JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

REVIEW ON GREEN SYNTHESIS OF SILVER NANOPARTICLES

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Abstract:
Silver nanoparticles has gain great attention in the research field in the recent years. It is due to their size, eco-friendly, cost effective, chemical stability, availability, biocompatibility, antimicrobial activity, antiviral activity, anticancer activity and many more therapeutic applications. Especially the green synthesis of nanoparticles has been most popular due to their high efficiency, non-pollutant and cost effective approach which helps in stabilizing the silver nanoparticles for vast applications. Their therapeutic applications have got a great advantage in the field due to the potential usage of phytochemicals in order to reduce and use them as antimicrobial and anticancer agents. This review provides an insight into the plant mediated synthesis of nanoparticles, methods of plant extract preparation, stabilization and characterization of nanoparticles, antibacterial and anticancer activities followed by their applications.

Key words:
Silver nanoparticles, green synthesis, nanoparticle characterization, antibacterial, anticancer.

1. INTRODUCTION:

With the advancement of science and technology universally, mankind has now touched upon a new branch of science termed Nanoscience or Nanotechnology. Nanotechnology is an emerging field which is referred to the manufacturing and manipulating the particle structure, shape and size which ranges from 1 - 100 nm [1]. The creation of such particles are known as nanoparticles (NPs). These particles are employed in various applications ; to name a few, medicine and pharmacy. Other fields of applications are in manufacturing materials, environmental, electronic, energy collection and mechanical industries due to their special physical and chemical properties [2]. NPs are found as carbon nanotubes, quantum dots, nanorods, nanocapsules, nanoemulsions, fullerenes, metallic NPs, ceramic NPs and polymer NPs [3,4]. As for metallic NPs, their exceptional properties have led to the creation of various methodologies for the synthesis of silver nanoparticles (AgNPs) from plant extracts which are of great interest for researchers to develop antimicrobial agents suitable for agricultural [5-7]. In addition, these initiatives are seen as low-cost processes that avoid products that generate toxicants and benefit agricultural activity. The concept of circular economics (i.e., the economy that gently decouples economic activity from the consumption of limited resources) plays an important role here which aims to balance economic process, resources sustainability and environmental protection [8]. Therefore the main challenge for biodiversity-rich countries and the scientists working in those countries is to gather and convert knowledge of biological resources into processes, compounds, methods and tools which are to be achieved with the sustainable use and exploitation of the biological diversity [9]. Due to this several strategies have been sought to contribute to the current field through the employment of green processes, like the creation of nanoparticles from plant extracts [10].

2. METAL OXIDES NANOPARTICLES:

There are numerous types of metal oxides nanoparticles composed of silver, gold, platinum, palladium, cerium, copper, nickel, selenium and iron. Their special physicochemical properties make them a potential material for industry and biomedical applications [11]. Various initiatives are being carried out in the field of agriculture to minimize the use of toxic chemicals. They can be done at low cost and provide various advantages to the producers [12]. There is additional evidence that, other than normal extricates, some nanoparticles, for example,

nanotubes, metal and metal oxides, and different varieties of polymer-stacked nanoparticles additionally have cancer prevention agent movement and can rummage the receptive nitrogen and responsive oxygen species (RNS/ROS) [13,14].

3. GREEN SYNTHESIS:

Green chemistry is the alternate methodology employed to reduce the utilization of environmentally harmful processes and products because of the severe consequences the planet is facing and also the limited time available to search out effective solutions [15,16]. To prepare one kg of AgNPs, the estimated cost would be around $4 million, while one kg of raw silver would cost around $14,000 [17]. The creation of nanobioreagents by extracting plant materials will be considered as a way to contribute to the field of bio-based materials [18]. Silver nanoparticles have a considerable surface zone which results into imperative biochemical reactivity, catalytic activity, and atomic behavior contrasted and greater particles having same compound organization [19].

4. SYNTHESIS OF NANOPARTICLES:

The development of NPs is accomplished through two stages: in the first, metal particles are decreased, and in the second one the agglomeration of colloidal suspension causing the development of the oligomeric groups [20]. The "green synthesis" or "biogenic synthesis" has acquired and more consideration as an eco-accommodating methodology helpful for synthesis of not just metal/metal oxide nanoparticles yet additionally the creation of other nanomaterials, for example, mixture materials, or an assortment of bioinspired materials [21]. Plant mediated biosynthesis is the best approach in green synthesis due to their rapid, eco-friendly, non-pathogenic, economical protocol and provides one step technique for synthesis and their low cost plant extract production and purification of synthesised nanoparticles makes them best in the business [22,23].

Plant extracts contain various compounds, which can be used as powerful reducing agents, stabilizers and precursor molecules for the formation of NPs [24,25]. To prepare the extracts, both the biomass of the whole plant and selected parts such as leaves, fruits, seeds and aerial parts can be used. The plant material can be fresh or dried and made as a powder. Different techniques are used to prepare the extracts, but most consistent method is conventional maceration with various solvents including water or mixtures of water and alcohol. The synthesis of nanoparticles is mediated by components extracted with reducing potential, including alkaloids, ascorbic acids, oxalic acids, carboxylic acids, glycosides, thiamine, polyols, tannins, terpenoids, polyphenols, phenols, flavonoids, polysaccharides, saponins, aminoacid, enzymes, vitamins and proteins, which have also been identified as stabilizers of nanoparticles [21,22,34].

Nanoparticles created by green combination strategies might be less steady contrasted with nanoparticles got as a result of chemical compound synthesis [26]. Due to the arrangement of AgNPs it has got huge interest in the possible application in areas such as catalysis [27], plasmonics [28], optoelectronics [29], biological sensors [30], antimicrobial activities [31], DNA sequencing [32], Surface-Enhanced Raman Scattering (SERS) [33], environmental change and contamination control [34], clean water technology , energy generation, data storage [35], and biomedical applications. The arrangement of NPs has given us striking advancements in the area of nanotechnology by exhibiting its latent capacity from the last decade [36].

5. METHODS FOR PLANT EXTRACT PREPARATION:

The extraction methods are the first step in separating the plant metabolites from the raw materials. In order to carry out an extraction process, some basic parameters must be taken into account, as these influence the quality of an extract [37]. Depending on the selected part of the plant material, as well as the solvents, they need to evaporate easily and not chemically modify the solutes. Since the end product retains traces of the solvent used, these must be small. In addition, when choosing an extraction method, the extraction time, the temperature, the pH of the solvent, the ratio between solvent and sample and the particle size of the raw materials must be taken into account in every laboratory [38].

5.1. Homogenization: It is the most widely used method for extraction, done by dried or wet extraction method. In the case of dried extraction method the dried plant samples are finely powdered and added to the solvent mixed for few minutes and kept in an orbital shaker for about 24 hours. For wet extraction process, the plant parts are chopped into small pieces, ground in a mortar and pestle and are added to a solvent and shaken in an orbital shaker for 24 hours and then filtered. The filtrate is used for the further analysis [39].

5.2. Serial Exhaustive Extraction: This method is processed with a different organic solvents from non-polar solvent like hexane to more polar solvent like methanol to extract a wide polarity range of compounds. The disadvantage is that thermolabile compounds cannot be extracted due to the high heat which leads to the degradation [40].

5.3. Soxhlet Extraction: This method is implemented when the compound is less soluble in the solvent and the impurities are soluble in the solvent. If the desired compound is highly soluble in the solvent then the impurities are removed by simple filtration. The major advantage in this method is that the solvent can be recycled and hence there is less wastage of the solvent. Similar to the serial exhaustive extraction method, in this method also, thermolabile compounds cannot be extracted [41].

5.4. Maceration: This is the best suitable method for the extraction of thermolabile compounds, where the whole plant or the powder can kept in the solvent for a certain period with frequent agitation until the soluble compounds are dissolved. Maceration is an ancient technique which was used by several traditional healers to get the whole extract without the loss of any secondary metabolites [42].

5.5. Decoction: This method is used to extract the heat stable and water soluble compounds. That means the extracted plant materials are boiled in the water for about 15 minutes and are cooled, filtered and are used for further analysis [43].

5.6. Infusion and Digestion: This method involves diluting the compounds in the solvents. The extract is prepared by macerating the compounds for a short period in cold or boiling water [44].

Another process called the digestion is done as maceration with a gentle heat applied. It is used when the elevated temperature do not interfere the solvent efficiency or the compounds [45].

5.7. Percolation: This process requires an instrument called percolator which is a narrow cone- shaped vessel with open ends. The ingredients are moistened with an appropriate amount of the specified menstrum and allowed to stand for approximately 4 hours in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstrum is added to form a shallow layer above the mass and the mixture is allowed to macerate in the closed percolator for 24 hours. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstrum is added as required, until the percolate measures about three quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient amount of menstrum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting [44].

5.8. Sonication: This method is processed with the help of ultrasound where the higher frequencies of 20 kHz – 2000 kHz are used which disrupts the cells and releases the constituents. Although the process is useful in some cases, like extraction of rauwolfi a root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules [46].

6. STABILIZATION OF NANOPARTICLES:

The adjustment of nanoparticles is essentially accomplished by electrostatic repugnance. Shockingly, this kind of adjustment is just viable with low ionic strength extricates where the shock is worked with by the profoundly scattered twofold layer. For the situation of high ionic strength, accumulation happens affected by solid van der Waals communications [47]. One more sort of adjustment is the formation of an extra hindrance on the outer layer of the NPs. Steric adjustment is given by proteins, in case they are parts of the concentrates, or by covering the surface with polymers like PEG or PVP (polyethylene glycol, polyvinylpyrrolidone). By and by, one can notice an expanding interest in investigations on the reactivity of nanoparticles contrasted with naturally visible articles and their cytotoxicity amassing in the body, which can produce Receptive Oxygen Species (ROS) [48].

A generally new space of examination is the utilization of nanoparticles with redox-active potential as free radical scavengers. For instance, cerium and yttrium oxides either go about as cell reinforcements [49] or can forestall the increment of ROS by emulating the action of the oxidative catalysts, catalase, or superoxide dismutase [50]. It has been demonstrated that AgNPs repress cell multiplication and adjust the action of cancer prevention agent catalysts [51].

7. CHARACTERIZATION OF SILVER NANOPARTICLES:

Characterization is the fundamental step in finding the size, shape, morphology, structure, surface chemistry, surface charge, dispersity and surface area of AgNPs. Such characterization methods are stated below [21].

7.1. UV-visible spectrophotometry: UV spectrophotometry is one of the most frequently used technique for characterizing synthesized nanoparticles, which is also used to monitor the stability and synthesis of AgNPs [52]. It is simple, easy, rapid, sensitive and selective for different types of nanoparticles [53-56]. This quantifies the amount of ultraviolet or visible radiation that is absorbed by a component in solution. UV-vis measures the ratio or the ratio function of the intensity of two light rays in the UV-visible range [56]. In AgNPs, the valence band and the conduction band are very close to one another in which the movement of electrons are free. Due to the combined oscillation of AgNP electrons in resonance with the incident light wave, these electrons generate a surface plasmon resonance band (SPR) [56-59]. The absorption spectrum of AgNP depends on the dielectric medium, morphology, shape, size and chemical environment of the synthesized nanoparticles [60]. Several studies have shown that AgNPs generate absorption bands around 200-800 nm in UV-visible wavelength spectra and were used to characterize nanoparticles with a range of 2-100 nm [61].

7.2. X-ray diffraction analysis (XRD): X-ray diffraction (XRD) is an analytical technique that has been used to study crystalline or polycrystalline structures [62], the quantitative dissolution of chemical compounds [63], the qualitative identification of different chemical species [64], the measurement of the degree of crystallinity [65], particle sizes [66] and so on. An X-ray beam is projected onto the crystal and the incident beam is scattered by the atoms, resulting in the diffraction patterns. Scattered X-rays interfere with each other. This interference could be observed using Bragg's law to find different properties of crystal or polycrystalline material [67]. Therefore, XRD can study the structural properties of various materials such as biomolecules, polymers, superconductors, glasses, etc. The analysis of these materials depends heavily on the formation of diffraction patterns, physical and chemical properties of glass [68]. In general, measurements are made in Angstroms (1 Angstrom = 0.1 nm), so X-ray diffraction is a primary characterization tool to obtain critical properties such as crystal structure, crystal phase identification, crystallite size (information on unit cell dimensions) and elongation [69]. XRD spectra determine the crystalline nature of silver nanoparticles through the general oxidation state of the particles as a function of time [70,1].

7.3. Scanning Electron Microscope (SEM): Among the various electron microscopic techniques, SEM is a surface imaging technique that can determine the particle shapes and surface morphology, sizes, and size distributions of synthesized NP on the micro (10^{-6}) and nano (10^{-9}) scale [71]. SEM uses a high-energy electron beam that is scanned across the surface of the AgNPs sample and then the observation of back scattered electrons provides properties of the sample [72]. Energy dispersion X-ray spectroscopy (EDX) is a chemical analysis method that is used in combination with SEM to determine the elemental composition of the AgNPs sample [73]. The sample during the electron beam bombardment and the EDS X-ray detector quantifies the relative frequency of the discharged X-rays in relation to their energy [74]. The disadvantage of the SEM is that it is not able to examine the internal structure of the sample, but it can provide valuable information about the particle level aggregation and purity [75].

7.4. Transmission Electron Microscope (TEM): The transmission electron microscope (TEM) is a powerful tool for characterizing nanoparticles. It is used to obtain quantitative parameters such as size distribution, particle size and morphology of the synthesized nanoparticles [76]. TEM uses an electron beam to interact with a sample and create an image on a photographic plate [77]. TEM is characterized by the detection and quantification of the chemical and electronic structure of individual nanoparticles. TEM has several advantages over SEM. It can offer superior spatial resolution and offers additional analytical measurements of nanoparticles [78].

7.5. Fourier transforms infrared spectroscopy (FTIR): FTIR spectroscopy is used to study the surface chemistry of metal nanoparticles and to find out whether biomolecules are involved in NP synthesis [79]. It can study the interactions that occur between the enzymes and the substrate during the catalytic process [80]. When infrared radiation penetrates the sample, some of the infrared radiation is absorbed by the sample and stays through it [81]. The resulting spectrum indicates absorption and transmission by creating a molecular fingerprint of the sample, there presents the identity of the sample [82]. FTIR is a suitable, valuable, inexpensive and simple non-invasive technique to investigate the role of biomolecules in the reduction of AgNO3 to silver [83].

7.6. Zeta potential measurement: The zeta potential measures the effective electrical charge on the surface of the NP and is a decisive parameter for characterizing the stability of AgNPs in aqueous suspensions [83]. Nanoparticles have a surface charge that attracts a layer of charged ions opposite the surface of the nanoparticle. The net electrical charge potential between the layers is known as the zeta potential of the nanoparticles and has values that are typically in the range of +100 mV to 100 mV [84]. The zeta potential is predictive for the nanoparticles stability [85]. Nanoparticles with a zeta potential size of less than 25 mV or more than

+25 mV tend to have a high stability [86]. A minimum zeta potential value of ± 30 mV is essential for a stable AgNP suspension. [87]. Dynamic light scattering is used to characterize the surface charge and the size and quality distribution of nanoparticles [88]. It is also very useful to know the polydispersity index of the manufactured nanoparticles [89].

8. ANTIBACTERIAL EFFECTS OF SILVER NANOPARTICLES:

The synthesized AgNPs have good antimicrobial potential and capable of restricting the replicative function of cells by affecting the membrane permeability and eventually causing cells to die by deteriorating the cell growth behavior of the pathogens [90]. Silver nanoparticles synthesized by green synthesis have strong foundation in the field of medication due to their rapidness, eco-friendliness and benignancy. These NP are free of polluters and are easy to scale-up [91]. Different types of coatings are used to decline the agglomeration and dissolution of bare AgNPs in solutions and the biological properties of the AgNPs are also depends on their coating[92-95]. The mechanism of action of AgNP against bacteria are as follows [96]:

i. Disabling the respiratory of chains

ii. Cell membrane disruption and leakage of its cellular contents

iii. Binding to functional group of proteins causing protein denaturation and cell death

iv. Blocking the DNA of replication

v. Denaturation of proteins and cell death through binding to functional groups of proteins.

The bacterial cellular membrane are negatively charged and AgNPs are positively charged and when the positively charged AgNPs accumulate on negatively charged membrane, it brings structural alterations in the membrane, which render more permeability to the bacterial cell membrane. Therefore uncontrolled transportation through cytoplasmic membrane leads to cell death [97]. AgNPs damages the genetic material inside the bacterial cell by binding, which results in inhibition of transcription and translation process[98]. This is also due to the soft acid nature of the silver which reacts with the soft bases like phosphorus and sulphur which are present in the cells and DNA [99,100]. Due to these properties, AgNPs are known to be an effective agent for killing a wide range of antibiotic resistant strains including Gram-negative and Gram-positive bacteria [101,102]. Besides, it has been proved that the nanoparticles at smaller size distribution possess higher rate of antibacterial activity against the pathogens compared to the larger size nanoparticles [103]. This is possible because the small size nanoparticles are capable of transporting into cells much faster than larger ones [104]. Furthermore, smaller size AgNPs have larger active surface area which is superior and ideal for interaction with various kinds of bacteria [105].

The following Table 1 indicates the action of AgNP against several strains of bacteria which exhibit the zone of inhibition indicated in millimeters.

Table 1: Antibacterial activity of Silver Nanoparticles

Sample	Plant parts used	Bacterial strain	Zone of Inhibition (mm)	Reference
Chamaemelum nobile	Whole plant	*E. coli* *S. typhimurium* *S. aureus* *B. subtilis*	15.1 14.3 13.0 14.3	106
Cassia fistala	Fruit	*S. aureus* *A. baumannii* *P. ceruginosa*	14.7 14.3 16.3	107
P. tripartita Sw	Leaf	*E. coli* *B. subtilis*	8.3 9.0	108

		S. typhi	16.3	
Pycnoporus sp	Whole plant	*S. aureus* *B. subtilis* *K. pneumonia* *E. coli*	16 17 18 18	109
Stevia rebaudiana	Leaf	*S. aureus* *E. coli* *E. faecalis*	15.3 12.7 10.7	110
Erythrina suberosa	Leaf	*S. aureus* *P. ceruginosa*	23 24	111
Dimocarpus logan lour	Peel	*B. subtilis* *S. aureus* *P. aeruginosa* *E. coli*	20 15 19 16	112
Origanum majorana	Mature Leaf	*S. aureus* *E. coli*	26 24	113
Origanum majorana	Tender leaf	*S. aureus* *E. coli*	20 18	113

9. ANTICANCER ACTIVITY OF SILVER NANOPARTICLES:

The AgNP can induce apoptosis-dependent programmed cell death in the absence of the tumor suppressor p53. 5-35 nm nanoparticles primarily could induce cell death through mitochondrial structure and functional targeting. Although the smaller AgNP are more cytotoxic, 5 and 35 nm was found to be ideal [114]. The cytotoxic characteristics of hybrid silver and AgNPs depends on the cell type. Higher cytotoxicity was recorded against tumor cells compared to non-cancerous fibroblasts. The metallic stimulation of fibroblast cells associated with tumor nanoparticles represents a typical therapeutic strategy. Since treatment with Ag and Ag hybrids suppresses the cancer cell by promoting the activity of fibroblasts associated with tumors. In addition, *in vivo* results demonstrated the ability of AgNPs and hybrids to inhibit metastatic spread of 4T1 tumor in mice. Incredibly, Ag hybrids can improve the therapeutic efficacy of intravenous doxorubicin treatment [115]. AgNP from dandelion of the common medicinal plant, *Taraxacum officinale* showed their high cytotoxic effect against human liver cancer cells (HepG2) [116]. The use of aqueous extract of *Commelina nudiflora L* [117] has shown reduced cell viability and increased cytotoxicity against colon cancer cells HCT116. The biofunctionalized AgNP synthesized in various plant extracts of guava and clove have shown a satisfactory anticancer effect against four different tumor cell lines; human colorectal adenocarcinoma, human kidney, human chronic myeloid, leukemia, human bone marrow and cervix [118]. AgNP developed using a phycocyanin protein pigment extracted from *Nostoc linckia* as a reducing agent showed effective cytotoxic activity against MCF7 [119]. The chemically synthesized AgNPs composites had promising anti-tumor activity against A549 (human lung cancer), HeLa (human cervical adenocarcinoma), MCF7 (human breast adenocarcinoma), MDAMB231 cells (human breast adenocarcinoma) and SKBR3 (adenocarcinoma of the human breast) [120]. The AgNP synthesized using ethanolic extract of rose petals (*Rosa indica*) showed potential anti-tumor activity against the human colon adenocarcinoma cancer cell line HCT 15 [117].

10. APPLICATIONS OF SILVER NANOPARTICLES:

Universally the applications of AgNPs are diverse. Each of them have been discussed below.

10.1. Medical: Nanoparticles are commercial used as antibacterial and antifugal agents and they have potential application in the fields of medical, such as dental cements [121], healing of burns and wound care [122], skin therapy and crucial in cosmetics [123], reconstructive orthopedic surgery and bone cements [124], medical devices and plastic catheters [125], target drug delivery [126], medical imaging, biolabeling and detection [127], cancer therapy [128], coating of hospital textiles such as face mask surgicals [129], gowns, coating of breath mask patient and ultrasonic detection [130], coating of implants for joint replacement [131], orthopedic and orthopedic fixations and implants [132]. AgNPs exhibits antiviral activity against human immunodeficiency virus-1, influenza virus (HIV-1), Herpes Simplex Virus HSV-2, monkey pox virus, hepatitis B, HSV-1 and respiratory syncytial virus [133].

10.2. Environmental Science: The AgNP find vast applications in air disinfection [134], drinking water purification [135], biological wastewater and groundwater disinfection, surface disinfection and waste water treatment [136].

10.3. Consumer goods: Apart from the biological applications AgNP finds a place in personal care products like textiles, paints and sunscreen cosmetics, toys and humidifiers, filters, kitchen utensils, toothpaste, washer systems, shampoo, bedding, deodorants and fabrics [137].

10.4. Food Industry: Applications in foods such as nutraceutical, food safety and food packing has gained popularity [138]. Metal oxide nanoparticles used in active packaging as sensors which is used as portable monitoring system for food analysis. Metals such as silver, gold, zinc are widely used in food packaging. These particles come into direct contact with the food material and migrate slowly and react with the organic substances present in the food. Inorder to provide beneficial characteristics to the final food packaging material, such as antimicrobial activity, enzyme immobilization, oxygen scavenging, mechanical strength, increased stability and shelf life of food and protection of food against humidity, temperature and other physiological factors [21]. Electronic components and transportation applications such as biosensors for food analysis, sensors, electrodes and integrated circuits has gained importance in the use of AgNPs [139].

CONCLUSION:

Green synthesis of silver nanoparticles has taken a lead over chemical and physical methods and arose its importance in the field of Nanobiotechnology to create simple, fast, cost effective, eco-friendly, and stable nanoparticles. Their applications in the fields of medicines, agriculture, electronics, automobile, environmental monitoring, consumer goods and personal care products, food, antimicrobial, antiviral and anticancer properties have increased exponentially. It was received well in the commercial market due to their size which possess unique propertis which is the biggest advantage that has made the nanobiotechnology as a broad spectrum of usage in the research field. In future there could be a possible outcome from the nanobiotechnology to make the impossible possible.

ACKNOWLEDGEMENT:

The authors are thankful to the host institution, Dr. N.G.P. Arts and Science College, Coimbatore, the management, Principal, Dean-Research and Development and Department of Biotechnology for their continued support and motivation. The communication number is DRNGPASC 2021-22 BS020

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