Uttar Pradesh Journal of Zoology

Volume 45, Issue 13, Page 348-364, 2024; Article no.UPJOZ.3598
ISSN: 0256-971X (P)

A Review on Plant Mediated Silver Nanoparticle Synthesis and its Antibacterial Applications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i134162

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3598

Review Article

Received: 04/04/2024
Accepted: 10/06/2024
Published: 17/06/2024

ABSTRACT

Nanotechnology is a developing field with numerous approaches for its synthesis, one of which is, the green synthesis of nanoparticles is gaining popularity due to its advantageous properties and unique applications in a wide range of fields. This environmentally friendly process of using plants

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***Cite as:** BalaKumaran, M.D., T. Spandana, T. Silambarasan, Iadalin Ryntathiang, S. Suresh, R. Santhi, and Mukesh Kumar Dharmalingam Jothinathan. 2024. "A Review on Plant Mediated Silver Nanoparticle Synthesis and Its Antibacterial Applications". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (13):348-64. https://doi.org/10.56557/upjoz/2024/v45i134162.*

has proven to be effective and quick in the synthesis of silver nanoparticles. In this study, current advances in the field of environmentally friendly synthesis of silver nanoparticles (AgNPs) utilizing a variety of plant extracts are summarized and discussed, along with the potential antibacterial uses of these materials. We discuss the impact of optimizing various parameters such as pH, temperature, time, and concentration on the synthesis process. Also covered is the mechanism of actively synthesized silver nanoparticles on infectious pathogens, with a focus on the recently used different plants for the synthesis of highly efficient antimicrobial green AgNPs.

Keywords: Silver nanoparticles; antibacterial; plant mediated synthesis; leaf extract.

1. INTRODUCTION

Nanotechnology involves the manipulation of matter at lengths between 1 and 100 nanometers and the creation of materials, tools, and systems based on these techniques. Materials are classified as either zero-dimensional, one-dimensional, two-dimensional, or three-dimensional based on their general shape. This scale results in new physical, chemical, and biological characteristics compared to those found at larger scales [1]. Nanomaterials have an exceptional surface area to volume ratio because of their size. In contrast to classical physics and chemistry, quantum mechanics govern nanomaterials [2]. By using nanomaterials, healthcare can be improved and negative manufacturing effects can be reduced by enabling mass production of items with improved functionality, drastically lower costs, and greener and more efficient manufacturing methods [3]. Over the past few years, nanotechnology has become an integral part of our lives.

Since nanoparticles are now being used in a growing number of products and applications, including pharmaceutical research, it is crucial to approach his cutting-edge technology from a comprehensive standpoint. Extensive research in the field of nanotechnology is currently being used in creating effective medications, and it has been recognized in the European Union (EU) as a Key Enabling Technology for the development of novel medical solutions that address unmet healthcare needs [4-6].

It has become a common topic in engineering, physics, and electronics in the past few decades as a multidisciplinary topic. However, the application of nanotechnology in the pharmaceutical and biomedical fields has not yet been fully explored. As nanotechnology is applied to medicine, there is growing hope that it will improve diagnosis, treatment, and prevention of illnesses. In response to escalating interest in nanotechnology's potential medical applications, a brand-new discipline called nanomedicine has emerged [7,8].

The use of nanotechnology has improved the quality, durability, safety, cleanness, and smartness of goods in the medical, communication, and daily living industries, and has had a profound impact on virtually all areas and industries [9]. Several products used on a daily basis are nanomaterials, such as sunscreen, cosmetics, sporting goods, tyres, and electronics [10].

1.1 Silver Nanoparticles

A major reason AgNPs have gained global attention is their distinct physical, chemical, and biological properties, which set them apart from other inorganic nanoparticles. These AgNPs have become innovative antibacterial agents due to their high surface area to volume ratio and distinctive characteristics. They possess a wide range of qualities that can be utilized in numerous scientific fields [11]. Several applications for AgNPs exist, notably in medicine and pharmacology, due to their low toxicity to human cells, great thermal stability, and low volatility [12]. A variety of applications have been demonstrated for AgNPs, including drug transport, detection and diagnosis, coating biomaterials and equipment, developing new antibacterial agents, and regenerating substances [13].

In recent years, AgNPs have gained increased interest for medical purposes due to their antibacterial properties [14]. Due to their broad-spectrum antibacterial capabilities, silver-based lotions and ointments, as well as biomedical goods, like wound dressings, have become commercially available for a variety of medical purposes [15]. In general, AgNPs are synthesized using physical and chemical methods, but the biological method is more cost-effective because it produces more AgNPs, is more yield-oriented, more solubility-oriented, is easy to apply, rapid, non-toxic, and reliable, and these green strategies are beneficial to produce well-defined sizes and morphologies under optimal conditions [16,17].

Because AgNPs possess distinct physical, chemical, and biological properties that have attracted global attention among other inorganic nanoparticles, they stand out among the others. Due to their high surface area to volume ratio and distinctive characteristics, AgNPs have become innovative antibacterial agents. A biogenic nanoparticle is typically synthesized from the bottom up, involving the cross-linking of atoms and molecules into a single molecule, and then the molecule self-assembles into a final product [18]. To assess the synthesised nanomaterials, numerous analytical methods have been used, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), and others [16,19,20].

Due to its potent antibacterial action, silver is most often used as an adjuvant therapy in wound care because it is an inert element that ionizes in nature when interacting with the surroundings, creating Ag^+ ions that have certain properties. Thus, silver compounds have been used to speed wound healing for centuries [21]. AgNPs in their purest form control the release of anti-inflammatory cytokines to expedite wound healing without causing further scarring. Myofibroblasts can be differentiated from fibroblasts to achieve this. The AgNP promotes epidermal reepithelialization and wound healing by increasing keratinocyte proliferation and relocation [22,23].

1.2 Synthesis of AgNPs

As a result of physical, chemical, and biological techniques used in silver nanoparticle synthesis, different shapes and sizes of AgNPs are formed, which are used in various fields. There are two major types of synthesis strategies: "top-down" and "bottom-up." As part of the top-down approach [24], lithography, laser ablation, mechanical milling, etching, sputtering, and others are used to break down a suitable bulk substance into fine particles (up to nanoscale). The bottom-up approach involves dissolving silver salts in a solvent, reducing silver ions to their component, and stabilizing the neutral AgNPs with stabilizing agents to prevent aggregation. Metal-based NPs are usually synthesized using the chemical reduction technique [25], which is better suited for the synthesis of NPs.

1.3 Physical Synthesis

In the physical synthesis method, particles are sized, and morphologies can be controlled. It uses a top-down approach to synthesize nanoparticles. Many decomposition processes fall under this category [26,27]. Among the most important physical approaches are evaporation-condensation and laser ablation. Another common method of producing AgNPs is laser ablation of metallic bulk materials in solution. Due to the absence of chemical reagents in solution, laser ablation produces uncontaminated metal colloids. This technique can therefore be used in subsequent applications to produce pure and uncontaminated metal colloids. In the past, Condensation/Evaporation has been used to synthesize Au, Ag, PbS, and fullerene nanoparticles in tube furnaces at a specific atmospheric pressure. There are, however, some drawbacks to this method, including the fact that it involves masons, masticating a large amount of energy, and increasing atmospheric temperatures near the source substance, as well as the fact that it takes several minutes for thermal stability to develop [28,29].

Physical synthesis methods have several advantages over chemical processes, including the absence of solvent contamination in thin films and a uniform distribution of nanoparticles. For the preparation of Ag-NPs [30], used laser ablation of silver plates in PVP aqueous solutions and laser irradiation of colloidal solutions. Another method (bottom approach) involves nanosphere lithography (NLS), a straightforward and low-cost technique for fabricating nanoparticle arrays and 2D nanoparticle structures [31].

1.4 Chemical Synthesis

Nanomaterials are highly influenced by their size, shape, and surface morphology when it comes to chemical, physical, optical, mechanical, and electronic properties. A variety of organic reducing agents, such as polyethylene glycol, sodium citrate, N-dimethylformamide, ascorbate, and sodium borohydride, are used as reducing agents to produce AgNPs. To reduce Ag^+, various organic reducing agents, including polyethylene glycol, sodium citrate, ascorbate, and sodium borohydride, are employed as reducing agents. This forms metallic silver, which then aggregates into oligomeric clusters and forms particles [32].

To prevent agglomeration of these dispersive nanoparticles, protective agents, such as capping agents, are critical to stabilizing them during preparation and protecting those that may be absorbed onto or bound to nanoparticle surfaces. To prevent particle sedimentation and agglomeration, surfactants such as thiols, amines, acids, and alcohols can be used. Several polymeric compounds have also been found to stabilize nanoparticles [33], including polyvinyl alcohol, poly vinylpyrrolidone, polyethylene glycol, and polymethacrylate. AgNPs have been synthesized with NaBH4 as a reducing agent. The formation of AgNO3+NaBH4 → Ag0+H2+B2H6+NaNO3 and AgNPs was observed by the formation of yellow colour, and the stability of these nanoparticles was assessed by changing parameters, such as pH, starch, temperature, etc. Starch served as a capping agent. This resulted in spherical AgNPs [34].

1.5 Biological Synthesis

To overcome the shortcomings of chemical methods, biological methods have emerged as viable alternatives. Researchers are increasingly focusing on green and biological bottom-up nanoparticle synthesis methods due to their less toxic effects and feasibility. Furthermore, these processes are also very cost-effective as well as environmentally friendly. In this approach, metal and metal oxide nanoparticles are synthesized using biological processes such as yeast, bacteria, and fungi [35].

The use of microorganisms such as bacteria, fungi, and plants can be regarded as an eco-friendly way of synthesis since they can be used to reduce metal salts and create nanoparticles of desired sizes and morphologies [36]. Microorganisms such as bacteria, yeast, fungi, and actinobacteria can either be classified as intracellular or extracellular synthesis of nanoparticles depending on where the nanoparticles are formed.

A culture medium containing substrates that are specific to the enzymes that act during the synthesis process may encourage fungi to produce and release these enzymes, resulting in a higher level of silver reduction and nanoparticles [37]. In recent years, the use of microbe cells to synthesize nanosized materials has emerged as an effective method for producing metal nanoparticles [38].

This implies that the development of green, cost-effective methods of synthesizing nanoparticles is crucial, as well as the use of natural microorganisms capable of producing nanoparticles, such as bacteria, fungi, and algae, to respond to green approaches. Because particle shape control is still a work in progress during chemical and physical nanoparticle synthesis, further investigation and improvement are needed to evaluate the stability of nanoparticles produced using bio-based methods. Furthermore, microorganism-facilitated production is inherently fragile, necessitating strict aseptic conditions, which are of no importance in the industrial context. Therefore, biological processes that are capable of strict particle shape control will provide significant advantages [38]. Consequently, due to the potential level of biohazard and the more complex procedure of maintaining cell cultures, plant extracts are considered preferable to microbes when it comes to synthesis of metal-based nanoparticles due to their lower biohazard and complexity [39].

Silver salt solution ($AgNO_3$) is chemically reduced with plant extracts to create AgNPs in a green synthesis process. The biological molecules present in plant extracts responsible for reducing silver ions into AgNPs were identified by FTIR analysis. In addition to acting as reducing agents during AgNP synthesis, certain active components in plant extracts also aid in capping and stabilizing nanoparticles [40-42]. Plants contain bioactive molecules such as enzymes, proteins, saponins, terpenoids, flavonoids, phenolic compounds, vitamins, polysaccharides, and organic acids [43]. AgNPs have been synthesized using plant extracts including leaves, flowers, seeds, bark, fruits, and roots [44-46].

As a result of reducing AgNPs with Jasminum officinale leaves extract, Elhawary and co-workers investigated how they can enhance the cytotoxicity of clean, green nanoparticles [47]. Plant-mediated AgNPs synthesis was also reported utilizing aqueous leaf extracts of *Azadirachta indica, Clitoria ternatea,* and *Solanum nigrum* where colorless to yellowish-brown nanoparticles were observed during the synthesis process. A variety of parameters, such as temperature, pH, and time, were used to optimize AgNPs synthesized from *Clitoria ternatea* and *Solanum nigrum* leaf extracts [48,49].

In another study, leaves of *B. globosa* were processed to form an aqueous extract, which was then used as a biomaterial for the generation of nanoparticles [40]. In addition to various bioactive components, Rao and Tang reported that leaf extracts of *E. japonica* were found to be rich in triterpenic acid, flavonoids, polysaccharides, and proteins which could also act as reducing and stabilizing agents in the synthesis process of AgNPs [42]. A faster synthesis reaction was observed when an aqueous extract of dried green *J. regia* leaf husks was mixed with silver nitrate solution, indicating the synthesis of AgNP [50]. In addition to the toxic chemicals used to synthesize and stabilize nanoparticles, by-products of these processes are unfriendly to the environment [49]. Chemical reactions and thermal decomposition of metal nanoparticles can produce toxic waste products and toxic chemicals, so bio-generation of metal nanoparticles using bacteria, fungi, or plant extracts is the preferred method for producing safe, clean, bio-friendly nanoparticles [51,52].

1.6 Plant Mediated AgNPs Synthesis

Many plant parts have been successfully used for efficient nanoparticle biosynthesis, including leaves, roots, calluses, peels, shoots, and so on, and found to be one of the major fields of research to synthesize nanoparticles using plant extracts [53-55]. Several nanoparticles have been synthesized from plants, including silver, gold, and copper oxides. In recent years, several researchers have been discovering that plants and plants-based components can be used to synthesize AgNPs as the process is rapid, eco-friendly, non-pathogenic, cost-effective, and provides a single step procedure for synthesizing AgNPs [56-57]. As compared to other biological processes, using plants to synthesize nanoparticles may have an advantage because the culture process is not as complex. It is through these bioactive compounds in plants that metal ions can be converted into biologically active nanoparticles following the standard biosynthesis pathway in an environmentally benign manner [58].

In most of the methods, plant extracts are mixed with a solution of metal salt at room temperature and then the reaction is conducted for a few minutes, with a change in the colour of the solution indicating the completion of the reaction. It involves bottom-up bioreduction, which produces nanoparticles from plant extracts. In plant material extract, silver ions interact electrostatically with proteins to trap Ag+ ions on the protein surface. By further reducing Ag+ ions, silver nuclei are formed, and AgNPs are formed at the nuclei [59]. The phytochemicals present in plant extracts play a key role in nanoparticle synthesis. A reducing agent can also serve as a capping and stabilizing agent, eliminating the need for an external agent [60]. A variety of factors must be taken into consideration for efficient synthesis, including the nature of the plant extract, its concentration, the concentration of the metal salt, the pH, temperature, and contact time [61] Over 45 traditionally used medicinal plants have been used to synthesize AgNPs, which are critically examined. To synthesize AgNPs in aqueous leaf extract that can effectively suppress bacterial growth, two commonly used Indonesian medicinal plants were used. Methanol extracts were used to synthesize AgNPs as biological reducing agents from *Senna alata* and *Senna hirsute* leaves [62]

Mohanta and co-workers synthesized biocompatible AgNPs using leaf extracts from *Senmecarpus anacardium, Glochidion lanceolarium*, *Bridelia retusa* and determine their sustainable biocompatibility [63] Leaf and petal extracts of various Ethiopian medicinal plants such as, *Moringa oleifera, Cucurbita maxima, Acorus calamus, Taraxacum officinale* and *Withania coagulans* were used to synthesize low-sized AgNPs via reduction, stabilization, and capping agent [56].

There have been numerous studies investigating the antioxidant activity of AgNPs synthesized from medicinal plants. A variety of plants, such as *Allium sativum, Abutilon indicum, Cassia occidentalis, Capsicum frutescens,* and *Zingiber officinale*, were used for the nanoparticles synthesis and found to be extremely beneficial [64]. Antibacterial activities of these water-based extracts were compared to those of conventional antibiotics. Aritonang et al. reported AgNPs using aqueous leaf extracts of medicinal plants, *Impatiens balsamina* and *Lantana camara,* and reported its antimicrobial efficiency [65].

Aqueous extracts of the whole plant from *Plumbago zeylanica* demonstrated the potential formation of AgNPs after 24 hours of incubation with silver nitrate solution [58]. Three plant extracts with potential roles in the synthesis and stabilization of AgNPs were analyzed using attenuated total reflective Fourier transform infrared to classify the phytoconstituents. The formation of AgNPs was observed microscopically and spectroscopically, while the phytoconstituents that likely played a role in synthesis were characterized qualitatively using the medicinally important plants such as *G. lanceolarium, B. retusa* and *S. anacardium* [66]. Table 1 displays the green synthesis of AgNPs from various plant parts [67-86].

Table 1. Green synthesis of AgNPs from various plant parts

Plant species	Size (nm)	Part of plant	Shape	References
Alstonia scholaris	50 nm	bark	Spherical	71
Ananas comosus	12 nm	leaf	Spherical	84
Annona muriciata	20-53 nm	Leaf	Spherical	80
Calotropis procera	19–45 nm	Plant	Spherical	76
Cocous nucifera	22 nm	flower	Spherical	85
Desmodium triflorum	10 nm	leaf	Spherical	74
Ficus carica	13 nm	leaves	-	86
Melia dubia	35 nm	Leaves	Spherical	72
Moria oleifera	11 nm	Leaf	Rectangle	78
Musa balbisiana	50 nm	Leaf	Spherical	67
Parthenium	20-50 nm	leaf	Spherical	68
Piper longum	46 nm	fruit	Spherical	73
Plukenetia volubilis	4-25 nm	leaf	Optical	75
Prosopis farcta	10.8 nm	Leaf	Spherical	69
Prunus yedoensis	20-70 nm	Leaf	Circular	83
Saraca indica	23 nm	Leaf	Spherical	81
Skimmia laureola	46 nm	Leaf	Hexagonal	77
Swietenia mahogany	50 nm	Leaves	-	82
Vitis vinifera	30–40 nm	Fruit	-	79
Ziziphus Jujuba	20-30 nm	leaf	Crystalline	70

1.7 Optimization

The size, shape, and morphological characteristics of nanoparticles are determined by chemical and physical factors that influence the synthesis of AgNPs.

1.8 Effect of pH on AgNPs Synthesis

pH is an important factor that affects nanoparticle synthesis by influencing the size and shape of the particles formed. As a result, these morphological changes can be controlled by adjusting the pH and demonstrating efficient activity and their stability can be influenced under the presence of acidic or basic conditions, optimized the synthesis of AgNPs from *Ziziphus Jujuba* leaf extract and it was observed that the ideal condition for AgNPs formation is neutral medium even though basic medium shows good results [87,70]. The maximum color intensity of AgNPs was observed at pH 9, and the AgNPs obtained were found to be monodispersed [88]. The optimal pH value for measuring the complete reduction of Ag^+ to Ag^0 during the synthesis of AgNPs using *Pinus eldarica* bark extract has been reported to be 7 [89]. AgNPs created from *Aegle marmelos* at a concentration of 6 mM, with the largest absorbance peak occurring between 400 and 420 nm. The pH of 7.0 was found to an optimal for maximum nanoparticle synthesis and minimal for acidic and alkaline conditions [90]. Furthermore, the plant *Tridax procumbent* synthesized AgNPs showed maximum absorption and a narrow peak at pH 9 with uniform size distribution, implying that the basic pH is favorable for AgNP synthesis [91]. The pH effects on silver nanoparticle synthesis were examined and found that alkaline pH produced the best dispersed small particles compared to acidic pH [92]. The stability of AgNPs was observed under different pH (2-8) conditions, with the particle size obtained at pH 8 being small and spherical in shape, implying that this also improves the reducing and stabilizing capability of the antioxidants in the given extract [93]. AgNPs derived from *Matoa* leaves were optimized at various pH levels, and it was discovered that out of pH 4, pH 5, and pH 11. The pH 11 was most effective, showing the presence of dark brown particles and a high absorption value; the particles were spherical and hexagonal in shape and ranged in size from 10 to 50nm [94]. Researchers found that the rate at which AgNPs are synthesized increases with increasing pH until pH 9 before decreasing [95].

1.9 Effect of Temperature on AgNPs Synthesis

The temperature is one of the most significant parameters that affect the formation of AgNPs during the synthesis process. The presence of a high temperature promotes the formation of nanoparticles that are spherical in shape. In contrast, the majority of nanotriangle production happens at lower temperatures. AgNPs can be synthesized at a variety of temperatures, although 70°C is optimal for producing small, uniformly distributed AgNPs [96]. At room temperature, the highest yield of biosynthesized AgNPs was discovered utilizing aqueous *Aloe barbadensis* leaf extract [97]. A temperature enhancement of 30°C to 60°C led to an increase in AgNPs synthesis using Mentha longifolia, whereas a temperature enhancement of 120°C led to a decrease in AgNPs synthesis. As a result, 60°C was found to be an ideal temperature for the process [98] AgNPs were tested at three different temperatures viz, 25°, 35°, and 45° Celsius. As the temperature rises, more AgNPs form, which results in the production of smaller particles [88] AgNPs was also investigated between 10°C and 100°C, the best yield was recorded at 40°C, with 100°C being the highest. Furthermore, even at a low temperature of 40°C, it was discovered that *Vitex agnus-castus* leaf extract may result in a rapid reduction of Ag^+ ions. In contrast, another study found that the efficient generation of AgNPs occurred when the temperature was between 60°C and 80°C [99]. The highest yield of biosynthesized AgNPs was obtained at 40˚C using *Hagenia abyssinica* plant leaf extract, which also demonstrated a high absorption spectrum at 402nm [100]. The synthesis of AgNPs using *Annona squamosa* peel extract was recorded at 25°C and 60°C. The optimal temperature for the formation of AgNPs is found to be 60°C due to higher absorption intensity [101]. There has been evidence that the synthesis of AgNPs can be carried out at 100°C with optimal results for the production of small-sized particles that are around 21nm in size [102] The formation of AgNPs increased as the temperature increased from 25°C to 80°C, as did the intensity of the SPR peak, and the particles obtained were more uniform and smaller in size at higher temperatures [42]. Temperature effects on *Cordia dichotoma-mediated* AgNPs were investigated by keeping $AgNO_3$ and extract concentration constantly at temperatures ranging from 4°C, 25°C, 40°C, 60°C, and 80°C. The highest levels of AgNPs formation were observed at 60°C and 80°C, while the lowest level of AgNPs formation was witnessed at 25°C [103].

1.10 Effect of $AgNO_3$ Concentration on AgNPs synthesis

Nanostructures are greatly influenced by their size and shape in addition to reaction kinetics, which is greatly affected by the concentration of silver salt in which they are formed. The synthesis of AgNPs increased from 1 mM to 3 mM, but additional increase in silver nitrate salt concentration caused the synthesis rate to decline, making 3mM the ideal concentration [98] The concentration of $AgNO_3$ was optimised from 0.1 mM to 2 mM. The scan data showed that the optimum $AgNO_3$ concentration was predicted to be 1mM because anything greater than this caused the reaction to become saturated [104]. According to Mittal and coworkers, the production of AgNPs rose as the metal salt concentration was raised from 0.5 to 4mM, further the concentration, absorbance found decreased, thus 4mM concentration found to be optimal [105]. The synthesis reaction was observed at several silver nitrate concentrations (0.25, 0.5, 0.75, 1, 2, and 5 mM), and the greatest yield was found at 1mM silver nitrate solution which considered optimal [106]. The concentration of salt required to carry out the reaction at optimum conditions is 0.5 mmol**,** which resulted in an increase in particle size as the concentration increased [107].

Various concentrations of silver nitrate ranging from 0.25 mM to 1.0 mM were investigated for effective AgNP synthesis, with 1.0 mM showing optimal results with the extract concentrations [108]. The effect of different silver nitrate concentrations on AgNPs was observed at 0.5, 1.0, 2.0, and 4.0 mM concentrations, and it was clear that the AgNPs were synthesized when the concentration was increased, as the SPR bands formed were more distinct, narrow, and stated uniform size distribution with spherical shape [42]. Among a range of silver nitrate concentrations evaluated, a concentration of 6 mM produced the greatest particle formation with a peak absorbance in the range of 400-420 nm [90].

1.11 Effect of Incubation Time on AgNPs Synthesis

The length of incubation time influences the quality and type of nanoparticles synthesised, which affects particle characteristics [74]. Long-term storage may cause particle aggregation in addition to particle shrinkage and growth [109]. The reaction mixture was incubated for 15 minutes to 24 hours, and the absorption spectra ranged from 390-440 nm. The peak after 15 mins of incubation indicated formation of polydisperse large particles. After 24 hours of incubation, a strong SPR peak at 418 nm was obtained, indicating that nanoparticle formation was enhanced [110]. Different time intervals were used to optimise the reaction process's time (10, 20, 40, 60, 80 and 100 min). It was observed that the solution had turned brown after 40 minutes, indicating the synthesis of AgNPs. With increasing time, nanoparticles were found to be synthesized more efficiently. As a result, the reaction was carried out for 100 minutes, and the AgNPs showed a characteristic peak at 430 nm [103]. There was a rapid formation of AgNPs within 5 minutes of the incubation period, and the intensity of the SPR peak gradually increased as the reaction time increased over time as the AgNPs formed. After 60 minutes, there was no increase in absorbance, indicating that formation had ceased. As a result, 60 minutes was the ideal time for the reaction to be completed [42]. The time-dependent formation of AgNPs began within 5 minutes and was completed in 1 hour, after which particle instability was observed due to precipitation. As a result, the longer the duration, the greater the synthesis of nanoparticles [111]. The effect of incubation time on AgNPs was observed at 120, 180 and 240 minute intervals. Within 10 minutes, the brown colour was obtained, and the colour intensity increased as the time passed [91].

While the other factors were kept at their optimal levels, AgNPs were evaluated for 0, 5, 10, 15, 20, 25 and 30 minutes, and dark brown colour became apparent within 20 minutes, indicating instant formation of AgNPs [112] noted a significant change in colour after 25 and 30 minutes compared to the colour after 20 minutes. As a result of the plant biomolecules present in *Shorea robusta* leaf extract, silver ions are reduced within 20 minutes of the application of the extract [113].

1.12 Antimicrobial Activity of AgNPs

AgNPs are utilised extensively, and their success depends on several physical parameters, including their size and form. Additionally, different silver nanoparticle concentrations have varying degrees of efficacy. AgNPs derived from plants are effective against a range of bacteria, fungus, and plant diseases. This paves the way for several applications of nanoparticles, particularly silver, in various fields [114]. It has been demonstrated that AgNPs have proved to be effective as antibacterial agents by overcoming bacterial resistance to several medicines. AgNPs are the most potent nanoparticles because of their high surface-to-volume ratios and crystalline structure. AgNPs were synthesized from *Carya illinoinensis* leaf extract and tested against four different organisms at varying concentrations using the agar well diffusion method. Compared to gram positive organisms like *S. aureus* and *L. monocytogenes*, AgNPs were significantly more effective against *E. coli* and *P. aeruginosa* [115] Researchers have investigated the antibacterial properties AGNPS synthesized using bark extracts of *Syzygium cumini against B. licheniformis, S. aureus, P. aeruginosa, E. coli, A. chroococcum* [116]. Activity was found to be greater against all the aforementioned organisms, but *Bacillus licheniformis* exhibited the greatest inhibition at low concentrations of AgNPs. *C. murale* leaf extract derived AgNPs have demonstrated the strongest antibacterial activity against *S. aureus* when compared to its leaf extract and silver nitrate, according to a different way of examining the impact of AgNPs on microorganisms known as the cup plate method [117].

When Ipomea carnea latex extract was applied at concentrations of 8 g/mL, 16 g/mL, and 24 g/mL, AgNPs showed strong antimicrobial activity against S aureus, with zones of inhibition of 13.33, 16.83, and 20.66, respectively, and followed by *S. epidermidis* and *E. coli.* There was no difference between streptomycin and AgNPs in terms of the zones of inhibition when they were compared [118].

Researchers have investigated the antimicrobial activity of AgNps using disc diffusion method against a variety of human pathogenic strains, including *K. pneumoniae, P. putida, E. coli B. subtilis*, *S. aureus* and *M. luteus.* The highest inhibition activity of AgNPs can be found at a concentration of 10 µg/ml against *E. coli* and *S. aureus.* µg/ml. With MIC and MBC values of 25 microgram/mL, the extract demonstrated good antimicrobial activity against, *P. putida* and *M.* luteus [119].

AgNPs synthesized from *Eucalyptus chapmaniana* leaf extract were evaluated for their antimicrobial activity against *K. pneumoniae, P. aeruginosa, S. aureus*, *E. coli, P. volgaris,* and *C. albicans*. The results showed a much greater antimicrobial activity against all the tested human pathogens. In addition to being dose-dependent, the antimicrobial effect was stronger against gram-positive bacteria than it was against gram-negative bacteria. Additionally, AgNPs found inhibit the growth of *C. albicans* effectively [120].

A study was conducted to evaluate the antibacterial potential of AgNPs that were derived from the medicinal plant *Boerhaavia* *diffusa* against pathogenic bacteria isolated from fish, namely *F. branchiophilum P. fluorescens* and *A. hydrophila*.

It was determined that the MIC value for a tested pathogen ranges from 3.12 µg/ml to 3.12 50 µg/ml, where greater activity was found against *F. branchiophilum* followed by *A. hydrophilla* and *P. fluorescence.*

The AgNPs exhibited maximum activity when treated against *F. branchiophylum* with a zone of inhibition of 15mm, followed by *A. hydrophilla* showing 14 mm and P. fluorescence recorded with 12 mm, however but no zone of inhibition was shown when treated against *Boerhaavia diffusa* leaf extract [121].

Saxena et al. tested the antibacterial activity of a new formulation of AgNPs synthesised from *Ficus benghalensis* leaf extract by using the broth microdilution method against Escherichia coli [122]. Another study conducted by Sankar and their co-workers confirmed that AgNPs derived from leaves of O. vulgare exhibited broad-spectrum antimicrobial activities against nine human pathogens [123].

Prakash et al. also investigated the role of *Mimusops elengi* leaves extracts in the synthesis of AgNPs through the process of biosynthesis. The synthesized AgNPs were found to be in the size ranging from 55 to 80 nm and they exhibit significant antibacterial properties against *K. pneumonia* [124].

Researchers have also investigated that ability of leaf extracts from various plants, as *S. tricobatum*, *O. tenuiflorum*, *C. asiatica* and *S. cumini* for the biological synthesis of AgNPs and also analyzed for their antimicrobial activity against, *B. subtilis*, *K. planticola* and other pathogenic bacterial species [125]. AgNPs antibacterial mechanism is not yet fully understood. However, there are several theories that could account for AgNPs' antibacterial properties. Possible explanations include the production of ROS because of the inactivation of respiratory enzymes, which in turn prompts the breakdown of the cell membrane and promotes the interaction of silver ions with proteins and DNA, which can stop important biological processes in the cell. The AgNPs also could continuously release silver ions. Ag^+ has the ability to kill bacteria by penetrating their cell walls. Silver cations released from AgNPs demonstrate potent antibacterial activity because of their positive charge and simplicity of interaction with the biomolecules on the S, P, and N-containing bacterial cell wall. This causes disruption which results in significant harm and toxicity. Additionally, the contact with negatively charged membranes prevents microbial development.

Urnukhsaikhan et al. claimed that AgNPs produced by Carduus crispus showed efficient inhibition of both Gram-positive and Gram-negative bacteria, suggesting that bacterial cell walls do not affect antibacterial activity [126].

Enerelt et al. claim that *Carduus crispus*-produced AgNPs showed efficient inhibition on both gram-positive and gram-negative bacteria, indicating that the antibacterial activity may not be affected by changes in bacterial cell walls. Although gram-negative bacteria are more susceptible to AgNPs due to thinner cell walls than gram positive bacteria [119,127-129].

2. CONCLUSION

To summarise, green methods of synthesis have supplanted the previously employed synthesis procedures for AgNP synthesis because they solve the drawbacks by being a non-toxic, affordable, and environmentally friendly natural alternative. Different plant parts and extracts are utilised to create silver nanoparticles, and they contain important phytochemicals which have proved effective in creating a range of AgNP sizes and shapes. These AgNPs are also stable since the plant's constituents act as stabilising and capping agents. Additionally, it has demonstrated exceptional antimicrobial qualities, which makes it a prime candidate for investigation in biomedical sciences. Although the actual mechanism by which AgNP induces its activity is unknown, it shows encouraging results against harmful microorganisms, expanding the range of their potential applications. To reap the benefits of these properties, the activity of AgNPs must be tailored by optimising their size, shape, and synthesis process, which also influences its antimicrobial activity. As a result, in various nanotechnology related aspects, silver nanoparticles are expected to play a significant role.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENT

The authors thank the Management and the Principal, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai for providing the necessary support to carry out this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3598