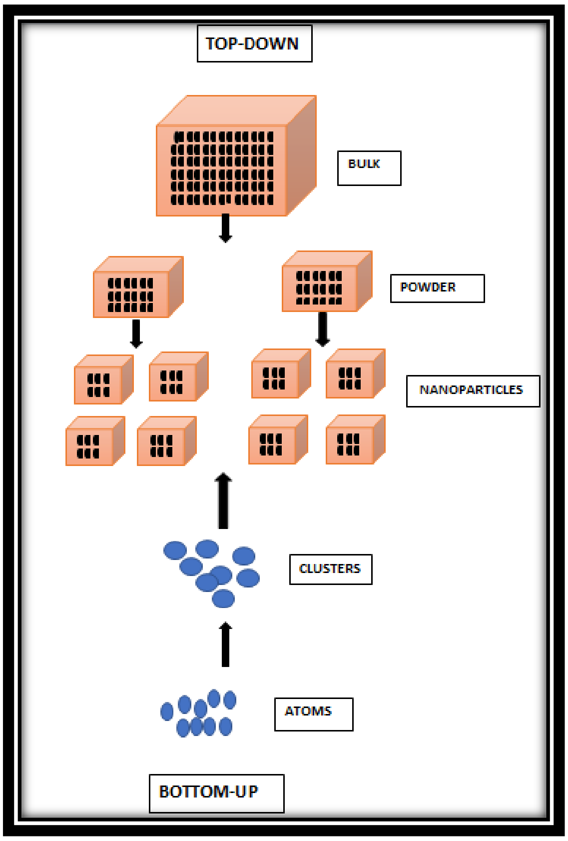

Figure 2: Representation of Top-down and Bottom-up approach for NPs synthesis.

Plants have biomolecules such as carbohydrates, proteins and coenzymes that have potential to reduce metal salts into nanoparticles. Like other biosynthesis processes, gold and silver nanoparticles were first to be investigated from the plant extracts. Various plants including Aloe vera (*Aloe barbadensis*), Lemon (*Citrus limon*), Oat (*Avena sativa*), Alfalfa (*Medicago sativa),* Tulsi (*Osimum sanctum)*, Coriander (*Coriandrum sativum*), Neem (*Azadirachta indica),* Mustard *(Brassica juncea*) and Lemon grass (*Cymbopogon flexuosus*) have been utilized to synthesize silver and gold nanoparticles.

A number of studies have reported biosynthesis of Au-NPs using different plants or plant extracts involving use of harmless bio-components from plants to carry out the reduction and capping of Au-NPs, with the reduction of waste generation and limiting the requirements for additional purification steps.

There are numerous bio-components that are present in plants such as flavonoids, phytosterols, quinones etc. which play a role in the synthesis of Au-NPs because of the functional groups that speed up the reduction and stabilization of Au-NPs.[1] Although nearly every part of plant has been reported to successfully carry out the synthesis of Au-NPs, leaves are most commonly used. The difference in the level of various compounds present in different plants and even in different parts of a plant affects the synthesis of Au-NPs. For example, a study has reported the effect of difference in level of phenolic contents present in leaves and fruit of *Garcinia mangostana* plant on the synthesis of Au-NPs. As the leaves are rich in phenolic content, so the rate of synthesis of Au-NPs was faster in the presence of leaves than fruit[2]. Similarly, the synthesis of gold nanoparticles using medicinal plants *Acorus calamus* and *Cassia auriculate* has also been reported.[3]

The study showed that silver (Ag) nanoparticles were produced from leaf extract of *Artemisia vulgaris* without the help of any external reducing or capping agent. The blackish- brown color was observed that showed the complete synthesis of nanoparticles. The Ag-nanoparticles that were extracted were approximately 25 nm in diameter when observed using Transmission Electron Microscopy (TEM)[4]. *Artemisia vulgaris* is also called mugwort which is highly used as herbal medicine to treat pain, relief itching and to boost energy**.**

Leaves of the *Terminalia arjuna* plant also carried out the synthesis of Au-NPs within 15 minutes. Au-NPs synthesized were 20–50 nm in size and had spherical morphology. The reactive compounds such as arjunetin, leucoanthoc-yanidins and hydrolysable tannins present in leaves of *Terminalia arjuna* contributed for the synthesis of Au-NPs.[5]

Leaves of olive plant and *Cassia auriculata c*ompleted the synthesis reaction of Au-NPs in 20 minutes and 10 minutes respectively. The active metabolites and biomolecules present in the leaves of the olive plant are proteins, apigenin-7-glucoside, oleuropein and luteolin-7-glucoside that resulted in the formation of spherical and anisotropic Au-NPs having size range between 50–100 nm**.** The major active substances like polysaccharides and flavonoids and Au-NPs synthesized from leaves of *Cassia auriculata* plant were 15–25 nm in size and had spherical and anisotropic morphology.[6]

On the other hand, gold (Au) nanoparticles were extracted from aqueous leaf extract of *Justica glauca*. It is a medicinal dicotyledonous plant belonging to Acanthaceae family and possesses great antibacterial properties. The reactive compounds were lignans (+)-pinoresinol, (+)-medioresinol, alkaloids, flavonoids, steroids (sitosterol-3-0-glucoside) and terpenoids reported to complete the synthesis reaction of Au-NPs in 60 mins. Au-NPs had spherical and hexagonal morphology and the extracted nanoparticles ranged between ~32.5±0.25 nm in size.[7]

Fresh aqueous leaf extract from *Morus alba* (mulberry) was used to extract silver nanoparticles. Silver nanoparticles formation was confirmed through the optical study that indicated the surface plasmon resonance ranging between 423-450 nm and the size of the nanoparticles was between 12 and 39 nm. *Morus alba* is not only economically worthy but medically important. It is used for the treatment of dizziness, insomnia, premature aging and DM2. It also provides a protective effect against atherosclerosis, liver and kidney disorders and inflammation.[8]

Mangifera indica leaves were used to synthesize spherical Au-NPs within 2 minutes of reaction time. The size of Au-NPs was found to be in the range of 17–20 nm. Terpenoids, flavonoids and thiamine are the active compounds present in mango fruit, which might have contributed to the synthesis of Au-NPs.[9]

Boiling of 10 grams freshly collected *Acalypha indica* leaves in 100 ml distilled water at 60 °C for 5 minutes yielded both silver (Ag) and gold (Au) nanoparticles. These nanoparticles ranged between the sizes of 20-30 nm and are great source of bio-reductants[10]. *Acalypha indica* is a weed plant that usually grows in wet, temperate and tropical regions. It is greatly used by the locals as a useful source of medicine for various therapeutic treatments such as anti-helmintic, anti-ulcer, bronchitis, asthma, wound healing, anti-bacterial and has many other applications.[11]

Apart from leaves, various other parts of plants including fruits, roots, stems, etc. have been used for the synthesis of Au-NPs. Banana peels synthesized Au-NPs of size 50 nm and mango peels synthesized Au-NPs of size ranging between 6.03±2.77 to 18.01±3.67 nm. It was observed that banana peels synthesized spherical shaped Au-NPs and the mango peel synthesized quasi-spherical shaped Au-NPs. The reaction time for both the processes took around 20 and 25 minutes respectively. Apart from these above-mentioned parts of plants, rhizomes of turmeric, seeds of cocoa[12], yam beans, ginger, pulp of green pepper, latex of *Hevea brasiliensis* bark of bay cedar, galls of zebra wood, effluent from palm oil mill and nuts of *Areca catechu* were found useful to carry out the synthesis of Au-NPs.

The fruits: The fruit of *Citrus maxima* synthesized spherical Au-NPs ranging around of 15–35 nm in size within 5 minutes of reaction time. Proteins, terpenes and ascorbic acid were the major compounds that acted as reducing agents during the reaction. Also the high phenolic content in *Sambucus nigra* (elderberry) was seen as the major factor in the synthesis of Au-NPs.[13]

The flowers: Flowers of *Lonicera Japonica* were observed to have amino acids as active compounds which successfully synthesized Au-NPs having triangular and tetrahedral morphology with the size range of 8 nm having reaction time of 60 minutes[14].Similarly, the flowers of the *Moringa oleifera* plant are known to synthesize Au-NPs of size 3–5 nm. It was reported that this plant had a high content of flavonoids, sterols, carotenoids, amino acids and phenols which were claimed to be responsible for carrying the reduction reaction during the synthesis process. Also various types of roses have been demonstrated to have the reducing ability for the Au-NPs synthesis.

Biosynthesis of nanoparticles from bacteria: Microorganisms have been reported as an excellent candidate for the synthesis of both intracellular and extracellular Au-NPs.[15] Bacterial species have been widely utilized for various commercial biotechnological applications such as bioremediation, genetic engineering and bioleaching[16]. Bacteria have the ability to reduce the metal ions and have potency for nanoparticles preparation.[17]

Prokaryotic bacteria and actinomycetes have been broadly used for synthesis of metallic nanoparticles. Synthesis of Au-NPs was achieved by the reduction of Au(III) ions at the surface of membrane and mycelia.[18] Micro-organisms possess certain reductase enzymes that can reduce metal salts to metallic nanoparticles having narrow size distributions and monodispersity. By the alteration of essential growth parameters, the shape and size of Au-NPs can be controlled.

Some examples of bacterial strains that have been extensively been exploited for the synthesis of bioreduced silver nanoparticles with distinct size or shape morphologies are- *Escherichia coli, Enterobacter cloacae, Lactobacillus casei, Bacillus cereus, Pseudomonas proteolytica, Bacillus amyloliquefaciens, Bacillus indicus, Bacillus cecembensis, Aeromonas sp. SH10, Geobacter spp., Arthrobacter gangotriensis, Corynebacterium sp. SH09* and *Shewanella oneidensis*. Similarly, for the preparation of gold nanoparticles, several bacterial species such as *Bacillus megaterium D01, Bacillus subtilis 168, Shewanella alga, Desulfovibrio desulfuricans, E. coli DH5a, Rhodopseudomonas capsulate and Plectonema boryanum UTEX 485* have been used.

The cell wall of bacteria which is negatively charged, can electrostatically interact with positively charged Au(III) ions. During the intracellular synthesis, enzymes and biomolecules carry out the synthesis of Au-NPs when the gold ions are transported inside the cell. On the other hand, during extracellular synthesis with the help of membrane enzymes, the gold ions are trapped on the cell membrane. These enzymes on the membrane are reductase enzymes that are secreted out by the microbial cell and can carry out the synthesis process even outside the bacterial cell. However, extracellular synthesis, is more fascinating as it does not require extra downstream processing steps which are required for the separation of nanoparticles from the intracellular matrix.

During the extracellular synthesis reaction, bacteria secrete NADPH-dependent enzymes which can reduce Au(III) ions to Au0 such as nitrate reductase secreted by *Pseudomonas denitrificans*. As a result, the action reductase enzyme diminished after the synthesis of Au-NPs[19]. It was reported that both NADH and NADH-dependent enzymes functioned as a scaffold or nucleating agent for the synthesis reaction[20]. During extracellular synthesis of Au-NPs, *Rhodopseudomonas* capsulate secreted NADH and NADH-dependent enzymes. The synthesis of Au-NPs was caused by the transfer of electrons from NADH carried by NADH-dependent enzyme which causes the reduction of Au(III) to Au0.

Ag-NPs activity against *Escherichia coli* was described. The formation of “pits” in bacterial cell wall and the accumulation of Ag-NPs in the cellular membrane led to an increase in permeability and eventually to the death of bacterial cells. They also tried to understand their mechanism of action[21]. So, presently there are three main explanations that have been proposed to describe the antibacterial activity:

- The direct interaction of Ag-NPs with the bacterial cell membrane leading to subsequent membrane damage and caused complications with components located inside the cells.[21]
- The interaction with thiol (−SH) groups and production of reactive oxygen species (ROS)[22].
- The release of silver ions inhibits the respiratory enzymes and also generates ROS.[23]

Silver nanoparticles were synthesized extracellularly by the bio-reduction of aqueous Ag^+ ions using culture supernatant of *Bacillus licheniformis*. *Bacillus licheniformis* belongs to a family of Gram-positive bacteria and is a spore-forming soil bacterium that is greatly used in biotechnology industry for the manufacture of enzymes, antibiotic, biochemicals and consumer products. The sizes of silver nanoparticles were approximately ~40nm. Moreover, it was found that well dispersed nanocrystals of silver of around ~50nm were produced using *Bacillus licheniformis.*[24]

It was found that *Bacillus subtilis* 168 was able to reduce Au+ ions to produce gold nanoparticles ranging between ~5-25 nm in size and are octahedral in shape. *Bacillus subtilis* also known as Hay bacillus or Grass bacillus is highly efficient in protein secretion system and adaptable metabolism widely used as a cell factory for microbial production of chemicals, enzymes and anti-microbial materials for industries, agriculture and medicine**.** The nanoparticles were synthesized within the bacterial cells by incubation of the cells along with gold chloride under circumambient temperature and pressure conditions**.** Gold particles can be synthesized both intra and extra cellularly whereas the silver nanocrystals were exclusively formed extracellularly.

A practical experiment was carried out successfully for the synthesis of gold nanoparticles from the bacterium *Rhodopseudomonas capsulata*. These nanoparticles were produced extracellularly ranging in size from 10-20 nm using NADH-dependent reductase.

Among the *Pseudomonas* genus, the strains of *Pseudomonas stutzeri* species showed the highest level of activity in the production of silver nanoparticles. Maximum amount of nanoparticles was formed extracellularly upon the addition of 100 mg/l of silver ions. It was observed that the shape and the size of the nanoparticles formed depend on the initial concentration of Ag+. Addition of silver ions to the concentration of 25-100 mg/l to the bacterial culture medium that leads to the appearance of the nanoparticles of the sizes ranging between 20nm - 300nm which then lead to the formation of agglomerates of sizes between 150nm–500 m.[25]

Biosynthesis of nanoparticles from fungi: Fungi have also been used as a biological source for the synthesis of Au-NPs. Fungi mediated biosynthesis of metallic nanoparticles is also a very efficient process for generating monodispersed nanoparticle shaving well-defined morphologies. Due to the presence of a variety of intracellular enzymes, they act as better biological agents for the preparation of metallic nanoparticles.[26]

Fungi secrete a number of biomolecules including metabolites and other extracellular enzymes such as hemicellulose, cell wall lytic enzyme β-1, acetyl xylem esterase, 3-glucanase etc. which play a very important role during the synthesis of nanoparticles.

As compared to bacteria, the competent fungi can synthesize larger amounts of nanoparticles[27]. Moreover, fungi have shown many merits over other organisms due to the presence of enzymes, proteins and reducing components on their cell surfaces[28]. Probably, mechanism for the synthesis of the metallic nanoparticles is due to enzymatic reduction taking place in the cell wall or inside the fungal cell. There are several fungal species that are used in synthesis of metallic nanoparticles such as silver and gold.

Antifungal activity of Ag-NPs remained less explored than the antibacterial activity. Numerous studies are done for the synthesis of gold nanoparticles using unicellular and multicellular fungi. *Fusarium oxysporum* was used in a study for the extracellular synthesis of gold and silver NPs by the reduction action of nitrate-dependent enzyme and shuttle quinone[29]. It has been reported that a fungal species *Verticillium* has carried out intracellular synthesis of Au-NPs. It was found that Ag-NPs were trapped in the cell membrane and the cell wall of fungi indicated that Au^{3+} ions were bio-reduced by the reduction action of reductase enzymes in fungi[30]. The biosynthesis of Au-NPs from *Phanerochaete chrysosporium* proved that enzyme laccase was secreted by the fungi for extracellular synthesis of Au-NPs and for intracellular synthesis, the responsible enzyme is ligninase enzyme.

Biosynthesis of silver nanoparticles was carried out extra cellularly using *Aspergillus fumigatus*. The process was quite fast and the silver nanocrystals were formed within few minutes of contact between silver ion and the cell filtrate. With the help of Transmission electron microscopy (TEM) micrograph, the well dispersed silver nanoparticles in the range of 5-25 nm were observed**.**

The fungus *Fusarium oxysporum* has the capability of producing silver nanoparticles of varied sizes ranging between 5-15 nm. These nanoparticles had been capped with the help of fungal proteins to make them remain in stable conditions. *Fusarium oxysporum* fungus also has the ability to synthesize nanoparticles extracellularly whereas fungus *Trichoderma reesei* could also be taken into consideration for the production of silver nanocrystals extracellularly, having size range of 5-20 nm but the process of synthesis of nanoparticles was appreciably slower when compared with *Aspergillus fumigatus* and *Fusarium oxysporum***.**

Tichoderma reesei is a non-pathogenic fungus for man. *Tichoderma reesei* has exhibited a long history of safe use in enzyme production on industrial scale. The cellulases and xylanases enzymes produced by this fungus are found in food, animal feed, pharmaceutical, textile, pulp and paper industries**.**

Gold nanoparticles were produced by the reduction of chloroauric acid by the fungal extract of novel strain *Tallaromyces flavus*. It was observed that crystalline, irregular and mostly flower shaped gold nanoparticles were formed having a mean hydrodynamic radius of 38.54 ± 10.34 nm.[31]

Applications

Nanoparticles as antivirals: Ag-NPs have received tremendous attention for their antibacterial activities, still the antiviral properties of metal nanoparticles remain undeveloped. These days, effective and safe antiviral therapies are available due to which significant improvement in lives of large numbers of patients is seen; nevertheless, viruses are still one of the leading causes of the diseases and increased death rates worldwide. The emerging and re-emerging of viral diseases are a major threat to human society and veterinary health. We have witnessed tough times because of viruses approximately one each year, with the majority of viruses originating from an animal reservoir and thus have ability to switch to a new host. The best known examples are SARS Corona virus, West Nile virus, Monkey Pox virus, Hantavirus, Nipah virus, Hendravirus, Chikungunya virus, pandemic influenza viruses of avian or swine origin and SARSCoV-19.

Of the many factors that are responsible, the changes in the local ecosystems disturb the balance between pathogen and principal host species, along with increase in urbanization and changes in the human behavior.[32] Three key aspects can be considered from the studies conducted so far on the antiviral properties of Ag-NPs;

- Antiviral activity has been demonstrated by Ag-NPs against number of viruses infecting both prokaryotic and eukaryotic organisms;
- Inhibition of viruses depends on the size of Ag-NPs (generally small Ag-NPs, 25 nm or less, resulted as more active in viral infectivity inhibition);
- The early infection might be the general time frame where Ag-NPs exert their antiviral activity, thus, impacting the rest of viral replication cycle.

Anti-HIV activity of selected nanoparticles was studied using the *in vitro* drug susceptibility assay having an endpoint determination of p24 antigen. The inhibition percentage of p24 antigen is co-related to the anti-HIV activity of the nanoparticles.

The nanoparticles showed greater activity in the test where interaction of viruses was allowed with nanoparticles before infecting the cells than the test where the cells, previously infected with viruses, were allowed to interact with nanoparticles. This may be due to the nanoparticles coating with charge stabilizers that may be of a larger size and hence cannot enter the cells. Similarly, demonstration by the uncoated gold particles at 4 ppm that were directly added to previously infected cells, also showed a higher percent inhibition of p24 (77.78%) than the test in which the virus was first interacted with the nanoparticles (55.56%).[33]

Nanoparticles role in cancer: Gold nanoparticles have been investigated for various cancer therapies and are sought as a potential alternative or adjunct to many non-selective chemotherapeutic agents, in order to improve therapeutic outcomes with reduction in undesirable side effects[34]. It has been demonstrated in multiple studies that the plasmonic gold nanoparticles have efficacy for the thermo-ablation of various cell types. The efficacy of gold nanoparticles for the thermal mediated induction of cellular death was studied by Iravani[35] wherein anti-CD8-labeled gold nanoparticles were used for the destruction of T-cells and selective targeting.

Photothermal therapy (PTT), also known as thermal ablation or optical hyperthermia is non-invasive and is widely used for cancer therapy due to benefits of real-time observation of tumor sites and photo-induced destruction of the tumor cells or tissues[36]. PTT uses materials with a high photothermal conversion efficiency, injected into the body which works by gathering near the tumor tissues by targeting recognition technology.[37,38]

PTT forms central application of gold nanoparticles in the cancer treatment. Gold nanoparticles absorb the incident photons and convert them to heat to destroy cancer cells. As a result of localised surface plasmon resonance, the gold nanoparticles have unique optical properties due to which they absorb light with extremely high efficiency (cross section at ~10 9 M^{-1} cm^{-1}), thus effective PTT is ensured at relatively low radiation energy. Tumor's abnormal vascular structure is inefficient in dissipating heat, thus tumors show more sensitivity to hyperthermia when compared with healthy tissues. When irradiated by light, the heat generated by gold nanoparticles causes denaturation of biomolecule, disruption of cellular membrane and kills tumor cells.

Many benefits of gold nanoparticles make them suitable for photothermal treatment of cancer such as:

- Au-NPs can be administered into the local area of tumor while minimizing the non-specific distribution.
- These nanoparticles can be activated via near-infrared laser light and so create the ability of deep penetration into biological tissues.
- To create multifaceted cancer PTT and for drug delivery systems, these nanoparticles are modulated.[39]

Gold is one of the noble metals characterized by its resistance to corrosion and oxidation. Because of evidence of gold's long-documented use in medicinal applications, its properties have been known for centuries. During the middle ages, colloidal gold was documented as a substance useful for treatment and diagnosis of diseases[40]. Recent advancements in nano-medicine have recognized the use of gold in the therapeutic delivery of drugs or as a therapeutic modality in itself. Colloid gold is covalently linked onto adenoviral vectors for selective cancer targeting and inducing hyperthermiable application of near-infrared laser light.[41]

In order to progress to clinical cancer treatment, it is necessary to have knowledge of the formulation and clinical trials to establish the nano-drug for treating cancerous cells. Biogenic Ag-NPs from *Vitex negundo* leaf extract showed the inhibition of proliferation of human colon cancer cell line HCT15, which indicated that Ag-NPs may exert anti-

https://doi.org/10.25303/2709rjce1450154

proliferative effects on the coloned cancer cell lines by suppressing its growth, arresting the G0/G1-phase, reducing DNA synthesis and inducing apoptosis.[42]

Anti-inflammatory and anti-fibrotic effect of nanoparticles: Typical hallmarks of the patient suffering from Covid-19 are severe inflammation and lung fibrosis. The induction of systemic as well as pulmonary inflammation is caused by cytokine storm by driving the pro-inflammatory signaling involved in lung fibrosis too[43-45]. Ag-NPs can be promising therapeutic candidate that have potential for anti-inflammatory and anti-fibrotic properties by virtue of their ability to abrogate the inflammatory cytokines by modulation of their transcriptional activity. Ag-NPs reduced the concentrations of inflammatory cytokines like interleukin (IL)-1β, IL-6, IL-17, transforming growth factor beta (TGF-β) and tumor necrosis factor alpha (TNF-α), in different pre-clinical animal models resulting in the reduction of inflammation and diminished fibrotic cascade by modulating NFκB and MAPKinase pathways.[46-48]

The impact of Ag-NPs on the inflammatory response has been studied well by the researchers[49]. Silver reacts with the immune cells and thus affects the suppression or stimulation of various pathological conditions. An increase in the size of Ag-NPs resulted in the increase of inflammatory cytokine secretion in rat's alveolar macrophages[50], toxicological effects on macrophage U937[51] and can also evoke an immune response in macrophages[52]. The repeated oral administration of Ag-NPs leads to an increased level of cytokine production, B-cell distribution and inflammatory cell infiltration in mice[53]. These nanoparticles were also used as adjuvants and showed effects *in vitro* and *in vivo* using model antigens of oval albumin (OVA) and bovine serum albumin (BSA).[54]

Ag-NPs also showed anti-fibrotic effects in a dextran sodium sulphate (DSS) induced colitis model. Ag-NPs decreased fibrosis and collagen deposition by the reduction in the expression of pro-fibrotic genes like Col 1a1 and Col 1a2 as evident from histological findings and were confirmed by mRNA expression studies. Thus, elevated levels of cytokines and cytokine mediated inflammation and fibrosis in the COVID-19 may be halted by Ag-NPs.

Conclusion

'Green synthesis' of metallic nanoparticles has always been a highly attractive research area over the last decade. The study aims to review the feasibility of green nano-synthesis in the rapidly advancing world where dependability on nano materials is increasing at a faster pace. There are many fields where green nanosynthesis can replace the chemical process of nano synthesis e.g. the use of green synthesis can replace the chemical processes for use of solar cells, removal of radioactive material, nanomedicines, in agriculture to protect plants and environment, for energy storage and many more.

By using the bio-based sources, the hazardous waste production is minimized and the green nanosynthesis being less consuming in terms of environment. Today when we are dealing with the extreme drug resistance, the green nanoparticles have demonstrated the potential to cope up with the situation.

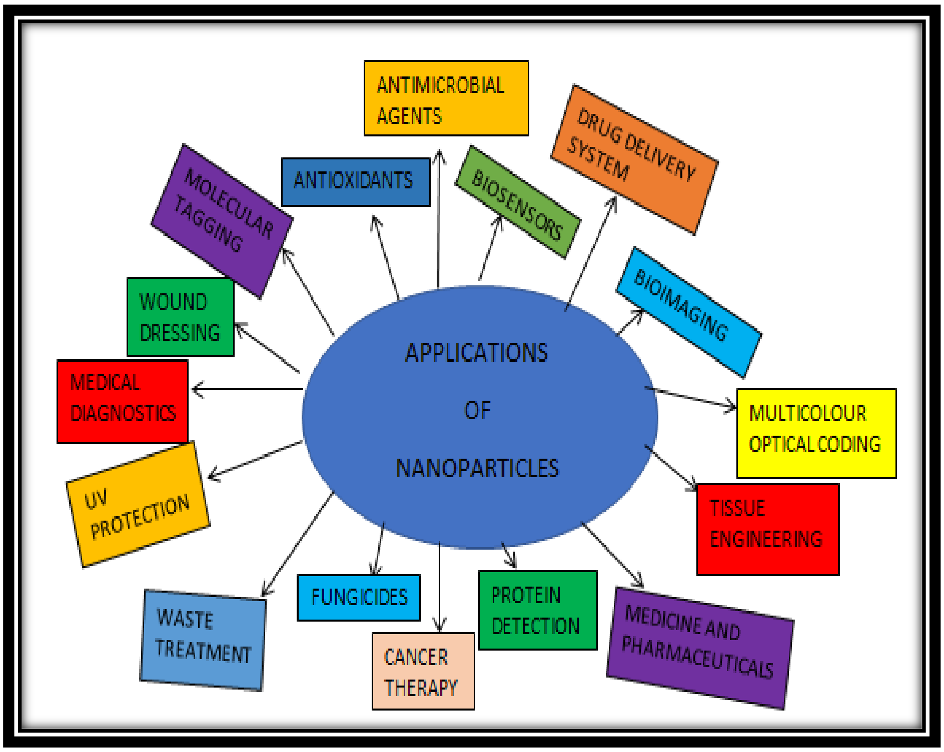

Figure 3: Representation of various applications of NPs.

Various natural extracts like bio-components like plant, bacteria, fungi and plant extracts have been employed as efficient resources in synthesis of nanoparticles. Among these, the plant extracts have proven to possess high efficiency as a stabilizing and reducing agent for nanoparticles synthesis. The basic premise is to test the safety of nanoparticles after they are put to market. For this the incorporation of safety assessment is to be done into the design and innovation stage of nonmaterial's development.

The future aim is to provide nanoparticles at a more cost effective range. The wide application of nanoparticles is due cytotoxicity. The solutions for many problems in various sectors can be sought through the worldwide development and study of practical usage of green nanosynthesis as many promising areas have been evaluated by different studies in this aspect.

References

1. Adriana F., Andras L.N., Bianca M., Corina T., Elena O., Luminita D., Mihai K., Nicoleta D., Razvan O. and Remus M., The effect of Sambucus nigra L. extract and phytosinthesized gold nanoparticles on diabetic rats, *Colloids and Surfaces B: Biointerfaces*, **150,** 10.1016/j.colsurfb **(2016)**

2. Ahmad A., Khan M.I., Kumar R., Ramani R., Sastry M., Senapati S. and Srinivas V., Published 6 June, *Nanotechnology*, **14(7),** DOI https://doi.org/10.1088/0957-4484/14/7/323 **(2003)**

3. Ahmad A., Khan M.I., Kumar R., Sastry M. and Senapati S., Extracellular biosynthesis of bimetallic Au-Ag alloy nanoparticles, *Small*, **1(5)**, 517-20, doi: 10.1002/smll.200400053 **(2005)**

4. Alkilany A.M. et al, The many faces of gold nanorods, *J. Phys. Chem. Lett.*, **1**, 2867–2875, doi: 10.1021/Jz100992x **(2010)**

5. Allawadhi P. et al, Nanoceria as a possible agent for the management of COVID-19, *Nano Today*, **35**, 100982 **(2020)**

6. Allawadhi P. et al, Potential of electric stimulation for the management of COVID-19, *Med. Hypotheses*, 144, 110259, 10.1016/j.mehy.2020.110259 **(2020)**

7. Anderson R.R., Joe E.K., Lin C.P., Pitsillides C.M. and Wei X., Selective cell targeting with light-absorbing microparticles and nanoparticles, *Biophys. J.*, **84,** 4023–4032, doi: 10.1016/S0006-3495(03)75128-5 **(2003)**

8. Arulvasu C., Babu G., Manikandan R., Prabhu D. and Srinivasan P., Biologically synthesized green silver nanoparticles from leaf extract of Vitex negundo L. induce growth-inhibitory effect on human colon cancer cell line HCT15, *Process Biochemistry*, **48(2),** 317-324, https://doi. org/10.1016/j.procbio.2012.12.013 **(2013)**

9. Arumugam A., Gopinath, K., Ilangovan R., Sankaranarayanan K., Venkatesh K.S. and Ilangovan R., Green synthesis of gold nanoparticles from leaf extract of *Terminalia arjuna*, for the enhanced mitotic cell division and pollen germination activity, *Industrial Crops and Products*, **50**, 737-742, https://doi.org/10.1016/j.indcrop.2013.08.060 **(2013)**

10. Badiee S.H., Manafi S., Noorani T., Pourali P., Rezaei A. and Yahyaei B., Biosynthesis of gold nanoparticles by two bacterial and fungal strains, Bacillus cereus and Fusarium oxysporum and assessment and comparison of their nanotoxicity in vitro by direct and indirect assays, *Electronic Journal of Biotechnology*, **29,** 86-93, https://doi.org/10.1016/j.ejbt.2017.07.005 **(2017)**

11. Bae E. et al, Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles, *Environ Toxicol Pharmacol*, **30(2)**, 162–168, doi:10.1016/j.etap.2010.05.004 **(2010)**

12. Balakumaran M.D., Kalaichelvan P.T., Krishnaraj C., Muthukumaran P. and Ramachandran R., Acalypha indica Linn: Biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells, *Biotechnol Rep*, **4,** 42-49, doi: 10.1016/j.btre.2014.08.002 **(2014)**

13. Banerjee M., Chattopadhyay A., Ghosh S.S., Mallick S. and Paul A., Novel Biogenic Silver Nanoparticle-Induced Reactive Oxygen Species Inhibit the Biofilm Formation and Virulence Activities of Methicillin-Resistant Staphylococcus aureus (MRSA) Strain, *Langmuir*, **26(8)**, 5901-5908, DOI: 10.1021/la9038528 **(2010)**

14. Barathy I.A., Dhas T.S., Gokavarapu S.D., Rajeswari A., Kapadia Z., Kumar V.G., Karthick V., Roy A., Shrestha T. and Sinha S., "Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent *Cassia auriculata*," *Colloids and Surfaces*, **87(1)**, 159-163, doi: 10.1016/j.colsurfb.2011.05.016 **(2011)**

15. Bickford L.R. et al, A new era for cancer treatment: gold-nanoparticle-mediated thermal therapies, *Small*, **7**, 169–183, doi: 10.1002/smll.201000134 **(2011)**

16. Bilal M., Gurunathan S., Kalimuthu K., Suresh B.R. and Venkataraman D., Biosynthesis of silver nanocrystals by Bacillus licheniformis, *Colloids Surf B Biointerfaces*, **65(1),** 150-3, doi: 10.1016/j.colsurfb.2008.02.018 **(2008)**

17. Bilal M., Iqbal M.N., Li C. and Rasheed T., Green biosynthesis of silver nanoparticles using leaves extract of *Artemisia vulgaris* and their potential biomedical applications, *Colloids and Surfaces B: Biointerfaces*, **158**, 408-415, https://doi.org/10.1016/j.colsurfb. 2017.07.020 **(2017)**

18. Carlson C. et al, Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species, *J Phys Chem B*, **112(43),** 13608–13619, doi:10.1021/ jp712087m **(2008)**

19. Chelladurai K., Emmanuel R., Ming C.S., Muthupandi K., Prakash P. and Selvakumar P., Green synthesis of gold nanoparticles and its application for the trace level determination of painter's colic, *RSC Advances*, DOI link: https://doi.org/10.1039/ C4RA14988B **(2015)**

20. Chen Y.L. et al, Augmented biosynthesis of cadmium sulfide nanoparticles by genetically engineered Escherichia coli, *Biotechnol Prog*, **25**, 12606, https://doi.org/10.1002/btpr.199 **(2009)**

21. Czaplicki S., Naczk M. and Zadernowski R., Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*), *Food Chemistry*, **112(3)**, 685-689, http://dx.doi.org/10.1016/j.foodchem.2008.06.030 **(2009)**

22. Dhas S.T. et al, Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent Cassia auriculata, *Colloids and Surfaces B: Biointerfaces*, **87(1),** 159-163, https://doi.org/10.1016/j.colsurfb.2011.05.016 **(2011)**

23. Eon L.S. and Kap L.D., Green Synthesis of Silver and Gold Nanoparticles Using Lonicera Japonica Flower Extract, *Bulletin of the Korean Chemical Society*, **33(8)**, 2609–2612, https://doi.org/10.5012/BKCS.2012.33.8.2609 **(2012)**

24. Everts M. et al, Covalently linked Au nanoparticles to a viral vector: potential for combined photothermal and gene cancer therapy, *Nano Lett.*, **6,** 587–591, doi: 10.1021/nl0500555 **(2006)**

25. Fazal S., Jayasree A., Koyakutty M., Menon D., Nair S.V. and Sasidharan S., Green Synthesis of Anisotropic Gold Nanoparticles for Photothermal Therapy of Cancer, *ACS Applied Materials and Interfaces*, **6(11),** 8080-8089, DOI: 10.1021/am500302Tc **(2014)**

26. Fletcher N.F. and Howard C.R., Emerging virus diseases: can we ever expect the unexpected?, *Emerg Microbes Infect*, **1(12)**, e46, doi:10.1038/emi.2012.47 **(2012)**

27. Gericke M. and Pinches A., Microbial production of gold nanoparticles, *Gold Bull.*, **39,** 22–8, https://doi.org/10.1007/BF03215529 **(2006)**

28. Ghosh A., Kumar R., Mukherjee P., Patra C.R. and Sastry M., *Chemistry of Materials*, **14(4)**, 1678-1684, DOI: 10.1021/cm010372m **(2002)**

29. Gonzalez D.A. et al, Silver nanoparticles reduce brain inflammation and related neurotoxicity through induction of H2S-synthesizing enzymes, *Sci. Rep.*, **7(1)**, 42871 **(2017)**

30. Goswami N., Ninan N. and Vasilev K., The impact of engineered silver nanomaterials on the immune system, *Nanomaterials*, **10(5)**, 967, doi:10.3390/nano10050967 **(2020)**

31. Gunasekaran M., Jeganathan K., Mubarakali D. and Thajuddin N., Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens, *Colloids Surf. B Biointerfaces*, **85**, 360–365, doi: 10.1016/j.colsurfb.2011.03.009 **(2011)**

32. Hao G., JiaCheng F., Linhong H., Ni W., Ning N., Xiaoxia W., Xirui H., Yinlan R., Yin S., Yu C. and Zefeng Z., Structures, bioactivities and future prospective of polysaccharides from Morus alba (white mulberry): A review, *Food Chemistry*, **245**, 10.1016/j.foodchem.2017.11.084 **(2017)**

33. Hebeish A. et al, Antimicrobial wound dressing and anti-inflammatory efficacy of silver nanoparticles, *Int. J. Biol. Macromol.*, **65**, 509-515 **(2014)**

34. Hirst D.G., Jain S. and O'Sullivan J.M., Gold nanoparticles as novel agents for cancer therapy, *Br. J. Radiol*., **85**, 101–113, doi: 10.1259/bjr/59448833 **(2012)**

35. Iravani S., Bacteria in nanoparticle synthesis: current status and future prospects, *Int Sch Res Not.*, 1–18, https://doi.org/10.1155/2014/359316 **(2014)**

36. Jahangirian H., Lee K.X., Rafiee M.R., Shameli K., Teow S.Y., Webster T.J. and Yew Y.P., Recent Developments in the Facile Bio-Synthesis of Gold Nanoparticles (AuNPs) and Their Biomedical Applications, *Int J Nanomedicine*, **15,** 275-300, doi: 10.2147/IJN.S233789 **(2020)**

37. Khurana A. et al, Yttrium oxide nanoparticles reduce the severity of acute pancreatitis caused by cerulein hyperstimulation, *Nanomed. Nanotechnol. Biol. Med.*, **18**, 54-65 **(2019)**

38. Kim H.W. et al, Label-free fluorescent mesoporous bioglass for drug delivery, optical triple-mode imaging and photothermal/photodynamic synergistic cancer therapy, *ACS Appl. Bio Mater*., 2218–2229, doi: 10.1021/acsabm.0c00050 **(2020)**

39. Lim D.H. et al, Size dependent macrophage responses and toxicological effects of Ag nanoparticles, *Chem Commun*, **47(15),** 4382, doi:10.1039/c1cc10357a **(2011)**

40. Liu J., Liu Y., Tang H., Wang H. and Xu Y., Evaluation of the adjuvant effect of silver nanoparticles both *in vitro* and *in vivo*, *Toxicol Lett.*, **219(1),** 42–48, doi:10.1016/j.toxlet.2013.02.010 **(2013)**

41. Martínez G.F. et al, Antibacterial activity, inflammatory response, coagulation and cytotoxicity effects of silver nanoparticles, *Nanomedicine Nanotechnol Biol Med.*, **8(3),** 328–336, doi:10.1016/j.nano.2011.06.014 **(2012)**

42. Mewada A., Oza G., Pandey S. and Sharon M., Extracellular Synthesis of Gold Nanoparticles Using Pseudomonas denitrificans and Comprehending its Stability, *Journal of Microbiology and Biotechnology Research*, **2**, 493-499 **(2012)**

43. Mohanpuria P., Rana N.K. and Yadav S.K., Biosynthesis of nanoparticles: technological concepts and future applications, *J Nanoparticle Res.*, **10,** 507–17 **(2008)**

44. Muhamad I.I., Nur H., Saidin S., Ya'akob H., Zahidin N.S. and Zulkifli R.M., A review of Acalypha indica L. (Euphorbiaceae) as traditional medicinal plant and its therapeutic potential, *Journal of Ethnopharmacology*, **207**, 146-173, https://doi.org/10.1016/j.jep.2017.06.019 **(2017)**

45. Narayanan K.B. and Sakthivel N., Synthesis and characterization of nano-gold composite using Cylindrocladium floridanum and its heterogeneous catalysis in the degradation of 4-nitrophenol, *J Hazard Mater*., **189**, 519–25, https://doi.org/10.1016/j.jhazmat.2011.02.069 **(2011)**

46. Oza G., Pandey S., Shah R. and Sharon M., Biogenic fabrication of gold nanoparticles using Halomonas salina, *J Microbiol Biotechnol Res*, **2(4),** 485-492 **(2012)**

47. Oza G., Pandey S. and Sharon M., Bio-nanotechnology: Concepts and Applications **(2012)**

48. Pal S., Song J.M. and Tak Y.K., Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli, *Applied and Environmental Microbiology*, **73(6),** 1712-1720 **(2007)**

49. Panda P.K., Pradhan N., Priyadarshini E. and Sukla L.B., Controlled Synthesis of Gold Nanoparticles Using Aspergillus terreus IF0 and Its Antibacterial Potential against Gram Negative Pathogenic Bacteria, *Journal of Nanotechnology*; 9 pages, https://doi.org/10.1155/2014/653198 **(2014)**

50. Philip D., Rapid green synthesis of spherical gold nanoparticles using Mangifera indica leaf, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **77(4),** 807-810 **(2010)**

51. Pricker S.P., Medical uses of gold compounds: past, present and future, *Gold Bull.*, **29**, 53–60, doi: 10.1007/BF03215464 **(1996)**

52. Rashidova S. et al, Microbial Synthesis of Silver Nanoparticles by Pseudomonas Sp., *Biotechnol Ind J*., **14(4),** 169 **(2018)**

53. Sondi I. and Sondi S., Silver Nanoparticles as Antimicrobial Agent: A Case Study on E. coli as a Model for Gram-Negative Bacteria, *Journal of Colloid and Interface Science*, **275**, 177-182, https://doi.org/10.1016/j.jcis.2004.02.012 **(2004)**

54. Wong K.K. et al, Further evidence of the anti-inflammatory effects of silver nanoparticles, *ChemMedChem*, **4(7)**, 1129-1135 **(2009)**.

(Received 19th January 2023, accepted 22nd February 2023)

*****