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REVIEW PAPER

Recent Advances in the Green Synthesis of Gold and Silver Nanostructures for Augmented Anti-Microbal Activity

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Abstract: *Green synthesis refers to the synthesis of nanoparticles using plants and microorganisms. It is preferred over conventional methods because it is sustainable, eco-friendly, cost-effective, and fast. The phytochemicals and enzymes present in plants and microorganisms respectively acts as the reducing and capping agent for the synthesis of nanoparticles. Phytochemicals and enzymes have the ability to reduce precursor metal ions into nanoparticles. As the conventional methods involve the use of high energy and toxic chemicals which are harmful to both environment and organisms, these synthesis methods are discouraged. Of the nanoparticles, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) have gained lots of attention owing to their multiple applications and less toxicity. In addition, various in-vitro studies have reported the antimicrobial activity of AgNPs and AuNPs against various microbes. This particular review portrays the methods of nanoparticles synthesis, components of green synthesis, mechanism of green synthesis, antimicrobial activity, other applications and various factors affecting the green synthesis of AgNPs and AuNPs.*

Keywords: *Green synthesis, Nanoparticles, AuNPs, AgNPs, Plants, Microorganisms, Antimicrobial activity.*

1. INTRODUCTION

The field of nanotechnology has gained tremendous importance in modern materials science [1]. The term "nanotechnology" was first coined by the American physicist Richard Feynman in 1959 [2]. However, the history dates back to the 9th century, when the artisans of Mesopotamia age utilized the gold nanoparticles (less than 100 nm) to produce glittering effects on pots and other utensils [3]. In general, nanotechnology refers to the application of science to control matter at the molecular stage [4]. It is also gaining importance in other areas of modern science like optics, mechanics, chemical industry, electronics, aeronautics, biomedical sciences, drug-gene delivery, catalysis, optoelectronic devices, energy science, and photo-electrochemical applications [4].

Recently, the utilization of nanoparticles, especially gold and silver nanoparticles, has accumulated significant spotlight in nanoscience owing to its multiple physical, chemical, and biological applications [5]. And nanoparticles are synthesized by two major processes top-down and bottom-up processes. The conventional synthesis methods include physical and chemical methods, which are highly discouraged owing to its high production cost, extensive usage of energy, production of chemical waste products that contaminate the environment, and excessive production of hazardous and toxic chemicals that are harmful to the organisms.

Due to its major setbacks, the green synthesis of nanoparticles has gained attention over conventional methods. The green synthesis method is safe, reliable, efficient, eco-friendly, and sustainable [6] (Fig. 1). Multiple applications of nanoparticles are credited for their very small size (<100 nm), distinct morphology, and extensive surface area [7-9].

Green synthesis is defined as reducing precursor metal or metal oxide ions into their respective nanoparticles by using the biological components. It is further divided into two major categories, plant-based synthesis and microorganism-based synthesis [6]. However, the exact mechanism of how nanoparticles are synthesized using the plant is not known. Still, it is claimed that phytochemicals present in the plant extract like ketones, aldehydes, flavones, amides, terpenes, carboxylic acids, phenol, and ascorbic acid reduce precursor metal salts into metal nanoparticles [10, 11]. The phytochemical present in plant extracts acts as both reducing and capping agents, external reducing and capping agents are not needed. While the intracellular enzyme present in microorganisms reduces the

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metal ions into their individual metal nanoparticles [12, 13, 14]. Green synthesis is dependent on various factors like the technique of preparation, the concentration of precursor metal salt, pH, temperature, pressure, time, preparation cost, shape, size, pore size, environment, the proximity of other nanoparticles, and other factors [15]. The NPs produced by green synthesis are used in nanomedicine, cancer treatment, antimicrobial agent, DNA analysis, targeted drug delivery, gene treatment, biosensors, and medical imaging [6, 16].

NPs are also associated with certain limitations or challenges such as stability in the hostile environment, bioaccumulations, toxicity, need for skilled operators, and the lack of understanding of the fundamental mechanism and modeling factors [6].

This review focuses on different methods of nanoparticles synthesis, types of green synthesis, its components, mechanism of plant and microorganism based-synthesis, factors affecting green synthesis, anti-microbial activity, other biomedical applications, and recent advances in the green synthesis of Au and Ag nanoparticles.

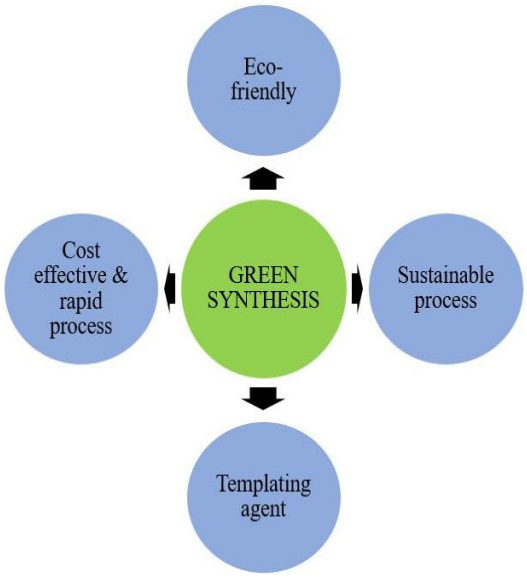

Fig. 1. Advantages of green synthesis

1.1. Different methods of NPs synthesis

The synthesis of nanoparticles is done by two major approaches, the top-down approach and the bottom- up approach [6]. The top-down approach involves the breaking down the bulk material into nanosized structures or particles. In a top- down approach, chemical and physical methods like chemical etching, laser ablation, mechanical milling, sputtering, electro-explosion, thermolysis, lithography, and pyrolysis reduces the precursor metal or metal oxide salt ions into nanoparticles [17]. The methodologies zed in the top-down approach are related to major drawback as they resulted in the formation of irregular surfaced nanoparticles. Which in turn results in modified physical and chemical properties. This approach also requires high energy, i.e., temperature and pressure and high production costs [18]. Different methods of nanoparticles synthesis are shown in Fig. 2.

The bottom-up approach is also known as the self-assembly approach. In this, the synthesis of nanoparticles is done by assembling smaller molecules or atoms, thus forming a larger structure (<100 nm).

The methods used in this approach include chemical vapor deposition, laser pyrolysis, spray pyrolysis, sol-gel process, atomic molecular condensation, and aerosol process [19]. It has gained more importance over the top-down approach as it can producehe top-down approach as it can produce the NPs with the homogenous chemical composition and less surface irregularity at a cheaper rate. However, this approach is also associated with its drawbacks. It involves reducing agents, capping agents, non-polar organic solvent and hazardous chemicals that produce chemical waste and toxic chemicals contaminating reducing agents, capping agents, non-polar organic solvent, and hazardous chemicals that produce chemical waste toxic chemicals contaminating the environment and posing biological hazards. Because of the drawbacks mentioned above, its use in medical and biomedical applications is restricted [20].

Due to the major drawbacks of the two approaches, the green synthesis of NPs has caught the attention of researchers as it is safe, eco-friendly, sustainable, efficient, and cheap compared to conventional chemical and physical methods [6].

Green synthesis involves using biological components to reduce the precursor metal salt ions into nanoparticles. The phytochemicals in plant extract and intracellular enzymes in an organism act as reducing and capping agents [12, 13, 14].

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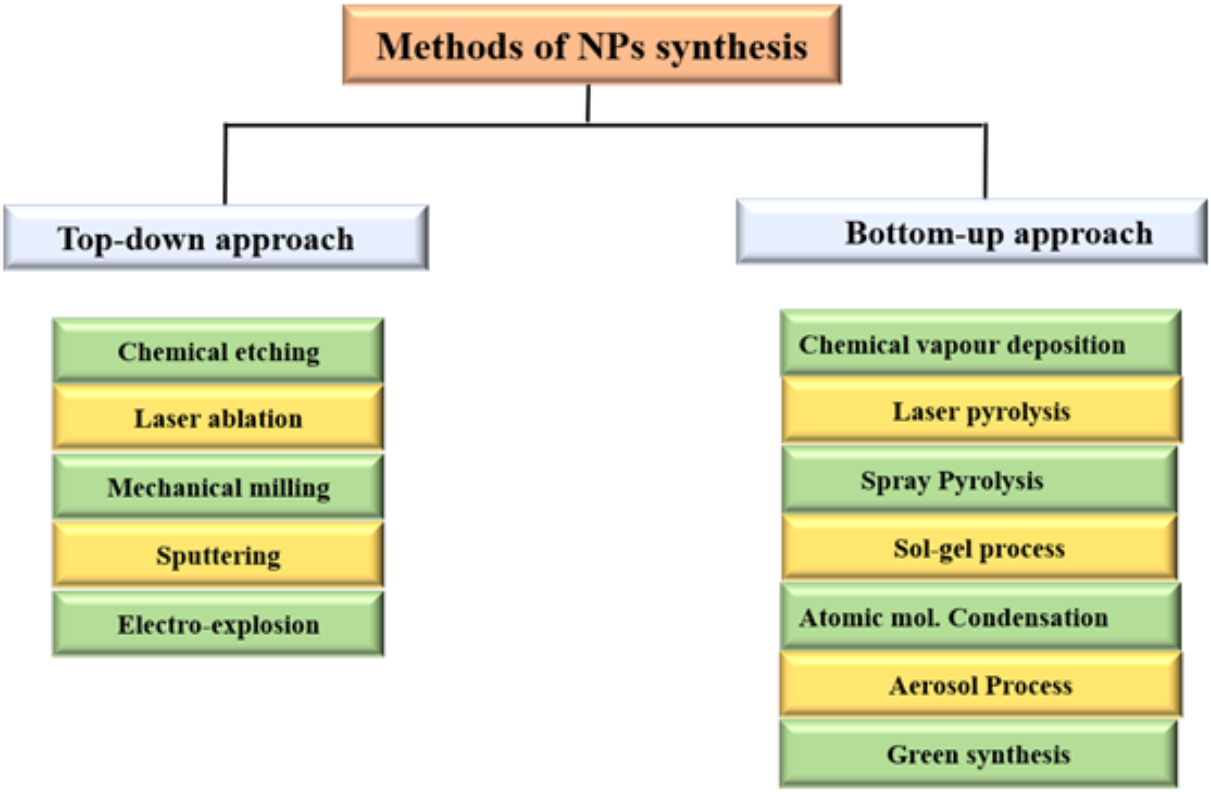

Fig. 2. Different methods of nanoparticles synthesis

1.2. Components used for green synthesis of NPs

The conventional method of green synthesis involves using a highly toxic reducing agent stabilizing agent, and requires a high amount of energy for the synthesis. In addition, it also produces lots of chemical waste, and toxic chemicals that are both detrimental to humans and the environment [18]. While green synthesis of nanoparticles is eco-friendly, rapid, and requires less energy, capable of large-scale productions, cheap and sustainable. And the components used for green synthesis are broadly divided into two main categories plant and microorganism [6]. Different components of green synthesis are shown in Fig. 3.

1.2.1. *Plant*

Plants have the innate potential to reduce precursor metal salt ions into respective nanoparticles. Various parts of the plant such as leaf, flower, fruit, bark, stem, and root extract are used to reduce metal and metal oxide ions.

Since this method is simple, eco-friendly, efficient, sustainable, and cost-effective, it is preferred over chemical and physical methods [6].

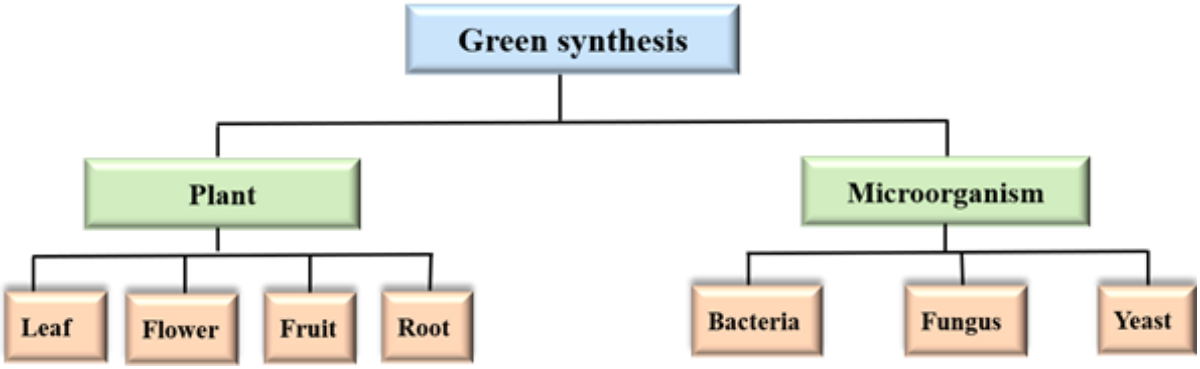

Fig. 3. Different biological components of green synthesis of nanoparticles.

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Plant extractions contain biological molecules such as enzymes, flavones, ascorbic acid, oxalic acid, carboxylic acid, terpenoids, glycosides, carbohydrates, polyols, thiamine, proteins, amino acids, polysaccharides, flavonoids and vitamins, which are environmentally benign. These molecules play a major role in reducing and stabilizing metal nanoparticles [10, 11].

Recently, researchers have shifted their focus to synthesise nanoparticles using plant as the phytochemical present in it acts as both reducing and capping agent along with biomolecules present in it whereby use of toxic chemicals are not required [12, 13, 14]. Many researches have been done to date on ex-vivo (outside living plant) synthesis of NPs, while synthesis can also be done in-vivo (inside living plant) by the reduction of absorbing precursor metal or metal oxide ions into soluble salt.

Gold and silver nanoparticles have gained more attention due to their efficiency and diverse applications in various fields. And the synthesis of Au and Ag nanoparticles was first investigated in plant extract-based synthesis [6]. Different plants used for the synthesis of Au and Ag nanoparticles and morphology and size are shown in table 1 and table 2, respectively.

1.2.2. *Microorganism*

This method involves using biological components, namely bacteria, fungus, and yeast. Other studies reported the synthesis via viruses, agricultural waste, food waste, microbes, and biopolymers [85].

1.2.2.1 Bacteria

The use of bacteria has gained importance in various fields like genetic engineering, targeted-drug delivery, bioremediation, and bioleaching [86]. Bacteria can reduce the precursor metal salt ions into metal nanoparticles. Bacteria as the biological components for synthesis has been a promising biological reductant for the preparation of nanoparticles [87].

Different varieties of bacterial species are made into use for the synthesis. Of various species used, actinomycetes and prokaryotic bacteria have been prominent for nanoparticle synthesis due to its versatility, easy manipulation, and efficiency [88].

Commonly used bacterial species for the synthesis of AgNPs includes Lactobacillus casei, E. coli, Aeromonas sp. SH10, Phaeocystis antarctica, Arthrobacter gangotriensis, Geobacter spp., Corynebacterium sp. SH09 and Shewanella oneidensis. Table 3 shows the bacterial species used to synthesize AgNPs with distinct sizes and morphology. For the preparation of AuNPs, different bacterial species such as Desulfovibrio desulfuricans, E. coli DH5a, Rhodo-pseudomonas capsulate, Bacillus megaterium D01, Bacillus subtilis 168, Plectonema boryanum UTEX 485, and Shewanella alga have been widely used. Table 4 shows different bacterial species used for the synthesis of AuNPs with distinct sizes and morphology.

1.2.2.1 Fungi

Besides bacteria, fungi also served as the efficient component for the synthesis of gold and silver nanoparticles with definite morphologies and sizes. Due to avariety of intracellular enzymes, fungi serve as the better synthesis component of metal and metal oxide nanoparticles [89]. The study also reported that competent fungi can be better than bacteria in terms of the amount and efficiency of synthesis of NPs [90].

Fungi-mediated synthesis of NPs has many advantages over other microorganisms due to the presence of enzymes, reductants, and proteins on the surface of their cell [91]. However, the actual mechanism of fungi-mediated synthesis of NPs is not known, but researchers and scholars believe that formation is due to enzymatic reduction in the cell wall or inside the fungal cell. Tables 3 and 4 summarize published research on the synthesis of silver and gold nanoparticles using fungi.

1.2.2.2 Yeast

Yeasts are single-celled microorganisms present in eukaryotic cells. A total of 1500 yeast species have been identified [92]. The successful synthesis of nanoparticles/ nanomaterials via yeast has been reported by numerous research groups. The biosynthesis of silver and gold nanoparticles by a silver-tolerant yeast strain and *Saccharomyces cerevisiae* broth has been reported [93]. Table 3 and 4 show the varieties of yeast species used to synthesize AgNPs and AuNPs with distinct size and morphology.

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Table 1. Synthesis of AuNPs using different plant sources

Sl. No	Plant source	Part Used	Morphology	Size (nm)	Ref.
1	*Abelmoschus esculentus*	Seed	Spherical	45– 75	(Jayaseelan *et al.*, 2013)
2	*Aerva lanata*	Leaf	Mostly Spherical	17.97	(Joseph *et al.*, 2015)
3	*Chenopodium album*	Leaf	Spherical	Various	(Dwivedi *et al.*, 2010)
4	*Cinnamomum zeylanicum*	Bark, Leaf	Nanoprisms & Spheres	25	(Smitha *et al.*, 2009)
5	*Eucommia ulmoides*	Bark	Spherical	16.4	(Guo *et al.*, 2015)
6	*Curcuma pseudomontana*	Seed	Spherical	20	(Muniyappan & Nagarajan, 2014)
7	*Geranium*	Leaf	Variable	8– 20	(Franco-Romano *et al.*, 2014)
8	*Hibiscus rosa sinensis*	Leaf	Variable	Various	(Philip, 2010)
9	Grape waste	Fruit	Spherical	20– 25	(Krishnaswamy *et al.*, 2014)
10	*Hovenia dulcis*	Fruit	Variable	15– 20	(Basavegowda *et al.*, 2014)
11	*J. sambac leaves*	Leaves	Spherical	20– 50	(Yallappa *et al.*, 2015)
12	*Krishna tulsi*	Leaf	Spherical	30	(Philip & Unni, 2011)
13	*Lansium domesticum*	Fruit peel	Triangular, Hexagonal	20– 40	(Shankar *et al.*, 2014)
14	*Mangifera indica*	Leaf	Spherical	17– 20	(Philip, 2010)
15	*Magnolia kobus*	Leaf	Variable	5– 300	(Song *et al.*, 2009)
16	*Maple leaf pine needle*	Leaf	Spherical	< 100	(Krishnaswamy & Orsat, 2015)
17	*Morinda citrifolia*	Root	Spherical	12.17– 38.26	(Suman *et al.*, 2014)
18	*Mentha piperita*	-	Spherical	150	(MubarakAli *et al.*, 2011)
19	*Cymbopogon sp*	Leaf	Spherical, triangular	200– 500	(Shankar *et al.*, 2005)
20	*Dioscorea bulbifer*	Tuber	Spherical	11– 30	(Ghosh *et al.*, 2011)
21	*Emblica officinalis*	Fruit	Polyhedral	15– 25	(Ankamwar *et al.*, 2005)
22	*Tamarindus indica*	Leaf	Triangular	20– 40	(Ankamwar *et al.*, 2005)
23	*Cinnamomum camphora*	Leaf	Spherical, triangular	55– 80	(Huang *et al.*, 2007)
24	*Sesbania*	-	Spherical	6– 20	(Sharma *et al.*, 2007)
25	*Cymbopogon flexuosus*	-	Triangular	-	(Shankar *et al.*, 2004)
26	*Salvia officinalis*	Whole plant	Spherical, triangular, truncated triangles, pentagons and hexagons.	4– 72	(Elia *et al.*, 2014)
27	*Pelargonium graveolens*	Leaf	Spherical rods, flat, sheets and triangular	21– 70	(Shankar *et al.*, 2003)
28	*Lippia citriodora*	Leaf	Spherical, triangular, truncated triangles, pentagons and hexagons	2.6– 50	(Shankar *et al.*, 2003)
30	*Phoenix dactylifera*	Leaf	Spherical	35– 45	(Zayed *et al.*, 2014)
31	*Pistacia integerrima*	gall	Spherical	20– 200	(Islam *et al.*, 2019)
32	*Pogostemon benghalensis (B) O. Ktz. leaf*	Leaf	Variable	10– 50	(Paul *et al.*, 2015)
33	*Solanum nigrum*	Leaf	Spherical	50	(Muthuvel *et al.*, 2014)
34	*Terminalia arjuna*	Leaf	Spherical	20– 50	(Gopinath *et al.*, 2013)
35	*Zingiber officinale*	Rhizome	Spherical	5– 15	(Kumar *et al.*, 2011)

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Table 2. Synthesis of AgNPs using different plant sources

Sl. No.	Plant source	Part Used	Morphology	Size (nm)	Ref
1	*Aerva lanata*	-	Mostly Spherical	18.62	(Joseph *et al.*, 2015)
2	*Artemisia nilagirica*	Whole plant	Predominantly Square	70– 90	(Vijayakumar *et al.*, 2013)
3	Banana peel	Fruit	Spherical	23.7	(Ibrahim, 2015)
4	Beetroot	Root	Spherical	15	(Bindu *et al.*, 2015)
5	*Boerhavia diffusa*	Whole plant	Spherical	25	(Kumar *et al.*, 2014)
6	*Cinnamon zeylanicum*	Bark	Variable	31– 40	(Sathishkumar *et al.*, 2009)
7	*Cocos nucifera*	Inflorescence	Spherical	22	(Mariselvam *et al.*, 2014)
8	*Coriandrum sativum*	seed	Spherical	13.09	(Nazeruddin *et al.*, 2014)
9	*Dalbergia spinosa*	Leaf	Spherical	18	(Muniyappan *et al.*, 2014)
10	*Delonix elata*	Leaf	Spherical	35-45	(Sathiya & Akilandeswari, 2014)
11	*Emblica officinalis*	Fruit	Spherical	15	(Ramesh *et al.*, 2015)
12	*Eucalyptus*	Leaf	Spherical	40-60	(Pourmortazavi *et al.*, 2015)
13	*Ficus carica*	Leaf	Spherical	13	(Ulug *et al.*, 2015)
14	*Gloriosa superba*	Leaf	Spherical	10-25	(Ashokkumar *et al.*, 2013)
15	*Hibiscus rosa sinensis*	-	Variable	various	(Philip, 2010)
16	*A. indicum*	Leaf	Spherical	7-17	(Ashokkumar *et al.*, 2015)
17	*J. sambac*	Leaf	Spherical	20-50	(Yallappa *et al.*, 2015)
18	Krishna tulsi	Leaf	Spherical	10– 20	(Philip & Unni, 2011)
19	*Lansium domesticum*	Fruit peel	Spherical	10-30	(Shankar *et al.*, 2014)
20	*Lantana camara* leaf	Leaf	Spherical	20-34	(Ajitha *et al.*, 2015)
21	*Lippia citriodora*	-	Spherical	15-30	(Cruz *et al.*, 2010)
22	*Mangifera indica*	Leaf	Spherical	20	(Philip, 2011)
23	Maple leaf	Leaf	Spherical	1-100	(Vivekanandhan *et al.*, 2014)
24	Neem gum	Gum	Spherical	< 30	(Velusamy *et al.*, 2015)
25	*Papaver somniferum*	-	Spherical	3200– 7600	(Vijayaraghavan *et al.*, 2012)
26	*Piper longum*	Leaf	Spherical	17.6– 41	(Jacob *et al.*, 2012)
27	*Piper nigrum*	-	Spherical	32-1	(Mohapatra *et al.*, 2015)
28	Tea leaf	Leaf	Spherical	20–90	(Sun *et al.*, 2014)
29	*Tephrosia purpurea*	Leaf	Spherical	20	(Ajitha *et al.*, 2014)
30	*Tribulus terrestris*	-	Spherical	16–28	(Gopinath *et al.*, 2012)
31	*Ziziphus jujuba*	Leaf	Variable	20–30	(Gavade *et al.*,2015)

Table 3. Synthesis of AgNPs using different microbial culture (94)

Sl.no	Microbial culture	Morphology	Size (nm)
Bacteria			
1	*Bacillus licheniformis*	Irregular	50
2	*Bacillus cereus*	Spherical	4–5
3	*Brevibacterium casei*	Spherical	10–50
4	*Corynebacterium glutamicum*	Irregular	5–50
5	*Escherichia coli*	Irregular	50
6	*Pediococcus pentosaceus*	Irregular	< 100
Fungi			
7	*Coriolus versicolor*	Spherical	25–75
8	*Cladosporium cladosporioides*	Spherical	10–100
9	*Aspergillus flavus*	Spherical	8.92
10	*Aspergillus terreus*	Spherical	1–20
11	*Fusarium oxysporum*	Spherical	5–50
12	*Fusarium oxysporum*	Irregular	5–15
13	*Fusarium oxysporum*	Spherical	20–50
14	*Fusarium semitectum*	Spherical	10–60
15	*Macrophomina phaseolina*	Spherical	5–40
16	*Penicillium fellutanum*	Spherical	5–25
17	*Penicillium nalgiovense*	Spherical	25 ± 2.8
18	*Aspergillus fumigatus*	Spherical	5–25
19	*Phaenerochaete chrysosporium*	Pyramidal	50–200
20	*Phoma glomerata*	Spherical	60–80

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21	*Pleurotus sajor-caju*	Spherical	30.5
22	*Trichoderma reesei*	Spherical	5–50
23	*Trichoderma viride*	Spherical	5–40
24	*Trichoderma viride*	Spherical	5–40
25	*Trichoderma viride*	Irregular	2–4
26	*Trichoderma asperellum*	Spherical	13–18
27	*Verticillium sp.*	Spherical	5–50
Yeast			
28	Yeast	Irregular, Polygonal	9–25

Table 4. Synthesis of AuNPs using different microbial culture [94]

Sl. No	Microbial culture	Morphology	Size (nm)
Bacteria			
1	*Brevibacterium casei*	Spherical	10–50
2	*Escherichia coli*	Triangular, Hexagons	20–30
3	*Pediococcus pentosaceus*	Spherical	< 100
4	*Plectonema boryanum*	Octahedral	10–6000
5	*Plectonema boryanum*	Cubic	10–25
6	*Pseudomonas aeruginosa*	Irregular	15–30
7	*Rhodococcus sp.*	Spherical	5–15
8	*Rhodopseudomonas capsulate*	Spherical	10–20
9	*Shewanella algae*	Irregular	10–20
10	*Shewanella oneidensis*	Spherical	< 100
11	*Ureibacillus thermosphaericus*	Irregular	50–70
Fungi			
12	*Aureobasidium pullulans*	Spherical	29 ± 6
13	*Aspergillus clavatus*	Triangular, Spherical	24.4 ± 11
14	*Alternaria alternata*	Spherical, Triangular	12 ± 5
15	*Aspergillus sydowii*	Spherical	8.7–15.6
16	*Aspergillus niger*	Polydispersed	10–20
17	*Aspergillus niger*	Spherical	12.8 ± 5.6
18	*Fusarium semitectum*	Spherical	10–80
19	*Fusarium oxysporum*	Spherical	2–50
20	*Hansenula anomala*	Irregular	14
21	*Helminthosporium solani*	Variable	2–70
22	*Hormoconis resinae*	Spherical	3–20
23	*Neurospora crassa*	Spherical	32
24	*Penicillium rugulosum*	Spherical	20–40
25	*Aspergillus oryzae var. viridis*	Mostly Spherical	10–60
26	*Penicillium brevicompactum*	Various	10–60
27	*Penicillium sp*	Spherical	30–50
28	*Phanerochaete chrysosporium*	Spherical	10–100
29	*Cylindrocladium floridanum*	Spherical	5–35
30	*Cylindrocladium floridanum*	Spherical	19.05
31	*Coriolus versicolor*	Spherical	20–100
32	*Rhizopus oryzae*	Spherical	16–25
33	*Colletotrichum sp*	Spherical	8–40
34	*Candida albicans*	Spherical	20–40
35	*Sclerotium rolfsii*	Spherical	25.2 ± 6.8
36	*Candida albicans*	Monodispersed, Spherical	5
37	*Epicoccum nigrum*	-	5-50

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38	*Trichoderma koningii*	Spherical	30–40
39	*Trichoderma koningii*	Spherical	10–14
40	*Verticillum luteoalbum*	Irregular	< 100
41	*Volvariella volvacea*	Triangular, Spherical	20–150
42	*Verticillium sp*	Irregular	< 100
43	*Verticillium sp*	Spherical	20 ± 8
44	*Yarrowia lipolytica*	Various	< 100
45	*Yarrowia lipolytica*	Triangles	15
Yeast			
46	*Yeast*	Irregular, Polygonal	9–25
47	*Candida utilis*	Irregular	< 100
48	*Saccharomyces cerevisiae*	Spherical	15–20

1.3. Probable mechanism of synthesis of Ag and Au Nanoparticles

1.3.1. *Microorganism based*

Various mechanisms for the synthesis of NPs using microorganisms have been reported. However, when simplified, the mechanism revolves around a similar concept where precursor metal or metal oxide salt ions are captured on the surface or inside microbial cells. With the assistance of extra or intracellular enzymes, the captured ions are then reduced into respective nanoparticles. One of the studies reported the synthesis of Au and Ag NPs via *Verticillium sp* [95], and the hypothesis stated that initially, Au and Ag ions were captured on the fungal cell surface with the help of electrostatic interaction between ions and negatively charged cell wall enzyme. Then, the captured gold and silver ions were bio-reduced into gold and silver nuclei, which then subsequently grew into nanoparticles. The two main aspects in the biosynthesis of nanoparticles are nicotinamide adenine dinucleotide (NADH) and NADH-dependent nitrate reductase. Another study demonstrated the synthesis of AgNPs via *B. licheniformis,* which concluded that nitrate reductase was responsible for the synthesis [96]. However, the exact mechanism for the production of metal salt ions and metal and metal oxide nanoparticles is still not known. The general mechanism of synthesis is shown in fig. 4.

1.3.2. *Plant based synthesis*

After maintaining the conditions suitable for the synthesis, such as phytochemicals, metal salt concentration, phytochemical concentration, pH, and temperature, the extract of the plant is mixed with metal precursor salt ions solution whereby synthesizing nanoparticles [96]. By maintaining the aforementioned conditions, the rate of nanoparticle formation, yield, and stability of nanoparticles can be controlled [97]. In comparison to fungi and bacteria, which need a longer incubation period, phytochemicals found in plant leaf extracts have an uncanny ability to reduce metal ions in a much shorter time [98].

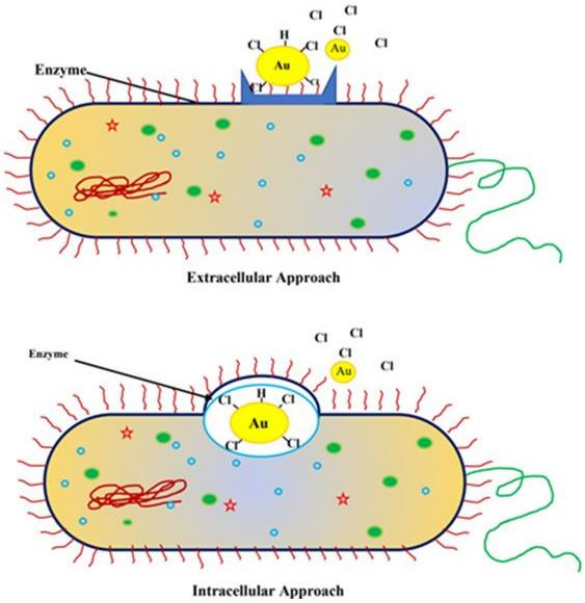

Fig. 4. Synthesis of AuNPs via different approaches using bacteria

In addition, plant leaf extracts are considered to be a good and safe source for metal and metal oxide nanoparticle synthesis. The plant extract has the potential to act as both a reducing and stabilizing agent for the synthesis process [12, 13, 14]. Within a few minutes, the reaction is completed. When the precursor solutions are

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mixed, the biochemical reduction of the salt begins almost immediately, and the generation of nanoparticles is usually indicated by a change in the color of the reaction solution. The mechanism for the synthesis of NPs is shown in figure 5.

Plant-based synthesis is primarily divided into three stages: activation phase, growth phase, and termination phase [99, 100]. The activation phase is the first step in the process of recovering metal ions from their salt precursors using plant metabolites, which are biomolecules with reduction abilities. Nucleation of the condensed metal atoms occurs after the metal ions are changed from their monovalent or divalent oxidation states to zero-valent states [101]. The activation phase is followed by the growth phase (Ostwald ripening), in which the separated metal atoms coalesce to form metal NPs, with further biological metal ion reduction taking place. Nanoparticles accumulate laterally as they expand, forming a variety of morphologies such as cubes, triangles, rods, spherical, hexagons, pentagons, and wires [102]. The increased thermodynamic stability of NPs occurs as the growth stage progresses, while extensive nucleation can cause aggregation of synthesized NPs, altering their morphologies. The termination phase (defining the final shape of NPs) is the final step in the green NP synthesis process. When the NPs are capped by plant metabolites, they get the most promising and stable morphology [94].

1.4. Clinical applications of gold and silver nanostructures/particles

Due to the extremely small size and multiple applications of NPs in the various fields, it has gained paramount importance in recent times [6]. NPs such as gold and silver have been recently used in bioimaging/ labeling, anticancer therapy, targeted drug delivery, agriculture, water treatment, nano fertilizers/ nano pesticides/ nano herbicides, catalytic activity, removal of pollutants, heavy metal ion sensing, acaricidal agent, anti-biofouling agent, anti-inflammatory, antileishmanial agent, antimicrobial agent, optics, mechanics, electronics and aeronautics, etc. [4, 6, 16]. The antimicrobial activity of NPs, can be divided into two categories: bactericidal and inhibitory. Bacterial cells die due to NPs activity in bactericidal action, whereas bacterial cell division is only prevented in inhibitory action without destroying any cells.

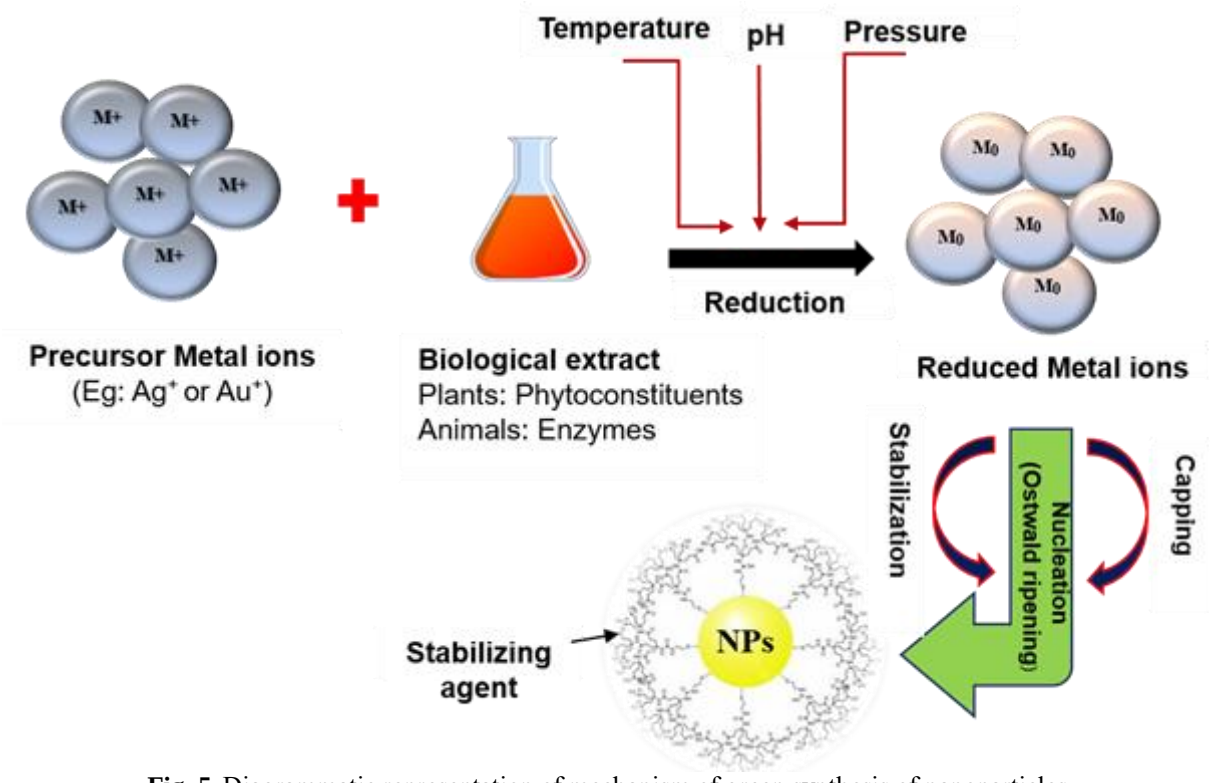

Fig. 5. Diagrammatic representation of mechanism of green synthesis of nanoparticles

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Based on these two types of actions, the disc diffusion method, minimum inhibitory concentration determination, and minimum bactericidal concentration determination are three commonly used methods to study the antibacterial properties of any material.

1.4.1. *Disc or Well Diffusion Method*

The disc diffusion approach is widely used to demonstrate the antimicrobial activity of AgNPs. Nanoparticles are coated on the surface of suitable absorbent materials with uniform size and disc shape in this process. The disc is positioned over the targeted microbe's surface during the analysis, and the antimicrobial activity of AgNPs is shown by the development of an inhibition zone around the disc. Instead of a disc, small disc-shaped pits are formed on the surface of the agar plate for filling the test solution in the well diffusion process. The plates are inoculated with microbes and incubated under clean conditions in both processes to obtain a clear inhibition zone. The diameter of the inhibition zone around the well or disc can easily visualize the effect of AgNPs on the selected microbes [20]. The researchers used *Datura stramonium* leaf extract to make AgNPs and tested their antibacterial activity using the well diffusion method against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. The antibacterial activity of AgNPs increases as the dose of stack solution increases, and better antibacterial activity of AgNPs was demonstrated against E. coli [103].

1.4.2. *Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC)*

The minimum inhibitory concentration (MIC) is characterized as a concentration of nanoparticles that causes 100 % visible growth of the targeted microbe after 24 hours of incubation. The MIC is calculated by watching the bacteria develop in culture tubes containing the same amount of bacterial culture but different concentrations of AgNPs. MIC is defined as the minimum amount of AgNPs required to inhibit the growth of the bacteria [20].
MBC was determined using by using a fixed amount of AgNPs, which is normally higher than the MIC value. AgNPs are applied to a nutrient medium containing a growing bacterial inoculum, and the bacteria's growth is observed using UV-Vis spectroscopy or a plate analyzer in terms of changes in the samples' optical density. Broth dilution measurements are also used for MIC and MBC analysis, in which a comparison of the result is made with the standard after the completion of the experiment [20].

1.4.3. *Antimicrobial activity of gold Nanoparticles (AuNPs)*

AuNPs are recently used to treat various diseases owing to their smaller size and greater therapeutic efficacy. In addition to biomedical applications of the AuNPs, it has also been used as antimicrobial agents in human, agriculture products, fertilizers, pathogens, and food. The antimicrobial activity of AuNPs is credited for its extremely small size, larger surface area, and its photothermic nature [5]. It is reported that the AuNPs interfere with the ionic interactions in the cell membrane, whereby the membrane gets damaged by pathogens. In addition, AuNPs often cause bacteria and fungi to die by increasing the expression of genes involved in the redox process [5]. However, the exact mechanism of antimicrobial activity of AuNPs is not still known. Some studies have also reported that it works by targeting sulfur or phosphorous base in the cell. When AuNPs are bound to a thiol group in an enzyme, the respiratory chain is disrupted, resulting in the production of a large number of free radicals and causing cell death. AuNPs, on the other hand, minimize ATP behaviors by reducing the interaction between tRNA and ribosomes [5]. It is also reported that AuNPs exhibit antimicrobial activity by hindering transmembrane hydrogen efflux. When the size of the NPs is much smaller than the size of the bacterial cell, however, a lower concentration of AuNPs will hinder bacterial growth by about 250-fold, causing the pathogen to die. NPs bind to the pathogen's cell wall and slow the cell process, causing death. By using the electrostatic force of attraction on the cell wall, AuNPs will attract DNA from bacteria. With the increased surface area and nano-size, the chances of cell death become higher. Table 5 shows the list of plants used for the synthesis of AuNPs and their antimicrobial activities [104].
AuNPs shows different action on gram-positive and gram-negative bacteria. Gram-positive bacteria have a thick peptidoglycan layer, whereas only a thin layer of peptidoglycan is present in gram-negative.

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Table 5. Plants used for the synthesis of AuNPs and its antimicrobial activity [104]

Sl. no	Plant name	Microorganisms tested	Method
1	*Acorus calamus*	*Escherichia coli, Staphylococcus aureus*	Agar well diffusion
2	*Trianthema decandra L.*	*S. aureus, Enterococcus faecalis, Streptococcus faecalis, E. coli, P. vulgaris, P. aeruginosa, B. subtilis, Yersinia enterocolitica, Klebsiella pneumoniae and C. albicans*	Well diffusion
3	Solanum lycopersicum	*S. aureus and P. aeruginosa*	Well diffusion
4	*Zizyphus mauritiana*	*S. aureus*	Luria medium
5	*Dracocephalum kotschyi*	*E. coli, Pseudomonas aeruginosa and Proteus vulgaris*	Agar cup-plate
6	*Mentha piperita*	*S. aureus and E. coli*	Müller-Hinton agar
7	*Solanum nigrum*	*Staphylococcus saprophyticus, Bacillus subtilis and E. coli*	Disc diffusion
8	*Nepenthes khasiana*	*E. coli, Bacillus, Candida albicans and Aspergillus niger*	Well diffusion
9	*Gloriosa superba*	*S. aureus, Streptococcus pneumoniae, Klebsiella pneumoniae and E. coli*	Disc diffusion
10	*Salicornia brachiate*	*Salmonella typhi, E. coli, P. aeruginosa and S. aureus*	Disc diffusion

As a result, the peptidoglycan in Gram-positive bacteria is very strong and can penetrate AuNPs through the cell wall, whereas Gram-negative bacteria have a thin layer that can easily cause cell death. In addition to the thickness of peptidoglycan and size of AuNPs, other factors such as concentration, capping agents, and purification are accounted for its antimicrobial activity. It was also reported that the AuNPs synthesized from the plant extract are superior or show greater inhibitory potential compared to those synthesized using the chemical method. The antimicrobial activity of green synthesized AuNPs could be attributed to the AuNPs' synergistic effect with plant extract [105-107].

1.4.4. *Silver Nanoparticles (AgNPs)*

AgNPs inhibits bacterial growth by suppressing respiratory enzymes and electron transporting unit by blocking the functioning of DNA. The respiratory and permeability functions of the cell membrane are hindered when silver ions are bound to the cell surface of microorganisms. Silver interacts closely with the membrane's surface and penetrates deep into the bacterial cell, causing DNA to lose its replicability and eventually die [108].

Many researchers have reported that AgNPs have more antimicrobial activity towards gram-negative bacteria than gram-positive because of thin peptidoglycan layers and a beta-barrel protein called porins present on the cell wall [109]. Furthermore, AgNPs with a high specific surface-to-volume ratio release Ag^{+}, increasing their contact with microorganisms, improving silver ion dissolution, and improving the biocidal effect. Factors such as pH, temperature, $AgNO_3$ concentration, and microorganism type affect the antimicrobial activity of AgNPs [20].

The study was carried out by Ruiz-Baltazar et al. to determine the antimicrobial properties of AgNPs synthesized via *Melissa officinalis* leaf extract against S. A and E. coli. The disc diffusion method and the MBC/MIC test were used to investigate the antibacterial activity. Both AgNPs solutions of various concentrations (ranging from 1 mM to 20 mM) were found to have an inhibitory effect. In both cases, the antibacterial properties of prepared AgNPs synthesized using a green synthesis method were verified by the inhibition zone results. In addition, it was also reported that with an increase in the AgNPs concentration, the antibacterial properties got enhanced, and better result was exhibited against *E. coli* than *S. aureus.* However, antibacterial properties were exhibited by AgNPs against both the microorganisms [110]. Photoinduced green synthesis and antimicrobial evaluation of polycaprolactone/ curcumin/ grape leaf extract-Ag hybrid nanoparticles (PCL/ Cur/ GLE-AgNPs) were reported by EL-Sherbiny et al. In comparison to GLE, GLE/ AgNPs, and PCL/ Cur/ GLE plain NPs, bacterial strains incubated with high concentrations of PCL/ Cur/ GLE-Ag hybrid NPs showed a high bactericidal

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impact. PCL/ Cur/ GLE-Ag hybrid NPs have a high surface area, enabling them to make direct contact with bacterial cells and free radicals formed by AgNPs, which can damage bacterial cell membranes [111].

The green synthesis of AgNPs from silver acetate was reported by Pethakamsetty et al., who used methanolic root extracts of Diospyros sylvatica (a member of the Ebenaceae family) as a reducing agent. The synthesized AgNPs were reported to exhibit mild activity against Proteus vulgaris and Streptococcus pyogenes, but moderate activity against *E. coli, Staphylococcus aureus,* and *Klebsiella pneumoniae.* However, *Bacillus subtilis, Bacillus pumilis, and Pseudomonas aeuriginosa* were shown the most activity by AgNPs [112]. The green synthesis of AgNPs by tissue extract from weaver ant larvae has been documented by Khamhaengpol and Siri (*Oecophylla smaragdina*). The synthesized AgNPs exhibited potential antibacterial activity against both *E. coli* and *S. aureus*. In contrast to other published literature, the MBC and MIC against both bacteria were found to be the lowest [113].

1.5. Factors affecting green synthesis

1.5.1. *Particular Method or Technique*

There are various methods to synthesize NPs. The methods or techniques include physical method (Mechanical procedure), chemical method (using chemicals), and biological method, which include the use of organic or inorganic chemicals, plants, and microorganisms for the synthesis of NPs. Each method is associated with certain benefits and limitations. Physical and chemical methods are associated with extensive use of energy and toxic chemicals, which are harmful to both people and the environment. Biological methods for nanoparticle synthesis, on the other hand, use nontoxic and environmentally safe materials in combination with green technology, making them more environmentally friendly and more suitable than conventional methods [121, 122].

1.5.2. *Effect of concentration on synthesis*

An increase in concentration increases the rate of collision of reactant particles and hence facilitates the rate of reduction of metal ions into metal nanoparticles. Although less concentration (3.5×10^{-6} M) of flavonoid derivatives has the ability to reduce metal ions, they do not have the ability to act as capping agents and stabilizers as compared to the high concentration (3.5×10^{-2} M) of phytochemical constituents (i.e., flavonoids) or enzymes. A higher concentration of flavonoid produces AuNPs with small size. It also indicates that when a high concentration of flavonoid derivatives was used, it stimulated strong interaction between the protective flavonoid derivatives and the surface of AuNPs, preventing AuNPs from sintering, thus resulting in size reduction of AuNPs [123].

1.5.3. *Choice of best organism*

In order to have an efficient and effective synthesis of NPs, researchers have given core importance to intrinsic properties of organisms such as enzymatic activities and biochemical pathways. It was also reported that plants with great potential for heavy metal accumulation and detoxification are the best candidates for the synthesis of metal nanoparticles [4].

Table 6. Plant extract-mediated green synthesis of AgNPs with antimicrobial activity.

Sl. no	Plant Extract	Targeted pathogens	Ref.
1	*Citrus limetta* peel	*Micrococcus luteus, Streptococcus mutans, Staphylococcus epidermidis, S. aureus, E. coli, Candida spp.*	(Dutta *et al.*, 2020)
2	*Luffa acutangula* leaf	*E. coli, Saccharomyces cerevisiae*	(Kaushal *et al.*, 2016)
3	*Parkia speciosa* leaf	*E. coli, S. aureus, Pseudomonas aeruginosa, Bacillus subtilis*	(Ravichandran *et al.*, 2019)
4	*A. indica* leaf	*S. aureus, E. coli.*	(Ahmed *et al.*, 2016)
5	*Gomphrena globosa* leaf	*S. aureus, B. subtilis, M. luteus, E. coli, P. aeruginosa, Klebsiella pneumoniae.*	(Tamilarasi & Meena, 2020)
6	*Pedalium murex* leaf	*E. coli, K. pneumonia, Micrococcus flavus, P. aeruginosa, B. subtilis, Bacillus pumilus, S. aureus*	(Anandalakshmi *et al.*, 2016)
7	*Musa acuminate* peel	*B. subtilis, S. aureus, P. aeruginosa, E. coli.*	(Ibrahim, 2015)

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1.5.4. *pH*

pH is the major factor that has a significant influence on the synthesis of NPs using the green synthesis techniques. Many researchers have discovered and reported that the pH of the solution medium has effects on the texture and size of the synthesized nanoparticles [124, 125]. As a result, changing the pH of the solution media will regulate nanoparticle size. A study demonstrated the effect of pH on the shape and size of the synthesized silver nanoparticle [126].

1.5.5. *Temperature*

Temperature is another significant factor that influences nanoparticle synthesis in all three methods, namely physical, chemical and biological methods (green synthesis). The physical method necessitates the maximum temperature (>350°C), while chemical methods necessitate temperatures below 350°C. In certain cases, green technology nanoparticle synthesis requires temperatures of less than 100°C or ambient temperature. The nature of the nanoparticles produced is determined by the temperature of the reaction medium [127].

1.5.6. *Pressure*

The pressure is an essential factor for the synthesis of NPs. The shape and size of the synthesized nanoparticles are influenced by the pressure applied to the reaction medium [128]. At ambient pressure, the rate of metal ion reduction using biological agents was reported to be much faster [129].

1.5.7. *Particle Shape and Size*

The size of nanoparticles plays an important role in determining their properties. The melting point of nanoparticles, for example, has been shown to decrease as their size approaches the nanometre scale [130]. Nanoparticles of various configurations have similar energies, making shape transformation easy. The type of energy typically used in nanoparticle analysis causes the nanoparticle to change shape [131]. The chemical properties of synthesized nanoparticles are greatly influenced by their complex nature and form. The type of energy typically used in nanoparticle analysis causes the nanoparticle to change shape. The chemical properties of synthesized nanoparticles are greatly influenced by their dynamic nature and form [132].

1.5.8. *Time*

The length of time the reaction medium is incubated has a significant impact on the quality and form of nanoparticles synthesized using green technology [133]. Similarly, the properties of synthesized nanoparticles changed over time and were heavily affected by factors such as the synthesis process, exposure to light, storage conditions, etc. [134, 135]. The study also reported that the green synthesis via plant extract is much faster compared to microorganisms (bacteria, fungi, yeast) as the latter requires a longer incubation period for the synthesis Variations in time may occur in a variety of ways, including particle accumulation as a result of long-term storage; particles can shrink or expand as a result of long-term storage; they may have a shelf life, and so on, all of which affect their potential [136].

1.5.9. *Preparation Cost*

The costs associated with nanoparticle synthesis must be regulated and monitored in order to promote the future application of nanoparticles in modern-day applications. As a result, the cost-effectiveness of the manufacturing process has a significant impact on nanoparticle synthesis. Despite the fact that the chemical method of synthesis produces a high yield in a limited amount of time, it is not cost-effective. As a result, chemical and physical synthesis of nanoparticles may be limited, whereas biological synthesis of nanoparticles is less expensive and can be done on a large scale [15].

1.5.10. *Pore Size*

The porosity of the synthesized nanoparticles significantly affects the quality and applications of the nanoparticles. The pore size of nanoparticles effects the drug loading and drug release characteristics. The larger the pore size, the drug loading capacity will be higher, release rate will be faster and so the therapeutic efficacy will be increased [137].

1.5.11. *Environment*

The nature of the synthesized nanoparticles is heavily influenced by the surrounding environment. A single nanoparticle can quickly become core-shell nanoparticles in many environments by absorbing materials or reacting with other materials in the environment through

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oxidation or corrosion [138]. The synthesized nanoparticles form a coating in a biological environment, making them thicker and larger in size [139]. The physical structure and chemistry of the synthesized nanoparticles are also affected by the environment. There are a few examples of how the environment affects the composition of nanoparticles that have been synthesized. As the atmosphere of the zinc sulfide nanoparticles was modified from wet to dry, the crystalline nature of the nanoparticles changed instantly. Another example also supports that the presence of peroxide in the solution modifies the chemical nature of cerium nitrate, which in turn indicates that the environment has an effect on the nature of NPs [134].

1.5.12. *Proximity*

In most cases, when an individual or isolated nanoparticles come into contact with or near the surface of other nanoparticles, their properties are altered. This shifting behavior of nanoparticles can be used to create more precise nanoparticles. The proximity effect of nanoparticles has numerous effects, including substrate interactions, particle charging, and nanoparticle magnetic properties [140].

1.5.13. *Other Factors*

The plants and microorganisms are rich in secondary metabolites, which have the ability to act as both reducing and stabilizing agents for the synthesis of NPs. But the composition of the various metabolites varies with the type of plant, part of the plant, and the procedure used for the extraction process [141]. Not unlike plants, various microorganisms produce different intracellular and extracellular enzymes that influence nanoparticle synthesis in different amounts [142]. Furthermore, the process used to purify the synthesized nanoparticles may have an effect on the quantity and consistency of the nanoparticles.

1.6. Recent advances

1.6.1. *Method or substrate used for green synthesis*

1.6.1.1 Biopolymers

Polymers synthesized by living organisms are called biopolymers. They can also be synthesized from renewable resources by polymerization. They are biodegradable polymers made up primarily of organic compounds. Proteins, carbohydrates, DNA, RNA, lipids, peptides, and polysaccharides are examples of biopolymers. Functional groups such as carboxylic, amide I and II, ketone, aldehyde present in the biopolymers assist in the production of NPs. [143-145]

Chitosan is one of the most commonly used biopolymers as a reducing agent in green chemistry. Deacetylation of chitin, a naturally occurring polymer, produces chitosan, a cellulose-like linear polysaccharide. It can be present in a wide variety of insects, fungi, yeasts, and marine invertebrates. [146]

The study was carried out by Esther et al. to investigate the impact of different preparation methods on the size and shape of NPs. In that study, two different preparation methods were employed. The first method involved dissolving chitosan in a boiling $HAuCl_4$ solution, while the second method involved dissolving a $HAuCl_4$ solution in hot boiling chitosan. The nucleation rate was rapid and standardized when the $HAuCl_4$ solution was applied directly to the boiling chitosan solution. However, the rate of nucleation was not uniform when chitosan solution was poured in hot $HAuCl_4$ as the metal ions were absorbed on the matrix and slowly reduced to the nuclei. NPs of various shapes and sizes were made [147]. In the presence of a plant-based extract, chitosan not only acted as a reducing agent, but also acted as a stabilizing agent.

Furthermore, Saha et al. conducted a study with chitosan and black pepper extract. When both chitosan and plant extract were used at the same time, the stability was improved. They suggested that chitosan acted primarily as a stabilizer by interacting electrostatically with the negatively charged AuNPs and positively charged polymeric network. [148]

1.6.1.2 Enzymes

Due to the numerous disadvantages of microbial bio-reduction processes, researchers have proposed that enzymes could be one of the best possible routes for the synthesis of NPs. They conclude that the existence of enzymes, proteins, carbohydrates, and bio membranes in microbes allows them to minimize AuNPs. However, studies have shown that it takes a longer incubation period for microbes and thus has a slow rate of synthesis compared to the plant-based synthesis [149]. Enzymes are also available

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in pure form on the market. Purification and processing of NPs would be made easier as a result of this. Nisin peptides, a class-Ia bacteriocin, can be used to synthesize AuNPs via an enzymatic path. *Lactococcus lactis* produces a peptide that contains 34 amino acids. The NPs were spherical in shape and measured 25 nm in diameter. XRD review validated this finding. [150].

1.6.1.3 Green synthesis from vitamins

Vitamin B2 (as reducing and capping agents) was used to create a green mixture of Ag and palladium nano-spheres, nanowires, and nano-rods. For the synthesis of nanowires and nanorods, vitamin B2 is used as a reducing agent. This is a novel approach in the field of green nanotechnology in that it proposes the use of natural agents in the development of the field, such as their effects on various tumor cells [151]. Ascorbic acid is used as a capping and reducing agent, and chitosan is used as a stabilizing agent. Since chitosan bonds with metal ions, the concentration of NPs is directly proportional to the chitosan concentration used [152].

The use of ascorbic acid as both a reducing and capping agent for the synthesis of NPs with uniform size was reported. Antioxidants that are water-soluble, such as ascorbic acid, seem to be responsible for the reduction of Ag NPs in *Desmodium triflorum*. Plants generate a significant amount of H^+ ions along with Nicotinamide adenine dinucleotide (NAD) during glycolysis, which acts as a powerful reducing agent and appears to be helpful in the formation of AgNPs. [153]

1.6.1.4 Microwave-assisted synthesi

By altering the parameters like surfactants, solvent, and metallic precursor, nanowires, tubes, and dendrites can be produced. Spherical NPs can also be produced by this synthetic method [154]. For the production of Ag NPs, carboxyl methyl cellulose sodium is used as a reducing and capping agent. MW union supports homogeneous heating and quick nucleation of noble metal NPs, in contrast to general heating treatment [155]. By using red grape pomace as a reducing agent, a quick NPs generation method (within seconds) for the synthesis of Ag, Au, palladium, and Pt in an aqueous medium by MW irradiation at 50 W was recorded [156].

1.6.2. *Recent applications of Au and Ag*

1.6.2.1 Water treatment

reduce the quality of pure drinking water and crop irrigation and, in turn, pose various health hazards to humans and other living organisms [157]. Due to the high surface area to volume ratio, enhanced catalytic activity, and chemical stability of green synthesized AgNPs and AuNPs, they serve as the best candidates for water treatment, purification, water monitoring, and agriculture wastewater treatment [158, 159]. Moreover, researchers have learned that NPs have great potential to detect toxic substances and heavy metals such as lead, cadmium, and mercury by incorporating sensors for quick detections [160, 161]. Pesticides present in the water are absorbed onto the NPs, retained on the surface, and then precipitate in the form of the complex. As a result, the water gets purified [162, 163]. The leaf extract of *Mussaenda glabrata* was used to synthesis AgNPs and AuNPs by Francis et al. to evaluate the antimicrobial activity against the pathogenic microorganisms. The NPs exhibited significant antimicrobial activity against the *E. coli, P. aeruginosa, Penicillium chrysogenum, and A.niger.* [164]

1.6.2.2 Targeted drug delivery

Due to the high surface area and greater biocompatibility of AuNPs, it has gained major attention in targeted drug delivery systems. NPs are bonded to the drug by either a covalent or non-covalent bond for its delivery at the targeted site. The drug release occurs by photothermal, triggered or ultrasonic method [165]. The drug release is dependent on parameters like pH, temperature, and biomolecule levels in the body. Targeted delivery using AuNPs has gained major attention in the treatment of cancer or tumor tissues than normal cells. Tumor cells, on average, are more temperature-sensitive than normal cells. As a result, drugs capped on AuNPs may be desorbed on the targeted spot during the heating phase, causing the drug and AuNPs to lose their bond. The drug release of glutathione-AuNPs in tumor cells is based on this theory [166]. Another method requires the deposition of layers of AuNPs on drugs using ultrasound radiation. The bond between AuNPs and drugs is broken when these layered AuNP drugs are exposed to ultrasound radiation. Finally, the drug will be released onto AuNPs' surface. One study reported

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that antibiotics coated with AuNPs demonstrated bacterial activity against multidrug-resistant bacteria [167]. AuNPs demonstrated potent antibacterial activity against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria using ampicillin-AuNPs as a result of this application. All of the research that used drug-coated AuNPs found that they greatly enhanced drug delivery while also improving performance for a variety of diseases. [167, 168]

1.6.2.3 Cancer treatment

Green synthesized NPs of different designs, compositions, shapes, and sizes have potential against many cancer cells lines through antimetastatic [169], antiproliferative [170] cytotoxicity [171], and apoptosis [172]. Once the tumor is attached to the main blood circulation system, NPs then exploit several features of the newly developed vasculature to effectively attack it. Tumor cells are supplied by blood capillaries that perfuse the tissue's cells, where NPs can passively accumulate or anchor to biomarkers overexpressed in tumor cells by targeting moieties. NPs may serve as therapeutic agents, enhancing radiotherapy, silencing genes, inducing hyperthermia, and delivering drugs to cause tumor cell death, as well as imaging enhancers or contrast agents to aid in real-time tracking of therapeutic effects.

Patra et al. used *Butea monosperma* leaf extract to synthesize Ag and AuNPs, in that leaf extract served as a stabilizing, capping, and reducing agent. The AgNPs and AuNPs generated are biocompatible with cancer cell lines and normal endothelial cells, as well as stable in the biological buffer. The synthesized NPs, in combination with doxorubicin, inhibited cancer cell growth better than the pristine compound [173].

1.6.2.4 Nano-fertilizers

To synchronize nutrient release with plant uptake, AuNPs and AgNPs-based nano fertilizers have been developed. This method helps to preserve soil fertility by reducing nutrient loss, soil and groundwater pollution, and chemical reactions between water, soil, and microorganisms that turn them into toxic substances for plants [174-176]. The study was carried out by Kang et al. to demonstrate the effect of AgNPs fertilizers on the red ginseng shoot. In that study, they applied 5 mg/L AgNPs fertilizer suspension to ginseng shoot 3 times per day at 14 days intervals. They discovered that the nano fertilizer had increased the ginsenoside content after harvesting [177].

Nano encapsulated herbicides and pesticides exhibited improved characteristics in terms of stability, solubility, specificity and permeability as the nano structure coating protects the active substance from early degradation and provides longer periods of pest control [l76]. Furthermore, phytopathogens that cause plant disease, such as bacteria and fungi, can be controlled by spraying an NPs solution directly on crops, seed, or foliage to prevent pathogen invasion [178]. El-Aziz et al. synthesized AgNPs from *Fusarium solani* in order to test their effect on grain-borne fungi. According to the findings, 4 percent sprayed NPs solutions resulted in a 0% frequency of fungal pathogens [179].

1.6.2.5 Metal sensing

Heavy metals such as Pb, Fe, Cd, Ni, Hg, Mn, Cr, Co, and Zn are the major pollutants in soil, air, and water. Some heavy metals such as lead, cadmium, copper, and mercury ions have the enormous potential to cause toxicity at trace ppm levels [180, 181]. As a result, identifying toxic metals in the biological and marine ecosystem has become critical for effective remediation [182, 183]. In multi-element analysis, traditional techniques focused on instrumental systems usually have excellent sensitivity. However, the experimental setups are costly, tedious, skill-required, and non-portable. Metallic nanoparticles have been preferred for the detection of heavy metal ions in contaminated water systems due to their tunable size and distance-dependent optical properties [184, 185]. The mercury and lead ions (Hg^{2+} and Pb^{2+}) were selectively sensed by AgNPs synthesized from fresh and dried mango leaves. In another study, AgNPs were synthesized from pepper seed extract and green tea extract to demonstrate selective sensing for Hg^{2+}, Pb^{2+}, and Zn^{2+} ions [186].

1.6.2.6 HIV treatment

The immune system of our body is capable of removing some viral infections itself. However, some of the infections such as herpes, hepatitis, HIV, are long-lasting, persistent, and cannot be

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removed. AuNPs are used as a delivery scheme for the improved effectiveness of the anti-HIV activity. The free AuNPs are unproductive against HIV infection. Kesarkar et al. (2012) [187] found that stabilized AuNPs in amino acid L-cysteine resulted in the enhanced azidothymidine delivery against the HIV-1Ba-L virus *in-vitro*.

AuNPs are being used as a delivery system to increase the efficacy of anti-HIV activity. AuNPs exhibit anti-HIV activities due to poly-anionic shallow, which allows binding with the positively charged amino acid at the binding position of glycoprotein GP120 (or gp120). The mechanism of inhibition takes place, and the reverse transcriptase enzyme of HIV-1 protein is blocked by AuNPs. A study carried out by Garrido et al. reported that conjugated AuNPs have better anti-HIV activities than free AuNPs, which don't exhibit anti-HIV activities.

1.7. Conclusion and future prospective

Green synthesis of nanoparticles has gained enormous importance in the field of nanoscience over the conventional methods of nanoparticles synthesis [1]. Green synthesis is an eco-friendly, cost-effective, energy-efficient, rapid and effective method. Generally, the synthesis of a nanoparticle is broadly categorized into two major divisions; top-down approach and bottom-up approach. Green synthesis is categorized under the bottom-up approach [6]. Green synthesis involves the use of plants and microorganisms for the synthesis of nanoparticles. However, in the green synthesis, synthesis via plant is preferred over microorganisms as the phytochemical present in the plant is an efficient and rapid process as extracellular and intracellular enzymes present in the microorganisms require a longer incubation period for the synthesis [133]. The studies have reported that intracellular and extracellular enzymes present in microorganisms have the ability to reduce precursor metal salt ions into their respective nanoparticles [87].

In terms of plant, the synthesis process is reported to proceed via three steps, namely activation phase, growth phase, and termination phase [99, 100]. Phytochemicals present in the plants also have the ability to reduce metal ions into nanoparticles. Owing to its high therapeutic efficacy and application in various fields, gold and silver nanoparticles have gained popularity over other nanoparticles. They are used in bioimaging/labeling, anticancer therapy, targeted drug delivery, agriculture, water treatment, nano fertilizers/ nano pesticides/ nano herbicides, catalytic activity, removal of pollutants, heavy metal ion sensing, acaricidal agent, anti-biofouling agent, anti-inflammatory, antileishmanial agent, antimicrobial agent, optics, mechanics, electronics and aeronautics [4, 6, 16]. The antimicrobial activity of gold and silver nanoparticles is determined by disc or well diffusion method, minimum inhibitory concentration, and minimum bactericidal concentration [20]. Various invitro studies have reported the antimicrobial activity of AgNPs and AuNPs against various microbes. Studies also found out that the antimicrobial activity was concentration-dependent [123]. In addition, studies have also reported that the AgNPs have less antimicrobial activity against gram-positive bacteria than gram-negative bacteria [109].

The green synthesis of gold and silver nanoparticles is affected by the parameters such as techniques or methods used, the concentration of substrate, pH, temperature, pressure, time, pore size, preparation cost, particle shape and size, pore size, types of reducing agent and capping agent used, the proximity of other nanoparticles, environment and other factors [15].

Recently, the green synthesis of NPs has gained major attention and importance in the field of nanoscience owing to its diverse applications and relatively small size. However, exact mechanisms for the synthesis are not clearly known. Further studies and research on AuNPs and AgNPs are needed to clearly understand the exact mechanisms of green synthesis. In addition, studies must be focused on the determination of main reducing components so that more precise and suitable methods can be established. Detailed synthesis mechanism should be mastered before implementing it into different fields. NPs hold a brighter future in the fields of medicine, technology, industry, and chemistry. Moreover, NPs hold the greatest potential in biomedical applications. Due to the multi-surface functionality of NPs, it will serve as the best candidate for the targeted delivery of drugs for the effective treatment of diseases with minimal side effects. Also, AuNPs are capable of emitting fluorescence, which can, in turn, be used as the biomarker for diseases. As a result, the disease can be effectively diagnosed. Most of the in-vitro

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studies concluded that they are non-toxic and effective. However, more in vivo studies are needed to be conducted to clearly understand the toxicity of NPs, duration of NPs in the body, time of excretion, and effects of residual NPs in the organism. Also, the dosage of NPs needs to be studied so that right and suitable quantities of NPs can be delivered to the patient. Therefore, more detailed and extensive studies and research are needed to be carried out to completely understand and harness the latent potential of AuNPs and AgNPs in the near future.

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