Review:

A Review on Green Synthesis, Antimicrobial Applications and Toxicity of Silver Nanoparticles Mediated by Plant Extract

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Abstract: *Nanotechnology explores nanoscale materials that can be used in a wide range of industries such as biotechnology, cosmetics, drug delivery, nanomedicine, and biosensors. Nanoparticles in diverse shapes and sizes can be prepared through physical, chemical, and biological methods. The employment of reducing agents, which will change their form, size range, level of stability, and interaction, is a crucial part thus employing a biological approach is necessary. Chemically generated metal oxide nanoparticles raise considerable issues owing to the usage of hazardous and poisonous chemicals, as well as the potential for conservational impairment. In contrast, the production of silver nanoparticles using the principal method of green synthesis has found a special place in research that is considered more environmentally approachable requiring the use to produce non-toxic nanomaterials. Plants and polymer materials have received a lot of interest in the preparation of nanoparticles since they are renewable and affordable. In this review, we present a comprehensive overview of more ecologically friendly synthesis techniques that use plant extracts to make silver nanoparticles and their application as antibacterial agents, as well as toxicity features based on the shape, size range, and phytochemical mechanism of plants.*

Keywords: *silver nanoparticles; green synthesis; biological method; size range; phytochemical mechanism*

INTRODUCTION

Nanotechnology is one of the disciplines that scientists are interested in today. Nanotechnology has been developed rapidly in the early twenty-first century, owing to the various applicable benefits in several fields of innovative products through nanotechnology techniques [1]. Nanotechnology is expected to replace processing technology and the significance of production that have a major impact on global environmental damage. Furthermore, this technology also reduces the length of the process to get useful results

[2]. It is estimated that green chemistry can deliver economically valuable results saving up to USD 65.5 billion by the end of 2020 [3]. The economic cycle (emissions and waste control, goods maintenance and resources used, and natural resources recovery) is always required to be able to produce balanced economic development, management and resources sustainability, and environmental conservation [4]. Nanotechnology is a technique for developing, producing, and using atomic and molecular materials with 1–100 nanometers in size [5]. One of the nanotechnologies that is used in everyday life is nanomaterial. Nanomaterials production through nanoparticle technology has currently been applied to various fields such as biomedicine [6], catalysts [7], biodiesel [8], military devices [9], cosmetics [10], food [11-13], packaging industry [14], agriculture [15-16], drug delivery systems [17], textile industry [18-19], renewable energy [8], and others.

One form of nanomaterial that is still being developed today is nanoparticles. Nanoparticles can be obtained through the synthesis process using organic or inorganic based materials. Carbon nanoparticles are an example of organic nanoparticles, while inorganic nanoparticles such as metal nanoparticles (gold, silver, copper, and aluminum) and semi-conductor nanoparticles (ZnO, ZnS, and CdS) [20-21]. However, their synthesis process still mostly uses chemicals that have a toxic impact on the environment and the possibility of side reactions produced. Challenge for developing countries that are rich in biodiversity is to utilize their bioresource for production processes and methods, which must be completed in line with long-term environmental stability by using resources found in nature [22]. As a result, several techniques have been used in this subject with modern green process engineering (MGPE), such as the manufacture of nanoparticles from a biological method in synthesis pathways [23]. The next challenge of nanoparticles themselves is how they are made. The precursors from natural materials have been developed using a variety of analytical techniques such as for the synthesis and design of nanoparticles of various size ranges, shapes and functions as expected [24-28]. The purpose of using precursors from natural materials is to be more environmentally friendly and reduce the toxic impact of the resulting side reactions [29]. The use of natural materials as a precursor for synthesizing nanoparticles such as fungi, algae, and bacteria has been reported and becomes an alternative because it has several advantages in its application such as lower energy use and processes. Technology that is both clean and safe without harmful chemicals use is critical to be considered [26,30-32]. In metal nanoparticles biosynthesis using plants, plant extracts containing metabolite compounds such as phenols, alkaloids, proteins, and saccharides are able to mediate the synthesis process and stabilize the size range of the nanoparticles formed [33]. One of the inorganic metal nanoparticles that are widely used is silver nanoparticles (AgNPs). The AgNPs application increase from day to day, this is due to the antibacterial properties possessed by AgNPs themselves [34]. Nevertheless, there have been very few publications about the toxicity properties of AgNPs to humans and their impact on health, especially the interaction of AgNPs against human biological systems [35]. Consequently, there are still many questions that remain a mystery about the utilization of AgNPs such as, which form of silver contributes more to giving toxicity properties, as AgNPs or in the form of silver ions. Therefore, in this review, the complete aspects of biosynthesis and the mechanisms of AgNPs derived from natural precursors such as plants, algae, and microbes along with their capability to act as antimicrobial agents and their mechanism of action are discussed.

■ SILVER NANOPARTICLES SYNTHESIS

The AgNPs can be obtained through physical, chemical, and biological synthesis processes. Two approaches are often used in the AgNPs preparation. The first approach is bottom-up and the second approach is top-down as shown in Fig. 1 [34,36]. Some examples of AgNPs synthesis by using chemical methods are sol-gel [37-38], hydrothermal [39-40], coprecipitation [40], microemulsions [41-42], and chemical reduction techniques [43-44]. Sol-gel synthesis is one of the most used approaches for producing AgNPs,

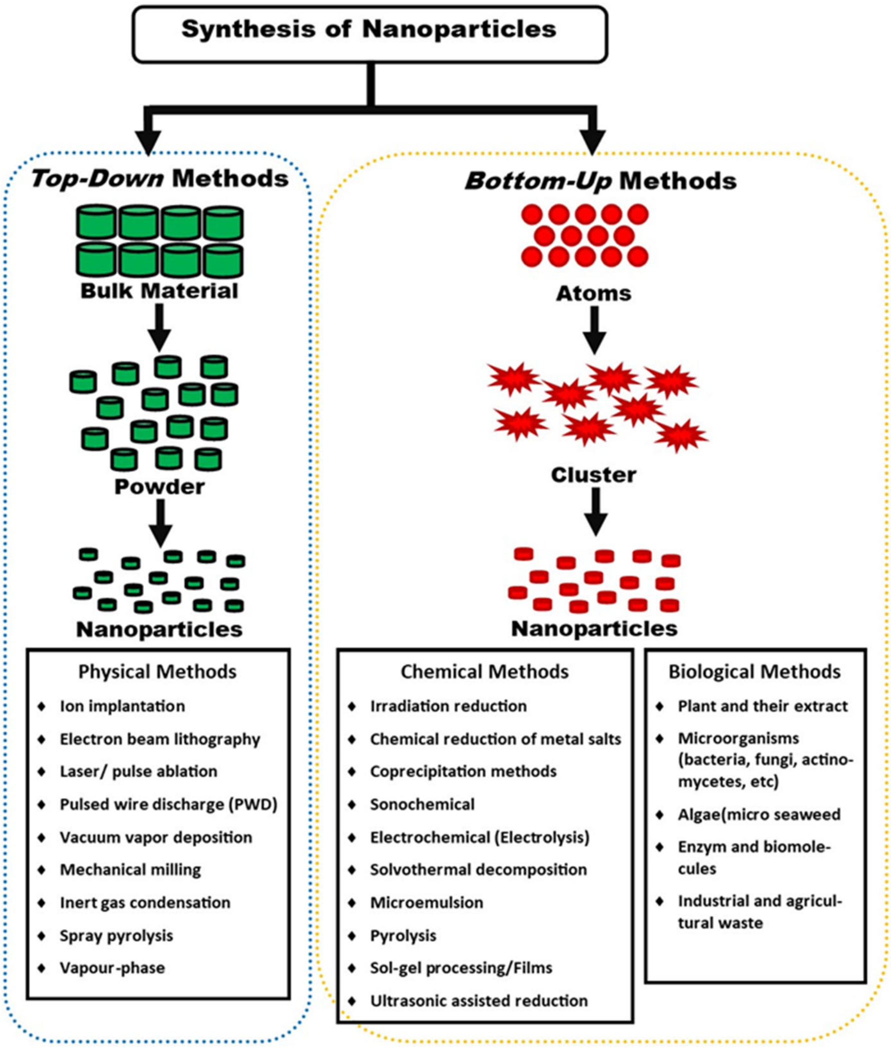

Fig 1. Various methods of approach in synthesizing nanoparticles

among the other chemical synthesis processes, because this method yields more product, has a simple procedure, and uses moderate temperatures [45]. Mostly, this method uses metal precursors for instance chloride [46], nitrate [47], acetylacetonates [48], acetate [49], sulfate [50], and oxalate [51] while chemical reducing agents such as citrate and polymer [52] which is used to avoid the formation of hydroxides and preserve the pH of the solution. Then, the residual solution can be heated up to 1000 °C to obtain the desired form of oxide nanoparticles. Silver metal can also be arranged by using physical methods to make it as nanoparticle size such as the vapor deposition method [53], plasma irradiation [54], and ultrasonic irradiation [55]. In general, this technique necessitates high energy and robust equipment to obtain AgNPs. Because it provides a simple, affordable, and environmentally friendly treatment method, biological synthesis (green synthesis) is the acceptable replacement for the above-mentioned procedures.

Nanoparticles come in a variety of forms, including spherical, cubic, needle, triangular, rod, fiber, and random shapes, allowing them to be used in a variety of industries, including device manufacture, electronics, optics, and biofuels [56]. The experimental conditions, the kinetics of interaction of metal ions with reducing agents and the adsorption processes of stabilizing agents with metal nanoparticles have all been shown to have a strong influence on the morphology, size, and stability of metal nanoparticles. The forms of nanoparticles can be seen in Fig. 2.

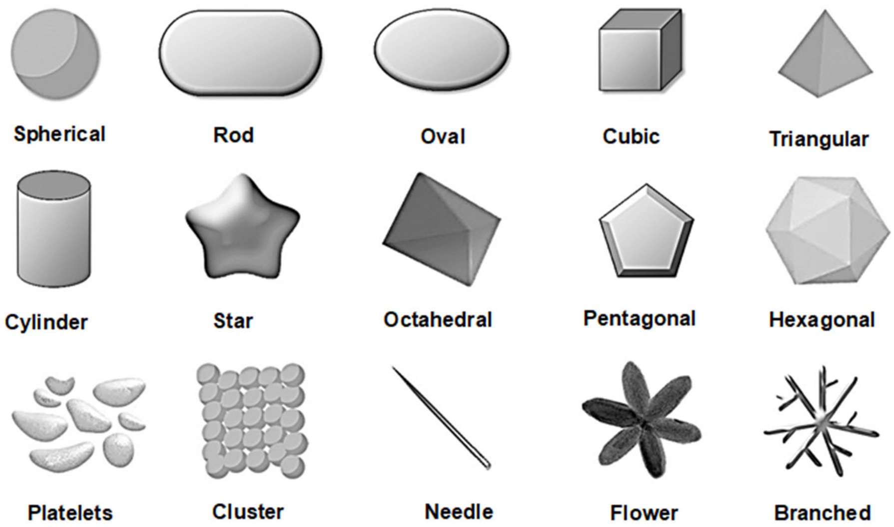

Fig 2. Variations of nanoparticles shape

■ GREEN SYNTHESIS (BIOLOGICAL METHOD)

In recent years, various techniques have been developed to synthesize AgNPs. These approaches are divided into three categories: physical, biological, and chemical processes [57]. However, among the three procedures, biological procedures offer many advantages because the processes are not complicated, harmless, and cost-effective [58]. Reducing and capping agents play an important role in AgNPs synthesis. Chemicals used in AgNPs synthesis procedures are physically and chemically considered hazardous and have high toxic levels, this is considered as the part responsible for the occurrence of environmental pollution problems. Biochemicals and microbes used in biological processes are considered not only is it safe for the environment, but it is also safe for untargeted critters. As a result, the biological process is the most suited and suggested method for AgNP synthesis [59]. There is currently an urgent need to develop sustainable procedures and methods for nanoparticles, as AgNP is needed for use in areas directly related to human activities [60]. We can establish a safe strategy for employing AgNP synthesis by improving our understanding of green synthesis and sustainable technology. The following five approaches can be roughly classified.

Polysaccharide Method

The AgNPs synthesis method using polysaccharides (cellulose) *in situ* was obtained from the extraction process of black ear fungus. Cellulose in the form of triple-helical cotton extracted was used as a reducing agent and stabilizer for AgNPs [61].

Tollens' Method

Tollens' reactions have the advantage of being easy to do in one stage of biosynthetic (one-pot synthesis). In this method, Tollens' reagents and reducing saccharides have an important role in the Ag^{+} ion reduction process, resulting in AgNPs with a high degree of the size range and shape of AgNPs. The size of the smallest AgNPs produced ranged from 5 to 8 nm when triazole sugars were used [62].

Irradiation Methods

The gamma irradiation method is quite suitable for synthesizing small metal NPs. This method can be carried out in the absence of a reducing agent at room temperature. Gamma irradiation uses a templating model to constrain the reaction loci to "virtual nanoreactors" or "nano molds" for control of particle size and shape or a template-free model [63].

Polyol Method

This method uses latex copolymer material in the liquid emulsion as a silver nitrate-reducing agent which is further added ethylene glycol as a stabilizer component of AgNPs formed [64].

Biological Methods

Various studies have shown in recent years that the biological synthesis of nanoparticles has great potential to be used as an environmentally friendly method that avoids the use of harmful substances and heavy metals and does not require a large amount of energy during the process compared to other chemical and physical methods. Stages of biological methods include the synthesis of nanoparticles by utilizing organisms such as plants and other microorganisms (bacteria, yeast, and fungi). The theory of biological synthesis that underpins many organisms is increasingly being developed to tolerate conditions of metal concentration. These microorganisms have the ability to convert more toxic chemicals and substances into less toxic components that are hazardous or even alter them into complex, non-toxic compounds [60]. The development of nanoparticles is the formation of "consequences" of the procedure of an organism into a specific metal at high concentrations. Metal nanoparticles biosynthesis may be split into two types "naturally": bioreduction and biosorption. Bioreduction reactions utilize biological raw materials to produce powerful metal ion models, which may be created by reducing various metals. With the aid of particular enzymes, oxidized metal ions will be decreased [65]. (b) Biosorption describes the ability of metal ions from water or soil samples that have been bonded to the organism bacteria and fungi to produce metal ions binding peptides or cell wall chains, which then form strong nanoparticle structures [66]. In the process of synthesizing nanoparticles using the green synthesis method, it has several advantages such as the use of non-toxic chemicals, the product of the synthesis has increased significantly, energy efficiency, the production process is cheaper, has good economic value, and the waste generated will be relatively low, so this process is more friendly to the environment. As a result, it can have a positive impact on human health and there will be less risk when the biosynthesis process is carried out. The biological synthesis scheme can be seen in Fig. 3.

■ GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PLANTS

This review is based on research articles and reviews taken from the reputation journal database using the word "biogenic plant nanoparticles, antibacterial" as keywords in the title search menu, abstracts, and keywords, over the past 5 years, from 2017 to 2021 (Searched on September 20, 2021) shown in Table 1.

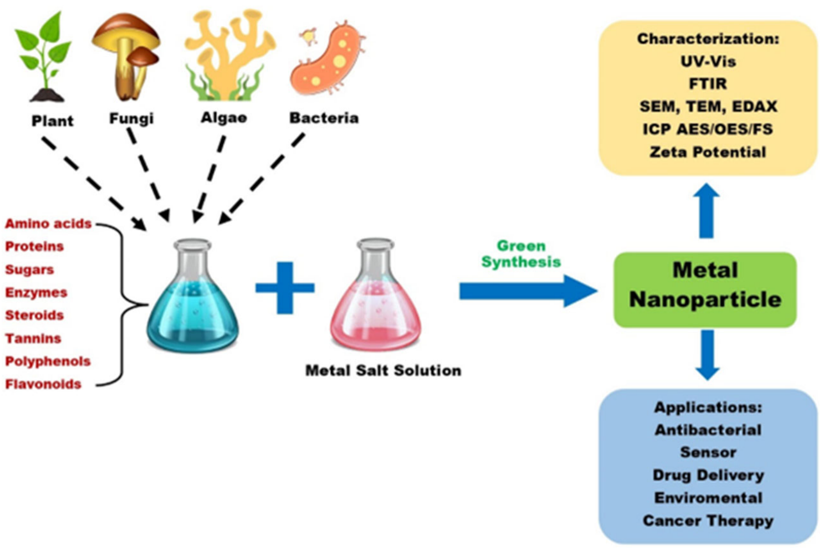

Fig 3. The biological synthesis scheme of metal nanoparticles

Table 1. Silver nanoparticles synthesis using plants and their applications as antibacterial

Plant Name	Size and morphology	Pathogen	Method	Conclusion	Ref.
Humulus lupulus	17 nm; spherical	*E. coli* and *S. aureus*	Agar well diffusion	Inhibition zone of bacterial growth at MIC = 201.88 and 213.19 μg/mL	[67]
Aquilegia pubiflora	19 nm; spherical	*E. coli*, *B. subtilis*, *K. pneumoniae*, *S. epidermidis*, *P. aeruginosa*, *A. fumigatus*, *A. flavus*, *M. racemosus*, *F. solani* and *A. niger*	Agar well diffusion	Inhibition zone of bacterial growth at 11 mm for *E. coli*, 10 mm for *B. subtilis*, 10 mm for *K. pneumonia*, 9 mm for *S. epidermidis*, 9 mm for *P. aeruginosa.* While in fungi 10 mm for *A. fumegatus*, 10 mm for *A. flavus*, 12 mm for *M. racemosus*, 10 mm for *F. solani*, and 13 mm for *A. niger*	[68]
Wynaadensis	14 and 25 nm; agglomerate	*M. luteus*, *E. coli*, *B. cereus* and *Salmonella* sp.	Disk diffusion	15 and 14 mm inhibition zone of bacterial growth at MIC = 800 μg/mL	[69]
Annona muricata	35 nm; spherical	*S. aureus*, *S. marcescens* and *P. aeruginosa*	Disk diffusion	Inhibiting at concentration of 100 μg/mL by 61.29% for *S. aureus*, 59.34% for *S. marcescens*, 54.42% for *P. aeruginosa*	[70]
Capparis zeylanica	23 nm; spherical	*S. epidermis*, *E. faecalis*, *S. parathyphi*, *S. dysenteriae*, *C. albicans*, and *A. niger*	Agar well diffusion	Inhibition zone of bacterial growth for *S. epidermis* (30 mm), for *E. faecalis* (26 mm), for *S. parathyphi* (23 mm), for *S. dysenteriae* (20 mm), for *C. albicans* (25 mm), and for *A. niger* (23 mm)	[71]
Allium sativum	10–50 nm; spherical	*P. aeruginosa*	Disk diffusion	MIC = 100 μg/mL, *P. aeruginosa* of 19.2 mm	[72]
Holoptelea integrifolia	32-38 nm; spherical	*E. coli* and *S. typhimurium*	Disk diffusion	MIC = 200 μg/mL with Inhibition zone of bacterial growth at 10 mm (*E. coli)* and MIC = 150 μg/mL, Inhibition zone of bacterial growth at 13 mm (*S. typhimurium*)	[73]
Calophyllum tomentosum	24 nm; spherical	*E. coli*, *P. aeruginosa*, *S. aureus,* and *K. aerogenes*	Disk diffusion	100 g/mL concentrations with a bacterial growth inhibition zone of 8 mm for *P. aeruginosa*, 7 mm for *E. coli*, 16 mm for *S. aureus*, 16 mm for *K. aerogenes*	[74]
Acacia cyanophylla	88 nm; spherical	*E. coli*	Microdilution broth	Against *E. coli* bacteria at MIC = 3.12–12.5 μg/mL	[75]
Conocarpus lancifolius	21 nm; spherical	*S. pneumonia*, *S. aureus*	Agar well diffusion	*S. pneumonia* (18 mm), *S. aureus* (24 mm)	[76]
Elephantopus scaber	37 nm; spherical	*B. subtilis*, *L. lactis*, *P. azurescens*, *P. aeruginosa*, *A. flavus*, and *A. penicillioides*	Agar well diffusion	Inhibition zone of bacterial and fungi growth at 16–24 mm, 11–12 mm	[77]
Green and black tea	10–20 nm; spherical	Methicillin- and vancomycin-resistant *S. aureus*	Disk diffusion and Broth dilutions	Inhibition zone of bacterial growth at 19–21 mm, at MIC = 8 μg/mL	[78]

Table 1. Silver nanoparticles synthesis using plants and their applications as antibacterial (*Continued*)

Plant Name	Size and morphology	Pathogen	Method	Conclusion	Ref.
Phyllanthus amarus	30–42 nm; Like a flower	*E. coli*, *Staphylococcus spp.*, *Bacillus spp.*, *Pseudomonas spp.*, *A. niger*, *A. flavus*, and *Penicillium spp.*	Disk diffusion	Inhibition zone of bacterial growth at 6–11 mm and 5–8 mm	[79]
Salvia leriifolia	27 nm; spherical	*P. aeruginosa*, *E. coli*, *C. freundii*, *E. aerogenes*, *A. baumannii*, *S. marcescens*, *K. pneumoniae*, and *S. pneumoniae*	Disk diffusion	Inhibited at concentrations of 67.9% (*P. aeruginosa*), 76.5% (*E. coli*), 84.7% (*E. aerogenes*), 101.4% (*A. baumannii*), 25.3% (*S. marcescens*), 191.8% (*K. pneumoniae*), and 141% (*S. pneumoniae*)	[80]
Psidium guajava	20–35 nm; spherical	*B. aryabhattai*, *B. megaterium*, *B. subtilis*, *A. creatinolyticus*, *E. coli*, *A. faecalis*, *S. cerevisiae*, *A. niger*, and *R. oryzae*	Agar well diffusion	At a concentration of 300 g/mL, the inhibition zone of bacterial growth at 19–22 mm, 23–26 mm, and 17–19 mm was observed	[37]
Alpinia katsumadai	12.6 nm; quasi-spherical	*S. aureus*, *E. coli*, and *P. aeruginosa*	Broth dilution	Against *S. aureus* and *E. coli* bacteria at MIC = 20 μg/mL, and at MIC = 40 μg/mL for *P. aeruginosa*. Inhibits bacterial growth at 20 μg/mL for *S. aureus* and *E. coli*, and at 40 μg/mL for *P. aeruginosa* by 65, 64 and 63% after 9 h of incubation	[81]
Nelumbo nucifera	12.9 nm; quasi-spherical	*S. aureus*, and *P. aeruginosa*	Broth dilution	MIC = 10 μg/mL for *S. aureus* and *P. aeruginosa*, at this concentration it effectively inhibits bacterial growth 100% after 10 h of incubation	[82]
Convolvulus arvensis	28 nm; spherical	*E. coli*, *S. aureus*, and *P. aeruginosa*	Disk diffusion and broth macro-dilution	17 mm bland zone for *E. coli* at high doses. For *S. aureus*, a concentration of 20 g/mL was used, while for *P. aeruginosa*, a concentration of 50 g/mL was used	[83]
Erythrina suberosa	15–34 nm; spherical	*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, *C. krusei*, *C. viswanathi*, and *T. mentagrophytes*	Disk diffusion and macro broth dilution	*E. coli*, *B. subtilis*, and *C. viswanathi* have no inhibitory zone, bacteria *S. aureus*, *P. aeruginosa*, *C. krusei*, and *T. mentagrophytes* in the 16–24 mm area 16.27–99.26 and 36–82.27%, respectively	[84]
Carthamus tinctorius L.	8.67 ± 4.7 nm; spherical	*S. aureus*, *P. fluorescens*	Spectrophoto metry	100% inhibits *S. aureus* at MIC 1.9 μg/mL and MIC 3.9 μg/mL. While *P. fluorescens* on MIC = 7.8 μg/mL and MLC = 15.6 μg/mL	[85]
Maclura pomifera	6–16 nm; spherical	*C. albicans*, *B. cereus*, *S. aureus*, *P. aeruginosa*, *E. coli*, *A. niger*	Disk diffusion	Inhibits *C. albicans* at MIC = 3.12 μg/mL, *B. cereus* at MIC = 6.25 μg/mL, *S. aureus* at MIC = 12.5 μg/mL, *P. aeruginosa* at MIC = 3.12 μg/mL, *E. coli* at MIC = 1.56 μg/mL, *A. niger* at MIC = 1.56 μg/mL	[86]

Table 1. Silver nanoparticles synthesis using plants and their applications as antibacterial (*Continued*)

Plant Name	Size and morphology	Pathogen	Method	Conclusion	Ref.
Paederia foetida Linn.	5–25 nm; spherical	*A. niger*, *S. aureus*, *B. cereus*, *E. coli*	Disk diffusion	Against *B. cereus* at 26.13%, *E. coli* at 26.02%, *S. aureus* at 25.43% and *A. niger* at 22.69%	[87]
Ricinus communis	8.96 nm; spherical	*S. aureus* and *P. aeruginosa*	Agar well diffusion	Zone of inhibition at concentration 500 ppm for *P. aeruginosa* are 14 mm and for *S. aureus* are 12 mm	[88]
Juniperus procera	15–34 nm; spherical	*B. subtilis*, *M. luteus*, *P. mirabilis*, *K. pneumoniae*, and *C. albicans*	Agar well diffusion	Against for *M. luteus* and *B. subtilis* at 28 mm, for *P. mirabilis* at 29 mm, for *K. pneumoniae* at 18 and for *C. albicans* at 24 mm	[89]
Lippia citriodora	10–45 nm; spherical	*E. coli*, *S. typhi*, *B. subtilis*, *S. aureus*, and *C. albicans*	Agar disk diffusion	Inhibits for *S. aureus* at 10 mm, *B. subtilis* at 19 mm, *S. typhi* at 20 mm, *E. coli* at 21 mm and *C. albicans* at 12 mm	[89]
Eucalyptus citriodora	17.51 mm; spherical	*A. baumannii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, and *C. albicans*	Disk diffusion	Inhibits *A. baumannii* at MIC = 0.04 μg/mL, *E. coli* at MIC = 0.04 μg/mL, *S. aureus* at MIC = 12.5 μg/mL, *K. pneumoniae* at MIC = 0.04 μg/mL, *P. aeruginosa* at MIC = 0.04 μg/mL, *E. faecalis* at MIC = 0.04 μg/mL, *S. aureus* at MIC = 0.09 μg/mL, *C. albicans* at MIC = 0.02 μg/mL	[90]

Based on the literature review, the use of *Paederia foetida* Linn. as a medium for synthesizing AgNPs gave the smallest size of 5–25 nm with a spherical shape. The AgNPs have the ability to penetrate the bacterial cell wall, alter the structure of cell membranes, and even cause cell death. Their effectiveness is based not only on their nanoscale size but also on their large surface area-to-volume ratio. They can increase the permeability of cell membranes, generate reactive oxygen species, and interfere with deoxyribonucleic acid replication by releasing silver ions [91].

Toxicity of AgNPs

AgNPs are commonly used in a variety of products due to their special activity and application as an antibacterial [92]. AgNPs are often used in electronic bio-sensing, clothing, cosmetics, sunscreens, and medical devices. However, a large number of *in vitro* studies have shown that AgNPs have toxic effects on various mammalian cell cultures. The production process and application of AgNPs will affect life by releasing nano-sized silver into the air, water, and soil environment so that it will cause direct exposure to humans. Embryos and fetuses will be more susceptible to environmental pollutants than adult humans [93]. Smaller AgNPs (between 5–45 nm) tend to be more toxic to human cells than larger AgNPs [34]. AgNPs have very low cell viability against colorectal carcinoma cell lines [94]. For animals that live in water, if AgNPs are present in the body of a zebrafish (*Danio rerio*) it can produce Ag^+ ion twice, this is because the precursor used as raw material loses the chelating agent during the synthesis process [95]. Benthic invertebrates and microbes are also very susceptible to exposure to AgNPs [96]. Research conducted by Jafir (2021) using AgNPs as an insecticide against pests that attack tobacco has proven to be effective in killing armyworms (*Spodoptera litura Fabr*) so it is likely to also affect similar animals [97]. In general, the smaller the nanoparticles the better the antimicrobial activity due to the increased surface contact with microbial cells. From the same size range, the antimicrobial activity of AgNPs can be sorted by shape and in order; triangle > pentagonal, hexagonal, cubic, nano bar > round. Triangles show the highest activity mainly due to better edges because of sharp edges and a dominant stable aspect. Hexagonal, cubic and nano-bar shapes have curved edges which might reduce their efficacy against microbes compared to triangular shape nanoparticles, whereas spherical nanoparticles have no sharp edges and mostly show the weakest antimicrobial effect [98].

■ CONCLUSION AND RECOMMENDATIONS

Biological synthesis methods (green synthesis) of AgNPs based on plant extracts with antimicrobial activity need to be considered as a possibility, either in whole or conjugated form. Different biological methods for the synthesis of AgNPs using phytochemical mechanism have advantages such as the resulting nanoparticles that are non-toxic, inexpensive, and environmentally friendly have been thoroughly investigated. The bacterial vulnerability of the resulting AgNPs to several morbific microbes has also been emphasized. The understanding of plant phytochemical mechanisms involved in the synthesis and inhibition of antimicrobials is still not completely comprehended. Moreover, controlling the biosynthetic form of AgNPs, which has many positive effects on their activity, remains largely unanswered this day even though the chemically synthesized method of AgNPs is already well known for controlling the shape of the resulting nanoparticles, it still has the potential to cause problems due to the number of problems. Many different phytochemicals are also present in plant extracts, making it difficult to systematically control the interaction with the resulting AgNPs. Therefore, a better understanding of each of the phytochemical mechanisms, their quantities and interactions will pave the way for the selective synthesis of biogenic nanoparticle forms.

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