Review Paper:

Biogenic Nanoparticles from Phytochemicals: A New Frontier in fighting Antimicrobial Resistance and Biofilm Formation

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Abstract

Biogenic manufacturing of nanoparticles is a highly efficient method that implements naturally occurring, non-toxic herbs to counteract the shortcomings of traditional physicochemical approaches. Plant synthesis of NPs is considered to be more beneficial when compared with microbial synthesis due to its several benefits such as high reliability, low imminent risk of contamination, inexpensive process and less time consumption. Secondary metabolites or phytochemicals released by plants serve a vital part during the biogenic synthesis of NPs. NPs possess a significant surface area, allowing them to accommodate the agents that act as reducing, stabilizing and capping agents, which prevent the aggregation of NPs. The increased risk of the development of drug-resistant organisms could be due to the overuse and inappropriate use of antimicrobials.

Once the microorganism develops drug resistance and the organism is pathogenic, then it is very much difficult to eliminate the pathogen by the use of antimicrobial agent. Plant-based nanoparticles have a novel application in treating the infection caused by multi-drug-resistant microorganisms due to their antimicrobial efficacy. It is estimated that biofilms are associated with approximately 65% of all human bacterial infections and biofilms formed on medical devices such as catheter can lead to persistent infections. Bacteria in biofilm are resistant to antibiotics and disinfectants, thus another approach for eradication of biofilm is the utilization of nanoparticles as an anti-biofilm agent on medical devices and prevention of the infection development.

Keywords: Nanoparticles, Biosynthesis, Antimicrobial, Drug-Resistant, Biofilm.

Introduction

The synthesis of biogenic nanoparticles involves an interdisciplinary approach related to biological and engineering technologies. Nanoparticles have several advantages due to their nano dimensions and peculiar properties[53]. The applications of nanoparticles when compared to bulk are different because of their considerably high surface area relative to volume, tunable porosity and controllable size and are thus responsible for their numerous applications[23]. The strategies involved in the nanoparticle synthesis involve two approaches: Bottom-Up and Top-Down. NPs can be manufactured using numerous processes including sol-gel, chemical processes, laser ablation and biogenic synthesis[64]. The classic physical and chemical techniques employed for the manufacturing of NPs had a disadvantage that it employs the usage of high pressure, high energy and hazardous compounds and hence is not an eco-friendly approach. Furthermore, it produces large-sized particles.

Low rate of production of NPs via physical techniques was also one of the main concerns. The chemical approach involves the usage of reducing agents which involving inorganic and organic chemicals which were responsible for environmental damage by releasing harmful by-products into the environment[32]. Biogenic fabrication of nanoparticles is a highly efficient method which implements naturally occurring, non-toxic herbs to counteract the shortcomings of traditional physicochemical approaches[13]. Biosynthesis of NPs is accomplished by plants, algae fungi and bacteria. Plants produce different phytochemicals which are accountable for the biosynthesis of NPs. Distinct components of plants such as stems, fruit, roots and leaves can be utilized for the manufacturing of NPs[40].

The plant extract comprises of various compounds including phenolics, terpenoids, flavonoids and others. These compounds act as reducing agents, converting metal ions into metal nanoparticles. The produced nanoparticles are coated with a shell that can be derived from either inorganic or organic sources [44]. Phenolics and polyphenols are the secondary metabolites which arise from phenylpropanoid pathway, responsible for the production of phenylpropanoids directly. Polyketide acetate/malonate pathway is responsible for the production of monomeric and polymeric phenols and polyphenols[55]. Under environmental stresses such as low temperature, nutrient deficiency and pathogenic infection, these phenolics act as defence compounds. Under stressful conditions, the plant phytochemicals are accountable for an increased production of oxidative species and free radicals in plants.

Apart from their function as reducing agents, the phytochemicals found in plants also function as capping and stabilizing agents during the biosynthesis of MNPs. Plant synthesis of NPs is considered to be more beneficial when

compared with microbial synthesis due to its several benefits such as high durability, minimal chances of contamination risk and reduced time requirement[41]. The increased risk in the development of drug-resistant pathogens could be the overuse and inappropriate use of antimicrobials. Once the microorganism develops the drug-resistance and the organism is pathogenic, then it is very much difficult to eliminate the pathogen by the use of antimicrobial agent.

Zinc oxide nanoparticles are categorised as "Generally Recognised as Safe" (GRAS) by US Food and Drug Administration (FDA)[7]. The utilization of nanoparticles presents a new and innovative approach to address the infection caused by microorganisms that are resistant to multiple drugs. Microbes that are attached to any surface which are often seen as a layer of slime are called biofilms. It is estimated that biofilms are associated with approximately 65% of all human bacterial infections. The microorganisms present in biofilm excrete exopolysaccharides (EPS) which are responsible for protecting microorganisms in biofilm from antibiotics and disinfectants. The metal NPs can restrict the progression of developed biofilms as well as planktonic bacteria.

The exact antimicrobial activity of NPs is not yet understood, it is thought and some reports suggest that they might interact with proteins and lipids followed by their entry into microbial cells, thus interaction of NPs with biological structures may kill the microbial cell and can also produce reactive oxygen species[94]. This review examines the process of biogenic synthesis of nanoparticles, which is facilitated by the phytochemicals found in plant extracts. It also investigates how changes in the concentration of these phytochemicals during nanoparticle synthesis alter the dimensions of the resulting nanoparticles.

Additionally, the study explores the application of these nanoparticles as antimicrobial agents against antibiotic-resistant microorganisms, which pose a substantial risk to human health. Furthermore, it investigates their potential in eradicating biofilms formed by pathogenic bacterial strains.

Biogenic Synthesis

When contrasting with chemical and physical means of synthesis of NPs, biosynthesis of NPs has several advantages, this method is more stable, eco-friendly and cost-effective which implements naturally occurring, non-toxic herbs to counteract the shortcomings of traditional physicochemical approaches. Biogenic fabrication of NPs is carried out by plants, fungi, algae and bacteria. Plants produce distinct kinds of phytochemicals which are accountable for the biosynthesis of NPs.

Distinct components of plants such as stem, fruit, root and leave can be utilized for the manufacturing of NPs [40]. Plant extract is thought to be more valuable when used in the manufacturing of nanoparticles in comparison to microbial synthesis due to its several benefits such as high durability, minimal risk of contamination, inexpensive process and minimal time consumption[41].

A substantial amount of phytochemicals available in the plant extract functions as stabilizing and reducing agents, which are responsible for reducing metal ions into metal NPs[90]. Different solvent systems can be used for the extraction of plant components for synthesizing nanoparticles such as methanol, chloroform, ethyl acetate etc. However, when the research is concentrated on the biogenic manufacturing of nanoparticles, then utilization of the toxic chemicals must be avoided, therefore water is preferred to be used as an aqueous solvent for the extraction of the plant components.

Arunachalan et al[12] extracted phytochemicals from *Chrysopogon zizanioides* by utilizing water as a solvent in order to produce gold and silver NPs. They concluded that water-soluble organic molecules such as phytosterols and alkaloids available in the plant extract of *Chrysopogon zizanioides* were primarily accountable for converting Ag and Au ions into Ag and Au NPs.

Phytochemicals available in the plant extract act as electron donors to the metal ions and reduce them to metal nanoparticles. The functional group of phytochemicals such as polyphenols, terpenoids and flavonoids, react with the metal ion by donating electrons which in turn activate nucleation. Siberian ginseng (*Eleutherococcus senticosus*), which is an oriental adaptogen, is used in the manufacturing of multifunctional Ag and Au nanoparticles. The analysis of Fourier Transform infrared revealed that protein and aromatic hydrocarbons were responsible for stabilizing and reducing silver NPs whereas phenolic compounds played a vital role in synthesizing and stabilizing gold NPs[1]. *Dalbergia sissoo* leaf extract was employed in the manufacturing of MgO NPs.

A number of metabolites are present in the plant including dalsissoside, kaempferol-3-O-rutinoside, sissooic acid, quercetin-3-O-rutinoside etc. are essential in the manufacturing of MgO NPs.[50] Titanium dioxide NPs were synthesized utilizing Aloe vera extract. Using an X-ray diffractometer, they identified the typical size of titanium dioxide NPs to be 20nm. By means of Transmission Electron Microscopy, the formed NPs were found to be crystalline in nature[65].

Diverse plant components such as stem, fruit, root, leave and flower are being used in the biosynthesis of NPs because of the existence of notable phytochemicals. For the manufacturing of NPs using plants, the part of the plant to be used for the biosynthesis was washed and boiled with distilled water followed by squeezing, filtering and addition of metallic salt. The solution color starts changing due to the production of metallic nanoparticles which could be analyzed with the aid of multiple techniques and could be extracted.

Role of phytochemicals in the biosynthesis of nanoparticles

Phenolics and polyphenols are the secondary metabolites which arise from phenylpropanoid pathway, responsible for the production of phenylpropanoids directly. Polyketide acetate/malonate pathway is responsible for the production of monomeric and polymeric phenols and polyphenols[55] . Distinct components of plants can produce primary and secondary metabolites such as polysaccharides, monosaccharides, organic acid, polyphenols, terpenoids and flavonoids, which are accountable for the phyto-fabrication of nanoparticles. Secondary metabolites or phytochemicals released by the plants play an essential part in the fabrication of NPs. Phytochemicals that are available in plant extract, act as an electron donor to the metal ion and reduce it to metal nanoparticles.

The functional group of phytochemicals such as polyphenols, terpenoids and flavonoids, reacts with the metal ion by donating electrons which in turn activates nucleation. These phytochemicals apart from acting as electron donors also act as a capping agents. NPs possess a significant surface area, allowing them to accommodate the agents that act as reducing, stabilizing and capping agents which prevent the aggregation of NPs [32]. Polysaccharides synthesized by plants also act as reducing substances during the manufacturing of NPs because of the presence of hemiacetal reducing end and hydroxyl group.

A systemic study was conducted to determine the approach of creating silver NPs through *Hypericum perforatum* plant extract. Extract of this plant consists of a number of secondary metabolites. However, in the extract, the reduction of Ag^+ ions was specifically facilitated by flavonoids and phenolic acids, while xanthones and phloroglucinols were used as capping agents. Additionally, naphthodianthrones played a dual role in both reducing and acting as capping agents[62]. Different tests can be performed to confirm the existence of various phytochemicals in the plant extract such as the flavonoid test. When NaOH is mixed in the plant extract, a bright yellow color develops, which confirms the presence of flavonoids.

Wagner's test is performed in order to confirm the presence of alkaloids, 1ml of 1.5%v/v HCl can be added to the extract and after adding Wagner's reagent, a brown color appears which provides evidence for the existence of alkaloids. A ferric chloride test can also be performed in which 5% natural ferric chloride is supplemented to the plant extract; the development of a bluish-black colored product verifies the presence of tannins. The frothing tests provide evidence of saponins. The plant extract can be diluted with water, development of a thick layer of foam confirms the presence of saponins.

In a study, *Artemisia absinthium* leaf extract was employed for the manufacturing of ZnO NPs. Phytochemicals available in *A. absinthium* leaf extract are terpenoids, flavonoids, polyphenols, sterols, acetylenes, coumarins which were identified using different tests. Tannins and flavonoids were the primary phytochemicals which serve as an agent to reduce and stabilize ZnO nanoparticles[3]. The role of phytochemicals available in the extract of *Citrus aurantifolia* fruit peel for the manufacturing of Ag NPs was identified using different phytochemical qualitative tests. These tests confirmed the existence of seven different phytochemicals in the extract: phenols, tannins, terpenoids, saponins, steroids, flavonoids and alkaloids. Thus, it was determined that *Citrus aurantifolia* is capable of being utilized in the massive-scale manufacturing of Ag NPs[59].

Tyagi[87] investigated the role of phytochemicals i.e. ascorbic acid and phenolic compounds in the manufacturing of Ag NPs. The ascorbic acid concentration was found to be 35.5088µg/gm and 38.88µg/gm in Marigold and Chenopodium leaf extracts respectively. The phenolic content was found to be 26.99µg/gm and 97.89µg/gm in Marigold and Chenopodium leaf extract respectively. Hence, it was determined that phenolics and ascorbic acid available in Marigold and Chenopodium leaf extract were in charge of Ag NPs biosynthesis. The phytochemicals available in *Myrtus communis* plant extract were identified and the presence of eleven phytochemical components was confirmed. These compounds act as reducing agents and biosynthesized ZnO NPs[5].

Function of phytochemicals in regulating nanoparticle size: The morphology and dimensions of the resulting NPs varied based on the quantity of extract used. For the manufacturing of NPs, it is necessary to have the most effective concentration and ideal physical properties. Initially, when there is a high extract concentration, before reaching the optimum concentration, the nanoparticles produced will be of smaller size. In a saturated state, the metal ion reduction occurs and the converse will occur once the optimum level of concentration is achieved. After achieving the optimum concentration, the increase in the amount of extract will not result in the production of smaller nanoparticles. The reason behind this is that the plant extract's electron transport reaches saturation, resulting in the saturation state[96]. The morphology and dimensions of the NPs can be controlled by regulating the quantity of extract.

The impact of the amount of Gum ghatti and the duration of reaction on the morphology and dimension of the biosynthesized Ag nanoparticles were observed. Ag NPs produced with the gum solution of 0.1% and 1mM $AgNO_3$, which were autoclaved for 30 min, were determined to be spherical in morphology and the average size was determined to be 5.7±0.2 nm. Ag NPs formed with 0.5% concentration of gum solution were observed to be polydisperse, spherical and aggregated and the average size was observed to be 31.6±21.7 nm. With a reaction time of 1hr, the morphology of Ag NPs was observed to be anisotropic with an average size of 27.2±11.5 nm [52]. It was

concluded that gum's capability for reduction rose as reaction time grew.

Fabrication of Ag and Au NPs was achieved by utilizing gallic acid as an antioxidant and tri-sodium citrate as a capping and reducing agent. An elevation in the amount of gallic acid caused a noticeable change in the color of the reaction mixture containing Ag NPs transitioned from a deep yellow to a lighter yellow shade, while in the reaction mixture containing Au nanoparticles, the color shifted from red to dark purple. This observation indicates a direct relationship between the amount of gallic acid and the size of the particles [49]. The impact of the concentration of extract of *Aegle marmelos* fruit pulp on the dimensions of Ag nanoparticles was analyzed. Surface plasmon absorption rises and the size of Ag nanoparticles decreases when the amount of extract increases[82].

The converse situation occurs due to the reason that exceeding the amount of the extract beyond the optimal level will not result in the manufacturing of smaller NPs. By adjusting various parameters such as temperature and pH, the nanoparticle size could be regulated. The controlled biosynthesis of Au nanoparticles was conducted using *Cucurbita pepo* and *Malva crispa*. pH was one of the major parameter for the production of gold NPs. In an unoptimized condition, poly dispersed silver NPs were formed having a size range from 1-100 nm. However, in an optimized condition, uniform size and shape of silver NPs were formed in both pumpkin and curled mallow plant extract[18].

Microbicidal application of biosynthesized nanoparticles against multi-drug-resistant microorganisms

The most significant implementation of metal nanoparticles is their use in antimicrobial activity as they are relatively effective against multi-drug-resistant microorganisms. The development of resistance against several antibiotics can be due to continuous exposure to certain antibiotics. Furthermore, some other factors such as lack of medical knowledge and inappropriate prescription of antibiotics, misuse and excessive utilization of antibiotics can also contribute to the emergence of multiple drug resistance.

Rather et al[66] in their study concluded that self-medication, over-dosage and taking medication from an uncertified medical professional are the major causes for the development of antibiotic resistance.

To overcome this problem, new methods or strategies which are effective against these multi-drug-resistant microorganism, are required. Once the microorganism develops the drug-resistance and the organism is pathogenic, then it is very much difficult to eliminate the pathogen by the use of antimicrobial agent. Use of nanoparticles has a novel application in treating the infection caused by multi-drug-resistant microorganisms.

Microbicidal efficacy of Ag Nanoparticles: Silver NPs are considered to be the most potent NPs to exhibit microbicidal efficacy against multi-drug resistant microorganisms. Silver NPs have demonstrated a significant ability to hinder the proliferation of bacteria. Silver's antibacterial properties have been historically known in Homeopathy and Ayurveda and were practiced in ancient times. With the use of nanotechnology, the size of silver can be decreased to the nanoscale and it is possible to control and enhance its ability to kill microorganisms. This can result in powerful antibacterial effects against bacteria that are resistant to multiple drugs [63]. The plant extract's utilization in the manufacturing of silver nanoparticles has been shown to possess potent bactericidal properties against a wide spectrum of Gram-negative and Gram-positive bacteria.

The difference in soil texture and climate may affect the phytochemical composition of the plant extract and this can result in the difference in antimicrobial efficiency of biosynthesized NPs. In a scientific study, Ag NPs manufacturing was carried out via carrot extract taken from three different regions with a difference in climate and altitude. Antibacterial property of Ag NPs against 7 pathogenic strains of bacteria and 3 macrolide-resistant clinical strains was evaluated. Their result indicated that with a rise in the concentration of Ag NPs, there was a rise in the antibacterial activity and NPs synthesized by carrot grown in different regions were shown to exhibit different antibacterial activity, thus indicating the role of climatic conditions [30].

Syzygium cumini is a medicinal plant which has been employed in the management of diabetes for decades. This plant can be employed in the manufacturing of Ag NPs as it is rich in phytochemicals which are required for the reduction and stabilization of NPs. Diksha et al[21] conducted a study where leaf extract of *Syzygium cumini* was employed in the manufacturing of Ag NPs and their antimicrobial activity was assessed. The bactericidal activity of Ag NPs was tested against nosocomial pathogens, *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* At a concentration of 25µg/mL and 20µg/mL for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, MIC was achieved which demonstrated them as a powerful bactericidal agent, effectively suppressing the bacterial cell proliferation.

The activity of bioactive Ag NPs manufactured using leaf extract of *Senna alexandrina* was evaluated as an antibacterial agent against the pathogenic bacteria that show resistance to multiple drugs, (Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis, Escherichia coli, Acinetobacter baumanii /haemolyticus*). Their result indicated that the MIC of silver NPs when compared with the standard antibiotic (Chloramphenicol) was found to be higher. The MIC of silver NPs was found to be 0.03 to 0.6 mg/mL, while the MBC of Silver NPs ranged from 0.06 to 2.5 mg/mL. Thus,

the study confirmed that these biosynthesized silver NPs act on both Gram-negative and Gram-positive bacteria and thus act as broad-spectrum antibacterial agent[4].

Lawsonia inermis which is commonly called as henna is cultivated in many parts of India. Historically this plant was used to treat various diseases such as rheumatoid arthritis, diabetes, headache, jaundice and fever and is also used as a coloring agent. The efficiency of henna in synthesizing Ag nanoparticles and the biocidal activity of biosynthesized Ag nanoparticles was examined. Silver NPs which were biosynthesized using leaves of *Lawsonia inermis* were tested against multi-drug resistant bacteria from urinary tract infection which include *Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Acinetobacter baumannii, Enterococcus faecalis, Staphylococcus arlettae and Klebsiella pneumoniae*. Their findings concluded that biosynthesized Silver NPs have better antibacterial properties compared to the plant extract and $AgNO_3$ which makes them beneficial to be used in medical area[69].

The compound responsible for the capping of NPs has a significant function in maintaining various physiochemical characteristics as well as the antibacterial activity of biosynthesized NPs.

Also, the type of capping agent chosen or the variations in the capping agent can either deteriorate or improve the antibacterial efficiency of the formed silver NPs. The bactericidal efficacy of silver NPs that were coated with different capping agents, was evaluated. The silver NPs coated with capping agent and bare silver NPs were examined against methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* The silver NPs that were coated with dextran (Dex) and carboxymethyl-dextran, demonstrated the highest efficiency against all the strains of bacteria because of their tiny size which intensifies their interaction with bacteria and better stability[31].

In a previous study by Amjad et al[10], fabrication of Silver NPs was done by employing the vegetable waste extract. For the evaluation of the antibacterial properties of Phyto fabricated silver NPs, they used two poultry pathogens, *Salmonella enteritidis* and *Salmonella gallinarum*. The zone of inhibition against *Salmonella gallinarum*, it was determined to be 31mm at 80 mg/mL and against *Salmonella enteritidis* was found to be 18mm at 80 mg/mL. It was also noted that with a rise in the amount of Ag NPs as a microbicidal agent, the zone of inhibition also increases. Their finding concluded that silver NPs showed more inhibitory effects against *Salmonella gallinarum* as compared to *Salmonella enteritidis*[10].

Microbicidal efficacy of copper Nanoparticles: Historically copper was employed in sterilization of water and wounds and in recent years, it is being utilized as a material in building hospitals due to its antimicrobial activity. Copper nanoparticles have broad-spectrum antimicrobial potential against Gram-negative and Gram-positive bacteria and thus can be employed as an alternative in therapeutics[72]. *Phyllanthus embilica,* commonly called Gooseberry is a plant rich in phytochemicals including polyphenols and ascorbic acid which functions as stabilizing and reducing agents for the manufacturing of NPs. Biogenic synthesis of Copper NPs by the reduction of $CuSO_4$ was executed by utilizing the aqueous extract of *Phyllanthus embilica.* The bactericidal property of the biosynthesized copper NPs was examined against *Staphylococcus aureus* and *Escherichia coli,* the two human pathogenic bacteria.

The formation of zone of inhibition confirmed that these biosynthesized copper NPs showed an excellent microbicidal activity. Also, it was demonstrated that the degree of inhibition was reliant upon the initial concentration of bacteria as well as on the concentration of copper NPs[17]. Secondary bacterial infection at the time of COVID-19 was responsible for majority of deaths during the pandemic. An increase in the utilization of medicines as a result of epidemic resulted in the emergence of multi-drug resistance. The synthesis of copper NPs was executed by employing aqueous leaf extract of *Fragaria ananassa* which is also known as strawberry.

The copper nanoparticles that were produced through biosynthesis were evaluated for their effectiveness against *Pseudomonas aeruginosa* and *Staphylococcus aureus* that were isolated from the sputum of patients of COVID-19. The zone of inhibition's diameter was measured as 22nm against *Staphylococcus aureus* and 12nm against *Pseudomonas aeruginosa.* Hence, the biocidal efficacy of bioactive copper NPs was observed to be greater against Gram-positive bacteria over Gram-negative bacteria[34]. Kaur et al[46] used *Punica granatum* to carry out the biosynthesis of copper NPs. The impact of these copper NPs was studied against some opportunistic pathogens, *Enterobacter aerogenes, Salmonella enterica, Micrococcus luteus* and *Pseudomonas aeruginosa.*

The inhibition zone's diameter of peel extract was observed to be less than that of copper NPs, thus it was assumed that the copper NPs showed higher affinity to the active groups located on the bacterial surface accountable for the microbicidal effect[46]. Copper NPs manufacturing employing rhizome extract of *Corallocarbus epigaeus* was studied and their biocidal efficiency against *Salmonella enterica* serovar typhi a multi-drug resistant microorganism was assessed. Minimum inhibition was determined at 2mM concentration and maximum at 10mM concentration of copper NPs. The human pathogen was effectively inhibited by the green synthetic copper NPs and is an effective antibacterial agent against multi-drug resistance bacteria[74].

Microbicidal efficacy of selenium NPs: Selenium exhibits different biological activities and is an important trace element. In fishes, selenium has an immense part in the

growth and protection. Sustainable biogenic manufacturing of selenium NPs was executed by utilizing the orange peel extract and their microbicidal activity was tested against microorganisms that are resistant to multiple drugs. Selenium NPs exhibited promising antibacterial property against all multi-drug resistant Gram-negative and Gram-positive bacteria. *Staphylococcus aureus* was discovered to be most susceptible to selenium NPs and its MIC was noted to be 25 µg/mL against MRSA. It has been found that selenium NPs possess strong bactericidal properties on bacteria that are resistant to multiple drugs. As a result, they can be employed in the field of medicine [70].

Selenium NPs synthesized using stem and root extract of *Blumea axillaris* were assessed for microbicidal activity against some aquatic pathogens, *Pseudomonas aeruginosa, Aeromonas hydrophila, Escherichia coli, Aeromonas hydrophila, Shigella, Salmonella and Enterococcus* species that were isolated from diseased fish. The selenium NPs possess an inhibitory effect against all aquatic pathogens and proved to be a potent antibacterial agent[20].

In a study, the creation of selenium NPs was executed using *Hibiscus esculentus* leaf extract. Their antibacterial efficacy was evaluated against 8 multi-drug resistant bacteria. The result revealed that the value of the minimum inhibitory concentration of bacteria decreased with an elevation in the amount of Selenium NPs. The highest inhibitory effect of Selenium NPs was shown against ATCC *Proteus mirabilis* and *Enterococcus faecalis* while these NPs showed moderate antibacterial efficacy against the pathogens that were isolated clinically having resistance to multiple drugs such as *E. faecalis* and two strains of *S. aureus* (MRSA and VRSA)[25]. Selenium NPs were bio-fabricated using 3 plant extracts *Malpighia emarginata, Allium cepa and Gymnanthemum amygdalinum.* These NPs were then assessed for their biocidal activity on some Gram-negative and Gram-positive bacteria.

Table 1
Synthesis of nanoparticles utilizing distinct plant parts and the existence of different phytochemicals within the plant extract responsible for the manufacturing of nanoparticles

Plant used	Part of the Plant used	Type of NP	NP synthesized	Size of NP	Phytochemical present
Krameria sp.[9]	Root	Metal	Cu	6.16 nm	Phenols
Chrysopogon zizanioides[22]	Roots	Metal	Ag	10-20nm	-
Berberis vulgaris[14]	Roots Leaves	Metal	Ag	30 to 70 nm	Flavones Terpenoves Amides Ketones Aldehydes Carboxylic acid
Morinda citrifolia[81]	Roots	Metal	Au	12.17-38.26 nm	Triterpenoids isoflavonoids flavonoids alkaloids proteins anthraquinones
Lepidium draba[15]	Roots	Metal	Ag	20–80 nm	Tannins Flavonoids Alkaloids Phenolics
Zingiber officinale[88]	Root	Metal	Ag Au	Au NPs - 5–20 nm Ag NPs -10–20 nm	Phenols Flavonoids Alkaloids
Mimosa pudica[61]	Root	Metal oxide	Fe_3O_4	67 nm	Mimosine Polyphenols Aliphatic amine
Astragalus tribuloides Delile[76]	Root	Metal	Ag	34.2 ± 8.0 nm	Phenolics Saponins Flavonoids Polysaccharides
Nigella sativa[54]	Seeds	Metal oxide	ZnO	50–100 nm	Flavonoids, Alkaloids, Quinones, Phenolics
Peganum	Seeds	Metal	Ag	11nm	Phenolics

Harmala[6]					Flavones Alkaloids Amino acid Terpenoids Polysaccharides Proteins
Myristica fragrans[77]	Seeds	Metal	Ag	7-20nm	Phenols Proteins
Alpinia Katsumadai[36]	Seeds	Metal	Ag	12.6nm	Proteins Flavonoids
Coriandrum sativum[60]	Seeds	Metal	Ag	13.09 nm	Polyphenols
Cydonia oblong[95]	Seeds	Metal	Ag	38nm	Carboxylic acid Ketones Amides Terpenoids Flavones Aldehydes
Eriobutria japonica[75]	Seeds	Metal oxide	ZnO	40.08nm	Proteins Phenolics Sugar Alcohol
Caesalpinia bonducella[80]	Seeds	Metal oxide	CuO	13.07 nm	β-carotene Flavonoids Phytosterinin Citrulline
Diospyros montana[16]	Stem bark	Metal	Ag	28nm	Alkaloids, Saponins, Glycosides, Steroids phenols, Tannins and Flavonoids
Eleutherococcus senticosus[1]	Stem bark	Metal	Ag and Au	126nm and 189nm	Phenolics
Jatropha curcas[37]	crude latex was obtained after cutting the stem	Metal oxide	TiO_2	25-100nm	Curcain, curcacycline A, curcacycline B
Boswellia ovalifoliolata[11]	Stem bark	Metal	Ag	30-40nm	Phenols Carboxylic acid Alkyl halides Alcohol Aliphatic amines
Boswellia Ovalifoliolata[84]	Stem bark	Metal oxide	ZnO	20.3nm	Phenols Carboxylic acid Alkyl halides Alcohol Aliphatic amines
Syzygium alternifolium[93]	Stem bark	Metal	Ag	44nm	Phenols Proteins
Cochlospermum religiosum[73]	Stem bark	Metal	Ag	20–35 nm	Proteins Carbohydrate Polyphenol
Alstonia scholaris[83]	Stem bark	Metal oxide	ZnO	26.2 nm	Alkaloids Carboxylic acid Terpenoids
Olive plant[8]	Leaf waste	Metal	Ag	28nm	Phenolic compounds including gallic acid, quercetin, catechin,

					punicalagin, ellagic acid
Ficus sycomorus[26]	Leaves	Metal	Ag	33 ± 1 nm	Saponins, Tannins, Phenolics, Alkaloid, Flavonoids
Psidium Guajava[58]	Leaves	Metal	Pt	113.6 nm	Vitamin E Sterols Carboxylic acid Phenols Amides Terpenoids Sterols
Artemisia herba-alba and *Morus alba*[2]	Leaves	Metal	Au	10-42nm	Tannins Polyphenols flavonoids
Citrus maxima[43]	Leaves Fruit Peel	Metal	Ag	Ag NPs synthesized by leaves- **396.0 ± 22.0** Ag NPs synthesized by fruit- **121.0 ± 5.4** Ag NPs synthesized by peel- **243.1 ± 30.0**	-
Olea europaea (common olive)[7]	Leaves	Metal oxide	ZnO	50nm	Polyphenols
Artemisia absinthium[3]	Leaves	Metal oxide	ZnO	18.77nm	Flavonoids, tannins
Clerodendrum infortunatum[57]	Leaves	Metal	Ag	27.67 nm	Saponins, Tannins, Alkaloid, Phenols, Flavonoid
Trigonella foenum-graceum[89]	Leaves	Metal oxide	MgO	13nm	Alkaloids, polyphenols, steroids, Saponins, steroidal sapinogens, flavonoids

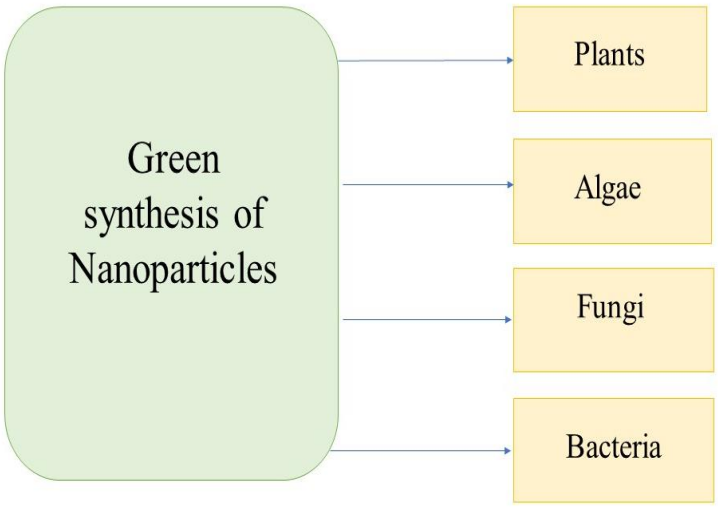

Fig. 1: Green synthesis of nanoparticles involves plants, algae, fungi and bacteria.

Table 2
Minimum inhibitory concentration (MIC), Maximum zone of inhibition of plant-based nanoparticles against multi-drug resistant microorganisms (MDRM)

Plant used	MDRM	Nanoparticle used against M/O	Minimum inhibitory concentration	Maximum Zone of inhibition
Psidium guajava[58]	*Bacillus cereus* (MCC 2243) *Escherichia coli* (MCC 2412) *Klebsiella pneumonia* (MCC 2716) *Pseudomonas aeruginosa* (MTCC 2453)	Platinum	10 µg/ml	*Bacillus cereus*- 8 ± 0.06 mm *Escherichia coli*- 18 ± 0.18 mm *Klebsiella pneumonia*- 15 ± 0.14 mm *Pseudomonas aeruginosa*- 14 ± 0.15 mm
Artemisia herba-alba and *Morus alba*[2]	*Escherichia coli* *Salmonella spp.*	Gold	*Escherichia coli*- 3.125 g/mL *Salmonella spp.* – 6.25 g/mL	*Escherichia coli* -23mm *Salmonella spp.* -16mm
Banana peel extract[86]	*Escherichia coli* *Enterobacter aerogenes* *Salmonella typhi* *Pseudomonas aeruginosa* *Serratia marcescens* *Klebsiella Pneumonia* *Proteus mirabilis*	Silver	-	*Escherichia coli*- 14mm *Proteus mirabilis*- 10mm *Salmonella typhi*- 16mm *Pseudomonas aeruginosa*- 17mm *Serratia marcescens*- 13mm *Klebsiella Pneumonia*- 17mm *Enterobacter aerogenes*- 15mm
Leaf extract of *Citrus maxima*[43]	*Bacillus subtilis (*MTCC 121) *Escherichia coli* MG1655 *Bacillus cereus* (MTCC 430) *Staphylococcus aureus* (MTCC 740) *Pseudomonas aeruginosa* (MTCC 741) *multi-drug resistant Pseudomonas aeruginosa* (ATCC 27853) *Klebsiella pneumoniae (*MTCC 3384) *Staphylococcus aureus (MRSA)* (ATCC 43300) *Salmonella enteritidis* (ATCC 13076)	Silver	*Escherichia coli* - 75 µg/ml *Bacillus subtilis*- 65µg/ml *Bacillus cereus*- 70 µg/ml *Klebsiella pneumoniae*- 60 µg/ml *Pseudomonas aeruginosa* - 55 µg/ml *Staphylococcus aureus*-55µg/ml	*Escherichia coli*- 14±1.3mm *Bacillus cereus* - 11±1.4mm *Staphylococcus aureus* - 12±1.1mm *Bacillus subtilis* - 12±1.5mm *Klebsiella pneumoniae*- 11±1.1mm *Pseudomonas aeruginosa*- 12±1.2mm
Olea europaea (common olive)[7]	*Pseudomonas aeruginosa*	ZnO	3mg/ml	-
Seed extract of *Peganum harmala*[6]	*Pseudomonas aeruginosa*	Silver	15.6 µg/ml	-

Leaves extract of *E. camaldulensis* (red gum)[28]	*Klebsiella pneumoniae*	Silver	15.625 µg/ml - 125 µg/ml.	-
Illicium verum fruit extract aloe vera leaves *Rosmarinus officinalis* leaves extract[29]	*Serratia marcescens* *Achromobacter denitrificans* *Yersinia enterocolitica* *Shigella flexneri* *Klebsiella aerogenes*	Magnetite	-	*Illicium verum synthesized magnetite NPs (2.5%)* *Serratia marcescens*- 6+1.8mm *Achromobacter denitrificans* - 10+1.0 *Yersinia enterocolitica*- 8+1.0 *Shigella flexneri* - 8+0.85 *Klebsiella aerogenes*- 6+1.0
Bark extract of *Cinnamomum zylinicum*[38]	*Pseudomonas aeruginosa,* *Acinetobacter baumanni,* *Klebsiella pneumoniae,* *Staphylococcus aureus*	Silver	*Pseudomonas aeruginosa*- 3.1±0.01µg/ml *Acinetobacter baumanni*- 5.7 ± 0.02 µg/ml *Klebsiella pneumonia*- 2.8 ± 0.03 µg/ml *Staphylococcus aureus*- 4.5 ± 0.04 µg/ml	*Pseudomonas aeruginosa*- 24mm *Acinetobacter baumanni*- 22mm *Klebsiella pneumonia*- 24mm *Staphylococcus aureus*- 25mm
Calvatia gigantea, Mycena leaiana[51]	*Escherichia coli,* *Enterobacter cloacae,* *Proteus mirabilis,* *Staphylococcus aureus,* *Klebsiella Pneumonia,* *Acinetobacter baumannii,* *Pseudomonas aeruginosa*	Silver	*Calvatia gigantea* synthesized Silver NPs- *Enterobacter cloacae*- 30 ± 0.929 mg/ml *Escherichia coli*- 20±0.113 mg/ml *Staphylococcus aureus*- 20 ± 0.209 mg/ml *Acinetobacter baumannii*- 30 ± 0.169 mg/ml *Klebsiella Pneumonia*- 20 ± 0.145 mg/ml *Pseudomonas aeruginosa*- 10 ± 0.181mg/ml *Mycena leaiana* synthesized silver NPs- *Enterobacter cloacae*- 40 ± 0.143 mg/ml *Escherichia coli*- 10± 0.831mg/ml *Staphylococcus aureus*- 30 ± 0.209 mg/ml *Acinetobacter baumannii*- 40 ± 0.163 mg/ml *Klebsiella*	*Calvatia gigantea* synthesized Silver NPs- *Enterobacter cloacae*- 13 ± 1.25mm *Escherichia coli*- 19 ± 2mm *Staphylococcus aureus*- 18 ± 1.73mm *Acinetobacter baumannii*- 13 ± 0.56mm *Klebsiella Pneumonia*- 18 ± 0.75mm *Pseudomonas aeruginosa*- 25 ± 1.52mm *Mycena leaiana* synthesized silver NPs *Enterobacter cloacae*- 9±0.62mm *Escherichia coli*- 15 ± 0.57mm *Staphylococcus aureus*- 14±1.45mm *Acinetobacter baumannii*- 10±1.36mm *Klebsiella Pneumonia*- 10±1.43mm *Pseudomonas aeruginosa*- 16± 1.52mm

			Pneumonia- 10 ± 0.651 mg/ml *Pseudomonas aeruginosa*- 30± 0.712 mg/ml	
Raphanus sativus[35]	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa Candida albicans, Bacillus subtilis*	Silver	*Candida albicans*- 0.06mg/ml *Staphylococcus aureus*- 0.03 mg/ml *Pseudomonas aeruginosa*- 0.12mg/ml *Escherichia coli*- 0.50mg/ml *Bacillus subtilis*- 0.25mg/ml	-
Streblus asper, Syzygium aromaticum, Cymbopogon citratus[45]	*Streptococcus*	Silver	*Streblus asper* Silver NPs- 0.265mg/ml *Syzygium aromaticum* Silver NPs- 0.064 mg/ml *Cymbopogon citratus*- 0.512mg/ml Mixed herbs- 0.064mg/ml	-
Nigella arvensis[27]	*Bacillus subtilis, Pseudomonas aeruginosa Staphylococcus aureus, Escherichia coli*	Silver	5.7 to 10.2µg/mL	*Bacillus subtilis*- 6-25mm *Escherichia coli*- 10-19mm Staphylococcus aureus- 8-10mm

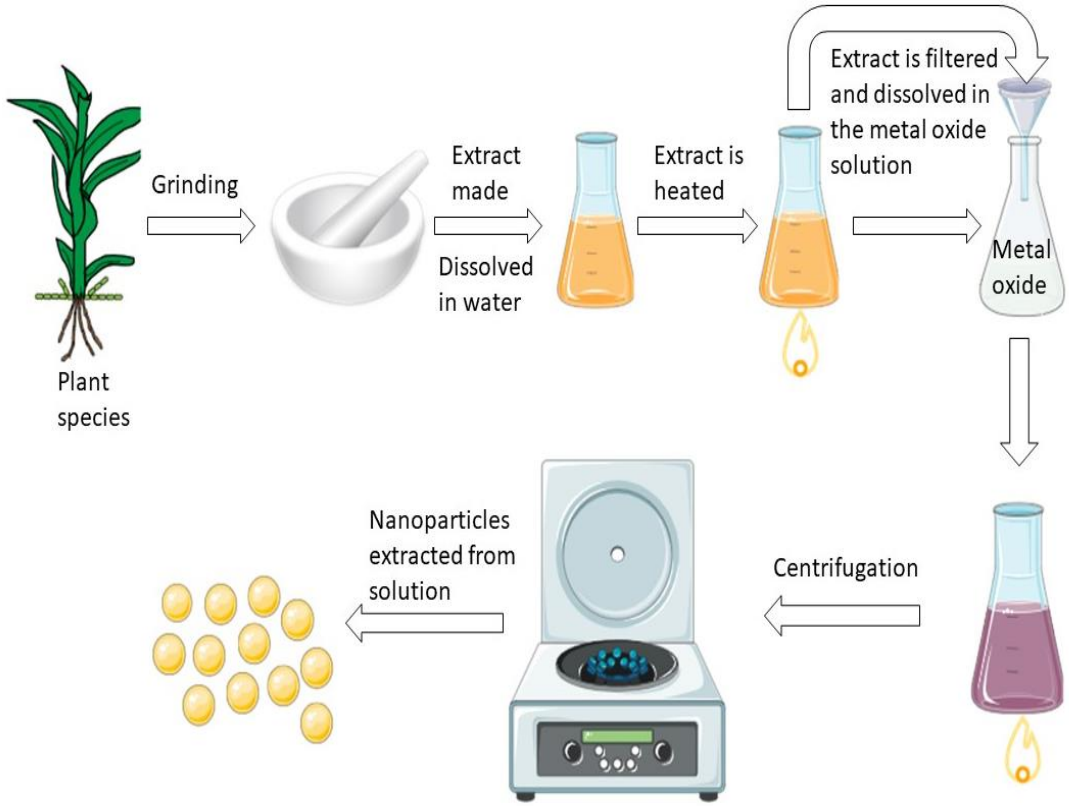

Fig. 2: Schematic diagram illustrating the steps involved during Nanoparticle synthesis using the plant extract.

The selenium NPs exhibited a suppressive impact against all Gram-positive bacteria. However, they were unable to demonstrate their capacity to suppress the proliferation of Gram-negative bacteria. The haemolytic activity of the NPs was found to be low which indicates low cytotoxicity. Hence the selenium NPs can be used in the place of antibiotics and other antimicrobial agents[79]

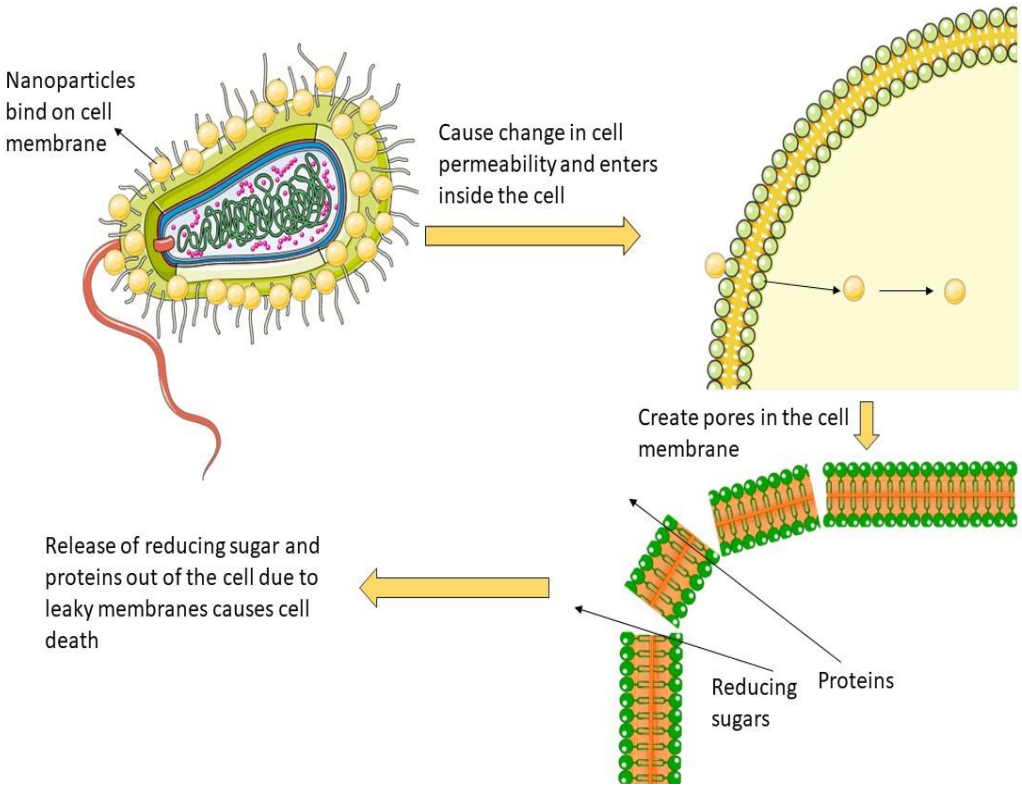

Fig. 3: Mechanism behind microbicidal activity of biosynthesized nanoparticles; The microorganism dies as a result of the nanoparticle's initial binding to the cell wall, which allows it to enter the bacterial cell and change its permeability. This allows proteins and reducing sugar to be released from the bacterial cell wall.

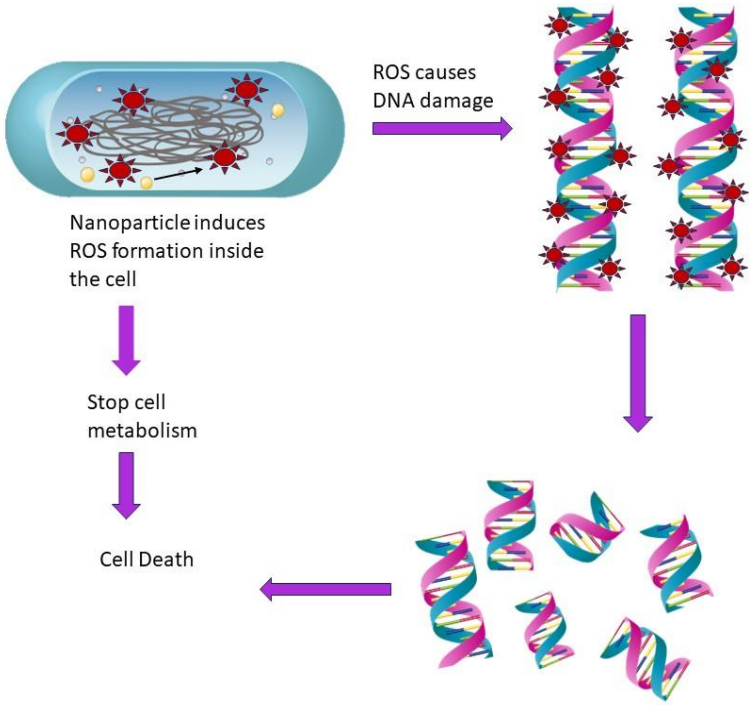

Fig. 4: Mechanism behind microbicidal activity of biosynthesized nanoparticles; nanoparticles within the bacterial cell induce the formation of reactive oxygen species which can damage the DNA and can also coagulate the cytoplasm that can cause the release of cytoplasmic content in the extracellular environment and thus induces cell death

Mechanism behind the antimicrobial activity of Bioactive Nanoparticles

Several mechanisms are reported that play a significant part in the microbicidal effect, however there is no evidence yet that can confirm the exact mechanism or concept that is behind the antimicrobial activity of biosynthesized NPs. Ag NPs can penetrate inside the bacterial cell by first binding to the bacterial cell wall and can thus cause the alteration in the cell membrane's permeability, which may finally result in cell death. In a scientific study, the manufacturing of Ag NPs utilizing the seed extract of *Tectona grandis* was executed. Their result illustrated that the change in the permeability of cell membrane was responsible for proteins and reducing sugar leakage from the bacterial cell. When the Silver NPs were incubated with bacteria with a rise in the incubation time, corresponding increase in the leakage of proteins and reducing sugar was observed. This observation was made at regular intervals. Silver NPs were responsible for change in the structure of the cell membranes, causing leaky cell membrane and thus release of proteins and reducing sugar causing cell death[67].

Another mechanism is the generation of free radicals by Ag NPs which possess the potential to harm the cell wall of bacteria making it porous and thus can cause bacterial cell death. When the Ag NPs enter in the cytoplasm of the bacterial cell, it induces ROS formation. Smaller, the size of NPs, more will be the induction of the formation of ROS as small-sized NPs can easily pass across the cell envelope of the bacterial cells and inside the cytoplasm can induce the formation of ROS[24,39]. An increase in the level of ROS can ultimately induce an elevation in oxidative stress which can damage the extracellular cell membrane and intracellular proteins, DNA etc. The cell membrane becomes permeable due to the damage of the cell envelope and is responsible for the release of proteins from the intracellular to the extracellular environment.

An investigation was executed to observe the effects of Ag NPs on two types of bacteria: *Staphylococcus aureus* which is a Gram-positive bacterium and *Escherichia coli* which is a Gram-negative bacterium. The formation of ROS, the activity of lactate dehydrogenase and protein leakage in bacterial cells were seen. The ROS formation was also responsible for the inactivation of the activity of lactate dehydrogenase. It was also observed that the amount of protein leaked was seen higher in *E. coli* compared to *S. aureus*, possibly due to the thickness of peptidoglycan which is higher in gram-positive bacteria in comparison to Gram-negative bacteria[56].

In a study, ROS formation by Ag NPs was assessed. The antibacterial efficacy of these coated Ag NPs was seen in both aerobic as well as in anaerobic conditions. Results confirmed that oxygen is required for the higher production of ROS and thus for higher antibacterial activity of silver NPs. Also, higher temperature induces an increase in the ROS formation, thus it can be noted that ROS formation is possibly the reason for antibacterial activity of silver NPs[92]. Another mechanism of microbicidal action of bioactive NPs can be DNA damage. Chen et al[19] investigated the antibacterial properties of Ag NPs that were incorporated into thermosensitive gel on *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa.*

The TEM analysis indicated that the thermosensitive gel may have caused the destruction of the bacterial cell membrane structure, so that the silver NPs could enter inside the bacterial cell. Further analysis confirmed that bands formed by the DNA of normal organisms were found to be lighter as compared to those treated with thermosensitive gel, which confirmed that the silver NPs that were incorporated into thermosensitive gel condensed their DNA. Further, they also coagulated the cytoplasm which resulted in the release of the cytoplasmic component to the extracellular environment and thus induces cell death. Vishnupriya et al[91] identified the interaction of Silver NPs with *Escherichia coli*. Through Raman spectroscopy, they identified the *in situ* interaction of silver NPs and internalization of silver NPs in *E. coli.*

The time-dependent study of Raman spectroscopy showed that with an increase in the incubation time, it was observed that DNA was degraded due to a reduction in the Raman signals of specific nucleotides, Thus Raman spectra indicated that the silver NPs degraded the DNA and proteins and are thus responsible for the cell death. However, there may be a possibility that bacterial cells may develop resistance against NPs. A report suggested that when *Pseudomonas putida* was exposed to Silver NPs, the bacterium exhibited a notable inhibition of silver NPs internalization by initiating change in the conformation of unsaturated fatty acid. The conversion of cis to trans conformation alters the fluidity of cell membrane, thereby impeding the penetration of silver NPs[33].

Impact of NPs synthesized via eco-friendly green approach on Biofilm formation

Microbes that are attached to any surface which are often seen as a layer of slime, is called biofilm. It is estimated that biofilms are associated with approximately 65% of all human bacterial infections. The microorganisms present in biofilm excrete exopolysaccharides (EPS) which are responsible for protecting microorganisms in biofilm from antibiotics and disinfectants. Biofilm formation by pathogenic multidrug-resistant bacteria can contribute to the development of deadly infections. Plant-based nanoparticles exhibit a potent anti-biofilm property and are capable of being utilized as an alternative to antibiotics against pathogenic multidrug-resistant microorganisms[71].

The research was conducted in which the potential of 70% ethanol, 0.5% sodium hypochlorite and selenium NPs in the eradication of planktonic and biofilm formed by *Acinetobacter baumannii* was assessed. They observed that 10 min of exposure of sodium hypochlorite, eradicated the

biofilm produced by 109 out of 111 isolates. 10 min of exposure to 70% ethanol was able to eradicate only 29 isolates out of 111. Hence, 70% ethanol must be restricted for hand disinfection while sodium hypochlorite can be considered for surface disinfection where the chances of biofilm formation are expected. The minimum concentration of selenium NPs that suppresses the proliferation of biofilm was found to be 0.15 mg/mL, which suggests that these selenium NPs can be used for the coating of medical devices which will act as a potent inhibitor of biofilm formation[78].

A person with Cystic fibrosis can develop an infection with *Pseudomonas aeruginosa.* Once a patient is getting repeated infection from *P. aeruginosa*, it is very difficult to eliminate it from the infected individual. Biosynthesis of ZnO NPs was executed using the extract of *Olea europaea* and their role in eradication of biofilm formed by *Pseudomonas aeruginosa* was studied. MIC was observed to be 3 mg/ml and MBC was observed to be 6 mg/ml. Inhibitory concentration of ZnO NPs in biofilm formation varied from strain to strain and ZnO NPs acted as a potent anti-biofilm and anti-growth compound which can be used in the biomedical sciences as an alternative to the antibiotics against antibiotic resistance microorganism[7].

Polymicrobial biofilms which consist of bacteria and fungi, are ineffective of antibiotics. So to combat these resistant pathogenic biofilms, alternative strategies are being developed. The effects of gold NPs that were manufactured using a bioactive compound were studied. β-Caryophyllene which is derived from different plant species, was studied. To evaluate the property of these β-Caryophyllene synthesized gold NPs, their anti-biofilm activity was tested on the biofilm formed by *Candida albicans* and *Staphylococcus aureus*. The gold NPs exhibited MIC of 512 µg/mL.

The findings indicated that at a concentration of 256 µg/mL, maximum suppression of biofilm formed by *Candida albicans* and *Staphylococcus aureus* was observed. The eradication of complete biofilm was observed at a significantly higher concentration when compared to MIC concentration. Thus, these gold NPs were found to be effective in combating the pathogenic biofilm formed by *S. aureus* and *C. albicans*[48]. Manganese dioxide NPs were synthesized using leave extracts of different plants: *Punica granatum, Matricaria chamomilla*, *Artemisia herba-alba Asso* and *Camellia sinensis.* The anti-biofilm activity of these Manganese NPs was investigated against different bacterial strains.

The greater ability to inhibit biofilm formation was seen by manganese dioxide NPs synthesized by *Punica granatum* and *Artemisia herba-alba asso*[68]. Biogenic synthesis of calcium oxide NPs was executed utilizing an edible fruit, *Ficus carica*. Bactericidal property of the NPs was evaluated against *Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae.* The highest diameter of the zone of inhibition was observed against *P. aeruginosa.*

It was also observed that calcium oxide NPs were effective in eradicating the mature biofilms as well as in preventing the formation of new biofilms. Hence, calcium oxide NPs can be used as a therapeutic agent in nanomedicines and can be used for the coating of medical devices showing an immense potential in eliminating resistant pathogenic biofilm formation[47]. *Crinum latifolium* leaves extract mediated manufacturing of Au NPs was achieved and these gold NPs were further evaluated for their anti-fungal, antibacterial and anti-biofilm forming activity. Anticandidal activity was observed by evaluating MIC which was observed to be in a range of 250 -500 mg/ml. Thus, gold NPs were observed to be promising biofilm inhibitor and potent antimicrobial agent[42].

Green synthesized NPs are more potent biofilm eradicators when compared to chemically synthesized NPs as reported by Swidan et al[85] that silver NPs were synthesized using ginger root extract and were found to be effective antimicrobial agent when compared with chemically synthesized silver NPs. SEM analysis of catheter segment revealed that ginger extract synthesized Ag NPs were found to prevent the enterococcal cells to adhere to the catheter more efficiently, surpassing the antibiofilm efficacy of chemically manufactured Ag NPs. Consequently, the green synthesized Ag NPs demonstrated superior antibiofilm efficiency compared to chemically synthesized silver NPs [85].

Future prospect

Nanoparticles are advantageous in many aspects and play a significant part in different fields such as biomedical sciences, electronic devices, medicine, imaging, environmental science, food packaging and preservation etc. However, it also serves to increase the risk to the health of living organisms. Inhalation of NPs present in the air can damage the lungs and can cause inflammation. Also, their overuse in inappropriate concentrations can cause the NPs to accumulate in the kidneys and liver. Thus, the negative aspects of nanoparticles are extremely little investigated, so the negative side should also be explored.

Due to their antimicrobial and biofilm eradication efficacy, they can be successfully used as a coating on medical devices for preventing biofilm formation but further analysis *in vivo* is required in employing these NPs in clinical practice concerning their safety, efficacy and long-term effects. Furthermore, it is important to remember that the majority of nanoparticle synthesis mentioned in the literature requires the destruction of entire plant or plant parts in addition to a significant amount of plant sources in order to prepare nanoparticles. Thus, the focus of research should be on using more readily available and local resources as well as extracts from various residues including agro- and bio-waste for synthesizing nanoparticles.

Conclusion

Comparing green synthesized nanoparticles to those synthesized using traditional physical and chemical methods, the former methods are highly preferred because of their unique properties which include microbicidal, antioxidant, anti-cancerous and anti-biofilm properties. Plants are able to perform the green synthesis of NPs due to the presence of a diverse range of phytochemicals. Their concentration used during the manufacturing of nanoparticles is crucial in regulating the morphology and dimensions of resulting NPs.

The NPs synthesized through biogenic means demonstrated a higher level of antimicrobial effectiveness when compared to NPs synthesized through chemical methods and successfully can be used as a therapeutic agent in nanomedicines and can be used for the coating of medical devices showing an immense potential in eliminating resistant pathogenic biofilm formation.

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