



## GREEN SYNTHESIS AND USAGE OF SILVER NANOPARTICLES: A REVIEW

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**ABSTRACT:** *Plants have been used in the synthesis of metallic nanoparticles because they are more ecofriendly. These plant extracts also allow a controlled synthesis. Organic chemical solvents are toxic and require extreme conditions during nanoparticle synthesis. Plant extracts function as stabilizing, capping or hydrolytic agents. The ZnO nanoparticles are of significant interest as they provide many practical applications worldwide. The most important application of ZnO nano particles would be as antibacterial agents. The increases surface area and smaller size of these particles make them an ideal anti-bacterial agent. In this review, the overview of green synthesis of ZnO nanoparticles along with their antimicrobial activity was also reviewed. The green synthesis of ZnO nanoparticles from Azadirachta indica, Aloe vera, Murraya koenigii and Anisochilus carnosus were also highlighted. In this review, the available published data on AgNPs synthesis, the effects of different factors, characterization methods, properties, and their application are compiled and critically analysed.*

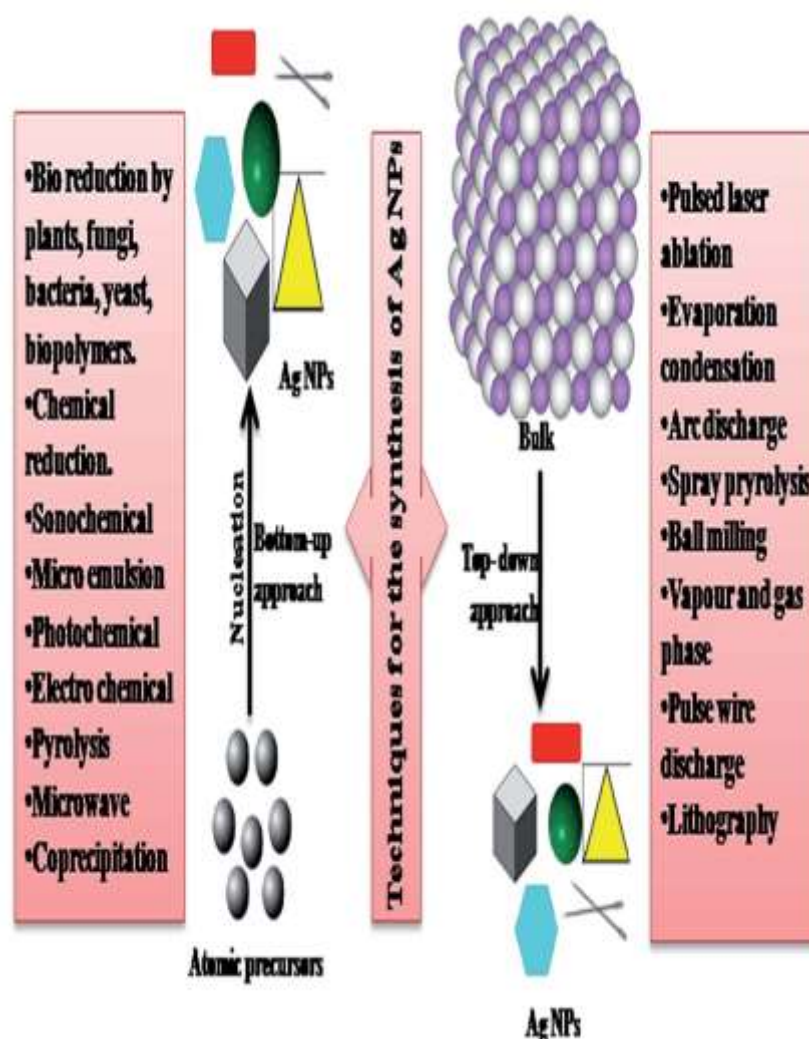
**KEYWORDS:** Green synthesis, nanoparticles, zinc oxide nanoparticles, antimicrobial activity.

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### 1 INTRODUCTION

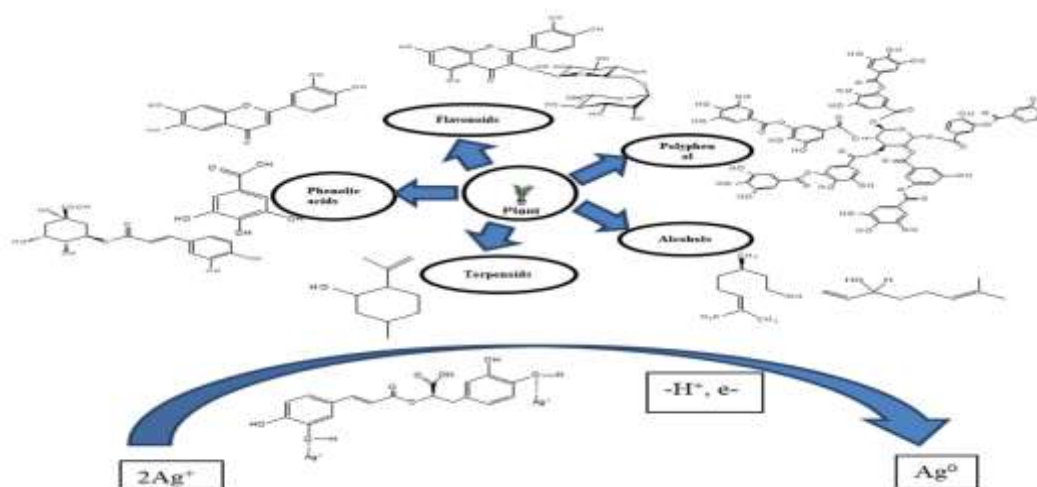
Nanoscience and nanotechnology are highly interdisciplinary branches conducted at the nanoscale, which is about 1 to 100 nm. The physicist Richard Feynman presented a talk entitled “There is Plenty of Room at the Bottom” on December 29, 1959 at the California Institute of Technology at a meeting of the American Physical Society Feynman RP. There's plenty of room at the bottom: an invitation to enter a new field of physics. In his keynote address, Feynman described manipulation technology at the atomic scale. A era decade, in his expedition of ultraprecise fabrication, Professor Norio Taniguchi framed the term nanotechnology. Nanotechnology has turned into a mainstream and vital innovation in recent years. Nanotechnology itself addresses NPs that are nuclear or atomic aggregates described by a size of under 100 nm. Nanotechnology alludes to the term for the assembling, depiction, control, and

utilization of structures to control the size and shape at the nanoscale. Materials in the nanoscale have exceptional contrasting properties to that of similar materials in bulk. These distinctions are due to the basic and physical properties of metal molecules and surface-to-volume proportion to nanotechnology progression, where countless nanomaterials display characteristic properties. Over the previous decade, few review concentrating on the green synthesis of AgNPs have been published. The majority of them concentrated on a few plants (aloe leaf, Coffea arabica seed, Trianthema decandra, cherry extract, Macrotyloma uniorum, and Rosa rugosa microbial sources), biopolymers (chitosan for synthesis of AgNPs. Several characterization procedures (DLS, UV-vis, FTIR, XRD, SEM, TEM and EDX) have been employed to investigate information regarding the source, shape, size and properties of AgNPs with respect to different applications. The present review, in contrast to the prior reviews, focuses on the synthetic methods, parameters, characterization techniques, applications, and anticipated antibacterial components from different green ways for the synthesis of AgNPs. [1,2,3,4]



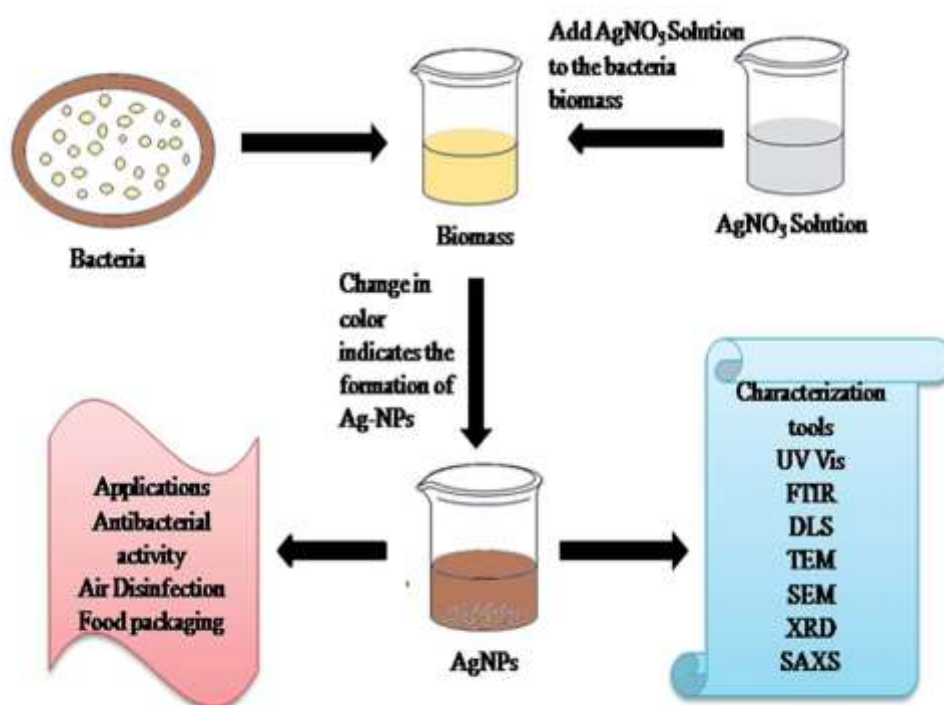
**Fig. 1 Representation of various techniques for the synthesis of NPs.**

The plant-based synthesis of AgNPs is generally adopted more compared to methods that use microorganisms since it can be improved easily, less bio-threatening and do not include the step of cell culture growth. All the parts of a plant (leaves, fruits, roots, seeds, and stems) contain biomolecules (e.g. Enzymes, alkaloids, polysaccharides, tannins, terpenoids, phenols, and vitamins), which are of great therapeutic value and, despite their complex structures, are good for the environment. Plant extract replaces all toxic chemicals such as sodium citrate and sodium borohydride ( $\text{NaBH}_4$ ). The extract from plants assists well in the synthesis of NPs due to the formation of AgNPs stabilized by the flavonoid and terpenoid components present in leaf broth, while the reduction of silver ions is favored by the polyol and water-soluble heterocyclic components of leaf broth. The extract of plant *Salvia spinosa* grown under in vitro conditions was used for the first time to synthesize AgNPs. The first report on the formation of AgNPs by a living plant system Alfalfa sprout was presented by GardeaTorresdey et al. (2003). Alfalfa roots can absorb Ag from agar medium and transfer them in the same oxidation state to the shoots of the plant. These Ag atoms are converted to AgNPs in the shoots. Harekrishna Bar et al. (2009) reported the use of the latex of *Jatropha curcas* as the reducing and capping agent to synthesize AgNPs. Sithara et al. synthesized AgNPs by using leaf extract of *Acalypha hispida* and these AgNPs were used for the detection of  $\text{Mn}^{2+}$  ions. Gavhane et al. (2012) reported the use of the extract of Neem and Triphala to synthesize AgNPs, which were characterized using EDX, TEM, and NTA. TEM and NTA revealed the size of the AgNPs was in the range of 43 nm to 59 nm and they were spherical in shape. Ahmad and Sharma (2012) utilized (pineapple juice) *Ananas comosus* as a stabilizing and reducing agent for the synthesis of AgNPs. Charusheela Ramteke et al. (2012) synthesized antibacterial AgNPs using the leaf extract of (Tulsi) *Ocimum sanctum*. Roy et al. (2014) used *Malus domestica* fruit extract as a reducing and capping agent to synthesize AgNPs with an average diameter of 20 nm. The formation of the NPs was characterized by UV-vis spectroscopy. [5,6,7]



**Fig. 2 Mechanism for the synthesis of AgNPs from plant sources.**

NPs of noble metals such as Ag and Gold have been synthesized utilizing either intra or extracellular inorganic materials created by bacteria. Fig. 3 shows the synthetic procedure for the synthesis of AgNPs from the biomass of bacteria. Slawson et al. (1992) reported that AgNPs are biocompatible in a few bacteria, which are Ag-resistant. Pooley (1982) reported that bacteria aggregate Ag on the bacterial cell walls, and recommended the use of bacteria to industrially recover Ag from ore. First, Klaus et al. (1999) synthesized AgNPs using the biomass of the *Pseudomonas stutzeri* AG259 bacteria (Ag resistant). The amount of AgNPs accumulated by the bacteria cells was up to 200 nm. Kalimuthu et al. (2008) reported the synthesis of AgNPs with a size of 50 nm by adding silver nitrate aqueous solution to the biomass of *B. licheniformis*. A whitish-yellow to brown color confirmed the formation of AgNPs stabilized by the nitrate of enzymes. Nanda and Saravanan (2009) also synthesized AgNPs utilizing culture supernatants of *Staphylococcus aureus*. [8,9]



**Fig. 3 Mechanism for the green synthesis of AgNPs from bacteria.**

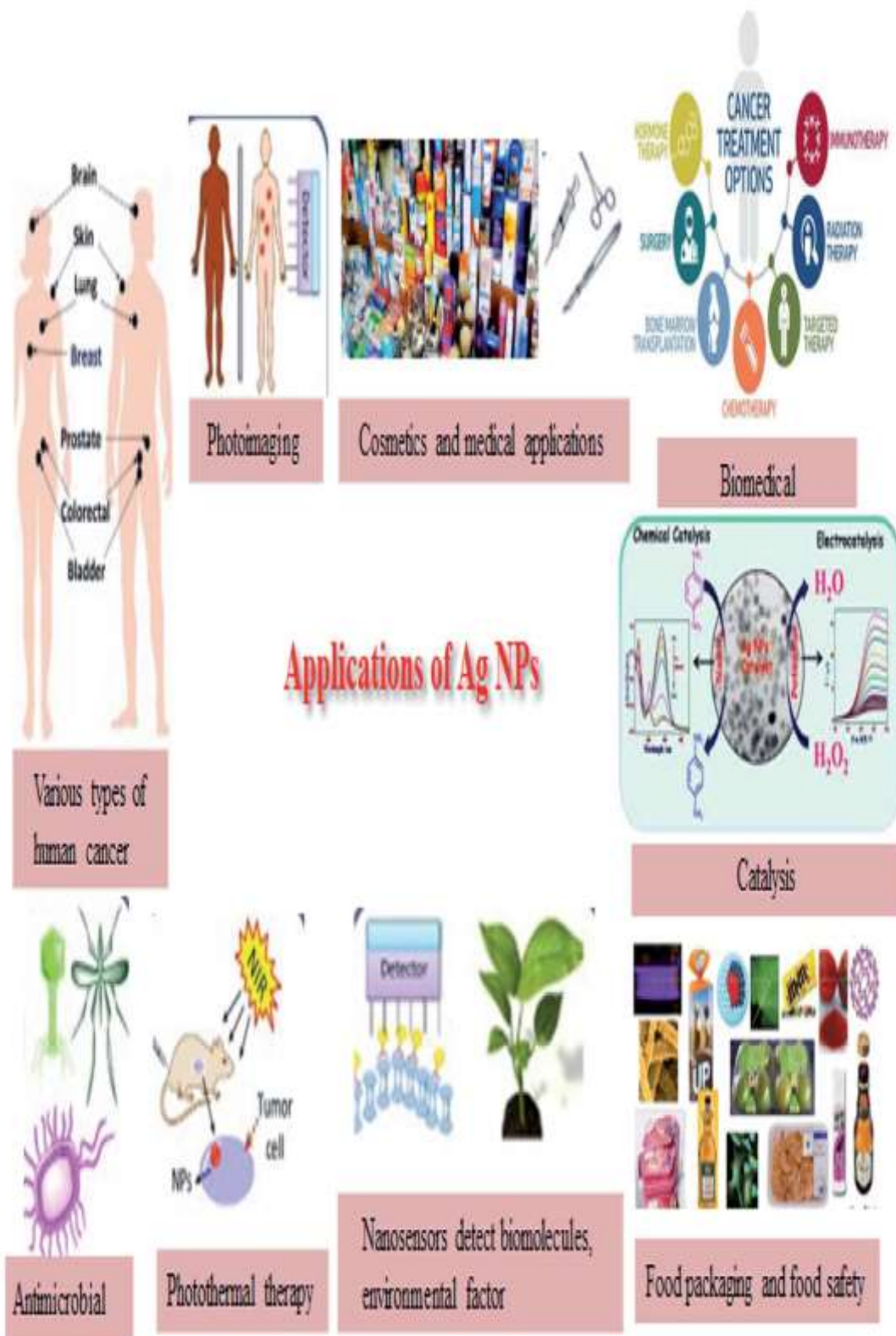


Fig. 4 Various applications of AgNPs.

**Table 1.** Medicinal Plants used for Synthesize of Silver Nano particles

S.No	Latin name	Common name	Family name	Part used	Reference(s)
1	Plumbago rosea	Koduveli	Plumbaginaceae	Root	[10]
2	Hemidesmus indicus	Mahali	Asclepiadaceae	Root	[11]
3	Smilax china	Pavu	Smilacaceae	Root	[12]
4	Melia azadirachta	Vepampattai	Meliaceae	Bark	[13]
5	Acorus calamus	Vasambu	Araceae	Rhizome	[14]
6	Andropogon muricatus	Vetiver	Poaceae	Root	[15]
7	Berberis aristata	Maramanjai	Berberidaceae	Wood	[16]
8	Cedrus deodara	Devadaru	Pinaceae	Wood	[17]
9	Celastrus paniculatus	Cherupunnari	Celastraceae	Seed	[18]
10	Coriandrum sativum	Dhaniya	Apiaceae	Fruit	[19]
11	Cuminum cyminum	Jeeraga	Apiaceae	Fruit	[20]
12	Embelia ribes	Vilangam	Myrsinaceae	Fruit	[21]
13	Glycyrrhiza glabra	Athi madhuram	Fabaceae	Root / rhizome	[22]
14	Holarrhena antidysenterica	Kodagapalari	Apocynaceae	Seed	[23]
15	Negella sativa	Karunjeeraga m	Apiaceae	Seed	[24]
16	Psoralea corylifolia	Karpokarasi	Fabaceae	Seed	[25]
17	Balsamodendron mukul	Gugulu	Burseraceae	Resin	[26]
18	Azadirachta indica	Neem	Meliaceae	Leaf	[27]

## 2 GREEN SYNTHESIS OF NANOPARTICLES

Lot of attention has been diverted to the green synthesis of metal nanoparticles using biological material as the reducing and stabilizing agents and due to the usage of ecofriendly, on-toxic and safe reagents during the biosynthesis process, green synthesis has been considered in the field of toxic chemical and physical methods (Moritz et al., 2013; Rajiv et al., 2013; Caruthers et al., 2007; Nath and Banerjee, 2013; Salam et al., 2012). In the biological method, plant extracts are used for controlled and precise synthesis of several metallic nanoparticles (Rajiv et al., 2013). High surface and a large fraction of surface atoms are responsible for the nanoparticles' atom-like behavior (Dijiken et al., 2000; Singhal et al., 2012). Despite the fact that conventional methods use less time for synthesizing nanoparticles, they contribute to environmental toxicity because they require toxic chemicals as capping agents. Green nanotechnology is an eco-friendly alternative and is cost effective (Chandran et al., 2000; Shankar et al., 2004; Huang et al., 2007) and utilizes proteins as natural capping agents. Synthesis of metal nanoparticles by plants utilize various secondary metabolites, enzymes, proteins and or other reducing agents. [28,29,30,31,32]

### 3 ZnO NANOPARTICLES

Zinc oxide (ZnO) are a class of inorganic metal oxides available and exhibit a wide range of nanostructures. Photocatalytic and photo oxidizing ability against chemical and biological species are used to characterize these metal oxides (Szabo, 2003). U.S. Food and Drug Administration have recognized ZnO as safe (Premanathan et al., 2016). Lower cost, UV blocking properties, high catalytic activity, large surface area, white appearance and their remarkable applications in the field of medicine and agriculture are the advantages of ZnO particles (Kairyte et al., 2013; Kumar et al., 2013; Kajbafvala et al., 2015). Recently, ZnO have been used extensively in environmental remediation and antibacterial activity (Kuriakose et al., 2013).[33,34,35,36,37,38]

ZnO nanoparticles exhibit strong strong antibacterial activity against high temperature and pressure resistant spores (Nicole et al., 2008; Neal, 2008). It is postulated that the generation of hydrogen peroxide or due to the electrostatic binding of the particles on the microbial surface contribute to the antimicrobial activity of ZnO nanoparticles (Zhang et al., 2007). Antibacterial activity of ZnO nanoparticles is of remarkable applications in designing microbial resistant articles (Sharma et al., 2010) for preserving food and wood products (Singhal et al., 2012), cosmetics, novel nanomedicines (Dijiken et al., 2000) wound dressing (Shalumon et al., 2021) and disinfecting agents. Photocatalytic activity of ZnO nanoparticles offers a promising method for waste water treatment (Reddy et al.,2012). Toxic water pollutants released from textile and dyeing industries by utilizing natural source of energy, sunlight are degraded by ZnO and exhibit photochemical reactivity. This could be because of the presence of many active sites and fabrication of hydroxyl radicals on ZnO surface (Baruah et al., 2019; Kajbafvala et al., 2020). Zinc oxide has vast applications in optical, piezo electric, magnetic, and gas sensing. They exhibit high catalytic efficiency, strong adsorption ability and used in sunscreens manufacture (Seshadri et al., 2004), ceramics and rubber processing, waste water treatment, and fungicide (Theodore, 2006; Wang et al., 2008). ZnO nanoparticles can absorb both UV-A and UV-B radiation and therefore offers better protection and improved opaqueness (Theodore, 2006). [39,40,41,42]

### 4 ANTIMICROBIAL ACTIVITY OF ZnO NANOPARTICLES

Understanding the mechanism of antibacterial effect of ZnO nanoparticles is necessary to make better use of these nanoparticles in food products and to develop nontoxic, antimicrobial derivatives but the mechanism is not very clear till date. Some studies have showed that morphology and oxidative stress are responsible for the antibacterial activity of zinc nanoparticles activity (Sourabh et al., 2014; Krishna et al., 2016). However, a few studies have suggested that the antibacterial activity might be because of the disruption of cell membrane activity [29,30]

Another mechanism might be because of the induction of intercellular reactive oxygen species, including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which is harmful to bacterial cells (Jones et al.,2018, Sawai, 2003). ZnO have also been reported to be activated by UV and visible light

in order to generate highly reactive oxygen species such as  $\text{OH}^-$ ,  $\text{H}_2\text{O}_2$ , and  $\text{O}_2^{2-}$ . These radicals and superoxides cannot penetrate into the cell membrane and are likely to remain on the cell surface, but  $\text{H}_2\text{O}_2$  penetrate into bacterial cells (Padmavathy and Vijayaraghavan, 2008).[31]

Yamamoto et al., 2000 stated that the presence of reactive oxygen species (ROS) generated by ZnO nanoparticles was responsible for their bactericidal activity. Zhang et al., 2010 further stated that chemical interactions between hydrogen peroxide and membrane proteins, or between other chemical species produced in the presence of ZnO nanoparticles and the outer lipid bilayer of bacteria could be responsible for the antibacterial behaviour of ZnO nanoparticles. The hydrogen peroxide which is produced enters the cell membrane of bacteria and kills them. The study also showed that bacterial growth is inhibited by nano-sized ZnO particles. Further, Padmavathy and Vijayaraghavan, 2008 also proposed that the bactericidal activity of ZnO nanoparticles was because of hydrogen peroxide generated by ZnO nanoparticles and the nanoparticles remain in contact with the dead bacteria thereby preventing further bacterial action and continue to generate and discharge hydrogen peroxide to the medium.[43,44]

Phototoxic effect is induced in the aqueous solution of ZnO nanoparticles under UV radiation and produce Reactive Oxygen Species such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and superoxide ions ( $\text{O}_2^-$ ) (Zhang et al., 2011). The active species penetrate into the cells and inhibit or kill microorganisms. This is used in bio nanotechnology and in bio nanomedicine for many antibacterial applications. Therefore, as ZnO absorbs UV light, enhancement of ZnO bioactivity is thought to be as a result of the produced free radicals (Seil et al., 2009)[45,46,47].

## 5 ZnO NANOPARTICLES SYNTHESIS

ZnO nanoparticles have been reported to be synthesized from many plant extracts. In *Azadirachta indica*, stabilizing agents for the nanoparticle synthesis are flavanones, terpenoids and reducing sugars, the constituents of the Neem leaf broth (Nath and Banerjee, 2013). It is suggested that the aldehyde groups are responsible for reduction of zinc oxide to zinc oxide nanoparticles and also stabilize the nanoparticles (Nath and Banerjee, 2017). Noorjahan et al., 2015 proposed a method to synthesize zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* and its characterization by FTIR and SEM analysis. It was seen that from FTIR analysis, alcohols, terpenoids ketones, aldehydes and carboxylic acid were surrounded by synthesized nanoparticles. SEM analysis showed stable Zinc oxide nanoflakes and spindle shaped nanoparticles. The size of the ZnO nanoparticles synthesized were found to be 50  $\mu\text{m}$ .

Elumalai and Velmurugan, 2015 reported the MIC, MBC and MFC values of prepared ZnO NPs against bacteria and fungi. Significant inhibition by the ZnO NPs was seen against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *P. mirabilis* and *E. coli* and fungi strains such as *C. albicans* and *C. tropicalis* with distinct differences in the susceptibility to ZnO NPs in a dose-dependent manner. Among them, *S. aureus* was found to be more susceptible to ZnO NPs.



The mean zones of inhibition ranged from  $9.8 \pm 0.76$  to  $23 \pm 0.50$  (mm). The highest mean zones of inhibition ranged from  $14.4 \pm 0.76$  to  $23 \pm 0.50$  (mm) against *S. aureus*. The MIC values ranged between 6.25 to 50 ( $\mu\text{g/mL}$ ) and MBC and MFC from 12.5 to 50 ( $\mu\text{g/mL}$ ). Antimicrobial activities of ZnO NPs increased with increase of concentrations (50, 100 and 200  $\mu\text{g/mL}$ ) and was considered to be due to the increase of  $\text{H}_2\text{O}_2$  concentration on the surface of ZnO.

Aloe vera has been stated to have immune-modulatory, anti-inflammatory, UV protective, antiprotozoal, and wound- and burn-healing promoting properties. Single crystalline triangular gold nanoparticle (~50-350 nm in size) and spherical silver nanoparticles (~15 nm in size) in high yield have been successfully synthesized. This synthesis is by the reaction of aqueous metal source ions (chloroaurate ions for Au and silver ions for Ag) with the extract of the Aloe vera plant. Aloe vera extract was used to synthesize Spherical zinc oxide nanoparticles and their optical properties were studied (Sangeetha et al., 2019).[48]

Lakshmi et al., 2012 have reported the antibacterial study of zinc oxide nanoparticles synthesized from Aloe vera hot extract (ZnO-AH), cold extract (ZnO-AC) and chemical method (ZnO-C) on six clinically isolated strains namely, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. Significant activity was seen in the zinc oxide particles synthesized by chemical method and particles obtained using Aloe vera cold extract. ZnO-AH showed lesser activity. There was a significant difference in the antibacterial activities of ZnO-AH and ZnO-AC though both synthesized in a similar manner. This variation was because of the size as the size of ZnO-AH is much more than that of ZnO-AC. The smaller the size of nanoparticles better is their activity (Yamamoto 2001a, Makhluaf et al., 2005).[49,50,51]

Mariam et al., 2014 reported a novel synthesis for  $\text{In}_2\text{O}_3$  and ZnO Nanoparticles with particle sizes in the range of 10 to 30 nm using indium nitrate and zinc nitrate solutions. They utilized A. vera extract as a solvent instead of organic solvents. The antibacterial and antifungal activities of the particles were studied using *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *E. coli*, and *S. typhi* and the fungal strains were *A. niger*, *A. flavus*, *A. fumigatus*, *Rhizopus indicus* and *Mucor indicus*. Highest inhibitory activity against the tested bacteria were displayed by the extracts with ZnO +  $\text{In}_2\text{O}_3$  A. vera. *A. niger* growth was also inhibited by the extract. It was concluded that ZnO nanoparticles mixed with A. vera were effective in inhibiting bacterial growth.

*Murraya koenigii* has been reported to have hypoglycemic (Khan et al., 2019) and anti-fungal effects (Das et al., 1965) and also against colon carcinogenesis (Khan et al., 2006). The plant has active agents like polyphenols and flavonoids which have strong roles in the synthesis and stabilization of metal NPs (Roy et al., 2020; Roy et al., 2021). Alam et al., 2022) reported that the contents of polyphenol and flavonoids present in the leaf of *M. koenigii* are 81.9mg Gallic acid equivalent  $\text{g}^{-1}$  and 39.98 mg of quercetin  $\text{g}^{-1}$ , respectively. These compounds act as reducing agents and as the stabilizing agents by adhering on the surface of the NPs formed, and thereby prevent their aggregation and control the particle size.

Elumalai et al., 2023 reported that to study the antimicrobial activity of the leaf extract of *Murraya koenigii* the bioassay was carried out using five bacterial strains such as *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *P. mirabilis* and two fungal strains such as *C. albicans* and *C. tropicalis* as per the disc diffusion and dilution technique. It was concluded that the zone of inhibition increased with increase in zinc oxide nanoparticle concentration and decrease in particle size. The ZnO-NPs were found to be effective for both *S. aureus* and *E. coli* and *P. aeruginosa*. [52]

## 6 CONCLUSION

The green synthesis of metal nanoparticles is an interesting subject of nanoscience. Also, of latest concern is the biosynthesis of metal nanoparticles using plants for the large-scale biosynthesis. Nanoparticles produced by plants are more stable and more varied in shape and size in comparison with those produced by other organisms. In this review, the synthesis of ZnO nanoparticles were reported. The ZnO nanoparticles have varied applications in all fields. Of special mention is the antimicrobial activity of ZnO nanoparticles. Lots of research work still need to be executed to understand the effect of time, temperature, light and other parameters regarding the phytoformation of Nanoparticles.. The enhanced bioactivity of ZnO nanoparticles is attributed to the higher surface area to volume ratio. The antimicrobial activity of ZnO nanoparticles were reported with respect to *Azadirachta indica*, *Aloe vera*, *Murraya koenigii* and *Anisochilus carnosus*. Therefore, based on the reported antibacterial and antifungal activity, it can be concluded that the ZnO nanoparticles constitute an effective antimicrobial agent against pathogenic microorganisms

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